

SIMD ABSTRACTS

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The 2007 Emmanuel Shapira SIMD Award

The Emmanuel Shapira SIMD Award was established in 2003 to recognize the best paper in the field of biochemical genetics and metabolism published in *Molecular Genetics and Metabolism* (MGM) by an SIMD member or member's trainee. It was named in memory of Emmanuel Shapira, M.D., Ph.D., one of the founders and most ardent supporters of the Society for many years. Dr. Shapira graduated with an M.D. from Hebrew University in Jerusalem and received his Ph.D. in immunochemistry at the Weizmann Institute of Science in Israel. He was a member of the Faculty of the Department of Pediatrics at Northwestern University Feinberg School of Medicine in Chicago, and later became Professor of Pediatrics and Pathology and Director of the Hayward Genetics Center at Tulane University in New Orleans.

Dr. Shapira's clinical and research interests were focused on inborn errors of metabolism. He made numerous contributions to the field of biochemical genetics and was a dedicated physician to his patients and a supportive and compassionate teacher to his students. His consistent participation in the annual meetings of the Society contributed both in knowledge and in spirit by making the meetings both scientifically stimulating and enjoyable. This award, that bears his name and the name of the Society that he cherished, is intended to recognize high-quality work in the field that he so loved. A \$1,000 prize is awarded annually to the first author of the winning paper chosen by a committee that includes several members of the SIMD and the Editor-in-Chief of MGM.

This year the winner of the Emmanuel Shapira award is Eric Goetzman, Ph.D. for his article entitled "Expression and characterization of mutations in human very long-chain acyl-CoA dehydrogenase using a prokaryotic system". E.S. Goetzman, Y. Wang, M. He, A.W. Mohsen, B.K. Ninness & J. Vockley: *Mol. Gen. & Metab.* 91(2), June 2007, p. 138–147. Dr. Goetzman is an Assistant Professor in the Department of Pediatrics at the University of Pittsburgh, Children's Hospital of Pittsburgh. He will give a brief oral presentation Tuesday, March 4th, 2008 at the Annual Meeting of the SIMD in Asilomar.

Past Winners of the Emmanuel Shapira Award:

- 2003 Elena Tartaglini, Ph.D.
 Judith C. Fleming, Ph.D.
- 2004 Gerard T. Berry, M.D.
- 2006 Randy Chandler, M.B.
 Melanie Gillingham, Ph.D., R.D.

The 2008 Neil Buist Award

The Neil Buist Award was established in 2004 in honor of Dr. Neil Buist, a former President of the SIMD, who served continuously for 26 years on the Board of Directors of the Society until his retirement in 2003. It is awarded annually to the trainee who gives the most outstanding oral presentation at the annual meeting. The winner is selected by a committee of SIMD members during the course of the meeting. A plaque honoring the awardee will be presented this year during the meeting.

Past winners are:

- 2004 Lina S. Correa-Cerro, M.D., Ph.D.
- 2005 Amanda Helip-Wooley, Ph.D.
- 2007 Miao He, Ph.D.

2008 TRAVEL AWARD WINNERS

The winners of the 2008 Travel awards are:

Ariel Brautbar, M.D.
Kristina Cusmano-Ozog, M.D.
Ralph DeBerardinis, M.D., Ph.D.
Shweta Dhar, M.D.
Marni Falk, M.D.
Miao He, Ph.D.
Brienne Howarth
James Lim, Ph.D.
Peter McGuire, M.S., M.D.
Sabrina Mitchell
Volkan Seyrantepe, Ph.D.
Ute Spiekerkoetter, M.D.
Joseph Thakuria, M.D.
Lisa Vincent, Ph.D.
Yudong Wang, Ph.D.

Each recipient received a travel award in the amount of \$1,000. Eleven winners of the 2008 Travel awards will give a brief oral presentation Monday afternoon, March 3, 2008 at the SIMD Annual meeting. Four others will present a poster at the SIMD Annual meeting. All have submitted abstracts which are printed in this issue.

Trainee travel awards were supported by a grant from the National Institute of Child Health and Human Development and the Office of Rare Diseases.

Abstracts

Invited Speakers

M. Eileen Dolan, Ph.D.*	Whole Genome Approaches to Genetic Variance in Human Metabolism: Lessons from Pharmacogenetics
Andrea Gropman, M.D.* Rodney Howell, M.D.	New Frontiers in Neuroimaging – Applications to Inborn Errors of Metabolism Research Collaboration and Consensus Development in Inborn Errors of Metabolism – Public and Private Resources and Opportunities
David Koeller, M.D.*	Carnitine Palmitoyl Transferase 1A Deficiency and Newborn Screening: Implications for Public Health
Don Mahuran, Ph.D.* David Mallott, M.D. Paul Pencharz, M.B., Ch.B., Ph.D.*	Lending a Helping Hand: Chaperones and Lysosomal Storage Disease When Should Psychiatry Consult the Metabolic Specialist? Recent advances in the determination of protein and amino acid requirements in humans - with a focus in inborn errors of amino acid metabolism
Piero Rinaldo, M.D., Ph.D.	Clinical Definition of Cutoff Values for Newborn Screening of Metabolic Disorders: An Evidence-Based, Collaborative Approach
Fran Rohr, M.S., R.D., L.D.* Jean-Marie Saudubray, M.D. Kenneth Setchell, Ph.D. Rani Singh, Ph.D., R.D.*	Building Consensus for Nutrition Therapy in Patients with IEM Clinical Approach to Liver Metabolic Diseases: An Overview Diagnosis and Treatment of Genetic Defects of Bile Acid Synthesis Protein Requirements in Patients with IEM: What's Needed to Support Optimal Growth and Clinical Outcome?"
Ron Sokol, M.D. Mendel Tuchman, M.D.*	Mitochondrial Hepatopathies: Emerging Genotypes and Clinical Phenotypes Setting up Multi-Institutional Network Research in Rare Diseases: The Urea Cycle Consortium
Jon Wolff, M.D.*	The Promise of siRNA therapy

*Abstract provided.

Whole Genome Approaches to Genetic Variance in Human Metabolism: Lessons From Pharmacogenetics. M.E. Dolan, C.M. Hartford, R.S. Huang, S. Duan, Department of Medicine, University of Chicago, Chicago, IL 60637, USA

The candidate gene approach has been traditionally utilized to determine the contribution of genetic variation to a particular phenotype, however, the sequencing of the human genome and the genetic resource provided by the International HapMap Project have allowed researchers to greatly expand their focus and perform genome-wide studies. In contrast to the candidate gene approach, a whole genome approach considers the entire genome without bias towards a particular gene or pathway. Advances in genomics technology including the SNP chip, gene expression platforms and the development of software to analyze large data sets generated by such platforms, have provided researchers with novel tools in the quest to find genetic variation contributing to human variation in quantitative traits. These tools have mainly been applied to find genetic variants associated with disease, gene expression or response to therapeutics. Although a major advantage of this approach is that it opens up the possibility of identifying previously unknown genetic variants that contribute to a specific phenotype, a caveat is the likelihood of false positive findings as a result of multiple testing. An important resource for investigating genetic variation comes from the International HapMap Project, an effort focused on characterizing common variations in DNA sequence among 4 different populations including 90 Utah residents with ancestry from northern and western Europe (CEU), 90 Yoruba in Ibadan, Nigeria (YRI), 45 Japanese in Tokyo, Japan (JPT), and 45 Han Chinese in Beijing, China (CHB). Gene expression on a whole genome scale has been performed with microarray platforms allowing for the identification of expression quantitative trait loci. Publicly available dense genotypic data on these samples can be used to perform a genome-wide interrogation of the regulatory mechanisms that underlie gene expression differences in humans. In addition, International HapMap lymphoblastoid cell lines have been used to study population and gender differences in susceptibility to chemotherapy-induced cytotoxicity and to identify genetic variants contributing to pharmacologic phenotypes. These studies illustrate that whole-genome approaches can be applied to the study of complex traits such as gene expression and susceptibility to drug-induced cytotoxicity in cell lines. Additional cellular phenotypes can be evaluated to identify the genetic basis for phenotypic differences within and among populations. This work was supported by NIH/NIGMS Grant GM61393.

New frontiers in neuroimaging. Andrea L. Gropman, Department of Neurology, Children's National Medical Center, Washington, D.C.

The inborn errors of metabolism may present as failure to thrive, damage to multiple organ systems and susceptibility to infections. Many of the inborn errors of metabolism result in significant damage to the developing central nervous system resulting in encephalopathy. The etiology of such injury has not been fully established in many disorders. Though shared mechanisms can be envisioned such as oxidative damage due to overactivation of NMDA receptors with subsequent glutamatergic damage, other causes such as energy depletion or inflammation are possible. Neuroimaging has emerged as a powerful clinical and research tool to study the brain in vivo. Several platforms exist to study neural networks underlying cognitive processes, diffusion of water in axons, and metabolism. For example, magnetic resonance imaging (MRI) has emerged as a powerful tool in the study of normal and abnormal brain structure, function, and biochemistry. In particular, functional MRI (fMRI) has come into its own as a tool to study normal and abnormal brain functions such as learning, memory, and motor learning, recovery from injury, as well as have the potential to delineate cognitive phenotypes observed in various inborn errors of metabolism. White matter microstructure can be studied using diffusion tensor imaging, which may allow abnormal white matter to be visualized prior to appearance of abnormalities on anatomic MRI. Magnetic resonance spectroscopy, a non-invasive method to study brain biochemistry, may allow for the delineation of regional metabolic changes as a result of disease progression and/or

therapeutic intervention. With MRI techniques, one can investigate the relationship between structure, function, genes, and behavior. The scope and limitations of these methods will be discussed in the context of valuable information they provide in the study of inborn errors of metabolism. Special emphasis will be placed on the role and feasibility of ^1H MRS and ^{13}C MRS to study compartmentalized processes occurring uniquely in neurons or astrocytes. Though technically complex, many of these modalities have or soon will move to the clinical arena. In addition, applications of this technology to the study of metabolism in other organ systems such as the muscle and liver will be discussed. As a result of this lecture, the participant will gain knowledge of the various imaging platforms available and how they might be used to study pathophysiology and guide clinical decision making in patients with inborn errors of metabolism.

Carnitine palmitoyltransferase 1A deficiency & newborn screening: Implications for public health. D.M. Koelle, Departments of Pediatrics, Molecular & Medical Genetics, Oregon Health & Science University, Portland, OR 97239, USA.

Following the implementation of expanded newborn screening in Alaska in October of 2003, we observed an unusually high incidence of carnitine palmitoyltransferase 1A (CPT1A) deficiency. Affected infants are identified by the presence of an increased ratio of free carnitine to C16 plus C18 acylcarnitines (C0/C16 + C18 > 100). As of February 2007, seventy-one Alaskan infants had been diagnosed with CPT1A deficiency. All of the affected infants were of Alaska Native heritage, and were homozygous for the same DNA sequence variant (c.1436C→T; P479L), which results in an 80% reduction in catalytic activity.

We found that many affected babies had normal acylcarnitines on both their first and second newborn screens, which are done during the first two days and at 14 days of life, respectively. These babies had been identified following a third screen that was requested to follow up on an unrelated abnormality (e.g. an abnormal thyroid study). Based upon this observation we hypothesized that MS/MS was not identifying all affected infants. To test this we compared the rates of ascertainment of acylcarnitine analysis, and DNA analysis for the presence of the c.1436C→T sequence variant. We observed that ~20% of Alaska Native infants were homozygous for the c.1436C→T sequence variant, but only about 2% had abnormal acylcarnitines. An additional 33% were heterozygous, and 46% did not carry the c.1436C→T sequence variant. Approximately 1% of both heterozygous and non-carrier infants had abnormal acylcarnitines (C0/C16 + C18 > 100).

The clinical consequences of CPT1A deficiency in Alaska Native infants and children are highly variable. Patients have presented with a Reye's like illness, seizures, hypoglycemia, and SIDS. However, it is clear that only a small fraction of homozygous affected infants are symptomatic. Furthermore, with such a high incidence within the population, it is difficult to determine whether CPT1A deficiency has any pathophysiologic role in the symptomatic patients. We are currently working with Alaska Native and Alaska Department of Health officials, and researchers at the University of Alaska, to assess the public health consequences of CPT1A deficiency in the Alaska Native population, and to develop State wide educational programs targeted at health care providers at all levels. Additional efforts will target the identification of risk factors and predictors of at risk infants. A reassessment of newborn screening for CPT1A deficiency is also ongoing.

Lending a helping hand: Chaperones and lysosomal storage disease. D.J. Mahuran^{1,2}, M. Tropak¹, G.H.B. Maegawa^{1,3}, J. Buttner¹, B. Rigat¹, J.T.R. Clarke^{1,3}. ¹Research Institute, Hospital For Sick Children, Toronto, Canada M5G 1X8; ²Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada; ³Institute of Medical Sciences, University of Toronto, Canada.

Objective: To provide an introduction to small molecule-based enzyme enhancement therapy (EET) for diseases associated with mutations (primarily missense) which negatively impact on the ability of the affected enzyme to obtain/retain its native fold. **Methods:** Two lysosomal storage

diseases, late-onset forms of GM2 gangliosidosis (Tay-Sachs and Sandhoff disease, LOTS) and type I Gaucher disease will be used as examples to describe the rationale of the approach and various screening techniques we have developed for identifying candidate compound from small molecule libraries, i.e. the Maybridge library of 50,000 “drug-like” molecules and the NINDS library of 1040 previously FDA-approved drugs. **Results:** For LOTS every compound that was a competitive inhibitor of the target wild-type enzyme (beta-hexosaminidase), i.e. a pharmacological chaperone, also stabilized it towards heat denaturation and was able to enhance the residual activity levels in patient cells; although levels of enhancement were mutation-specific. The best inhibitors were also the best heat-stabilizers and chaperones. From these observations three assays were developed; enzyme inhibition, heat stabilization and a direct readout of enhancement in live cells; for screening libraries with the Gaucher enzyme, beta-glucocerebrosidase. These results were less straightforward. The best inhibitors were not always the best chaperones. Additionally a new class of compounds were found that directly enhanced the specific activity of the enzyme, both in the test tube and in cells, without stabilizing it against heat denaturation. Screening of the NINDS library resulted in “hits” for both enzymes; pyrimethamine (an anti malarial drug) for LOTS and Ambroxol (an expectorant) for Gaucher. Based on the LOTS data the FDA has approved plans for a multi-center Phase I/II clinical trial of pyrimethamine. **Conclusions:** EET is a promising new approach for the treatment of some lysosomal storage diseases and may have even broader clinical applications. Small molecules have the advantage of often being able to cross the blood brain barrier and can usually be given orally. Additionally their use does not preclude co-administration of other types of therapy, such as enzyme replacement therapy or substrate reduction therapy. Ultimately a combination of therapeutic approaches may prove the most effective.

Recent advances in the determination of protein and amino acid requirements in humans—With a focus on inborn errors of amino acid metabolism. B. Paul Pencharz. Departments of Paediatrics and Nutritional Sciences, University of Toronto, Ont., Canada.

The requirements for dietary amino acids are determined by the response of an outcome variable to graded levels of the test amino acid ranging from well below to well above the requirement level. We have extensively applied two-phase linear regression cross-over analysis to define a break-point which is a measurement of mean requirement. Until recently amino acid requirements were based on studies which used nitrogen balance as the end-point. However in adults and children, nitrogen balance is an insensitive parameter. Our group introduced the indicator amino acid oxidation (IAAO) technique as a novel method of determining amino acid requirements in humans based on earlier studies in piglets, lead by Dr. Henry Bayley. This method is based on the assumption that since there is no storage of essential amino acids when one is limiting all the other essential amino acids are oxidized. So, when an essential amino acid (phenylalanine, lysine and leucine have been used) is used as an indicator, the indicator is either oxidized or is incorporated into protein (protein synthesis). Hence oxidation which is measured as the appearance of the tracer in breath as $^{13}\text{CO}_2$ is inversely proportional to whole body protein synthesis. IAAO has become recognized as the “gold standard method to determine amino acid requirements. A modification of IAAO has been made to extend the studies to 24 h in order to incorporate fed and fasted periods, which has also been called 24 h indicator oxidation/balance. No surprisingly requirements from the 24 h method are not different from the original IAAO approach.

All of the dietary essential amino acid except for histidine have been determined using IAAO. Since our approach is also minimally invasive we have also applied it to children. We have shown in 6–10 year old children that the requirement estimates are essentially identical to the adult requirement and hence are an estimate of maintenance requirement. Therefore for a child a growth component has to be added to the maintenance value. We have also been able to apply IAAO to define

requirements in patients with Maple Syrup Urine Disease and Phenylketonuria as well as children with liver disease.

Building consensus for nutrition therapy in patients with inherited metabolic disorders. F. Rohrer¹, D.M. Frazier², S. Van Calcar³. ¹Children’s Hospital Boston Metabolism Program, USA; ²University of North Carolina, Division of Genetics and Metabolism, USA; ³University of Wisconsin-Madison Biochemical Genetics Program.

Information regarding specific nutrition therapy for many of the disorders identified through expanded newborn screening is sparse. Eight dietitians in the professional organization, Genetic Metabolic Dietitians International, conducted a consensus process to determine best practices in nutritional management of selected fatty acid oxidation disorders. The consensus process for very long-chain acyl-coA dehydrogenase deficiency (VLCADD) and medium-chain acyl-coA dehydrogenase deficiency (MCADD) included a review of the literature to identify treatment strategies and the strength of the association between treatment strategies and positive outcomes as well as areas where treatment practices are based on expert opinion versus evidence and more research is needed. A summary of treatment guidelines was drafted and reviewed by experts in the field of metabolism and includes practical information on establishing and monitoring diets for VLCADD and MCADD. The summary is available as a web-based interactive resource (www.gmdi.org) where practitioners may comment online about the guidelines and suggest changes based on their experiences. A dietitian serves as an editor of each guideline. The editor periodically reviews the comments as well as newly published literature and revises the guidelines as needed. The web page serves as a dynamic repository of information and treatment options for fatty acid oxidation disorders and can be modified as more evidence regarding treatment becomes available.

Protein requirements in patients with IEM: what’s needed to support optimal growth and clinical outcome? R.H. Singh. Emory University School of Medicine, Department of Human Genetics, Decatur, GA, USA.

Objective: To review factors impacting protein requirements in patients with inborn errors of metabolism using review of literature and clinical experience. **Content:** Protein intake must be adequate to cover amino acid and nitrogen requirement for optimal growth and maintenance. Recommended protein intake for patients with inborn errors of metabolism (IEM) has been an area of international controversy over the last several decades. The current recommended dietary allowances of protein for healthy individuals in the US are derived from multiple clinical studies. Recommendations for protein vary depending on life stage and gender. The protein RDA for healthy adults is 0.8 g/kg/d which is usually lower than actual intakes based on National Health and Nutrition Examination Survey (NHANES) data. Evaluation of protein requirements in various aminoacidopathies is complicated by the fact that up to 60–80% of the dietary protein may be provided by medical foods which are composed of free amino acids. Free amino acids are utilized differently than amino acids from intact protein, and thus dietary recommendations may need to be adjusted for these differences. Although concern has been expressed that the protein recommendations for patients in the US utilizing medical foods are excessive, the limited data that are available have shown improved compartmental growth and outcomes has been positively associated with higher intact protein intake for patients taking medical foods. Additionally, improved growth has been positively associated with prescribed natural protein. While a case has been made for an adjusted RDA based on nitrogen content of amino acids in medical foods for maintenance requirements, other factors like energy intake, adaptation and methodological factors can make the assessment of protein amino acid needs inherently difficult. **Conclusions:** Higher protein intake consisting of a combination of medical food and natural protein appears to be related to improved outcomes. Systematic studies utilizing new methodologies available for the determination of how the use of free amino acids differs from the use of amino acids in intact protein may help define protein requirements of patients with IEM.

Setting up a multi-institutional research network in rare diseases: The urea cycle consortium. M. Tuchman. Urea Cycle Disorders Consortium. Children's National Medical Center, Washington, DC 20010, USA.

The Rare Disease Clinical Research Network (RDCRN) has been funded by the NIH as a collaborative grant (U54) since 2003 following the Rare Disease Act which became public law in 2002. The RDCRN currently consists of 10 rare disease research consortia and a Data Technology Coordinating Center for the Network. Its unified goals are to: (1) perform collaborative clinical research in rare diseases; (2) train clinical investigators in rare disease research; (3) establish a centralized data repository and data sharing for rare diseases. The Urea Cycle Disorders Consortium (UCDC), one of the consortia within the RDCRN, focuses on the 8 known inherited urea cycle disorders each of which is caused by a deficiency of a urea cycle protein. The central research project within all RDCRN consortia, including the UCDC, is a natural history longitudinal study. The main objective of the UCDC longitudinal study is to determine the relative frequency, and clinical and laboratory characteristics of patients with urea cycle disorders (UCD) in the United States. Here we present a cross sectional evaluation of participants with urea cycle disorders who have registered and/or enrolled in the longitudinal study. Patients with urea cycle disorders have been enrolled into the study in one of 8 UCDC consortium sites across the US. Baseline assessment at the time of enrollment included a detailed medical history, physical examination, laboratory tests and neuropsychological testing and quality of life questionnaire. All information and results were submitted electronically to the DTCC which stored and helped analyze the data. As of October 31, 2007, 171 patients with confirmed UCD and 11 with highly likely diagnoses of UCD have been enrolled. By the same time, 204 self-reported UCD patients in the US have registered with the DTCC contact registry, and of those, 51 (25%) have enrolled in the study. Ornithine transcarbamylase (OTC) deficiency was the most frequent UCD among both the registered and enrolled groups (54% and 56% respectively), followed by argininosuccinic aciduria (ASA, 17% and 14%) and citrullinemia (8% and 14%). Among OTC deficient enrolled participants, 9% were males with an acute neonatal presentation, 18% were males with late onset disease, 22% were symptomatic OTC-deficient heterozygous females and 49% were asymptomatic heterozygous females. All symptomatic patients have been treated with a protein restricted diet. Among enrolled patients, 34% have been treated with Na-phenylbutyrate (Buphenyl) with a much small proportion (5%) treated with Na-benzoate. Among patients with symptomatic OTC deficiency, 78% have been given L-citrulline, while 62% of patients with citrullinemia or ASA have been treated with L-arginine. Additional observations from the longitudinal study will be discussed. The establish-

ment of the RDCRN allows for the first time a comprehensive analysis of rare inherited urea cycle disorders, their frequencies and clinical characteristics and current practices with respect to diagnosis and treatment.

The promise of siRNA therapy. Jon A. Wolff. University of Wisconsin-Madison, Madison, WI, USA.

SiRNA's powerful ability to silence specific genes has generated great interest in its use as a research tool and therapeutic agent for a wide spectrum of disorders that include cancer, infectious disease, neurologic disorders, dominant genetic disorders and metabolic conditions. However, an essential component of these siRNA-based discovery and therapeutic efforts is the safe and efficient *in vivo* delivery of siRNA to the appropriate target cell. SiRNA delivery like the delivery of other nucleic acids fall into two major categories, viral and non-viral. Viral vectors are derived from viruses by the use of recombinant DNA techniques and are most favored because they can efficiently deliver genes into a variety of cells directly *in vivo*. However, issues related to production, safety and immune response need to be addressed so that their clinical potential can be fully realized.

Non-viral delivery may be subdivided into synthetic delivery vehicles and physical methods such as hydrodynamic and electroporation. While synthetic delivery vehicles (SDV's) are widely used to deliver nucleic acids to cells in culture, the challenge has been to develop SDV's that enable *in vivo* delivery. Often the elements that enable *in vivo* targeting, such as PEG groups, inhibit endosomal release. A promising avenue to improve their efficacy *in vivo* is to create SDV's that are chemically-dynamic in that delivery is enabled by the cleavage of chemical bonds upon exposure to various physiological environments or external stimuli. This chemically-dynamic approach employs masked endosomolytic agents (MEA's) that rely upon chemical bond cleavage to unmask a compound's endosomolytic activity. Namely, when the MEA enters the acidic environment of the endosome, a pH-labile bond is broken releasing the agent's endosomolytic capability.

We have incorporated MEA's into a new vehicle for the delivery of siRNA to cells *in vivo*, which we have named Dynamic PolyConjugates. Using this delivery system and simple, low pressure tail vein injections, we have demonstrated effective knockdown of several endogenous genes in rodent liver such as apolipoprotein B (*apoB*). Fatty livers (steatosis) were observed in animals receiving siRNA against apoB, a clear phenotypic change. The delivery system was well tolerated with no significant changes in serum liver enzyme or cytokine levels. Dynamic PolyConjugates are a modular platform system so other ligands could be easily incorporated to enable targeting to other cells, an avenue that is under investigation.

Travel Award Recipients/Short Oral Presentations

1. Ariel Brautbar, M.D. The mitochondrial G13513A mutation frequency in a Leigh like disease cohort: clinical, biochemical and molecular features of six new patients
2. Kristina Cusmano-Ozog, M.D. Outcomes Among Newborns with Abnormal C5-OH on Expanded Newborn Screening in California
3. Ralph DeBerardinis, M.D., Ph.D. Regulation of cellular metabolism by manipulating signal transduction pathways: Akt and c-Myc promote an anabolic phenotype favoring cell growth
4. Miao He, Ph.D. Mutations in the *SC4MOL* gene cause autosomal recessive psoriasiform dermatitis, revealing a novel mechanism for the pathogenesis of psoriasis
UCYCLYD FELLOW
5. Brianne Howarth In vitro effect of miglustat on phosphotransferase deficient I-cells
6. James Lim, Ph.D. Detection of Remethylation Disorders in Dried Blood Spots by Combined MS/MS and LC/MS/MS Methods
7. Peter McGuire, M.S., M.D. Urinary measures of oxidative stress in Inborn Errors of Metabolism
8. Andrew Palladino, M.D. Quantitation of Tissue Acyl-CoAs in Beta-Oxidation Defects using Tandem Mass Spectrometry
UCYCLYD FELLOW
9. Volkan Seyrantepe, Ph.D. Targeted disruption of the Neu4 sialidase gene results in mice with lysosomal storage and abnormal ganglioside catabolism
10. Ute Spiekerkoetter, M.D. Carnitine supplementation induces acylcarnitine production in tissues of very long-chain acyl-CoA dehydrogenase-deficient mice, without replenishing low free carnitine
11. Lisa Vincent, Ph.D. Rescuing a Common Mutation in Hermansky-Pudlak Syndrome Type 1 Using Exon Skipping
12. Yudong Wang, Ph.D. Study of the biophysical relationship between mitochondrial fatty acid oxidation and oxidative phosphorylation.

1. The mitochondrial G13513A mutation frequency in a Leigh like disease cohort: Clinical, biochemical and molecular features of six new patients.

A. Brautbar¹, J. Wang¹, J.E. Abdenur², R.C. Chang², J.A. Thomas³, T. Grebe⁴, C. Lim⁴, B.H. Graham¹, L.J. Wong¹. ¹Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA; ²Division of Metabolic Disorders, Children's Hospital of Orange County, Orange, CA, USA; ³University of Colorado Health Sciences Center and The Children's Hospital, Denver, CO, USA; ⁴Phoenix Genetics Program, St. Joseph's Hospital, Phoenix, AZ, USA.

The mitochondrial G13513A(D393N) mutation in the ND5 subunit of the respiratory chain complex I was initially described in association with MELAS syndrome. Recent observations have linked this mutation to patients with Leigh and Leigh like disease. Approximately 16 patients with Leigh and Leigh like disease, positive for the G13513A mutation have been described to date.

In this study we screened for the G13513A mutation in a cohort of 262 patients with Leigh and Leigh like disease. The mutation was found in a total of 6 patients. Although the G13513A mutation has been originally reported with MELAS syndrome, of the 88 patients with MELAS disease criteria screened for both the G13513A and G13514A mutation, one patient was found positive for the G13514A, but none, for the G13513A mutation. Of the 56 patients with Complex I deficiency none were positive for the G13513A mutation.

We studied the clinical, biochemical, and molecular phenotype of the patients who carry the G13513A mutation. Interestingly, positive patients for the G13513A mutation presented with variable clinical courses that ranged from severe neurological involvement to mild hypotonia and developmental delay. None had dysmorphic features or cardiac conduction abnormalities as had been previously published. Low mutation heteroplasmy in the range of 20–40% was observed in all six patients. This was consistent with the previously reported low heteroplasmy of this mutation in some of the patients with the G13513A mutation and complex I deficiency. However, normal complex I activity was observed in two patients in our cohort, whose muscle biopsies were available for respiratory chain enzyme analyses. As most patients with Leigh like disease and the G13513A mutation have been described with complex I deficiency, this report adds to the few other patients with normal respiratory complex function previously reported. We conclude that in any patient with Leigh or Leigh like disease, testing for the G13513A mutation is clinically relevant and low mutant load may be considered pathogenic, regardless of clinical respiratory chain analysis.

2. Outcomes among newborns with abnormal C₅-OH on expanded newborn screening in California. K.P. Cusmano-Ozog¹, F. Lorey², L. Feuchtbauer², V.T. Sweet¹, T.M. Cowan³, G.M. Enns¹.

¹Department of Pediatrics, Division of Medical Genetics, Stanford University, Stanford, CA, USA; ²Genetic Disease Branch, California Department of Public Health, Richmond, CA, USA; ³Department of Pathology, Stanford University, Stanford, CA, USA.

Elevated newborn screening (NBS) levels of C₅-OH acylcarnitine can be associated with 3-methylcrotonyl-CoA carboxylase deficiency (3MCCD), β -ketothiolase deficiency, methylglutaconic aciduria, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) lyase deficiency and holocarboxylase synthetase deficiency (HCS), as well as maternal 3MCCD in apparently healthy mothers. Biotinidase deficiency (BD) is also rarely identified. Earlier NBS reports indicate the frequency of 3MCCD may be as high as 1/50,000 newborns (Baumgartner 2001), with the other disorders (excluding BD) being much more rare. The purpose of this study is to characterize the diagnostic outcomes among newborns with abnormal C₅-OH screening results in the California NBS population. *Methods:* A retrospective review of the California NBS Program database was performed for the period July 7, 2005–July 6, 2007, representing 1,109,400 newborns. For babies with C₅-OH levels greater than the screening cutoff (1.2 μ M), results of follow-up studies and/or diagnostic outcomes reported by statewide metabolic specialists were

compiled. *Results:* Of the 1,109,400 babies evaluated, 101 had C₅-OH levels greater than 1.2 μ M. Follow-up biochemical and/or enzymatic testing confirmed a diagnosis of 3MCCD in 31 babies. An additional 10 cases were secondary to maternal 3MCCD, two to HCS and one to BD. Screening levels of C₅-OH were indistinguishable between cases of newborn and maternal 3MCCD. No disorder was identified in the remaining 57 cases, including no cases of β -ketothiolase deficiency, methylglutaconic aciduria or HMG-CoA lyase deficiency. Based on these results, the estimated incidence of 3MCCD in California is estimated at 1/36,000 live births, HCS at 1/555,000 and each of the other disorders (excluding BD) at <1/1,100,000. Maternal 3MCCD was identified in approximately 1/111,000 newborns screened, representing one-third the number of newborn 3MCCD cases. *Conclusions:* Following a positive C₅-OH screening result, a metabolic abnormality was identified in 44 of the 101 cases, with 3MCCD as the most common disorder. One of every four identified cases of 3MCCD was maternal in origin. As NBS C₅-OH levels could not differentiate newborn from maternal 3MCCD, follow up testing should be performed in both mother and child. Although mothers identified with 3MCCD through newborn screening may be asymptomatic, a complete evaluation and carnitine supplementation should be considered. Long-term follow up is needed to elucidate the natural history of these disorders so that more clear management guidelines may be formulated for both infants and mothers.

3. Regulation of cellular metabolism by manipulating signal transduction pathways: Akt and c-Myc promote an anabolic phenotype favoring cell growth. Ralph J. DeBerardinis^{1,2}, Nabil Sayed¹, Xiaoyong Zhang³, Anthony Mancuso², Steven B. McMahon³, Craig B. Thompson².

¹Department of Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA; ²Abramson Family Cancer Research Institute, University of Pennsylvania, Philadelphia, PA 19104, USA; ³Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA 19107, USA.

Objective: Most therapies for inborn errors of metabolism (IEMs) aim to maximize nutritional management and prevent catabolism. However, cellular metabolism is orchestrated primarily by growth factor signal transduction. This suggests that intervening in signaling pathways can provide a novel therapeutic approach, possibly stabilizing anabolism and growth. We studied the effects of Akt and c-Myc, two downstream effectors of growth factor signaling, on the metabolic activities that support lipid synthesis in growing cells. *Methods:* We used radioactive tracers, NMR spectroscopy and mass spectrometry to examine metabolism in proliferating cells. We used inhibitors of the Akt pathway, conditionally active c-Myc alleles and RNA interference to study the role of Akt and c-Myc in cell metabolism. Transcriptional effects of c-Myc were determined using quantitative RT-PCR. *Results:* During growth factor stimulation, human glioblastoma cells rapidly consumed glucose and glutamine. Glucose was the preferred carbon source for fatty acid synthesis whereas glutamine metabolism supplied both the anaplerotic flux and the NADPH needed to synthesize fatty acids. The majority of glutamine carbon and nitrogen was secreted rather than incorporated into macromolecules. Inhibition of Akt limited glucose utilization and cell proliferation but did not affect glutamine utilization. By contrast, enhanced c-Myc activity in fibroblasts increased glutamine consumption, glutaminase activity, ammoniogenesis and triglyceride synthesis. These effects were accompanied by increased gene expression of nutrient transporters and enzymes for glutamine metabolism and fatty acid desaturation. RNA interference against c-Myc suppressed these genes and decreased proliferation. *Conclusions:* In proliferating cells, Akt and c-Myc can exert complementary effects culminating in a metabolic platform that facilitates lipid biosynthesis. The data suggest that Akt exerts control over glucose metabolism while c-Myc's effects on transcription direct glutamine utilization. A deeper understanding of the interplay between signal transduction and anabolic metabolism may lead to new therapeutic opportunities in IEM patients in whom catabolic states are life-threatening.

4. Mutations in the *SC4MOL* gene cause autosomal recessive psoriasisform dermatitis, revealing a novel mechanism for the pathogenesis of psoriasis.

M. He¹, L.E. Kratz², J.J. Miche¹, A.N. Vallejo^{1,3,4}, L. Ferris¹, R.I. Kelley², J. Hoover¹, K.M. Gibson¹, J. Vockley^{1,3}. ¹Children's Hospital of Pittsburgh, University of Pittsburgh, School of Medicine, Pittsburgh, PA, USA; ²The Kennedy Krieger Institute, Baltimore, MD, USA; ³Department of Pediatrics, University of Pittsburgh, School of Medicine, Pittsburgh, PA, USA; ⁴Department of Immunology, University of Pittsburgh, School of Medicine, Pittsburgh, PA, USA.

