1. SIMD **SIMD business**

2. SIMD **SIMD business**

3. Long term follow-up of survivors of "metabolic stroke" associated with methylmalonic aciduria. D.R. Adams[a], J.L. Sloan[a], A.L. Gropman[c], E.H. Baker[b], C.P. Venditti[a]. [a]NHGRI, National Institutes of Health, Bethesda, MD, USA; [b]Clinical Center, National Institutes of Health, Bethesda, MD, USA; [c]Department of Neurology, Children's National Medical Center, Washington DC, USA.

Objective: The natural history of the ''metabolic strokes'' associated with methylmalonic aciduria has been described in children but less so in surviving adults. We used neurologic examination, CNS imaging and magnetic resonance spectroscopy to study a group of seven adult survivors of metabolic stroke associated with methylmalonic aciduria. *Methods:* Seven patients aged 19–33 years were admitted for inpatient evaluation. CNS imaging and spectroscopy were performed using a 3 Telsa magnet to maximize spectroscopic sensitivity and resolve j-couples resonances. Data for each participant was evaluated by a group of specialists with expertise including imaging, neurology and biochemical genetics. *Results:* Five out of seven patients had initial strokes that could be identified as a discrete historical event. Among those patients, the median time from stroke to NIH admission was 18 years. The remaining two patients had one or more metabolic decompensations (including coma) followed by later MRI evidence that a stroke was likely to have occurred. Our patient group demonstrated heterogenous clinical and imaging findings. Both pyramidal and extrapyramidal manifestations were observed clinically, with poor apparent correlation between neurological signs and MRI changes. Brain imaging results included basal ganglia abnormalities, and unanticipated examples of leukodystrophy, leukoencephalopathy and possible Wallerian degeneration involving corticospinal tracts. The residual stroke-associated lesions were variable in size, and asymmetrical in 4/7 patients. MR spectroscopy demonstrated metabolite concentrations consistent with abnormalities in gray and white matter areas. *Conclusions:* ''Metabolic stroke'' associated with methylmalonic aciduria has a variety of possible long-term outcomes. In addition to the well-described cystic encephalomalacia seen in the basal ganglia, methionine-synthase independent white matter metabolism may also be perturbed and chronic degeneration seems likely. The poor correlation of both pyramidal and extrapyramidal neurologic findings with the structural changes seen on MRI suggests that the sequellae of MMA-associated ''metabolic strokes'' are complex. Spectroscopic studies using labeled precursors and neuroactive markers in affected patients and animal models will be needed for a more complete understanding of the neurological manifestations observed in the patients as will diffusion tensor imaging to evaluate white matter microstructural changes that are not appreciated on routine anatomic imaging.

4. Toxic intravenous arginine overdose in a girl with symptomatic OTC. G.L. Arnold. University of Rochester School of Medicine and Dentistry, Rochester, NY, USA.

Objective: Describe the findings associated with an accidental toxic overdose of intravenous arginine. *Case report:* An 11 month old girl was diagnosed with OTC after an episode of hyperammonemia associated with an intercurrent illness. She had several subsequent additional episodes of hyperammonemia, often requiring treatment with intravenous arginine and sodium phenylacetate/sodium benzoate; hyperammonemic episodes were typically rather quickly responsive to treatment but commonly associated with modestly elevated transaminases (up to 500–2000 U/L). At age 3 she was seen at a regional hospital for hyperammonemia and treated with intravenous glucose and arginine before and during transport. However unlike prior events in which ammonia and mental status improved rapidly during this treatment, on arrival to the tertiary medical center this time she had an ammonia of 351 μmol/L, chloride of 121 μmol/L, bicarbonate of 19, BUN of 23 mg/dl, ALT of 4900 U/L, and worsening mental status and neurological exam. This picture prompted a prompted a careful review of transfer records, where an accidental IV arginine overdose of 30 cc/kg of 10% arginine hydrochloride over 2 h was discovered The arginine was initiated in the referring hospital and completed during the transport. Plasma arginine measured approximately 4 h after the arginine infusion was completed was 283 μmol/L with an accompanying ammonia of 408 μmol/L. Over the next several hours the patient began to show rapid improvement in mental status and neurologic examination; ammonia after the next 4 h (8 h after infusion) was 164 and arginine was 99, and at 4 h after that (12 h after infusion) ammonia was 49 with arginine of 61. The patient tolerated the episode without evidence of sequelae. However her hyperammonemia remained brittle over the next year and she underwent orthotopic liver transplant with good outcome. In this case the source of the error was a miscommunication between nursing and pharmacy—the pharmacy sent up the entire 300 cc bottle of arginine HCl intending that only 20 cc be administered, with the remainder available to begin maintenance dosing during transport. However, the nurse believed the bottle contained the desired volume of drug diluted with 300 cc of saline and administered the entire 300 cc as a 2 h loading dose. *Discussion:* An overdose of 30 cc/kg of 10% arginine caused worsening hyperammonemia accompanied by hyperchloremia and mild acidosis. The excess arginine was metabolized within eight hours. Although this event was short lived and without permanent sequelae in this female OTC patient, such an overdose might have more severe consequences in a patient with less native urea cycle enzyme activity.

5. Mutation analysis on confirmed cases of 2-methylbutyryl Co-A dehydrogenase (SBCAD) deficiency in Hmong infants identified by newborn screening using tandem mass spectrometry. M.W. Baker[c,d], S.C. Van Calcar[b,c], T. Litsheim[d], G. Hoffman[d], J. Vockley[f], W.J. Rhead[e], J.A. Wolff[b,c], M.S. Durkin[a]. [a]Department of Population Health Sciences, University of Wisconsin, Madison, Wisconsin, USA; [b]Department of Pediatrics and Medical Genetics, University of Wisconsin, Madison, Wisconsin, USA; [c]Waisman Center, University of Wisconsin, Madison, Wisconsin, USA; [d]Wisconsin State Laboratory of Hygiene, University of Wisconsin, Madison, Wisconsin, USA; [e]Departments of Pediatrics and Pathology, Medical College of Wisconsin, Milwaukee, Wisconsin, USA; [f]Department of Pediatrics, University of Pittsburgh School of Medicine, Children's Hospital of Pittsburgh, Pittsburgh, Pennsylvania, USA.

SBCAD deficiency is a disorder of l-isoleucine metabolism recently found in high frequency in the Hmong . A common mutation at 1165 A > G in ACADSB gene has been identified in this ethnic group. This study reports on 16 additional cases found to be homozygous for this mutation. *Method:* Genomic DNA was extracted from a 1/8'' dry blood spot, and underwent PCR reaction to generate the 260 bp fragments flanking ACADSB 1165 A > G site. The PCR products were then incubated with restriction enzyme BtsCI at 50 °C for 2 h. The wild type fragments were cut into two fragments of 201 and 59 bp. The ACADSB 1165 A > G mutation abolishes the BtsCI recognition site and the PCR products remained 260 bp. Analysis was completed on filter paper spots from 16 Hmong and 2 non-Hmong infants with biochemically confirmed SCBADD. Ten controls were randomly selected from newborn screening cards of infants of known Hmong descent but not identified with this disorder. *Results:* All 16 SBCADD cases were homozygous for the ACADSB 1165 A > G mutation. Controls were either wild type (9 cases) or heterozygote (1 case) for this mutation. Analysis from confirmed SBCADD cases in 2 non-Hmong infants were wild type for this mutation. *Conclusion:* In 16 Hmong infants identified by MS/MS with SBCADD, all were homozygote for 1165 A > G of ACADSB gene. It is likely that the gene ACADSB position 1165 is an exonic splicing enhancer, and the mutation at this position causes the splicing error in the pre-mRNA processing. This identical mutation was previously identified in 3 infants of Hmong descent. These results suggest this is a common mutation in this ethnic group. Further study is needed to identify the clinical significance of this finding.

6. Metabolomic study of disorders of propionate metabolism. William R. Wikoff[a], Jon A. Gangoiti[b], Gary Siuzdak[a], Bruce A. Barshop[b]. [a]Department of Molecular Biology and The Center for Mass Spectrometry, The Scripps Research Institute, USA; [b]Division of Genetics, Biochemical Genetics Laboratory, Department of Pediatrics, UCSD School of Medicine, La Jolla, CA, USA.

A global mass spectrometry-based approach to metabolomics was used to characterize plasma samples from patients with methyl-malonic acidemia (MMA) and propionic acidemia (PA). The screening platform was designed to be comprehensive, using a novel nonlinear data alignment process and online database to identify features which vary significantly between disease and normals. The study included samples normal adults ($n = 3$) with and without carnitine supplementation, from normal children without ($n = 12$) and with ($n = 3$) carnitine, and children with MMA ($n = 15$) and PA ($n = 9$). Methanolic plasma extracts were injected onto a reverse phase capillary HPLC interfaced to an electrospray time-of-flight mass spectrometer, and data collected from *m/z* 75 to 1000. The program XCMS (C.A. Smith et al., Anal. Chem. 78:779–87, 2006) was used to integrate chromatographic peaks, assign the peaks into groups, and use the grouped data for a non-linear correction in the time domain. Compounds with significant differences were selected auto-matically in an untargeted manner, without prior consideration of identity, but a posteriori identification of a number was possible. Among compounds differing between patient (MMA and PA) samples and controls, the most significant was identified as propionyl carnitine (t-*test value,* $t = 1.3 \times 10^{-18}$), a well-known marker for the disorders. Other acylcarnitine metabolites also showed significant differentiation, including C14 ($t = 6.0 \times 10^{-8}$), C6:1 (or methyl-C5:1) ($t = 6.4 \times 10^{-7}$), C6- ($t = 9.1 \times 10^{-6}$), and C5:1 ($t = 1.4 \times 10^{-3}$). However, despite efforts for comprehensiveness, C4DC was notably not detected (although readily measured in conventional acylcarnitine analysis of the samples, with average 0.56 μM for MMA (and $t = 2.4 \times 10^{-9}$ compared to control). γ-Butyrobetaine was in creased (13.3-fold, $t = 3.3 \times 10^{-3}$ for MMA + PA overall), but in control range for some and highly increased in a subset. All patients were taking carnitine, and γ-butyrobetaine might arise from enteric bacterial metabolism rather than endogenous carnitine synthesis. There were also significant differences between MMA and PA patients; at $p = 0.005$, there were 154, and using $p = 0.001$, there were 76 compounds (~2.2% of observed peaks) significantly different. Those features included isovalerylcarnitine (increased in PA relative to MMA with a *t-test* value of 9.7×10^{-8}), and unidentified ions of mass 177.10 (increased in MMA) and 386.33 (increased in PA) with *t*-test values between 1.5 and 2.8×10^{-5}. A wide array of metabolites are affected in organic acidemias and a complex cascade of metabolic effects may result from the knockout of a single enzyme. Metabolomics may expand the range of perturbed metabolites associated with human disease, and has the potential to increase insight into the ramifications of metabolic disease.

7. Enhanced clinic management system using a sharepoint website. D.G. Hook, J.A. Bartley. UC Irvine Medical Center, Department of Pediatrics, Division of Genetics and Metabolism, Orange, CA 92868, USA.

Objective: Improve patient care and health outcomes through better clinic management of time, personnel, and resources. *Methods:* An electronic clinic workspace was developed using Microsoft Office Sharepoint 2003 software in the University of California Irvine (UCI) Metabolic Specialty Care Center. Sharepoint was developed to provide industry with an electronic platform to connect people and projects. The application integrates programs and processes, allows collaboration, and the ability to work offsite. The clinic's webpage is located on a server behind the UCI's firewall. Only invited members may access the site and activity may be monitored. Using Infopath, another Microsoft program, electronic patient care documentation and forms were developed and deployed. All patient documents are centrally stored, managed, and accessed. Using Sharepoint increased overall quality of patient care, improved consistency of patient care, and enhanced ease of access to patients' documents. Redundant entry of patient data is reduced by the population of varied forms through single entry of specific data. Clinic work is coordinated with shared calendars, task lists, alerts, and notifications. The electronic workspace allows clinic members to provide patient care while working in satellite clinics. Members may fax prescriptions, access patient documentation and information, while continuing to provide direct patient care in offsite clinics. *Results:* The documentation process was streamlined through the Sharepoint website which reduced work effort by as much as 80%. This has lead to a greater amount of time spent on direct patient care, an increase in patient services such as the authorization of medical foods, consistent work-product among clinic members, and reduction in training time of new members. *Conclusions:* The UC Irvine Metabolic Specialty Care Center Sharepoint website connects clinic members, patient care work processes, and information. It greatly enhances patient care through greater efficiency, increased quality of documentation, and the ability to work offsite.

8. Assessment of tetrahydrobiopterin (BH_4)-responsiveness in phenylketonuria. Betina Fiege, Nenad Blau. Division of Metabolism and Molecular Pediatrics and Division of Clinical Chemistry and Biochemistry, University Children's Hospital, Zurich, Switzerland.

Aim of the study was to determine the prevalence and identify subjects with phenylketonuria (PKU) responsive to 6R-tetrahydrobiopterin (BH_4) and to establish selection criteria for potential treatment with BH_4. Blood phenylalanine levels from 557 newborns and infants with various degrees of PKU (blood phenylalanine 301–4743 mmol/L) challenged with BH_4 (20 mg/kg bw) were analyzed at 8 and 24 h after BH_4 administration. The two modalities were compared for phenylalanine reduction. The overall prevalence of BH_4-responsiveness within patients with PKU for the blood phenylalanine reduction of 20, 30, 40, and 50% was 48, 38, 31, and 24%, respectively, using the 8 h modus, and 55, 46, 41, and 33%, respectively, using the 24 h modus. Using the 30% cut-off, BH_4-responsiveness was similar regardless of the two modalities in patients with mild hyperphenylalaninemia (79–83% responders), mild PKU (49–60% responders), and classical PKU (7–10% responders).

In summary, BH_4-responsiveness is more prevalent than initially assumed, particularly in patients with mild hyperphenylalaninemia and mild PKU. Depending on the severity of hyperphenylalaninemia, selection criteria for the potential treatment with BH_4 may range between 20 and 40% of blood phenylalanine reduction after 24 h.

9. Localization of glucocerebrosidase and α-synuclein in hippocmpal neurons from a gba null mouse. Y. Blech-Hermoni, O. Goker-Alpan, E. Goldin, M.E. LaMarca, E. Sidransky. Medical Genetics Branch, NHGRI, NIH, Bethesda, MD 20814, USA.

Objective: Gaucher disease (GD) is the most common lysosomal storage disease. It is an autosomal recessive disorder characterized by the accumulation of glucocerebroside (GC), due to mutations in the gene (Gba) encoding the lysosomal hydrolase glucocerebrosidase (GCase). A growing number of reports suggest an association between GD and parkinsonism. In particularly, a disproportionate number of PD patients have been found to be carriers of GD mutations, leading to the hypothesis that a mechanistic relationship between misfolded GCase and mutant (A53T) α-synuclein, previously described in PD, results in the pathological aggregation of α -synuclein. Although there is no common phenotype or genotype characterizing the human cases, we set out to observe the effects of co-expression of mutant (L444P and N370S) GCase and mutant (A53T) α-synuclein. *Method:* A mouse model exists in which gba has been knocked out. Although homozygous knockout mice express no residual enzyme activity, they also die perinatally. We therefore developed a cellular model of hippocampal neurons from homozygous null embryos. In our model, primary neuronal cultures were established from gba−/− mouse embryonic hippocampal neurons. These neurons were co-transfected with A53T α-synuclein (Scna) and wild-type/L444P/N370S human Gba. Forty-eight hours post-transfection, the neurons were fixed and immuno-stained for GCase and α-synuclein. *Direction*: We hope to be able

to more closely recapitulate the human genotype by expressing Gba on a null background. Furthermore, we intend to investigate the expression of A53T α-synuclein in hippocampal neurons of mouse embryos homozygous for the L444P or N370S Gba and, conversely, the expression of mutant Gba in hippocampal neurons from mice homozygous for A53T Scna.

10. Newborn screening for lysosomal storage diseases—A reality? O.A. Bodamer, A. Mühl. Division of Biochemical Genetics, University Children's Hospital Vienna, Austria.

Neonatal screening programmes for inborn errors of metabolism are implemented throughout the world. Disorders include defects of fatty acid oxidation, defects of protein metabolism, galactosemia, hypothyroidism and others. Ideally, the natural history of such disorders is well understood while early diagnosis and treatment results in significant reduction of otherwise high morbidity and mortality. With the advent of novel treatment modalities in lysosomal storage diseases (LSD) such as bone marrow transplantation and/or enzyme replacement therapies, newborn screening for LSD has become a focus point. From a technological perspective high-throughput newborn screening for LSD may be feasible using different analytical approaches. Among these, screening by tandem-mass spectrometry using unique, specific substrates and internal standards seems to be the most promising method as enzyme activities can be readily measured in dry blood spots from neonatal filter cards. This technique may be incorporated into existing neonatal screening programmes using tandem mass spectrometry. Prior to implementation of neonatal screening programmes for LSD, pilot studies have to demonstrate its technical feasibility, sensitivity and specificity. In addition, strategies for confirmatory testing, treatment, follow-up care and scientific evaluation have to be defined and agreed upon at an international level.

11. Clinical and molecular spectrum of Alpers syndrome patients with POLG1 mutations. N. Brunetti-Pierri, Q. Zhang, P.C. Chou, C.K. Truong, J. Wang, E.S. Schmitt, W.J. Craigen, L.-J. Wong. Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA.

Objective: We studied at the clinical, biochemical and molecular level a large cohort of patients affected with Alpers syndrome, an autosomal recessive, early-onset fatal disease characterized by intractable seizures, global neurological deterioration, and hepatic failure. Alpers syndrome is due to mutations in the POLG1 gene encoding for DNA polymerase gamma required for mitochondrial DNA biosynthesis. So far, about 30 mutations have been associated with Alpers syndrome (http://dir-apps.niehs.nih.gov/polg). POLG1 mutations are responsible for a variety of mitochondrial diseases, including also dominant and recessive forms of progressive external ophthalmoplegia (PEO), Parkinsonism, juvenile spinocerebellar ataxia-epilepsy syndrome, sensory ataxia with neuropathy, dysarthria and ophthalmoparesis (SANDO). *Methods:* Clinical, biochemical and molecular data from the 19 patients with Alpers syndrome were collected. *Results:* The age of disease onset in our cohort ranged from 2 months to 22 years. Almost all patients presented with intractable seizures and liver problems became often evident only later. Based on the initial presentation including an intense seizure activity, several patients were thought to be affected with either Angelman or Rett syndrome. 6/19 patients died before 2 years of age with liver failure. Mitochondrial respiratory chain enzyme analysis on liver and muscle, available in a subgroup of patients, revealed reduced activities in the RC complexes containing mitochondrial encoded protein subunits. Real time quantitative PCR analysis demonstrated mtDNA depletion. The molecular analysis of POLG1 gene revealed, as previously reported, that the A467T is the most common mutations being present in 10 out of the 19 patients (53%). We identified 10 novel mutations, 8 missense mutations (G11D, L83P, H110Y, S305R, R853Q, G888S, R1138C, K1191R), one deletion (c.1270 del CT) and one insertion (c.2544-2545 ins GC). The mutation K1191R appeared to be de novo. Interestingly, in 3 patients POLG1 mutations were found only on one allele. While heterozygous mutations in POLG1 have been previously reported in patients with PEO, they have not been previously reported with Alpers syndrome. Mutations in a different gene giving Alpers-like syndrome may be considered in these cases. Alternatively, synergistic heterozygosity involving heterozygous mutation in another gene functioning in the same pathway may be responsible for the disease. *Conclusion:* The identification of novel mutations and the finding of a de novo mutation further support the hypothesis that POLG1 is particularly prone to mutations. In addition, our data indicate that patients carrying POLG1 mutations may not express at presentation the full spectrum of Alpers phenotype. Given the clinical heterogeneity of patients with Alpers syndrome, POLG1 molecular analysis is warranted in young patients presenting with intractable seizures and dysfunction in energy metabolism.

