Program for SIMD Annual Meeting  
February 27–March 2, 2011 Asilomar Conference Center, Pacific Grove, CA

**Sunday, February 27, 2011**

1:00–6:00 PM  
Meeting registration — Phoebe A. Hearst Social Hall

3:00–9:00 PM  
Poster board and vendor set up — Fred Farr Forum and Kiln

3:00–8:00 PM  
Asilomar check in — Phoebe A. Hearst Social Hall

3:00–6:00 PM  
**SIMD Board meeting — Scripps**

6:00 PM–8:00 PM  
Dinner and Welcome reception — Crocker Dining Hall

**Scientific Session 1**  
8:00 AM–12:15 PM  
**Education for metabolic disorders — Merrill Hall**

Moderator: Mark Korson M.D.

8:00–8:20 AM  
The NAMA experience  
Jerry Vockley, M.D., Ph.D.  
*University of Pittsburgh, Pittsburgh, PA*

8:20–8:50 AM  
Laboratory training in inborn errors of metabolism  
Tina Cowan, Ph.D.  
*Stanford University SOM, Palo Alto, CA*

8:50–9:20 AM  
Metabolic Outreach Service: A comprehensive approach to metabolic education, care and recruitment  
Mark Korson, M.D.  
*Tufts University School of Medicine, Boston, MA*

**Monday, February 28, 2011**

7:00 AM–8:00 AM  
Breakfast — Crocker Dining hall

8:00 AM–5:30 PM  
Registration — Merrill Hall  
Poster set up — Fred Farr Forum and Kiln

**Scientific Session 2**  
8:00 AM–12:15 PM  
**Disorders of the carnitine cycle and fatty acid oxidation — Merrill Hall**

Moderator: Nicola Longo, M.D., Ph.D.

8:00–8:30 AM  
Carnitine and carnitine palmitoyl transferase II deficiency  
Nicola Longo, M.D., Ph.D.  
*University of Utah, Salt Lake City, UT*

8:30–9:00 AM  
Hypoglycemia in children: Interactions between the endocrine system, fatty acid oxidation and ketone bodies (SCHAD, CACT, CPT-1 and HMG-CoA synthase deficiency)  
Charles Stanley, M.D.  
*Children’s Hospital of Philadelphia, Philadelphia, PA*
9:00–9:30 AM  Challenges in the diagnosis and management of VLCAD and trifunctional protein deficiencies
Cary Harding, M.D.
Oregon Health & Science University, Portland, OR

9:30–9:50 AM  Genotype–phenotype–metabolite correlations in MCAD deficiency
Georgianne Arnold, M.D.
University of Pittsburgh, Pittsburgh, PA

9:50–10:05 AM  Short chain acyl-CoA dehydrogenase deficiency and other rare disorders of metabolism with elevated C4-carnitine detected by tandem mass spectroscopy newborn screening
Dwight Koebert, M.D., Ph.D.
Duke University Medical Center, Durham, NC

10:05–10:45 AM  AM coffee break — Exhibits open — Fred Farr Forum and Kiln

Scientific Session 3
10:45 AM–12:00 PM  GMDI SESSION: Advances in medical foods — Merrill Hall
Moderator: Amy C Cunningham M.S., L.D.N., R.D.

10:45–11:00 AM  Medical foods: History and future directions
Dianne Frazier, Ph.D., R.D.
University of North Carolina at Chapel Hill, Chapel Hill, NC

11:00–11:20 AM  Glycomacropeptides — GMP: A new option for PKU diet management
Sandy Van Calcar — Ph.D., R.D.
Waisman Center, University of Wisconsin-Madison, Madison, WI

11:20–11:40 AM  Competitive inhibition of large neutral amino acids as an alternate treatment modality in inborn errors
Steve Yannicelli, Ph.D., R.D.
Nutricia North America, Valencia, CA

11:40–12:00 noon  Panel discussion: How do we make a choice?
Dianne Frazier Ph.D., R.D., Fran Rohr M.P.H, R.D., Amy C. Cunningham M.S., R.D.

12:00–1:00 PM  Lunch — Crocker Dining Hall

Scientific Session 4
1:15–5:15 PM  Session 4 Contributed Papers— Merrill Hall
Presentations from Travel Award Winners, the SIMD Hyperion and Ucyclyd Fellows, and the 2010 Shapira Award Winner
15 presentations — 15 min each

1:15–1:30 PM  Novel application of bortezomib in abrogating antibodies against ERT in Pompe disease: A strategy for broad clinical application in diseases treated with therapeutic proteins
Suhrad Banugaria, M.B.B.S.
Duke University Medical Center, Durham, NC

1:30–1:45 PM  Substrate oxidation and cardiac workload during exercise in long chain fatty acid oxidation disorders
Annie Behrend, M.S., RD.
Oregon Health & Science University, Portland, OR

1:45–2:00 PM  Detailed phenotype and long-term outcome of “early-onset” cblC disease
Nuria Carrillo-Carrasco, M.D.
NHGRI/NIH, Bethesda, MD

2:00–2:15 PM  Biochemical, molecular, and clinical characteristics of children with short chain acyl-CoA dehydrogenase deficiency detected via newborn screen in the state of California
Natalie Gallant, M.D.
University of California at Los Angeles, Los Angeles, CA

2:15–2:30 PM  Rescue of medium-chain acyl-CoA dehydrogenase protein activity by small molecule compounds and synthetic peptides: Implications for future treatment
Heejung Kang, MSc.
University of Pittsburgh, Pittsburgh, PA
2:30–2:45 PM  Muscle targeted transgene expression rescues the lethal phenotype of Mut knockout mice
Irini Manoli, Ph.D.
NHGRI/NIH, Bethesda, MD

2:45–3:00 PM  Presentation from 2010 Hyperion Fellow
Nitric oxide production in subjects with MELAS syndrome and the effect of arginine and citrulline supplementation: Interim results
Ayman El-Hattab, M.D.
Baylor College of Medicine, Houston, TX

3:00–3:30 PM  PM coffee break — Fred Farr Forum and Kiln

3:30–3:45 PM  A breakthrough toward establishing a murine model for n-acetylglutamate synthase (NAGS) deficiency
Khulkar Mirzozoda
Children’s National Medical Center, Washington, DC

3:45–4:00 PM  Exome sequencing and ex-vivo metabolic flux analysis as a diagnostic platform for inborn errors of metabolism
Andrew Mullen
The University of Texas Southwestern Medical Center, Dallas, TX

4:00–4:15 PM  The emerging phenotype of long-term infantile Pompe survivors on enzyme replacement therapy
Sean Prater, M.Res.
Duke University Medical Center, Durham, NC

4:15–4:30 PM  Combined screening for lysophosphatidyl cholines and acyl carnitines in dried blood spots
Yana Sandlers, M.Sc., Ph.D.
Kennedy Krieger Institute, Johns Hopkins University SOM, Baltimore, MD

4:30–4:45 PM  Long chain acyl-CoA dehydrogenase deficiency: A new inborn error of metabolism manifesting as congenital surfactant deficiency
Kristen Suhrie, M.D.
The Children’s Hospital of Pittsburgh, Pittsburgh, PA

4:45–5:00 PM  Murine very-long-chain acyl-CoA dehydrogenase (VLCAD) cardiac-specific knockout reveals essential role in response to cold stress
Dingding Xiong, M.D., Ph.D.
Cincinnati Children’s Hospital Medical Center, Cincinnati, OH

5:00–5:15 PM  Presentation from 2010 Ucyclyd Fellow
Tandem mass spectrometry for the diagnosis of free sialic acid disorders
Lidong Zhai, Ph.D.
University of Alabama at Birmingham, Birmingham, AL

5:15–5:30 PM  Presentation from the 2010 Emmanuel Shapira Award Winner
Allelic diversity in MCAD deficiency: The biochemical classification of 54 variants identified during 5 years of ACADM sequencing
Emily H. Smith, Ph.D.
Mayo Clinic, Rochester, MN

6:00–7:00 PM  Dinner — Crocker Dining Hall

7:15–9:30 PM  Exhibits open — Fred Farr Forum and Kiln
Posters Attended by Authors — Fred Farr Forum, Kiln, Afterglow living room and Hearth living room
Wine and dessert served

Tuesday, March 1, 2011
7:00 AM–8:00 AM  Breakfast — Crocker Dining hall

8:00 AM–12:00 PM  Registration — Merrill Hall
Scientific Session 5
8:00–10:00 AM

Homocystinuria and disorders of methionine metabolism — Merrill Hall
Moderator: Jan P. Kraus Ph.D.