Psoriasis is a common chronic inflammatory and hyperproliferative skin disease that affects 2% of the population and exhibits a strong genetic component in family and mapping studies. We have recently reported the identification of mutations in *SC4MOL*, a gene in the *PSORS9* region on 4q31-34 as the cause of autosomal recessive psoriasis, microcephaly, and developmental delay. This gene encodes a novel sterol C4 methyl oxidase. 4-methyl and 4,4'-dimethylsterols, the direct substrates for sterol C₄ methyl oxidase, are highly elevated in patient plasma and skin. Immune abnormalities were demonstrated in patient, including unusually high population of immature B cells, antigen presenting granulocytes and chronic activation in T cells. The serum cytokine levels in the patient were atypical. The proinflammatory cytokines such as TNF- α is not detectable despite her severe inflammatory skin lesion and she has a modestly high INF- γ and low IL-4, suggesting induction of a T helper-1 pathway. This is consistent with her chronic T cell activation pattern, but does not explain her high serum IgE and IgA levels. Interestingly, mild elevation of methylsterols and immunologic abnormalities in immunocytes and serum cytokines were also detected in the father, an obligate mutation carrier, who exhibits a mild inflammatory arthritis. Although IL-2 is only mildly elevated in patient, IL-2 receptor (CD25) expression in the granulocytes from the patient and her father is increased 30- and 20-fold, respectively, comparing to the controls, suggests cytokine hypersensitivity. In agree with this, patient skin fibroblast showed 10-fold increase in IL-6 production upon TNF- α induction, while IL-6 production in control was unchanged. In our cellular proliferation studies, the growth of the fibroblasts from the patient is 5 times greater than control when grown in low cholesterol media, a condition under which *de novo* cholesterol is induced and the methylsterols in the media peaked. This increase in cell growth decreased when methylsterol production was inhibited pharmacologically. This suggests the inflammatory and hyperproliferative skin disorder in our patient is primarily associated with the elevation of methylsterols in the blood, which are sterols known play a role in nuclear and cytoplasmic maturation in cell cycles. Our findings reveal a novel aspect in the pathogenesis of psoriasis and other autoimmune diseases, and we identified *SC4MOL* as a strong candidate gene for common psoriasis in human.

5. In vitro effect of miglustat on phosphotransferase deficient I-cells. B.S. Howarth¹, D.Y. Hou¹, A.A. Khan², M.P. Potter¹. ¹McMaster University, Hamilton, Ont., Canada; ²University of Calgary, Calgary, Alta., Canada.

Objective: I-cell disease is a lysosomal storage disorder resulting from the deficiency of *N*-acetylglucosaminyl-1-phosphotransferase that leads to improper processing and secondary lysosomal deficiency of many lysosomal enzymes. It is characterized by accumulation of glycosphingolipids and other compounds in body tissues, with lysosomal inclusions found in cells of affected individuals (so-called I-cells). The imino sugar *N*-butyl-deoxynojirimycin (miglustat), currently approved for use in Gaucher disease, can decrease storage of glycosphingolipids by inhibition glucosylceramide synthase, the first step in the biosynthetic pathway for glycosphingolipids. Additionally, miglustat may have a "chaperone-like" activity that can increase the activity of mutant lysosomal enzymes by imino sugars. **Methods:** The effect of miglustat on the cell inclusions from I-cell fibroblasts was tested by co-culture. I-cell fibroblasts were cultured in 20% DMEM (with or without miglustat at 5, 10 or 50 μ M) in 2 cm plates containing a cover-slip for 6 days, medium was changed on the third day. After 6 days, cells were stained in 0.1% Toluidine Blue for 1 h for initial studies (0 or 10 μ M miglustat) or

1% Toluidine Blue for 2 h for further studies (0, 5 or 50 μ M miglustat). Randomly selected cells were scored for inclusions by 2 independent methods: first by manual scoring of the cell area containing inclusions (expressed as a percent of the total cell area) and then by gray scale analysis of each cell, with lower numbers representing darker images (Photoshop 7.0). **Results:** The initial screening showed a significant reduction of mean inclusion area with miglustat at 10 μ M. Mean inclusion area was $17.2 \pm 9.7\%$ versus $31.1 \pm 18.8\%$ in control cells with no miglustat ($p = 0.004$). Gray scale analysis showed a significant increase in mean scores (i.e. lighter staining cells) in 10 μ M versus 0 μ M miglustat (220 ± 14 vs. 202 ± 19 , $p = 0.009$). No significant decrease in mean inclusion area was detected between 0 and 5 μ M or 0 and 50 μ M miglustat groups, but gray scale analysis showed a significant lightening of the cells in the 5 μ M group or 50 μ M group (195 ± 17 and 185 ± 27 , $p = 0.01$ and 0.02 , respectively) vs. controls (150 ± 24). There was some decrease in cell viability at 50 μ M miglustat. **Conclusion:** Initial experiments indicate that miglustat at 5, 10 or 50 μ M can reduce inclusions in cultured fibroblasts from an I-cell deficient patient. Preliminary experiments did not show a clear dose-response effect. Further studies with other I-cell fibroblast lines are necessary, and the underlying mechanism (glucosylceramide synthase inhibition and/or chaperone activity) will be studied.

6. Detection of remethylation disorders in dried blood spots by combined MS/MS and LC/MS/MS methods. J. Lim¹, D. Gavrilov¹, D. Matern¹, P. Rinaldo¹, C. Turgeon¹, J.E. Abdenur², F. Lorey³, A. Bergum⁴, D. Day-Salvatore⁵, J. Bernstein⁶, S. Baumgart⁷, S. Tortorelli¹. ¹Biochemical Genetics Laboratory, Mayo Clinic College of Medicine, Rochester, MN 55905, USA; ²Division of Metabolic Disorders, Children's Hospital of Orange County, CA 92868, USA; ³California Department of Public Health, Richmond, CA 94804, USA; ⁴Inborn Errors of Metabolism Laboratory, Trenton, NJ 08625, USA; ⁵Institute for Genetics Medicine, St. Peter's University Hospital, NJ 08901, USA; ⁶Lucile Packard Children's Hospital, Palo Alto, CA 94301, USA; ⁷Children's National Medical Center, Washington DC 20010, USA.

Objective: The severe form of methylene tetrahydrofolate reductase (MTHFR) deficiency is a rare inborn error of folate metabolism commonly associated with severe neurological manifestations. The defect is in the conversion of methylene-THF (tetrahydrofolate) to methyl-THF resulting in hyperhomocysteinemia and hypomethioninemia. Treatment with betaine has been reported anecdotally to be beneficial, but only when initiated shortly after birth. Although HCY is not one of the metabolites currently measured in dried blood spots by primary screening using MS/MS, we investigated the use of a low cutoff for methionine (Met) as a surrogate indicator for MTHFR and other remethylation disorders, followed by a 2nd tier test for homocysteine. The hypothesis behind this study originated from a confirmed MTHFR deficient case who was found to have a Met concentration of 4 μ mol/L and a HCY level of 157 μ mol/L (abnormal >15) in the original NBS blood spot. **Methods:** A two tier strategy was implemented in January 2007 where all specimens with a Met level lower than 8 μ mol/L were reflexed to a 2nd tier test for HCY determination by LC-MS/MS. For clinical validation, NBS blood spots from 2 confirmed MTHFR, 1 Cbl G, and 2 additional cases with the expected biochemical phenotype but still pending confirmatory diagnosis, were obtained and analyzed. They were all ascertained clinically. **Results:** In the prospective testing, 68,020 newborns have been screened for low methionine levels by MS/MS. Of these, 147 (0.22%) have Met <8 μ mol/L. Currently, none have tested positive for elevated HCY. In the 3 confirmed cases studied, the Met concentration range was 4.68–8.09 μ mol/L (1st percentile = 11 μ mol/L, $N = 262,405$), the HCY levels detected by the 2nd tier test were markedly elevated (41.7–73.7 μ mol/L; abnormal <15). In the 2 unconfirmed cases, the methionine levels were 4.18 and 5.52 μ mol/L and the HCY concentrations were 64.4 and 74.9 μ mol/L, respectively. To maximize sensitivity, Met values <1 percentile (11 μ mol/L) coupled with a Met/Phe ratio <0.18 will also be reflexed to the 2nd tier test. **Conclusions:** Expanding our two tier testing strategy for the early detection of MTHFR deficiency, and possibly Cbl E and G disorders, might improve the

prognosis of this group of diseases since early intervention could lead to an improved outcome. Current data suggest this approach is feasible and the impact on the case load and labor/time requirement is manageable with minimal additional resources.

7. Urinary measures of oxidative stress in inborn errors of metabolism. P.J. Mc Guire^{1,2}, A. Parikh¹, G.A. Diaz^{1,2}. ¹Department of Genetics and Genomic Sciences, Mount Sinai Medical Center, New York, NY 10029, USA; ²Department of Pediatrics, Mount Sinai Medical Center, New York, NY 10029, USA.

Objective: Oxidative stress has been documented in various inborn errors of metabolism (IEM) including PKU, organic acidemias, mitochondrial disease and MSUD using various markers. The current study focuses on establishing the levels of urinary markers of oxidative stress in a broad group of IEM as compared to controls. **Methods:** Urine isoprostanes (lipid peroxidation), di-tyrosine (tyrosine oxidation) and advanced glycation endproducts (glyco-oxidation) were measured in subjects with IEM (HCY 6, MSUD 6, OA 15 {PA 6, CblC 6, IVA 1, MMA 1, holocarboxylase synthase 1}, UCD 9 {ASL 1, Cit 1, OTC 7}). Control subjects unaffected with IEM but acutely ill were enrolled during pediatric emergency department visits. 69 samples were collected from 36 subjects with IEM during clinic visits and hospital inpatient stays. Single samples were collected from 26 control subjects. Urine creatinine determinations for standardization were done using a Beckman Creatinine Analyzer. Samples with dilute urine (creatinine <20 mg/dL) were excluded from the analysis. Urine isoprostanes were determined using an ELISA-based assay (Oxford Biomed EA85). Urine di-tyrosine and AGE measurements were determined by direct spectrofluorometric assay of urine. **Results:** As a group, subjects with IEM displayed significantly greater mean levels of urinary isoprostanes ($p < 0.0002$) and di-tyrosine ($p < 0.0013$). The most marked increases in lipid peroxidation were observed in subjects with HCY ($p < 0.0227$), OA ($p < 0.0006$) and UCD ($p < 0.0001$). Within the OA group, PA ($p < 0.0075$) and CblC ($p < 0.0001$) showed significant increases in isoprostane levels. A $>2\times$ increase in tyrosine oxidation was observed in MSUD ($p < 0.0072$) and OA ($p < 0.0001$). Within the OA group, PA ($p < 0.0036$) and CblC ($p < 0.0001$) showed significant increases in tyrosine oxidation. HCY ($p < 0.1793$) and UCD ($p < 0.7549$) had similar levels of tyrosine oxidation as compared to controls. Glyco-oxidation levels by AGE determination did not show any differences between IEM as a whole and control subjects ($p < 0.1814$). However, when considered individually, CblC samples displayed higher levels of glyco-oxidation ($p < 0.0126$). **Conclusions:** A subset of IEM display higher levels of oxidative stress markers than acutely ill controls in a marker-specific fashion. Elevated levels of lipid peroxidation were observed in HCY, OA and UCD. Elevated levels of tyrosine oxidation were observed in MSUD and OA. Interestingly, elevated levels of all three markers were observed in CblC. These results suggest that markers of oxidative stress are abnormally elevated in a set IEM with generally severe clinical outcomes. If confirmed and extended, these data may provide a rationale for antioxidant supplementation in targeted IEM and a potential tool for determining response to antioxidant therapy.

8. Quantitation of tissue Acyl-CoAs in beta-oxidation defects using tandem mass spectrometry. A.A. Palladino¹, J. Chen², S.B. Narayan², A.W. Strauss³, P.A. Wood⁴, M.J. Bennett⁵, C.A. Stanley⁶. ¹Children's Hospital of Philadelphia, Division of Endocrinology, USA; ²Children's Hospital of Philadelphia, Pathology and Laboratory Medicine, USA; ³Cincinnati Children's Hospital Medical Center, Department of Pediatrics, USA; ⁴University of Alabama at Birmingham, Department of Genetics, UK; ⁵University of Pennsylvania, Children's Hospital of Philadelphia, Pathology and Laboratory Medicine, USA; ⁶University of Pennsylvania, Children's Hospital of Philadelphia, Division of Endocrinology, USA.

Objective: The primary accumulating metabolites in fatty acid oxidation defects are intramitochondrial acyl-CoAs. Typically we measure secondary metabolites such as acylcarnitines, acylglycines and dicarboxylic acids

to study these defects. Methods have not been available for tissue acyl-CoA measurement. Therefore we have developed a method to measure fatty acyl-CoA species that are present in various tissues of mice with fatty acid oxidation defects using tandem mass spectrometry. **Methods:** We have developed a method for the isolation and measurement of multiple chain-length acyl-CoA species in mouse tissues using direct injection-electrospray ionization tandem mass spectrometry. Following the addition of internal standards of 5 nmol [$C^{13}C_2$] acetyl-CoA and 2.5 nmol [C_{17}] heptadecanoic CoA, tissue samples are twice homogenized in methanol:chloroform (2:1), and then undergo phase separation with equal volumes of chloroform and ammonium formate. The upper aqueous layer is saved and washed with an additional volume of chloroform. Methanol is removed by evaporation under a steady stream of nitrogen at room temperature. Solid phase extraction is then performed using a Phenomenex Strata-X weak anion column. The column is conditioned with methanol then water. Samples are loaded followed by a 2% formic acid wash, then a methanol wash. Acyl-CoAs are eluted with 2% ammonium hydroxide then 5% ammonium hydroxide. The pooled eluates are dried down under a steady stream of nitrogen and reconstituted in 100 μ L 5% methanol and loaded onto the HPLC injector. No column is used in the HPLC system, but a C18 guard column is used to prevent backpressure. Data is acquired using the 506.9 neutral loss scan using the multiple reaction-monitoring (MRM) mode using a Waters Quattro Ultima mass spectrometer. **Results:** Our preliminary studies have indicated that this method can identify all long-, medium-, and short-chain acyl-CoA species in mouse liver and muscle including 3-hydroxy and 3-keto species. We are currently applying this method to the analysis of liver from wild type and SCHAD KO mice that have undergone 24-h fasts. Initial data shows a higher ratio of C_4 , C_6 , and C_8 CoAs in the SCHAD KO liver compared to that of the WT. **Conclusions:** We expect to find that we are able to detect the buildup of fatty acyl-CoA metabolites in the various tissues of mice with other defects of fatty acid oxidation including LCHAD, TFP, SCAD, VLCAD, LCAD, MCAD, CPT-I, and SCHAD deficiencies.

9. Targeted disruption of the Neu4 sialidase gene results in mice with lysosomal storage and abnormal ganglioside catabolism. Volkan Seyrantepe¹, Maryssa Canuel², Jibin Zeng², Stéphane Carpentier³, Aureo Caqueret¹, Sergio Marchesini⁴, Claudia Zwingmann⁵, Jacques Michaud¹, Carlos R. Morales², Thierry Levade³, Alexey V. Pshzhetsky^{1,2}. ¹Department of Medical Genetics, CHU Sainte-Justine, University of Montreal, Montreal, Que., Canada; ²Department of Anatomy and Cell Biology, Faculty of Medicine, McGill University, Montreal, Que., Canada; ³Laboratoire de Biochimie "Maladies Métaboliques", INSERM U.858, CHU Toulouse, France; ⁴Department of Biomedical Sciences and Biotechnologies, University of Brescia, Italy; ⁵CHUM, University of Montreal, Montreal, Que., Canada.

Sialidases are glycohydrolytic enzymes that cleave terminal sialic acid residues from a variety of sialoglycoconjugates such as gangliosides. Four different mammalian sialidases have been classified on the basis of their subcellular localization as lysosomal (Neu1 and Neu4), cytosolic (Neu2) and plasma membrane-associated (Neu3). We have previously shown that Neu4 is an ubiquitously expressed mammalian sialidase with considerable activity towards gangliosides at acidic pH. To investigate whether Neu4 sialidase is involved in ganglioside catabolism, we transiently expressed it in α -hexosaminidase A-deficient neuroglia cells from a human Tay-Sachs patient and demonstrated the correction of storage due to the clearance of accumulated GM2 ganglioside. To further clarify the biological role of Neu4 sialidase, we have generated a stable loss-of-function phenotype in cultured HeLa cells and mice with targeted disruption of the *Neu4* gene by homologous recombination. Neu4 sialidase silenced HeLa cells showed reduced activity against gangliosides and had large heterogeneous lysosomes containing lamellar structures. *Neu4*^{-/-} mice showed no apparent difference in size and reproductive ability compared with wild-type littermates. Pathological examination of the *Neu4*^{-/-} mice did not show any changes in the visceral organs; however the microscopic investigation of tissue sections revealed a marked vacuolisation of the lungs and spleen consistent with the lysosomal storage phenotype. We found significantly

increased levels of sialylated gangliosides (GD1a, GD1b and GT1b) in the liver, lung and spleen of the *Neu4^{-/-}* mice by the thin-layer chromatography. In the brain in addition to the increased level of GD1a, a markedly decreased level of GM1 ganglioside was observed in the *Neu4^{-/-}* mice suggesting that Neu4 may be important for desialylation of gangliosides in brain neurons consistent with the *in situ* hybridization data. Increased level of cholesterol, phosphatidylcholine, ceramide and polyunsaturated fatty acids were also detected in the lungs of *Neu4^{-/-}* mice by the high-resolution NMR spectroscopy. Overall, our findings provide definitive evidence for the involvement of Neu4 sialidase in ganglioside metabolism and implicate the Neu4 knockout mice as a valuable tool to explore the degradation pathway of gangliosides.

10. Carnitine supplementation induces acylcarnitine production in tissues of very long-chain acyl-CoA dehydrogenase-deficient mice, without replenishing low free carnitine. Ute Spiekeroetter, Frank ter Veld, Ertan Mayatepek, Sonja Primassin. Department of General Pediatrics, Heinrich-Heine-University, Duesseldorf, Germany.

Objective: Deficiency of very long-chain acyl-CoA dehydrogenase (VLCAD) results in accumulation of long-chain acylcarnitines in blood and tissues. In addition, secondary “carnitine deficiency” has been observed. The clinical symptoms in VLCAD-deficiency are especially attributed to the accumulation of long-chain acylcarnitines. The role of a low free carnitine in disease pathogenesis and a carnitine supplementation have widely been discussed. The mouse model of VLCAD-deficiency (VLCAD^{-/-}) exhibits a similar clinical and biochemical phenotype to those observed in humans. In these mice, the effects of a carnitine supplementation were studied. **Methods:** VLCAD^{-/-} mice were fed with carnitine dissolved in drinking water (about 200 mg kg⁻¹ day⁻¹). Carnitine, acylcarnitines and γ -butyrobetaine (the immediate carnitine precursor in endogenous carnitine biosynthesis) were measured in blood and tissues from VLCAD^{-/-} and wild-type mice with and without carnitine supplementation. Measurements were performed under resting conditions, after exercise and after 24 h of regeneration. **Results:** Long-chain acylcarnitine production was significantly induced in tissues from VLCAD^{-/-} mice with supplementation of carnitine. However, despite carnitine supplementation, free carnitine was still low in skeletal muscle after exercise. Concomitantly, liver carnitine was significantly increased and γ -butyrobetaine significantly decreased. After 24 h of regeneration, carnitine concentrations in skeletal muscle completely replenished to initial values with and without carnitine supplementation. **Conclusion:** The present study demonstrates that carnitine supplementation results in significant accumulation of possibly toxic acylcarnitines in tissues. The expected prevention of “carnitine deficiency” could not be confirmed. The principle mechanism regulating carnitine homeostasis appears to be endogenous carnitine biosynthesis, even in situations of increased carnitine demand such as in VLCAD-deficiency.

11. Rescuing a common mutation in Hermansky-Pudlak syndrome Type 1 using exon skipping. L.M. Vincent¹, T. Blake², R. Hess¹, W.A. Gahl¹, M. Huizing¹. ¹Medical Genetics Branch, NHGRI NIH, Bethesda, MD, USA; ²Genetics and Molecular Biology Branch, NHGRI NIH, Bethesda, MD, USA.

Hermansky-Pudlak syndrome (HPS) is characterized by oculocutaneous albinism, a bleeding diathesis, and other systemic complications, including pulmonary fibrosis and granulomatous colitis due to defects in intracellular protein trafficking. Type-1 HPS (HPS-1), the most common subtype of HPS, occurs among Puerto-Ricans and results from a 16-bp duplication in exon 15 of the *HPS1* gene. Given the recent advances of

therapeutic exon skipping (e.g., for Duchenne Muscular Dystrophy), we sought to utilize anti-sense morpholino oligonucleotides to induce in-frame exon skipping of exon 15 from the pre-mRNA of *HPS1*. We chose the zebrafish (zf) model system for its well-developed use of translation-blocking or splice-altering morpholinos and the time-efficiency and ease of producing and scoring of a hypo- or normal pigmentation phenotype. Preliminary results of embryonic injection of a morpholino overlying the *zfHPS1* gene start site (AUG) in exon 3 (human exon 3) resulted in a mild hypopigmented phenotype observable at post-fertilization day 3, most likely due to abolished translation of the *zfHPS1* protein. Preliminary results using a morpholino homologous to the 3' or 5' regions of zf exon 14 (human exon 15) failed to produce correct and consistent exon skipping. Interestingly, a mixture of these two morpholinos resulted in the inclusion of the proximal introns of zf exon 14. We are now pursuing the targeting of internal exonic sequence and predicted exonic splicing enhancer sequences in zf exon 14 as well as other in-frame exons with mutations known to cause HPS-1. Further investigation into targeted exon skipping could potentially result in clinical applications for the treatment of systematic complications associated with HPS-1 in patients with mutations in in-frame exons.

12. Study of the biophysical relationship between mitochondrial fatty acid oxidation and oxidative phosphorylation. Yudong Wang, Jerry Vockley. Children's Hospital of Pittsburgh, Division of Medical Genetics, and the University of Pittsburgh School of Medicine, Department of Pediatrics. Pittsburgh, PA, USA.

Fatty acid β -oxidation (FAO) and oxidative phosphorylation (OXPHOS) are both energy related metabolic pathways located in mitochondria. Reducing equivalents from FAO in the form of reduced co-enzyme Q (QH2) and NADH enter OXPHOS as substrates of complexes III and I, respectively. Genetic disorders of FAO and OXPHOS are among the most frequent inborn errors of metabolism in humans. Importantly, many patients with genetic disorders of OXPHOS also show evidence impairment of FAO for reasons that are not known. Thus we have begun to examine the physical relationship between these two pathways. Rat liver or skeletal muscle mitochondria were isolated, the mitochondrial membranes were gently dissolved with a mild detergent, and the resultant extracts were subjected to blue native acrylamide gel electrophoresis to separate OXPHOS complexes and supercomplexes. The gels were then transferred to nylon membranes and probed via western blotting with various antisera to α -oxidation enzymes. Extracts were also subjected to sucrose density centrifugation and analyzed by blue native gel electrophoresis or enzymatic assays. VLCAD, LCAD, LCHAD, MCAD, SCAD, ETF, and ETFDH were all found to be associated with respiratory chain supercomplexes in different patterns. When palmitoyl-CoA was added to the sucrose gradient fractions containing respiratory chain supercomplexes in the presence of KCN, cytochrome C was reduced. Cytochrome c reduction could be completely inhibited by myxothiazol (a complex III inhibitor) and MAP (an inhibitor of the long chain acyl-CoA dehydrogenases), and only partially inhibited by rotenone (a complex I inhibitor). Thus reducing equivalents generated by acyl-CoA dehydrogenase and the 3-hydroxyacyl-CoA dehydrogenase steps can each reduce cytochrome c. HPLC analysis of sucrose gradient fractions containing the linked FAO-OXPHOS complexes showed that palmitoyl- and octanoyl-CoA were completely oxidized without the appearance of intermediate α -oxidation products, indicative of metabolic channeling. These results provide direct evidence of physical interaction between α -oxidation and OXPHOS. Moreover, we have shown that our preparation contains a complete, functional, multiprotein α -oxidation complex that supports metabolic channeling of physiologic substrates.

Poster List

Four of our poster presentations are submitted by travel award winners. They are Shweta Dhar, M.D., Marni Falk, M.D., Sabrina Mitchell and Joseph Thakuria, M.D. Please join the SIMD in congratulating them.

1. SIMD BUSINESS
2. SIMD BUSINESS
3. David Adams Juvenile G_{M1} gangliosidosis: Clinical Summary of a Cohort of Seven Affected Patients
4. Fatma Al-Jasmi Computer-Assisted Teaching of Mucopolysaccharidosis by Patient Management Problems
5. Fathiya Al Murshedi Elevated Propionylcarnitine on Newborn Screening and Vitamin B12 Levels
6. Maryam Banikazemi Preliminary Results of a Phase 2 Clinical Trial of Genz-112638 in Patients with Type 1 Gaucher Disease
7. Leticia Belmont Molecular Studies of the CTNS Gene in Mexican Families with Cystinosis
8. Gerry T. Berry The Biochemical Effects of Liver Transplantation in an Infant with Propionic Acidemia and Biliary Cirrhosis
9. Gerry T. Berry Menke Disease Phenocopy in a Female with Normal ATP7A Coding Sequence
10. Amy Blake Description of Two Patients with Clinical Phenotypes Consistent with a Defect in the SUCLA2 Gene
11. Nenad Blau Variable Outcome of Tetrahydrobiopterin Deficiency: Effect of the Time of Diagnosis and Treatment
12. Wim Blom Complementary Techniques for the Analysis of Amino Acids
13. Sarah J. Boldt Elevated Ferritin in Classic Galactosemia – a Sequela of Liver Disease or a “New” Association with Hypoglycosylated Transferrin?
14. Ruben Bonilla Guerrero Genotype Dependent Variations of Galactose-1-Phosphate Concentrations in Galactosemic Patients
15. Catherine Brunel-Guitton Enzyme Replacement Therapy in Gaucher Disease: What Should we Use as Maintenance Dosage?
16. Barbara K. Burton Identification of Sapropterin-Responsive Phenylketonuria (PKU) Patients (pts) in a Single PKU Clinic During an Expanded Access Program
17. Ljubica Caldovic Large Variations in mRNA and Protein Expression of Urea Cycle Genes in the Liver and Extrahepatic Tissues
18. Philippe M. Campeau Twins with Ethylmalonic Encephalopathy and Different Clinical Evolutions
19. Donald H. Chace A Metabolic Examination of Infants on TPN used in the Care of Premature Infants with a Correlation to Observations in Newborn Screening
20. Randy Chandler Genetic Therapy Rescues a Neonatal Lethal Murine Model of mut⁰ Methylmalonic Acidemia
21. Richard C. Chang Gamma Polymerase Deficiency Presenting as Glycogen Storage Disease
22. Kimberly A. Chapman A False Positive Newborn Screen: Goat’s Milk Acidopathy
23. Amelia Chappelle Project DOCC
24. Jeff Chinsky Clinical Spectrum of Mutations in SLC4A4, Encoding the Sodium Bicarbonate Cotransporter Protein NBCe1
25. Donald L. Coppock Resource for Cell Lines and DNA Samples from Propionic Acidemia in the NIGMS Human Genetic Cell Repository
26. Ellen Crushell The Lebers Hereditary Optic Neuropathy Mutation T14484C Can Cause Leigh – Like Disease
27. Aditi Dagli Ethylmalonic Encephalopathy with a Novel ETHE1 Mutation Diagnosed on Newborn Screening
28. Jane DeLuca A Case Report of a Patient with Dual Diagnoses: 3-Methylcrotonyl Carboxylase Deficiency and Schizencephaly
29. Shweta Dhar *TRAVEL AWARD WINNER* GAMT Deficiency should be Considered in Patients with Nonspecific Developmental Delay, Seizures including Myoclonic Epilepsy, and Extrapyrmidal Signs
30. Patricia I. Dickson Intrathecal Recombinant Human α -L-Iduronidase Alleviates Spinal Cord Compression Symptoms and is Well-Tolerated in Attenuated MPS I Patients
31. David Dimmock Simultaneous Detection of Mitochondrial DNA Depletion and Single Exon Deletion in DGUOK Using Array CGH
32. David Dimmock Management and Clinical Outcome of 8 Patients Identified with Mildly Elevated Citrulline
33. Marni Falk In vivo assessment of relative mitochondrial oxidant levels in *C. elegans* mitochondrial mutants
34. Marni Falk Primary Coenzyme Q Deficiency in *Pdss2* Mutant Mice Causes Isolated Renal Disease
35. W. Andrew Faucett An Improved Model for Moving Genetic Tests from Research to Clinical Testing
36. John J. Flanagan Pharmacological Chaperones for the Treatment of Lysosomal Storage Disorders
37. Rebecca L. Forst Novel Mutations in the Fukuyama Congenital Muscular Dystrophy (FCMD) Gene Associated with a Mild Phenotype
38. Diane M. Frazier Maternal 3-MCC Deficiency as a Model for Long Term Outcome
39. E.H. Giannini Development of a disease severity scoring system for patients with Pompe disease
40. K. Michael Gibson Aberrant Thermoregulation in the Mevalonate Kinase-Deficient (Mvk^{+/-}) Hyper-IgD Mouse Model
41. Melanie B. Gillingham Insulin Sensitivity in Subjects with VLCAD or LCHAD Deficiency
42. Andrea L. Gropman Diffusion Tensor Imaging Detects Areas of Abnormal White Matter Microstructure in Patients with Partial OTCD
43. Andrea L. Gropman ¹³C MRS Study of Ornithine Transcarbamylase Deficiency (OTCD)
44. Marjan Huizing 3-Methylglutaconic Aciduria Type III: Insights into the OPA3 Protein
45. Wendy J. Introne Tolerance of Elevated Tyrosine Levels in Patients with Alkaptonuria Receiving Nitisinone
46. Phil M. James Menke Disease Phenocopy in a Female with Normal ATP7A Coding Sequence
47. Reene N. Jethva Clinical Outcomes of Infants with Short-Chain Acyl-Coenzyme A Dehydrogenase Deficiency Detected by Newborn Screening

48. Jessica Joines The M405V Mutation in GCDH Can Cause Clinically Typical GA1, False-Negative Newborn Screens, Normal Glutaric Acid, and Variable 3-Hydroxyglutaric Acid in Serum and Urine
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1. SIMD business**2. SIMD business**

3. Juvenile G_{M1} gangliosidosis: Clinical summary of a cohort of seven affected patients. D. Adams¹, C.P. Morgan^{2,6}, B. Brooks³, S. Yang⁴, C.J. Tiffi^{1,2,4}. ¹National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA; ²National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, USA; ³National Eye Institute, National Institutes of Health, Bethesda, MD, USA; ⁴Childrens National Medical Center, Department of Genetics and Metabolism, Washington D.C., USA.

Objective: We present clinical data for a cohort of seven patients with juvenile-onset G_{M1} gangliosidosis. *Methods:* Individual patients were seen at the National Institutes of Health and/or Children's National Medical Center. History and Physical Examinations were supplemented with past clinical data. Additional evaluation including neuro-imaging, developmental assessment, ophthalmologic evaluation, plain-film X-rays, EEGs and molecular analysis were performed as possible for individual families/patients. *Results:* Ages of patients in our cohort ranged from 3½ to 18 years. The initial presentations for probands occurred at ages ranging from 18 months to 6 years with an age of initial diagnosis ranging from 3 to 13 years. Common medical findings at diagnosis included failure to thrive and failure to achieve developmental milestones. Seizures, developmental regression and boney abnormalities were observed in some patients, as was a prominent loss of social interaction and restricted affect. For two patients, serial brain MRIs were available and showed cortical volume loss over time. Ophthalmologic examination did not find any examples of a cherry red macula. Molecular data collected for 4 of the 7 patients showed a variable pattern of mutations including examples of known populations-specific mutations, and, in one case an infantile-associated mutation plus an adult-onset-associated mutation. Miglustat therapy was ongoing in 4 of 7 patients. While the efficacy of such therapy is not yet clear from our cohort, the known side effects of weight loss and diarrhea were manageable and had not required cessation of therapy. *Conclusions:* Clinical presentations among persons affected with juvenile G_{M1} gangliosidosis are variable and non-specific contributing to a pattern of late diagnosis. Presenting symptoms include delayed, or failed, achievement of developmental milestones and failure to thrive. The clinical variability present in our cohort is likely partially attributable to variation in residual enzyme activity and we are in the process of comparing enzyme activity with clinical observations. Juvenile onset G_{M1} gangliosidosis is an uncommon variant of a rare disease. As such there is limited natural history data available for affected persons and their physicians. We are actively recruiting families willing to participate in an ongoing natural history protocol at the NIH (identifier NCT00029965). An understanding of the expected course of G_{M1} gangliosidosis will provide a basis for the future evaluation of therapeutic agents.

4. Computer-assisted teaching of mucopolysaccharidosis by patient management problems. F. Al-Jasmi, L. Moldovan, J.T.R. Clarke. Division of Clinical and Metabolic Genetics. The Hospital for Sick Children, Toronto, Canada.

Background: Early diagnosis is critical in the management of mucopolysaccharidosis. Educating the general physician and paediatrician are the key for the best management since they are the frontier to recognize those cases. Computer-assisted patient management problems (PMP) are well-know educational device and it has been used extensively in teaching in other areas, such as the management of chronic illness in adults and psychosocial aspects of practice. However; it has never been applied systematically to the teaching of inborn error of metabolism. *Aim:* To develop interactive teaching software for independent learning about the management of mucopolysaccharidosis, by general physicians and postgraduate medical trainees. *Method and result:* The software consists

of two main parts: the e-book and the clinic. The e-book contains six sections: overview, biochemistry, genetics, clinical manifestations, diagnosis and management. The e book is visual illustrative with basic and clinical knowledge that is delivered to the trainee in simple and attractive way. All references are hyperlinked to PubMed and websites, for those needing further information or clarification. The e-clinic is the interactive portion of the software. Within an environment resembling a real clinic, the trainee will be instructed to carry out a standard history, examine the patient, and order appropriate tests. The software provides real patient data, such as urine MPS, radiograph, and TLC plate. The trainee will be then questioned and provided with a final score, along with performance feedback. While a trainee is working on a case, he/she can access the e-book through the book icon. *Conclusion:* Our software is an educational tool for independent learning. It adopts the PMP through the e-clinic as well as it provides the trainee with comprehensive and thorough information through the illustrative e-book.

5. Elevated propionylcarnitine on newborn screening and vitamin B12 levels.

F. Al Murshedi, F. Al Jasmi, E. Crushell, A. Feigenbaum. Division of Clinical and Metabolic Genetics, SickKids and University of Toronto, Toronto, Ont., Canada.

Background: Screening for elevated blood propionylcarnitine (C3) level was incorporated into the expanded newborn screening (NBS) program of Ontario in August 2006. An elevated C3 level may suggest the following diagnoses: Methylmalonic Acidaemia (MMA), Propionic Acidaemia (PA) or disturbances of cobalamin metabolism. *Hypothesis:* In a significant number of neonates with elevated C3 on NBS in our population, the cause is vitamin B12 deficiency. *Methods:* The records of all babies reported to our centre as having elevated C3 >8 imol/l by the central screening laboratory for our province from September 1st 2006–October 31st 2007, were reviewed. *Results:* 59 neonates (26 male) were reported to have an elevated C3 on NBS. Two babies were already clinically diagnosed with MMA at time of reporting and another baby had died from complications of prematurity. On follow-up of the remaining 56 babies; all had normal repeat plasma C3 level. 1/56 urine organic acids showed trace MMA (71 mmol/mol Creat). Total homocysteine (Hcy) levels were slightly elevated in four children at 12, 12.5, 13.2 and 15.9, respectively, (normal <11 mmol/l). 55/56 had vitamin B12 levels recorded: vitamin B12 levels were deficient (<200 pmol/l) in 23 babies, a further 10 had vitamin B12 levels in the range 200–253. None of the 51 babies who had a CBC recorded had evidence of macrocytosis. Three mothers were known to have B12 deficiency. The cohort was multiracial and where ethnicity was recorded (*n* = 55), nearly half were from the Indian subcontinent (23). Incomplete data was available on maternal diet during pregnancy. Few were recorded as being vegan or vegetarian. *Conclusions:* Low neonatal vitamin B12 level is a common cause of elevated C3 on NBS, we presume this is secondary to maternal Vitamin B12 deficiency but this needs further study. Vitamin B12 level is the best identifier of this, rather than MMA, Hcy or CBC findings. The long-term consequences of vitamin B12 deficiency in these babies, especially if breast fed by a B12 deficient mother, deserves study and intervention. We recommend that dietary history of mother and vitamin B12 level be performed at the first step follow up of an elevated C3 level detected on NBS.