12. Four families with pyruvate dehydrogenase deficiency due to E1-β (PDHB) mutations. K. Okajima[a], L.G. Korotchkina[b], D.S. Kerr[a]. [a]Center for Inherited Disorders of Energy Metabolism (CIDEM), Department of Pediatrics, Case Western Reserve University, Cleveland, OH, USA; [b]Department of Biochemistry, School of Medicine and Biomedical Sciences, State University of New York at Buffalo, Buffalo, NY, USA.

Pyruvate dehydrogenase complex (PDC) deficiencies are a major cause of primary lactic acidosis. About 60% of cases result from mutations of the gene for the E1-α subunit (*PDHA1*), with fewer cases resulting from mutations in genes for E3, E3-binding protein, E2, and the E1-β subunit (*PDHB*). We have found 4 cases of *PDHB* mutations among 69 analyzed cases of PDC deficiency, including a 5 year old male (consanguineous, homozygous R36C); a neonatal female who died soon after birth, (compound heterozygous C306R/D319V), a 26 year old female. (heterozygous for I142M/W165S), and a 3 month old female (consanguineous, homozygous Y132S) who is a sibling of a previously published case (Brown et al., Hum. Genet 115 (2004) 123–27). No *PDHA1* mutations were detected in these patients. Their ethnic background is diverse (Caucasian, Arab, and African American descent). All cases had lactic acidosis and developmental delay, $\frac{3}{4}$ had agenesis of corpus callosum, seizures and hypotonia, and one died within the first year of life. These clinical findings are similar to those of *PDHA1* mutations, except consanguinity was found only in *PDHB* families. All cases were diagnosed by low PDC activity, with normal E2 and E3 activities. PDC activity in lymphocytes from 6 parents is normal, who all proved to be heterozygous carriers for the respective mutations. Two patients who have been on a ketogenic diet since diagnosis appear to have more favorable outcomes. Computer analysis predicts that: R36C affects the interaction of several amino acids resulting in conformational change, I142M affects stability of the beta chain, W165S affects hydrophobic interaction between subunits changing conformation of the active TPP site, C306R affects conformation around a K ion affecting stability of beta subunit, D319 is in the vicinity of C306, and Y132C affects interaction between the two beta subunits. These residues are conserved in *PDHB* across species; Y132 is conserved in other TPP enzymes. These observations support the conclusion that these are all pathogenic mutations.

13. Polarographic and enzymatic defect in mitochondrial complex III caused by BCS1L mutations in a girl with clinical features of both Bjørnstad syndrome and GRACILE syndrome. Bruce H. Cohen[a], Bruce A. Barshop[b], J. Travis Hinson[c,d], Charles L. Hoppel[e], J.G. Seidman[c,d], Christine E. Seidman[c,d]. [a] Department of Pediatrics, The Neuroscience Institute, The Cleveland Clinic Foundation, USA; [b]University of California, San Diego, USA; [c]Department of Genetics Harvard Medical School, USA; [d]The Howard Hughes Medical Institute, USA; [e]Division of Clinical Pharmacology, Case Western Reserve University, USA.

Introduction: BCS1L encodes for a 419 amino acid inner mitochondrial membrane chaperone protein presumed to facilitate insertion of Rieske Fe/S protein into complex III precursors. We describe the polarographic

and respiratory chain enzymatic features of a 4 year old girl with Bjørnstad syndrome (SNHL and pili torti) and several features of GRACILE syndrome (growth retardation, aminoaciduria, cholestasis, iron overload, lactic acidosis and early death), who was found to have mutations in the BCS1L gene and a defect in complex III of the electron transport chain (ETC). *Methods:* DNA was amplified and sequenced, and sequence variants were confirmed by restriction-enzyme digestion and polymorphisms identified in NIH and Celera databases. Measurements of oxidative rates were measured under normal ADP (state 3) and ADP-limited (state 4), high-ADP concentrations as well as uncoupled conditions; performed on freshly isolated muscle mitochondria. ETC enzyme activity was measured in freshly isolated mitochondria and whole muscle homogenate. *Results:* State III oxidation rate of duroquinol (complex III substrate) with rotenone was 388 natoms oxygen/min/mg protein (control mean 588 ± 74, control range 433–766). The oxidation rate did not improve with the addition of high concentrations of ADP: 264 (control mean 564 ± 75, range 206–779). Enzymatic activity of complex III (decylubiquinol-cytochrome c reductase) was 671 nmol/min/mg mitochondrial protein (control mean 4512 ± 1527, control range 1417–8498), and of complex I + III (rotenone-sensitive NADH-cytochrome *c* reductase) was 51 (mean 1377 ± 554, range 307–2658); significantly reduced to 15 and 4% of control values. Two novel BCS1L mutations (G35R and R184C) were identified. *Discussion/Conclusion:* Mutations in the BCS1L gene may cause a wide spectrum of disease, which depends on the extent to which BCS1L mutations decrease respirasome electron transport activity and increased reactive oxygen species. This patient demonstrates novel mutations in this gene that result in the clinical features of Bjørnstad syndrome and GRACILE Syndrome. The predicted functional and enzymatic function of mitochondrial complex III activity was markedly reduced in this patient, demonstrating consistency in clinical presentation, genetic findings, protein function and enzyme deficiency.

14. Result of follow up in 125 phenylketonuric children in Chile. V. Cornejo, G. Castro, E. Fernández, J.F. Cabello, A. De la Parra, A. Valiente, M. Colombo, E. Raimann. INTA, Universidad de Chile, Chile.

Introduction: Since 1992, Chile started a National Neonatal Screening Program for Phenylketonuria and Congenital Hypothyroidism in all the Public Health Maternities. The organization of this Program is based on a Committee constituted by the Ministry of Health, INTA, University of Chile and the Occidental Health Service. *Methodology:* The method used for the determination of phenylalanine is fluorometric test and all positive results for PKU are sent to INTA, University of Chile where the diagnosis is confirmed by tandem mass spectrometry. *Results:* We present 125 Classical PKU diagnosed in average at 18 ± 10.4 days of life, with plasma Phe levels of 20 ± 8 mg % and tyrosine levels 0.9 mg %. The incidence for classical PKU is 1:15.600 and 1:9.940 for Hyperphenylalaninemia (171 children). Sixty one children are male and 64 female. The age is less than 12 months in 8%, 24% is between 1 and 4 years old, 4% is between 4 and 10 years old and 28% is older than 10 years. The Phe intake tolerated in 55% is <20 mg/k/day and in 3% the Phe intake is over 50 mg/kg/day. 87% has a normal nutritional status and 14% is obese. The intellectual quotient is normal in 90% of PKU children. *Conclusions:* It is important to start neonatal screening, but the follow up program is the most important step for preventing mental retardation.

15. A dose optimization study of aldurazyme® (laronidase) in patients with mucopolysaccharidosis I (MPS I). G.F. Cox[a], R. Giugliani[b], A.M. Martins[c], E.R. Valadares[d], J.T.R. Clarke[e], J.E.C. Goes[f], M.A. Worden[a], M. Sidman[a], E.D. Kakkis[g]. [a]Genzyme Corporation, Cambridge, MA, USA; [b]Hospital de Clinical de Porto Alegre, Porto Alegre, Brazil; [c]Universidade Federal de Sao Paulo, Sao Paulo, Brazil; [d]Universidade Federal de Minas Gerais, Belo Horizonte, Brazil; [e]The Hospital for Sick Children, Toronto, CA; [f]Hospital Infantil Joana de Gusmao, Florianopolis, Brazil; [g]BioMarin Pharmaceutical, Novato, CA, USA.

Objective: To evaluate the pharmacodynamics and safety of different laronidase dose regimens. Methods: A 26-week, randomized, open-label, multicenter, multinational, study in 33 MPS I patients receiving 1 of 4 dose regimens of laronidase: 0.58 mg/kg (100 U/kg) IV qw (labeled dose); 1.2 mg/kg (200 U/kg) IV qw; 1.2 mg/kg (200 U/kg) IV q2w; and 1.8 mg/kg (300 U/kg) IV q2w. *Results:* Patients were 53% female, 62% Caucasian, and 26% Hurler. Mean age was 8.7 yr (range 1.4–20.7 yr). The 4 treatment groups showed similar pharmacodynamic responses after 26 weeks of treatment with only small differences that tended to favor the higher doses over the labeled dose. Mean urinary GAG reduction was 58% for the labeled dose vs. 63–67% for the other doses. Mean liver volume reduction was 26% for the labeled dose vs. 31–32% for the other doses; however, this difference was not apparent when only patients with abnormal baseline liver volumes were considered. Mean change in 6MWT distance was +7 m for the labeled dose group vs. −12 ± 52 m for the other doses. Laronidase was well-tolerated and had an acceptable safety profile in all dose groups. One 4-yr-old Hurler patient with acute bronchitis died of respiratory failure 18 h after receiving her first 1.2 mg/kg dose of laronidase: her death was considered possibly (Sponsor) or remotely (Investigator) related to laronidase. Thirteen of 33 (39%) patients experienced a total of 75 infusion-associated reactions (IARs), the most common of which were pyrexia (21%), vomiting (15%), rash (15%), and urticaria (12%). Most IARs were mild, easily managed, and decreased in frequency over time. Patients who received greater amounts of laronidase per 2-week period (1.8 or 2.4 mg/kg) experienced more IARs than those who received the labeled dose equivalent (1.2 mg/kg), but the difference was attributable to a few patients. Anti-laronidase IgG antibodies were detected in 32 of 33 (97%) patients. Seroconversion occurred faster in the qw than the q2w regimens though peak titers were similar. No patient tested IgE-positive. *Conclusions:* The 0.58 mg/kg qw laronidase dose regimen provided near-maximal reductions in lysosomal storage (urinary GAG level and liver volume) and the best benefit-to-risk ratio. The 1.2 mg/kg q2w regimen may provide an acceptable alternative for patients with difficulty receiving qw infusions, but the long-term effects are unknown.

16. Early identification and aggressive treatment of cobalamin C disease. K. Cusmano-Ozog[a], M. Martin[b], E. Nicholas[b], S. Packman[b], D.S. Rosenblatt[c], T.M. Cowan[a,d], G.M. Enns[a]. [a]Department of Pediatrics, Division of Medical Genetics, Stanford University, Stanford, CA, USA; [b]Department of Pediatrics, Division of Medical Genetics, University of California, San Francisco, CA. USA; [d]Department of Human Genetics and Division of Medical Genetics, Department of Medicine, McGill University, Montreal, Que., Canada; [c]Department of Pathology, Stanford University School of Medicine, Stanford, CA, USA.

Cobalamin C (cblC) disease, the most common inborn error of vitamin B12 metabolism, is characterized by elevated levels of methylmalonic acid (MMA) and homocysteine (Hcy). Typical findings in early onset disease include mental retardation, nystagmus, visual impairment and neurodegeneration despite treatment with parenteral B12 0.5–1 mg 3×/week or 0.5 mg daily. Since July 2005, we have identified six patients by expanded newborn screening with biochemical findings consistent with cblC. This was confirmed by complementation studies in four patients, with confirmatory studies ongoing in two. Targeted mutation analysis of the MMACHC gene revealed one patient to be homozygous for 482G > A (R161Q), one heterozygous for 271dupA (R91KfsX14), and a third heterozygous for 609G > A (W203X). Further DNA studies are ongoing. Four of the six patients were asymptomatic at presentation; one had mild hypotonia, which has since resolved. The final one, for whom treatment was delayed until age 1 month, presented with moderate hypotonia and seizures, which have resolved. Following the initial screening result and confirmatory biochemical studies, aggressive treatment was started with daily intramuscular injections of hydroxocobalamin 1 mg, and oral supplementation of folic acid 1 mg, l-carnitine 100 mg/kg/day, and betaine–HCl 300 mg/kg/day. One patient has also required methionine

supplementation. Propimex formula was started in four cases, but was later discontinued when growth and metabolic parameters were found to be stable on the above treatment. In all patients, serum MMA and Hcy dropped to normal or near-normal levels within three weeks of initiating therapy: MMA pretreatment 40–254 μM (mean 131.29 ± 83, controls <0.3) and post treatment 0.41–8.58 μM (mean 2.32 ± 3.16); Hcy pretreatment 58–250 μmol/L (mean 121.85 ± 99, controls <14) and post treatment 5.4–70.1 μmol/L (mean 27.06 ± 24). Five of the six patients are now less than 8 months and have normal development; two of these have had a normal ophthalmologic exam at 6 months. The patient in whom treatment was delayed was found to have nystagmus and decreased visual acuity at age 9 months, but essentially normal development at one year. Given the ranges of abnormal metabolites and clinical presentations, cblC disease appears to comprise a spectrum. Additional studies including genotype-phenotype correlation are needed. Long term follow up with longitudinal ophthalmologic and developmental evaluations is essential to determine if this aggressive treatment regimen started at an early age alters the natural history of this condition.

17. Profiling metabolic activity in glioblastoma cells: Efforts to integrate signal transduction and intermediary metabolism in a model of cell proliferation. Ralph J. DeBerardinis[a,b], Anthony Mancuso[a], Suzanne Wehrli[b], Craig B. Thompson[a]. [a]Cancer Biology, University of Pennsylvania, USA; [b]Child Development, Rehabilitation Medicine and Metabolic Disease, The Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA.

During tumorigenesis and other situations of cell proliferation, cells undergo a metabolic 'transformation' characterized by high rates of glycolysis and macromolecular biosynthesis. This transformation is triggered by activation of signal transduction pathways, which reorchestrate metabolic fluxes into a platform that supports cell growth. A major goal in cancer research is to better characterize the metabolic activities of proliferating cells and to integrate them fully with modern understanding of signal transduction. Here we studied signal transduction and metabolism in a human glioblastoma cell line, SF188. In these cells, serum stimulation of the phosphatidylinositol 3′ kinase (PI3K)/Akt signaling pathway promoted glucose utilization, lipid synthesis and cell proliferation. We used a combination of techniques, including real-time metabolic analysis by ^{13}C nuclear magnetic resonance spectroscopy, to further study the metabolism of abundant nutrients in these cells. The results showed that glioblastoma cells exhibit pronounced aerobic glycolysis (the 'Warburg effect') during proliferation, with robust glucose consumption and synthesis of lactate. A fraction of pyruvate from glycolysis entered the tricarboxylic acid (TCA) cycle via pyruvate dehydrogenase. The cells did not completely oxidize this pyruvate, because isotopomer analysis of mitochondrial metabolites revealed a large efflux of intermediates from the TCA cycle. We determined that lipid synthesis accounted for part of this efflux, and that glucose contributed at least 60% of the carbon for de novo fatty acid synthesis, a required activity for tumor cell growth. Together, these data implied a non-glucose source of anaplerosis to support ongoing TCA function during growth. By perfusing cells with both ^{13}C-labeled glucose and ^{13}C-labeled glutamine, we determined that glutamine provided more than 80% of the anaplerotic flux in glioblastoma cells. Therefore, growth of glioblastoma cells involves catabolism of both glucose and glutamine; glucose provides carbon for fatty acid synthesis while glutamine maintains TCA cycle activity. These studies are the foundation of a comprehensive profiling of intermediary metabolism during cell proliferation, and provide one explanation for the high rate of glucose and glutamine consumption long known to characterize tumor growth.

18. The clinical features and molecular genetics of deoxyguanosine kinase deficiency. D.P. Dimmock[a], Q. Zhang[a], J. Shieh[b], P.-C. Chou[a], C. Truong[a], E. Schmitt[a], M. Sifry-Plat[c], C.H. Ficicioglu[d], G.M. Enns[e], E.M. Arch[f], N. Longo[g], M.H. Lipson[c], W.J. Craigen[a], L.-J. Wong[a]. [a]Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA; [b]Medical Genetics, J. David Gladstone Institute at UCSF, San Francisco, CA, USA; [c]Deptartment of Medical Genetics, Kaiser Permanente, Sacramento, CA, USA; [d]Department of Metabolism, Children's Hospital Philadelphia, Philadelphia, PA, USA; [e]Division of Medical Genetics, Stanford University School of Medicine, Stanford, CA, USA; [f]Massachusetts General Hospital, Boston, MA, USA; [g]Department of Peds, Div Med Genetics, University of Utah, Salt Lake City, UT, USA.

Background: Deoxyguanosine kinase (DGK; MIM 601465) is a nuclear gene that along with thymidine kinase-2 (TK2; MIM 188250) salvages deoxyribonucleotides (dNTPs) for mtDNA synthesis. Deficiency of either of these genes causes a mitochondrial depletion syndrome. *Results:* We have identified 6 separate families with mutations in the DGK coding region. In all patients there is disturbance of hepatic function. This has typically presented with significant elevations in ALT and AST. Patients have cholestasis with a normal gamma GT. Hypoglycemia is a significant feature of this illness in many of the patients. Liver biopsy typically shows lipid accumulation, fibrosis and may show increased or normal numbers of mitochondria on electron microscopy. In 4 of the 6 families the probands have also presented with significant failure to thrive. Although hypotonia and nystagmus are common findings, neurological dysfunction was not seen in 2 siblings identified in our series. This is consistent with other published cases. A total of 11 mutations have been identified in 6 families. 10 of these 11 mutations are novel. Electron transport chain activities and mtDNA content are considerably reduced in both muscle and liver when compared with controls. *Conclusions:* Mitochondrial depletion caused by mutations in DGK should be considered in children with hepatic dysfunction or cholestasis even without neurological findings. Full gene sequencing is warranted if DGK mutations are suspected.