8:00–8:40 AM
Disorders of homocysteine transsulfuration and remethylation:
Introduction and classic homocystinuria
Jan P. Kraus, Ph.D.
University of Colorado, Aurora, CO

8:40–9:10 AM
Clinical characterization of patients with various forms of homocysteine elevation in blood or urine
David Rosenblatt, M.D.
McGill University, Montreal, Quebec

9:10–9:30 AM
Biochemistry and genetics of homocysteine remethylation disorders
Brian Fowler, Ph.D.
University Children’s Hospital Basel (UKBB), Basel, Switzerland

9:30–9:50 AM
Folate metabolism and MTHFR deficiency
Rima Rozen, Ph.D., F.R.S.C., F.C.C.M.G.
McGill University, Montreal, Quebec

9:50–10:10 AM
The genetic hypermethioninemias
S. Harvey Mudd, M.D.
NIMH/LGCB, Bethesda, MD

10:10–10:40 AM
AM coffee break — Exhibits open — Fred Farr Forum and Kiln

Scientific Session 6
10:40 AM–12:00 PM

Cofactor deficiencies affecting the brain — Merrill Hall
Moderator: Barry Wolf, M.D., Ph.D.

10:40–11:20 AM
Biotin holocarboxylase synthetase, biotinidase deficiency and biotin transporter disorders:
What do we know and what is still left to learn?
Barry Wolf, M.D., Ph.D.
Henry Ford Hospital, Detroit, MI

11:20–11:40 AM
Cerebral folate deficiency
Keith Hyland Ph.D.
Medical Neurogenetics, Atlanta, GA

11:40 AM–12:00 PM
Molybdenum cofactor and sulfite oxidase deficiency
Günter Schwarz, Ph.D.
Institute of Biochemistry and Center for Molecular Medicine,
Cologne University, Koeln, Germany

12:00–1:00 PM
Lunch — Crocker Dining Hall

1:00–6:00 PM
Free afternoon

1:00–6:00 PM
Posters and exhibits on display — Fred Farr Forum and Kiln

1:00–4:00 PM
NAMA Faculty Meeting — Triton

4:00–6:00 PM
NAMA Reception — by invitation only — Scripps

6:00–7:00 PM
Dinner — Crocker Dining Hall

7:30–8:00 PM
Invited Address — Merrill Hall
The NIH Undiagnosed Diseases Program
William A. Gahl, M.D., Ph.D., Past-President, SIMD
Clinical Director, NHGRI/Director, NIH Undiagnosed Diseases Program

8:00–9:30 PM
SIMD Business Meeting and Award Presentations
2010 Emmanuel Shapira SIMD Award (First Author of Best Publication in MGM)
Neil Buist Award (Best Oral Presentation by a Trainee)
### Wednesday, March 2, 2011

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<td>7:00–8:00 AM</td>
<td>Breakfast — Crocker Dining Hall</td>
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<tr>
<td><strong>Scientific Session 7</strong></td>
<td>Clinical trials and patient registries in inborn errors of metabolism — Merrill Hall</td>
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<tr>
<td>8:00–10:00 AM</td>
<td><strong>Moderator:</strong> Douglas Kerr M.D., Ph.D.</td>
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<tr>
<td>8:00–8:30 AM</td>
<td>Why are there no proven therapies for genetic mitochondrial diseases?</td>
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<td><strong>Peter Stacpoole,</strong> Ph.D., M.D.</td>
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<td>University of Florida, Gainesville, FL</td>
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<tr>
<td>8:30–9:00 AM</td>
<td>Liver cell transplantation (LCT) in urea cycle defects</td>
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<td><strong>Georg F. Hoffmann,</strong> M.D., Ph.D.</td>
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<td>University of Heidelberg, Heidelberg, Germany</td>
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<tr>
<td>9:00–9:30 AM</td>
<td>Clinical trials in urea cycle disorders</td>
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<td><strong>Sandesh Sreenath-Nagamani,</strong> M.D.</td>
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<td>Baylor College of Medicine, Houston, TX</td>
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<td>9:30–10:00 AM</td>
<td>New therapies for lysosomal storage disorders (LSDs)</td>
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<td><strong>Greg Grabowski,</strong> M.D.</td>
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<td>Cincinnati Children's Hospital, Cincinnati, OH</td>
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<td>10:00–10:30 AM</td>
<td>AM coffee break — Fred Farr Forum and Kiln</td>
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<td><strong>Scientific Session 8</strong></td>
<td>Registries and long-term outcome of metabolic disorders — Merrill Hall</td>
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<td>10:30 AM–11:45 AM</td>
<td><strong>Moderator:</strong> Lorenzo Botto M.D.</td>
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<td>10:30 AM–10:55 AM</td>
<td>Phenylketonuria registry</td>
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<td><strong>Shideh Moﬁdi,</strong> M.S., R.D., C.S.P.</td>
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<td>Maria Fareri Children’s Hospital — Westchester Medical Center, Valhalla, NY</td>
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<td>10:55 AM–11:20 AM</td>
<td>Surveillance programs for metabolic disorders: Clinical and</td>
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<td>public health aspects</td>
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<td><strong>Lorenzo Botto,</strong> M.D.</td>
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<td>University of Utah, Salt Lake City, UT</td>
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<td>11:20 AM–11:45 AM</td>
<td>Update on the urea cycle registry</td>
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<td><strong>Marshall Summar,</strong> M.D.</td>
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<td>Children's National Medical Center, Washington, DC</td>
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<td>11:45–12:00 noon</td>
<td>Closing comments and meeting adjourns</td>
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<td><strong>Nicola Longo,</strong> M.D., Ph.D.</td>
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<td>University of Utah, Salt Lake City, UT</td>
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ATTENTION SIMD MEMBERS:

Dues payments can now be made online at www.simd.org. Please check to see if your dues are current.

2011 dues notices will be sent out in May 2011.
The 2010 Emmanuel Shapira SIMD Award

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

Dr. Shapira's clinical and research interests were focused on inborn errors of metabolism. He made numerous contributions to the field of biochemical genetics and was a dedicated physician to his patients and a supportive and compassionate teacher to his students. His consistent participation in the annual meetings of the Society contributed both in knowledge and in spirit by making the meetings both scientifically stimulating and enjoyable. This award, that bears his name and the name of the Society that he cherished, is intended to recognize high-quality work in the field that he so loved. A $1000 prize is awarded annually to the first author of the winning paper chosen by a committee that includes several members of the SIMD and the Editor-in-Chief of MGM.

This year the winner of the Emmanuel Shapira award is Emily H. Smith, Ph.D. for her article entitled “Allelic diversity in MCAD deficiency: The biochemical classification of 54 variants identified during 5 years of ACADM sequencing”, Emily H. Smith, Cheryl Thomas, David McHugh, Dimitar Gavrilov, Kimiyo Raymond, Piero Rinaldo, Silvia Tortorelli, Dietrich Matern, W. Edward Highsmith and Devin Oglesgee: Mol. Gen. & Metab. 100 (3), July 2010, p. 241-250. Dr. Smith is a Clinical Molecular Genetics Fellow in the Clinical Molecular Genetics Laboratory at the Mayo Clinic in Rochester, MN. She will give a brief oral presentation at the annual meeting on Monday, February 28, 2011.