6. Preliminary results of a phase 2 clinical trial of Genz-112638 in patients with Type 1 vGaucher disease.

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Introduction: Genz-112638 is a novel oral small molecule inhibitor of glucosylceramide synthase for the treatment of type 1 Gaucher disease (GD1). **Objective:** To assess the efficacy, safety, and pharmacokinetics of Genz-112638 in patients with mild to moderate GD1. **Methods:** An ongoing open-label Phase 2 clinical trial of Genz-112638 (50 or 100 mg bid orally) enrolled patients with GD1 at medical centers in Israel, North America, Russia, and South America. The main efficacy endpoints of the study included changes in hemoglobin level, platelet level, and spleen volume after 52 weeks of Genz-112638 administration. An extension study will follow. **Results:** To date, 26 weeks of follow-up data are available for 8 patients receiving Genz-112638. All 5 males and 3 females (age range: 19–33y) were Caucasian; 7 were of non-Jewish descent. Plasma glucosylceramide levels normalized in all 5 patients for whom data were available. The mean change in hemoglobin from baseline was 1.3 ± 0.5 g/dL and the mean percentage change in platelet level from baseline was $42.7 \pm 20.7\%$, with all patients showing increases following 6 months of therapy. The mean percentage changes from baseline for spleen and liver volume were $-29.8 \pm 7.9\%$ and $-10.1 \pm 6.3\%$, respectively, with all patients showing reductions in organ size following 6 months of therapy. One drug-related adverse event was reported and was mild and transient in nature. **Conclusions:** Initial observations suggest that Genz-112638 may represent a safe, effective, and convenient oral therapy for patients with GD1. Based upon the clinical results obtained thus far, clinical development of Genz-112638 will proceed in ongoing and additional clinical trials.

7. Molecular studies of the CTNS gene in Mexican families with cystinosis.

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Background: The cystinosis gene (*CTNS*) maps to 17p13, spans 23 kb of genomic sequence and is composed of 12 exons. *CTNS* has a mRNA of 2.6 kb encoding a 367 aminoacid protein called cystinosin. The 57 kb deletion is the most common mutation detected in 60–70% North European alleles. **Objective:** Characterization of mutations of *CTNS* gene responsible of cystinosis in a sample of Mexican patients. **Materials and methods:** We studied 11 patients with a confirmed diagnosis of cystinosis in 9 families. DNA was obtained from leukocytes of the patients and their relatives. Initially we searched for the 57 kb deletion by performing duplex PCR. Subsequently DNA of patients with one (heterozygous) or no 57 kb deletion was studied by a non-isotopic technique of SSCP of exons 3–12 of the *CTNS* gene, followed by direct sequencing of fragments with anomalous electrophoretic pattern. **RESULTS:** Most of patients presented infantile cystinosis, previous four reported mutations c.985-986insA ($n = 2$ alleles), c.357-360delGACT ($n = 1$ allele), a small deletion that comprises exon 4–5 ($n = 1$ allele) and c.537-557del21pb ($n = 1$ allele). In our study we identified three mutations not yet described and with a deleterious effect predicted in cystinosin: c.379delC (one allele), c.986C>G (one allele) and c.1090-1093delACCAinsCG (one allele). Distribution of genotypes was as follows: two severe mutations were identified in four patients with the infantile nephropathic form; the patient with the juvenile form had a c.537-557del21pb/c.1090-1093delACCAinsCG genotype, in which the former has been described too in patients with the juvenile nephropathic form. Two patients with the infantile nephropathic form have a heterozygous compound genotype 57 kb deletion/X and c.357-360delGACT/X, where X means an unknown mutation to date. Finally two patients with the infantile nephropathic form do not show anomalous electrophoretic patterns in the SSCP analysis of exons 3–12 of the *CTNS* gene, so the responsible mutations in these patients are unknown to date. **Results:** This is the first molecular study of the *CTNS* gene in a Mexican population. In our study we identified 6 single nucleotide polymorphisms, three of them previously reported, and three not yet reported.

8. The biochemical effects of liver transplantation in an infant with propionic acidemia and biliary cirrhosis.

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Hecht¹, M.M. Jonas³, C. Lillehei⁴, H.B. Kim⁴.¹Division of Genetics, Department of Pediatrics, Children's Hospital Boston, USA; ²Division of Neurology, Department of Pediatrics, Children's Hospital Boston, USA; ³Division of Gastroenterology, Department of Pediatrics, Children's Hospital Boston, USA; ⁴Department of Surgery, Department of Pediatrics, Children's Hospital Boston, USA.

We report a 1-year-old male infant with propionic acidemia who underwent a liver transplantation at 5 months of age because of biliary cirrhosis. Presentation was at day 3 with poor feeding, lethargy, hypothermia and hyperammonemia (339 $\mu\text{mol/L}$). Newborn screening tests revealed an elevated level of C3-carnitine ester; repeat plasma level was 7.06 $\mu\text{mol/L}$. Plasma glycine was 354 $\mu\text{mol/L}$ and glutamine 328 $\mu\text{mol/L}$ and there was increased urinary excretion of 3-hydroxypropionate, methylcitrate, tiglylglycine and lactate. Patient underwent hemodialysis, in addition to treatment with intravenous glucose, sodium bicarbonate and carnitine. Following resolution of the acute decompensation, the patient was maintained on a low protein diet (1.3 g protein/kg/d) and medical food (1.2 g Ile/Val/Met/Thr-free AA/kg/d). Congenital abnormalities included agenesis of the corpus callosum, undescended testes and small ASD and VSD. A brain MRI showed small collections of blood along the falx, the dura in the occipital regions, left frontal region and along the tentorial leaflets. There was a small focus of hemorrhage/ischemic injury within the periventricular white matter and at the right germinal matrix. A mild T2 signal abnormality was present in the right basal ganglia. The MRS revealed a small lactate peak. A karyotype was normal and a chromosomal microarray analysis uncovered only a familial variant. Prior to discharge from NICU, the infant was noted to be jaundiced. Subsequently, he developed hepatomegaly with cholestasis. As the cholestatic liver disease worsened, the infant failed to grow, the plasma total 2-methylcitrate levels were 8073–10,324 nmol/L (NI: 60–228) and the plasma C3-acylcarnitine was 17 $\mu\text{mol/L}$. After a successful liver transplantation, the initial plasma 2-methylcitrate levels were reduced to 4104–5656 nmol/L. Eventually, during periods of stress, it ranged from 14,748–16,987 nmol/L, while C3-acylcarnitine reached 49 μM . Diet was liberalized to 1.6 g natural protein/kg. Following a repeat brain MRI at 1 year of age, which was normal aside from the agenesis of the corpus callosum, a lumbar puncture was performed for assessment of propionate metabolites and lactate in CSF. The purpose of this work is to review the effect of liver transplantation on the biochemical phenotype in a patient with severe propionic acidemia with probable prenatal onset of disease.

9. Menke disease phenocopy in a female with normal ATP7A coding sequence.

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We report a 17-month-old female with poor growth, developmental delay, hypotonia, truncal ataxia, decreased serum copper 11–52 $\mu\text{g/dl}$ [nl: 75–145], and ceruloplasmin 130–170 mg/L [nl: 220–660]. Physical examination revealed an alert, animated, playful infant with weight <1 percentile, length 5 percentile, head circumference 10 percentile, lax smooth skin, decreased subcutaneous tissue, uneven islands of light pigmentation in lower extremities, slight wiry appearance to hair, costochondral protuberances, hyperflexion of knees and hips and 3 + deep tendon reflexes in lower extremities. Additional studies included a normal plasma lactate, CK and catecholamines; normal urine $\hat{\alpha}$ -microglobulin and normal karyotype (46, XX). Wormian bones were present on skull radiograph and bone age was delayed. Cerebellar atrophy, enlargement of extra-axial spaces and marked tortuosity of arteries of the Circle of Willis were seen on MRI/A. Pathologic examination of hair showed periodic narrowing of the hair shaft with thinning of the cortex and reduction/absence of the medulla consistent with monilethrix. The sequencing in both forward and reverse directions of the 23 exons of the ATP7A gene did not detect any deleterious changes. Additionally, Western analysis to quantitate ATP7A protein in the patient's cultured lymphoblasts and sequencing of the copper uptake gene hCTR1 and the

copper chaperone ATOX1 are all in progress. We conclude that this female patient has a phenotype that resembles Menke disease but is without pili torti and abnormal catecholamine levels, and has a normal coding region sequence of the ATP7A gene.

10. Description of two patients with clinical phenotypes consistent with a defect in the SUCLA2 gene. A. Blake¹, E.R. Vanden Heuvel², G.M. Rice², J.A. Wolff^{1,2,3}. ¹University of Wisconsin Madison - School of Medicine and Public Health, Department of Pediatrics, Madison, WI 53792, USA; ²Waisman Center, Madison, WI 53705, USA; ³Department of Medical Genetics, University of Wisconsin Madison, Madison, WI 53705, USA.

Mitochondrial diseases can be caused by defects in genes that encode proteins that function in the mitochondria or by defects in the mitochondrial DNA. Defects in genes coding for respiratory chain enzyme complexes and numerous secondary abnormalities such as mitochondrial DNA depletion syndromes have been previously identified. In addition to these relatively more common disorders, tricarboxylic acid cycle defects with accumulation of methylmalonic acid have also been shown to cause depletion of mtDNA. Two studies identified patients in the Faroe Islands population that were found to have mitochondrial encephalopathy and elevated methylmalonic acid. Mutation analysis in patients from both studies revealed mutations in the SUCLA2 gene. We identified two unrelated patients from Wisconsin who presented with hypotonia, failure to thrive, developmental delay and elevated MMA that was unresponsive to treatment with vitamin B12. One patient has congenital sensorineural hearing loss. Both patients had abnormalities in the basal ganglia on MRI and increased succinyl carnitine in the urine and plasma. The clinical presentations and laboratory findings of these two patients are consistent with a defect in succinyl CoA synthase. Mutation analysis of the SUCLA2 gene in these patients will be performed and will be presented.

11. Variable outcome of tetrahydrobiopterin deficiency: Effect of the time of diagnosis and treatment. Leandra Jäggi¹, Marcel R. Zurflüh¹, Agnes Schuler², Alberto Ponzone³, Francesco Porta³, Laura Fiori⁴, Marcello Giovannini⁴, René Santer⁵, Georg F. Hoffmann⁶, Hans Ibel⁷, Udo Wendel⁸, Diana Ballhausen⁹, Matthias R. Baumgartner¹⁰, Nenad Blau¹. ¹Division of Clinical Chemistry and Biochemistry, University Children's Hospital, Zürich, Switzerland; ²Buda Children's Hospital, PKU Laboratory, Budapest, Hungary; ³Department of Pediatrics, University of Torino, Turin, Italy; ⁴Department of Pediatrics, San Paolo Hospital, University of Milan, Milan, Italy; ⁵Department of Pediatrics, University Medical Center, Hamburg-Eppendorf, Hamburg, Germany; ⁶Division of Metabolic Disorders, Department of Pediatrics, University of Heidelberg, Heidelberg, Germany; ⁷Werneck, Germany; ⁸Department of Pediatrics, University of Düsseldorf, Düsseldorf, Germany; ⁹Division of Molecular Pediatrics, Centre Hospitalier Universitaire Vaudois Clinique Infantile, Lausanne, Switzerland; ¹⁰Division of Metabolism and Molecular Pediatrics, University Children's Hospital, Zürich, Switzerland.

We describe the treatment, the clinical, and biochemical findings and the outcome of 26 patients with 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency and 10 patients with dihydropteridine reductase (DHPR) deficiency. These are the two most common forms of the autosomal-recessively inherited tetrahydrobiopterin (BH₄) deficiency. Time of diagnosis, dosage of BH₄ and neurotransmitter precursors, folinic acid substitution, and levels of 5-hydroxyindoleacetic acid (5HIAA) and homovanillic acid (HVA) in cerebrospinal fluid (CSF) are essential parameters in the follow-up of patients. Unfortunately, treatment protocols vary greatly among patients and clinical centers, and CSF investigations and outcome assessments are not always available. 17 patients with PTPS deficiency and 4 patients with DHPR deficiency were diagnosed within 2 months after birth. In 14 patients with PTPS deficiency (54%; 9 early and 5 late diagnosed) and 2 patients with DHPR deficiency (20%; all early diagnosed) no developmental delay is observed, while in 10 patients

with PTPS deficiency (38%; 6 early and 4 late diagnosed) and 8 patients with DHPR deficiency (80%; 2 early and 6 late diagnosed) development was delayed. Two PTPS-deficient patients died in the newborn period. DHPR deficiency seems to be more severe than PTPS deficiency and it is clearly the onset of treatment that determines the outcome. Our data suggest that diagnosis within the first month of life is essential for a good outcome and that low CSF 5HIAA and HVA values in CSF could be an indicator for the ongoing developmental impairment, even in the absence of neurological symptoms.

12. Complementary techniques for the analysis of amino acids. W. Blom. Ansynth Service BV, Berkel en Rodenrijs, The Netherlands.

Recent technological advances in the field of tandem mass spectrometry (MS/MS) mean that this technique, which is already widely used for screening, is now also increasingly used for quantitative amino acid analysis. However traditional ion exchange techniques using ninhydrin post-column derivatisation should not be completely dismissed as they still provide valuable information for the diagnosis of inherited metabolic disorders, particularly as these systems are now even more robust and enable to routinely perform quantitative analysis of up to 50 amino acids and related compounds in a single run. Indeed, with the right chromatographic conditions, such systems will provide adequate separation between peaks and allow less common amino acids or drug metabolites to be separated thus facilitating data interpretation of full amino acids profiles of complex biological samples such as urines. Homocitrulline, saccharopine, pipercolic acid or allo-isoleucine are only examples of some of the amino acids that can be separated within one run thus enabling certain metabolic disorders to be differentiated. Dedicated amino acid systems are therefore complementary to MS/MS techniques and offer a useful tool to confirm positive screens.

13. Elevated ferritin in classic galactosemia—A sequela of liver disease or a “New” association with hypoglycosylated transferrin? S.J. Boldt¹, K.M. Camp², N.R. Dobson², G.T. Berry³. ¹National Capital Consortium Pediatric Residency Program, Bethesda, MD 20889, USA; ²Department of Pediatrics, Walter Reed Army Medical Center, Washington DC 20307, USA; ³Division of Genetics, Department of Pediatrics, Children's Hospital, Boston MA 02215, USA.

A term 9-day-old Caucasian male presented with persistent indirect and direct hyperbilirubinemia, hyperammonemia, and transaminitis. He was admitted to the hospital for phototherapy on day of life four with a total bilirubin of 20.8 mg/dL. His hyperbilirubinemia persisted with an increasing direct component, and he subsequently developed elevated liver enzymes, a coagulopathy, and compensated metabolic acidosis with an elevated lactate and ammonia. Urine reducing substances were positive, but this was attributed to antibiotics being given at the time of collection. The differential diagnosis at this time included infection, inborn errors of metabolism, cholestatic hepatitis, and biliary atresia and he was switched from breast milk to a protein hydrolysate formula. Further laboratory studies demonstrated an elevated ferritin level (1909 ng/mL, normal is 22–322 ng/mL) with a low transferrin level (<80 mg/dL, normal is 215–365 mg/dL), adding neonatal hemochromatosis to the differential diagnosis. An abdominal ultrasound, head ultrasound and ophthalmologic exam were unremarkable, and the infant had no evidence of excessive iron storage on liver MRI or salivary gland biopsy. During this evaluation, the infant was given several breast milk feedings but within several hours, urine reducing substances were positive. Due to severe liver dysfunction and suspected galactosemia, he was then switched to an elemental formula. The newborn screen was positive for galactosemia; confirmatory testing revealed reduced galactose 1-phosphate uridylyltransferase activity and homozygosity for the Q188R mutation. Elimination of galactose from the infant's diet rapidly resolved his hepatic dysfunction and led to a significant decrease in ferritin levels. The elevated ferritin level in this patient complicated management since it raised concern for neonatal hemochromatosis. Subsequent glycosylation analysis of the infant's plasma transferrin revealed a mild increased amount of hypoglycosylated-transferrin. We question whether abnormally glycosylated transferrin

may lead to aberrant intercellular trafficking of iron, and sequestration within the reticuloendothelial system. A review of the literature did not demonstrate any evidence correlating elevated ferritin levels with galactosemia. If other patients with classic galactosemia are found to have elevated ferritin levels, further research into galactose toxicity, abnormal transferrin metabolism and iron storage mechanisms is warranted.

14. Genotype dependent variations of galactose-1-phosphate concentrations in galactosemic patients. Ruben Bonilla Guerrero¹, Sara J. Minnich¹, Kimiyo Raymond¹, Dietrich Matern¹, Amber M. McDonald², W. Edward Highsmith², John F. O'Brien¹. ¹Biochemical Genetics Laboratory, Mayo Clinic Rochester, MN 55905, USA; ²Molecular Genetics Laboratory; Mayo Clinic Rochester, MN 55905, USA.

Genotype (# of patients)	GALT U/g Hb	GALIP mean $\mu\text{mol/g Hb}$ (range)
Q188R/Q188R ($n = 18$)	0–1.4	0.7 (0.4–1.2)
N314D/Q188R ($n = 115$)	1.8–9.5	0.1 (0–1.8)
N314D/S135L ($n = 13$)	4.2–7.8	0.1 (0–0.9)
Q188R/S135L ($n = 6$)	0.25–0.91	0.4 (0.3–0.5)

Background: Galactose-1-phosphate uridylyltransferase (GALT) is essential in human infants for the metabolism of lactose as their primary source of carbohydrates. Ingestion of galactose in infants with classic galactosemia with near or total absence of enzyme activity results typically in poor feeding, weight loss, severe liver dysfunction, coagulopathy, and often sepsis. Treatment consists of withdrawal of galactose from the diet. Ovarian failure and chronic neurological deficits represent long term complications. Catalytic activity of GALT varies depending on the mutation present and is more severe in mutations such as Q188R that affect the enzyme's active site. Erythrocyte galactose-1-phosphate (GALIP) accumulates in GALT deficiency and is considered to be a toxic metabolite. Therefore, determination of GALIP levels is used to assess treatment success and concentrations below $<0.5 \mu\text{mol/g Hb}$ are considered optimal management. Whether this goal is achievable in patients with classic galactosemia is uncertain. **Objective:** To determine the range of GALIP levels observed in patients with classic galactosemia after institution of a galactose free diet. **Methods:** We reviewed GALIP concentrations of at least 2-month-old patients with treated GALT deficiency and known genotypes whose samples were submitted periodically for up to 7 years. **Results:** Only 27% of GALIP values in Q188R homozygotes were $<0.5 \mu\text{mol/g Hb}$. We found that 68% of the GALIP values in DG patients were well within the recommended treatment range with most being equivalent to those seen in non-galactosemic infants. Notably, we do observe GALIP levels above the target range in some normal infants. Furthermore, sporadic elevations of GALIP in DG genotypes may be because of sampling close to feeding and prior to dietary restriction. **Conclusions:** Published guidelines suggest that patients with GALIP below $<0.5 \mu\text{mol/g Hb}$ are adequately restricted. Our data (table) demonstrate that GALIP concentrations within the target range are difficult to attain in specific genotypes, particularly when the patients grow older and formula feedings are replaced by solid food.

15. Enzyme replacement therapy in gaucher disease: What should we use as maintenance dosage? Catherine Brunel-Guitton¹, Georges-Étienne Rivard², Grant Mitchell¹, Marie Lambert¹. ¹Service de Génétique Médicale, Département de Pédiatrie, CHU Sainte-Justine, Université de Montréal, Que., Canada H3T 1C5; ²Service d'Hémo-oncologie, Département de Pédiatrie, CHU Sainte-Justine, Université de Montréal, Que., Canada H3T 1C5.

Objective: Retrospectively evaluate the efficiency of low-dosage maintenance enzyme therapy in children with Gaucher disease. Therapeutic goals were: (1) maintenance of normal hemoglobin; (2) no dependence on transfusions; (3) no complaint of fatigue or dyspnea and improvement of quality of life; (4) if moderate thrombocytopenia, increase of 1.5–2 times in the 1st year, then maintenance to the lower limit of the

reference range; if severe thrombocytopenia, increase of 1.5 times in the 1st year, then continuous increase during years 2–5; (5) prevention of splenectomy and spleen infarcts; (6) reduction of liver size to 1–1.5 normal, with a reduction of 20–30% during years 1–2 and 30–40% during years 3–5; (7) reduction of spleen size to <2 –8 times normal, with a reduction of 30–50% during year 1 and 50–60% during years 2–5; (8) alleviation of GI symptoms; (9) reduction of bone pain in years 1–2; (10) prevention of bone crisis, osteonecrosis and fractures. (11) increase in bone density after 2 years; (12) return and maintenance of growth velocity in the first 3 years and avoidance of pubertal delay; (13) prevention of pulmonary complications. **Methods:** Medical records of 10 pediatric patients were retrospectively reviewed. **Results:** None had splenectomy. Genotypes were: N370S/N370S, $n = 1$; N370S/W184G, $n = 1$; N370S/L444P, $n = 4$; L444P/W223, $n = 1$ and L444P/L444P, $n = 3$. Median age at diagnostic was 3.2 years (9 months–9.9 years) and at initiation of treatment, 6.3 years (1.6–13.4 years). Median duration of follow-up before treatment was 2.5 years (2–7 years) and on treatment, 7 years (2–15 years). Mean dosage at initiation was 67 U/kg/month (30–104 U/kg/month), every 2 weeks. 3 of 10 patients had initiation dose <60 U/kg/month (30–54 U/kg/month). Seven of 10 patients (including one L444P homozygote) received 30–35 U/kg/month for maintenance, for a median period of 4 years (3–5 years), one received 46 U/kg/month (L444P/W184G) and two L444P homozygotes received 72 and 90 U/kg/month, respectively. All patients, save one L444P homozygote, achieved and maintained therapeutic goals, including maintenance of a normal bone density. One other patient, who had also Down syndrome did not show improvement on bone density. The other two L444P homozygotes responded more slowly to treatment. However, objectives were achieved and maintained. **Conclusions:** Our data suggest that once therapeutic goals are achieved, a maintenance dosage of 30–35 U/kg/month given every 2 weeks may be adequate for most pediatric patients with Gaucher disease. L444P homozygotes had a more severe disease and might require a higher maintenance dosage.

16. Identification of sapropterin-responsive phenylketonuria (PKU) patients (pts) in a single PKU clinic during an expanded access program. H. Bausell, D. Hartung, R. Katz, BK Burton. Children's Memorial Hospital and Northwestern University Feinberg School of Medicine, Chicago, IL, USA.

Methods: Sapropterin dihydrochloride (Kuvan™, Biomarin Pharmaceutical Inc., Novato, CA) was offered to all eligible patients in the PKU Clinic at Children's Memorial Hospital in Chicago, during an expanded access program. 32 pts were tested for responsiveness. Pts were 8–30 yrs of age; the mean baseline phenylalanine level was 874 $\mu\text{mol/L}$ (range 252–1692); most pts were on some type of medical food and dietary phe restriction. Pts were started on a single daily dose of 20 mg/kg/day and phe levels were obtained at 1, 7 and 14 days along with diet records. No dietary changes were made. A positive response was a decline of greater than 30% in the blood phe level. **Results:** Two pts. discontinued the medication after obtaining the 1 day phe level due to GI side effects. One, a likely non-responder, complained of diarrhea. A second pt., whose phe level had decreased by 23% at 1 day, complained of nausea and vomiting. A third pt. complained of nausea and heartburn after demonstrating responsiveness but was successfully maintained on the medication by reducing the dose and gradually increasing it in a stepwise fashion. Subsequently, the drug was routinely given with food and there were no further complaints. Of the 30 pts who continued beyond 48 h of treatment, 15 were responders while one pt. who did not meet the 30% criterion was felt to have a "clinically significant response" when her baseline phe of 1380 $\mu\text{mol/L}$ declined to 990 $\mu\text{mol/L}$ at 14 days (–28%). 12 pts were non-responders and the drug was discontinued at 2 weeks. In 2 cases, responsiveness was impossible to assess due to erratic blood phe levels and/or poor diet records. Mutation data was available for 17/32 pts and will be presented. **Summary:** Over 50% of unselected patients with PKU of all degrees of severity over 8 years of age responded to sapropterin dihydrochloride at a dose of 20 mg/kg/day. The medication is well-tolerated when given with food in a single daily dose.

17. Large variations in mRNA and protein expression of urea cycle genes in the liver and extrahepatic tissues. Ljubica Caldovic, Himani Majumdar, Juan Cabrera-Luque, Hiroki Morizono, Mendel Tuchman. Children's National Medical Center, Research Center for Genetic Medicine, Washington DC, USA.

In mammals, the primary known function of the urea cycle is to protect the brain from the toxic effects of ammonia by conversion into urea in the liver. Urea cycle enzymes are also expressed in the intestines and kidneys, where they catalyze biosynthesis of arginine. Northern blots of mouse tissues showed *N*-acetylglutamate synthase (NAGS) expression in spleen, testis, and, as expected, in the liver and intestine. Western blotting confirmed the presence of NAGS protein in all four tissues. Two prominent NAGS bands were detected in the liver. The most abundant species was comparable in size to recombinant “mature” NAGS (NAGS-M). The next smaller, and less abundant, band had a similar size to the “conserved” segment of NAGS (NAGS-C). NAGS-C was the only species seen in the intestine, spleen and testis. The expression of NAGS, carbamylphosphate synthase 1 (CPS1) and ornithine transcarbamylase (OTC) mRNA was then examined in 14 mouse tissues using quantitative RT-PCR. Significant NAGS expression was seen in the kidney, ovary, spleen, testis and stomach. CPS1 and OTC mRNA were highly expressed in the liver and intestine, but significant expression was also observed in the testis and stomach. Western blotting was used to examine expression of the NAGS, CPS1 and OTC proteins in eight extrahepatic tissues from four inbred strains of mice and the abundance of these three proteins was found to be dependent on the genetic background of the mouse. Quantitative Western blotting was used to more accurately measure abundance of the NAGS-M and NAGS-C in the livers and intestines harvested from different mouse strains. The abundance of NAGS-M in the liver was the highest in the FVB strain and the lowest in the 129SvEv; the difference between two strains was approximately 8-fold. The abundance of NAGS-C in the liver and the ratio of NAGS-M to NAGS-C varied with respect to mouse strain. Variability of NAGS-C expression was also observed in the intestines harvested from different strains. This suggests a natural variation in expression levels of the urea cycle in the liver as well as other tissues that may partially be due to genetic background. Understanding this variability is important for a better understanding of the natural progression of urea cycle disorders, the side effects of medications, and the possible role of urea cycle enzymes in development and adaptation to a changing environment.

18. Twins with ethylmalonic encephalopathy and different clinical evolutions. Philippe M. Campeau¹, Denis Cyr², Bernard Lemieux², Nicole Pigeon³, Joe T.R. Clarke^{2,4}. ¹Department of Human Genetics, McGill University, Montreal, Que., Canada; ²Medical Genetics Service, Sherbrooke University Hospital, Sherbrooke, Que., Canada; ³Department of Pediatrics, Sherbrooke University, Sherbrooke, Que., Canada; ⁴Division of clinical and metabolic genetics, the hospital for sick children and University of Toronto, Toronto, Ont., Canada.

Ethylmalonic encephalopathy is a recently described inborn error of metabolism clinically characterized by developmental delay and regression, recurrent petechiae, orthostatic acrocyanosis and chronic diarrhea. We describe monozygotic twins presenting with hypotonia in infancy and diagnosed with ethylmalonic encephalopathy based on biochemical findings. They are compound heterozygous for missense mutations in *ETHE1*. They are now 10 years of age, have severe axial hypotonia but never displayed petechiae, orthostatic acrocyanosis nor chronic diarrhea. Their clinical courses differ markedly; one had an episode of coma at three and a half years of age, now has spastic paraparesis and cannot speak. The other can freely use her upper extremities, her pyramidal syndrome being mostly limited to the lower extremities, and can speak two languages. MRI changes affecting the white matter, corpus callosum and basal ganglia were seen in both patients. These cases illustrate the clinical heterogeneity of ethylmalonic encephalopathy, even in monozygotic twins.

19. A Metabolic examination of infants on TPN used in the care of premature infants with a correlation to observations in newborn screening Donald H. Chace¹, Reese Clark², Alan Spitzer². ¹Pediatrix Analytical, 90 Emerson Lane, Bridgeville, PA, USA; ²Pediatrix Medical Group, Sunrise, FL, USA.

Newborn Screening programs have been aware of a higher incidence of false positive results for numerous metabolites in the premature infant population at its very beginnings when the “PKU” test was first introduced. Elevated amino acids have been observed for more than 40 years. It has never been clear whether elevated analytes such as phenylalanine or methionine were due to prematurity, its treatment or both. We recently concluded a study designed to evaluate the impact of intravenous amino acids (TPN) at two different concentrations (high and low) on growth of premature infants. As part of this study we performed a MS/MS based metabolic profile (amino acids, acylcarnitine and thyroxine) on samples obtained on day 1, 7 and 28. Infants did not receive IV nutrition before the 1st sample was collected, where at their maximum TPN doses at day 7 and were on complete enteral feeds by day 28.

One hundred twenty-two neonates were divided into 2 groups (64 in the 3.5 g/kg/day group and 58 in the 2.5 g/kg/day group). Results suggests there was no significant difference in growth by day 28 after birth (median weight gain = 12.9 vs. 11.4 g/kg/day, 3.5 and 2.5 maximum dose groups respectively $p = 0.6$). However, we did observe changes in concentrations of various metabolites in both groups. On day 7, several serum amino-acid levels (Alanine, Arginine, Glutamate, Leucine-Isoleucine, Methionine, Ornithine, Phenylalanine, Serine, Tyrosine, and Valine) were higher in the 3.5 compared to the 2.5 maximum dose group; none of the measured amino-acids were lower. In addition, blood urea nitrogen was higher in the higher dose group. We also observed increases in selected acylcarnitines related to organic acid metabolism and administration of lipid supplements.

In contrast to the 1960's with the limited panel of amino acid metabolites, MS/MS analysis enables multiple analyte determination. This information together with clinical studies can be helpful in improving characterization of MS/MS NBS profiles and perhaps reducing or better characterizing presumptive positive results. These data will be presented and compared to median newborn data from routine newborn screening (day 1–3) and older infants (>day 7).

20. Genetic therapy rescues a neonatal lethal murine model of *mut*⁰ methylmalonic acidemia. Randy J. Chandler, Charles P. Venditti. Genetic Diseases Research Branch, National Human Genome Research Branch, National Institutes of Health, Bethesda, MD, USA.

Methylmalonic acidemia (MMA), an autosomal recessive metabolic disorder, is most often caused by mutations in methylmalonyl-CoA mutase (MUT). Severely affected patients typically present in crisis within the first two days of life and can perish despite intervention. Survivors follow an unstable metabolic course punctuated by various complications throughout life. Elective liver transplantation can prevent frequent decompensation but does not normalize methylmalonic acid levels. Potential alternatives to liver transplantation include hepatocyte-directed gene and cell therapies. We have used a murine model of *mut*⁰-MMA with uniform neonatal lethality of the *Mut*^{-/-} pups in the first 48 h of life, to assess the efficacy of viral mediated gene therapy. Newborn *Mut*^{-/-} pups and control littermates received either intramuscular or intrahepatic injections of an adenovirus carrying the *Mut* gene expressed under the control of the CMV promoter. All of the *Mut*^{-/-} pups injected via the intramuscular route perished within the first 48 h of birth. However, more than 50% of the *Mut*^{-/-} pups that received intrahepatic injections survived beyond weaning (day 15). The treated mutants had methylmalonyl-CoA mutase mRNA, enzyme protein, and decreased metabolite levels. These results demonstrate that adenoviral mediated, hepatic methylmalonyl-CoA mutase expression can rescue *Mut*^{-/-} pups from neonatal mortality. These experiments represent the first successful viral gene delivery in any lethal murine model of organic acidemia, specifically; they provide proof of principle for the efficacy of liver-directed gene delivery in methylmalonic acidemia. Preliminary genetic therapy experiments using an adeno-

associated virus with a CBA promoter driving the expression of murine methylmalonyl-CoA mutase via hepatic injection indicate that long-term rescue and expression are possible.

21. Gamma polymerase deficiency presenting as glycogen storage disease.

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Introduction: Alpers syndrome due to mitochondrial DNA polymerase α defect, presents in toddlers with ataxia, progressive encephalopathy, developmental delays, myopathy, hypo/hypertonia, progressing to intractable myoclonic epilepsy and liver failure. Intermittent hypoglycemia has been reported but the etiology is unclear. **Aim:** To report a patient with Alpers syndrome with initial presentation mimicking a glycogen storage disease. **Patient:** A 4-month-old female presented with 2 episodes of early morning listlessness. Initial work-up was negative. At 9 mo of age a fasting morning blood glucose was 44 mg/dl while patient was asymptomatic. The patient was electively admitted for a work-up. Physical exam, growth and neurodevelopment were normal. At 8 h of fasting, blood glucose dropped to 27 mg/dl with normal electrolytes and growth hormone, elevated AST (293), ALT (151) and low insulin. Lactic acid was 1.6 mMol and alanine was 175 μ Mol (nl 246–486) indicating intact gluconeogenesis. B-OH butyric was high and organic acids showed ketosis, demonstrating normal lipolysis. Acylcarnitines showed non-diagnostic mild elevations and fibroblast fatty acid oxidation (FAO) studies were normal. Cortisol was inappropriately low (7.3 μ g/dL). MRI of brain was normal. The patient was treated with cortef[®] and frequent feedings supplemented with glucose polymers. Despite steroids treatment, patient became hypoglycemic when night time feedings were spaced. Subsequent fasting studies confirmed the initial findings, in addition, a post-prandial sample showed elevated lactate and borderline high blood glucose. Later on, patient had unexplained altered mental status. A mitochondrial disease was suspected and she underwent muscle and liver biopsies. At 14 months the patient developed myoclonic seizures and MRI showed brain atrophy. POLG deficiency was suspected. The patient progressively developed encephalopathy, intractable myoclonus and liver failure. Support was withdrawn. **Results:** Glycogen storage disease type 0 was ruled out by full sequence of GYS2 gene. Liver pathology showed fibrosis and nonspecific inflammatory infiltrates. Glycogen content was in the upper normal range, glucose-6-phosphatase was normal, branching enzyme activity was low and debrancher activity was undetectable, but sequence of AGL gene was normal. Muscle biopsy showed <1% COX negative fibers, but was otherwise normal including respiratory chain activity. Sequencing of POLG gene revealed A467T and G848S mutations. **Conclusions:** Secondary defects in glycogenolysis may cause hypoglycemia in Alpers syndrome, while gluconeogenesis and FAO are intact. Cortisol deficiency does not appear to be the cause of hypoglycemia in this patient.