19. Glycerol kinase overexpression perturbs pathways of glucose catabolism: Implications for understanding complexity in inborn errors of metabolism. K.M. Dipple[a,b,c,d], J.S. He[a], J.C. Liao[d,e], G. Sriram[a,e]. [a]Department of Human genetics, David Geffen School Medicine at UCLA, USA; [b]Department of Pediatrics, David Geffen School of Medicine at UCLA, USA; [c]Mattel Children's Hospital at UCLA, USA; [d]Biomedical Engineering Interdepartmental Program, Henry Samueli School of Engineering and Applied Science, UCLA, Los Angeles, CA, USA; [e]Department of Chemical and Biomolecular Engineering, Henry Samueli School of Engineering and Applied Science, UCLA, Los Angeles, CA, USA.

Glycerol kinase (GK) has several diverse cellular functions in mammalian cells including phosporylation of glycerol. Glycerol kinase deficiency (GKD) is a complex, X-linked inborn error of metabolism (IEM), wherein no genotype-phenotype correlation has been observed. Metabolic flux has been hypothesized to play a role in the complexity of GKD, therefore, we investigated compared the flux analysis between wild type and two GK-overexpressing H4IIE rat hepatoma cell lines. We quantified fluxes in pathways of glucose catabolism by using stable isotope labeling, isotopomer measurements and analysis, and mathematical metabolic network modeling. We mathematically designed a stable isotope carbon source mixture to precisely measure fluxes in primary metabolic pathways. Metabolites from H4IIE cells that were grown on this mixture were analyzed by gas chromatography-mass spectrometry. The ensuing isotopomer measurements were computationally interpreted by comprehensive isotopomer balancing and mathematical metabolic network modeling to obtain values of fluxes through primary metabolic pathways. Glucose-6-phosphate dehydrogenase activity was measured by an enzymatic assay. The GK-overexpressing cell lines exhibited significantly different growth and carbon source utilization compared to the wild type cells. In addition, the GK overexpressing cells had significantly different ^{13}C isotopomer abundances compared to the wild type. Interpretation of the isotopomer abundances and flux evaluation using isotopomer balancing revealed that the flux of the pentose phosphate pathway in the GK-overexpressing cell lines was two-fold higher than that in the wild type, in addition to smaller flux changes in other pathways. In addition, we determined that the

activity of glucose-6-phosphate dehydrogenase, the rate-limiting enzyme of the pentose phosphate pathway, was 1.8-fold higher in the GK-overexpressing cell lines compared to the wild type. This substantiates the results of isotopomer-based flux analysis and shows that GK has effects on metabolic flux in other pathways which may be due in part to its moonlighting functions. Such investigations can be valuable toward dissecting the biochemistry and elucidating the pathology of GKD and other IEMs which is especially relevant with the advent of expanded newborn screening for detection of IEMs.

20. Autosomal dominant polycystic kidney disease (ADPKD) Mimicking autosomal recessive polycystic kidney disease (ARPKD) with congenital hepatic fibrosis (CHF) and portal hypertension (PH). Esperanza E. Font-Montgomery[a], Iclal Ocak[b], Peter Choyke[b], Theo Heller[c], Robert Kleta[a], Hailey Edwards[a], Parvathi Mohan[d], Lisa Guay-Woodford[e], Kailash Daryanani[f], Zenaide Quezado[g], William A. Gahl[a], Meral Gunay-Aygun[a]. [a]Medical Genetics Branch, NHGRI, NIH, Bethesda, MD, USA; [b]National Cancer Institute, NIH, Bethesda, MD, USA; [c]NIDDK, NIH, Bethesda, MD, USA; [d]Children's National Medical Center, Washington, DC, USA; [e]University of Alabama, Birmingham AL, USA; [f]Clinical Center, NIH, Bethesda, MD, USA.

ADPKD and ARPKD are the most common forms of PKD, a leading cause of end stage kidney disease. The characteristics of liver disease differ in ARPKD and ADPKD. All ARPKD patients have CHF often complicated with PH and a subset of them also exhibit Caroli's syndrome (CS), predisposing to cholangitis. In contrast, liver cysts in ADPKD are relatively benign; typically not associated with PH or cholangitis. Although it is generally adult onset, ADPKD can present in childhood and can be mistaken for ARPKD, which has its onset in utero, or in early infancy but can manifest later. Sorting out the correct diagnosis by molecular analysis is arduous, since the PKD genes are large, with complicated transcription profiles. In our ongoing ARPKD natural history protocol (www.clinicaltrials.gov, trial NCT00068224), we identified five families with overlapping features of ARPKD and ADPKD. In family 1, the 48-year-old father received kidney transplantation at age 47 due to ADPKD without liver involvement. His 9-year-old daughter, who presented at age 4 years with esophageal variceal bleeding due to PH, had both PKD and CHF. At NIH, his sons (14 and 12) were diagnosed with PKD with CHF/PH and PKD without liver involvement, respectively. In Family 2, the female proband (33) had kidney cysts typical of ADPKD, but carried a diagnosis of "ARPKD" based on a liver biopsy at 6 months which revealed CHF. Her daughter (16) and son (14) both had PKD without liver involvement. In family 3, the male proband (43) presented at age 39 with cholangitis and was diagnosed with PH and CS. His kidneys were normal. His mother had multiple kidney cysts without liver involvement. In Family 4, the proband (13) carried a diagnosis of ARPKD because of CHF/PH and PKD detected at 6 months. Her asymptomatic maternal grandmother was diagnosed with CHF at age 76, based on liver biopsy. However, her asymptomatic mother had normal kidney and liver imaging. In Family 5, the proband (5) presented at birth with classical ARPKD findings. NIH evaluation of her mother disclosed numerous small hepatic cysts. These cases illustrate the clinical overlap between these two disorders and the variability of the phenotype within and among families. Mutation analysis and further family studies might help explain the potential causes of this overlap and variability.

21. Chromatographic resolution and tandem MS measurement of the leucine isomers associated with the monitoring of MSUD patients. Scott Freeto[a], Donald Mason[a], Jie Chen[b], Robert Scott[b], Srinivas Narayan[b], Michael Bennett[b]. [a]Waters Corporation-Beverly, MA 01915, USA; [b]Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA.

Maple Syrup Urine Disease (MSUD) is the result of a genetic defect of the branched-chain α-keto acid dehydrogenase enzyme system. This severe metabolic defect is characterized by an accumulation of branched-chain α-keto acids and their respective branched-chain amino acids leucine, isoleucine and valine. The presence of alloisoleucine is considered pathognomic for MSUD. Monitoring of therapeutic dietary intervention of patients with MSUD requires accurate measurement of these amino acids, particularly leucine in blood. The ideal methodology for measurement should be sufficiently rapid to provide immediate response while the patient is still in the clinic/emergency room. The 3 isomeric forms of leucine have identical molecular weights and product ions, making direct injection into a tandem mass spectrometer unsuitable. We present here the results of a study of amino acid measurement in MSUD and phenylketonuria (PKU) patients with a rapid (6 min) separation using the ACQUTY UPLC™ and tandem mass spectrometry in positive MRM mode. Concentrations of the individual amino acids are calculated using peak area ratios: (area under AA peak/area under internal standard peak) x concentration of internal standard—a process similar to that used by neonatal screening laboratories. For isoleucine and alloisoleucine a relative response factor is generated to accurately quantify these AAs in the absence of isotopically labeled internal standards. An analysis time of six (6) minutes and an injection-to-injection cycle time of nine (9) minutes is used for the analysis—significantly shorter than conventional methods. Split samples were also analyzed on a Beckman 6300 and the results compared by correlation analysis. For all AAs analyzed (Phe, Tyr, Met, Val, Allo, Ile and Leu) the correlation coefficient (r) between methods is >0.96. We demonstrate that UPLC-MS/MS technology provides a rapid means of measuring plasma amino acids and may be beneficial in the differential diagnosis and long-term clinical management of patients with MSUD.

22. The work-up of persistent hyperinsulinemic hypoglycemia in a newborn infant—Interesting genetic implications. P. Galvin-Parton[a], T.A. Wilson[a], A. Lane[a], J. Weiss[a], M. Puangco[a], W.K. Seltzer[b]. [a]Department of Pediatrics, SUNY at Stony Brook, Children's Medical Center at Stony Brook, USA; [b]Athena Diagnostics, Worcester, MA, USA.

Congenital hyperinsulinism (CHI) is the most frequent cause of infantile hyperinsulinemic hypoglycemia. Mutations in any one of five genes have been associated with CHI. Diffuse and focal forms of the disease have been described. Treatment can either be conservative medical management or require surgery. Focal disease requires a limited pancreatectomy while diffuse disease requires a near-total pancreatectomy. We describe a male infant who was delivered at 32 weeks gestation following a pregnancy complicated by polyhydramnios. Birth weight was 7 lb. 1 oz. By day #4, infant was noted to have severe hyperinsulinemic hypoglycemia. He failed to respond to medical management. At 8 weeks of age he underwent a subtotal pancreatectomy and eventually required a total pancreatectomy. The clinical course of our patient is now further complicated by resulting diabetes. Echocardiogram initially revealed a cardiomyopathy that has improved with treatment. Brain imaging studies have been abnormal and he is neurologically impaired. Recently, molecular analysis has revealed a mutation in his ABCC8 gene. There are interesting implications depending on whether there are two copies of the gene mutation or a single copy. In cases of single gene mutation, it becomes important to identify whether the mutation was transmitted through the paternal or maternal line. These studies may predict a definitive pattern of inheritance as well as the form of the disease. This would provide families with information regarding recurrent risks and the potential of prenatal diagnosis. Future siblings would benefit from the morbidity associated with delays in diagnosis and treatment.

23. Dried blood spot assay for Pompe disease: Diagnostic experience of the Duke Biochemical Genetics Laboratory. J.L. Goldstein, S.P. Young, P.S. Kishnani, M. Changela, J. Dai, D. Bali. Division of Medical Genetics, Biochemical Genetics Laboratory, Duke University, RTP, NC 27709, USA.

Pompe Disease (Glycogen Storage Disease type II) is a rare, progressive, and often fatal muscular condition caused by deficiency of the lysosomal enzyme acid α-glucosidase (GAA). Clinical presentation varies from a rapidly progressive infantile form to a more slowly progressive late-onset

form. The approval of Myozyme replacement therapy as a treatment necessitates early diagnosis. We recently modified and validated an assay measuring GAA activity in dried blood spots (DBS), based on the method of Chamoles and coworkers. This assay is quicker and less invasive than typical methods for measuring GAA activity in skin fibroblasts and muscle. *Objective:* To evaluate the diagnostic experience of our laboratory with the GAA DBS assay, performed on a clinical basis in our laboratory over a 6 month period. *Methods:* Patients referred for testing were divided into 2 groups, according to age. Group 1 were infantile patients, <12 months of age ($n = 28$) and group 2 were individuals $\geqslant 1$ year of age ($n = 93$). The number of patients in each group that had deficient GAA activity in dried blood spots was determined. The percentage of patients referred from different specialty clinics was also determined. *Results:* We received 121 blood samples for GAA activity testing. Of these, 31 (26%) had reduced GAA enzyme activity, suggestive of Pompe disease. Eight of the 28 infantile patients (29%) and 23 of the 93 group 2 patients (25%) had deficient GAA activity in DBS. Thirteen of these affected patients were further investigated in our laboratory by skin fibroblast or muscle GAA activity, DNA mutation analysis, or urinary Hex_4 analysis as a biomarker of glycogen storage. Of the total 121 patients, 13 (11%) patients were referred from pediatric clinics, 11 (9%) from neuromuscular clinics, 10 (8%) from general genetics clinics, and the majority (72%) came from other referral testing centers. *Conclusions:* GAA activity determination in DBS is a valuable, reliable, relatively non-invasive and specific method for the diagnosis of Pompe disease and has the added advantage of rapid turnaround time. We consider the DBS assay, with acarbose as an inhibitor of maltose-glucoamylase (MGA), to be a valuable diagnostic tool. A limitation of this assay is the inability to accurately assess the degree of residual enzyme activity to aid in predicting phenotype. A systematic study should be performed to investigate whether second tier testing is necessary for confirmation of diagnosis and if the additional information this would provide would impact the clinical management of Pompe disease.

24. Screen for suppressors of VDAC mutant phenotypes in Drosophila: A model for mitochondrial dysfunction and disease. B.H. Graham[a], E.P. Alesii[a], Z. Li[a], W.J. Craigen[a,b]. [a]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA; [b]Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA.

Voltage-dependent anion channels (VDACs) are a family of small pore-forming proteins of the mitochondrial outer membrane found in all eukaryotes. VDACs play an important role in the regulated flux of metabolites between the cytosolic and mitochondrial compartments and three distinct mammalian isoforms have been identified. The specific physiologic and potential pathophysiologic roles of the various isoforms are not understood, but animal and cell culture experiments suggest that the various isoforms function in apoptosis, learning and reproduction. In Drosophila melanogaster, porin is the ubiquitously expressed VDAC isoform. Analysis of flies homozygous for hypomorphic mutant alleles of porin reveal abnormal phenotypes remarkably reminiscent of mouse VDAC mutant phenotypes including partial lethality, neuromuscular dysfunction manifested by increased sensitivity to mechanical stress (''bang'' sensitivity) and by synaptic abnormalities, and male infertility. In order to better understand VDAC's functional roles, a genetic screen to identify suppressors of increased bang sensitivity and male infertility has been initiated. A series of deletions covering approximately 38% of the genome (93% of chromosome 3) have been crossed into a homozygous mutant porin background and assessed for suppression of these phenotypes. From this pilot screen, several deficiencies that suppress bang sensitivity and/or male infertility have been identified. For the two deficiencies that demonstrate the strongest suppression, testing of overlapping deletions for suppression to further map the critical regions are ongoing. Interestingly, the deficiency that is the best suppressor of bang sensitivity in the porin mutant background also suppresses increased bang sensitivity observed in P element hypomorphic mutants of two independent predicted orthologs of human mitochondrial disease genes: SDHB (subunit of Complex II of the mitochondrial respiratory chain) and ATPAF2 (assembly factor of Complex V). This analysis of porin mutant phenotypes validates Drosophila as a model for mitochondrial dysfunction that is relevant to mammals. The identification of suppressor loci for mutant mitochondrial phenotypes in Drosophila should provide insights into mitochondrial function and pathophysiology that can be extended into studies in mammalian systems as well as potentially identify novel candidate therapeutic targets for mitochondrial diseases.

25. Neuroimaging findings post renal transplant in an adult with cobalamin-responsive methylmalonic academia. Andrea L. Gropman[a,b], Kevin. O'Brien[b], Jennifer Sloan[a], Eva Baker[d], Charles P. Venditti[a]. [a]Genetic Disease Research Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA; [b]Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA; [c]Department of Neurology, Children's National Medical Center and the George Washington University of the Health Sciences, Washington, DC, USA; [d]Clinical Center Radiology, National Institutes of Health, Bethesda, MD, USA.

Methylmalonic acidemia (MMAemia) represents a group of autosomal recessive inborn errors of branched chain amino acid metabolism due to a deficiency of the methylmalonyl-CoA mutase enzyme, or its cofactor cobalamin (vitamin B12). MMA clinically presents as lethargy, vomiting, and dehydration, commonly with onset in the newborn period. Affected infants demonstrate metabolic acidosis, ketosis and ketonuria, hyperammonemia, and hyperglycinemia. First described in the late 1960s, dietary management with protein restriction and B12 has remained the mainstay of therapy. Current management relies on these principles with the addition of carnitine and antibiotics. However, a relatively recent treatment for inborn errors of metabolism is organ transplantation. A major complication of B12 unresponsive MMAemia is renal failure, and a few patients have received transplantation. Other patients have undergone liver transplantation to prevent the recurrent life threatening metabolic acidosis that is the hallmark of this disorder. Little information is available regarding the clinical and neurological outcomes or metabolic parameters of patients after transplantation, nor are there guidelines that advise as to when indications for transplantation are met. We therefore describe a single patient with cblA (MMAA) deficiency requiring renal transplantation and describe her metabolic parameters and neuroimaging findings.

26. Preliminary experience with functional MRI (fMRI) detects altered neural networks subserving executive function and attention in subjects with partial ornithine transcarbamylase deficiency (OTC). Andrea L. Gropman[a,b], Ayichew Hailu[b], Rebecca Seltzer[b], Stanley T. Fricke[b], John VanMeter[b], M. Layne Kalbfleish[c], and the Urea Cycle Rare disorders Consortium[*]. [a]Department of Neurology, Children's National Medical Center Washington, DC, USA; [b]Department of Neurology/Neuroscience, Georgetown University, Washington, DC, USA; [c]Krasnow Institute for Advanced Study, George Mason University, Fairfax, VA, USA.

Objective: Ornithine transcarbamylase deficiency (OTC), an X-linked disorder, is the most common of the urea cycle disorders. Preliminary neurocognitive assessment in women heterozygous for OTC have demonstrated weaknesses in attention/executive function. fMRI is based on the principle that the MRI signal changes in response to changes in the magnetic character of the intravascular contents. Since deoxygenated hemoglobin is more paramagnetic than oxygenated hemoglobin, it acts as an endogenous intravascular paramagnetic contrast agent. During increased neural activity, there is an elevation of cerebral blood flow, which is greater than that required by local oxygen consumption. On a magnetic susceptibility T2*-weighted image, this results in increased signal intensity, which, as in positron emission tomography (PET), allows estimation of task-related neural activation when compared with a baseline image. fMRI has been used extensively to examine neural

networks subserving attention memory, and language in patients with epilepsy, ADHD, Alzheimer disorder, and TBI, but rarely as a tool to assess alterations in neural networks that may underlie cognitive changes seen in inborn errors of metabolism. *Methods:* Ten adult subjects with partial OTC (8 females and 2 males) and eight controls consented to participate in this IRB approved study. Subjects performed a modified flanker task which probes frontal lobe function, executive function and attention. All data were acquired on a Siemens Trio 3.0T scanner using echo-planar imaging sequences. Data were spatially normalized and corrected for head motion and artifacts. A fixed effects model was used to analyze activation maps. Statistical parametric analysis using p value of <0.05 was considered significant. *Results:* Activation maps revealed decreased activation in the frontal lobes as compared to controls. OTC subjects demonstrated parietal lobe activation suggesting recruitment of additional brain areas to compensate for perturbation of neural networks involved in frontal lobe function. *Conclusions:* Although preliminary, this study suggests that subjects with OTC exhibit differential activation patterns as compared to controls when performing a task that probes executive function. This is in agreement with earlier cognitive testing. Additionally, this study suggests that fMRI may be a useful tool to monitor the cognitive and neurological consequences of inborn errors of metabolism and may provide a biomarker for early recognition of neurological damage and potential response to therapies.

27. Preliminary experience with ^{1}H Magnetic resonance spectroscopy at 3T detects altered brain metabolism in subjects with partial ornithine transcarbamylase deficiency (OTC). Andrea L. Gropman[a,b], Ayichew Hailu[b], Rebecca Seltzer[b], Stanley T. Fricke[b], John VanMeter[b], and the Urea Cycle Rare disorders Consortium[*]. [a]Department of Neurology, Children's National Medical Center Washington, DC, USA; [b]Department of Neurology/Neuroscience, Georgetown University, Washington, DC, USA.