Past Winners of the Emmanuel Shapira Award:

2003 — Elena Tartaglini, Ph.D.
        Judith C. Fleming, Ph.D.
2004 — Gerard T. Berry, M.D.
2006 — Randy Chandler
        Melanie Gillingham, Ph.D., R.D.
2007 — Eric Goetzman, Ph.D.
2008 — no award given
2009 — Georgianne Arnold, M.D.

The 2011 Neil Buist Award

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

Past winners are:

2004 — Lina S. Correa-Cerro, M.D., Ph.D.
2005 — Amanda Helip-Wooley, Ph.D.
2007 — Miaoo He, Ph.D.
2008 — Ralph DeBerardinis, M.D., Ph.D.
2009 — no award given this year due to ICIEM conference
2010 — Sander M. Houton, Ph.D.
2011 Travel Award Winners

The winners of the 2011 Travel awards are:

Suhrad G. Banugeria
Annie Behrend
Nuria Carrillo-Carrasco
Natalie Gallant
Heejung Kang
Irini Manoli
Khulkar Mizozoda
Andrew Mullen
Sean Prater
Yana Sandlers
Kristen Suhrie
Dingding Xiong

Each recipient received a travel award in the amount of $1000. All winners will give a brief oral presentation on Monday afternoon, February 28, 2011 at the SIMD Annual meeting. All have submitted abstracts which are printed in this issue.

Trainee travel awards were supported in part by a grant from the National Institutes of Health, The Eunice Kennedy Shriver National Institute of Child Health and Human Development grant 5R13HDO62129.
Travel Award Recipients/SIMD Fellows
Short Oral Presentations
To be presented Monday, February 28, 2011

1. **Novel application of bortezomib in abrogating antibodies against ERT in Pompe disease: A strategy for broad clinical application in diseases treated with therapeutic proteins**


| 1 | Suhrad Banugaria, M.B.B.S. | Novel application of bortezomib in abrogating antibodies against ERT in Pompe disease: A strategy for broad clinical application in diseases treated with therapeutic proteins |
| 2 | Annie Behrend, M.S., R.D. | Substrate oxidation and cardiac workload during exercise in long chain fatty acid oxidation disorders |
| 3 | Nuria Carrillo-Carrasco, M.D. | Detailed phenotype and long-term outcome of “early-onset” chILD disease |
| 4 | Natalie Gallant, M.D. | Biochemical, molecular, and clinical characteristics of children with short chain acyl-CoA dehydrogenase deficiency detected via newborn screen in the state of California |
| 5 | Heejung Kang, M.Sc. | Rescue of medium-chain acyl-CoA dehydrogenase protein activity by small molecule compounds and synthetic peptides: Implications for future treatment |
| 6 | Irini Manoli, Ph.D. | Muscle targeted transgene expression rescues the lethal phenotype of Mut knockout mice |
| 7 | Khulkar Mirzozoda | A breakthrough toward establishing a murine model for n-acetylglutamate synthase (NAGS) deficiency |
| 8 | Andrew Mullen | Exome sequencing and ex vivo metabolic flux analysis as a diagnostic platform for inborn errors of metabolism |
| 9 | Sean Prater, M.Res. | The emerging phenotype of long-term infantile Pompe survivors on enzyme replacement therapy |
| 10 | Yana Sandlers, MSc., Ph.D. | Combined screening for lysophosphatidyl cholines and acyl carnitines in dried blood spots |
| 11 | Kristen Suhrrie, M.D. | Long chain acyl-CoA dehydrogenase deficiency: A new inborn error of metabolism manifesting as congenital surfactant deficiency |
| 12 | Dingding Xiong, M.D., Ph.D. | Murine very-long-chain acyl-CoA dehydrogenase (VLCAD) cardiac-specific knockout reveals essential role in response to cold stress |

**Background:** Enzyme replacement therapy (ERT) is now available for a multitude of disorders. However, therapeutic enzymes are potentially immunogenic, inducing antibodies, leading to its decreased efficacy. Pompe disease (PD) is a lysosomal disorder caused by a deficiency of acid alpha-glucosidase. Infantile PD is characterized by cardiomyopathy, hypotonia, respiratory insufficiency and death by age 1–2 years. Since the availability of ERT with alglucosidase alfa, the history of this disease has changed, with prolonged survival and enhanced quality of life. However, the immune response to ERT remains a challenge. Tolerance-inducing therapies have been successful in at-risk (CRIM-negative) patients in the naive setting or after early exposure to ERT. Drugs that target B cells and T cells such as rituximab and methotrexate have been used successfully to prevent development of antibody titers. Once high-titer antibodies are formed, tolerization therapy has uniformly failed to lower antibodies. Notably lacking are drugs that target long-lived plasma cells, whose elimination is vital in reversing entrenched immune responses. Bortezomib, an FDA-approved therapy for multiple myeloma, is a 26S proteasome inhibitor which targets antibody-producing plasma cells.

**Methods:** We report data from Pompe knock-out mice involving the use of bortezomib in the setting of a well-established immune response. We also report clinical data from a Pompe infant with an entrenched immune response treated with bortezomib in combination with other immunomodulatory agents (rituximab and methotrexate).

**Results:** After administration of 4 doses of bortezomib, there was a significant reduction in anti-rhGAA antibody titers in Pompe KO mice. Subsequent administration resulted in an even further decline. Dramatically, the first use of bortezomib in Pompe infant suppressed the high antibody titers developed due to long-term ERT exposure. Bortezomib was well-tolerated without side-effects and a clinical benefit was noted. Antibody titer in Patient 1 had dropped from 1:204,800 to 1:6400. Along with the reduction in the antibodies, there was a significant improvement in terms of cardiac and motor status. With rising antibody titer, left ventricular mass index (LVMI) which had increased from 74 g/m² to 360 g/m² (normal — 65 g/m²) then dropped to 104 g/m² following immunomodulation with bortezomib.

**Conclusions:** This response to plasma cell depletion with bortezomib is unprecedented in the treatment of infants with Pompe disease following the formation of high titer anti-GAA antibodies. We are currently accruing additional data on experience with bortezomib in combination with other agents to suppress and induce tolerance in the setting of an established immune response in preclinical and clinical setting. The novel use of bortezomib to reverse an entrenched immune response in patients with Pompe disease is a breakthrough in the field. It has implications for other diseases treated with therapeutic proteins such as hemophilia, mucopolysaccharidosis and kidney failure, where the immune response is a challenge and results in clinical decline.
2. Substrate oxidation and cardiac workload during exercise in long chain fatty acid oxidation disorders

Behrend AM¹,², Harding C¹, Elliot D³, Sahn D⁴, Shoemaker J⁵, Matern D⁶, Gillingham MB¹,²

¹Department of Medical & Molecular Genetics, Oregon Health & Science University, Portland, OR, USA
²Graduate Programs of Human Nutrition, Oregon Health & Science University, Portland, OR, USA
³Department of Health Promotion & Sports Medicine, Oregon Health & Science University, Portland, OR, USA
⁴Pediatric Cardiology, Oregon Health & Science University, Portland, OR, USA
⁵Biochemistry & Molecular Biology, St Louis University, St Louis, MO, USA
⁶Biochemical Genetics, Mayo Clinic, Rochester, MN, USA

Background: Inherited disorders of long-chain fatty acid oxidation (FAO) inhibit the ability to oxidize long-chain fatty acids (LCFAs) for energy production. A frequent complication of FAO disorders is rhabdomyolysis, which often follows a bout of exercise and leads to exercise avoidance in some individuals. Medium-chain triglyceride (MCT) supplementation bypasses the block in long-chain FAO, and may provide an alternative energy substrate to the exercising muscle, reducing the risk of adverse metabolic events.