22. A false positive newborn screen: Goat's milk acidopathy.

Kimberly A. Chapman¹, Jaya Ganesh², Can Ficicioglu². ¹The Children's Hospital of Philadelphia, Department of Genetics, USA; ²The Children's Hospital of Philadelphia, Department of Development of Rehabilitation, Section of Metabolism, USA.

With the increase in number of diseases in newborn screening (NBS) due to inclusion of mass spectrometry, an increase in false positive cases is noted (Tarini 2006). This short report describes a positive NBS result for Maple Syrup Urine Disease (MSUD) secondary to goat milk consumption in a newborn. Our case (KW) initially had NBS at 36 h of life that showed an elevated phenylalanine (2.5 mg/dL; nl < 2.3 mg/dL) and so the filter paper was repeated showing elevated valine and leucine levels (637 nmol/mL nl: 93–321 nmol/mL and 381 nmol/mL nl: 42–188 nmol/mL) at 12 days of age. Upon presentation, at 12 days of age, this infant was growing and feeding well without lethargy. Infant had been started on undiluted

goat's milk, feeding every 3–4 hours since birth due to a family history of cow's milk intolerance. Initial testing was consistent with metabolic acidosis (bicarbonate 17 mmol/L), elevated blood urea nitrogen (BUN) of 34 mg/dL (nl: 20–24 mg/dL), no alloisoleucine but twice normal valine, leucine and isoleucine levels (505 nmol/mL, 249 nmol/mL, and 120 nmol/mL nl: 17–106 nmol/mL). Electrolytes and acidosis resolved on soy formula and hydration overnight. As a result, findings were felt to be consistent with goat's milk acidosis that was secondary to a protein and acid load in excess of the filtering capacity of the infant's kidneys. Harrison et al. described a similar patient in 1979 who also resolved with hydration and more appropriate feeding regimen (Harrison et al. 1979). This report adds to the list of possible dietary causes such as goat milk for false positive newborn screens.

23. Project DOCC. A. Chappelle¹, P. Furlong², K. Clapp³, J. McInerney⁴, S.F. Terry¹, D. Appell⁵, M. Hoffman⁶. ¹Genetic Alliance, Washington, DC 20011, USA; ²Parent Project Muscular Dystrophy, Middletown, OH 45042, USA; ³Fragile X Research Association, Newburyport, MA 01950, USA; ⁴National Coalition for Health Professional Education in Genetics, Lutherville, MD 21093, USA; ⁵Hermansky-Pudlak Syndrome Network, Oyster Bay, NY 11771, USA; ⁶Project DOCC, Great Neck, NY 11021, USA.

Objective: Few physicians and other healthcare providers have an in-depth understanding of how individuals and their families cope with genetic diseases, and the chronic care needs that cascade from them, on a daily basis. Project DOCC (Delivery of Chronic Care) improves the quality of care for chronically ill children and adults by educating healthcare providers about their special needs from a family caregiver's perspective. It promotes understanding of the issues involved in caring for a family member living with special health care needs and puts the family at the center of the healthcare system. Project DOCC accomplishes this goal by enlisting individuals and families affected by chronic disorders as educators. Each Project DOCC presentation is a panel of individuals and family members who live with chronic diseases, and Genetic Alliance has been working with Project DOCC to develop a repertoire that includes important genetic diseases. The first combined GA/Project DOCC panel will include discussion of the lived experience of Duchenne and Becker Muscular Dystrophy and Fragile X Syndrome, including issues such as access to education, collaborating with healthcare providers, the importance of support organizations, and what it is like to care for the family member on a daily basis. We present Project DOCC as a model and a potential partner for professionals working on strategies to improve education about and understanding of all aspects of metabolic disease.

24. Clinical spectrum of mutations in *SLC4A4*, encoding the sodium bicarbonate cotransporter protein NBCe1.

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There have now been 9 mutations in *SLC4A4*, which encodes the electrogenic Na⁺/HCO₃⁻ cotransporter NBCe1, associated with human clinical disease. Two human isoforms have been described, kNBCe1 (kidney) and pNBCe1 (pancreatic), produced by alternative promoter usage (exon 3) and demonstrating variable tissue expression in vertebrates. The common clinical presentation of patients with mutations in *SLC4A4* appears to be proximal renal tubular acidosis and ophthalmologic disease (cataracts, glaucoma, band keratopathy), with other individual comorbidities. We describe here a patient whose clinical course over two decades suggests involvement in many of the tissues in which both kNBCe1 and pNBCe1 appear to be expressed. His mutation in exon 18 of *SLC4A4* due to homozygous deletion of nucleotide 2311A produces a novel frame shift, predicting a premature nonsense (stop) codon and demonstrates absence of functional activity in microinjected oocyte studies (Eur J Physiol 448:438–444 (2004)). His clinical manifestations include: *Renal:* Proximal

tubular acidosis with mild chronic renal insufficiency, late onset development of nephrocalcinosis and cortical macrocysts; *Ophthalmologic*: Lens cataracts requiring replacement, glaucoma, corneal band keratopathy, Peter anomaly; *Pancreatic*: chronic mild hyperamylasemia with one episode of atypical acute pancreatitis; *Bonelskeletal growth/teeth*: Short stature responsive to human growth hormone, moderate scoliosis, dental enamel defects; *Central nervous system*: Bilateral basal ganglia calcifications but normal intellectual development and normal movement functions. The relationship between the clinical spectrum observed in this and other patients with *SLC44A* mutations and the isoform expression patterns observed in vertebrate studies will be discussed. Since these patients may present in childhood with unexplained acidosis affecting growth, the comorbidities associated with this rare condition should be kept in mind during early diagnostic evaluation.

25. Resource for cell lines and DNA samples from propionic acidemia in the NIGMS human genetic cell repository. D.L. Coppock¹, B.A. Frederick¹, J. Franks², LH Toji¹. ¹Coriell Institute for Medical Research, Camden, NJ 08103 USA; ²Propionic Acidemia Foundation, Highland Park, IL 60035 USA.

Objective: The NIGMS Human Genetic Cell Repository and the Propionic Acidemia Foundation (PAF) are cooperating to establish a resource for the study of propionic acidemia, a rare metabolic disorder. Cell lines and DNA samples accompanied by excellent clinical and laboratory data are critical to a deeper understanding of the disorder. The goals of the collaboration are to (1) develop a detailed questionnaire for all subjects, (2) establish a protocol to collect a blood sample (or submit an existing cell line) for processing into a lymphoblastoid line, (3) establish a protocol to permit recontacting the submitter periodically for clinical updates while preserving de-identification and 4) collect at least 50 probands with first degree relatives where possible. *Results*: The Propionic Acidemia Foundation worked with its Medical Advisory Board to develop a detailed questionnaire to be answered by the parents and/or physician for each of the probands. Working with the IRB, Repository staff developed a procedure whereby the voluntary health organization acts as an intermediary for the collection of clinical information to accompany the blood samples or cell lines submitted to the Repository for processing. The PAF assigns a PAF code to the materials submitted for each subject; no personal identifiers accompany the submissions to the Repository. The Repository assigns an accession number to each sample. The consent gives the subject the option of being recontacted through the PAF to provide updated clinical information at designated intervals. In such cases the PAF would recontact the subjects and obtain updated clinical information on the status of the patients. New samples would not be submitted at this time. At the date of this report 22 samples from 7 families and 3 individual probands have been submitted. Many of these samples will be available to the scientific community in the spring of 2008. *SUMMARY*: The Repository has developed a process to establish a resource for cell lines, DNA and longitudinal clinical data for a variety of disorders by working through voluntary disease organizations. The first of these is for propionic acidemia. This offers the scientific community a unique resource for the study of rare diseases.

26. The Lebers hereditary optic neuropathy mutation T14484C can cause Leigh-like disease. E. Crushell¹, B. Robinson², S. Blaser³, S. Murray⁴, P.J. Ainsworth², A. Feigenbaum¹. ¹Division of Clinical and Metabolic Genetics, Canada; ²Mitochondrial Metabolism Laboratory, Research Institute, Canada; ³Neuroradiology, Sickkids, Toronto, Canada; ⁴Department of Paediatrics, Sudbury Reg Hosp, Sudbury, Canada; ⁵Molecular Genetics Laboratory, London Health Sciences Centre, Canada.

Introduction: Leigh-like encephalomyopathy has been reported in three adult males with previously diagnosed Lebers Hereditary Optic Neuropathy (LHON) however LHON mutations have not been reported as a cause of Leigh disease in children without visual signs. *Case report*: A 7-year-old girl has been followed since age 20 months with global developmental delay and ataxia. The family history is significant for LHON affecting her mother (who

has visual failure and Multiple Sclerosis - like demyelinating lesions on MRI of Brain), a maternal aunt and her son. The family is French Canadian and mother has 95% homoplasmy in blood for the 14484 T>C ND6 mtDNA LHON mutation. On examination, growth and muscle bulk were normal, tone and reflexes were increased in lower limbs with reduced distal power (4/5). Speech was slurred and there was a broad-based ataxic gait. Cardiology assessment (including echocardiograph and EKG) was normal and yearly ophthalmological assessments have been normal. The blood lactate was elevated at 3.1 mmol/l. The CSF lactate was 2.5 mmol/l (normal <2.5), total and free Carnitine levels were low at 17 and 16 mmol/L, respectively. MRI of brain at ages 2 and 6 years showed bilateral T2 bright signal in putamen symmetrically and on MR spectroscopy a lactate peak was seen in the left putamen consistent with a mitochondrial disease. Skin fibroblasts showed normal mitochondrial Complex II, III and IV enzyme activity and normal cellular lactate: pyruvate ratio. The family was reluctant to proceed to muscle biopsy. Blood mtDNA analysis identified the familial mtDNA 14484 T>C mutation to be >95% homoplasmic in blood. Other metabolic investigations did not reveal another cause of the MRI changes. The patient was commenced on Coenzyme Q10, Carnitine and antioxidants. On follow up, the patient's development continues to slowly progress and there is persistent ataxia and lower limb spasticity. *Conclusions*: It is likely that Leigh-like disease without optic atrophy falls within the phenotypic spectrum of LHON mutations and should be part of counseling in families.

27. Ethylmalonic encephalopathy with a novel ETHE1 mutation diagnosed on newborn screening. A.I. Dagli¹, P.K. Edwards¹, H.J. Stalker¹, M.K. Maisenbacher¹, M.R. Wallace², M.N. Burch², V. Tiranti³, D. Gavrilov⁴, P. Rinaldo⁴, L.S. Martin⁵, B.A. Heese¹. ¹Division of Genetics, Department of Pediatrics, University of Florida, Gainesville, USA; ²Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, USA; ³Division of Molecular Neurogenetics, IRCCS Foundation Neurological Institute, Milano, Italy; ⁴Biochemical Genetics Laboratory, Mayo Clinic College of Medicine, Rochester, MN, USA; ⁵Division of Genetics, Nemours Children's Clinic, Jacksonville, FL, USA.

Ethylmalonic encephalopathy (EE) is an autosomal recessive disorder characterized by severe neurological sequelae, orthostatic acrocyanosis, petechiae and chronic diarrhea. It is lethal in infancy or early childhood. Our patient is an Hispanic female who presented with an abnormal newborn screen with an elevation of butyrylcarnitine (C₄). A confirmatory acylcarnitine profile was abnormal with elevated butyrylcarnitine (C₄) at 3.19 nmol/ml (normal values <1.06 nmol/ml) and isovalerylcarnitine (C₅) at 1.40 nmol/ml (normal value <0.63 nmol/ml). Urine organic acid analysis was significant for elevation of ethylmalonic acid, 2-methylbutyrylglycine and isovalerylglycine. Urine acylglycine analysis showed an elevated ethylmalonic acid at 172 micrograms/mg of creatinine (normal <20). Other elevated acylglycines included butyrylglycine and isovalerylglycine. Of note, methylsuccinic acid was not found to be elevated. In addition, urine acylcarnitine testing showed an elevated C₄ acylcarnitine. Molecular testing for the ETHE1, the gene responsible for EE, found that the patient was a compound heterozygote for two mutations. One was a previously described mutation 487 C > T in exon 4 (Tiranti V et al. 2004) leading to a R163W amino acid change. The other mutation is a novel mutation 455 C > T, also in exon 4, leading to a T152I amino acid change in a highly conserved amino acid residue. Our patient was well and thriving with a normal clinical exam and no evidence of neurological abnormalities, petechiae or diarrhea at 2 weeks, 6 weeks and 4 months of age. As there is no proven medical management for patients with EE, thus, none was recommended at the time. At 6 months of age the infant presented with seizures and since then she has had progressive neurological deterioration.

This case emphasizes the value of newborn screening in diagnosis of EE. In our patient the diagnosis would have been delayed as she did not have skin manifestations or diarrhea and had a delayed neurological presentation. The mutation 455 C > T has not been previously reported. We conclude that this mutation is pathogenic.

- 28. A case report of a patient with dual diagnoses: 3-Methylcrotonyl carboxylase deficiency and schizencephaly.** J.M. DeLuca, G.L. Arnold, E. Blakely. Inherited Metabolic Treatment Center, Golisano Children's Hospital at Strong Memorial, University of Rochester, Rochester, NY 14642, USA.

Background: 3-methylcrotonyl CoA carboxylase deficiency (3MCC) is an autosomal recessive disorder of leucine catabolism characterized by elevated levels of plasma 3-hydroxyisovaleryl- carnitine (C₅OH), urinary 3-methylcrotonyl glycine (3-MCG) and 3-hydroxyisovaleric acid (3-HIVA). Most infants diagnosed by newborn screening appear asymptomatic, but fasting intolerance, hypotonia, mental retardation, failure to thrive and acute metabolic crises have been described in affected patients. We report a child with 3MCC deficiency detected on newborn screen who appeared normal at birth, but who was found to have schizencephaly on investigation for progressive neurological abnormalities, a finding not previously reported in 3-MCC deficiency. **Case report:** This full-term female infant, born to non-consanguineous parents, had modestly elevated C₅OH, 0.85 imol/L, on newborn screening. On confirmatory testing, C₅OH was moderately elevated at 0.24 imol/L, and 3-MCG was increased at 13.3 mg/g UCr. Urine organic acids and free/total carnitine were normal. Early growth and development were unremarkable. At about 6 months of age, the patient demonstrated failure to thrive and neurological regression without an apparent precipitating illness or event, exhibiting poor truncal tone, scissoring, poor head control, and decreased gross motor strength. Her social and emotional development was nearly normal for age. Lymphocyte assay demonstrated very low carboxylase activity at 9 pmol/min/mg/protein. The patient began a leucine-restricted formula, which did not improve either her failure to thrive or neurological issues. Chromosomes, comparative genome array, lysosomal enzyme screening, and ophthalmology exam were normal. A cranial MRI revealed bilateral, open lip schizencephaly and pachy/polymicrogyria. Seizures and failure to thrive are commonly described in schizencephaly; this child has not manifest seizures, and growth improved after discontinuation of the leucine restricted diet such that growth and head circumference remain below (but parallel to) the 5th percentile. **Discussion:** This patient's neurological deterioration and poor outcome prompted further evaluations to determine other causes for these problems beyond 3MCC. As 3-MCC deficiency may be relatively common, and this prenatal origin cerebral defect has not been previously reported in 3-MCC deficiency, we believe their co-occurrence is more likely to be coincident than causal.

- 29. GAMT deficiency should be considered in patients with nonspecific developmental delay, seizures including myoclonic epilepsy, and extrapyramidal signs.** S. Dhar¹, F. Scaglia¹, F. Li¹, B.A. Barshop², C. Eng¹, R.H. Haas², T. Lotze³, B. Maranda⁴, W. O'Brien¹, L. Smith⁵, M. Willis², L.J. Wong¹. ¹Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA; ²Department of Biochemical Genetics, University of California, San Diego, CA, USA; ³Department of Pediatric Neurology, Baylor College of Medicine, Houston, TX, USA; ⁴Service de genetique medicale, Departement de Pediatrie, CHUL-CHUQ, Universite Laval, Que., Canada; ⁵Department of Genetics, UMKC School of Medicine, Kansas City, MO, USA.

Guanidinoacetate methyltransferase (GAMT) deficiency is an autosomal recessive disorder of creatine biosynthesis. This disease is diagnosed by the demonstration of excessive amounts of guanidinoacetate (GAA) in body fluids, the deficiency of creatine/phosphocreatine in the brain, and the presence of mutations in the GAMT gene. It is a largely underdiagnosed disorder. Since its initial discovery in 1994, only 29 patients from 20 families have been reported worldwide. We present here 5 additional cases of GAMT deficiency along with their clinical, biochemical and molecular data. The age at diagnosis of our patients ranges from 10 months to 13 years. They exhibit developmental delay associated with marked speech impairment. The mean age of onset of clinical symptoms is from infancy to 3 years, but one of our patients developed seizures at age 5, which progressed to myoclonic epilepsy at age 8 years. Biochemical and magnetic resonance spectroscopy data are consistent with low creatine and elevated GAA, which have shown improvement with treatment. We also

identified two novel mutations including c.37ins26 and c.522G>A (p.W174X). All of our patients had the c.327G>A splice-site mutation on one of their alleles with one patient being homozygous for this mutation. This mutation appears to be pan-ethnic and our data further adds to the evidence that the c.327G>A splice-site mutation is a hotspot in the spectrum of GAMT mutations. Our example also suggests that it would be prudent to consider GAMT deficiency in the differential diagnosis of juvenile progressive myoclonic epilepsy with Unverricht Lundborg syndrome. As more patients are reported, the prevalence of this fairly treatable disorder will become known and guidelines for prenatal diagnosis, newborn screening, presymptomatic testing, and treatment will have to be formulated. With the availability of relatively inexpensive and easy tests such as measuring levels of creatine and GAA, as well as variability in ages of onset of clinical features, as seen in our patient, screening for GAMT deficiency is warranted in the differential diagnosis of any child with developmental delay, seizures and extrapyramidal signs and symptoms. Diagnosis can be easily confirmed by sequencing of the GAMT gene.

- 30. Intrathecal recombinant human α -L-iduronidase alleviates spinal cord compression symptoms and is well-tolerated in attenuated MPS I patients.** P.I. Dickson¹, D. Naylor², A. Mlikotic³, A. Victoroff¹, A. Chen², M. Passage¹, S. Le¹. MPS I Intrathecal Research Collaborative, ¹Division of Medical Genetics, LA Biomed at Harbor-UCLA, USA; ²Department of Neurology, Harbor-UCLA Medical Center, USA; ³Division of Neuro-radiology, Harbor-UCLA Medical Center, Torrance, CA, USA.

Objective: Intrathecal recombinant human α -L-iduronidase (IT rhIDU) reduces meningeal glycosaminoglycan levels in canine MPS I (Kakkis et al., 2004; Dickson et al., 2007). MPS I patients with spinal cord compression due to thickened meninges may benefit from IT rhIDU. **Methods:** Two attenuated MPS I subjects age 24 and 31 years with symptomatic cord compression received IT rhIDU as part of a clinical trial. One additional patient age 13 years received IT rhIDU off-study. 1.74 mg rhIDU in Elliotts B artificial CSF (total volume 9 mL) was administered into the lumbar or cisternal CSF once per month for four doses. The off-study patient was subsequently treated at ~3 month intervals for an additional 4 doses. **Results:** Subjective improvement was noted in all patients in at least 3 major symptoms, including lower extremity mobility, lower extremity pain, back and/or neck pain, urine and fecal incontinence, restless legs, numbness/tingling of the feet, fatigue, and reduced hand use. Neurologic examination improved in all patients, including improvements in pain and temperature asymmetries, strength, deep tendon reflexes, and range of motion. One patient had slight improvements in scored measures of functionality. Spinal MRI has not shown change. Somatosensory evoked potentials were normal in 2 patients and absent in 1 patient at baseline and did not change. IT rhIDU was well-tolerated, and adverse experiences were in all cases manageable and preferable to surgical decompression of the cord. The most common adverse events were headache and local pain at the injection site. One patient developed an elevated CSF leukocyte count (37 cells/mm³) without clinical meningitis, which responded to a short course of oral steroids and a 2-month interval between injections. Two subjects with hydrocephalus and implanted shunts at study entry had isolated, transient elevations in CSF opening pressure (to 26 cm H₂O). Four serious adverse events have occurred in study subjects to date and have not been related to study drug. **Conclusion:** Preliminary results of IT rhIDU in MPS I patients show that it is safe and may alleviate signs and symptoms of spinal cord compression.

- 31. Simultaneous detection of mitochondrial DNA depletion and single exon deletion in DGUOK using array CGH.** Ni-Chung Lee^{1,2}, David Dimmock³, Wuh-Liang Hwu^{1,2}, Lin-Ya Tang³, Wen-Chang Huang⁴, A.Craig Chinault³, Lee-Jun C. Wong³. ¹Department of Medical Genetics, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan; ²Department of Pediatrics, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan; ³Department of

Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA; ⁴Department of Pathology, National Taiwan University Hospital, Taipei.

Intragenic exonic deletions that cannot be detected by direct DNA sequencing are a common cause of Mendelian disease. Array-based comparative genomic hybridization (aCGH) has now been widely used for the clinical diagnosis of large chromosomal deletions, but not small deletions or analysis of the mitochondrial genome.

We have developed an oligonucleotide-based microarray that provides high-density coverage of the entire mitochondrial genome and nuclear genes related to mitochondrial disorders. Using this oligoarray, an intragenic heterozygous deletion of a small exon of the deoxyguanosine kinase (*DGUOK*) gene was detected in a patient with mitochondrial DNA (mtDNA) depletion syndrome in whom a heterozygous missense mutation, c.679G>A (p.E227K) in the *DGUOK* gene had been identified by direct DNA sequencing. We were then able to retrospectively diagnose an older sibling who died of liver failure with the same mutations. The same oligoarray revealed severe mtDNA depletion in the liver. Real time quantitative PCR analysis confirmed hepatic mtDNA depletion (6% of control). This report demonstrates the clinical utility of this oligoarray in the detection of DNA copy number changes in both the mitochondrial and nuclear genomes, thus, greatly improving the molecular diagnosis of mitochondrial disorders caused by nuclear genes involved in mtDNA biosynthesis.

32. Management and clinical outcome of 8 patients identified with mildly elevated citrulline. D. P. Dimmock¹, Pamela Trapani², Annette Feigenbaum³, Catherine E. Keegan⁴, Jess Thoene⁴, Stephen Cederman⁵, James Gibson⁵, Michael J. Gambello⁶, Keith Vaux⁷, Patricia Ward¹, William O'Brien¹, Ping Fang¹. ¹Baylor College of Medicine, Houston, TX, USA; ²Medical College of Wisconsin, Milwaukee, WI, USA; ³The Hospital For Sick Children, Toronto, Ont., Canada; ⁴University of Michigan, Ann Arbor, MI, USA; ⁵UT Health Science Center, San Antonio, TX, USA; ⁶UT Health Science Center, Houston, TX, USA; ⁷UCSD, San Diego, CA, USA.

Argininosuccinate synthetase (ASS) catalyzes the third step in the urea cycle, the conversion of citrulline and aspartate into argininosuccinic acid with the cleavage of ATP into AMP and PPi. Deficiency of the enzyme results in Classic citrullinemia (type I). This disorder is typically associated with elevations in citrulline above 1000 microM. And is associated with significant long term neurological disability.

Several recent papers have investigated the mutation spectrum in patients with mildly elevated citrulline on newborn screening. However long term response to therapy in these patients has not been documented. Classification of the disease to date has been based on citrulline levels and presence or absence of ASS protein. However another patient has been described with a poor neurological outcome in spite of only modestly elevated levels of citrulline. Therefore it seems that the natural history of these mild cases is not well understood. This leads to significant therapeutic dilemmas. Additionally mild elevations in citrulline in the newborn period are also associated with citrin deficiency (citrullinemia type 2), argininosuccinate lyase deficiency and pyruvate carboxylase deficiency and these disorders must be considered in the differential diagnosis. Clinical sequence analysis of the ASS gene has recently become available in the US. In this paper we explore the value of molecular and enzymatic information in conjunction with clinical reporting and psychometric analysis to direct clinical management decisions and genetic counseling.

33. In vivo assessment of relative mitochondrial oxidant levels in *C. elegans* mitochondrial mutants. M.J. Falk¹, R. Lightfoot², S. Dingley¹, M. Rao¹, E. Polyak¹, H. Ischiropoulos². ¹Divisions of Human Genetics, The Children's Hospital of Philadelphia and University of Pennsylvania, Philadelphia, PA, USA; ²Neonatology, Department of Pediatrics, The Children's Hospital of Philadelphia and University of Pennsylvania, Philadelphia, PA, USA.

The mitochondrial respiratory chain is associated with oxidant production and altered longevity in *C. elegans*. These associations have been made largely using *in vitro* markers of oxidant damage. To better assess individual mitochondrial component involvement in oxidant species generation, we developed an *in vivo* method to ascertain changes in *C. elegans*' mitochondrial superoxide levels. **Methods:** Synchronous *C. elegans* young adults mutant for complexes I (*gas-1*), II (*mev-1*), III (*isp-1*), the insulin receptor (*daf-2*), or mitochondrial manganese superoxide dismutase (*sod-3*) were fed 10 uM Mitosox Red (a mitochondrial matrix superoxide indicator dye) with or without oxidant stressors (Paraquat or Antimycin A). Terminal pharyngeal bulb mean intensity in living worms was quantified by fluorescence microscopy following 24 hour exposures. *sod-3* relative gene expression was also assessed to determine the response of the major mitochondrial superoxide scavenging enzyme to primary mitochondrial dysfunction and oxidizing agents. **Results:** A significant increase in steady-state superoxide levels was detected in *gas-1* (9.1%, $p < 0.0001$) and *sod-3* (63.7%, $p < 0.0001$) when compared with wildtype (N2). Significantly increased superoxide levels were observed in all mutants in comparison with N2 upon exposure to Paraquat (*gas-1* 55.6%, $p < 0.0001$; *mev-1* 40.3%, $p < 0.0001$; *isp-1* 14.5%, $p < 0.0001$, *sod-3* 60.6%, $p < 0.0001$), with the exception of *daf-2*. Similarly, a lethal dose of Antimycin A for *sod-3* resulted in no significant increase in superoxide levels in N2, *isp-1*, or *daf-2*. Intrastrain comparisons with and without Paraquat demonstrated significantly increased superoxide levels only in *gas-1* (37.8%, $p < 0.001$) and *mev-1* (34.6%, $p < 0.0001$). RT-qPCR analysis demonstrated a 10- to 20-fold upregulation of mitochondrial mnSOD (*sod-3*) in *isp-1* and *daf-2* as compared to a 3- to 5-fold upregulation in *gas-1* and *mev-1*, regardless of paraquat exposure. Relative expression of a second mitochondrial mnSOD homologue in *C. elegans* (*sod-2*) demonstrated no change in *gas-1*, and only 2- to 3-fold changes in *isp-1* and *daf-2*. **Conclusions:** Terminal pharyngeal bulb fluorescence intensity quantitation of Mitosox Red-fed *C. elegans* is a sensitive and specific method of assessing *in vivo* steady-state mitochondrial superoxide levels. The results suggest the short-lived complex I and II mutants have increased sensitivity to oxidant stress and relatively decreased capacity to scavenge oxidant species. Among the long-lived complex III and insulin receptor mutants, superoxide levels do not increase substantially with oxidant stress; this is likely related to their dramatically increased superoxide scavenging capacity. In addition, *sod-3* appears to be the predominant mitochondrial superoxide dismutase responding to oxidant species stress in *C. elegans*.

34. Primary coenzyme Q deficiency in *Pdss2* mutant mice causes isolated renal disease. M.J. Falk¹, M. Peng², Z. Zhang², V.H. Haase⁴, R. King⁵, E. Polyak¹, W.W. Hancock³, DL Gasser⁴. ¹Divisions of Human Genetics, The Children's Hospital of Philadelphia and University of Pennsylvania, Philadelphia, PA, USA; ²Biomedical Informatics, Department of Pediatrics, The Children's Hospital of Philadelphia and University of Pennsylvania, Philadelphia, PA, USA; ³Department of Pathology & Laboratory Medicine, The Children's Hospital of Philadelphia and University of Pennsylvania, Philadelphia, PA, USA; ⁴Departments of Genetics, ⁵Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA, USA.

Coenzyme Q (CoQ) is an essential electron carrier in the mitochondrial respiratory chain whose deficiency has been implicated in a wide variety of human mitochondrial disease manifestations. Its multi-step synthesis involves the addition of an isoprenoid side chain to a benzoquinone, a reaction which requires the gene products of *PDSS1* and *PDSS2*. Homozygous mutations in either of these genes lead to severe neuromuscular disease in humans, as well as nephrotic syndrome in the case of *PDSS2* deficiency. Using missense and conditional knockout mutants of the mouse homolog, *Pdss2*, we show that a presumed autoimmune kidney disease in mice is instead attributable to the mitochondrial CoQ biosynthesis defect. Furthermore, we show that disease manifestations originate specifically in glomerular podocytes, as renal disease is seen in *Podocin^{Cre};Pdss2^{loxP/loxP}* knockout mice but not in conditional knockouts targeted to renal tubular epithelium, monocytes, or hepatocytes. *Pdss2^{kd/kd}* missense mutants have no

overt extra-renal disease manifestations, despite having significant functional impairment of integrated respiratory capacity in freshly isolated liver mitochondria. Similarly, no overt disease manifestations are present in *Albumin^{Cre}, Pdss2^{loxP/loxP}* liver-specific conditional knockout mice despite demonstration of >90% downregulation of *Pdss2* by real-time qPCR and significant functional impairment of integrated respiratory capacity in freshly isolated liver mitochondria. Interestingly, global genome expression analysis in liver isolated from *Albumin^{Cre}, Pdss2^{loxP/loxP}* knockout mice demonstrates a similar pattern of cellular adaptive responses at the level of biochemical pathways as were previously identified in a *C. elegans* model of primary mitochondrial dysfunction (Falk et al., 2007 SIMD abstract; Falk et al, *Molecular Genetics and Medicine*, in press). These data suggest that disease manifestations of CoQ deficiency relate to tissue-specific respiratory capacity thresholds, with glomerular podocytes displaying the greatest sensitivity to *Pdss2* impairment. Therapeutic trials are ongoing to assess the response of renal disease to dietary coenzyme Q10 or antioxidant supplementation. In addition, the identification of consistent expression patterns of cellular response to primary mitochondrial dysfunction in both nematode and mammalian models permits insight into the complex pathogenesis underlying primary mitochondrial disease and may enable the development of a metabolomic profiling diagnostic tool applicable to human mitochondrial disease.

35. An improved model for moving genetic tests from research to clinical testing. W. Andrew Faucett, Emory University School of Medicine, USA.

Abstract: The NIH Office of Rare Diseases' Collaboration, Education and Test Translation (CETT) Program has improved and increased the translation of genetic tests from research laboratories to clinical laboratories. In this model tests are translated by a collaborative group consisting of a clinical laboratory, researcher, and research laboratory and disease specific advocacy group. Each collaborative group also develops a plan to collect clinical data necessary for test result interpretation which is linked with mutation data and posted in a publicly accessible database. Research laboratories lack the necessary federal CLIA certification and are not prepared to provide the full extent of clinical services needed by the clinical/patient community. Research laboratories often have long turn-around times, lack sufficient quality control systems, lack staff trained in providing and explaining clinical results, and cannot support testing as a clinical service. When tests move from the research setting to the clinical setting important clinical and mutation data are no longer accessible for future research which may limit understanding of the rare disease manifestations and natural history. The expertise provided by the research laboratory and/or clinical researcher is needed to interpret newly identified mutations, recessive mutation combinations not seen during the research testing phase, or variants of unknown significance. Many researchers are not aware of clinical laboratories that are willing and able to translate testing developed in their research laboratory. The CETT Program has resulted in over 25 tests moving successfully from research to clinical testing. To date most tests have used various molecular testing methodologies, but the Program is open to funding and review for all testing platforms. Several tests have used an existing biochemical screening test in the testing paradigm and the CETT Program has funded the development of the clinical molecular test. The Program has shown that the same level of quality assurance and quality control can be provided for low volume rare disease testing.

36. Pharmacological chaperones for the treatment of lysosomal storage disorders. J.J. Flanagan, R. Khanna, A.C. Powe, X. Wu, E.R. Benjamin, W. Liang, R. Soska, K. Tang, L. Pelligrino, Y. Lun, S. Sitaraman, D. Palling, B.A. Wustman, K.J. Valenzano, H.V. Do, D.J. Lockhart. Amicus Therapeutics, Inc., 6 Cedar Brook Drive, Cranbury, NJ 08512, USA.

Lysosomal storage disorders are caused by mutations in genes that encode for enzymes responsible for performing critical lysosomal functions. The majority of the mutations lead to the production of

less stable proteins that are inefficiently trafficked to lysosomes. We are developing pharmacological chaperones for the treatment of Fabry, Gaucher, and Pompe diseases. Pharmacological chaperones are small molecules designed to interact with the target protein to stabilize and prevent premature degradation. Stabilization by binding of pharmacological chaperones improves protein trafficking and results in higher lysosome-specific enzymatic activity. We have demonstrated that binding of pharmacological chaperones to mutant forms of the lysosomal enzymes increases protein levels and activity in both patient-derived cell lines and in transiently transfected COS-7 cells expressing mutant forms of the enzymes. AT1001 administration to Fabry transgenic mice resulted in a reduction of the lipid, globotriaosylceramide, in plasma and tissues such as heart, skin, and kidney. Taken together, these in vitro and in vivo results demonstrate the potential of using pharmacological chaperones for the treatment of lysosomal storage disorders. Currently, we have three pharmacological chaperones in clinical trials: AT1001 for Fabry disease, AT2101 for Gaucher disease, and AT2220 for Pompe disease.

37. Novel mutations in the fukuyama congenital muscular dystrophy (FCMD) gene associated with a mild phenotype. R.L. Forst¹, T.L. Winder², S.A. Moore³, S.G. Romansky⁴, K. King Covault¹, J.E. Abdenur¹. ¹Division of Metabolic Disorders, Children's Hospital of Orange County, Orange, CA, USA; ²Prevention Genetics, Marshfield, WI, USA; ³Department of Pathology, University of Iowa, Iowa City, USA; ⁴Department of Pathology, Long Beach Memorial Medical Center, Long Beach, CA, USA.