Objective: OTC, an X-linked disorder, is the most common of the urea cycle disorders. Patients with neonatal onset present in the first few days of life with lethargy progressing to hyperammonemica coma, whereas the manifestations of partial deficiency are more variable with respect to age of onset and neurological symptoms. *Methods:* Ten adult subjects with partial OTC (8 females and 2 males) and 8 age matched controls provided consent for this IRB approved study. All subjects were studied under similar conditions (morning, postabsorptive state), although disease severity and time from last hyperammonemic episode varied. We investigated the metabolite concentrations of tNAA, tCreatinine, choline, lactate, myoinositol, and the glutamate/glutamine system in parietal white matter, thalamus, and posterior cingulate regions. ^{1}H MRS was performed on a 3T Siemens Trio system with a phased array head coil using a volume localized PRESS sequence with echo time (TE) of 30 ms, repetition time (TR) of 1500 ms, 192 transients, spectral width of 3 kHz, and 1 k complex data points, with a voxel of 2.0 cm on edge (8 ml) centered at the various points of interest. All in vivo peak areas were analyzed by the built-in Siemens peak fitting routine to assess relative differences in brain chemistry using the patient as his own standard. The data were Fourier transformed and processed in the frequency domain with LC Model' a user independent method using a custom basis set comprised of single model chemical phantoms. *Results:* Significant differences in biochemical markers were seen in OTC patients versus controls. Specifically, thalamic glutamine levels were elevated in both symptomatic and asymptomatic females relative to controls and myoinositol was decreased in all OTC subjects. In symptomatic females, NAA, the neuronal marker, was significantly decreased compared to either asymptomatic heterozygotes or age matched controls, suggesting gray matter damage occurs over time with more significant disease. *Conclusions:* This study represents the largest series of OTC subjects studied to date using^{1}H MRS at 3T the benefits of which are to maximize spectroscopic sensitivity and resolve j-coupled resonances. Several biochemical markers distinguish partial OTC subjects from controls and these changes were also seen in subjects with less significant disease. This is consistent with previous findings in the literature, and furthermore, suggests that peripheral markers of perturbed metabolism cannot adequately serve as indicators of central nervous system damage due to underlying inborn errors of metabolism.

28. Niemann–Pick type C disease, Alzheimer's disease, apolipoprotein E, and amyloid precursor protein: Does an alteration in the cholesterol-enriched microdomain lipid environment result in formation of neurofibrillary tangles? R.A. Heidenreich, D. Jelinek, W.S. Garver. Section of Medical and Molecular Genetics, Department of Pediatrics, Steele Children's Research Center University of Arizona School of Medicine Tucson, AZ, USA.

Niemann–Pick type C disease (NPC) is a rare inborn error of intracellular cholesterol trafficking. It is characterized clinically by steady neurodegeneration typically beginning around 3–5 years of age and death during the second decade of life. Mutations in two genes, NPC1 and NPC2, are causative with 95% of cases due to defects in NPC1. The function of these two proteins in intracellular cholesterol trafficking remains undefined. The major biochemical finding in NPC disease is massive accumulation of unesterified cholesterol in the late endosomal/lysosomal compartment derived from uptake of low-density lipoprotein (LDL). Pathologic examination of the NPC brain has described neurofibrillary tangles (NFT) similar to those found in Alzheimer's disease; amyloid plaques have not, however, been found in NPC disease. This pathologic finding raises the possibility of a common mechanism for formation of NFT in NPC and Alzheimer disease. We have previously described an accumulation of unesterified cholesterol in cholesterol-enriched microdomains (CEM) in NPC disease. On proteomic analysis of CEM from murine liver, apolipoprotein E (apoE) was identified as a major protein in 2-D gels. Immunoblot analysis confirmed the localization of a fraction of cellular apoE to CEM. Further investigation by immunoblot analysis also found amyloid precursor protein (APP) in CEM. Investigations in normal human fibroblasts showed that the amount of apoE in CEM decreased in fibroblasts incubated in the presence of LDL, whereas the concentration of unesterified cholesterol in CEM increased suggesting that apoE may function in the intracellular trafficking of CEM cholesterol. The apolipoprotein E4 (apoE4) allele is a major risk factor for development of Alzheimer's disease although the mechanism remains undefined at this time. Our identification of apoE in CEM raises the possibility that apoE4 may (1) alter the lipid environment of CEM, or (2) that the structure of apolipoprotein E4 may be sensitive to the lipid microenvironment of CEM. Alterations of the CEM lipid microenvironment may then alter APP proteolysis resulting in the accumulation of NFT in both NPC disease and Alzheimer disease.

29. Hermansky pudlak proteins interact within biogenesis of lysosome-related organelle complex-2. P.K. Held, W. Westbroek, A. Helip-Wooley, M. Ayub, M. Huizing, W.A. Gahl. Section of Human Biochemical Genetics, Medical Genetics Branch, NHGRI, NIH, Bethesda, MD 20892, USA.

Hermansky pudlak (HPS) is a rare, genetically heterogeneous autosomal recessive disease characterized by oculocutaneous albanism and bleeding diathesis. The clinical features result from a defect in the biogenesis of lysome-related organelles, such as melanosomes in melanocytes and dense bodies in platelets. Eight genes have been identified that associate with different types of HPS in humans. HPS-3, HPS-5, and HPS-6 generally have mild hypopigmentation with little or no bleeding, while HPS-1 and HPS-4 have severe albinism, bleeding, and early death from pulmonary fibrosis. The correlation between disease severity and subtype is based upon HPS protein interaction. Different HPS proteins interact with one another in the Biogenesis of Lysosome-related Organelles Complexes (BLOCs). HPS-3, HPS-5, and HPS-6 associate to form BLOC-2, but the mode of protein interaction and the overall function of BLOC-2 is largely unknown. We employed a mammalian interaction system, MAmmalian Protein–Protein Interaction Trap (MAPPIT), to elucidate the mode of interaction between HPS proteins in BLOC-2. HPS-6 protein directly interacts with the longer splice form of HPS-5 and to a lesser

extent with the shortened splice form. The long form of HPS-5 has a putative WD40 domain at its N-terminal site. This domain is interrupted in the shortened splice form of HPS-5 and therefore may effect protein-protein interactions with HPS-6 or potentially other proteins. Additional studies have demonstrated that post-translational modifications to HPS-5 also effect interaction with HPS-6. Elucidating the mode of interaction between HPS proteins is critical to understanding how BLOCs are formed and whether additional proteins exist within the different complexes. Previously, we demonstrated that HPS-3 contains a putative clathrin-binding domain that is essential for the accurate localization of cargo targeted to the melanosome. Melanogenic cargo (early endosomes and vesicles) appeared to be perinuclearly clustered in HPS-3, HPS-5, and HPS-6 melanocytes. Therefore, it is likely that BLOC-2 proteins assist in either the budding or trafficking of clathrin-coated vesicles from the early endosome to the developing melanosomes. We are currently investigating the role of HPS-5 and HPS-6 in vesicle budding and/or trafficking.

30. Identification of novel mutations in the medium chain acyl-CoA dehydrogenase gene in a heterogenous population. Lindsey A. Herrel, Chonan Tokunaga, Zaza Khuchua, Arnold W. Strauss. Department of Pediatrics, Vanderbilt University, Nashville, TN, USA.

Background: Medium chain acyl-CoA dehydrogenase (MCAD) deficiency is the most common inborn error in fatty acid oxidation. Up to 85% of symptomatic Caucasian patients are homozygous for the common mutation, A985G, which can result in high morbidity and mortality. Other genotypes have rarely been reported in non-Caucasian populations. *Objective:* The goal of this study was to discover mutations other than A985G that are present in a heterogenous population and provide further clinical information regarding these cases. *Methods:* Patients with an abnormal tandem mass spectrometry (MS/MS) newborn screen with elevated acyl-carnitines usually with one or no A985G alleles by the standard *Pst*I restriction analysis were sent for molecular studies. After DNA isolation and PCR amplification, direct sequencing of the coding regions of the MCAD gene in these patients revealed a wide array of mutations. Mutant MCAD protein expression was quantified by immunoblotting. Follow-up information on these patients was gathered to provide further clinical insight. *Results:* Of 252 patient samples sequenced, 28 patients were homozygous or compound heterozygous for non-A985G MCAD mutations. An additional 119 individuals carried one non-A985G mutated allele, 49 of whom were relatives of ascertained newborns. Twenty-one individuals with homozygous non-A985G mutations reported origin in locations outside northern Europe, including India, Japan, Korea, Mexico, Palestine, Lebanon, and Albania. A particularly interesting missense mutation, G443A, altering arginine-123 to lysine, occurred in three unrelated, homozygous patients born in the US. with Mexican or Latino ancestry. Additionally, G443A was discovered in five patients as a heterozygous mutation, three of whom are related to other patients with the mutation. *Conclusions:* Although the A985G mutations represent a majority of MCAD deficiency in Caucasians, novel mutations are common among non-Caucasian populations in the US G443A likely represents a common MCAD mutation among the Hispanic/Latino population. Further investigation with expanded newborn screening, MCAD gene sequencing and close follow-up will help determine the clinical significance and prevalence of these mutations in the population.

31. Gluconeogenesis in fatty acid oxidation-deficient rat hepatoma cells and mouse primary hepatocytes. Sander M. Houten[a,b], Maxim Boek[a], Albert K. Groen[c], Ronald J.A. Wanders[a,b]. Laboratory Genetic Metabolic Diseases, [a]Department of Clinical Chemistry, Academic Medical Center, Amsterdam, The Netherlands; [b]Department of Pediatrics, Academic Medical Center, Amsterdam, The Netherlands; [c]Medical Biochemistry, Academic Medical Center, Amsterdam, The Netherlands.

Mitochondrial fatty acid β-oxidation (FAO) deficiencies are a group of clinically and biochemically heterogeneous inherited metabolic diseases. A typical clinical feature of these FAO deficiencies is hypoketotic hypoglycemia, which is provoked by illness combined with a period of prolonged fasting. In addition, liver disease with hyperammonemia and cerebral edema may develop (Reye-like syndrome). During the last decade the diagnostic tools to identify FAO defects and our knowledge of the deficient FAO enzymes have expanded tremendously. The underlying mechanisms of most clinical manifestations, however, have not been resolved at present. In our study, we have investigated whether the fasting-induced hypoglycemia occurring in FAO disorders may be due to decreased hepatic production of glucose (gluconeogenesis). To this end we have used rat hepatoma cells (FAO and H4-II-E-C3 cells) and mouse primary hepatocytes as a model system to study the effect of a block in FAO on gluconeogenesis. We show that glucose production from lactate can be stimulated by the addition of fatty acids in rat hepatoma cells as well as mouse primary hepatocytes. When compared with mouse primary hepatocytes, the rate of gluconeogenesis in hepatoma cells is relatively low. This can be attributed to the lower mitochondrial content of the hepatoma cells and as a consequence lower activity of the rate-limiting enzyme in gluconeogenesis, pyruvate carboxylase. We furthermore show that inhibition of FAO using l-aminocarnitine results in decreased gluconeogenesis. Interestingly, this effect is less pronounced when relatively high, non physiological lactate concentrations are used. The decreased gluconeogenesis may result from I) low acetyl-CoA levels, and consequently insufficient activation of pyruvate carboxylase, and/or II) low ATP levels to drive gluconeogenesis. Current studies focus on the measurement acetyl-CoA and ATP levels and the confirmation of our results in mouse models with a genetic FAO defect. In conclusion, our results indicate that the fasting-induced hypoglycaemia observed in patients suffering from a FAO disorder may result from decreased gluconeogenesis, however, increased utilization of glucose in peripheral tissues cannot be ruled out as a contributing factor.

32. Analysis of conserved regulatory elements in the glucocerebrosidase gene locus. K.S. Hruska[a], M.E. LaMarca[a], S.G. Ziegler[a], B. Stubblefield[a], M.E. Portnoy[b], E.D. Green[b], E. Sidransky[a]. [a]Medical Genetics Branch, National Human Genome Research Institute/NIH, Bethesda, MD 20892, USA; [b]Genome Technology Branch, National Human Genome Research Institute/NIH, Bethesda, MD 20892, USA.

While some regulatory elements in the promoter and first exon of the human glucocerebrosidase gene (GBA) have been reported previously, the availability of sequenced genomes from diverse species has simplified functional analyses and greatly expanded their scope. An alignment of the 39.4 kb segment of human genomic sequence that encompasses GBA, metaxin (MTX1) and their respective pseudogenes with orthologous sequence from nine mammals was examined for multi-species conserved sequences (MCSs) using WebMCS. Two non-exonic MCSs were identified in the 3' region of GBA; one 25-bp MCS represents a known thrombospondin enhancer within intron 6 of MTX1, while a second 25-bp MCS appears to be novel. Utilizing the Genomatix Suite applications, a shared framework of three transcription factor-binding sites (TFBSs) was identified within this MCS in nine of the ten species examined, excluding mouse. Similarly, a framework of five TFBSs was predicted in the 5' promoter region of GBA in human and four species, and other possible motifs were identified further upstream. A 5.8 kb genomic fragment containing the potential 5′ regulatory elements and exons 1–3 of GBA has been subcloned in-frame with a luciferase reporter gene, generating an active luciferase that includes the signal peptide of GBA. The reporter construct also fuses the luciferase cDNA with the stop codon of GBA and 2.3 kb of 3′ sequence containing the novel downstream MCS. The eight identified TFBSs are being systematically mutated and tested in vitro for their effects on the transcriptional regulation of GBA.

33. Allele-specific silencing of the dominant disorder sialuria by small interfering RNA. M. Huizing, E. Klootwijk, P.J. Savelkou, C. Ciccone, D. Krasnewich, W.A. Gahl. Medical Genetics Branch, NHGRI, NIH, Bethesda, USA.

Sialuria is a rare autosomal dominant disorder characterized by variable clinical symptoms, including mild hepatomegaly and developmental delay. Patients have a single missense mutation (heterozygous) involving the allosteric site of the rate-limiting enzyme of sialic acid biosynthesis, UDP-GlcNAc 2-epimerase/ManNAc kinase, encoded by the GNE gene. This results in loss of feedback inhibition of UDP-GlcNAc 2-epimerase activity by CMP-sialic acid, and, consequently, overproduction of sialic acid. Since dominantly inherited disease alleles are attractive therapeutic targets for allele-specific silencing mediated by RNA interference (RNAi), we employed this method in fibroblasts of sialuria patients. Small interfering RNA (siRNA) was designed to specifically target a sialuria GNE missense mutation (c.797G > A, R266Q). This siRNA was transfected into patients' fibroblasts and the extent of silencing was assessed after 48 h. After silencing, allele-specific real-time PCR analysis demonstrated that expression of the mutant GNE transcript was decreased by 71 ± 3 (SD)% ($n = 3$). Furthermore, HPLC analysis of fibroblast extracts showed that sialic acid levels decreased 59 ± 15% ($n = 3$) after silencing. Finally, UDP-GlcNAc 2-epimerase enzymatic activity measurements showed a 41 ± 6% ($n = 3$) recovery of feedback inhibition by CMP-sialic acid. These allele-specific RNAi therapeutics in sialuria provide an example of how dominant-negative mutations can be corrected through elimination of specific mutant transcripts. In addition, these results demonstrate that RNAi can provide in vitro correction of the underlying metabolic defect in a human inborn error of metabolism.

34. Can a common SNP in the organic anion transporter MRP4/ABCC4 influence homogentisic acid secretion and the severity of ochronosis in alkaptonuria? Michael A. Kayser[a], Pim Suwannarat[b], Wendy Introne[a], Howard A. Austin[b], Maya Tuchman[a], Bradford Tinloy[a], Cornelia Klein[b], K. O'Brien[a], Isa Bernardini[a], William A. Gahl[a], Robert Kleta[a]. [a]SHBG, MGB, NHGRI; [b]NIDDK, NIH, Bethesda, MD, USA.

Alkaptonuria is a rare metabolic disorder of tyrosine catabolism in which the organic compound homogentisic acid (HGA) and its metabolites bind to connective tissue and cause darkened cartilage (ochronosis), joint destruction, and cardiac valve deterioration. In our investigation of more than 90 alkaptonuria patients, we previously identified four women with an ochronotic phenotype but normal HGA excretion. All had been treated with the tetracycline derivative minocycline. Tetracyclines are secreted into the urine by proximal tubular organic anion transporters. These transporters recognize a variety of drugs, xenobiotics, and organic acids, including *p*-aminohippurate (PAH). We had sequenced all coding exons of the known organic ion transporter genes, i.e., *OAT1*, *OAT3*, *OAT4*, and *MRP4*, in two of our four pseudo-ochronotic patients. Both patients exhibited a homozygous intronic acceptor splice site mutation, IVS2-5T > C, in *MRP4. PAH* clearance was abnormally low in the one patient we studied, reflecting the deleterious effect of this mutation on organic anion transport. We generated a restriction site in *MRP4* to screen for IVS2-5T > C. In 136 alleles from patients with alkaptonuria, we found the expected frequency of wild type (70%) and minor (30%) alleles. We propose that this common *MRP4 SNP, IVS2-5T > C*, can modulate the clinical severity of alkaptonuria, since HGA secretion depends upon an intact organic anion transporter. To pursue this, we measured plasma HGA levels in our alkaptonuria patients and correlated them with the presence of the *MRP4 SNP*. Our preliminary data support a potential influence of this SNP on plasma HGA levels. Our findings point toward a gene product that modifies the severity of alkaptonuria and could have a major impact on the clinical course of other organic acidurias, in which documented variability among affected siblings currently has no explanation.

35. Management of difficult infusion related reactions in a young patient with mucopolysaccharidosis type VI on Naglazyme therapy. K.H. Kim[a,b], C. Decker[c], B.K. Burton[a,b]. [a]Division of Genetics, Birth Defects and Metabolism, Children's Memorial Hospital, Chicago, IL 60614, USA; [b]Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA; [c]BioMarin Pharmaceutical Inc., Novato, CA 94949, USA.