Objectives: To determine the influence of isocaloric MCT vs. carbohydrate (CHO) supplementation prior to exercise on substrate oxidation and cardiac workload during exercise in participants with carnitine palmitoyltransferase 2 (CPT2), very long-chain acyl-CoA dehydrogenase (VLCAD) and long-chain 3-hydroxyacyl CoA dehydrogenase (LCHAD) deficiencies.

Design and methods: Two 45-minute, moderate intensity treadmill exercise studies were completed by subjects (n = 11) in a randomized cross-over design. An isocaloric oral dose of CHO (1 g/kg lean body mass (LBM)) or MCT-oil (0.5 g/kg LBM) was administered prior to exercise; hemodynamic and metabolic indices were assessed during exertion. Changes in exercise tolerance and cardiac workload were compared between interventions.

Results: A significant decrease in respiratory exchange ratio (RER), and steady state heart rate was observed during the exercise test pretreated with MCT. A significant increase in circulating ketone bodies and a simultaneous decrease in the generation of glycolytic intermediates was observed in serum following the exercise test pretreated with MCT. As expected, plasma free fatty acid (FFA) concentrations rose similarly during both exercise tests.

Conclusions: MCT supplementation prior to exercise in subjects with LCHAD, CPT2 or VLCAD deficiency increased the oxidation of medium chain fats by the liver and potentially by the exercising muscle during that bout of exercise. The decrease in heart rate with no change in systolic blood pressure lowered the double product estimate of cardiac ejection fraction for the same VO₂ and amount of work performed when compared to CHO supplementation. We propose that MCT may expand the usable energy supply, particularly in the form of ketone bodies, and during a period of increased workload improve the oxidative capacity of the heart. This has not been reported in normal athletes consuming MCT and we believe that this effect is unique to subjects with an inherited defect in long-chain FAO. In practice, MCT supplementation of approximately 0.3–0.4 g/kg total body weight mixed with a CHO containing beverage will allow subjects with a long-chain FAO disorder to safely exercise at a moderate intensity for up to an hour.

3. Detailed phenotype and long-term outcome of “early-onset” chlC disease

N. Carrillo-Carrasco¹, J. Sloan¹, I. Manoli¹, N. Hauser¹, W.M. Zein², A. Gropman³, P. Tanpaiboon⁴, J. Graf⁵, E. Baker³, J. Snow³, S.M. Paul³, B. Brooks², C.P. Venditti¹

¹National Human Genome Research Institute, NIH, USA
²National Eye Institute, NIH, USA
³Clinical Research Center, NIH, USA
⁴Children's National Medical Center, USA
Background: Combined methylmalonic acidemia and homocystinuria, cblC type, is a multi-systemic disorder that causes growth impairment, developmental delay, neurologic, ophthalmologic and cardiovascular abnormalities, and decreases the lifespan of affected patients. It is caused by mutations in the MMACHC gene resulting in impaired intracellular synthesis of adenosylcobalamin and methylcobalamin, cofactors for the methylmalonyl-CoA mutase and methionine synthase enzymes.

Methods: We evaluated 22 patients with early-onset cblC disease (age range, 1 to 27 years) through NIH study 04-HG-0127 (http://clinicaltrials.gov identifier: NCT00078078) “Clinical and Basic Investigations of Methylmalonic Acidemia and Related Disorders” between 2004 and 2010. All patients had complementation studies and sequencing of the MMACHC gene.

Results: The diagnosis was made at the median age of 2.7 months based on an abnormal newborn screening (n = 3) or clinical suspicion because of poor feeding and failure to thrive (n = 18), or progressive encephalopathy (n = 8). Patients diagnosed after 2 months of age also had seizures (n = 2), visual concerns (n = 3) and megaloblastic anemia (n = 1). Two patients were diagnosed prenatally because of an affected sibling. Clinical investigations found intellectual disabilities in all patients ranging from ADHD and mild developmental delay to severe impairment. A characteristic eye disease consisting of maculopathy and retinal degeneration, and in some patients, optic nerve atrophy was present in various stages in almost all patients. The median visual acuity was 20/500 (profound low vision range). Neurologic manifestations included a seizure disorder in 18%, infantile spasms (n = 3) and ischemic stroke (n = 1). Although growth improved after the initiation of treatment, 26% of patients had short stature; decreased growth was associated with protein-restriction, (odds ratio = 7.5), but not with MMA or tHcy levels. Other findings include microcephaly in 37%; decreased bone mineral density with an average z score of −1.62 with 4 patients in the osteoporotic range; patients had borderline (n = 6) and elevated (n = 6) triglycerides with an average level of 225 mg/dl (range 75–1028 mg/dl); intermittent transaminitis (n = 8), and mild hepatomegaly (n = 4) or fatty infiltration of the liver (n = 6) seen on ultrasound. Cardiac abnormalities were found in 6 patients and included Ebstein's anomaly (n = 1), global hypokinesis (n = 2) or hypertrabeculation of the left ventricle (n = 2), and left ventricular noncompaction associated with hypydrosis (n = 1). Management strategies were highly variable; all received parenteral OHCbl (IM or SQ, with different dosing and frequency of administration), betaine and carnitine, while 77% received folate or folic acid and 27% aspirin; other supplements included methionine (n = 3), valine (n = 1), methylcobalamin (n = 1), choline (n = 1), and creatine (n = 1). Protein intake was restricted in 41% of patients to an average of 16.02 g/kg/d (range: 0.49–151 g/kg), C3 of 2.75 g/mol/l (range: 0.59–11.41 g/mol/l), tHcy of 70.8 μmol/l (range: 22–134 μmol/l) and methionine of 22 μmol/l (4–53 μmol/l). Patients with higher plasma B12 levels had lower tHcy (r = −0.53, p < 0.0001) and MMA (r = −0.38, p = 0.005) levels.

Conclusion: Detailed clinical characterization of patients with cblC disease will establish a baseline to evaluate the efficacy of interventions such as newborn screening, optimal OHCbl and betaine dosing, nutritional management and other therapies that might improve the outcome of this inborn error of metabolism.

4. Biochemical, molecular, and clinical characteristics of children with short chain acyl-CoA dehydrogenase deficiency detected via newborn screen in the state of California

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Background: Short-chain acyl-CoA dehydrogenase deficiency (SCADD) is an autosomal recessive inborn error of mitochondrial fatty acid oxidation with highly variable biochemical, genetic, and clinical characteristics. SCADD has been associated with accumulation of butyryl-CoA byproducts, including butyrylcarnitine (C4), butyrylglycine, ethylmalonic acid (EMA), and methylsuccinic acid (MS) in body fluid and tissues. Differences in genotype frequency have been shown between patients diagnosed clinically vs. those diagnosed via newborn screen (NBS). Moreover, while patients diagnosed clinically have a variable clinical presentation including developmental delay, ketotic hypoglycemia, epilepsy and behavioral disorders, studies suggest patients diagnosed via NBS are largely asymptomatic. Scant information is published about the biochemical, genetic and clinical outcome of SCADD patients diagnosed via NBS.

Methods: We collected California NBS, follow-up biochemical levels, and ACADS mutation data from September, 2005 through April, 2010. We retrospectively reviewed available data on SCADD cases diagnosed by NBS from three newborn screen referral centers for clinical outcomes.