Background: The dystroglycanopathies comprise a clinically and genetically heterogeneous group of muscular dystrophies. They are characterized by deficient glycosylation of α -dystroglycan secondary to mutations in at least six genes: POMT1, POMGNT1, POMT2, FKRP, FCMD, and LARGE. Mutations in FCMD, the gene for fukutin, have primarily been identified among patients with classic FCMD, a disorder characterized by muscle weakness and hypotonia in early infancy, severe mental retardation and neuronal migrational defects. Survival is usually less than 20 years. Prior to the identification of the gene mutated in this disorder, diagnosis was based on clinical symptoms and muscle pathology. FCMD has been reported almost exclusively in Japan due to the presence of a founder mutation. **AIM:** To report two brothers of mixed Caucasian and Japanese ancestry, with normal intelligence and mild muscle weakness, who were found to be compound heterozygotes for two novel FCMD mutations. **Patients:** The proband was referred at 13 months of age for failure to thrive and elevated transaminases. Physical exam, including muscle tone, was normal. Creatine kinase (CK) was markedly elevated 3110 U/L (nl <174). Work-up for metabolic myopathies was negative. Symptoms of muscle weakness were noted at 21 months of age and the patient was subsequently referred for muscle biopsy. **Results:** Muscle pathology revealed wide variation in fiber size and a number of degenerating and regenerating fibers, strongly consistent with a muscular dystrophy. A dystrophinopathy was ruled out. Immunofluorescence demonstrated a reduction in γ -dystroglycan staining, suggestive of a defect of γ -dystroglycan glycosylation. Sequencing of the FCMD gene led to the identification of two previously unreported mutations: 340G>A/527T>C. Both mutations are predicted to be pathogenic due to their disruption of highly conserved amino acid residues. The patient is now over 3 years of age. His muscle weakness has progressed, but no neurological, ocular or cardiac abnormalities have been identified. A brain MRI was recommended, but has not been performed. The proband's older sibling was evaluated at 3 y and 11 m, due to his brother's diagnosis. He had normal intellectual development. Physical exam revealed mild muscle weakness, calf hypertrophy, decreased DTR's and partial Gower's sign. CK was 5487 U/L. Subsequent ophthalmologic and cardiac evaluations were normal. **Conclusions:** Our results provide further evidence for allelic heterogeneity and the presence of milder phenotypes, including normal intelligence, in FCMD. We also provide additional information for the occurrence of this disorder in non-Japanese populations.

38. Maternal 3-MCC deficiency as a model for long term outcome. D.M. Frazier¹, J. Koepke¹, T. Wood², T. Pritchett¹. ¹Division of Genetics and Metabolism, Department of Pediatrics, University of North Carolina, Chapel Hill NC 27599, USA; ²Greenwood Genetics Center, Greenwood SC 29646, USA.

Objective: Women who were first diagnosed with 3-methyl crotonyl CoA carboxylase (3-MCC) deficiency when their newborns had elevations of 3-OH isovaleryl carnitine (C₅OH) on their MS/MMS newborn screen were studied to learn if there are negative consequences of having no intervention through early adulthood. **Methods:** Four women, between the ages of 24–43 were enrolled in the study. They agreed to travel to the Genetics Center, have a physical exam, give a complete family and medical history, keep dietary records and have clinical testing. These tests included: urine organic acids, plasma acyl carnitine profile, carnitine, CK, plasma amino acids and 3-MCC mutation analysis. If they were found to be carnitine deficient, they agree to take carnitine (~50 mg/kg) for three months and keep a clinical diary. At the end of this period, they were re-interviewed regarding any changes in their symptoms, diet, etc. **Results:** On entrance to the study, plasma free carnitine levels ranged from 3 to 13 micromolar. Plasma acylcarnitine profiles and urine organic acids were consistent with 3-MCC deficiency, and CK levels were normal. Two women had three mutations previously described and one novel mutation. The two unrelated Latino women were homozygous for mutations which have not been reported elsewhere. Clinical symptoms included easy fatigability, joint and muscle pain, headaches, dry hair/skin/nails, and poor short term memory. Free carnitine levels normalized after 4 weeks on the supplement. Clinic symptoms improved significantly. **Conclusion:** In this small group of untreated 3-MCC deficient adult women, the symptoms were similar and appeared to be alleviated with carnitine supplementation. Entering the study, the women's typical dietary protein intake was somewhat lower than average, but was not modified during the study. Decisions regarding the exclusion of 3-MCC deficiency from the MS/MS screening panel should wait until a large multi center study of similar untreated adults can be completed.

39. Development of a disease severity scoring system for patients with Pompe disease. E.H. Giannini¹, K. Berger², A. van der Ploeg³, L. Case⁴, C. Dandrea⁵, P. Kishnani⁴, D. Marsden⁷. ¹Cincinnati Children's Hospital, Cincinnati, OH, USA; ²NYU School of Medicine, New York, NY, USA; ³Sophia Children's Hospital, Rotterdam, The Netherlands; ⁴Duke University Medical Center, Durham, NC USA; ⁵Genzyme Corporation, Cambridge, MA, USA.

Introduction: A Disease Severity Scoring System (DS3) measures disease burden in patients. It consists of critical health domains, each described by relevant clinical assessment(s) quantified via reliable, valid and feasible methods. DS3s are particularly useful in rare, heterogeneous diseases in which evaluating severity and prognosis is difficult. Properly configured, a DS3 provides inter- and intra-patient comparisons through time across critical organ systems. Development is underway for Pompe disease, a rare, autosomal recessive, heterogeneous, neuromuscular disorder. **Description:** A panel of Pompe experts was assembled to identify critical Pompe disease health domains. A broader "Delphi" physician group was consulted to capture standard medical practice(s) for severity measurement within each critical domain. Selected domains were: Cardiac, Respiratory, Proximal Muscle, Physician Reported Outcomes and Patient Reported Outcomes. Within each domain, 1–2 clinical assessments were identified. To test this preliminary model, 9 cases from the Pompe Registry representing a severity spectrum were scored. **Results:** Results were compared to results from a blinded small expert group assessment of the cases using a scale similar to the Clinical Global Impression (CGI) Severity scale, yielding a 0.93 coefficient of correlation, indicating preliminary DS3 consistency with expert opinion, suggesting preliminary DS3 validity, reliability and relevance. Preliminary validity and reliability testing are being completed by the use of standardized methods. **Conclusion:** Preliminary results indicate the Pompe DS3 model will help standardize disease terminology and highlight key clinical assessments to quantify disease severity. Ultimately this tool can evolve

into a universal disease "staging" system that permits more exact prediction of important disease outcomes and identify the need for specific medical interventions.

40. Aberrant thermoregulation in the mevalonate kinase-deficient (Mvk^{+/-}) hyper-IgD mouse model. E.J. Hager^{1,4}, C. Croniger⁶, F. Allen⁶, K.M. Gibson^{1,2,3,4,5}. ¹Department of Pediatrics, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA; ²Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA; ³Department of Human Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA; ⁴Children's Hospital of Pittsburgh, University of Pittsburgh Medical Center, Pittsburgh, PA, USA; ⁵Biochemical Genetics Laboratory, University of Pittsburgh Medical Center, Pittsburgh, PA, USA; ⁶Department of Nutrition and Mouse Metabolic Phenotyping Center, Case Western Reserve University School of Medicine, Cleveland, OH, USA.

Objectives: Deficiency of mevalonate kinase (Mvk) in the early pathway of cholesterol synthesis results in two human disorders (e.g., severe mevalonic aciduria and hyper-IgD syndrome with periodic fever), which overlap in metabolic and molecular phenotypes yet differ immunologically. Loss of both Mvk alleles in mouse appears lethal; conversely, Mvk^{+/-} animals survive with decreased liver enzyme activity, elevated tissue/blood mevalonate, increased serum IgD levels and immune dysfunction¹. Thus far, penetrance in Mvk^{+/-} mice appears to be incomplete. In the current study, we asked whether decreased Mvk activity and hyper-IgD syndrome associated with altered thermoregulation and increased interleukin-6 (IL-6), a key proinflammatory cytokine that induces fever. **Methods:** Subjects were 12-week-old female mice ($n = 2$, Mvk^{+/-}; $n = 2$, Mvk^{+/+}). Mice were implanted with mini-mitters that measured core body temperature over a 48-h span at 10 min intervals at 70 °F ambient temperature with alternating 12 h cycles of light and dark. Animals were fed *ad libitum*. Serum IL-6 levels were quantified by standard ELISA methodology. **Results:** Body temperatures of both wild-type and Mvk^{+/-} mice fluctuated rhythmically about a mean temperature, 35.1 and 36.0 °C, respectively, with Mvk^{+/-} mice almost a full degree higher than Mvk^{+/+} mice. In Mvk^{+/+} mice, the oscillations ranged between 34–36 °C having a maximal 2° in amplitude, while the Mvk^{+/-} mice ranged between 34–38 °C having a maximal 4° in amplitude. The temperature rhythmicity pattern of Mvk^{+/+} mice showed a rapid fluctuation frequency having an average period length of oscillation of 0.5 min, whereas the Mvk^{+/-} mice temperature patterns had a prolonged average periodicity of 0.9 min, thus a slower fluctuation frequency almost twice as long as that of Mvk^{+/+}. Forty-four percent of Mvk^{+/-} animals manifested serum IL-6 titers up to 1:200, whereas only 8% of Mvk^{+/+} animals had a measurable IL-6 titer. **Conclusions:** Emerging evidence suggests that IL-6 is an important endocrine factor transmitting information on peripheral inflammation to the central nervous system via the hypothalamic-pituitary-adrenal axis². Our findings that Mvk^{+/-} mice appear to have thermoregulatory differences that associate with elevated IL-6 support this observation. Our preliminary findings further suggest that Mvk^{+/-} mice represent a valid model of human hyper-IgD syndrome with periodic fever. ¹Hager et al. *J Inherit Metab Dis*, *in press*. ²Hopkins SJ (2007) *Acta Biomed* 78 Suppl 1: 231-247. Supported in part by NIH/NIDDK U24DK76169 to the Case Western Reserve University MMP.

41. Insulin sensitivity in subjects with VLCAD or LCHAD deficiency. M.B. Gillingham¹, J.Q. Purnell², C.O. Harding¹. ¹Department of Molecular and Medical Genetics, Oregon Health & Science University, Portland, Oregon 97239, USA; ²The Center for the Study of Body Weight Regulation, Oregon Health & Science University, Portland, Oregon 97239, USA.

Despite the rising epidemic of obesity, insulin resistance, and type 2 diabetes, the precise metabolic connection between increased adiposity and insulin resistance is still unknown. A causative role for increased mitochondrial fatty acid oxidation (FAO) flux in the development of diet-

induced, obesity-associated insulin resistance has been proposed. If this hypothesis is true, then decreasing FAO flux may prevent insulin resistance. Consistent with this hypothesis, there are no literature reports of subjects with inherited disorders of long-chain FAO developing insulin resistance despite exhibiting impaired FAO flux and an increased incidence of obesity. To explore this hypothesis, we measured fasting glucose and insulin levels in nine subjects with LCHAD deficiency, estimated insulin resistance using a homeostatic assessment model (HOMA), and found no evidence of insulin resistance despite high total body adiposity. Furthermore, we measured body composition by DEXA, intrahepatic and intramuscular lipid by magnetic resonance spectroscopy (MRS) and estimated insulin sensitivity by oral glucose tolerance test in subjects with VLCAD or LCHAD deficiency compared to controls. Subjects with long-chain FAO disorders had increased muscular lipid deposition (primarily extramyocellular lipid deposition) but no apparent increase in hepatic lipid content. Plasma glucose clearance and insulin concentrations following a 75 g oral glucose load were similar between subjects and controls. The data suggest subjects with VLCAD and LCHAD deficiency may have normal insulin sensitivity despite increased total and muscular lipid deposition.

42. Diffusion tensor imaging detects areas of abnormal white matter microstructure in patients with partial OTCD. Andrea L. Gropman^{1,2}, Ilana L. Kahn¹, Rebecca Seltzer³, John Van Meter³, and the Urea Cycle Rare Disorders Consortium. ¹George Washington University Medical School, Washington DC, USA; ²Children's National Medical Center, Washington, DC, USA; ³Georgetown University Center for Functional and Molecular Imaging, Washington, DC, USA.

Aim: Use diffusion tensor imaging (DTI) to compare white matter microstructure in OTCD with controls and correlate with cognitive deficits. *Background:* Males with late onset OTCD show deficits in executive function and motor planning. A study of heterozygous females found a nonverbal learning disability associated with white matter or subcortical dysfunction. Previous autopsy and neuroimaging studies are also consistent with a pattern of neuronal injury leading to white matter damage. However, the extent to which the deficits involve specific pathways in the brain is unknown. *Methods:* Using our previous multimodal imaging findings to guide our current work, we used DTI to assess white matter integrity. 13 subjects with partial OTCD and 12 control subjects (age range 19–59 years) participated in this study. Subjects were recruited as part of a NIH funded Rare Diseases Clinical Research Center. MRI was performed using a 3T whole-body MRI scanner (Siemens Magnetom Trio) and an eight channel phased array head coil. A DTI sequence was acquired four times using an EPI sequence with two diffusion weighted gradients of $b = 0$ and 1000 s/mm² applied in 30 orthogonal directions. 55 axial interleaved slices were acquired with a 2.5mm³ spatial resolution (TE = 100, TR = 7700). From the raw data, the diffusion tensor was calculated. The degree of anisotropy was calculated for each acquisition from the eigenvalues of the diffusion tensor using the fractional anisotropy (FA) metric. Diffusion indices in these ROIs were quantified and compared between groups by using the Mann–Whitney rank-sum test. *Results:* FA of the cingulum, superior frontal, and supplemental and motor white matter were significantly lower in subjects with partial OTCD indicating changes in white matter microstructure in these regions. This fits with the cognitive phenotype in which there are impairments in executive function. There was an inverse relationship between FA and disease severity scores (a measure of previous hyperammonemic episodes, coma, and elevated ICP). Fiber tracking disclosed abnormalities in corpus callosum fiber integrity with blunting of anterior directed fibers. *Discussion:* OTC is the only X linked disorder of ammonia metabolism. MRI scans are often normal in patients who have late onset disease, are heterozygotes, or are stable and not in hyperammonemic crisis. In our study, DTI images were more sensitive than FSE T2-weighted images for detecting abnormalities in the normal-appearing white matter. In addition, DTI images can provide additional quantitative MR parameters for assessing and monitoring patients over time and their response to diet and therapies.

43. ¹³C MRS study of ornithine transcarbamylase deficiency (OTCD). Andrea L. Gropman¹, Napapon Sailasuta², Larry Robertson³, Kent Harris², Peter S. Allen⁴, Brian D. Ross². ¹Department of Neurology, Children's National Medical Center, Washington, DC, USA; ²Huntington Medical Research Institutes, Pasadena, CA, USA; ³Spin Dynamics, South Pasadena, CA, USA; ⁴Department of Biomedical Engineering, University of Alberta, Edmonton, CA, USA.

Aim: To investigate glutamine and glutamate turnover in OTCD. *Background:* The etiology of brain injury in OTCD is not fully known. ¹H MRS studies show elevations in glutamine and decreases in myo-inositol and choline in patients. Myo-inositol concentration inversely correlates with disease severity. ¹H MRS is a sensitive tool to detect biochemical abnormalities in individual patients; specificity suffers from complex peak patterns due to J-coupling. ¹³C MRS can reliably quantitate distinct signals from Glu to Gln. ¹³C metabolic flux measurements have proved useful in grading severity of a related brain disorder, hepatic encephalopathy (HE). *Methods:* Three subjects with partial OTCD were studied with quantitative ¹H and ¹³C MRS and compared to control, unaffected subjects. ¹³C MRS was performed on a 1.5 Signa GE Scanner equipped with a stand alone broadband decoupling hardware. Axial T2 weighted MR images and localized quantitative proton spectra (PRESS TE/TR 35/1.5 ms) were acquired with the standard GE head coil from a 12.5 ml volume of mixed gray and white matter of the occipital region. Proton decoupled ¹³C spectra were acquired with a half volume surface coil using a 4 kHz excitation bandwidth and WALTZ 4 decoupling bandwidth 1000 Hz centered at 2.7 ppm in the proton spectrum. Natural abundance ¹³C MRS was obtained in 5 min blocks for 20–30 min. Intravenous low dose infusion of 0.23 g/kg body weight of 99% 1-¹³C glucose (20% W/V) over 10 min was commenced and unlocalized ¹³C MRS acquisition continued over 120 min. Myo-inositol which does not become enriched, was used as an internal reference for quantification of other ¹³C metabolites. *Results:* 1-¹³C glucose appeared in the ¹³C brain spectra of all subjects. Enrichment of C1–thru C₅ of glutamate and glutamine occurred, as expected from normal operation of turns 1, 2 and 3 of the cerebral TCA cycle. Enriched ¹³C HCO₃⁻, observed in all patients and controls after 60 min, confirmed complete glucose oxidation. While preliminary, interpretation indicates normal glucose uptake and further metabolism through the neuronal glial glutamate-glutamine cycle in patients with OTCD; excess enrichment in glutamine C4 and C2 is consistent with abnormality in glial gln synthesis in OTCD. *Discussion:* Using ¹³C MRS we confirm that OTCD subjects metabolize administered ¹³C glucose through three turns of the neuronal TCA-cycle. Excess ¹³C glutamine is consistent with the anticipated abnormalities in glial glutamate metabolism. Further elucidation of flux rates may identify subtle differences between symptomatic and asymptomatic OTCD carriers.

44. 3-Methylglutaconic aciduria type III: Insights into the OPA3 protein. M. Huizing¹, W. Pei², H. Dorward¹, L. Ly¹, E. Klootwijk¹, C.A. Wassif³, F.D. Porter³, W.A. Gahl¹, B. Feldman², Y. Anikster⁴. ¹Human Biochemical Genetics, Medical Genetic Branch, NHGRI, NIH, Bethesda, USA; ²Vertebrate Embryology Section, Medical Genetic Branch, NHGRI, NIH, Bethesda, USA; ³Program in Developmental Endocrinology and Genetics, NICHD, NIH, Bethesda, USA; ⁴Metabolic Disease Unit, Sheba Medical Center, Israel.

Type III 3-methylglutaconic aciduria is a neuro-ophthalmologic syndrome of early-onset bilateral optic atrophy, later-onset spasticity, and extrapyramidal dysfunction. Urinary excretion of 3-methylglutaconic acid (3MGC) and of 3-methylglutaric acid (3MGR) is markedly increased. In 2001, we identified the causative gene, *OPA3*, consisting of two exons coding for a 179-amino acid protein. Here we report that *OPA3* also produces a novel transcript consisting of the common exon 1 spliced directly to a third exon, skipping exon 2 altogether. This transcript is conserved in mammals, and exon 3 closely resembles exon 2, suggesting an evolutionary segmental duplication. Molecular analysis of 25 patients with unexplained optic atrophy and/or increased urinary 3MGC and 3MGR did not reveal exon 3 mutations.

The OPA3 protein, whose function remains unknown, contains an N-terminal mitochondrial leader sequence and targeting signal and a putative C-terminal peroxisomal targeting signal. Since increased 3MGC and 3MGR levels can result from defects in either the mitochondrion (e.g., leucine degradation) or the peroxisome (e.g., mevalonate shunting), we investigated intracellular localization of OPA3. Normal fibroblasts were electroporated with Green Fluorescent Protein tagged OPA3 fusion proteins (ex 1–2 and ex 1–3 transcripts) with or without mutated mitochondrial and/or peroxisomal targeting signals. Confocal microscopy clearly demonstrated a mitochondrial localization for both OPA3 transcripts (ex 1–2 and ex 1–3). However, the ex 1–2 transcript also localized to peroxisomes. This rare dual localization could help elucidate the biochemical function of OPA3. To further explore OPA3 function, we created zebrafish models using antisense morpholinos. The mutant fish showed unique features, including small eyes with severely affected optic nerves, delayed development, kinked tails, spastic movements, and increased levels of 3MGC and 3MGR - all consistent with the human condition. These zebrafish models offer promise in further elucidating the biochemical function of OPA3 and assisting in the development of possible therapies for this devastating disorder.

45. Tolerance of elevated tyrosine levels in patients with alkaptonuria receiving nitisinone. W.J. Inrone¹, M.A. Kayser², E.T. Tsilou³, K.E. O'Brien¹, J. Bryant¹, I. Bernardini⁴, W.A. Gahl^{1,4}. ¹OCD, NHGRI, NIH, Bethesda, MD, USA; ²Warren Clinic Center for Genetics and Center for Genetic Testing at St. Francis, Tulsa, OK, USA; ³NEI, NIH, Bethesda, MD, USA; ⁴MGB, NHGRI, NIH, Bethesda, MD, USA.

Alkaptonuria, a rare disorder of tyrosine degradation, is due to deficiency of homogentisate 1,2-dioxygenase. Homogentisic acid (HGA) accumulates, becomes oxidized, and binds to connective tissue causing darkened urine, ochronosis, joint destruction, and cardiac valve deterioration. In 2002, nitisinone (Orfadin[®]), a potent reversible inhibitor of *p*-hydroxyphenylpyruvate dioxygenase, was approved for the treatment of hereditary tyrosinemia type I (HT-I), a disorder in which nitisinone has been well tolerated and life-saving. As a potent inhibitor of the second step in the tyrosine degradation pathway, nitisinone raises plasma tyrosine levels, causing concerns about complications, such as corneal crystals, erosions and opacities. Dietary restriction of tyrosine and phenylalanine has been advised. In 2005, we initiated a long-term clinical trial investigating the use of nitisinone in patients with alkaptonuria. Forty patients have been enrolled with twenty of the patients being randomized to receive treatment with nitisinone. Plasma tyrosine levels are measured every 4 months. After administration of nitisinone, tyrosine levels rose to an average of 770 μ M (range 357–1528 μ M). Despite this 10-fold increase, remarkably few complications have been reported. One patient with alkaptonuria receiving nitisinone developed ocular symptoms of eye irritation and pain. On slit lamp examination, the classical branching subepithelial corneal opacity previously described in HT-I patients was observed. On two separate measurements, his tyrosine levels were 615 μ M and 574 μ M. The lesion completely resolved with discontinuation of the nitisinone; however, his symptoms recurred with reinitiation of therapy and he was unable to continue use of the medication. None of the other patients with alkaptonuria receiving nitisinone has developed ocular symptoms, and ophthalmologic examinations have revealed no evidence of corneal pathology. We conclude that the marked hypertyrosinemia associated with nitisinone is surprisingly well tolerated by our adult patients with alkaptonuria. There are, however, patients who will not tolerate the medication, and plasma tyrosine levels do not predict the occurrence of side effects.

46. Menke disease phenocopy in a female with normal ATP7A coding sequence. P.M. James¹, C. Ryan², E.C. Engle², J.R. Tang³, S.G. Kaler³, G.T. Berry¹. ¹Division of Genetics, Department of Pediatrics, Children's Hospital Boston, USA; ²Division of Neurology, Department of Pediatrics, Children's Hospital Boston, USA; ³Unit on Pediatric Genetics, Laboratory of Clinical Genomics, NIH, USA.

We report a 17-month-old female with poor growth, developmental delay, hypotonia, truncal ataxia, decreased serum copper 11–52 μ g/dl [nl: 75–145], and ceruloplasmin 130–170 mg/L [nl: 220–660]. Physical examination revealed an alert, animated, playful infant with weight <1 percentile, length 5 percentile, head circumference 10 percentile, lax smooth skin, decreased subcutaneous tissue, uneven islands of light pigmentation in lower extremities, slight wiry appearance to hair, costochondral protuberances, hyperflexion of knees and hips and 3 + deep tendon reflexes in lower extremities. Additional studies included a normal plasma lactate, CK and catecholamines; normal urine α_2 -microglobulin and normal karyotype (46, XX). Wormian bones were present on skull radiograph and bone age was delayed. Cerebellar atrophy, enlargement of extra-axial spaces and marked tortuosity of arteries of the Circle of Willis were seen on MRI/A. Pathologic examination of hair showed periodic narrowing of the hair shaft with thinning of the cortex and reduction/absence of the medulla consistent with monilethrix. The sequencing in both forward and reverse directions of the 23 exons of the ATP7A gene did not detect any deleterious changes. Additionally, Western analysis to quantitate ATP7A protein in the patient's cultured lymphoblasts and sequencing of the copper uptake gene hCTR1 and the copper chaperone ATOX1 are all in progress. We conclude that this female patient has a phenotype that resembles Menke disease but is without pili torti and abnormal catecholamine levels, and has a normal coding region sequence of the ATP7A gene.

47. Clinical outcomes of infants with short-chain acyl-coenzyme A dehydrogenase deficiency detected by newborn screening. R.N. Jethva, C. Ficicioglu. Division of Child Development, Rehabilitation, and Metabolic Disease, The Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA.

Numerous inborn errors of metabolism can be detected through MS/MS-based newborn screening. Although it is widely accepted that early diagnosis has the potential to benefit many patients with metabolic disorders, the need for early detection of short-chain acyl-coenzyme A dehydrogenase deficiency (SCADD) warrants further investigation. SCADD is a fatty acid oxidation disorder with variable expressivity and incomplete penetrance. Genotype-phenotype correlations are poorly defined due to a high prevalence of susceptibility polymorphisms and relatively fewer mutations. Furthermore, many individuals who are homozygotes or compound heterozygotes are asymptomatic. Although possible modifiers for clinical presentation are being explored, SCADD may also be a benign biochemical phenomenon that is found randomly in patients with non-specific findings merely because of the distribution of genotype variants. Few investigators have examined long-term outcomes in infants diagnosed via newborn screening, a strategy that may provide insight into the disorder's true course. Our institution has followed twelve patients with butyrylcarnitine elevations at birth over a range of several weeks to over four years. These patients had biochemical and molecular evidence suggestive of SCADD. Biochemical results included elevated plasma butyrylcarnitine and urinary ethylmalonate in eleven cases. Molecular results included two 625G > A polymorphism homozygotes, a 625G > A polymorphism heterozygote, a 529T > C homozygote, two 529T > C heterozygotes, a 511C > T polymorphism heterozygote, a 319C > T homozygote, and a compound heterozygote with 529T > C and 1095G > T mutations. Three infants are awaiting sequencing studies. Clinically available molecular testing was limited to exons 5 and 6 for several years; thus, patients who are listed as heterozygotes are likely to actually be compound heterozygotes if they had classic metabolic findings. One male patient had speech delay, but all of the others had no developmental or significant health concerns. The delayed patient had three siblings, all of whom were 625G > A homozygotes. One of his sisters also had speech delay, both parents had a history of speech delay, and his father had a mild learning disability. *Conclusion:* In an effort to understand the natural course of SCADD, we report several infants and family members who fit the biochemical and genotypic definitions. Among these patients, only one sibling pair developed speech concerns, for which there was a strong family history. Although controlled studies with

adequate power are necessary to further explore this trend, these cases suggest the possibility that SCADD may not be a true disease.

48. The M405V mutation in GCDH can cause clinically typical GA1, false-negative newborn screens, normal glutaric acid, and variable 3-hydroxyglutaric acid in serum and urine. C. Greene¹, E. Strovel¹, J. Joines¹, E. Spector², G. Scharer², M. Woontner², S.I. Goodman². ¹Department of Pediatrics, University of Maryland School of Medicine, Baltimore, USA; ²University of Colorado School of Medicine, Denver, USA.

Glutaric acidemia type I (GA1) is due to recessively inherited deficiency of glutaryl-CoA dehydrogenase (GCDH) and often causes acute striatal necrosis in childhood. The rationale for newborn screening—usually by measuring glutarylcarnitine by tandem mass spectrometry—is that presymptomatic diagnosis and treatment often prevents neurological disease. Previously a patient with the M405V and V400M mutations has been reported who presented with acute striatal necrosis and whose serum and urine organic acids were often normal. A newborn screen was not done, but presumably would have been normal [R.C. Gallagher et al., *Mol. Genet. Metab.* 86 (2005) 417]. We report here clinical and laboratory data on six additional GA1 patients heterozygous for the M405V mutation. Two of the six patients were screened for GA1 as newborns; one of them tested positive (C5DC 0.48 μ M, upper limit of normal up to 0.27) and the other negative (0.13 μ M). The latter child and the four who were not screened all developed acute striatal necrosis during the first year of life. The infant who tested positive at birth had normal urine organic acids, normal blood spot acylcarnitine profile, and normal quantitative urine glutaric acid at one week of age. However a small amount (11 μ g/mg creat) of 3-hydroxyglutaric was detected on quantitative analysis. Based on this finding, molecular analysis was performed and a diagnosis was made. The genotype in this patient was found to be E414K/M405V. Genetic testing of this patient's parents confirmed that these mutations are in trans. The infant, now 13 months, remains asymptomatic with a birth and current head circumference at the 50th percentile. The second mutant allele (and % residual activity for each patient) in the remaining five patients were: Q333X (15), L174P (5), R402W (4), C115Y (7) and A293T (10). All six present diagnostic challenges. Serum glutaric acid levels were always less than 100 ng/ml (normal < 250), and 3-hydroxyglutaric was detected (or elevated) only intermittently in serum or urine. These results show conclusively that some GA1 patients will be missed by MS/MS newborn screening for glutarylcarnitine. In addition, when newborn screen is positive or clinical symptoms suggest GA1, normal urine organic acids and blood acylcarnitine profile cannot exclude diagnosis and in some situations enzyme or mutational analysis will be necessary.

49. Apoptotic cell death is increased in fibroblasts from pyruvate dehydrogenase deficient individuals. S. Judge, D. Pourang, P.W. Stacpoole. Department of Medicine, University of Florida, Gainesville, FL 32610, USA.

The pyruvate dehydrogenase complex (PDC) is a multienzyme complex located in the inner mitochondrial membrane that catalyzes the oxidative decarboxylation of pyruvate to acetyl CoA. Congenital defects in PDC result in lactic acidosis, neurological degeneration, and early death. However, there has been little investigation into the molecular and biochemical consequences of PDC deficiency. Using a water-soluble tetrazolium compound (MTS) that is reduced to a colored formazan product in metabolically active cells, we found that PDC-deficient fibroblasts had a decrease ($p < 0.05$) in the amount of formazan product as measured by the absorbance at 490 nm compared to control cells, suggesting reduced mitochondrial metabolism in PDC-deficient fibroblasts. Mitochondrial membrane potential is believed to be an early apoptotic event and can be assessed using the fluorescent dye JC-1. In healthy cells, JC-1 enters the negatively charged mitochondria and forms aggregates that fluoresce orange-red. In apoptotic cells, in which the membrane potential has collapsed, JC-1 remains in the cytosol and fluoresces green. A higher percentage of PDC-deficient fibroblasts exhibited green fluorescence compared to controls ($5.7 \pm 1.2\%$ vs. $2.0 \pm$

0.5% ; $p < 0.05$). The number of TUNEL positive cells was also increased in fibroblasts from PDC-deficient patients compared to controls ($18.8 \pm 3.7\%$ vs. $9.0 \pm 0.7\%$; $p < 0.05$). Annexin V has a high binding affinity for phosphatidylserine (PS) which translocates from the inner to the outer surface of the cell membrane during apoptosis. The percent of cells staining positive for Annexin V was almost 2-fold greater ($p < 0.05$) and the expression of the anti-apoptotic Bcl-2 protein was lower ($p < 0.01$) in PDC-deficient fibroblasts. Because the accumulation of reactive oxygen species (ROS) may initiate apoptosis, we used the fluorescent probe 5-(and-6-)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate (CM-H₂DCFDA) to measure intracellular ROS production. PDC-deficient cells exhibited a 1.9-fold increase ($p < 0.05$) in DCF fluorescence compared to control fibroblasts. Additionally, both activity ($p = 0.01$) and protein expression ($p = 0.002$) of the mitochondrial antioxidant enzyme manganese superoxide dismutase (MnSOD) were reduced in PDC-deficient cells. In conclusion, the apparent increase in intracellular ROS combined with a diminished capacity to scavenge ROS provides a potential mechanism for the increase in apoptotic cell death in PDC-deficient fibroblasts. Apoptotic cell death may be a major contributing factor to the pathophysiology of PDC deficiency, especially in those tissues that are highly oxidative and postmitotic.

50. Reference material needs assessment for biochemical genetic testing. L. Kalman¹, B.A. Barshop², M. Blitzer³, T. Cowan⁴, C. Greene³. ¹Centers for Disease Control and Prevention, Atlanta, GA, USA; ²UCSD-Rady Children's Hospital, San Diego, CA, USA; ³University of Maryland School of Medicine, Baltimore, MD, USA; ⁴Stanford University Medical Center, Stanford, CA, USA.

Background: The "Quality, Access, and Sustainability of Biochemical Genetic Testing" working meeting was held on Oct. 6–7, 2006, in Atlanta, GA, as part of a collaborative effort to improve the availability, sustainability, and quality assurance measures for biochemical genetic tests. This meeting, (<http://www.cdc.gov/dls/genetics/qualityaccess/default.aspx>) was organized by CDC, ORD-NIH, SIMD, ASHG, ACMG, HRSA, the Genetic Alliance, Emory University, and several other key groups. The meeting participants discussed the current lack of reference materials (quality control, proficiency testing and test development/validation) for biochemical genetic testing and the need to improve the availability of such materials. *Method:* To address the availability of reference materials, the U.S. Centers for Disease Control and Prevention (CDC)-based Genetic Testing Reference Materials Coordination Program (GeT-RM <http://www.cdc.gov/dls/genetics/qcmaterials/default.aspx>) and SIMD are planning to conduct a survey of clinical biochemical genetic laboratory directors to identify currently available reference materials and to assess urgent reference and proficiency testing material needs. *Results:* The data from the survey will be used to develop a database of existing, publicly available, biochemical genetic reference materials. This information will be posted on the GeT-RM and SIMD websites. Data collected from the survey will also be used to identify unmet reference material needs and to guide subsequent efforts to acquire, store and characterize reference materials for biochemical genetic tests. *Conclusions:* These materials, which are to be publicly available, will be useful for quality control, research, proficiency testing, and test development/validation and will help to improve and assure the continued quality and availability of biochemical genetic testing.

51. The genetic testing reference materials coordination program (GeT-RM)—a sustainable community process to improve availability of appropriate reference materials for genetic testing. L. Kalman. Centers for Disease Control and Prevention, Atlanta, GA 30333, USA.

Background: Expansion of genetic testing in clinical and public health practice has increased the need for appropriate and characterized cell line/genomic DNA reference materials for quality assurance, test validation, proficiency testing, and development of new tests. However, despite the growing test volume and the rapidly increasing number of tests offered, necessary reference materials (RM) are not available for the vast majority

of tests. **Method:** The U.S. Centers for Disease Control and Prevention (CDC), with members of the genetic testing community, has developed a program to improve public availability of reference materials and facilitate information exchange and communication on reference materials development, contribution, characterization, distribution, and needs assessment. This CDC-based Genetic Testing Reference Materials Coordination Program (GeT-RM) provides continuing support and coordination to improve genetic testing reference material availability. The GeT-RM Program (1) facilitates the identification, procurement, characterization and availability of needed RM; (2) facilitates exchange of quality control and RM-related information; and (3) explores collaborative efforts for ongoing needs monitoring and materials development. **Results:** GeT-RM has characterized RM for Huntington disease, Fragile X, disorders on the Ashkenazi Jewish Panel (Bloom syndrome, f. dysautonomia, Canavan disease, Niemann-Pick disease, Tay-Sachs disease, Gaucher disease, Glycogen storage disease type 1a, Fanconi anemia and mucopolidosis type IV). GeT-RM is currently characterizing reference materials for cystic fibrosis and a number of other genetic tests, including many newborn screening disorders. The GeT-RM program also collects genetic information from other sources about publicly available cell lines/DNA with clinically important mutations, including many pharmacogenetic polymorphisms, which may be useful reference materials. This information is posted on the program website. To date, the GeT-RM has focused its efforts on DNA based testing for inherited genetic disorders. However, there is a similar lack of RM for other areas of genetics, including molecular oncology, molecular infectious disease testing and biochemical genetic testing. To address these needs the GeT-RM, together with the genetics community, relevant professional organizations and government agencies, is working to provide information about currently available RM and considering mechanisms to address RM needs for these areas. **Conclusions:** The increased availability of characterized RM, which can be used for quality assurance, proficiency testing, test development and research, will help to improve the quality and accuracy of genetic testing. More information is available at the GeT-RM website, <http://www.cdc.gov/dls/genetics/qcmaterials/default.aspx>.