Background: Recombinant human arylsulfatase B (rhASB) was approved for use by the Food and Drug Administration in May 2005 and is currently marketed as Naglazyme (galsulfase). Infusions of rhASB were well tolerated during the clinical trials. The majority of infusion associated reactions (IARs) were judged to be mild to moderate and were successfully managed by interrupting the infusion or slowing the rate of infusion and/or the administration of antihistamines, antipyretics, corticosteroids, or oxygen. The typical adverse events observed during infusions included rash, urticaria, headache, hypotension, nausea, and vomiting. Very few serious or severe adverse events occurred and the majority were felt to be related to the patient's underlying condition. *Case report:* We report on our patient with severe mucopolysaccharidosis type VI who initiated enzyme replacement therapy with rhASB at three years of age. He developed significant IARs after four infusions of rhASB. The observed reactions included urticaria, facial swelling, stridor, and oxygen destaturation. All reactions resolved with interruption of therapy and administration of antihistamines and corticosteroids. Even with antihistamine pretreatment, our patient could not tolerate subsequent infusions that were progressively lengthened to 10 h by slowing the infusion rate from the typical 4 h schedule. We, therefore, devised a protocol by which the patient was treated with oral prednisolone 2 mg/kg the day before each infusion, followed by methylprednisolone 1 mg/kg IV and diphenhydramine 1.25 mg/kg IV one hour before each infusion. The infusions were given initially over 16 h with no IARs observed. Over the next several months, the patient's infusion rate was slowly increased and the premedications were weaned. The patient is now on a four hour infusion schedule with only oral antihistamine as premedication. After one year of therapy, he exhibits clinical improvement including resolution of his hepatomegaly and an increased range of motion of his shoulders and elbows. *Conclusion:* Enzyme replacement therapy does not need to be discontinued even in the face of difficult IARs. We demonstrate that by significantly reducing the rate of infusions and adjusting the premedication regimen, rhASB infusions can continue with no further occurrence of infusion related reactions.

36. Highly efficacious gene therapy in glycogen storage disease type Ia (GSD-Ia) with a double-stranded AAV vector. D.D. Koeberl[a], B. Sun[a], C. Pinto[b], T. Brown[b], A. Bird[a], Y.T. Chen[a]. [a]Division of Medical Genetics, Department of Pediatrics, Duke University Medical Center, Durham, NC, USA; [b]College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA.

New therapy is needed to prevent long-term complications in glycogen storage disease type Ia (GSD-Ia). Patients with GSD-Ia succumb to hepatocellular carcinoma, in addition to life-threatening pulmonary hypertension, kidney failure, and pancreatitis. As the limitations of dietary therapy with uncooked cornstarch have become apparent, liver transplantation has been increasingly recommended for adults with GSD-Ia. Yet liver transplantation remains invasive, prone to failure, and not generally available. Therefore, gene therapy has been advocated as an alternative new therapy in GSD-Ia. We have pursued gene therapy with adeno-associated virus (AAV) vectors in GSD-Ia mice and dogs. The animal models for GSD-Ia feature severe complications, including high mortality, growth retardation, hypoglycemia, and hyperlipidemia. These abnormalities have been corrected with a double-stranded AAV vector encoding human glucose-6-phosphatase that was pseudotyped as AAV8. The double-stranded AAV vector was administered intravenously to 2-week-old GSD-Ia mice (10^{13} vector particles/kg body weight). Survival was prolonged to >5 months following vector administration, in contrast to untreated GSD-Ia mice that survived for <3 weeks. Although GSD-Ia mice were initially growth-retarded, treated mice increased 4-fold in weight to normal size. Hypoglycemia during fasting was completely corrected by 2 weeks following treatment, which surpassed the benefit of previous AAV vectors. Hyperlipemia was normalized. Importantly, the number of vector particles administered was reduced >100-fold without

compromising survival or biochemical correction of treated mice. Finally, the AAV vector completely corrected hypoglycemia during fasting and normalized growth in three GSD-Ia dogs, which surpasses all previous gene therapy results in the canine model for GSD-Ia. These early preclinical data have demonstrated efficacy and feasibility that could justify a clinical trial of AAV vector-mediated gene therapy in GSD-Ia.

37. Plasma branched-chain amino acid concentrations are decreased by sodium phenylbutyrate with no change in their appearance rate or protein turnover as measured by stable isotope tracers. B.C. Lanpher[a], J. Marini[c], F. Scaglia[a], S. Carter[a], P.J. Garlick[c], F. Jahoor[a,d], B. Lee[a,b]. [a]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, USA; [b]Howard Hughes Medical Institute, Chevy Chase, MD 20815, USA; [c]Department of Animal Sciences, University of Illinois Urbana-Champagne, Urbana, IL 61801, USA; [d]Children's Nutritional Research Center, Houston, TX 77030, USA.

Objective: We have observed that patients taking sodium phenylbutyrate as therapy for urea cycle disorders have a clinically significant decrease in circulating levels of branched chain amino acids. In this study, we attempt to further understand this effect with multiple stable isotope tracers. *Methods:* Seven healthy controls were studied using a multiple primed-constant infusion of stable-isotope tracers at baseline and after 2 days of sodium phenylbutyrate treatment (10 mg/m^2/d). In each case, after 2 days of dietary stabilization (0.6 g protein/kg/day), infusions were conducted over 10 h. Blood, urine, and breath samples were collected just prior to and during the infusions. *Results:* Urea synthesis decreased by 24 ± 7 μmol/kg/h ($p < 0.05$) in treated subjects, while glutamine flux increased by 40 ± 9 μmol/kg/h ($p < 0.05$). Also, BCAA levels decreased significantly in subjects on phenylbutyrate. Fasting leucine levels dropped from 106 ± 33 to 64 ± 18 μmol/L ($p < 0.01$). Isoleucine dropped from 54 ± 9 to 33 ± 10 μmol/L ($p < 0.01$). Valine dropped from 204 ± 24 to 139 ± 32 μmol/L ($p < 0.01$). There were no significant changes in the concentration of other amino acids, including threonine, serine, methionine, phenylalanine, and lysine. There was no demonstrable change in the appearance rate of leucine (104.9 ± 4 μmol/kg/h at baseline, 110.0 ± 21 μmol/kg/h on treatment, $p = 0.53$). There was also no change in whole-body protein oxidation as measured by the appearance of labeled carbon dioxide from $^{13}C_1$-leucine in expired breath of participants ($p = 0.27$). *Conclusions:* At this level of protein intake in control subjects, sodium phenybutyrate achieves its desired effect on urea synthesis, while the decrease in BCAA does not appear to adversely affect total body protein catabolism. An important question will be whether this will similarly hold patients with inborn errors of metabolism who may have less capacity for adaptation to a nitrogen load.

38. Evidence of dysregulated insulin secretion in mice with global knockout of short-chain 3-hydroxy acyl-CoA dehydrogenase. C. Li[a], P. Chen[a], S.B. Narayan[a], W. Qin[b], J. Chen[a], F.M. Matschinsky[b], A.W. Strauss[c], M.J. Bennett[a], C.A. Stanley[a]. [a]The Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA; [b]School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA; [c]Vanderbilt Children's Hospital and Vanderbilt University Medical Center, Nashville, TN 37232, USA.

Congenital hyperinsulinism (HI) is the most common cause of hypoglycemia in infants and children. A newly discovered genetic form of HI is associated with recessive inactivating mutations of the mitochondrial fatty acid β-oxidation enzyme, short-chain L-3-hydroxy fatty acyl-CoA dehydrogenase (SCHAD). To explore the mechanism of this unusual fatty acid oxidation defect, we have begun studies of insulin secretion in SCHAD knockout mice. Pancreatic islets were isolated by collagenase digestion and cultured in 10 mM glucose for 3 days. Insulin secretion was studied by islet perifusion; cytosolic calcium responses were measured by dual-wave length fluorescence microscopy. In vivo, SCHAD−/− mice had mild hypoglycemia compared to +/+ controls (98 ± 4 mg/dL vs. 136 ± 6 mg/dL, $p < 0.001$, $n = 12$). Similar to children with SCHAD deficiency, −/− mice had increased plasma levels of 3-OH-butyrylcarnitine (0.61 ± 0.08 vs. 0.16 ± 0.02 μmol/L, $p < 0.01$, $n = 3$). In perifusion studies using ramp stimulation with glucose (0–25 mM in 50 min), isolated SCHAD−/− islets had a slightly lower glucose threshold (6 mM vs. 8 mM), but similar maximum insulin secretion compared to +/+ control islets. Addition of 1 mM octanoate further lowered the threshold for glucose stimulation in SCHAD−/− islets to 3.5 mM, but had no effect in +/+ islets. SCHAD−/− islets had normal basal cytosolic Ca^{2+}; however, stimulation with glucose produced an earlier rise in cytosolic calcium in −/− islets compared to +/+ islets. These studies indicate that SCHAD-/- islets display abnormalities in regulation of insulin secretion consistent with the reports of hyperinsulinism in children with SCHAD deficiency. The results suggest that SCHAD deficiency causes increased sensitivity to glucose stimulated insulin release; this abnormality in insulin regulation is enhanced in the presence of a medium chain fatty acid substrate for the enzyme. Further studies to identify the mechanism of islet dysregulation in SCHAD−/− islets are in progress.

39. A new prospective multicenter study of treatment and outcome in urea cycle disorders (UCDs). Uta Lichter-Konecki and the Members of the UCD-Consortium. Children's National Medical Center (CNMC), Washington, DC, USA.

Objective: To conduct a longitudinal multidisciplinary investigation of the natural history, morbidity, current state of the art treatment, and mortality in people with urea cycle disorders (UCDs). Research questions regard the prevalence of morbid indicators of disease severity, correlations between various biomarkers and disease severity and progression, and the safety and efficacy of currently used and new UCD therapies. *Methods:* This study is conducted at CNMC and 7 collaborating centers (Baylor College of Medicine, UCLA, Children's Hospital of Philadelphia, Vanderbilt University, Yale University, Mt. Sinai School of Medicine, University Hospitals of Cleveland). The RDCRC established a contact registry and launched a longitudinal study at all 8 sites to determine the natural history of UCDs. It also approved a study to determine the effect of sodium phenylbutyrate (Buphenyl) treatment on markers of morbidity in argininosuccinic aciduria (ASA). Additional components are (i) studies: a clinical trial of an investigational new drug, *N*-carbamyl-L-glutamate, for treatment of these disorders; a demonstration project for measuring *in vivo* ureagenesis in UCD using ^{13}C acetate that will be important for classification of patients and for evaluation of treatment efficacy; a neuroimaging study to assess neural mechanisms of Injury in Inborn Errors of Urea Metabolism; an incidence, prevalence, and case fatality study, a pilot study regarding cytokines as biomarkers of metabolic crisis in individuals with urea cycle defects; a study about the safety and efficacy of hypothermia treatment in hyperammonemic encephalopathy; a study of the role of nitric oxide in ASA and a study regarding DNA polymorphisms in urea cycle disorders (ii) training of graduate students, pediatric residents, clinical fellows and junior faculty members in the field of inborn errors of metabolism; and (iii) development and maintenance of a UCD website. This initiative is undertaken in close collaboration with the National Urea Cycle Disorders Foundation (NUCDF), the leading public advocacy organization for these diseases. *Results:* Two hundred and twelve individuals are registered in the UCD contact registry to date. Seventy-eight subjects have been enrolled in the Longitudinal Study with a baseline evaluation. Subjects include patients of SIMD physicians, those that have contacted us through the contact registry and at the NUCDF annual meeting, and those identified through NUCDF recruitment efforts and collaborations with metabolic centers around the country. Funded by a NIH Rare Diseases Clinical Research Center Grant (5U54RR019453), the Kettering Foundation, and the O'Malley Family Foundation.

40. Treating a patient with severe early-onset, non-dysmorphic carnitine palmitoyltransferase II (CPTII) deficiency. Janet Isaacs, Uta Lichter-Konecki. Children's National Medical Center (CNMC), Washington, DC, USA.

Objective: To device a dietary therapy for a child with severe early-onset, non-dysmorphic CPTII deficiency whose two older siblings had succumbed to the disease in the first 5 weeks of life. *Methods:* To provide the minimum amount of essential fatty acids required for age, the diet was composed of a formula very low in long-chain fats (tolerex powder, water and MCT oil) and oral walnut oil. Tolerex was selected because it was the lowest in fat and previously used in similar conditions. High energy was achieved from high carbohydrates and medium chain triglycerides. Total fats were slowly elevated from 4% of total calories to 45% of total calories, with 6% total calories from long-chain fats and the remainder of the lipids from medium-chain triglycerides. Oral walnut oil was used as a supplemental source of linoleic acid (LA) and alpha-linolenic acid (ALA) to provide 500 mg/100 cal LA and 50 mg/100 cal ALA in infancy, and increased over time with growth. *Results:* The patient was born at 39 weeks gestation. The pregnancy was complicated by ultrasonographic (US) evidence for cardiomegaly and hepatosplenomegaly at 37 weeks gestation. Because of the US findings and prior death of two siblings, the child was transferred to our NICU on the first day of life for observation. He went into severe metabolic crisis with an ammonia level >1200 μmol/l, when lipid intake (soybean oil emulsion) reached 2 g/kg. Diagnosis was confirmed by tandem mass spectrometry (David Millington), fibroblast assay (Michael Bennett), and molecular genetics (Georgirene D. Vladutiu). Hemodialysis, high carbohydrate and low fat intake (0.5 g/kg/day) rescued the child and dietary management was initiated. The combination of Tolerex, walnut oil and MCT oil used, allowed maintaining essential fatty acids within the normal range and normal growth but weight and head circumference remained below the third percentile. Developmentally, he started walking at 14 months of age and at 16 months of age he walked stably, spoke 2 words, could feed himself, and used a pincer grasp. *Conclusion:* The combination of Tolerex, MCT oil and walnut oil we employed may allow to raise a child with severe early-onset, non-dysmorphic CPTII deficiency. We were concerned about his head circumference which remained below the third percentile but he reached developmental milestones in time. Supplementation with preformed arachidonic acids and DHA was considered. He succumbed to severe, acute cardiomyopathy in a metabolic crisis following an RSV infection.

41. Glycosylation affects membrane maturation of the OCTN2 carnitine transporter. N. Longo, C. Amat di San Filippo. Division of Medical Genetics, Departments of Pediatrics and Pathology, University of Utah, Salt Lake City, UT 84132, USA.

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. Exceptions are P46S and R83L, 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. We tested whether glycosylation of OCTN2 was required for maturation to the plasma membrane. *Methods:* The three putative glycosylation sites (N57, N64, and N91) of OCTN2 were substituted using site-directed mutagenesis by glutamine (Q) and their effect on carnitine transport and subcellular localization was determined by kinetic analysis and confocal microscopy. *Results:* Substitution of the three putative glycosylation sites with glutamine (Q) decreased mildly carnitine transport when single sites were substituted. By contrast, simultaneous substitution of N57 and N64 caused a marked decline in carnitine transport that was fully abolished 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 three 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 maturation of OCTN2 carnitine transporters to the plasma membrane and suggest that P46S and R83L cause primary carnitine deficiency by impairing OCTN2 glycosylation.

42. Physician reported outcomes of enzyme replacement therapy in older, severely affected patients with Pompe disease. A.T. Van der Ploeg[a], D.L. Marsden[b]. [a]Division of Metabolic Diseases and Genetics, Erasmus MC-Sophia, 3015 GJ Rotterdam, The Netherlands; [b]Genzyme Corporation, Cambridge, MA 02142, USA.

Subjective: Pompe disease, due to a deficiency in lysosomal acid α-glucosidase, results in progressive skeletal muscle weakness and respiratory insufficiency leading to substantially decreased quality of life and often early death. Clinical trials in severely affected infants showed that ERT was safe and effective. There is currently limited outcomes data in older patients. We reviewed the physician reported outcomes of severely affected juvenile and adult patients who were treated with recombinant human acid α-glucosidase (ERT). *Methods:* Physician narrative reports describing outcomes for 18 juvenile and adult patients with severe Pompe disease who were enrolled in an extension phase of an early clinical trial (3) or a compassionate use program (15) because they did not meet inclusion criteria for a currently ongoing clinical trial, were reviewed. Mean age at ERT initiation was 30.8 ± 14.3 years ($N = 18$); treatment duration ranged from 8 to 75.6 months. At baseline, all patients were wheelchair bound. Seventeen patients required respiratory assistance by invasive ($N = 9$), non-invasive ($N = 7$), a combination of invasive and non-invasive ($N = 1$) ventilation. They received a starting dose of 10 mg/kg weekly or 20 mg/kg bi-weekly ERT infusions. *Results:* Most patients showed signs and symptoms of advanced stage Pompe disease prior to ERT. Ten patients demonstrated improvements in respiratory function, including a 50% reduction in required ventilation for one patient. Motor function improved for 13 of 18 patients, and stabilized in the remaining 5 patients; no declines in muscle strength or tone were noted. Almost all (15/16) patients reported positive improvements in their quality of life since commencing ERT. Treatment was well-tolerated, with only one report of a transient infusion-associated reaction (chills) during the first infusions. *Conclusions:* Enzyme replacement therapy for juvenile and adult patients with severe Pompe disease is associated with gains in both respiratory and motor function. Intervention earlier in the disease course was associated with greater improvement in clinical parameters. Overall, patients were satisfied with their treatment, and reported positive improvements in their quality of life regardless of the magnitude of clinical gains or baseline disease involvement. For rare diseases, all forms of clinical information, including physician reported outcomes, can provide meaningful outcomes data.

43. Response to treatment with Myozyme in juvenile patients with Pompe disease. A.T. Van der Ploeg[a], D.L. Marsden[b]. [a]Division of Metabolic Diseases and Genetics, Erasmus MC-Sophia, 3015 GJ Rotterdam, The Netherlands; [b]Genzyme Corporation, Cambridge, MA 02142, USA.

Subjective: Pompe disease, due to a deficiency in acid-α glucosidase, which causes intralysosmal storage of glycogen, can present from soon after birth with severe, rapidly progressive cardio-respiratory failure and death, usually by 1 year of age. Older patients can present at any time after age 1 with a progressive neuromuscular disease, usually with little or no cardiac involvement, leading to death from respiratory failure. A subset of patients aged 5–15 years at presentation may progress more rapidly than older patients. We present preliminary clinical trial data for 74 weeks of enzyme replacement therapy with Myozyme (alglucosidase alfa, rhGAA) in 5 patients, aged 5–15 years. *Methods:* Prospective, open-label, single arm, single-centre study (Rotterdam). All patients were freely ambulatory and ventilator-free except one patient who required nocturnal ventilation with BiPAP. Age at treatment-onset was median 12 years with range 5–15 years. All were treated with Myozyme 20 mg/kg intravenously every 2

weeks for 74 weeks. Assessments were for respiratory function by measured Forced Vital Capacity (FVC) in sitting position, FVC in supine position; for muscle strength by Manual Muscle Testing (MMT) and Hand Held Dynamometry; for muscle function by 6-Minute Walk Test (MWT) at comfortable and fast speed timed tests; for safety evaluation by reported adverse events, infusion-associated reactions, and anti-rhGAA IgG antibody titers. *Results:* Preliminary results show that 2 patients had normal FVC at baseline and remained stable; 3 patients had reduced FVC at baseline which progressively improved; all patients had improved muscle strength compared to baseline (MMT, combined hip and shoulder scores) and improved endurance (6MWT). Treatment was well-tolerated by all patients. No infusion-associated reactions were reported. 5/5 patients developed anti-rhGAA IgG antibody titers during first 38 weeks. Titers ranged from 1/100–1/6400. No in vitro inhibitory antibody activity was found. *Conclusion:* Preliminary data in 5 patients with later onset Pompe disease (aged 5–15 years) indicate that treatment with Myozyme 20 mg/kg every other week leads to improvements in respiratory function and motor function after 74 weeks (38 infusions) of treatment, without any evidence of progression of neuromuscular symptoms. Treatment was well-tolerated by all patients.