Results: During the study period, 2,632,058 newborns were screened and 74 confirmed SCADD cases were identified. No correlations between initial (NBS) C4 value and follow-up biochemical markers (C4, EMA or MS levels) were found in the 74 cases studied. We found significant correlation between follow-up C4 vs. urine EMA (R²=0.51, p = 0.0001) and follow-up C4 vs. urine MS (R²=0.45, p < 0.0001). Of 21 cases where ACADS gene sequencing was performed: 6 had two or more disease-causing mutations; 7 were compound heterozygotes for a deleterious mutation and common polymorphism; 7 were homoyzogous for the common polymorphism c.625G>A; and 1 was heterozygous for c.625G>A. A statistically significant increase in mean EMA level was noted in patients with two or more deleterious mutations vs. mutation heterozygotes (p = 0.02) or common polymorphism homoyzogotes (p = 0.005). Clinical outcome data was available in 31 patients. None developed epilepsy or behavioral disorders, and only one patient had isolated speech delay. Over time, parents refused long-term follow-up in a significant number of cases (12/31). Hypoglycemia (blood glucose ≤ 60 mg/dl) occurred in two patients, both solely in the neonatal period: one with meconium aspiration and one instance of hypoglycemia; the other with central apnea, poor feeding, and hypotonia. The latter, a c.625G>A polymorphism homozygote, has had persistent elevations in both short- and medium-chain acylcarnitines; diagnostic workup is ongoing.

Conclusions: Our study examines the largest series to date of SCADD patients identified via NBS. Our results suggest that biochemical confirmatory tests may be useful to differentiate patients with common polymorphisms from those with disease-causing mutations. Even in those patients with disease-causing mutations, SCADD diagnosed via NBS appears to present largely as a benign condition.
5. Rescue of medium-chain acyl-CoA dehydrogenase protein activity by small molecule compounds and synthetic peptides: Implications for future treatment

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Medium chain acyl-CoA dehydrogenase deficiency (MCADD) is an inborn error of fatty acid metabolism, rivaling phenylalanine hydroxylase deficiency (PKU) as the most common biochemical genetic disorder in the United States. The overall frequency of the disease has been estimated at 1:8500 to 1:17,000 in Caucasians of mostly Northern European ancestry. MCADD patients are asymptomatic at birth but are at risk for episodes of acute, life threatening metabolic decompensation. Historically, episodes first occurred between 3 and 24 months of age in association with physiologic stress such as fasting or infection, fever, or strenuous exercise. The mortality rate during an acute crisis in previously undiagnosed patients can be as high as 20%. With the introduction of expanded newborn screening via tandem mass spectrometry, MCADD can now be identified pre-symptomatically, nearly eliminating mortality due to this disease. However, treatment requires lifelong dietary monitoring, and significant morbidity still occurs due to hospitalizations for IV glucose therapy in the face of reduced oral intake during illnesses. Thus a medication capable of relieving the metabolic block would be of great benefit to these patients. A single point mutation in the MCAD gene (G985A) has been identified in 90% of the alleles in deficient patients. This mutation substitutes a Glu for a Lys at position 304. This amino acid change in turn leads to impaired folding and/or stability of MCAD in mitochondria and eliminates most MCAD activity. Co-expression of this mutant protein with bacterial chaperonins in E. coli stabilized the mutant enzyme allowing us to determine its crystal structure. To stabilize the mutant MCAD in vivo and establish proof of principle for development of a medication, we attempted to mimic the bacterial chaperonin effect on recombinant mutant MCAD stability. Lymphoblasts homozygous for the common mutation were cultured with small molecule compounds, namely, DMSO, glycerol, betaine, trimethylamine N-oxide (TMAO), and L-proline for 2 days. MCAD activity was tested with the highly sensitive and specific electron transfer flavoprotein (ETF) fluorescence reduction assay. Incubation in the presence of two of the compounds, TMAO and glycerol, significantly increased MCAD activity. The enhancement of MCAD activity differed in lymphoblasts from two different patients. In the case of TMAO treatment, MCAD activity increased 5.5 and 35 fold, respectively, compared to control, and glycerol yielded 4 and 20 fold increases, respectively. In addition, synthetic peptides designed to target the docking site of MCAD with its endogenous electron acceptor ETF were tested for ability to stabilize the purified mutant enzyme in vitro. One peptide was found to enhance mutant MCAD activity three fold compared to the mutant MCAD alone. These results suggest that activity of the common MCAD mutant protein can be rescued by chemical chaperonins or synthetic peptides and offers insight into the development of a treatment for MCADD.

6. Muscle targeted transgene expression rescues the lethal phenotype of Mut knockout mice

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Background: Methylmalonic acidemia (MMA) is caused by deficiency of the mitochondrial enzyme methylmalonyl-CoA mutase (Mut) and results in elevations of methylmalonic acid in tissues and body fluids. Studies in knockout mice (Mut−/−) and transplanted MMA patients have suggested that a large portion of circulating methylmalonic acid derives from extrahepatic organs, particularly the skeletal muscle.

Methods: To examine the effects of restoring skeletal muscle expression of the Mut enzyme on the Mut−/− phenotype and gain insight into the efficacy of skeletal muscle-targeted therapies for MMA, we generated mice that express the Mut cDNA under the control of an insulated, muscle-specific promoter (Mut−/−;TgMut+) and studied the effects on survival, growth, metabolism, and organ-specific pathology.

Results: Mut−/−;TgIns-MCK-Mut+ mice were born in Mendelian proportions, showed 83% survival past day of life 60 (n = 40), and achieved >40% the weight of their heterozygous littermates at 1 year. Mut RNA and protein expression in Mut−/−;TgIns-MCK-Mut+ mice exceeded that seen in wild-type mice in the muscle and the heart, but was absent in the liver and kidneys. Plasma MMA levels at 3 months of age were 269 ± 76 μM (n = 5) in Mut−/−;TgIns-MCK-Mut+ mice fed a high fat and carbohydrate diet, compared to <5 μM (n = 5) in controls and >1000 μM in Mut−/− mice (n = 9). To further assess transgene function, we measured the oxidation of 1-13C propionate into 13CO2. The Mut−/−;TgIns-MCK-Mut+ mice metabolized 18.4 ± 3.6% of the label in 25 min, compared to 50.7 ± 9.8% in Mut+/− and 13.1 ± 3.7% in Mut−/−. Significant liver pathology, characterized by megamitochondria formation and decreased respiratory chain complex IV activity, was present in the Mut−/−;TgIns-MCK-Mut+ mice. Similar changes were noted in the tubular epithelial cells and were associated with a decreased glomerular filtration rate as measured by inulin clearance (n = 4).

Conclusions: Selective muscle expression of the Mut enzyme resulted in near uniform rescue of the neonatal lethal phenotype and a reduction in metabolite levels displayed by Mut−/− mice, but was unable to prevent liver and kidney pathology. This new murine model demonstrates that targeted correction of skeletal muscle can augment metabolism in MMA, but cell-autonomous and/or toxic metabolic effects may mediate hepatic and renal tubular pathology. Mut−/−;TgIns-MCK-Mut+ mice present a novel platform for the testing of liver- and/or kidney-directed gene and cell therapy for MMA, an often lethal disorder of organic acid metabolism with limited therapeutic options.

7. A breakthrough toward establishing a murine model for N-acetylglutamate synthase (NAGS) deficiency

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Background: We have recently created a novel mouse model of NAGS deficiency using the VelociGene gene ablation method via the NIH-funded KOMP resource. The entire NAGS gene has been replaced with an expression cassette consisting of the lacZ gene and a neomycin selection marker. In patients with NAGS deficiency, treatment with N-carbamylglutamate (NCG) fully restores ureagenesis by activating CPS1. Therefore, following heterozygous mating, we have attempted to rescue homozygous NAGS deficient mice by providing NCG to the pregnant females in drinking water during pregnancy. NCG, administered this way at a modest dose (~100 mg/kg/d), extended the life of homozygous pups for a period of 60 to 120 h after birth, while untreated mice died within 24 h after birth. We have subsequently been able to extend the lifespan of, at least, one NAGSko homozygous mouse pass weaning age (28 days) using a combination of NCG and l-citrulline.