52. Alglucosidase alfa in infants and children with advanced pompe disease.

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Introduction: Pompe disease is caused by a deficiency of acid alpha-glucosidase (GAA). Severe GAA deficiency manifests during infancy with rapidly progressing muscle weakness, hypotonia, cardiomyopathy, and failure to thrive. Most patients die from cardio-respiratory failure within 1 year. **Methods:** Two open-label, multinational, multicenter studies (S1 and S2) were conducted in patients =6 months (S1 $n = 18$) or >6–36 months (S2 $n = 21$) of age with cardiomyopathy [abnormal left ventricular mass (LVM) by echo] and GAA = 1% of normal mean activity in fibroblasts. In S1, which excluded patients on ventilator support at baseline, patients were randomized to alglucosidase alfa intravenously at 20 or 40 mg/kg qow. In S2, which included patients on ventilator support, treatment began at 20 mg/kg qow. **Results:** In S1, mean age \pm SD at first symptoms was 1.6 ± 1.8 months and at first infusion 5.1 ± 2.0 months. In S2, first symptoms occurred at 3.9 ± 2.8 months and first infusion at 15.7 ± 11.0 months. Median duration of treatment was 121 weeks (range 60–150) in S1; 120 weeks (range < 1–168) in S2. Eleven of 39 patients died (unrelated to treatment); 16 of 34 became invasively ventilated. Cox regression analyses comparing similar untreated historical controls ($n = 61$ for S1; $n = 84$ for S2) indicated that treatment at =6 months old reduced the risk of death by 95%, the risk of death or invasive ventilation by 91%, and the risk of death or any form of ventilation by 87% (all with $p < 0.0001$). In children >6–36 months old, treatment reduced the risk of death by 79% ($p = 0.0009$) and the

risk of death or invasive ventilation by 58% ($p = 0.02$). A sustained decrease in LVM occurred in 94% (S1) and 81% (S2) of patients. Normal weight and length percentiles were observed in >80%. Clinically significant motor gains (including independent ambulation) occurred in 61% of patients. Infusion-associated reactions (all managed successfully) occurred in 56% of patients; IgG antibodies developed in 92%. Low IgG titers or a trend towards decreasing titers occurred with continued treatment in 74% of those who seroconverted (range: seronegative to 6,400). Notably, two null GAA mutations and high, sustained IgG titers were frequently found in those who died, needed ventilation, and/or had minimal motor response. **Conclusions:** In these patients, alglucosidase alfa significantly prolonged survival and invasive-ventilation-free survival, compared with historical controls. LVM, growth and motor development also markedly improved. Clinical response optimized by treatment at early stages of disease progression highlights the importance of rapid clinical recognition and diagnosis.

53. The pompe registry: Centralized data collection to track the natural course of pompe disease.

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Introduction: Pompe disease is a rare, progressive, and often fatal muscle disease, which manifests as a clinical spectrum that varies with respect to age at onset, rate of disease progression, and extent of organ involvement. The underlying pathology is a deficiency of acid alpha-glucosidase (GAA) that hydrolyzes lysosomal glycogen. **Description:** To gain a better understand of the natural course of Pompe disease, a global, observational Registry was developed to collect anonymous, longitudinal data on Pompe patients. **Preliminary data overview:** As of September, 2007, 400 patients from 23 countries are enrolled in the Pompe Registry; the majority of which (71%) are Caucasian. 78/400 (20%) of enrolled patients are infants (typically with cardiomyopathy, profound skeletal and respiratory muscle weakness, and death within the first year of life) with a median age at diagnosis of 4.0 months. 238/400 (60%) of enrolled patients have late-onset disease (typically without cardiac involvement, but with progressive proximal skeletal and respiratory muscle weakness and longer survival) with a median age at diagnosis of 34.5 years. The median age of symptom onset was 2.0 months for infants and 26.3 years for the older patients. Of the patients genotyped, 54% (76/140) expressed the IVS1-13T>>G mutation. **Summary:** Preliminary Registry data show that the (median) range of time from symptom onset to diagnosis represents a significant lag (consistent with published literature), which is often due to misdiagnosis and highlights the need for greater disease awareness. The overall objective of the Pompe Registry is to increase disease understanding across patient phenotypes/genotypes, medical disciplines and regional disease management norms and monitor the impact of treatment and other disease support methods over time.

54. Development of criteria for the confirmation of diagnoses detected by expanded newborn screening.

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The Newborn Screening Standardization Work Group of the NY-MAC for Genetics and Newborn Screening (NBS) Services has assumed the task of developing a dialogue and the tools to standardize newborn screening within our region. One of the tools that has been developed is the newborn screening diagnosis and confirmation table that we are presenting here.

This table presents our understanding of the diagnosis and confirmation of each of the disorders currently on the New York State (NYS) newborn screening panel and some of the outcomes of screening as obtained from NYS. Among our concerns is that patients are correctly assigned to their diagnoses. A “metabolite diagnosis” only may not be an acceptable level of diagnostic confirmation for many disorders and so it is important that diagnoses are correctly resolved. This is especially true for patients who remain essentially asymptomatic and yet are being given a specific diagnosis, resulting in medicalizing a healthy patient. In addition, since they are continually followed through their life span, they carry a lifelong diagnosis with lifelong follow up. Accepting “metabolite diagnoses” also affects the newborn screening process itself, potentially leading to more false positives because patients who have not been fully evaluated, but have been given diagnoses may have lower levels of the marker compounds on the newborn screen. This causes the NBS program to maintain higher cut-offs, when true diagnoses may allow cut-off values to be reevaluated.

We are distributing this table among the metabolic community for additional feedback and suggestions. This will help refine the table and update the information presented. Our aim is to make this available as an online document with links to the most current information on the disorders and where diagnostic testing can be offered.

55. Altered lipid metabolism in young children with phenylketonuria (PKU)

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Objective: The objective of this study was to determine if children with phenylketonuria (PKU) have lower plasma and erythrocyte essential fatty acid concentrations than healthy, sex- and age-matched control subjects. The study also investigated whether the variation in plasma and erythrocyte fatty acid concentrations could be explained by variation in type and amount of dietary fat consumed. **Methods:** Twenty-one subjects (=6 years of age) with PKU and 23 healthy, sex- and age-matched (± 1 year) control children participated in the study. Dietary intake of energy, macronutrients, saturated fat, monounsaturated fat and polyunsaturated fat were calculated from three, separate 24-h diet recall telephone interviews. Absolute fatty acid concentrations in total plasma lipid and in total erythrocyte lipid were determined by gas chromatography mass spectrometry (GCMS). Dietary intake and fatty acid concentrations in total plasma lipid, and in total erythrocyte lipid were compared for subjects with PKU and control subjects by independent samples *t*-test. **Results:** Subjects with PKU had a significantly lower intake of dietary protein and significantly higher intake of dietary polyunsaturated fat compared to controls. Subjects with PKU had significantly higher concentrations in total plasma lipid of C14:0, C18:0, C18:1, linoleic acid (LA), α -linolenic acid, and the sum of the ω -6, monounsaturated and polyunsaturated fatty acids compared to controls, but significantly lower concentrations in total erythrocyte lipid of C14:0, C16:0, C18:0, LA, arachidonic acid (ARA), and the sum of the ω -3, ω -6, saturated and polyunsaturated fatty acids. Age was negatively correlated with ARA and docohexaenoic acid (DHA) concentrations in total plasma lipid and in total erythrocyte lipid in subjects with PKU, but not in control subjects. Multivariate linear regression analysis found group (PKU/Control) to be the most significant predictor of fatty acid concentrations in total erythrocyte lipid. **Conclusions:** Despite a higher intake of dietary polyunsaturated fat and higher concentrations of fatty acids in total plasma lipid, subjects with PKU had lower concentrations of fatty acids in total erythrocyte lipid compared to controls. This data suggests that young children with PKU may have altered fatty acid metabolism.

56. γ -Glutamylcysteine co-migrates at the peak typically assigned to L-aspartate in ion-exchange chromatography-based amino acid analysis.

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Background: Glutathione is a tripeptide that plays a crucial role in free radical scavenging, oxidative injury, and cellular homeostasis. γ -glutamylcysteine (γ -GC) is an immediate precursor during glutathione biosynthesis that is converted to glutathione by glutathione synthetase. **Objective:** To determine plasma concentration of γ -GC in healthy human individuals. **Methods:** All samples underwent acidification and protein precipitation and were subsequently assayed using a Hitachi L-8800 High Performance Amino Acid Analyzer, a method that relies on anion-exchange chromatography and ninhydrin derivitization. **Results:** Using a standard protocol, we determined that γ -GC elutes at 8.5 min, corresponding with the elution time for L-aspartate. To resolve these two compounds, we acidified the chromatography buffer to pH 2.9. Under this acidified condition, we determined that L-aspartate elutes at 9.4 min whereas γ -GC elutes at 10.8 min. We then studied plasma samples from healthy individuals using both a standard and an acidified protocol. Addition of L-aspartate or γ -GC to the plasma samples confirmed the applicability of the previous results to plasma samples. Under the standard amino acid analysis protocol, a compound elutes at 8.5 min, corresponding to both γ -GC and L-aspartate. Using the acidified conditions to resolve L-aspartate and γ -GC, we determined that there is no detectable L-aspartate present in plasma. In contrast, γ -GC is present in plasma at 5–8 μ M concentrations. **Conclusions:** L-aspartate elutes simultaneously with γ -GC under standard conditions using ion-exchange chromatography. Slight acidification of the chromatography buffer allows adequate resolution of these two compounds. Analysis of human plasma samples under these modified conditions demonstrates that the concentration of L-aspartate is below the level of detection in plasma whereas the concentration of γ -GC is 5–8 μ M. Our findings indicate that, under the standard conditions of amino acid analysis, the plasma compound that elutes at the time expected for L-aspartate is exclusively γ -GC.

57. Effect of glycosylation on activity and membrane maturation of the OCTN2 carnitine transporter.

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Objective: Primary carnitine deficiency is a disorder of fatty acid oxidation caused by mutations in the Na⁺-dependent carnitine/organic cation transporter OCTN2. Most missense mutations identified in patients with primary carnitine deficiency affect predicted transmembrane domains or intracellular loops of the transporter. P46S and R83L are located in an extracellular loop close to putative glycosylation sites (N57, N64, and N91) of OCTN2. Analysis by confocal microscopy indicated that P46S and R83L impaired maturation of the transporter to the plasma membrane, possibly affecting glycosylation. We tested whether OCTN2 was indeed glycosylated and glycosylation was required for function. **Methods:** The 3 putative glycosylation sites (N57, N64, and N91) of OCTN2 were substituted using site-directed mutagenesis by glutamine (Q) and their effect on glycosylation, carnitine transport and subcellular localization was determined by Western blot analysis, kinetic analysis and confocal microscopy. **Results:** Substitution of the three putative glycosylation sites with glutamine increased the gel mobility of the OCTN2 carnitine transporter up to a mobility comparable to that of the wild-type transporter treated with peptide-N-glycosidase F when all 3 sites were substituted. P46S and R83L transporter had increased mobility, similar to that of deglycosylated transporters. Carnitine transport activity decreased mildly when single sites were substituted. Simultaneous substitution of multiple glycosylation sites caused a marked decline in carnitine transport up to its full ablation when all three glycosylation sites were substituted by glutamine (N57Q/N64Q/N91Q). Analysis by confocal microscopy indicated that glutamine substitutions caused progressive retention of OCTN2 transporters in the cytoplasm, up to full retention (such as that observed with R83L) when all 3 glycosylation sites were substituted. Correlation between carnitine transport and number of transporters on the plasma membrane indicated a significant, but not perfect relationship. Kinetic analysis indicated that the substitution of glycosylation sites also affected the affinity of the transporter toward carnitine. Therefore, substitution of

glycosylation sites affects both maturation of OCTN2 transporters to the plasma membrane and substrate recognition. *Conclusions:* These results indicate that glycosylation is essential for the function and maturation of OCTN2 carnitine transporters to the plasma membrane.

58. Ethnic differences in prevalence rates for metabolic disorders: Implications for national prevalence rates. F. Lorey¹, L. Feuchtbaum¹. ¹Genetic Disease Screening Program, CA Department of Public Health, Richmond, CA, USA.

Objective: With the addition of tandem mass spectrometry to most states' newborn screening programs, a great deal of knowledge has been gained relating to prevalence rates. While several national groups are claiming some rates are being found to be rarer than previously reported, others contend that numbers are large enough now that expected prevalence rates can be established. Large multi-state data collections are identifying more reliable cutoffs, confidence intervals, or methodological improvements to optimize success; however in many cases one of the most significant reasons for differences in prevalence rates is not methodological, but demographic. *Methods:* California began ms/ms screening in July of 2005 and by November, 2007, over 1.3 million newborns have been screened. California's ethnic makeup is quite dissimilar to most states, and currently Hispanic births are more than 51% of births. Caucasian non-Hispanic births are only 28% of the total, while Asian births comprise 12% of the total. Significant numbers of screens of over 20 ethnicities (as required by California law) have been performed. Racial/ethnic coding is obtained by self identification (parents) and multi-racial identification is recorded. *Results:* Although some ethnic cells are limited by small numbers, overall the data lend themselves to some interesting findings. For example, Cbl C Disease, previously reported in the 1:200,000–1/300,000 range, is 1/78,000 births (17 cases) or 1/55,000 Hispanic births, a significant difference. Conversely, the single Caucasian case indicates a prevalence of 1:314,000 Caucasian births (similar to previously published overall rates) *Conclusions:* Ethnic or demographic factors can significantly alter expected prevalence rates of metabolic disorders detected by NBS. Sample rates for MCADD are provided below, but findings for all detected disorders will be presented at this conference.

59. Treatment of ADHD with tetrahydrobiopterin (BH4). R. Matalon¹, G. Bhatia¹, K. Michals-Matalon², W. Zinser³, S. Tyring⁴. ¹University of Texas Medical Branch, Galveston, USA; ²University of Houston, Houston, USA; ³University of Texas of South Western, Dallas, USA; ⁴University of Texas Health Sc. Center, Houston, USA.

Objectives: Attention deficit disorders with or without hyperactivity, ADD/ADHD are associated with inadequate levels of neurotransmitters. In order to assess the effect of Tetrahydrobiopterin (BH4), a co-factor for dopamine and serotonin synthesis, subjects with ADD/ADHD were given BH4 and change in the profile of ADD/ADHD was examined. *Methods:* Seven individuals with ADD/ADHD, 5 males and 2 females were studied. Conner Continuous Performance Test (CCPT), a computerized system for evaluating ADD, was administered before, during and after treatment with BH4. CCPT is sensitive enough to examine attention, concentration, impulsiveness and other features of ADD/ADHD. Five individuals, 4 males and 1 female had been on stimulant therapy which was suspended 4 weeks prior to BH4 trial; because these patients did not feel they improved on the prescribed medications. The other two had no therapy prior to the study. Most patients received 100 mg of BH4 daily in a single dose, while one patient was given 350 mg

daily. Dosage was approximated to 5mg/kg. Prior to treatment, CCPT was administered, then after 1 week, 2, 3 and 4 weeks while patients remained on BH4. CCPT was followed after discontinuation of BH4. *Results:* After one week, 4 of the 5 male patients had significant improvement on CCPT while one male patient showed no change. Two of the female patients showed moderate improvement. CCPT returned to baseline 1–2 weeks after BH4 was discontinued. The patient who received 350 mg BH4 daily continued to show benefit for 4 weeks before returning to baseline. *Conclusion:* Treatment of ADD/ADHD with BH4 shows improvement using a single dose in 6 out of the 7 patients in the trial. Dosage response has not been studied and was varied only in one patient. The preliminary data in this study are encouraging and further work is needed to establish the optimal level of BH4 and the length of time to achieve positive response.

60. Experience with long term use of LNAA in the treatment of phenylketonuria. K. Michals-Matalon¹, G. Bhatia², J. Grady², S. Tyring³, R. Matalon². ¹University of Houston, Houston, USA; ²University of Texas Medical Branch, Galveston, USA; ³University of Texas Health Sc. Center, Houston, USA.

Objective: Short term treatment trials of Phenylketonuria (PKU) with large neutral amino acids (LNAA) done in our centers resulted in lowering of blood Phenylalanine (PKU). These trials have been reported, but were not followed by long term studies. The purpose of this trial is to examine long term safety, efficacy and acceptability of LNAA tablets, and to find out whether the effect of lowering blood Phe with LNAA (NeoPhe) is sustained over a one year period. *Methods:* Four patients with classical Phenylketonuria (PKU), three females and one male, ages 25–38 years, were enrolled in the long term trial. Patients were not taking medical food for more than ten years. Their mean blood Phe level was 1507 µmol/L. Patients were given NeoPhe tablets, 0.5 g/kg/day and were instructed to divide the pills equally with the three meals. Blood amino acids and Phe were determined once a month. *Results:* The mean blood Phe levels declined for each of the subjects during the study period: 642, 707, 899 and 869 µmol/L, from the mean level of 1507 µmol/L. The mean change from pre-and during NeoPhe trial was statistically significant (paired *t*-test: *P* = 0.002). Patients reached blood Phe level within the NIH Consensus Conference recommendations. None of the patients gained or lost any weight beyond minor fluctuations of +/- 0.2 kg. The acceptability of the pills was monitored during the monthly visit and through a check-list given to the patients for any complaints. There were no reports of abdominal discomfort, nausea or change in bowel movements. All patients felt encouraged by the drop of their blood Phe concentrations, and indicated that they felt "more focused" at work and asked to continue to be on NeoPhe beyond the trial period. *Conclusions:* The data from the four patients show that LNAA can be used to lower blood Phe in patients with PKU and can be taken for a long time without any adverse side effects. Future studies should include larger number of patients and also include neuropsychological tests to document improvement in such parameters as executive functioning and concentration.

61. Effective and affordable 1st tier newborn screening (NBS) for tyrosinemia type I (TYR-I). C. Turgeon, M.J. Magera, P. Allard*, S. Tortorelli, D. Gavrilov, P. Rinaldo, D. Matern. Biochemical Genetics Laboratory, Mayo Clinic College of Medicine, Rochester, MN, USA; *Hôpital Sainte-Justine, Montreal, Canada.

Background: TYR-I is a severe disorder causing early death if left untreated. NBS is problematic because tyrosine is a nonspecific marker for

Ethnicity	Screens	MCADD Cases	Prevalence	Ethnicity	Screens	MCADD Cases	Prevalence
Black	63,222	2	1/31,611	Multiple—all	81,402	2	1/40,700
Hispanic	637,589	25	1/28,981	Native Amer.	1366	1	1/1366
White	313,906	26	1/12,000	Asian	106,523	0	—
Other	16,950	1	1/16,950	Total	1,248,569	57	1/21,900

TYR-I and so far the determination of the diagnostic marker, succinylacetone (SUAC), required a separate 1st tier or only partially effective 2nd tier analysis based on tyrosine level. To overcome these problems we developed a new assay that simultaneously determines acylcarnitines (AC), amino acids (AA), and SUAC in dried blood spots by flow injection tandem mass spectrometry (MS/MS). *Methods:* 3/16" DBS punches are extracted with 300 μ L methanol containing AA and AC internal standards (IS). This extract is derivatized with butanol-HCl. Meanwhile, SUAC is extracted from the residual filter paper with 100 μ L of a 15mM hydrazine solution containing $^{13}\text{C}_5$ -SUAC as IS. The derivatized aliquots are then combined in acetonitrile for traditional MS/MS analysis of AC and AA now including additional MRM experiments for SUAC (m/z 155 to 137) and its IS (m/z 160 to 142). Analysis time is 2.5 min. *Results:* SUAC was found to be elevated in the retrospective analysis of original NBS samples of ten TYR-I patients (range: 13–81 $\mu\text{mol/L}$) with Tyr levels of 90–293 $\mu\text{mol/L}$ (mean: 183) compared to 13,500 controls (SUAC mean: 1.25; 99.5th percentile: 2.65). *Conclusion:* The inclusion of SUAC analysis into the routine analysis of AC and AA allows for rapid, cost effective NBS for TYR-I with no tangible risk of false negative results.

62. Essential fatty acid status in treated patients with MSUD. Laura M. Mazer¹, Sarah H.L. Yi², Rani H. Singh^{2,3}. ¹Emory University School of Medicine, Atlanta, GA, USA; ²Nutrition and Health Sciences Program, Graduate Division of Biological and Biomedical Sciences, Emory University, Atlanta, GA, USA; ³Department of Human Genetics, Emory University School of Medicine, Decatur, GA, USA.

Objective: To evaluate the dietary and biochemical status of omega-3 fatty acids in patients with maple syrup urine disease (MSUD). *Content:* Patients with MSUD are unable to metabolize the branched-chain amino acids (BCAAs) valine, isoleucine and leucine, and require lifelong dietary therapy to restrict their intake in order to prevent poor neurological outcomes and even death. Since the majority of their protein and other nutrient needs are typically derived from synthetic BCAA-free medical foods, it is important to evaluate patients for potential nutrient inadequacies. Neither intakes nor biochemical status of omega-3 fatty acids in MSUD patients has been evaluated. Given the diet restriction and the importance of omega-3 fatty acids, particularly docosahexaenoic acid (DHA), in neurological development, it is critical to evaluate the status of these fatty acids in patients with MSUD. The objective of this study was to evaluate the dietary and fatty acid status of patients with MSUD attending a metabolic camp at Emory University in Atlanta, Georgia. Healthy controls of similar age and sex of the patients were selected from existing laboratory normal data. The fatty acid status in plasma and erythrocytes was analyzed using gas chromatography-mass spectroscopy. Although patients had high concentrations of plasma and erythrocyte alpha-linolenic acid (aLNA) they had significantly lower concentrations of plasma docosahexaenoic acid (DHA) compared to controls ($p < 0.01$). Concentrations of erythrocyte DHA, however, were similar to controls. These results indicate a need to differentiate between aLNA and DHA when supplementing metabolic foods, as well as a need for careful evaluation to avoid potential dietary inadequacies in MSUD patients. *Conclusions:* These data suggest lower plasma DHA concentrations in females with MSUD, however the implication of these low concentrations warrants further investigation.

63. Sequencing of ACADM from dried blood spots in neonates with positive NBS results for MCADD in whom confirmatory testing is purportedly normal. S.E. McCandless¹, R. Chandrasekar², S. Linard², W. Becker², L. Rice¹. ¹Department of Genetics, Case Western Reserve University, University Hospitals Case Medical Center, Cleveland, OH, USA; ²Ohio Department of Health, Newborn Screening Program, Reynoldsburg, OH, USA.

Medium chain acyl-CoA dehydrogenase deficiency (MCADD) is one of the most common disorders identified by newborn screening (NBS) programs. NBS for MCADD uses measurement of octanoylcarnitine (C8) from dried blood spots. In the state of Ohio, as in many places, primary care

providers, with or without consultation from a metabolic specialist, may perform confirmatory testing, with the final diagnostic decision returned to the state. Confirmatory testing may involve measurement of metabolites, mutation screening, or sequencing. We now report sequencing results for infants said to have "false positive" NBS results for MCADD. *Methods:* Dried blood spots (DBS) were obtained from all 17 available NBS cards identified as "false positive" by NBS between April 2003 and Feb 2006 in Ohio ($N = 20$, thus 3 had no DBS available). DNA extracted from DBS was screened for the common A985G mutation in exon 11 of the ACADM gene, using a specific restriction digest method, followed by sequencing of the 12 exons, intron-exon junctions, and several hundred base pairs of the 5' untranslated region. *Results:* The cut-off value for C8 used was 0.7 $\mu\text{mol/L}$. The mean C8 for the 17 infants was 0.91 (95CI 0.77–1.15), much lower than the mean value for confirmed cases. Ten of the 17 were premature weighing <1200 g, the rest were normal sized and full term. A variety of previously reported common SNPs were identified. Five infants, all full term with appropriate birth weight, were heterozygous for the common A985G mutation; one of those also has a novel sequence change identified in exon 9 that predicts a PRO to LEU change at residue 258 of the protein. The clinical significance of the variant is unknown. That child was and had an NBS C8 of 2.0, more than double the mean for the group. Unfortunately, the study design did not provide clinical outcome data, but the child is not known to have presented clinically in 3.5 years of life. *Conclusions:* Sequencing of ACADM in 17 neonates with "false positive" elevated C8 on NBS identified one infant with two sequence changes, one known to be disease causing, the other novel, raising the possibility that some infants with two mutations may be reported as normal at "follow-up". Full term infants with "false positive" screens were likely to be carriers (5/7). State registries should ask for documentation of confirmatory testing to avoid misdiagnosis by primary care providers. ACADM alleles that appear to be less clinically significant are well described, but the clinical utility of sequencing all infants with abnormal NBS for MCADD, as is suggested for VLCADD, deserves further investigation.

64. Effect of simvastatin on plasma sterols and urinary mevalonate in Smith-Lemli-Opitz syndrome (SLOS). L.S. Merckens¹, A.S. Pappu², J.M. Jordan², J.A. Penfield¹, W.E. Connor², R.D. Steiner^{1,3}. ¹Department of Pediatrics, Oregon Health & Science University, Portland, OR 97239, USA; ²Department of Medicine, Oregon Health & Science University, Portland, OR 97239, USA; ³Department of Molecular & Medical Genetics, Oregon Health & Science University, Portland, OR 97239, USA.

Objective: Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive disorder, due to a defect in the last step of cholesterol synthesis, 7-dehydrocholesterol reductase (DHCR7). The resultant low cholesterol and/or high 7-dehydrocholesterol (7DHC) in plasma and tissues may be responsible for the pathology. Treatment goals include increasing the cholesterol concentrations and decreasing the 7DHC concentrations. We evaluated 2 potentially therapeutic approaches to decrease flux through the cholesterol synthesis pathway in an attempt to inhibit 7DHC accumulation: high cholesterol diet versus high cholesterol diet with simvastatin, an HMGCoA reductase (HMGCoAR) inhibitor. *Methods:* In 10 subjects, we tracked changes in plasma sterols by GC after (1) a low cholesterol diet (4 weeks), (2) a high cholesterol diet (at least 0.4 year) and (3) a high cholesterol diet with simvastatin (0.1–0.5 mg/kg for at least 0.5 year). Five of the subjects discontinued the statin while remaining on high cholesterol diet: 2 for very mild increase in liver enzymes and 3 due to a decrease in plasma cholesterol >27%. Urinary mevalonate as a measure of HMG CoAR activity and flux through the cholesterol synthetic pathway was measured by a radioenzymatic method. Differences were tested with paired *t*-tests. *Results:* High cholesterol diet increased plasma cholesterol compared to low cholesterol diet ($+24 \pm 23$ mg/dL (mean \pm SD) $p < 0.02$), but the addition of statins resulted in a decrease of plasma cholesterol (-15.5 ± 21 mg/dL, $p < .04$). Although the concentration of 7DHC on the high cholesterol diet relative to the low cholesterol diet was unchanged; addition of statins had the desired effect of decreasing 7DHC (-3.6 ± 3.6 , $p < .02$). Urinary

mevalonate trended towards a decrease with a high cholesterol diet (-587 ± 1026 ng/mg creatinine) and trended towards a further decrease with statin (-308 ± 353 ng/mg creatinine $p = .06$). After statin was discontinued ($n = 5$) plasma cholesterol ($+38$ mg/dL $p < .04$) and 7DHC ($+3.4$ mg/dL, $p = .06$) increased with a parallel increase in urinary mevalonate ($+141$ ng/mg creatinine) to values similar those in original high cholesterol diet. **Conclusions:** Although the high cholesterol diet with statins was effective in lowering the plasma 7DHC, cholesterol also decreased. In contrast, in an SLOS mouse model, simvastatin decreases 7DHC with no change in cholesterol. There may be an optimal dose that maintains cholesterol while preventing the accumulation of 7DHC, or this may be a species difference. Until the effects of simvastatin in SLOS *in vivo* are better understood, this medication should be used with caution in this setting.

65. Correlation of *in vitro* acylcarnitine profile with Western blot in very long-chain acyl-CoA dehydrogenase deficiency (VLCADD). J.L. Merritt¹, D.B. Schowalter², J. Daniels², D. Matern³. ¹Department of Pediatrics, University of Washington, Children's Hospital and Regional Medical Center, Seattle, WA, USA; ²Department of Medical Genetics, Mayo Clinic College of Medicine, Rochester, MN, USA; ³Department of Laboratory Medicine and Pathology, Mayo Clinic College of Medicine, Rochester, MN, USA.

Objective: The implementation of acylcarnitine profiling in expanded newborn screening programs brought about an increasing number of children with biochemical evidence of VLCADD. Limited information is available on the relationship between the acylcarnitine profile, protein expression, and clinical presentation. **Methods:** We reviewed eighteen individuals diagnosed with VLCADD referred for acylcarnitines analysis by *in vitro* fatty acid oxidation probe assay. Fibroblasts were incubated with palmitate and L-carnitine for 72 h and quantified by tandem mass spectrometry. Protein expression was demonstrated through the use of a novel anti-human VLCAD antibody. Western blot analysis demonstrated a 70 kDa band consistent with wild-type human VLCAD at varying intensities. **Results:** Nine patients had had abnormal newborn screening tests. Two patients were detected due to clinical symptoms. Four had developed symptoms consistent with the neonatal cardiac dysfunction form of VLCADD and four were asymptomatic. No clinical information was available on seven individuals. All probe assays demonstrated elevations of C14, C14:1, C16, and C16:1 species. The highest concentrations were measured in three of the four deceased children. No relationship was observed between those detected through newborn screening and acylcarnitine concentration. While a general pattern of lower acylcarnitines concentrations correlating with increased band intensity on Western blot was seen, this was not consistent. **Conclusions:** Comparison of acylcarnitine concentration and intensity of band on Western blot revealed no constant relationship with clinical phenotype or detection on newborn screening. Comprehensive evaluation including *in vitro* probe assay and Western blot are useful in confirming a diagnosis, but they do not predict clinical presentation or outcome. Longitudinal study is warranted to further understand the correlation between genotype and phenotype.

66. Mice null for very long chain acyl-CoA dehydrogenase (VLCAD) have upregulated immune functions, while mice null for long chain acyl-CoA dehydrogenase (LCAD) have upregulated expression of oxysterol associated genes including bile acid responsive LRH1. Stephanie J. Mihalik¹, Zuzana Swigonova¹, Liqun Tian², Phillip Wood², Jerry Vockley¹. ¹Department of Pediatrics, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA; ²University of Alabama Birmingham School of Medicine, Birmingham, AL, USA.

Mice null for LCAD and for VLCAD appear healthy and grow normally. When fed regular mouse chow, they are of normal weight. However, when cold-stressed, these same animals become hypoglycemic and die. To further investigate how LCAD and VLCAD null mice adapt to living with these fatty acid oxidation blocks, we performed

expression array studies on skeletal muscle, liver, brown fat, and cardiac left ventricle from unstressed animals. Subjects were 6 week old males fed a low phytoestrogen diet. Serum samples were taken from a second set of animals using identical conditions. VLCAD null mice had upregulated cytokine and MHC1 associated genes including C3. Serum cytokine analyses showed increases in TNF α , IL4, and IL5 in VLCAD null but not in LCAD null or control animals. In contrast, the LCAD null animals had upregulated oxysterol associated genes including LHR1, a major player in bile acid and cholesterol synthesis. The LCAD mice also had altered genes associated with nitric oxide metabolism. In serum, their citrulline levels were significantly elevated ($P < 0.001$), consistent with enhanced nitric oxide synthesis relative to both VLCAD and control mice. Muscle from LCAD mice also had significant alterations in genes associated with serine, tyrosine and tryptophan metabolism. Subsequently, serum levels of serine and tyrosine were found to be significantly lower in the LCAD mice relative to the controls and VLCAD mice. The MS/MS amino acid analysis did not assess tryptophan. Thus, despite the fact that LCAD and VLCAD seem to have similar and even overlapping functions, array results and subsequent physiologic measures showed that the two genotypes have major differences in their biochemical and expression profiles.

67. Plasma peptide tyrosine tyrosine (PYY) levels are increased in urea cycle disorder patients. S. Mitchell¹, D. Murdock^{2,1}, The Urea Cycle Disorders Consortium, M. Summar^{3,1}. ¹Center for Human Genetics, Vanderbilt University, Nashville, TN, USA; ²Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN, USA; ³Department of Pediatrics, Vanderbilt Children Hospital, Nashville, TN, USA.

Peptide tyrosine tyrosine (PYY) and N-acetylglutamate synthase (NAGS) are divergently transcribed and separated by less than 200 base pairs in humans; an arrangement that is consistent with coordinate regulation. PYY is a neuropeptide that inhibits gut motility and digestive enzyme secretion from the pancreas, and is a key component in the regulation of appetite, inducing feelings of satiety postprandially. NAGS plays an important role in ureagenesis providing the necessary co-factor for carbamoyl phosphate synthetase 1 (CPS1), which catalyzes the first, and rate-limiting, step of the urea cycle. The urea cycle is the primary means of nitrogen metabolism in ureotelic organisms. Nitrogen, in the form of ammonia, is converted to urea and is eliminated through the kidneys. There are inherited metabolic disorders associated with deficiencies of each of the urea cycle enzymes resulting in aberrant nitrogen clearance and elevated ammonia levels. It is also well-documented that poor appetite is associated with urea cycle disorder patients. Decreased appetite is normally attributed to cerebral edema and dysregulation of neurotransmitters resulting from the elevations in ammonia levels and other nitrogen trafficking molecules like glutamine. However, if PYY and NAGS are regulated in a coordinate manner due to their physical proximity, then increased levels of PYY may underlie a significant part of the anorexia observed in hyperammonemic patients. We hypothesize that patients with urea cycle defects and upregulation of NAGS transcription will show increases in PYY production. Using an ELISA, we measured plasma PYY levels in a group of urea cycle disorder patients ($n = 37$). The average plasma PYY level was 48.8 pmol/L. Plasma PYY levels ranged from 10.5–249.5 pmol/L. The upper end of this range is dramatically higher than ranges reported for normal fasting and postprandial plasma PYY levels, 12.1 pmol/L and 29.9 pmol/L, respectively. We plan to further investigate these findings; as the increased expression of PYY may result in appetite suppression in these individuals leading to catabolism and in turn, to an increase in ammonia levels, thus creating a negative feedback loop where NAGS and PYY are continually upregulated. If clinical observations in urea cycle disorder patients are directly relevant to the transcriptional regulation of this gene pair, then PYY becomes a potential therapeutic target in the management of patients suffering from urea cycle disorders and other diseases involving disrupted nitrogen metabolism.

68. Homocitrullinuria in two children in the absence of additional evidence of HHH syndrome. R.A. Mooney¹, J.M. DeLuca², N.A. Hall¹, K.B. McCall¹, G.L. Arnold². ¹Department of Pathology and Laboratory Medicine, University of Rochester, Rochester, NY 14642, USA; ²Golisano Children's Hospital at Strong Memorial, University of Rochester, Rochester, NY 14642, USA.