44. Domino liver transplant in a patient with intermediate maple syrup urine disease. M.M. Martin[a], K. Weisiger[a], C. Zlatunich[a], W. Packman[b], I. Mehta[a], T. Cowan[c], J. Roberts[d], N. Bass[e], S. Packman[a]. [a]Department of Pediatrics, University of California, San Francisco, USA; [b]Pacific Graduate School of Psychology; [c]Department of Pathology, Stanford University; [d]Department of Surgery, University of California, San Francisco; [e]Department of Medicine, University of California, San Francisco.

Maple syrup urine disease (MSUD) is an autosomal recessive disorder caused by decreased activity of branched-chain α-ketoacid dehydrogenase (primarily expressed in muscle and liver), leading to the accumulation of branched chain amino acids (BCAA) and corresponding α-ketoacids (BCKA). Treatment consists of a low BCAA diet, thiamine for those who prove responsive, and management of acute metabolic episodes, for which patients are continuously at risk. It has been believed that patients under good dietary and biochemical control could be expected to have a good neurological prognosis, with liver transplant reserved for those with severe liver disease. However, recent reports suggest that patients with classic MSUD may in fact have mild-to-moderate neurocognitive dysfunction despite adequate control. In this setting, we report the second patient with MSUD, and the first with intermediate type, to undergo domino liver transplant. The patient is 27 y o male diagnosed with intermediate MSUD at age 10 mos, after presenting in a ketoacidotic coma. He was maintained on a diet low in BCAAs, with plasma leucine levels between 300 and 600 μ, when he was well. DNA analysis revealed two mutations in the E1α gene: a missense mutation in exon 9 and a single nucleotide insertion in exon 2. *In vivo* oxidation analysis showed him to be thiamine non-responsive. Over the years, he had numerous hospitalizations for mild-to-severe metabolic decompensation, and white matter changes were seen on brain MRI. At age 26 y, he requested evaluation for liver transplant, due to chronic fatigue and difficulty concentrating. Pre-transplant testing revealed that his cognitive functioning is approximately 1 SD < mean. In psychosocial functioning, he reported minimal difficulty in daily living skills; however, his level of distress was elevated (i.e., he endorsed feelings of inadequacy, anxiety, depression, and social alienation). He received a cadaveric liver transplant and his native liver was donated to another patient. Two mos post-transplant, he remains stable on an unrestricted diet, with normal BCAA levels, allo-isoleucine <12 μM, and trace BCKA elevations. Reports from the patient and parent indicate improved energy and concentration. The domino recipient is a 58 y o female with end-stage liver disease (alcoholic cirrhosis). Prior to transplant there were no elevation in the BCAA, and allo-isoleucine was absent. These chemistries remained normal post-transplant.

There have now been several cases of non-exigent liver transplant in patients with classic MSUD, in an attempt to minimize or eliminate long-term neurological deficits. In the present case, patient and parental reports indicate that our patient has experienced improvement in his neurocognitive functioning. Formal post-transplant psychosocial and cognitive testing will be performed once his clinical course stabilizes. Biochemical studies post-transplant show no plasma BCAA elevations on an unrestricted diet. Accordingly, liver transplant may well decrease his risk of metabolic intoxication, and improve his quality of life. This transplant was performed for a disease under good metabolic control, but in which long term neurodevelopmental defects are only now becoming recognized. However, since the natural history of the disease and the actual long term benefits of transplant remain unknown, the potential risks and benefits of liver transplant should be carefully weighed in all such patients.

45. Quantitative method for the determination of carnitine and acylcarnitines in biological matrices by high performance liquid chromatography/mass spectrometry. Paul E. Minkler[a], Maria S.K. Stoll[a], Stephen T. Ingalls[a], Shuming Yang[a], Janos Kerner[c], Charles L. Hoppel[a,b]. [a]Department of Pharmacology, Case Western Reserve University School of Medicine, Cleveland, OH 44106, USA; [b]Department of Medicine, Case Western Reserve University School of Medicine, Cleveland, OH 44106, USA; [c]Department of Nutrition, Case Western Reserve University School of Medicine, Cleveland, OH 44106, USA.

Objective: A method for the quantitative measurement of carnitine and specific acylcarnitines in urine, plasma, skeletal muscle, and bloodspots by HPLC/MS is presented. For acylcarnitines, this procedure chromatographically resolves and therefore distinguishes among acylcarnitine constitutional isomers (e.g. octanoyl- from valproyl-), and it also distinguishes acylcarnitines from isobaric contaminants. Therefore, false positives from isobaric contaminants are eliminated. *Methods:* Carnitine and acylcarnitines were isolated from biological matrices by silica gel cation-exchange solid phase extraction, derivatized with pentafluorophenacyl trifluoromethanesulfonate, chromatographed by sequential ion-exchange/reversed-phase HPLC, and detected by electrospray ionization-mass spectrometry using a quadrupole ion trap instrument. Carnitine was detected by MS/MS selected reaction monitoring. Acylcarnitines were detected using full scan MS/MS spectra. Multiple-point standard curves were generated for carnitine (with d_3-carnitine as the internal standard). Acylcarnitine standards were synthesized, and multiple-point standard curves were generated for 42 acylcarnitines: acetyl-, propionyl-, butyryl-, isobutyryl-, valeryl-, isovaleryl-, 3-hydroxy-isovaleryl-, 2-methyl-butyryl-, tigloyl-, 3-methyl-crotonyl-, hexanoyl-, 4-methyl-valeryl-, phenylacetyl-, phenylpropionyl-, 4-phenyl-butyryl-, benzoyl-, 4-methyl-hexanoyl-, octanoyl-, valproyl-, cis-3,4-methylene-heptanoyl-, 4-methyl-octanoyl-, decanoyl-, cis-4-decenoyl-, cis-3,4-methylene-nonanoyl-, 5-decynoyl-, lauroyl-, trans-2-dodecenoyl-, myristoyl-, trans-2-tetradecenoyl-, palmitoyl-, palmitoleoyl-, trans-2-hexadecenoyl-, stearoyl-, oleoyl-, linoleoyl-, succinyl-, methyl-malonyl-, ethyl-malonyl-, glutaroyl-, adipoyl-, 3-methyl-glutaroyl-, suberoyl-, and sebacoylcarnitine (internal standards used were d_6-acetyl-, d_3-propionyl-, undecanoyl-, undecanedioyl-, and heptadecanoylcarnitine). *Results:* Method validation studies were performed demonstrating the accuracy, precision, and reproducibility of the method. Examples of urine, plasma, skeletal muscle, and bloodspot carnitine and acylcarnitine quantification using this procedure are shown. *Conclusions:* This procedure was applied to different biological matrices leading to unambiguous identification and accurate quantification of carnitine and acylcarnitines in specimens of interest for research and diagnostic purposes.

46. Characterization of mutations in human *N*-acetylglutamate synthase using bioinformatic and crystallographic approaches. H. Morizono, L. Caldovic, D. Shi, M. Tuchman. Children's National Medical Center, Washington, DC 20010, USA.

Objective: *N*-Acetylglutamate synthase (NAGS) deficiency, an autosomal recessive disorder, is the last urea cycle disorder for which molecular testing became available. In order to better understand how mutations may result in functional deficits of NAGS, we mapped the mutations onto a homology model of the enzyme based on shared similarity to *N*-acetylglutamate kinase (NAGK), and to proteins that have a GNAT

(GCN5-related *N*-acetyltransferase) fold. A still better model would be an X-ray crystallographic structure of the enzyme, however, vertebrate NAGS appears to readily denature, but we identified a closely related bacterial homolog from *Xanthomonas campestris* that was more stable and have used it for crystallization studies. *Methods:* Mutations were identified in patients by sequencing samples provided to us, or by a literature search for NAGS deficiency. Hidden Markov Model profiles were created for NAGK and for GNAT proteins whose structures were available and the human NAGS amino acid sequence was aligned to the profiles. These alignments were used for building homology models with MODELLER. The *X. campestris* NAGS has been expressed and purified as a selenomethionine derivative and crystallized. Crystals were analyzed at the Advanced Photon Source, and preliminary diffraction data has been collected. *Results:* To date, 21 mutations and three polymorphisms have been identified in the NAGS gene. The deleterious effects of eight mutations were confirmed in expression studies. Mutations on the NAGS gene are distributed throughout the gene. No mutations have been found in the putative mitochondrial targeting signal or the variable segment of NAGS that is encoded by exon 1. The homology model is in two parts, corresponding to the acetylglutamate kinase domain and the GNAT domain. Mutations affecting the Km of NAGS for glutamate are found near the predicted glutamate-binding site. Mutations affecting the arginine response appear to surround the predicted arginine-binding site. Model building using the *X.* campestris NAGS electron density maps has been complicated by the numerous breaks in the density, but two distinct domains are visible that appear to be the NAGK and GNAT domains. Docking of the two parts of the homology model into the density map is in progress. *Conclusions:* The homology model of NAGS appears to reasonably describe the structural and functional basis for several mutations, and may be improved by providing additional spatial restrains using the available low resolution density maps.

47. Role of arginine and protein-protein interactions in regulation of the urea cycle. Ljubica Caldovic, Nantaporn Haskins, Himani Majumdar, Qiuhao Qu, Hiroki Morizono, Mendel Tuchman. Children's Research Institute, Children's National Medical Center, The George Washington University, Washington, DC, USA.

N-Acetylglutamate (NAG) is an essential allosteric activator of carbamylphosphate synthetase I (CPSI), the first and rate limiting enzyme of the urea cycle. Formation of NAG is catalyzed by *N*-acetylglutamate synthase (NAGS; EC 2.3.1.1). In the presence of arginine, the enzymatic activity of mammalian NAGS increases, while it inibits the activity of the homologous microbial enzymes. We examined the evolution of the arginine–NAGS interaction to gain a better understanding of the role arginine may play in regulation of the urea cycle. We also examined if the three mitochondrial enzymes of the urea cycle, NAGS, CPSI and ornithine transcarbamylase (OTC), form a multiprotein complex; this could explain channeling of the urea cycle substrates that was inferred by other researchers and the high efficiency of the urea cycle in converting ammonia to urea. We used co-immunoprecipitation and Western blotting to show that NAGS, CPSI and OTC interact in vivo and are currently examining if the interaction between NAGS and CPSI alters the biochemical properties of both enzymes, such as the response to arginine of NAGS and the affinities for substrates of either enzymes. To better understand the role of arginine in the function of NAGS, we cloned fish NAGS genes and examined the arginine response of the corresponding proteins. Enzymatic activity of fish NAGS is partially inhibited by arginine; This suggests that the effect of arginine on NAGS activity changed from inhibition to activation along with the change of CPSIII to CPSI as animals moved from the sea to land. Based on the similarity between mammalian NAGS and bacterial *N*-acetylglutamate kinases (NAGK) with known three-dimensional structures we identified candidate residues that interact with arginine in mouse NAGS and engineered amino acid substitutions of these residues: F121C, E354A, G360P and G362S. The E354A and G362S substitutions completely abolish the activation of mouse NAGS by arginine, while the G360P and F121C mutant proteins are only partially activated by arginine. These results provide functional identification of arginine-binding amino acids and indicate that they are conserved across species in spite of the disparate arginine effect on NAGS across species.

48. Silver-Russell syndrome, UPD 7 and mitochondrial dysfunction. Sumit Parikh[a], Michelle Puchowicz[b], Charles Hoppel[b], Bruce Cohen[a]. [a]Center for Pediatric Neurology, Cleveland Clinic Neurosciences Institute, Cleveland, OH, USA; [b]Center for Inherited Diseases of Metabolism, Case School of Medicine, Cleveland, OH, USA.

Introduction: Silver-Russell syndrome (SRS, OMIM 180860) is a disorder that predominantly affects somatic growth. The diagnosis is made clinically using a predefined set of parameters. Approximately 10% of individuals with the clinical phenotype of SRS have maternal disomy of chromosome 7 (K. Hannula, 2002). In the past, metabolic derangements have been noted in children with SRS including hypoglycemia, lactic acidosis, basal ganglia abnormalities, and renal tubular acidosis. (A.L. Cazgan, 1994; R. Alvarenga, 1995; P.J. Willems, 1988) *Results:* We are reporting the first Silver-Russell patient with both maternal disomy of chromosome 7 and mitochondrial dysfunction. This patient had abnormal accumulation of tricarboxylic acid cycle intermediates in urine, elevated C14:1 and long-chain acylcarnitines in plasma, renal tubular acidosis, and aminoaciduria. Polarographic study of freshly isolated skeletal muscle mitochondria (SMM) demonstrated a greater than 2 standard deviation (SD) reduction in ADP-stimulated state-3 oxidation of TMPD + ascorbate (complex IV) and complex I, II, and III substrates. Oxidation of fatty acid oxidation substrates palmitoyl-l-carnitine + malate, octanoate + malate, and acetyl-l-carnitine + malate were also reduced more than 2 SD's. Spectrophotometric electron transport chain analysis of fresh SMM, fresh intact skeletal muscle and skin fibroblasts was normal. Mitochondrial DNA point mutation and Southern blot analysis in blood and Complex 4 nuclear mutation testing in muscle (SCO1, SCO2, and SURF1) was negative. Skin fibroblast PDC testing was normal. Skin fibroblast lactate and pyruvate levels are normal. *Conclusions:* We suspect the mitochondrial dysfunction is likely secondary in SRS, due to the primary genetic defect. Since the genes involved in SRS are not known, and the vast majority of nuclear gene defects in primary mitochondrial disease have not been identified, this finding can not be proven. Due to the previously reported metabolic abnormalities in SRS patients, as cited above, and the findings in our patient, it may be worthwhile sending metabolic screening on individuals with a SRS phenotype. Our patient has made significant subjective developmental progress (parental and physician observation) once starting a low fat diet, l-carnitine, coenzyme Q10, sodium citrate and riboflavin. Treatment of metabolic and mitochondrial abnormalities in SRS patients may improve growth and development.

49. Analysis and separation of plasma glutarylcarnitine by UPLC-MS/MS. A. Liu[a], R. Guymon[b], D.M. Johnson[c], M. Pasquali[a,b,d]. [a]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT 84108, USA; [b]ARUP Laboratories, Salt Lake City, UT 84108, USA; [c]Department of Chemical Pathology, Women's and Children's Hospital, North Adelaide, Australia; [d]Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT 84108, USA.

Objective: The diagnosis of glutaric acidemia type I (GA-1, glutaryl-CoA dehydrogenase deficiency) relies on increased excretion of glutaric and 3-hydroxyglutaric acids in urine and increased glutarylcarnitine in plasma and urine. Reliable methods for the quantitation of glutarylcarnitine are critical for screening and diagnosis of GA-1, since affected patients have variable elevations of the characteristic metabolites. Tandem mass spectrometry (MS/MS) identifies acylcarnitine species based on their *m/z* (mass/charge) ratio and cannot separate isomers or other species with the same *m/z* ratio. Glutarylcarnitine (C5-DC), methylsuccinylcarnitine, hydroxydecanoylcarnitine (C10-OH) all produce a 388/85 ion transition by MS/MS parent ion scan. We have developed a quantitative method for the separation of glutarylcarnitine from other species and quantitation by stable isotope dilution and UPLC-MS/MS. *Methods:* We analyzed plasma

specimens collected from normal controls, patients with GA-1, and patients receiving carnitine and other dietary supplements, with MS/MS alone and with UPLC-MS/MS. Plasma samples were extracted using acidified acetonitrile (formic acid 0.3%) and analyzed using UPLC-MS/MS (Waters Quattro Premier with Acquity UPLC™ using Acquity UPLC™ BEH C18 column, 2.1 × 50 mm, 1.7 mm pore size). Glutarylcarnitine was monitored in MRM mode, using d3-glutarylcarnitine as internal standard. *Results:* Analysis of acylcarnitines by MS/MS resulted in three group of patients: (a) patients with GA-1 and elevated glutarylcarnitine (plus other species); (b) normal controls with concentrations of glutarylcarnitine (plus other species) in the normal range; (c) patients who did not have GA-1 with elevated glutarylcarnitine (plus other species). Using UPLC-MS/MS, glutarylcarnitine was chromatographically well separated from methylsuccinylcarnitine, C10–OH-carnitine, and 6 other species, all producing 388/85 ion transitions and therefore interfering with the quantitation of glutarylcarnitine by MS/MS. Interestingly, these other species were particularly elevated in patients receiving carnitine and other dietary supplements suggesting that they could be of dietary origin. *Conclusions:* UPLC-MS/MS can distinguish glutarylcarnitine from other species with the same *m/z* (mass/charge) ratio. This method could be used as a second tier test to confirm elevated glutarylcarnitine levels in newborn screening, plasma or urine samples of patients with suspected GA-1.

50. Sterol precursors accumulate in detergent-resistant membranes in SLOS and CDPX2 patients. Dinesh Rakheja[a,b], Richard L. Boriack[a], Srinivas B. Narayan[c]. [a]Department of Pathology, Children's Medical Center, Dallas, TX, USA; [b] Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX, USA; [c]Department of Pathology and Laboratory Medicine, The Children's Hospital of Philadelphia, Philadelphia, PA, USA.

Disruption of post-squalene cholesterol biosynthesis leads to systemic fetal dysmorphogenesis, now believed to be caused by interruption of the hedgehog signaling pathway. In this study, we demonstrate the accumulation of cholesterol precursors in detergent-resistant membranes prepared from liver tissues of two severely affected infants with cholesterol biosynthetic disorders. 8-dehydrocholesterol and 7-dehydrocholesterol were identified in the detergent-resistant membranes of an infant with severe Smith–Lemli–Opitz Syndrome (SLOS); and cholest-8(9)-ene-3β-ol was identified in the detergent-resistant membranes of an infant with severe X-linked dominant chondrodysplasia punctata (CDPX2) (Table 1). Interestingly, the absolute amounts of cholesterol were not decreased in whole liver homogenate and detergent-resistant membranes of the latter infant, raising the question whether cholesterol deficiency or sterol precursor accumulations are the cause for the lethal malformations. The role of altered lipid raft environment in cholesterol biosynthetic disorders should be studied to further understand the pathophysiology of these disorders of fetal dysmorphogenesis.