Methods: After conception, heterozygous females diet has been supplemented with NCG delivered in drinking water at 1.5 mg/ml. One week before delivery, heterozygous females have been given intraperitoneal (IP) injections of NCG combined with citrulline at 180 mg/kg/day and 300 mg/kg/day respectively. At birth and thereafter, pups have been given a daily IP dose of NCG and citrulline until 16 days of age. At this age the homozygous pup has been maintained by a combination of NCG treatment in drinking water (at 1.5 mg/ml) and a daily dose of NCG and citrulline of 180 mg/kg/day and 300 mg/kg/day respectively, delivered in the form of powdered chow.

Results: By treating the pups right after birth as described above we have extended the lifespan of a NAGSko mice considerably in comparison with the previous attempts to rescue these mice. So far we have extended the lifespan of a homozygous NAGSko female for 12 days while a homozygous NAGSko male has survived pass weaning age.

Conclusions: We have shown that a combination of NCG and l-citrulline allows survival of NAGSko mice post 24 h of age. This model recapitulates clinical observations in human and seems to be suitable model of the human disorder.

8. Exome sequencing and ex-vivo metabolic flux analysis as a diagnostic platform for inborn errors of metabolism

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Background: Inborn Errors of Metabolism (IEM) are an important class of human disease. Current difficulties in the field include the inability to identify causative molecular defects in a subset of individuals and an incomplete understanding of known disorders. We have developed ex-vivo methods to quantify metabolic fluxes in primary human skin fibroblasts, allowing us to characterize metabolic disturbances using an efficient analytical platform based on mass spectrometry. Additionally, we have combined this with exome sequencing as a novel diagnostic paradigm for IEMs and have applied this approach to two siblings with an undiagnosed mitochondrial myopathy.

Results: Two siblings were brought to the UT Southwestern Neuromuscular center with symptoms of early muscle fatigue and exercise intolerance. Baseline metabolic testing was uninformative and a muscle biopsy revealed a low oxidative capacity and mitochondrial proliferation. A cycle exercise test revealed profound increases in blood pyruvate with increasing workload while lactate levels rose at a rate indistinguishable from normal controls. As a result, the patients displayed an extremely low lactate/pyruvate ratio, suggesting a failure to oxidize pyruvate and/or a defect in converting pyruvate to lactate (Figure 1 A, B, C). All conventional biochemical and genetic tests including tests for PDH and LDH deficiency have failed to assign a diagnosis. To identify the causative genetic defect we carried out exome sequencing and ex-vivo metabolic profiling with primary skin fibroblasts. These patients-derived cells have allowed us to monitor the metabolism of isotopically-labeled glucose by Gas Chromatography–Mass Spectrometry (GC/MS). Using this method, we have shown that the ability of glucose derived pyruvate to enrich the tricarboxylic acid cycle intermediate citrate is greatly reduced in patient cells, consistent with the oxidative defect seen in the muscle (Fig. 2, A). Concomitant exome sequencing revealed a number of genes containing mutations in both patients. Loss of function experiments using stable and transient gene silencing in control skin fibroblasts allowed us to prioritize further analysis of the candidate genes (Fig. 2, B). Ongoing work will attempt to correct the oxidative defect in the patient cells by expressing wild-type alleles of the candidate genes.

Figure 1. Cycle exercise test in controls and two patients. (A and B) Concentrations of lactate and pyruvate in the blood of control subjects and brother/sister with undiagnosed myopathy. (C) Lactate/pyruvate ratio.
Conclusion: We have identified an unusual mitochondrial myopathy and are using exome sequencing and ex vivo flux profiling to identify the genetic defect and characterize its effect in fibroblasts. We hope this approach will allow us to identify the causative genetic defects responsible for other IEMs and to better characterize the metabolic effects of known disorders.

9. The emerging phenotype of long-term infantile Pompe survivors on enzyme replacement therapy

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Background: The natural history of untreated infantile Pompe disease is limited as the median age of death in this lethal disorder is approximately 12.7 months. With the advent of enzyme replacement therapy (ERT) with alglucosidase alfa (Myozyme®), the clinical course of the disease has improved significantly. Improved survival has also led to a number of unanswered questions regarding the clinical course of treated disease. Currently, there is a paucity of data in the published literature pertaining to the physical and cognitive phenotype of older infantile Pompe survivors.

Methods: We report a series of 9 (7 M, 2 F) of the oldest infantile Pompe survivors treated with ERT ranging from 4 to 11 years of age. All patients had symptom onset within the first 6 months of life and were noted to have severe cardiomyopathy.

Results: Median age at ERT initiation was 4.6 months (range, 0.3 to 6.3 months). Patients received alglucosidase alfa at 20 to 40 mg/kg biweekly and all patients tolerated ERT. At most recent follow-up, 7/9 patients were independently ambulatory without assistive devices. None of the 9 patients required invasive ventilation or supplemental oxygen. All 9 patients were following their typical growth curves. The majority of patients had some residual motor weakness: 8/9 and 7/9 patients had neck flexor and dorsiflexor weakness, respectively; all 9 required AFOs. Additional notable findings included myopathic facies (9/9), hypernasal speech (8/9), velopharyngeal weakness (8/8), sensorineural hearing loss (6/9), osteopenia (4/8) and ptosis (3/9). Despite the existence of speech and/or gross motor delays (found in a total 8/9 patients), these children remain cognitively intact. Further, although there are some phenotypic similarities shared with late-onset patients, there also exist key distinguishing features.

Conclusions: This is the largest cohort of long-term survivors of infantile Pompe disease and provides a contextual basis for the emerging phenotype and relevant issues in clinical management. Continued systematic follow-up is needed to better characterize this emerging phenotype and to allow for improved patient management.

10. Combined screening for lysophosphatidyl cholines and acyl carnitines in dried blood spots

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Background: While measurement of acyl carnitines from dried blood spots is routinely performed by many state newborn screening laboratories, newborn screening for X-linked adrenoleukodystrophy (X-ALD) and peroxisomal biogenesis disorders (PBD) has not been implemented. Recently W. Hubbard et al. demonstrated that 26:0-lysophosphatidyl choline (26:0-lyso PC) is a target diagnostic metabolite for X-ALD and other peroxisomal disorders [1, 2].

In this study, we developed a new one step extraction procedure that simultaneously extracts the acyl carnitines and the lyso PCs from dried blood spots. Further analysis of these metabolites has been performed by two different LC–MS/MS methods.

Methods: 1/8 inch DBS punches were extracted with 100 μl of methanol containing isotope labeled acyl carnitines and D4-26:0-lyso PC as internal standards. A 50 μl aliquot of the extract was directly used for LC–MS/MS. A second aliquot was derivatized with butanol-HCl and processed for acyl carnitine measurements according to the slightly modified published methods. 26:0-lyso PC was analyzed by positive ESI LC–MS/MS. The multiple transition reaction (MRM) monitored for detection and quantification of 26:0-lysoPC was chosen as a transition from molecular ion [M + H]+ at m/z 636 to the choline fragment at m/z 104. Chromatographic resolution of target 26:0-lyso PC was achieved in 7.5 min on an Agilent Zorbax-XDB-C8 column, 3.5 μ, 4.6 × 50 mm, with a gradient of solvents using water, methanol and chloroform. For high throughput we used a Waters X-Terra-C8 column, 1.0 × 50 mm, 3.5 μ and an isocratic elution using a mixture of water, acetonitrile and chloroform to resolve the lyso PCs in 2 min.

Acyl carnitines were analyzed by rapid flow injection analysis through positive ESI LC–MS/MS. The method doesn't involve chromatographic separation and has been performed as a precursor ion scan of m/z 85. Each injection takes only few minutes and data was processed by Chemoview software application (ABSCIEX).
Results: The analysis of DBS from X-ALD or PBD (Fig. 1) reveals abnormal levels of 26:0 lyso-PC. Corresponding acyl carnitine profiles were measured (Fig. 1). No difference was found in the acyl carnitine profile between original and lyso PC combined extraction procedures.