Background: Homocitrullinuria is strongly associated with a defect in the mitochondrial ornithine transporter (*ORNT1*) that is manifest as Hyperornithinemia-Hyperammonemia-Homocitrullinuria (HHH) syndrome. Clinical signs and symptoms vary between affected individuals, but can include episodic vomiting and lethargy after high protein feedings, infantile spasms/seizures, spasticity, developmental delay, and episodic confusion/ataxia. We report the transient identification of homocitrulline in the urine of two infants who lacked additional biochemical evidence of HHH syndrome. **CASE Reports:** Urine amino acid analyses on two infants, ages 21 days and 9 months, revealed large apparent increases in methionine. Suspecting a co-migrating compound, a modified separation technique isolated and identified the compound as homocitrulline. Plasma amino acid analysis on each infant revealed very modest elevations in ornithine: 73 and 75 μM (nl 21–67) and no other specific abnormalities. Follow-up testing 4 weeks later was normal on both infants. Case 1—A 21 day old was referred for a newborn screen positive for citrulline (1.61 mg%, cut-off <0.90). The infant was from mixed ethnic background (not French Canadian). He was fed exclusively on Enfamil with Lipil. He was evaluated for “shaking behaviors” noted by mother, but EEG and neurological evaluation were normal. Plasma ammonia was normal on NBS follow-up. Follow-up lab testing was normal and the infant appears well. Case 2—A 9 month old with Down syndrome was evaluated for documented infantile spasms. The child was from a mixed ethnic background (not French Canadian). At time of lab testing, the patient had not yet begun ACTH or other anticonvulsant medications. His diet consisted of a Target brand infant lipil-containing formula and oatmeal cereal. Plasma ammonia was 23 μM (nl 10–47). **Discussion:** The origin of the apparent homocitrullinuria is unclear. The cases were ascertained approximately 4 weeks apart, thus seem unlikely to represent a processing contaminant. One mother reports microwaving the formula; both deny boiling formula. In summary, transient homocitrullinuria was identified in two cases without hyperammonemia, encephalopathy, or clotting disorders. Only long-term follow-up will determine if this biochemical abnormality precedes clinical presentation of HHH syndrome.

69. Preliminary findings from the sapropterin expanded access program for PKU. B. Burton¹, S. Turbeville², E. Jurecki², P. Pallansch², A. Schatz², K. DeMarco², A. Volz², R. Cady², H. Nicely². ¹Children's Memorial Hospital, Feinburg School of Medicine, Northwestern University, Chicago, IL, USA; ²BioMarin Pharmaceutical Inc., Novato, CA 94949, USA.

Background: The Sapropterin Expanded Access Program (SEAP) is an FDA-approved program providing sapropterin dihydrochloride, prior to launch of drug (KuvanTM), to patients with hyperphenylalaninemia (HPA) due to phenylketonuria (PKU), aged = 9 years, who did not participate in sapropterin clinical trials nor were enrolled in compassionate/temporary use programs. **Objective:** The SEAP provides data including adverse-events (AEs) documented by treating physicians using Medical Dictionary for Regulatory Activities (MedDRA) terminology. All safety and program-retention data will be analyzed and described. **Methods:** Confirmed PKU patients will be enrolled in the SEAP from commencement of the program in June, 2007 until approximately February, 2008 or two months after commercialization of drug (Kuvan). Patients will receive a daily dosage of sapropterin dihydrochloride between 5 mg/kg/day and 20 mg/kg/day for variable lengths of time. Throughout the SEAP, time on drug, safety data pertaining to AEs such as MedDRA terms, severity, seriousness, relatedness to drug, action required and outcome will be analyzed. **Results:** As of November 6, 2007, a total of approximately 111 patients began therapy = 30 days, (between June, 2007 and October 6, 2007). Twenty of 111 (18%) patients who discontinued therapy were withdrawn for either non-response (12 patients; 11%); or non-compliance

(4 patients; 3.6%), or AEs (4 patients; 3.6%: nausea [2], vomiting [1], diarrhea [1], fatigue [1], abdominal cramps [1] and abdominal pain [1]). For all patients enrolled during SEAP up to October 6, 2007, AEs occurred in 22 of 111 (20%) patients, including 4 patients who withdrew from the SEAP. All AEs were mild to moderate in severity, belonged mostly to the gastrointestinal system order class, most of which were possibly related to sapropterin dihydrochloride. **Conclusions:** Discussion and definitive conclusions of the final set of results will be presented. Preliminary findings suggest that 91 (82%) of patients who were initiated on drug before October 6, 2007, were retained on drug up to November 6, 2007, whereas 20 (18%) of patients withdrew for reasons associated with non-response to drug, or due to non-compliance or due to AEs. Sapropterin dihydrochloride was well tolerated at doses between 5 and 20 mg/kg/day with mostly mild to moderate gastrointestinal-related AEs.

70. The bioavailability of KuvanTM (sapropterin dihydrochloride) from intact or dissolved tablets administered with or without food to healthy volunteers. D. Musson¹, C. O'Neill¹, W. Kramer², E. Foehr¹, F. Bieberdorf³, S. Kim¹, A. Dorenbaum¹, S. Turbeville¹, H. Nicely¹. ¹BioMarin Pharmaceutical Inc., Novato, CA 94949, USA; ²Kramer Consulting LLC North Potomac, MD, USA; ³CEDRA Clinical Research LLC, Austin, TX, USA.

Background and objective: Phenylketonuria (PKU) is an autosomal recessive metabolic disorder in which mutations of the phenylalanine-4-hydroxylase (PAH) gene result in reduced hydroxylation of phenylalanine (Phe) to tyrosine. Consequently abnormally high levels of Phe and toxic metabolites accumulate in blood, brain and other tissues, leading to severe neurological manifestations and other problems. KuvanTM is a synthetic tetrahydrobiopterin (BH4), which is a cofactor for PAH, which was recently tested in clinical trials to evaluate its safety in healthy volunteers and efficacy for use in reducing Phe levels in PKU patients. Here, we report the results of a Phase I trial conducted in healthy volunteers to investigate pharmacokinetic data for Kuvan. **Methods:** The relative bioavailability of Kuvan was evaluated in three regimens: (1) swallowed as an intact tablet; (2) dissolved in water; and (3) taken as an intact tablet with food, in a Phase I three-treatment, three-period study. This study was conducted as a crossover design in 30 healthy subjects between the ages of 18 and 50 years of age at a dose of 10 mg/kg. Plasma BH4 levels were determined using a LC/MS/MS method. The plasma BH4 levels were analyzed for pharmacokinetic parameters using non-compartmental modeling. **Results:** Systemic exposure for the swallowed intact tablet was about 40% greater than dissolved tablet. The AUC(0-t) values for swallowed intact ingestion and dissolved ingestion were 474 ± 235 and 337 ± 186 ng-h/mL, respectively; the C_{max} values were 84.1 ± 42.2 and 63.0 ± 28.5 ng/mL, respectively. Regarding the effect of food, exposure was found to be about 40% greater when administering the intact tablet with a high calorie, high fat meal than without food. The AUC(0-t) and C_{max} values for intact tablet taken with food were 635 ± 246 ng-h/mL and 105 ± 32.1 ng/mL, respectively. **Conclusion:** Both the ingestion of intact Kuvan tablet in comparison to dissolved tablet, and ingestion of the intact tablet with food, increased the systemic exposure of Kuvan.

71. Phenylalanine metabolism disorders and breastfeeding: Experience of two Mexican patients. Z. Olivares, M. Contreras, C. Ramírez, M. Pérez, L. Belmont, M. Vela. Unidad de Genética de la Nutrición, Instituto de Investigaciones Biomédicas-UNAM, Instituto Nacional de Pediatría-SS, Ciudad de México, México. irmzazil@yahoo.com.mx.

Introduction: Breastfeeding success in phenylalanine disorders (PD) has been reported since 1981, nevertheless in the usual practice, newborns with these diseases frequently receive infant formula instead of human milk. **Methods:** A diet with phenylalanine-free formula and human milk was calculated based on phenylalanine (Phe) initial serum concentrations and according to the individual nutritional requirements, to two patients with confirmed PD diagnosis. The diet was modified based on the metabolic response, weight and age. **Results:** In a female patient and male one, treatment was started at 27 and 9 days of age respectively, the girl with

46.40 mg/dl of blood Phe, and the boy with 6.4. They received Phe-free diet the former, and with 62 mg of Phe/kg of body weight the latter, serum Phe attained normal range (1 mg/dl and 0.9 mg/dl respectively), and human milk is reintroduced, combined with Phe-free formula. The patients have received this diet for 12 and 7 months each one, showing Phe serum average values of 3.9 and 1.02 mg/dl respectively. Growth and development of both infants has been adequate, according to normal population growth charts. **Conclusions:** It is possible to practice breastfeeding in PD and get the advantages of its benefits, besides its usefulness in the management of the disease thanks to its lower protein content, compared to the infant formulae.

72. Psychological aspects of patients with niemann pick disease, type B. W. Packman¹, S.L. Henderson², J. Wallace¹, K. Hatheway¹, S. Packman³. ¹Pacific Graduate School of Psychology, Palo Alto, California, USA; ²University of California, Davis, Sacramento, California, USA; ³University of California San Francisco, San Francisco, California, USA.

Objective: The present study investigates the psychosocial consequences of Niemann Pick Disease, type B (NPD) on patients and families. The study explores the relationship between psychological distress, psychosocial development, and the transition to adulthood in the context of a chronic illness. Given the limited research on patients with NPD, it is vital to gain greater understanding of how this disease affects the psychosocial functioning of patients and their families. **Methods:** The participants were patients with NPD type B between the ages of 13–18 (adolescent group) and their parents; and patients 19 and older (adult group). The total sample consisted of 17 participants. All participants were administered a semi-structured interview, which elicited information in seven domains: (1) life experiences with respect to NPD; (2) feelings about having NPD; (3) relationships with family and friends; (4) developmental history and schooling; (5) college; (6) employment; and (7) medical experiences in the context of NPD. A qualitative case study methodology was used to explore and examine the psychosocial adjustment of patients with NPD. Six cases were chosen for qualitative analysis. Objective self-report measures were also administered, including the Brief Symptom Inventory; quality of life measures (PedsQL 4.0 and SF-36); the PedsQL, Family Impact Module; and the Measures of Psychosocial Development. **Results:** Five major findings emerged from the interviews: (a) all participants identified limited physical activity, social isolation, and peer rejection as significant stressors; (b) these stressors had a specific impact during the age span of 10–16 years; (c) parents and adult patients expressed significant frustration regarding the lack of information and treatment available; (d) patients described close family relationships as a way of coping with the illness; and (e) adult patients identified early medical experiences as having a considerable psychological impact. A secondary analysis was conducted consisting of a multidimensional measurement of psychosocial adjustment using objective self-report measures. Although most of the participants showed relatively good adjustment, areas of psychosocial maladjustment were revealed including unresolved developmental crises and health-related quality of life concerns in the areas of social, school and physical functioning. **Conclusion:** The information from this study offers health care providers a more comprehensive view of their patients with NPD and of the resources required by their patients.

73. Psychological aspects of patients with fabry disease. W. Packman¹, T. Wilson-Crosbie¹, M. Needham¹, S. Packman². ¹Pacific Graduate School of Psychology, Palo Alto, California, USA; ²University of California San Francisco, San Francisco, California, USA.

Objective: The purpose of this study was to examine the psychological profile or personality features of patients with Fabry Disease (FD) and the impact of FD on psychosocial functioning. This study represents the first time that the Minnesota Multiphasic Personality Inventories (MMPI) have been used to describe the psychological complications of patients with FD. Patients with FD were also compared to patients diagnosed with Gaucher Disease (GD), Chronic Heart Disease (CRHD) and chronic pain. **Methods:** Participants were patients with FD ($n = 28$; ages 18–58 years) who had been referred to or evaluated at the University of

California, San Francisco Genetics Clinic and Lysosomal Disease Center. Assessment measures included the MMPI-2 and a demographic questionnaire. The MMPI-2 is a self-report instrument composed of 567 true or false statements and multiple validity, clinical, content, and supplementary scales. The demographic questionnaire collected information on medical history, symptom description, pain history, and enzyme replacement therapy history. **Results:** FD patients scored significantly higher than the normative sample on seven MMPI-2 clinical scales (HS, D, Hy, Pd, Pa, Pt, Sc) and two validity scales (L, F). Profiles of patients with FD were then compared to profiles of patients with Gaucher Disease (GD), Chronic Heart Disease (CRHD), and chronic pain. The FD patients had significantly higher scores than the GD patients and CRHD patients on several clinical, validity, supplementary and content scales. Of note, no significant differences were found between the FD patients and the chronic pain patients on any of the clinical, validity, supplementary and content scales; however, the chronic pain patients evidenced higher elevations on two clinical scales. **Conclusion:** The results underscore the fact that FD patients hurt physically and reported numerous physical symptoms. Coping with physical discomfort and pain issues, their concerns appear to be health-related, rather than psychological in nature. Our results highlight the clinical significance of understanding FD patients' physical as well as psychological needs when making treatment decisions. Further, making treatment decisions based on immediate stressors, physical health concerns, and psychological well-being will facilitate both clinical intervention and facilitate patients' coping with FD in positive ways.

74. Effect of prolonged storage on the concentration of amino acids and acylcarnitines in blood spots. L. Richardson², N.J. Morlan¹, P. Dobrowolski², M. Pasquali^{1,2}. ¹Department of Pathology, University of Utah, Salt Lake City, UT 84108, USA; ²ARUP Laboratories at the University of Utah, Salt Lake City, UT 84108, USA.

Background: Blood spots used for newborn screening are often stored for subsequent use for unlimited time. No systematic studies have been done to evaluate the effect of prolonged storage at different temperature on the analytes measured in these blood spots. **Objective:** Evaluate changes in the concentration of amino acids and acylcarnitines measured by MS/MS in blood spotted on filter paper stored at room temperature, 4, –20, –70 °C. **Methods:** Whole blood was spiked with different concentrations of amino acids and acylcarnitines and spotted on filter paper. Five levels of concentrations were used. The filter papers were stored under vacuum and in bags with desiccant under 4 different storage conditions for up to 2 years. Amino acid and acylcarnitine concentrations were measured by MS/MS after methanol extraction at different time intervals. ANOVA was used to calculate the significance of the regression obtained by plotting the concentrations of the analytes vs. time. **Results:** We have observed a significant ($p < 0.05$) change in concentrations of several acylcarnitines when the filter paper was stored at room temperature. The change in concentrations for the majority of the amino acids was not significant. No significant changes in the concentration of acylcarnitines or amino acids were observed when samples were stored at –20 or –70 °C. Samples stored under vacuum behaved similarly to samples stored in bags with desiccant. **Conclusions:** Storage of blood spots for a prolonged period of time (up to two years) can result in significant change in concentrations of acylcarnitines, depending on the temperature. Storage at room temperature results in the highest change, while storage at –20 and –70 °C results in the lowest change. Results obtained from blood spots stored under vacuum were not significantly different from those obtained from blood spots stored in bags with a desiccant. Storage of blood spots under vacuum will considerably reduce the space necessary for storage.

75. Identification of two cases of mild glutaric aciduria type II (MAD deficiency). Laura Pollard¹, Nolan Williams², Sarah Young³, Elaine Spector⁴, Kathy Tomashits⁵, Robyn Porter⁵, Kenton Holden^{1,2}, Richard Schroer¹, Tim Wood¹. ¹Greenwood Genetic Center, Greenwood SC, USA; ²Medical University of South Carolina, Charleston

SC, USA; ³Duke University Medical Center, USA; ⁴University of Colorado Denver, School of Medicine, USA; ⁵South Carolina Department of Health and Environmental Control, USA.

Glutaric aciduria type II (Multiple acyl CoA dehydrogenase deficiency, OMIM #231680) is a rare disorder of fatty acid and organic acid metabolism. A deficiency of one of three proteins (alpha or beta subunit of EFT and ETF dehydrogenase) is known to cause GAI. The severe and lethal neonatal form presents with severe nonketotic hypoglycemia, metabolic acidosis, hypotonia and hepatomegaly, and may exhibit dysmorphic features and renal cysts. The milder later onset form presents with lethargy, vomiting, hypoglycemia and acidosis. Diagnosis of GAI is usually made based on a characteristic urine organic acid pattern and multiple acylcarnitines may also be elevated. We present two cases of mild glutaric aciduria type II. The first case is a 5 year old Hispanic male with two hospitalizations for nonketotic hypoglycemia progressing to coma. During recovery he had slowly resolving mutism, ataxia, hypotonia and strabismus. The patient had no prior history of metabolic crisis or repeat episodes of hypoglycemia. Initial studies from samples taken between the two crises showed normal urine organic acids and low total carnitine with slight elevations of medium chain acylcarnitines. Repeat plasma acylcarnitines (with normal total carnitine level) and an *in vitro* probe assay were consistent with glutaric aciduria type II. Mutation analysis identified a previously described homozygous change (p. P27S) in the *ETFDH* gene. The patient has not experienced metabolic crisis for two years following carnitine therapy; however, he remains hypotonic with residual strabismus. The second case, an asymptomatic 13 month old male, was identified through S.C. expanded newborn screening. Follow-up plasma acylcarnitines were consistent with GAI. Urine organic acids and acylglycines have shown persistent elevations of ethylmalonic acid with slight elevations of glutaric acid and episodic elevations of hexanoylglycine and methylsuccinic acid. An *in vitro* probe assay also showed elevations consistent with glutaric aciduria type II. Molecular studies are currently underway. The patient has been treated with riboflavin and carnitine and remains asymptomatic. The cases highlight the mild, episodic phenotype associated with late onset GAI and demonstrate the challenge in diagnosing this milder form due to the variation in laboratory findings compared with “classical” glutaric aciduria type II.

76. Long-term developmental outcomes in duarte galactosemia. K.K. Powell^{1,2}, K. Van Naarden Braun², R.H. Singh³, R.S. Olney⁴, S.K. Shapira⁴, M. Yeargin-Allsopp². ¹Association for University Centers on Disabilities (AUCD), ²Centers for Disease Control and Prevention (CDC), Developmental Disabilities Branch, ³Emory University, ⁴CDC Pediatric Genetics Team.

Objective: Examine developmental outcomes of a population-based sample of children with Duarte galactosemia (DG). **Background:** Compared to classical galactosemia, DG or variant galactosemia has a much milder phenotype although a previous study found a risk for speech and language problems similar to some findings of verbal dyspraxia in classical galactosemia. This study contributes to the limited information on long-term outcomes in DG. **Methods:** Children who were identified with DG by the Georgia Newborn Screening Program and born in the five-county metropolitan Atlanta area during 1988–2002 were linked to (1) the Metropolitan Atlanta Developmental Disabilities Surveillance Program (MADDSP), an ongoing, population-based surveillance system for selected developmental disabilities (intellectual disabilities (IQ < 70), cerebral palsy, hearing loss, vision impairment and autism) among 8 year-olds and (2) the Special Education Database of Metropolitan Atlanta, electronic files identifying 3–10 year-old children who received special education services in 1 of the 9 public school systems in the study area. Clinical genetics records were reviewed to determine metabolic control. **Results:** From 688,036 live births, 91 children were identified with DG. Five children were identified as having received special education services with the following exceptionalities: 1 behavior disorder, 2 learning disorders and 2 speech/language disorder. None were found to have any of the five categories of MADDSP developmental disabilities. Clinical genetics records were found for 80% of children. For children identified in special education, newborn screen GAL-

1-P levels ranged from 13 to 30 mg/dl. At first clinic visit to confirm the diagnosis, 2 had >10mg/dl GAL-1-P (high), 2 had 3.5–10mg/dl GAL-1-P (moderate) and 1 had <1 mg/dl GAL-1-P (normal), and during treatment of restricted lactose through 1 year, 3 had excellent control, 1 had fair control and 1 had unknown control based on GAL-1-P levels. Among children not in special education, at first clinic visit, 53% had normal levels, 12% 1–3.4 mg/dl GAL-1-P (mildly-elevated), 19% moderately-elevated, 10% high levels, and 6% unknown and during treatment 82% had excellent control, 10% good, 1% fair and 6% unknown. Future review of medical genetics records will be conducted to determine if receipt of special education services has been attributed to DG or to other causes. **Conclusions:** Despite treatment until 1 yr, select developmental issues associated with special education have been found in some children with DG. The initial GAL-1-P level and duration of elevation prior to treatment might play a role in developmental outcomes. Further studies are needed to evaluate these findings.

77. Tissue localization of fatty aldehyde dehydrogenase activity in mice: Implications for Sjögren-Larsson syndrome. Joseph V. Lamar¹, M. Anita Jennings², William B. Rizzo¹. ¹Departments of Pediatrics, University of Nebraska Medical Center, Omaha, NE, USA; ²Genetics, Anatomy and Cell Biology, University of Nebraska Medical Center, Omaha, NE, USA.

Objectives: Sjögren-Larsson syndrome (SLS) is an inborn error of metabolism characterized by ichthyosis, mental retardation and spasticity. The disease is caused by mutations in the *ALDH3A2* gene encoding fatty aldehyde dehydrogenase (FALDH), an enzyme that catalyzes the oxidation of aliphatic aldehydes to fatty acids. Little is known about the tissue localization of FALDH and why its deficiency in SLS chiefly affects the skin and nervous system. To better understand the role of FALDH deficiency in SLS, we investigated the cellular localization of this enzyme in mice. **Methods:** FALDH activity was detected in frozen tissue sections using a histochemical staining method with decanal as substrate. To clearly establish that staining activity was due to FALDH and not other aldehyde dehydrogenase enzymes, tissue sections from wild-type mice (*Aldh3a2*^{+/+}) were compared to FALDH-deficient genetic knockout mice (*Aldh3a2*^{-/-}). **Results:** In wild-type liver, hepatocytes showed a strikingly intense centrilobular staining pattern interspersed with regions of relatively low staining. In the small intestine, FALDH activity was concentrated in the epithelial cells of the intestinal villi. In the kidney, enzyme activity was high in the renal tubules and very low in glomeruli. Enzyme staining in brain was most prominent in the granular layer of the cerebellum, whereas the cerebral white matter showed relatively little enzyme activity. The skin showed scattered staining of dermal fibroblasts; intense staining in the sebaceous glands and hair follicles; and greater staining of the epidermal basal cells compared to the spinous and granular layers. No staining of the stratum corneum was seen. Compared to wild-type animals, there was a severe deficiency of FALDH staining in all tissues from *Aldh3a2*^{-/-} mice except for the epidermis, where it was only mildly reduced. This indicates the presence of other epidermal aldehyde dehydrogenase enzymes that oxidize decanal. **Conclusions:** Our results in *Aldh3a2*^{-/-} mice suggest that widespread deficiency of FALDH occurs in patients with SLS, even in tissues such as liver, kidney and intestine that are not known to be clinically affected.

78. Crystal structures of N-acetylglutamate synthase provide insights into catalytic and regulatory mechanisms. D. Shi¹, V. Sagar², Z. Jin³, X. Yu¹, L. Caldovic¹, H. Morizono¹, N.M. Allewell², M. Tuchman¹. ¹Children's National Medical Center, Washington, DC 20010, USA; ²University of Maryland, College Park, MD 20742, USA; ³Argonne National Laboratory, Argonne, IL60439, USA.

N-Acetylglutamate synthase (NAGS) catalyzes the first committed step of the arginine biosynthetic pathway to convert L-glutamate to *N*-acetyl-L-glutamate in micro-organisms and plant. In mammals, NAGS produces *N*-acetyl-L-glutamate (NAG), an obligatory allosteric activator of carbamyl phosphate synthase I (CPSI) in the urea cycle. NAGS deficiency in human leads to hyperammonemia owing to the decreased activity of CPSI

deprived of its cofactor NAG. NAGS seems to act as the principal regulator of both the urea cycle in mammals and the arginine biosynthetic pathway in micro-organisms and plants. However, no crystal structures have been reported for this protein from any organism due to its instability and poor crystallization. We report herein the crystal structures of NAGS from *Neisseria gonorrhoeae* in the inactive *T*-state bound with its feedback allosteric inhibitor L-arginine and in the active *R*-state complexed with acetyl-co-enzyme A (AcCoA), or CoA and NAG. These structures reveal that NAGS consists of two separately folded domains, an amino acid kinase (AAK) domain and an *N*-acetyltransferase (NAT) domain. The kinase domain has a fold similar to other members of the AAK family while the NAT domain has a fold similar to GCN5-related NAT proteins. The monomers form a hexameric ring that consists of a trimer of dimers with inner and outer ring diameters of ~20 and ~100 Å, respectively, and a height of 110 Å. The catalytic sites are located within the NAT domains and the mode of binding of AcCoA and CoA is similar to other NAT family proteins. NAG interacts within the active site in the NAT domain with two arginines (Arg316 and Arg425), to anchor the substrate in its proper position. Comparison of active *R*- and inactive *T*-state structures indicates that binding of arginine to the AAK domain induces a large global conformational changes in the enzyme. The hexameric structure becomes ~20 Å shorter and ~10 Å larger in diameter. Specifically, the NAT domain rotates ~109° to rearrange the interaction between AAK and NAT domains. This rearrangement disrupts the interactions of the adjacent AAK domain with AcCoA hampering its binding and thus inhibiting catalytic activity. These findings provide new insights into the catalytic and inhibition mechanism of NAGS. These first NAGS solved structures also allow modeling of human NAGS and enable better understanding of the molecular basis of NAGS deficiency.

79. The long term impact of tetrahydrobiopterin therapy in phenylketonuria:

Dietary and nutritional implications. Rani H. Singh. Department of Human Genetics, Emory University School of Medicine, Decatur, GA, USA.

Objective: While improvements in plasma phenylalanine (Phe) concentrations have been the primary outcome measure of tetrahydrobiopterin (BH4) responsiveness thus far, the implications for diet and nutrition status are lacking. The objective of this study is to investigate the impact of BH4 on Phe tolerance, long-term dietary patterns, medical food continuation and nutritional status. **Methods:** At the Emory Genetics Clinic, 7 of 9 children with well-controlled phenylketonuria (PKU) responded to a dose of 20 mg/kg/day of BH4 (sapropterin dihydrochloride) with a =30% decrease in plasma Phe concentrations after 8 days ($p = 0.014$). Six of the responders were enrolled in a 6-month follow-up study to evaluate further the impact of BH4 on Phe tolerance and nutritional status. Maximum dietary Phe tolerance was determined by progressively increasing milk or egg powder over a six-week period while maintaining plasma Phe concentrations between 120 and 360 $\mu\text{mol/L}$. Subsequently, protein from medical food was decreased by 25% each week provided that plasma Phe concentrations and nutrition status markers remained within the therapeutic range and the average protein intake met or exceeded US Dietary Reference Intakes (DRIs). **Results:** Six weeks: Dietary Phe tolerance increased to a mean \pm SD of 1380 ± 395 mg/d (baseline 575 mg/d \pm 215) ($p = 0.001$). Six months: Mean plasma Phe concentrations persisted within the therapeutic range of 120–360 $\mu\text{mol/L}$ throughout the 6-month follow-up period while the mean dietary Phe tolerance was 1595 ± 615 mg/d. Four of the six patients were able to completely eliminate medical food from their diet, while the remaining two took medical food below baseline intakes. While mean total protein intake did not significantly decrease and continued to exceed DRIs for each patient, vitamin and mineral supplementation was required for those who discontinued formula to meet micronutrient DRIs. There was no significant change in mean energy intake; weight percentiles; and concentrations of prealbumin, hemoglobin and hematocrit. **Conclusions:** These results demonstrate the need to systematically reduce medical food to maintain nutrient adequacy of the diet while maintaining

plasma Phe levels within therapeutic range and to personalize diet recommendations. Vitamin and mineral supplementation may be necessary, particularly if medical food has been discontinued.

80. 3-Hydroxyisobutyric aciduria: A case report. U. Garg¹, D. Scott¹, A. Atherton², L.D. Smith². ¹Section of Pathology, Children's Mercy Hospitals and Clinics, Kansas City, MO 64108, USA; ²Section of Clinical Genetics, Dysmorphology and Metabolism, Children's Mercy Hospitals and Clinics, Kansas City, MO 64108, USA.

We present the case of a child with mild dysmorphic features (relative microcephaly, midface hypoplasia, hypotelorism, bulbous nose and small jaw) and mild developmental delays. He walked at 17.5 months and had speech delay. Urine organic acid profile was remarkable for increased 3-hydroxybutyric, 3-hydroxypropionic, and 2-ethyl-3-hydroxypropionic acids. Urine amino acid profile was remarkable for increased 3-aminoisobutyric and 3-aminopropionic acids. These results are suggestive of methylmalonic semialdehyde dehydrogenase deficiency, a rare inborn error of valine metabolism. Management was primarily dietary with a low protein diet and BCAD1 with isoleucine and leucine supplementation along with carnitine therapy. Fasting was avoided and implementation of prompt interventions when ill was also done. No recurrent episodes of ketoacidosis were documented. Improvement in linear growth was seen although relative microcephaly persisted. Development, including speech improved. At 26 months of age, he developed a mild febrile illness for which he received Tylenol. No other interventions were performed at the local hospital despite availability of an emergency letter and protocol. He was found comatose and hypoglycemic after initially appearing to have medically improved. He was emergently transported to a tertiary care center and upon admission to the intensive care unit was noted to have severe cerebral edema consistent with a hypoxicemic ischemic event. Resuscitative efforts were complicated by disseminated intravascular coagulopathy and were unsuccessful. An autopsy was performed which was significant for centrilobular hepatic necrosis. Brain sections were most significant for changes associated with cerebral edema but rare microcalcifications were noted in the frontal lobes. The cause of death was reported to be hepatoencephalopathy. This case highlights diagnosis and treatment of this rare disorder but also emphasizes the need for aggressive interventions for even mild illnesses in children affected with this disorder.

81. A novel mutation identified in a patient with clinical features of hyper IgD syndrome (HIDS).

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K.W. is a 3 year old female with a history of recurrent episodes of fever, vomiting, and diarrhea with an associated rash every 3 weeks since 2 weeks of age. These episodes typically start with a fever followed by 4–5 bouts of emesis, which then lead to loose stools and the development of a rash. She does not improve with symptomatic treatment. Comprehensive evaluations from pediatric allergy, dermatology, rheumatology, gastroenterology and metabolic disorders clinics were performed. She had numerous normal laboratory studies obtained when ill and well including Immunoglobulin panel (including IgD levels), complement panel, chemistries, and complete blood counts. Urine organic acids were analyzed twice during illness and qualitatively reported as “no abnormalities found”. Subsequently, urine mevalonic acid quantification was requested revealing a significantly elevated level of mevalonic acid, 48 $\mu\text{g/mg}$ creatinine (nl 0.25 ± 0.14 $\mu\text{g/mg}$ creatinine) suggesting the diagnosis of HIDS. At this point, the patient was given steroids for acute attacks and begun on antioxidant therapy to include co-enzyme Q10, vitamin C and vitamin E. Mevalonic kinase (*MVK*) gene analysis was then performed which initially identified one of the common HIDS mutations (V377I) in exon 10. With the clinical phenotype suggesting this disorder but only one common mutation found, full gene sequencing was performed which identified a heterozygous nucleotide change of C > T in exon 9 (p.Q302X) of the *MVK* gene. This nonsense mutation has not been previously reported in the literature, but

is predicted to result in nonsense mediated mRNA decay or production of a truncated protein. This case stresses the importance of quantitatively testing urine mevalonic acid in a patient who has clinical symptoms suggestive of HIDS even when IgD levels and urine organic acid analysis are normal.

82. Effects of exercise on cardiac metabolism, function and morphology and in very long-chain acyl-CoA dehydrogenase knock-out mice. Ute Spiekerkoetter¹, Christoph Jacoby², Sonja Primassin¹, Alexander Vogt¹, Juergen Schrader², Ertan Mayatepek¹, Ulrich Floegel², Frank ter Veld¹. ¹Department of General Pediatrics, Heinrich-Heine-University, Duesseldorf, Germany; ²Department of Cardiovascular Physiology, Heinrich-Heine-University, Duesseldorf, Germany.

Objective: Since implementation of newborn screening programs for fatty acid oxidation defects, the majority of patients is asymptomatic at time of diagnosis and during the first years of follow-up. However, physical exercise can trigger severe rhabdomyolysis in later life. The effects of exercise on cardiac function and morphology have not been systematically studied. **Methods:** The present study investigates the effects of forced treadmill exercise on cardiac function, energy metabolism and heart morphology of very long-chain acyl-CoA dehydrogenase (VLCAD) knock-out mice. VLCAD-deficient (VLCAD^{-/-}) mice and wild-type (WT) mice were subjected to 2 weeks of forced treadmill exercise. In order to determine possible peroxisomal and mitochondrial proliferation, catalase and citrate synthase activities were determined in heart, skeletal muscle and liver. High-resolution in-vivo nuclear magnetic resonance imaging was used to study cardiac function and morphology in non-exercised and exercised mice. **Results:** After exercise, no increase in peroxisomal catalase activity was observed in muscle, liver and heart. Mitochondrial citrate synthase activity, however, was significantly increased in heart from exercised VLCAD^{-/-} mice as compared to exercised WT mice. Under resting conditions, cardiac output was impaired in VLCAD^{-/-} mice, as reflected by a significant decrease in stroke volume. In addition, VLCAD^{-/-} mice were unable to perform the exercise protocol designed for WT mice and treadmill belt speed had to be reduced during the protocol. After a 2-week exercise challenge, VLCAD^{-/-} mice displayed significant cardiac wall hypertrophy of 14%. Changes in cardiac morphology in VLCAD^{-/-} heart were accompanied by a significant increase in cardiac output, as compared to exercised WT mice and resting VLCAD^{-/-} mice. **Conclusion:** Physical exercise is impaired in VLCAD^{-/-} mice and exercise training of already 2 weeks induces signs of cardiomyopathy. The observed increase in mitochondrial citrate synthase activity in heart suggests further mitochondrial proliferation and may well represent an adaptive response to compensate for impaired oxidative phosphorylation in VLCAD-deficiency. Nevertheless, cardiac hypertrophy due to short-term exercise may compensate the observed decreased cardiac output under resting conditions.

83. A method for comprehensive molecular screening of exonic regions for metabolic disorders through polymerase colony sequencing. J.V. Thakuria^{1,2}, G.T. Berry^{1,2}, G.M. Church². ¹Children's Hospital Boston, Boston, MA, USA; ²Harvard Medical School, Boston, MA, USA.

Advances in polymerase colony sequencing ("polony" sequencing) provide a novel opportunity for comprehensive screening of exons in genes known to cause metabolic disorders with far reaching implications in diagnostic capabilities, second tier screening options for abnormal newborn screens, elucidation of potential therapeutic targets, and identification of disease modifier polymorphisms.

Out of the approximately 22,000 known human genes, less than 1400 of these are available either through academic centers or industry for diagnostic sequencing. From this comprehensive list of genes available for diagnostic sequencing, we have manually curated a comprehensive list of over 250 genes involved in metabolic disorders for exonic sequencing by polymerase colony sequencing. Assuming an average of 10 exons per gene, and an average exon size of 500 bp, the targeted sequencing area will be approximately 1.25 Mb in size or 31.25 Mb for 25X coverage to insure accuracy of sequencing with short read lengths.

Polony clusters are one micron wide and one femtoliter in volume allowing billions to fit on one slide. Millions of such beads, each bearing identical copies of template on each bead, can be placed in individual wells or immobilized by a gel where sequencing is performed on them simultaneously by fluorescent-signal detection by digital microscope. Error rates are less than one per 3 million base pairs with only 7X coverage and throughput clocks in at 42 kbp/s. Current methods represent at least a 9-fold reduction in price over conventional sequencing. Immediate applications include rapid molecular diagnoses of less prevalent biochemical genetic mutations, thus obviating the need for traditional single gene first tier exon-by-exon sequencing.