Table 1
Sterols, expressed as μg per μg protein, detected by gas chromatography/mass spectrometry

	Sterols in whole liver homogenate			Sterols in detergent-resistant membranes		
	Controls	SLOS	CDPX2	Controls	SLOS	CDPX2
Cholesterol	1.505	0.111	2.961	0.712	0.299	0.734
Desmosterol	0.033	—	—	—	—	—
8-Dehydrocholesterol	—	0.444	0.002	—	0.442	—
7-Dehydrocholesterol	—	0.462	—	—	0.812	—
Cholest-8(9)-ene-3β-ol	—	—	0.139	—	—	0.028
Lathosterol	0.015	0.004	0.045	0.026	—	0.008

51. Worldwide experience in newborn screening for medium-chain Acyl-CoA dehydrogenase deficiency (MCAD). William J. Rhead. Medical College of Wisconsin.

As judged by tandem mass spectrometry blood spot screening, MCAD incidence is 1/14,600 (CI 95%: 1/13,500–1/15,900) in almost 8.2 million newborns worldwide and is 2- to 3-fold higher than that identified in the same populations after clinical presentation. In mass screened newborn populations, the 985 A>G (K329E) mutation accounts for 54–90% of disease alleles, with homozygotes representing about 47–80% of MCAD cases. Worldwide, octanoyl-(C_8)-carnitine levels are an effective primary screen for MCAD in newborns. Newborns homozygous for the 985 A>G mutation have higher octanoyl-(C_8)-carnitine levels than do 985 A>G compound heterozygotes and other genotypes. Time of sampling after birth also significantly affects octanoyl-(C_8)-carnitine levels in MCAD newborns. Tandem mass spectrometry newborn blood spot screening for MCAD is accurate, effective, reduces morbidity and mortality and merits expansion to other populations worldwide.

52. Cardiomyopathy as the presenting feature in a 15-year-old boy with propionic acidemia. Amy White, William J. Rhead, Dieter Matern, Joseph Cava, Jan Kraus, Magdalena Ugarte. Children's Hospital of Wisconsin, Mayo Clinic, University of Colorado, and Universidad Autonoma de Madrid.

A 15-year-old boy was well and normally active until diagnosed with a cardiomyopathy. He was a competitive high school tennis player until shortly before diagnosis and had no symptoms of cardiac insufficiency. The family history was remarkable for a seven year-old sister who died of an idiopathic cardiomyopathy 20 years earlier. Mitochondrial vitamin and cofactor cocktail therapy did not improve his initial cardiac function, nor did high dose carnitine supplementation. He was not carnitine deficient. He required donor cardiac transplant. During his pretransplant workup, an elevated propionyl-carnitine level was detected. Acyl-carnitine analysis in skin fibroblasts revealed massively elevated propionyl-carnitine, similar to that seen in severe, neonatal propionic acidemia. Direct assay of propionyl-CoA carboxylase (PCC) revealed 4% residual activity. Mutation analysis demonstrated that he had G188R and N536D substitutions in PCC, resulting from c.562 G>A and c.1606 A>G mutations, respectively. These have been observed in other propionic acidemia patients and represent both severe and mild mutations. Since the transplant, he has been maintained on a normal diet with carnitine and biotin supplementation, as well as immunosuppressive medications. Acyl-carnitine analysis of his cardiac tissue is underway at Mayo Clinic. He is a very unique case of propionic acidemia with globally normal health and cardiovascular function before developing an acute cardiomyopathy. His younger sibling presumably had the same disorder, although we have no explanation for her presenting at a much earlier age. This is a rare, unusual and detectable form of metabolic cardiomyopathy that may be amenable to medical therapy if detected before irreversible cardiac damage has occurred.

53. Glutaric acidemia type I and the expanded newborn screening program: Recommendations for follow-up of abnormal screening results. G. Scharer[a], M. Woontner[b], E. Savino[a], E. Spector[c], S.I. Goodman[b]. [a]Department of Pediatrics, Division of Clinical Genetics and Metabolism, UCDHSC, Denver, CO, USA; [b]Department of Pediatrics, University of Colorado School of Medicine, Denver, CO, USA; [c]DNA Diagnostics Laboratory, UCDHSC, Aurora, CO, USA.

Glutaric acidemia type I (GA-I) is a rare metabolic disorder caused by mutations in the glutaryl CoA dehydrogenase (GCDH) gene; and is characterized chemically by urinary excretion of glutaric and 3-hydroxyglutaric acids, and clinically by disabling dystonia in childhood due to acute or chronic striatal necrosis. The abnormal organic aciduria is easily detected in some patients (high excretors), but in others organic aciduria is mild, intermittent, or absent. Genotype appears to predict the excretor phenotype, but not clinical severity. Treatment of patients before the onset of symptoms with carnitine, diet, and riboflavin prevents neurological disease in about two-thirds of affected children, and presymptomatic

diagnosis in newborns is possible by demonstrating increased C-5 dicarboxylic carnitine ester in blood by tandem mass spectrometry (MS/MS). Urine organic acid analysis and measurement of glutaric and 3-hydroxyglutaric acids are indicated in all patients with even minimal increases of the C-5 dicarboxylic carnitine ester. Even if these studies are negative, babies with subtle or atypical features of GA-I should probably have the disease excluded by DNA analysis or enzyme assay on cultured fibroblasts. Such features would include unexplained or progressive macrocephaly, movement disorder/mild dystonia, CNS or retinal hemorrhages, seizures, and radiologic evidence of fronto-temporal cortical atrophy, lesions in the caudate or putamen, or periventricular white matter changes.

The hope-expectation-hypothesis is that GA-I infants likely to be missed by newborn screening are the low excretors that are difficult to diagnose even after the appearance of symptoms, and that those detected by elevated blood glutarylcarnitine in newborn blood spots will be relatively simple to confirm using this algorithm. Further, because some patients will not be detected by MS/MS, it is important that the diagnosis be considered in patients with appropriate clinical signs and symptoms, even if they had been screened for the condition as newborns.

We believe that this algorithm will allow for maximal sensitivity/specificity of newborn screening for this condition, and for comprehensive data collection to better assess the true prevalence of the disease and the risk of complications associated with various disease-causing mutations.

54. Cellular and tissue localization of globotriaosylceramide in Fabry disease. H. Askari[a], C.R. Kaneski[a], C. Semino-Mora[b], A. Ang[c], B. Wustman[d], R. Schiffmann[a]. [a]Developmental and Metabolic Neurology Branco, NINDS, National Institutes of Health, Bethesda, MD, USA; [b]Laboratory of Gastrointestinal and Liver Studies, Uniformed Services University of the Health Sciences, Bethesda, MD, USA; [c]HistoRx, Inc., New Haven, CT, USA; [d]Amicus Therapeutics, Inc., Cranbury, NJ, USA.

Objective: To describe cellular and subcellular localization of globotriaosylceramide in order to assess potential abnormal interactions of globotriaosylceramide leading to disease in Fabry patients. *Methods:* We used an anti-globotriaosylceramide monoclonal antibody (Seikagaku, Tokyo, Japan) for immunogold electron microscopy immunohistochemistry, and immunofluorescence studies of tissues from patients on long-term enzyme replacement therapy (ERT) and normal controls. Anti-LAMP1, anti-calreticulin, and DAPI were used in immunofluorescence studies as markers of the lysosomes, ER, and nucleus, respectively. Toluidine blue, hematoxylin eosin, and Luxol fast blue staining were done as well. *Results:* Immunoreactivity for globotriaosylceramide was present in all organs examined: heart, kidney, brain, intestines, adrenal gland, aorta, skin, liver, and spleen. The cellular pattern and distribution of globotriaosylceramide varied between organs and cell types. In the brain, positive immunoreactivity was found only in the parahippocampal region. In all organs examined, globotriaosylceramide immunostaining was present in the cell membranes and cytoplasm of endothelial cells, even in the absence of lysosomal inclusions. Immunofluorescence immunolabeling of heart and kidney tissues from a Fabry patient showed colocalization of globotriaosylceramide with lysosomal, ER, and nuclear markers. Immunogold electron microscopy confirmed the presence of globotriaosylceramide in the cell membrane, lysosomes, ER, nuclear membrane and nucleus of vascular endothelial cells and fibroblasts even in the absence of lysosomal inclusions. Cultured fibroblasts from patients showed similar findings. Immunolabeling of organ tissues and cultured fibroblasts from three unaffected controls was uniformly negative for globotriaosylceramide by immunohistochemistry and electron microscopy. *Conclusions:* A substantial amount globotriaosylceramide immunoreactivity remains in cells and tissues even after years of ERT in Fabry disease. For the first time we demonstrate the presence of accumulated globotriaosylceramide in extralysosomal regions including in the cell membrane, ER and nucleus. These findings are crucial for the understanding of disease mechanism and suggest the use of immunostaining for globotriaosylceramide as a means to assess response to novel specific therapies.

55. Pyruvate dehydrogenase complex deficiency due to abnormal stability of the E1β subunit. Zongchao Han[a], Li Zhong[a], Arun Srivastava[a,b,c], Peter W. Stacpoole[c,d,e]. [a]Departments of Pediatrics (Division of Cellular and Molecular Therapy), University of Florida College of Medicine, Gainesville, FL 32610, USA; [b]Department of Molecular Genetics and Microbiology, University of Florida College of Medicine, Gainesville, FL 32610, USA; [c]The General Clinical Research Center, University of Florida College of Medicine, Gainesville, FL 32610, USA; [d]Department of Medicine (Division of Endocrinology and Metabolism), University of Florida College of Medicine, Gainesville, FL 32610, USA; [e]Department of Biochemistry and Molecular, University of Florida College of Medicine, Gainesville, FL 32610, USA.

We describe a new case of pyruvate dehydrogenase (PDH) complex (PDC) deficiency associated with the PDH E1β subunit (PDHB) gene. The patient was a 5-year-old girl with severe developmental delay, microcephaly, and agenesis of the corpus callosum. She had mild hyperlactatemia and moderately elevated lactate levels in her cerebrospinal fluid. Her cultured skin fibroblasts demonstrated a 55% reduction in PDC activity and markedly decreased immunoreactivity for PDHB protein, compared to healthy controls. The sequence of the total cDNA corresponding to the patient's PHDA and PDHB genes revealed no pathological mutations. The relative expression level of PDHB mRNA and the rate of transcription and translation of the PDHB gene were normal. In contrast, PDC activity could be restored and the rate of degradation of the patient's E1β protein decreased by the proteasome inhibitor MG132 and by Tyr23, a specific inhibitor of epidermal growth factor receptor–protein tyrosine kinase (EGFR-PTK). However, only Tyr 23 treatment restored E1β protein levels to those found in cells from healthy subjects or from patients with PDH E1α deficiency. We also found that the patient's cells contained a high basal level of the tyrosine phosphorylated form of the EGFR, although the total EGFR protein level was similar to that found in cells from Elα deficient patients and healthy subjects. These data indicate that PDC deficiency in this patient involves a post-translational modification that may be due, in part, to increased turnover of the E1β protein following activation of EGFR-PTK by autophosphorylation at its tyrosine residues. This likely results in enhanced ubiquitination of the E1β protein, leading to proteasome-mediated degradation.

Keywords: Pyruvate dehydrogenase deficiency; Mitochondria; Proteasome; EGFR-PTK; Ubiquitin.

56. Genetic mutation profile of isovaleric acidemia patients in Thailand. P. Suwannarat, P. Kodcharin, N. Chongviriyaphan, Wattanasirichaigoon. Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.

Objective: Isovaleric acidemia is an autosomal recessive disorder caused by deficiency of isovaleryl-CoA dehydrogenase. We describe the clinical presentation and course of two Thai patients and report the first mutation analysis of the IVD gene in Thai patients. *Methods:* Patient 1 presented at three days of age with poor feeding, hypoglycemia and metabolic acidosis. Urine organic acids analysis revealed the presence of 3-OH-isovalerate and isovalerylglycine. Patient 2 had poor feeding and seizures at 10 days of age. Mild metabolic acidosis and ketosis resolved with intravenous fluid resuscitation. He was referred due to a peculiar body odor at 17 days of age. Acylcarnitine analysis by MS/MS showed increased isovaleryl carnitine. Urine organic acid analysis showed 3-OH-isovalerate and isovalerylglycine. DNA analysis: genomic DNA was extracted by standard methods. Each exon of the IVD gene was PCR amplified and subjected to direct sequencing. *Results:* At 14 months of age, Patient 1 has two episodes of mild decompensation associated with acute gastroenteritis and has mild speech delay. He is homozygous for the Arg50Pro mutation. At 10 months of age, Patient 2 has normal growth and development and has had no

further episodes of decompensation. He is homozygous for the Gly120Arg mutation. *Conclusions:* We found two previously described mutations, however we did not find the common Ala282Val which is associated with a mild phenotype. Long term follow-up will provide additional genotype–phenotype information. The majority of mutations in the IVD gene have been reported among Caucasians. Mutation analysis in other populations will provide additional information on the spectrum of mutations in this gene.

57. Second tier testing for the differential diagnosis of SCAD versus IBCD and IVA versus 2MBCD by UPLC-MS/MS of acylcarnitines. Sabrina Forni, Linda Alvarado, Xiaowei Fu, Lawrence Sweetman. Institute of Metabolic Disease, Baylor Research Institute, 3812 Elm Street, Dallas, TX 75226, USA.

Second tier diagnostic tests based on the chromatographic separation of C4 and C5 acylcarnitine isomers can provide fast differential diagnosis of deficiencies of SCAD (Short-Chain Acyl-CoA Dehydrogenase) versus IBCD (Isobutyryl-CoA Dehydrogenase) and IVA (Isovaleric Aciduria) versus deficiency of 2MBCD (2-Methylbutyryl-CoA Dehydrogenase). Newborn screening and subsequent acylcarnitine profiles by flow-injection MS/MS cannot distinguish compounds of the same mass (geometric isomers) and therefore liquid chromatographic separation before MS/MS is required. Stable isotope dilution UPLC-MS/MS methods have been developed for the simultaneous determination of isobutyryl and butyryl L-carnitine, and for isovaleryl carnitine, D and L 2-methylbutyryl L-carnitine diastereomers as well as valeryl L-carnitine and pivaloyl L-carnitine in dried blood spots and plasma. Dried blood spots are extracted with methanol containing stable isotope internal standards and plasma proteins are precipitated with methanol containing stable isotope internal standards and butyl esters formed. Liquid chromatographic separation of acylcarnitine butyl esters is achieved with methanol/water gradients with a C18 BEH, 1 × 100 mm, 1.7 μm UPLC column, at 60 °C, with a run time of less than 10 min. The isomers are detected and quantified with a Quattro Premier MS/MS by positive ESI using MRM transitions from protonated molecular precursor ions of acylcarnitines and stable isotope labeled acylcarnitine internal standards to the common product ion at *m/z* 85. Reference ranges for isobutyryl carnitine, butyryl carnitine, isovaleryl carnitine, and 2-methylbutyryl carnitine isomers for normal newborns and infants and concentrations of these isomers for patients with confirmed metabolic disorders will be presented.

58. The identification and characterization of glucocerebrosidase activators and inhibitors as potential therapeutic agents for Gaucher disease. Daniel Urban[b], Wei Zheng[a], Aashish Goswami[b], Ozlem Goker-Alpan[b], Ehud Golding[b], Janak Padia[a], Jim Inglese[a], Chris Austin[a], Ellen Sidransky[b]. [a]NIH Chemical Genomics Center, National Human Genome Research Institute, NIH; [b]Medical Genetics Branch, National Human Genome Research Institute, NIH.

Gaucher disease is caused by an inherited deficiency of the lysosomal enzyme glucocerebrosidase (GC). It has been suggested that chemical chaperones might correct the misfolding of the mutant enzyme and thus restore its function. Using quantitative high throughput screening (qHTS) of a compound collection of over 60,000 chemicals, we identified 299 inhibitors and 56 activators, with a hit rate of 0.57%. Amount these were one structure class of GC activators and three classes of GC inhibitors. Twelve compounds were found to have AC50 values at less than 0.5 micromolar concentrations. The best inhibitors included sulfonamides, quinolines, and triazines. In order to characterize the mechanism of action for these compounds and to determine their selectivity profiles, we performed enzyme kinetics assays using fire different lysosomal hydrolases. We found that both the GC activators and inhibitors identified in our screening were highly selective for GC and not the other enzymes tested, and showed better selectivity than existing GC inhibitors. The identification of these selective compounds confirms the power of qHTS, which may ultimately lead to the development of small molecule drugs that can be tested as therapeutic agents for Gaucher disease.

59. Retrospective genotyping of newborn screening cards for the P479L carnitine palmitoyltransferase (CPT1) variant: Correlation with acylcarnitine profiles and estimation of incidence in British Columbia. G. Sinclair[a], J. Ma[a], P. MacLeod[b], L. Arbour[b], H. Vallance[a]. [a]Departments of Pathology, University of British Columbia, Vancouver, Canada; [b]Medical Genetics, University of British Columbia, Vancouver, Canada.

Introduction: A common variant of CPTI deficiency (P479L) first identified in the Canadian Inuit population has also been identified in 21 First Nations children in British Columbia. All BC cases presented with one or more of the following: hypoglycemia, liver disease, and sudden unexpected death. Several of the parents were P479L homozygous suggesting a high frequency of this allele in BC. The objective of this study was to assess the optimal acylcarnitine parameters to identify the CPTI P479L variant from newborn screening blood spots cards and then to estimate the incidence of this CPTI variant in the BC population. *Method:* Archived acylcarnitine data from the 2004 birth year ($n = 41{,}900$) was mined as a function of either the C0/C16 + C18 ratio or free carnitine (C0) values. For C0/C16 + C18, a total of 158 cards were randomly chosen from above means + 6SD cutoff ($N = 246$). For C0, a stratified random sample ($n = 279$) was collected above means + 1SD ($N = 5464$). Test performance was determined by ROC analysis. A Taqman allelic discrimination assay on an ABI real-time PCR instrument was used to genotype the blood spot cards for the P479L variant and this genotype was used to define affected (P479L homozygous) and unaffected (P479L heterozygous or non-carrier) for the subsequent ROC analysis. *Results:* ROC analysis of the sample selected by C0/C16 + C18 ratio revealed an area under the curve (AUC_{ROC}) of 0.493 ± 0.081 suggesting a non-discriminatory test. Inspection of the data revealed a high proportion of false positives from cards with elevated ratios due to very low total carnitine levels. For C0 sampling, a total of 62 individuals out of 279 sampled were homozygous for P479L. The ROC for C0 alone revealed an $AUC_{ROC} = 0.860 \pm 0.033$. Analysis of the full acylcarnitine spectrum, however, revealed that the most discriminatory test was the ratio C0/C18:1 + C18:2 with an $AUC_{ROC} = 0.961 \pm 0.016$. Based on this data, a C0/C18:1 + C18:2 ratio cutoff of 28, had a specificity of 0.84 and sensitivity of 0.95 for identifying P479L homozygotes. *Conclusions:* Applying this cutoff to the population as a whole and correcting for the sensitivity and specificity of the test, we estimated that ~500 P479L homozygous individuals were born in BC in 2004. A further study is underway to determine if the P479L mutation is in fact confined to First Nations and to clarify the clinical significance of this common and potentially treatable genetic variant.