Figure 1. A. Representative TIC chromatogram of lyso PCs profile from DBS. B. MRM extracted ion chromatogram from control sample. C. MRM extracted ion chromatogram from PBD affected sample. D. Acyl carnitine profile from control DBS. E. Acyl carnitine profile from PBD affected DBS. (* )- acyl carnitines internal standards.

Conclusion: We developed a new method that combines extraction of acyl carnitines and lysophosphatidyl cholines in one step. Our results demonstrate that the method is highly sensitive, reproducible with high extraction yields and can be applied and automated as a high throughput method for simultaneous extraction and screen for peroxisomal disorders, fatty acids oxidation disorders and organic acidurias.

References

11. Long chain acyl-CoA dehydrogenase deficiency: A new inborn error of metabolism manifesting as congenital surfactant deficiency

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Background: Long Chain Acyl-CoA dehydrogenase (LCAD) was one of the first enzymes described in the acyl-CoA dehydrogenase (ACAD) family of enzymes. While other members of the ACAD family are involved in energy and branched chain amino acid catabolism, the physiologic role of LCAD has remained elusive. To date, no cases of deficiency have been reported. We recently demonstrated that LCAD is highly expressed in type II pneumocytes, so we hypothesized that a patient with LCAD deficiency would present with congenital surfactant deficiency. LCAD is also highly expressed in thyroid, kidney, heart, and prostate, leading us to suspect that a deficiency might also lead to dysfunction of these organs as well.

Objective: Identify a case of LCAD deficiency in patients with a phenotype of congenital surfactant deficiency.

Design/methods: Term infants (>37 weeks gestation) with unexplained respiratory distress from birth were evaluated for known causes of congenital surfactant deficiency including surfactant protein B and C as well as ABCA3 deficiencies and were confirmed to be normal. Skin fibroblast from patient and control cells were cultured and examined for LCAD antigen and enzyme activity with the specific substrate 2,6 dimethylheptanoic acid. Genetic studies were then performed.

Results: Fibroblasts from one patient with profound congenital surfactant deficiency, hypothyroidism, hypertension, and pericardial effusion had no LCAD activity. LCAD activity was easily detected in extracts from control cells and could be inactivated with antibodies to LCAD. Immunostaining of patient cells and control cells were cultured and examined for LCAD antigen and enzyme activity with the specific substrate 2,6 dimethylheptanoic acid. Genetic studies were then performed.

Utilization of this penultimate free bile acid precursor by LCAD implicates this enzyme in the biosynthesis of cholestenoic acid, an important regulator of thyroid function.
Conclusions: This is the first report of LCAD deficiency, presenting with a unique combination of congenital surfactant deficiency and hypothyroidism. The clinical and biochemical abnormalities in this patient highlight the role of LCAD as a biosynthetic rather than a catabolic enzyme. Further studies of patients with congenital surfactant deficiency of unknown origin will be necessary to demonstrate the frequency of LCAD deficiency.

12. Murine very-long-chain acyl-CoA dehydrogenase (VLCAD) cardiac-specific knockout reveals essential role in response to cold stress

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Background: Patients with VLCAD deficiency often present with sudden death, cardiomyopathy, skeletal myopathy, and hypoketotic, hypoglycemic metabolic crises in response to the stresses of fasting or exercise. Global VLCAD knockout mice exhibit similar phenotypes, with rapid development of hypoglycemia, hypothermia, and death following fasting in the cold, and are susceptible to cardiac arrhythmia.

Methods: To delineate the cardiac contribution, we generated a Cre-inducible cardiac specific knockout mouse line.

Results: Although generally healthy with normal cardiac function by echocardiography under standard conditions, following exposure to the cold (4°C) and fasting, knockout animals (n = 3) become moribund within four hours after being placed in the cold with fasting. Controls, including cardiac specific VLCAD heterozygous animals (n = 3) and wild-type mice (n = 3) survived and were asymptomatic. Knockout animals exhibited reduced cardiac function with fractional shortening of 21 ± 1 as compared to 29 ± 4 in controls. In contrast to the global knockout animals, the cardiac-specific knockout animals did not develop hypoglycemia. Glucose levels in heart-specific knockout animals were 146 ± 42; 113 ± 15 in heterozygous animals and 111 ± 13 in wild-type mice.

Conclusions: Cardiac VLCAD is essential in mice for normal cardiac function in response to stress.

SIMD Fellows

1. Nitric oxide production in subjects with MELAS syndrome and the effect of arginine and citrulline supplementation: Interim results

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Background: The mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome is one of the most frequent maternally inherited mitochondrial disorders. The pathogenesis of stroke-like episodes remains unclear. It is believed that endothelial dysfunction leads to nitric oxide (NO) deficiency and ischemic events. Both arginine and citrulline act as NO precursors.

Methods: In this study we aim to measure NO production rate using a stable isotope infusion technique and plasma concentrations of NO metabolites (nitrite and nitrate, NOx), arginine, and citrulline in 15 control subjects in a single admission and in 15 subjects with MELAS before and after arginine and citrulline supplementation. The goals are to determine whether NO production is lower in subjects with MELAS, whether arginine and citrulline supplementation will increase NO production, and whether citrulline will increase NO production more substantially than arginine supplementation due to the fact that arginine requires a transporter to enter the cell and the action of arginase on the intracellular arginine pool.

Results: To date, seven control subjects and seven subjects with MELAS syndrome have completed the study. Subjects with MELAS syndrome have lower plasma arginine (62.1 ± 2.4 vs 74 ± 4.2 μM, P = 0.01) and citrulline (24.4 ± 1.9 vs 28.1 ± 1.3 μM, P = 0.08). No significant differences between the two groups were found in plasma NOx and the NO production rate. In subjects with MELAS, arginine supplementation resulted in increase in plasma arginine (62.5 ± 3.2 → 158.2 ± 11.3 μM, P < 0.001) and citrulline (24.8 ± 3.5 → 28.9 ± 3.2 μM, P < 0.05). However, citrulline supplementation resulted in more significant increase in plasma arginine (63.2 ± 2.7 → 218.5 ± 23.9 μM, P < 0.001) and citrulline (27.2 ± 3.2 → 138.1 ± 33.0 μM, P = 0.01). Plasma NOx concentration trended higher after arginine supplementation (19.59 ± 2.1 → 21.1 ± 1.8 μM, P = 0.15) and after citrulline (18.7 ± 2.1 → 21.2 ± 1.8 μM, P = 0.09); however, these changes did not reach statistical significance. The NO production rate increased after arginine supplementation (0.074 ± 0.016 → 0.123 ± 0.026 μmol/kg/h, P < 0.05); however, the increment was more significant after citrulline supplementation (0.075 ± 0.018 → 0.516 ± 0.16 μmol/kg/h, P < 0.05).

Conclusion: The interim analysis reveals that plasma arginine is lower in subjects with MELAS, while there is a trend for plasma citrulline to be lower. In comparison to arginine, citrulline supplementation in subjects with MELAS results in a higher increase in plasma arginine and citrulline concentrations, and NO production rate. Ultimately, the completion of this study will shed light on better therapeutic strategies for the management of stroke-like episodes in subjects with MELAS syndrome.