84. Dietary glycomacropeptide (GMP) supports growth and reduces the concentrations of phenylalanine in plasma and brain in the PKU mouse. S.C. Van Calcar¹, A.K. Hull², X. Liu², M. Etzel³, D.M. Ney². ¹Biochemical Genetics Program, Waisman Center, USA; ²Department of Nutritional Sciences, University of Wisconsin-Madison, 53706, USA; ³Department of Food Science, University of Wisconsin-Madison, 53706, USA.

Current treatment recommendations for phenylketonuria (PKU) include a life-long phenylalanine (phe)-restricted diet. However, compliance with diet after 10 years of age is often poor and new approaches are needed to improve dietary treatment options. Glycomacropeptide (GMP) is an abundant protein found in the whey fraction as a byproduct of cheese production. Pure GMP is the only known dietary protein that is free of aromatic amino acids, including phe. Threonine and isoleucine are elevated 2 to 3 times in GMP compared to a reference protein. This unique amino acid profile makes GMP uniquely suited as a potential protein source for the treatment of PKU. Our aim was to assess how ingestion of diets containing GMP support growth and impact the concentrations of amino acids in plasma and brain of mice with a deficiency of phenylalanine hydroxylase (PAH), the *Pah*^{enu2} mouse (PKU mouse). Experiments were conducted in 4–6 week old wild type (C57Bl/6) and PKU mice fed diets containing 20% protein from casein, amino acids, or GMP supplemented with limiting indispensable amino acids (IAA). PKU mice fed the GMP diet showed gain in body weight, feed efficiency and a protein efficiency ratio that were not significantly different compared to the amino acid diet. The concentrations of isoleucine and threonine in plasma showed a significant 2- to 3-fold increase for wild type and PKU mice fed GMP compared to casein or amino acid diets, respectively. PKU mice fed the GMP diet showed significant decreases in the concentration of phe in plasma (11% decrease) and in five regions of brain (20% decrease) compared to the amino acid diet. In summary, PKU mice fed GMP showed comparable growth and reduced concentrations of phe in plasma and brain compared with an amino acid diet. These data support continued research to assess the efficacy of GMP supplemented with IAA as an alternative source of dietary protein for individuals with PKU.

85. Pleiotropic regulation of biotin in eukaryotes. Velazquez-Arellano Antonio, Ortega-Cuellar Daniel, Hernandez-Mendoza Armando, Gonzalez-Alvarez Rafael. Nutritional Genetics, Instituto de Investigaciones Biomedicas, UNAM & Instituto Nacional de Pediatría, Mexico City, DF, USA.

The best characterized function of biotin in metabolism is as a prosthetic group of biotin-dependent enzymes. This vitamin is carrier of CO₂ in carboxylation, decarboxylation, and transcarboxylation reactions. In addition, evidences have been provided that biotin also plays a role in gene expression in prototrophic organisms as bacteria, fungi, plants and in biotin auxotrophic-organism, including mammals. In *Escherichia coli*, biotin is a co-regulator of the *bio* operon, whereas in mammals, biotin regulates the expression of biotin protein-ligase (BPL), biotin-dependent carboxylases and biotin transporters.

More enigmatic are the regulatory effects of biotin in proteins unrelated to its function as a prosthetic group (carboxyl transfer). Some of these

proteins are involved in carbohydrate metabolism. In rats, biotin induces glucokinase expression (GK) and represses phosphoenolpyruvate carboxykinase (PEPCK). These effects result in stimulation of glycolysis and inhibition of gluconeogenesis. Even more recently, some data indicate that biotin effects are mediated by soluble guanylate cyclase (sGC) and the cGMP-dependent protein kinase (PKG).

We are now studying the pleiotropic effects of biotin as a regulatory molecule in three organisms (yeast, *Saccharomyces cerevisiae*, nematode, *Caenorhabditis elegans* and zebra fish *Danio rerio*). Our data show that, as occurs in rats and mammalian cell lines, biotin starvation decreases mRNAs level of BPL and one hexokinase in *C. elegans* and *D. rerio*. In contrast, both genes increase their expression following biotin deficiency in *S. cerevisiae*. In addition we explored whether sGC-PKG signalling pathway is involved in this regulation. Our results indicate that BPL is regulated by the same signalling pathway in both *C. elegans* and *D. rerio*. However, this effect does not appear to be involved in hexokinase regulation, suggesting that the signalling pathway for both genes is different. In conclusion, our results suggest that the signaling pathway for the regulation of BPL could be present in the common ancestor from the nematodes, fishes and rats approximately 520 millions years ago. (Supported by PAPIIT IN228605 from DGAPA-UNAM).

86. Variability of blood phenylalanine and its relationship to children with PKU. V. Anastasoae, L. Kurzius, P. Forbes, S. Waitsbren. Children's Hospital Boston Metabolism Program. 300 Longwood Avenue Boston, MA 02115, USA.

A meta-analysis of 40 studies confirmed that in children with phenylketonuria (PKU), mean lifetime blood phenylalanine levels are significantly correlated with IQ ($r = -0.34$). A similar correlation ($r = -0.35$) was found between IQ and mean exposure during 0–12 years of age. Most of the studies in the meta-analysis, however, included children who had discontinued the phenylalanine restricted diet. None examined the impact of fluctuations in metabolic control in continuously treated children. This is important because new therapies may increase stability in blood phenylalanine levels. The question has arisen whether these therapies are beneficial in children whose blood phenylalanine levels are generally within the recommended range 2–6 mg/dl. In this study, we described the relationship between IQ and two parameters of metabolic control: (1) mean blood phenylalanine level of all reported specimens for each subjects, and (2) variability of the blood phenylalanine level as indicated by the standard deviation of blood phenylalanine levels for each subject. Analyses were performed using lifetime phenylalanine levels and levels during 3 periods (0–6 years, 0–10 years, and >10 years of age). The most recent IQ for each child was used in the correlation analyses. Data were collected from medical records on all 46 children born between 1999 and 2006 with early and continuously treated PKU followed at the Metabolism Program at Children's Hospital Boston. The mean age of the children at the time of their most recent IQ test was $7.5 \pm .32(2.92-15.5)$ and their mean IQ was $103 \pm 14(68-143)$. The lifetime blood phenylalanine level in these children was $5.2 \pm 2.2(2.1-14.2)$. The mean standard deviation of blood phenylalanine levels was $3.03 \pm 1.2(1.6-5.6)$. The correlation between mean lifetime blood phenylalanine levels and IQ was -0.08 ($p = 0.34$) while the correlation between standard deviation of blood phenylalanine levels and IQ was -0.38 ($p = 0.04$). The mean blood phenylalanine level was not significantly correlated with IQ during critical periods (0–6 years or 0–12 years of age). The correlation between IQ and standard deviation of blood phenylalanine levels was higher than the correlation between IQ and mean blood phenylalanine levels at each age period. These results indicate that stability of blood phenylalanine levels may be more important to cognitive functioning than overall exposure to phenylalanine in early and continuously treated PKU. In treating PKU attention should be given to variability in blood phenylalanine levels as well as maintenance of phenylalanine levels within the recommended range.

87. Two mtDNA mutations 14487T > C (M63V, ND6) and 12297T > C (tRNA Leu) in a leigh syndrome family. J. Wang¹, A. Brautbar¹, A. Chan², T. Dzwiniel², F. Li¹, B.H. Graham¹, L.J. Wong^{1*}. ¹Department

of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA; ²Department of Medical Genetics, University of Alberta, Edmonton, AB, Canada.

Background: Leigh syndrome is an early-onset progressive neurodegenerative disorder associated with defects in mitochondrial energy metabolism. Mitochondrial respiratory chain complex I deficiency is an important and frequent cause of Leigh syndrome. We described a 10-month-old girl with developmental delay, hypotonia, right exotropia, and lactic acidemia with concomitant hyperalanemia. Brain MRI showed characteristic abnormal hyperintensities bilaterally in the basal ganglia and other areas. Biochemical analysis revealed complex I deficiency in patient's muscle (20% of normal control). There is also a family history of a maternal uncle and a maternal aunt who died with clinical diagnosis of Leigh syndrome at age 15 years and 13 month old, respectively. Based on this constellation of clinical features, this patient was diagnosed as having Leigh syndrome. **Method:** Whole mitochondrial genome sequencing of the proband's muscle DNA was performed. Allele specific oligo (ASO) and amplification refractory mutation system (ARMS) quantitative PCR were used to detect and quantify mutations in various non-invasive tissues of the proband and matrilineal relatives. **Results:** Sequencing of the mitochondrial whole genome detected two novel mutations: 14487T > C (M63V in ND6) and 12297T > C in tRNA Leu (CUN) gene. Both mutations are nearly homoplasmic in the proband's muscle specimen. These two mutations were also detected in the blood, urine, hair follicles, and buccal swap samples of all matrilineal relatives tested including her brother, mother, maternal grandmother, aunt, and cousin. All individuals tested were nearly homoplasmic for 12297T > C mutation, but had different degrees of heteroplasmy for the 14487T > C mutation. **Conclusion:** The methionine at amino acid position 63 of ND6 subunit is evolutionarily highly conserved. This mutation is located right next to M62V, a known mutation causing LHON, and has been reported to have a specific defect of complex I on its assembly and stability. We conclude that 14487T > C is the primary mutation to cause Leigh syndrome in this family, and needs a high threshold to present symptoms. The 12297T > C is located at the anticodon loop of tRNA Leu(UUR), right next to the triplet anticodon. It has been reported in two unrelated Italian patients with dilated cardiomyopathy. For the family members that we have tested with nearly homoplasmic 12297T > C, none of them has clinical signs or symptoms of cardiomyopathy. The clinical significance of 12297T > C in this family is not clear at this point. There may be some co-effect between these two mutations on disease expression.

88. Pulmonary fabry disease associated with lysosomal storage of globotriaosylceramide in lung tissue. Raymond Wang¹, John Abe², Arthur Cohen³, William Wilcox⁴. ¹Children's Hospital of Orange County, Orange, CA, USA; ²Peninsula Pulmonary Medical Associates, Torrance, CA, USA; ³Department of Pathology and Laboratory Medicine, Cedars-Sinai Medical Center, Los Angeles, CA, USA; ⁴Department of Pediatrics, Cedars-Sinai Medical Center, Los Angeles, CA, USA.

Background: Fabry disease is an X-linked glycosphingolipidosis caused by a deficiency of the lysosomal enzyme α -galactosidase A. Symptoms in hemizygous males and heterozygous females are due to lysosomal storage of globotriaosylceramide (GL₃) in the central and peripheral nervous system, vascular endothelium, cardiac valves and myocytes, gastrointestinal tract, and renal epithelium. Pulmonary involvement is also a recognized manifestation of Fabry disease, but pathological evidence of pulmonary lysosomal storage is scant. **Case report:** We report at 52-year old female with a G43R α -gal A mutation who was ascertained at age 37 years after her son was diagnosed with Fabry disease. Her symptoms include a cerebrovascular accident at age 43, acroparesthesias, hypohidrosis, temperature intolerance, decreased vibration sense of the distal extremities, concentric left ventricular hypertrophy, trace mitral valve regurgitation, 2+ aortic valve regurgitation, and microalbuminuria. Her plasma α -gal A level was 3.9 nmol/h/mg protein (normal 12 ± 4.2).

Two and a half years prior to presentation, she took part in an exercise test research protocol and her pulmonary function tests were normal. She began to experience a dry, nonproductive cough that persisted despite

treatment with antibiotics and bronchodilators. Spirometry demonstrated a mixed restrictive/obstructive pattern (FVC 70% predicted, FEV₁ 76% predicted, FEF_{25–75} 72% predicted, TLC 82% predicted) and suggested an infiltrative defect in gas exchange (DLCO 51% predicted). “Ground-glass” pulmonary interstitial infiltrates were found on plain radiography and computerized tomography. She underwent an open lung biopsy that demonstrated peribronchiolar fibrosis and smooth muscle hyperplasia. Prominent inclusion bodies of the bronchiolar/arteriolar smooth muscle and endothelium were present. Electron microscopy indicated the inclusion bodies were lamellated zebra bodies consistent with GL₃ storage. Enzyme replacement therapy (ERT) with recombinant human α -galactosidase A was instituted. Since initiation of therapy, she occasionally has a dry cough but her pulmonary function tests have remained stable in the past 39 months. **Conclusions:** This report demonstrates a female patient with Fabry disease with pulmonary symptoms, deterioration of pulmonary function test parameters, and pathological evidence of GL₃ storage in pulmonary smooth muscle and endothelium. Considering two previous reports of inclusion bodies in bronchiolar epithelium obtained from either bronchoalveolar lavage or induced sputum, it is reasonable to conclude that pulmonary involvement in Fabry disease is in fact due to GL₃ storage. The patient’s post-ERT spirometry results suggest that ERT is capable of stabilizing pulmonary Fabry disease.

89. Utility of oligonucleotide array-based CGH for detection of intragenic deletions. Lee-Jun C. Wong^{1,+}, David Dimmock^{1,+}, Michael Gerahty², Richard Quan³, Uta Lichter-Konecki⁴, Jing Wang¹, NiChung Lee⁵, Ellen K. Brundage¹, Fernando Scaglia¹, A. Craig Chinault^{1,*}. ¹Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA; ²Genetics, Children’s Hospital of Eastern Ontario, Ottawa, Ontario, Canada; ³UC Davis Medical Center, Sacramento, CA, USA; ⁴Children’s National Medical Center, Washington, DC, USA; ⁵Medical Genetics and Pediatrics, National Taiwan University Hospital and College of Medicine, Taipei, Taiwan.

Background: Although direct DNA sequencing is the method of choice for the detection of point mutations, it does not detect intragenic exonic deletions. We hypothesized that oligoarray based-comparative genomic hybridization (oligo aCGH), which is now clinically available for detecting large chromosomal deletions and duplications, could be utilized to screen for intragenic deletions in conjunction with direct DNA sequencing, particularly in recessive conditions where only one heterozygous mutant allele is detected. **Methods:** A custom designed oligonucleotide based microarray was constructed to provide high density coverage of the genes of interest. The performance of the array was characterized in 109 control samples. Sequencing of genomic DNA breakpoints and qPCR analysis were used to confirm oligo aCGH data. For multigene deletions, analysis on a 244K whole genome oligo array was performed to confirm the deletion and map endpoints. **Results:** Oligo aCGH identified intragenic exonic deletions in three cases: a heterozygous single exon deletion of 4.5 kb in the *SLC25A13* gene on chromosome 7q21.3 leading to citrin deficiency, a homozygous 10.5 kb deletion of exons 13 to 17 in the *ABC11* gene on chromosome 2q31.1 in a patient with progressive familial intrahepatic cholestasis II (PFIC II) and a heterozygous 1.8 kb deletion of exon 4 of the *DGUOK* gene in a patient with hepatocerebral form of mitochondrial DNA depletion syndrome. In addition, we found a heterozygous 7.4 Mb deletion spanning a region distal to the *DMD* gene through exon 4 of the *OTC* gene on the X chromosome in a female infant with OTC deficiency and a second heterozygous 10 Mb deletion on X-chromosome extending from a position on Xp distal to *DMD* to a sequence on the entromeric side of the *OTC* gene in another girl with OTC deficiency. **Conclusions:** These cases illustrate the successful utilization of custom oligonucleotide arrays to detect either whole gene deletions or intragenic exonic deletions in nuclear genes that are involved in the pathogenesis of metabolic and mitochondrial disorders.

90. Application of oligonucleotide aCGH to the diagnosis of mitochondrial DNA deletion and depletion syndromes. A. Craig Chinault¹, Chad Shaw¹, Qing Zhang¹, Jing Wang¹, Lin-Ya Tang¹, Lee-Jun C. Wong^{1,*}.

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Background: To date the use of oligonucleotide array-based comparative genomic hybridization (oligo aCGH) for copy number analysis has focused on nuclear chromosomes without inclusion of the mitochondrial genome. Mitochondrial disorders are a group of clinically and genetically heterogeneous diseases for which molecular diagnosis has been a challenge. Since molecular defects in nuclear genes can severely affect the mitochondrial DNA (mtDNA) integrity and copy number, an oligonucleotide array containing probes from both the mitochondrial genome and the coding regions of a group of nuclear genes would be valuable for the simultaneous evaluation of nuclear and mitochondrial copy number changes that are relevant to mitochondrial disorders. **Methods:** A custom designed oligonucleotide based microarray was constructed to provide high density coverage of the nuclear genes involved in the biogenesis and the regulation and maintenance of mitochondrial function. The array also contains ~6400 probes covering both strands of the 16.6 kb mitochondrial genome. The performance of this array was characterized in more than 100 control samples and at least 30 samples with known mtDNA deletions. The oligo aCGH results were confirmed by sequencing to determine the DNA deletion breakpoints and by qPCR analysis to verify mtDNA depletion. **Results:** All samples with previously known deletions were clearly detected and the deletion breakpoints were correctly identified by the oligo aCGH. A previously unidentified deletion sample was also discovered. Extent of mtDNA depletion or percentage of deletion heteroplasmy in mitochondrial deletion cases can be estimated from the relative hybridization intensity values of the patient DNA and the age and tissue-matched reference DNA using a computational program we developed to implement the heteroplasmy and segmentation analysis. The power of this assay was particularly evident in two samples with confirmed molecular diagnosis of hepatic mtDNA depletion syndrome, one of which was shown to carry an intragenic deletion in the *DGUOK* gene. **Conclusions:** Our results demonstrate the strength of this custom oligonucleotide array for simultaneous detection of both mtDNA copy depletion and internal deletions, with elucidation of the deletion breakpoints and the percentage of deletion mutant heteroplasmy, as well as detection of intragenic nuclear gene deletions responsible for mtDNA depletion, if present.

91. Phenylalanine (Phe) control in patients with phenylketonuria (PKU) consuming a novel metabolic medical food (Add Ins™). H. Shaw, R.H. Singh, S. Yannicelli. SHS International, Liverpool, England, UK; Emory University, Atlanta, GA, USA; Nutricia North America, Gaithersburg, MD, USA.

Background: Amino acid-based medical food products are effective in nutrition management of phenylketonuria (PKU), however, long term compliance can be poor. A flavorless novel medical food (Add Ins™) was developed, and contains free amino acids, excluding phenylalanine (PHE), encapsulated in a lipid coating. These coated amino acids (CAAs) were designed to be incorporated into low protein foods in the PHE-restricted diet. **Objectives:** Primary outcome was quantitation of plasma amino acid concentrations and protein status indices; secondary outcome variables were to assess compliance using an acceptability questionnaire and dietary intakes compared to current medical food. Serum lipids profiles were also quantitated. **Methods:** Ten patients with PKU replaced at least 1/3 of their medical food requirements with CAAs for 28 days. Baseline, 2 and 4 week data were analyzed. **Results:** Patients (16 ± 7 yrs) were prescribed on average 2 sachets (1 sachet contains 10g protein equivalents) daily of CAAs. There was no significant difference in mean baseline (24.3kg/m² + 7.9) and post intervention Body Mass Index results (23.1kg/m² + 7.3) (*p* = 0.70). Mean baseline plasma Phe concentration (587 ± 443 μmol/L) did not statistically differ at 28 days (586 ± 322 μmol/L). Plasma tyrosine (14 and 28 days), protein status indices and serum lipid concentrations (28 day) were not statistically different from baseline and were in normal reference ranges for age. **Acceptance:** one major limitation identified by patients included the gritty texture of CAAs which required preparation of foods to mask texture, thus making it inconvenient for some patients. Overall, 70% of study completers rated taste of CAAs, when added to

foods, as “good”, “very good” or “excellent”. Additional comments included ‘it’s simple and easy’, ‘food was the taste, rather than the product’, and ‘no detectable odor, which is good’. **Conclusion:** CAAs were found to be safe and effective in supporting normal nutrition status indices as part of a PHE-restricted diet and may help to “normalize” diet regimen in patients with PKU. CAAs may help compliance as an alternative flavorless and flexible medical food compared to traditional powders or liquids. Acceptability comments were helpful in identifying the best types and amounts of low protein foods to which CAAs can be successfully added to the diet.

^aCAAs and *Add Ins* are known as *Phlexy-10 Add Ins* in USA.

92. Quantification of 2,3-dinor-F₂-isoprostanes as a biomarker of oxidative stress in urine by UPLC-LC-ESI-MS/MS: A convenient method for high-throughput analysis. Judit Sztáray^{1*}, Dora Il'yasova², Sarah P. Young¹, David S. Millington¹. ¹Division of Medical Genetics, Department of Pediatrics, Duke University Medical Center, Durham, USA; ²Department of Community and Family Medicine, Duke Comprehensive Cancer Center, Duke University Medical Center, Durham, USA.

Background: F₂-Isoprostanes are considered a useful indicator of oxidative status. Because chronic oxidative stress has been implicated in many illnesses, etiological and outcome research in human populations requires high-throughput non-invasive measurements of these compounds in biological fluids. Our goal was to develop and validate a method for measurements of urinary metabolites of F₂-isoprostanes applicable to large-scale epidemiological studies. **Methods:** Spot urine specimens were collected on two occasions at 8 week intervals from 20 non-institutionalized volunteers (10 males, 10 females). Urine volumes equivalent to 0.2 mg creatinine were spiked with a fixed amount of stable isotope-labeled internal standard, ²H₄-PGF_{2 α , purified by single-stage SPE cartridge, then analyzed by a novel UPLC LC-ESI-MS/MS targeted to detect 2,3-dinor-F₂-isoprostanes, one of the two primary metabolites of F₂-isoprostanes (F₂-IsoPs). Applying a fast chromatographic gradient between 95% aqueous and 100% organic phase the isomers of F₂-IsoPs have been collapsed into a single peak and resulted in a 3 minute run time per assay (Figure 1.). **Results:** The assay was linear over the range of 3.0–60 ng/mL. The limit of quantification was 3.0 ng/mL, the limit of detection was 0.3 ng/mL at a signal-to-noise ratio of 3. Intra- and interday precision of the assay were within acceptable limits. Urinary levels of 2,3-dinor-F₂-IsoPs in the 40 urine samples ranged from 4.4 to 140 ng/mg creatinine, (mean±sd 29.4 ± 29.8). The mean urinary concentration of 2,3-dinor-F₂-IsoPs at week 1 and 8 was similar (29.7 ± 34.6 and 29.0 ± 24.9 ng/mg creatinine, respectively). The mean concentration for the male participants (n = 10)}

was significantly lower ($P < 0.05$) than that of the females (n = 10) (22.2 ± 27.3 and 36.5 ± 28.6 ng/mg creatinine.). **Conclusions:** This new assay allows rapid and reproducible quantification of a urinary F₂-isoprostane metabolite that may be used for monitoring patients with increased oxidative stress, such as mitochondrial disorders or Down syndrome.

93. Long-term monitoring of patients with infantile-onset pompe disease using a urinary tetrasaccharide biomarker. S.P. Young, H. Zhang, D. Bali, P. Kishnani, D.S. Millington. Pediatric Medical Genetics, Duke University Medical Center, Durham, NC, USA.

We previously reported the usefulness of monitoring the excretion of a urinary glucose tetrasaccharide (Hex₄), as a marker of glycogen storage, in patients with infantile Pompe disease receiving recombinant human acid- α -glucosidase (rhGAA) as enzyme replacement therapy (ERT). Results from the extension phase of a clinical trial of patients receiving ERT from age ≤ 6 months further supports the role of this biomarker in a) differentiating patients who respond with an improvement in motor function during the early phase of treatment from patients with minimal or no gains, and b) differentiating patients who maintain a good response in the 2nd and 3rd year of treatment from those who suffer a subsequent decline after one year. The 18 patients enrolled were categorized into three groups based on their motor function response to treatment. The first group (n = 7) made motor gains within the first year of ERT and all were walking independently and ventilator free by the end of the study (116 weeks). The second group (n = 6) also made motor gains (sitting, n = 3, standing, n = 2 or walking, n = 1) by one year of ERT, but did not make further improvements or regressed and became ventilator dependent during the extension phase. The third group (n = 5) made no or minimal motor gains throughout the study and became ventilator dependent. Mean Hex₄ levels (Fig. 1A) at baseline were significantly higher ($p = 0.03$) in this third group compared with patients in groups 1 and 2, suggesting an increased disease burden at the start of therapy. Mean Hex₄ values correlated with the motor response at all time points, being the most elevated in group 3 and least elevated in group 1 (Fig. 1A). Group 1 patients had a decrease in mean Hex₄ as early as week 4 of ERT to within or just above the normal range. These low levels of Hex₄ were sustained in 4 of these 7 patients throughout the study, whereas 3 showed increases after 52 weeks ERT. However, these 3 patients showed no clinical decline by the end of the study. Group 2 mean Hex₄ levels showed a similar trend as the good responder group for the 1st year of ERT, but this was followed by an increase for all 6 patients. Hex₄ levels in group 3 patients did not normalize, but remained elevated and showed an increasing trend. In contrast, enzyme markers of skeletal muscle damage (CK and AST) did

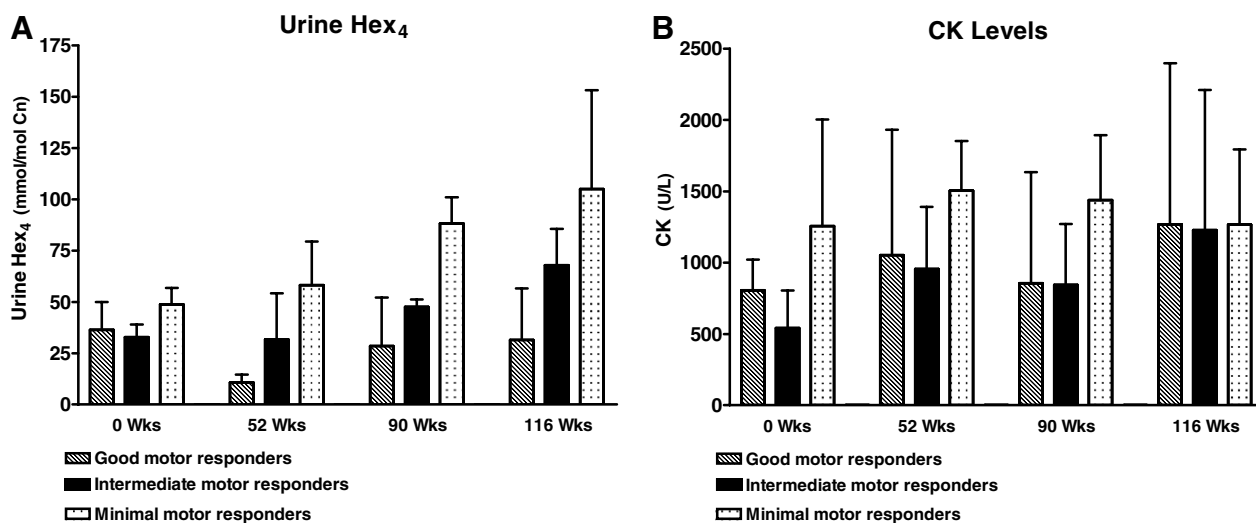


Fig. 1. Mean (SD) urine Hex₄ (A) and CK (B) levels in patients with good (group 1, n = 7), intermediate (group 2, n = 6) and minimal (group 3, n = 5) motor response to ERT with rhGAA over time.

not correlate as closely with the different motor responses as the Hex₄ biomarker, and tended to remain elevated at a constant level for most patients (Fig. 1B). Overall, our results show the importance of assessing the disease burden at regular intervals by monitoring urinary Hex₄ levels in patients with infantile Pompe disease receiving ERT.

94. Validation and extension of the urease method for urine organic and amino acid analysis. V. Young¹, S. Lo², J. Shoemaker³, A. Thomas⁴, W.J. Rhead⁵. ¹Children's Hospital of Wisconsin, Laboratory MS 701, Milwaukee, WI, 53226, USA; ²Children's Hospital of Wisconsin, Laboratory MS 701, Milwaukee, WI, 53226, USA; ^{3,4}Metabolic Screening Lab, St. Louis, MO, 63104, USA, ⁵Medical College of Wisconsin, Department of Pediatrics, Milwaukee, WI, 53226, USA.

Urine organic acid analysis by a GCMS method represents the standard of analysis for detection and quantitation of acidic amino acid and fatty acid metabolites in urine. Unfortunately, many neutral and positively charged compounds of interest to metabolic physicians and biochemical geneticists are not detected using the classical variants of this technique. However, pretreatment of biologic fluid samples with urease permit TMS derivatization of virtually all organic molecules in urine and other body fluids. We have adapted the published methods of Shoemaker et al (J. Chrom. Biomed. Appl. 562 (1991) 125–138) and expanded the range of compounds and metabolites analyzed and quantitated. Our Agilent 5973 GCMS is calibrated with 11 deuterated organic acid, amino acid and carbohydrate internal standards. Individual calibration curves for all analyzed and quantitated metabolites are established and integrated using the Agilent ChemStation Program. Metabolite identities are confirmed both by using NIST, St. Louis University School of Medicine Metabolic Screening Laboratory and/or Pitt/Sweetman libraries, and by direct comparison with the mass spectra of the pure compound analyzed in our own instrument. Using this technique, we can currently quantitate 54 organic acid metabolites, 37 amino acids, 8 acylglycines, 9 sugars and carbohydrates, 7 neurotransmitters and 6 purines and pyrimidines during a single 45 minute run. Work is underway to add an additional 25 to 30 metabolites to our panel, including pentoses, S-sulfocysteine, dipeptides, and vitamin and peroxisomal metabolites. We also have demonstrated several instances where this approach had clear advantages over traditional organic acid analysis in newborn screening follow-up. While the advantages of this approach are outlined above, disadvantages include a more laborious sample preparation than for the traditional organic acid GCMS analyses (currently 25–30 samples/week) and analysis of a highly complicated elution profile with many overlapping peaks, often requiring confirmation of peak identity and individual mass spectra. However, in our view, the utility of the information gained largely outweighs these inconveniences. This technique clearly invites serious consideration by the biochemical genetic and metabolic disease communities.

95. Evaluation of performance metrics of TMS newborn screening in Georgia. Chunli Yu¹, Adrya Stembridge¹, Patricia Z. Page¹, Paul M. Fernhoff¹, David H. Ledbetter¹, Rani H. Singh¹. Division of Medical Genetics, Department of Human Genetics, Emory University School of Medicine, Atlanta, GA¹.

Introductions: Effective Jan 1, 2007, Georgia expanded the newborn screening (NBS) panel from 10 to 28 mandated disorders (ACMG core panel with exception of hearing screen) plus an additional 25 reportable disorders. All expanded disorders except for Cystic Fibrosis are screened by TMS. Nine months of experience is summarized focusing on the performance metrics evaluation of TMS screening. **Methods:** GA DHR NBS data, Emory Follow Up NBS data, and Region 4 TMS analyte database were used as reference tools. Percentile of analyte values, analyte cut off, false positive rate and detection rate were monitored and compared to the region 4 national data on the monthly basis. **Results:** From January 2 through September 30, 2007, 112, 446 newborns in GA were screened. Thirty-one cases were confirmed by TMS, 11 of which benefited from expansion. Overall performance metrics by TMS showed: a detection rate of 1:3627, a false positive rate (FPR) of 0.81% and a

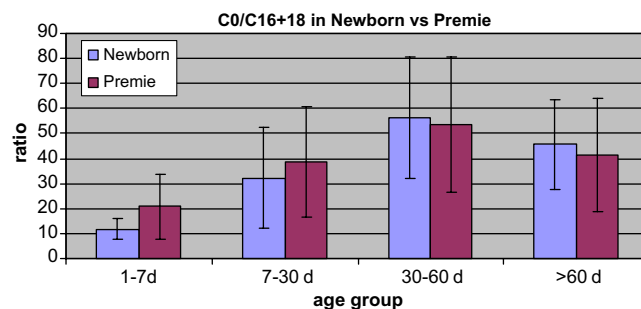


Figure. Trends in C0/C16+18 with age in newborn period.

positive predictive value (PPV) of 3% indicating lower than average analytical performance when compared with the reference tools. The 5 most referred analytes were C0 (H), methionine, C18:1, arginine and tyrosine with no confirmed cases. The highest false positive screen was CPT IA. We performed detailed analyses of C0, C16, C18 and C0/C16+18 in both full term and premature newborns. Data indicated the decline of C16 and C18 and the inverse increase of C0/C16+18 with age (see Fig. 1 below). Based on these observations, C0/C16+18 was proposed as the primary analyte for CPT I A along with age specific cut offs in both full term and premature newborns. This resulted in an improvement of PPV to 5% and FPR to 0.6%. This exemplified the importance of monitoring and evaluating analytical performance and the resultant improvement toward the NBS system. This strategy has been further utilized to approach other problematic analytical issues with NBS system. **Conclusion:** NBS by TMS allows detection of more than 50 metabolic disorders from one single analysis but also creates challenges in post analytical processes. Systematic monitoring and evaluation of performance metrics is critical in identifying problems and achieving satisfactory targets for performance metrics.

96. Sudden death in MCADD despite newborn screening: Biochemical and clinical high risk factors. Roman Yusupov¹, David N. Finegold², Edwin Naylor³, Inderneel Sahai⁴, Harvey Levy¹. ¹Division of Genetics and Metabolism, Children's Hospital Boston and Department of Pediatrics, Harvard Med Sch Boston, MA 02115, USA; ²Department of Pediatrics, Children's Hosp, Pittsburgh, PA 15213, USA; ³Pediatrix (formerly NeoGen Screening Inc.), Pittsburgh, PA 15220, USA; ⁴New England Newborn Screening Program, Boston, MA 02130, USA.

Medium chain acyl-CoA dehydrogenase deficiency (MCADD) is a fatty acid oxidation disorder that can present with hypoketotic hypoglycemia, vomiting and lethargy during an acute illness in an otherwise healthy child. This can result in sudden death. Avoidance of sudden death by immediate preventive therapy during an acute illness is the primary reason for including MCADD in newborn screening (NBS). However, we have studied 4 children who died suddenly between the ages of 7 months to 3 ½ years despite previous diagnosis of MCADD by NBS. In all of the cases the NBS octanoylcarnitine (C8) level was markedly elevated at 13.7–24.8 µM. Two cases were homozygous for the A985G MCAD mutation (A985G/A985G) while the other two cases were heterozygous (A985G/other). In 3 of the 4 cases the child vomited the day before sudden death while in the 4th case vomiting had occurred several days before death (It is not known whether the vomiting was still present just before death). Three of the 4 cases were found dead in the early morning at home while the 4th case died in the hospital.

We reviewed the literature of sudden death in MCADD and accepted 18 case reports in which there was convincing diagnosis of MCADD and good clinical information. All such cases were preceded by vomiting, presumably associated with acute viral illness, within 1-3 days of sudden death.

Based on these observations we postulate that:

- Those at highest risk for sudden death had markedly elevated C8 in NBS.

- Not all cases of MCADD at highest risk for sudden death are homozygous for the A985G mutation. However, whether at least one copy of the A985G mutation is required for high risk of sudden death is unclear.
- Vomiting seems to be the key symptom leading to sudden death in MCADD.
- There may be lack of appropriate concern by both parents and physicians in the face of minor acute illness in an otherwise normally growing and developing child with MCADD. In one of the cases the parent did not think it was important enough to seek medical care right away. In another case the child was given IV fluids in the ER but was sent home and found dead several hours later.