60. Acceptable low-phenylalanine foods and beverages can be made with glycomacropeptide from cheese whey for individuals with PKU. S.C. Van Calcar[a], K. Lim[b], K. Nelson[b], S.T. Gleason[a], D.M. Ney[c]. [a]Waisman Center, University of Wisconsin, Madison, WI 53706, USA; [b]Wisconsin Center for Dairy Research, University of Wisconsin, Madison, WI 53706, USA; [c]Department of Nutritional Sciences, University of Wisconsin, Madison, WI 53706, USA.

Glycomacropeptide (GMP) is a whey protein produced during cheese making when bovine kappa (κ)-casein is cleaved by chymosin into para-κ-casein, which remains with the curd, and GMP, which remains with the whey. Pure GMP contains elevated amounts of threonine and isoleucine and no aromatic amino acids including phenylalanine (phe). The objective of this study was to make a variety of palatable, low-phe foods and beverages with GMP and to assess their acceptability by conducting consumer sensory studies in subjects with PKU. Foods and beverages containing GMP (BioPURE-GMP™, Davisco Foods International, Inc., Le Sueur, MN) were developed at the Food Applications Laboratory, Wisconsin Center for Dairy Research. Informed consent was obtained from each subject prior to the sensory studies. PKU subjects ($n = 35$, ages

12–22 years) tasted six products made from GMP and two commercial products during summer PKU camp in 2004 and 2005. Foods and beverages were rated using a five-point hedonic scale (1 = dislike very much to 5 = like very much). Independent *t*-test was performed to analyze mean acceptability in five categories including appearance, odor, taste, texture, and overall acceptability. Values are means ± standard deviations. Among the foods and beverages, GMP strawberry pudding was the most acceptable (overall score of 4.4 ± 0.6). Other foods in order of overall acceptability were GMP snack crackers (3.6 ± 1.4), GMP chocolate beverage (3.5 ± 0.9), GMP sports beverage (3.3 ± 1.1), GMP strawberry fruit leather (2.9 ± 0.8), low protein crackers (2.9 ± 1.3), and GMP in apple juice (2.6 ± 1.1). An amino acid-based chocolate beverage was the least acceptable in PKU subjects (2.2 ± 1.5). PKU subjects rated the taste, odor, and appearance of GMP snack crackers as significantly more acceptable compared to commercial low protein crackers ($P \leqslant 0.05$). PKU subjects rated the odor and appearance of a GMP sports beverage as significantly more acceptable compared to apple juice supplemented with GMP. These data demonstrate that a variety of palatable, low-phenylalanine foods and beverages can be made with GMP as a potential protein source for individuals with PKU. Studies in individuals with PKU are ongoing in our research group to establish the safety and efficacy of GMP in the nutritional management of PKU. Supported by NIH DK071534.

61. Finding Twinkle in the eyes of a 71-year-old lady. J.L.K. Van Hove[a], C. Rice[a], V. Cunningham[a], L.-J.C. Wong[b]. [a]Department of Pediatrics, University of Colorado at Denver Health Sciences Center, Denver, CO, USA; [b]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA.

Progressive external ophthalmoplegia (PEO) is often caused by a mitochondrial disorder. The molecular basis for this can be a deletion in mitochondrial DNA, a point mutation in a mtDNA tRNA gene, or a disorder characterized by multiple mtDNA deletions, which is often caused by a mutation in one of the genes ANT1, POLG1, or C10orf2. *Case report:* A 71-year-old lady was referred for investigation of a probable mitochondrial disorder. She had developed progressive external ophthalmoplegia since the age of 55 years. A muscle biopsy done at the age of 63 years showed a few ragged red fibers. She also had cataracts, adult-onset diabetes mellitus, paresthesias in her fingers and toes, cognitive defects with short-term memory problems, sensorineural hearing loss, and mild ataxia. She had chronic progressive muscle weakness particularly of the limb girdle, and uses CPAP at night for oxygen desaturation during sleep. On physical exam, she had complete ophthalmoplegia, muscle weakness with positive Trendelenburg sign, and ataxic gait. MRI showed scattered white matter changes unchanged over 6 years. *Methods:* Muscle tissue was examined for deletions by Southern blot. Point mutations were analyzed by dot blot analysis. MtDNA was sequenced. The genes POLG1, ANT1, and C10orf2 were sequenced. *Results:* Neither dot-blot analysis nor sequencing of mtDNA showed a pathogenic mutation. Southern blot did not show deletions in mtDNA. Sequencing of the nuclear genes showed an R303Q mutation in the C10orf2 gene encoding for the Twinkle protein. *Discussion:* The mutation R303Q has not been reported before, but a similar mutation R303W has been reported. Mutations in Twinkle have been reported in older patients >50 years of age with PEO, whereas other causes tend to present at an earlier age. *Conclusion:* In older patients with PEO and other symptoms associated with mitochondrial disease, the Twinkle gene should be analyzed regardless of the presence or absence of recognizable deletions on Southern blot in muscle, and even in the absence of a family history of PEO.

62. Outcomes and complications of CPT1A deficiency observed during the long-term follow up of 4 cases. Nithiwat Vatanavicharn[a], Denise Salazar[b], Pertchoui B. Mekikian[a], William R. Wilcox[a,c]. [a]Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA; [b]Department of Biochemical Genetics, Quest Diagnostics Nichols Institute, San Juan Capristano, CA, USA; [c]Department of Pediatrics, UCLA School of Medicine, Los Angeles, CA, USA.

Deficiency of hepatic carnitine palmitoyl transferase (CPT-1A) is a rare autosomal recessive disease of long-chain fatty acid transport into the mitochondria. Approximately 30 cases have been reported, and most clinical manifestations develop in infancy or early childhood. We follow four cases of CPT-1A deficiency. Case 1 was a product of a non-consanguineous East Indian couple. He had several episodes of encephalopathy and metabolic acidosis since 7 months of age. At 19 years of age, he had an episode of encephalopathy, hyperammonemia, and severe intrahepatic cholestasis with otherwise relatively normal liver functions. A brain MRI showed bilateral, symmetrical T2-weighted hyperintensities of the basal ganglia and brain stem. After recovery, he had a resting tremor, but intelligence was unaffected. CPT-1 activity in cultured fibroblasts was 9% of normal. Case 2 is a product of a non-consanguineous Hispanic couple. Her first episode of encephalopathy, hypoglycemia, and hyperammonemia occurred at 14 months of age, and she had distal renal tubular acidosis (RTA) that resolved with medium-chain triglyceride (MCT) oil supplementation. CPT-1 activity in cultured fibroblasts was 11% of normal (reported by Falik-Borenstein et al., 1992). She has had episodes of pancreatitis without hypertriglyceridemia since 10 years of age. At 19 years of age, she had an episode of encephalopathy, hyperammonemia, pancreatitis, elevated liver transaminases, cholestasis, and macrocytic anemia. A magnetic resonance cholangiopancreaticography showed mild dilatation of the distal pancreatic duct. She had a homozygous 298C > T substitution in the CPT-1A gene, predicted to cause premature truncation of the protein (Gobin et al., 2002). Cases 3 and 4 are identical twins born to a consanguineous Hispanic couple. Both had a first episode of encephalopathy, hypoglycemia, hyperammonemia, and distal RTA at 7 months of age. They had persistent RTA and recurrent episodes of lethargy, with or without hypoglycemia, during intercurrent illnesses in spite of MCT supplementation. CPT-1 activity was 3% of normal control in the lymphocytes and 26% of normal control in the liver. Mutation analysis identified the same homozygous mutation as found in Case 2, on the same ancestral haplotype. Both families originate from the Mexican state of Durango. Individuals with CPT-1A deficiency are at risk for metabolic decompensation, severe cholestasis, recurrent pancreatitis, and chronic RTA in spite of supplementation with MCT oil.

63. Enzymatic analysis of MCAD, VLCAD, and glutaryl-CoA dehydrogenase in lymphocytes with implication for neonatal sceening. R.J.A. Wanders, J.P.N. Ruiter, M. Duran, F.A. Wijburg, H.R. Waterham. University of Amsterdam, Academic Medical Center, Department of Pediatrics, Emma Children's Hospital, The Netherlands, Department of Clinical Chemistry, Lab of Genetic Metabolic Diseases, Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands.

Neonatal screening for a range of inborn errors of metabolism is being introduced in an increasing number of countries around the world. Discrimination between false- and true positives requires the availability of straight forward and unequivocal methods of detection. We have focused on the generation of simplified procedures for the enzymatic analysis of medium chain acyl-CoA dehydrogenase (MCAD) and very-long-chain acyl-CoA dehydrogenase (VLCAD) in lymphocytes, using ferricenium hexafluorophosphate as electron acceptor, and phenylpropionyl-CoA, and palmitoyl-CoA as specific substrate for MCAD and VLCAD, respectively, at least in fibroblasts. To this end we have developed HPLC-based methods, which allow unequivocal identification of MCAD and VLCAD deficiency in lymphocytes of newborns. The feasibility of the two methods has recently been tested in practice in neonatal screening programs with excelent results [Derks et al., submitted for publicaton; Spiekerkoetter et al., Pediatrics (2006) 118 (3) 1065–1069]. The same methodology has also been used for glutaryl-CoA dehydrogenase and the validity of the assay has been tested in lymphocytes from proven GA1 patients with excellent discrimination between patients and controls.

64. HEM dysplasia and ichthyosis are laminopathies rather than inborn errors of cholesterol synthesis. C.A. Wassif[a], K.E. Brownson[a], W.K. Wilson[b], M.F. Starost[c], F.D. Porter[a]. [a]HDB, NICHD, Bethesda, MD, USA; [b]Rice University, Houston, TX, USA; [c]NIH, OD, ORS, Bethesda, MD, USA.

Mutations of the lamin B receptor (LBR) cause both HEM dysplasia in humans and Ichthyosis in mice. LBR is a bifunctional protein with an amino terminal lamin B binding domain and carboxyl terminal sterol Δ^{14}-reductase domain. Although only a minor accumulation of precursor sterols was observed in HEM dysplasia patients, it has been proposed that LBR is the primary sterol Δ^{14}-reductase, and that impaired sterol Δ^{14}-reduction underlies HEM dysplasia. However, a second protein, Dhcr14, also has sterol Δ^{14}-reductase activity. We thus hypothesized that LBR and Dhcr14 could be redundant with respect to sterol Δ^{14}-reduction. To test this idea, we obtained Ichthyosis mice (Lbr−/−) and disrupted Dhcr14 in mouse embryonic stem cells to produce Dhcr14−/− mice. Dhcr14−/− mice are phenotypically normal. To test for a digenic phenotype, we bred Lbr and Dhcr14 heterozygous mice to obtain compound mutant mice. Lbr−/−:Dhcr14−/− mice die soon after implantation. Lbr−/−:Dhcr14+/− mice die in utero or soon after birth. In contrast, Lbr+/−:Dhcr14−/− mice appear normal at birth, but by 10 days of age they are growth retarded and neurologically abnormal (ataxia and tremors). Pathological evaluation demonstrated vacuolation and swelling of the myelin sheaths in the spinal cord consistent with a demyelinating process in Lbr+/−:Dhcr14−/− mice. This was not observed in either Lbr−/− or Dhcr14−/− mice. In contrast to Lbr−/− mice, Lbr+/-:Dhcr14−/− and Dhcr14−/− mice have normal skin. Also, in contrast to Lbr−/− mice, Dhcr14−/− mice do not have Pelger-Huët anomaly. Gas Chromatography/Mass Spectroscopy (GC/MS) was used to characterize liver and brain cortex sterols from 10-day-old mice. Liver sterols were normal in Lbr−/−, Dhcr14−/−, and Lbr+/−:Dhcr14−/− mice. However, significantly elevated levels (50% of total sterols) of cholesta-8,14-dien-3β-ol and cholesta-8,14,24-trien-3β-ol were found in brain tissue from 10-day-old Lbr+/−:Dhcr14−/− mice. The identity of these precursor sterols was confirmed by NMR analysis. A much smaller and transitory increase in these precursor sterols was observed in Lbr−/− and Dhcr14−/− mice. Minor elevations (0–3% of total sterols) were observed in brain from 10-day-old Lbr−/− mice, and a moderate elevation (15% of total sterols) was observed in brain from 10-day-old Dhcr14−/− mice. GC/MS analyses of sterols from both brain and liver tissue obtained from 21-day-old Lbr−/− and Dhcr14−/− mice were normal. Expression analysis of Lbr and Dhcr14 in liver tissue supports the redundant nature of these two proteins with respect to sterol Δ^{14}-reduction. Our data support the idea that HEM dysplasia and Ichthyosis phenotypes are due to impaired lamin B receptor function rather than impaired sterol Δ^{14}-reduction. Impaired sterol Δ^{14}-reduction gives rise to a novel murine phenotype for which a corresponding human disorder has yet to be identified.

65. A lethal autosomal dominant defect of mitochondrial and peroxisomal fission. Hans R. Waterham[a], Janet Koster[a], Carlo W.T. van Roermund[a], Petra A.W. Mooijer[a], Ronald J.A. Wanders[a], James V. Leonard[b]. [a]Laboratory Genetic Metabolic Diseases (F0-224), Academic Medical Center, University of Amsterdam, The Netherlands; [b]Department of Pediatrics, Institute of Child Health, University College London, UK.

Mitochondria form a dynamic network which is subject to continuous fusion and fission processes for which several proteins involved have been identified. We report a deceased newborn female who had microcephaly, abnormal brain development, optic atrophy, and hypoplasia, failure to thrive, persistent lactic academia, and raised plasma very long-chain fatty acids. Extensive laboratory investigations revealed a defect in the fission of both mitochondria and peroxisomes due to a single heterozygous, but dominant negative mutation in the DLP1 gene. DLP1 codes for the dynamin-like protein DLP1, previously shown to be involved in the fission of these two organelles. Overexpression of mutant DLP1 in control fibroblasts resulted in the aberrant mitochondrial phenotype, whereas overexpression of wild-type DLP1 in fibroblasts of the patient reversed the aberrant mitochondrial phenotype to normal. The autosomal dominant inheritance of the observed defect was confirmed by the absence of the mutation in the DLP1 genes of the patient's parents. Our finding represents the first patient from a new class of diseases with a combined defect in both mitochondria and peroxisomes.

66. Creatine transporter deficiency: Repeat urine testing and false positives. Tim Wood; Judy Haley; Harold Taylor. Greenwood Genetic Center, Greenwood South Carolina, USA.

Creatine transporter (CrT) deficiency is an X-linked disorder caused by mutations in the SLC6A8 gene located at Xq28. Affected males present with mental retardation, hypotonia and seizures. Carrier females may be normal or have a milder clinical course. Diagnostic testing begins with urine screening or cerebral proton MRS studies followed by confirmatory DNA or cellular assays. Elevations in urinary creatine have been identified in all affected males; however, false elevations can occur. Obtaining a repeat first void urine sample may be a relatively quick, less invasive method to identify false positives and limit expensive follow up testing. To evaluate this hypothesis, multiple random urine samples were collected from two males with confirmed CrT deficiency. All 14 samples showed significant elevations (ranging from 1595 to 2965 mmol creatine/mol creatinine; normal range 5–560 mmol creatine/mol creatinine) suggesting that urinary creatine levels in affected males are consistently elevated and do not reach into the normal range. We also evaluated this hypothesis by repeat sampling of four males with initial elevations. Urinary creatine elevations persisted in two patients while values in the remaining two returned to normal. Follow up molecular testing in all four patients identified mutations in only the two patients with consistent elevations. These data along with the lack of any reports of affected males with normal urinary creatine levels suggest that repeat urine testing maybe a cost effective method to identify false positives and streamline follow up testing for CrT deficiency.

67. Hepatic mtDNA depletion syndrome caused by novel mutations in MPV17 gene encoding a mitochondrial inner membrane protein. Q. Zhang[a], N. Yazigi[b], E.S. Schmitt[a], D. Kerr[c], C.L. Hoppel[c], P.C. Chou[a], J. Wang[a], M.A. Puchowicz[c], D. Adams[d], N. Leslie[b], E.E. Baldwin[e], R.G. Boles[e,f], W.J. Craigen[a], L.J. Wong[a]. [a]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA; [b]Department of Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA; [c]The Center for Inherited Disorders of Energy Metabolism, Case Western Reserve University School of Medicine, and Rainbow Babies and Children's Hospital, Cleveland, OH, USA; [d]Department of Pediatrics, Section of Genetics and Metabolism, Albany Medical Center, Albany, New York, USA; [e]Division of Medical Genetics, The Saban Research Institute, Children's Hospital, Los Angeles, CA, USA; [f]Department of Pediatrics, University of Southern California, Keck School of Medicine, Los Angeles, CA, USA.

Background: A growing number of patients with respiratory chain (RC) dysfunction due to mitochondrial DNA (mtDNA) depletion have been reported. The defective genes identified are mostly involved in the biosynthesis of mtDNA. The onset of disease usually occurs during the first months of life with a fatal outcome. The molecular defects in at least three nuclear genes are known to cause mtDNA depletion in liver with central nervous system involvement. These include DNA polymerase gamma (POLG), deoxyguanosine kinase (DGUOK), and MPV17. Mutations in POLG gene have been associated with Alpers syndrome, which is characterized by intractable seizures and liver failure. DGUOK deficiency causes more tissue-specific fatal infantile liver disease and encephalopathy. Mutations in MPV17, encoding an inner mitochondrial membrane protein, were recently reported in patients with infantile hepatic mtDNA depletion. *Objective:* In order to understand the importance of the MPV17 gene in the molecular etiology of patients with liver failure and an apparent mitochondrial respiratory chain disorder, we sequenced the coding exons of the gene

in 80 patients. *Results:* Four patients with deleterious mutations were identified. To date, only four MPV17 mutations have been reported in three unrelated families with infantile hepatic mtDNA depletion and in five American Navajo families with neurohepatopathy. Three of our patients with MPV17 mutations presented at infancy with liver failure and died early. One patient had liver transplantation at age 5 months and is still living at age 7 months. Sequence analysis of the MPV17 gene revealed a homozygous nonsense mutation, W69X, in two Jordanian siblings, a homozygous missense mutation, R50W, in a Mexican patient, and compound heterozygous microdeletions in a European Caucasian patient. Three of the mutations are novel. Electron transport chain enzymes in affected tissues were deficient, and mtDNA copy numbers were below 20% of mean. *Conclusions:* Our results suggest that mutations in the MPV17 gene occur in all ethnic groups and may not be uncommon in patients with infantile hepatic mtDNA deletion syndrome.