2. Tandem mass spectrometry for the diagnosis of free sialic acid disorders

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Lyosomal free sialic acid storage diseases (LSASDs) are caused by dysfunction of the lysosomal transport protein, sialin (SLC17A5). Sialin deficiency is associated with two clinical phenotypes; Salla disease and Infantile free Sialic acid Storage Disease (ISSD). Clinical manifestations of
ISSD include severe developmental delay, coarse facies, hepatosplenomegaly, cardiomegaly, skeletal abnormalities, and death within the first four years of life. Laboratory investigation of suspected LSASD typically begins with measurement of urinary SA. However, SA levels are not always elevated in patients with confirmed sialin deficiency and the standard thiobarbituric acid assay is susceptible to interference. Therefore, it is important to establish a more effective diagnostic approach for these conditions, which may ultimately facilitate earlier therapeutic intervention and improved outcomes. SLC17A5 also mediates vesicular transport of at least 6 additional small molecules in the CNS, including aspartate and glutamate, and sialin deficiency has been correlated with increased levels of N-acetylaspartate (NAA) and N-acetylaspartylglutamate (NAAG). Therefore, we are investigating whether LC–MSMS analysis of multiple biomarkers (SA, NAA, and NAAG) may provide a more effective initial approach for the diagnosis of LSASDs.
**ABSTRACTS**

**Invited Speakers**

**Name**
- Georgianne Arnold, M.D.*
- Lorenzo D. Botto, M.D.*
- Tina Cowan, Ph.D.
- Brian Fowler, Ph.D.*
- Dianne Frazier, Ph.D., M.P.H., R.D.*
- William A. Gahl, M.D., Ph.D.*
- Gregory A. Grabowski, M.D.*
- Cary O. Harding, M.D.*
- Georg F. Hoffmann, M.D., Ph.D.*
- Keith Hyland, Ph.D.*
- Dwight Koeberl, M.D., Ph.D.*
- Mark S. Korson, M.D.*
- Jan P. Kraus, Ph.D.*
- Nicola Longo, M.D., Ph.D.*
- Shideh Mofidi, M.S., R.D., C.S.P.
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**Title of abstract**
- Genotype–phenotype–metabolite correlations in MCAD deficiency
- Surveillance programs for metabolic disorders: Clinical and public health aspects
- Laboratory training in inborn errors of metabolism
- Biochemistry and genetics of homocysteine remethylation disorders
- Medical foods: History and future directions
- The NIH Undiagnosed Diseases Program
- New therapies for lysosomal storage disorders (LSDs)
- Challenges in the diagnosis and management of VLCAD and trifunctional protein deficiencies
- Liver cell transplantation (LCT) in urea cycle defects
- Cerebral folate deficiency
- Short chain acyl-CoA dehydrogenase deficiency and other rare disorders of metabolism with elevated C4-carnitine detected by tandem mass spectroscopy newborn screening
- Metabolic Outreach Service: A comprehensive approach to metabolic education, care and recruitment
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- Carnitine and carnitine palmityl transferase II deficiency
- Phenylketonuria registry
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- Clinical characterization of patients with various forms of homocyst(e)ine elevation in blood or urine
- Folate metabolism and MTHFR deficiency
- Molybdenum cofactor and sulfite oxidase deficiency
- Clinical trials in urea cycle disorders
- Why are there no proven therapies for genetic mitochondrial diseases?
- Hypoglycemia in children: Interactions between the endocrine system, fatty acid oxidation and ketone bodies (SCHAD, CACT, CPT-1 and HMG-CoA synthase deficiency)
- Update on the urea cycle registry
- Glycomacropeptides (GMP): A new option for PKU diet management
- The NAMA experience
- Biotin holocarboxylase synthetase, biotinidase deficiency and biotin transporter disorders: What do we know and what is still left to learn?
- Competitive inhibition of large neutral amino acids as an alternate treatment modality in inborn errors

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**Genotype–phenotype–metabolite correlations in MCAD deficiency**

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Although the outcome of children diagnosed with MCAD deficiency by newborn screening is greatly improved, some degree of morbidity and mortality remains. In addition, controversy remains regarding the extent of dietary intervention indicated in well infants, such as awakening for feedings at night (vs the effects of interfering with normal hunger mechanism and feed–fast cycles), restricting dietary fat, adding bedtime cornstarch, or supplementing with carnitine.

Prior fasting studies in affected infants and children suggest a wide range of fasting tolerance in this disorder, thus it would be helpful clinically to identify markers that might be predictive of higher risk or shorter fasting tolerance that indicate a need for a higher degree of intervention. Efforts to identify genotype-phenotype correlations have met with mixed success. However, preliminary data suggest that severe phenotypes are more likely to be related to more severe underlying mutations and to greater metabolic derangements. We will review the current status of genotype-phenotype-metabolite correlations and their implications for patient care and future research in this disease.

**Surveillance programs for metabolic disorders: Clinical and public health aspects**

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By incorporating increasingly larger numbers of metabolic disorders into newborn screening, health professionals and the public face many opportunities and challenges. Opportunities include, among others, understanding the natural and modified history of these conditions, the
Medical foods: History and future directions

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Changing the biochemical environment through dietary intervention is the premise for designing medical foods to treat inborn errors of metabolism (IEM).

Early attempts at dietary treatment focused on eliminating foods that had high concentrations of the “offending” substrate. At first, this was done without the knowledge that many of these substrates, most of which are amino acids, are essential nutrients and must be provided at some level in the diet. These treatments then evolved into restriction, rather than elimination, diets. The concept of medical foods, aka metabolic formulas, originated to meet the need of providing adequate protein (and other nutrients) for growth, while restricting the offending amino acid.
The further development and refinement of these medical foods paralleled the advances in understanding the biochemical nature of inborn errors of metabolism, their natural history, and nutritional requirements of both affected and unaffected individuals over the life cycle.

Quality of life considerations have played a large part in the evolution of medical foods. Once the most serious manifestations of the particular IEM were controlled by restricting the substrate, other nutritional requirements became apparent and were addressed with improvements in the medical foods. Some of nutritional requirements were related to the iatrogenic deficiency of nutrients due to the artificial nature of the medical foods, and others to additional requirements secondary to the IEM itself. Finally, modifications have been made to improve palatability, versatility, and adaptability to concurrent medical needs.

Medical food development is painstaking and costly, the shelf life is limited and production cannot benefit from the economy of scale. Although they are not natural foods, neither are they recognized by third party payers as medication. This is the focus of a concerted effort by advocacy groups for individuals with IEM, and should continue to be supported by all who work with this patient population.

Also included in the toolbox for treating IEM are: natural foods, whose nutrient contribution to the restricted diet can be more easily calculated through the use of recently designed web-based computer programs; low protein specialty foods that are continually being developed to mimic their higher protein counterparts; treatment adjuncts, e.g. Kuvan, for phenylketonuria (PKU); development of a naturally occurring intact protein, glycomacropeptide (GMP) with very low phenylalanine content as a nutrient source for PKU; and the use of large neutral amino acids in the treatment of PKU and maple syrup urine disease.

Medical foods remain crucial to both treatment of IEMs and providing an optimal nutrient environment to allow growth and development.

The NIH Undiagnosed Diseases Program

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The NIH Undiagnosed Diseases Program (UDP), supported by the Office of Rare Disease Research, the NHGRI, and the NIH Clinical Center, was established to diagnose patients who have long sought a diagnosis, and to discover new diseases and insights into their physiology, cell biology, and biochemistry. Since its inception in May of 2008, the UDP has reviewed more than 1300 medical records, seen over 280 patients, and made approximately 30 diagnoses, including some ultra-rare metabolic diseases. As of 2010, one new disease exposing a novel mechanism for vascular calcification has been discovered. The Program provides hope to a desperate population by offering access to comprehensive and coordinated specialty examinations and state-of-the-art genetic evaluations.

New therapies for lysosomal storage disorders (LSDs)

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The advent of enzyme therapy (ET) for Gaucher disease type 1 two decades ago ushered in a new era in the treatment of lysosomal storage diseases. Now ETs are FDA approved for six LSDs with early to late-stage development for an additional five diseases. These ETs have focused primarily on the visceral manifestations of the LSDs, but current efforts are being directed to ET for primary CNS diseases. In addition, these developments and their commercial successes have fostered alternative therapeutic modalities including substrate synthesis inhibition and in situ enzyme enhancement, as well as development of new manufacturing platforms that might decrease the costs of biotherapeutics. Indeed, the next few years hold the promise for 3–5 therapeutics for Gaucher disease alone, and the potential for direct treatment of CNS LSDs. This presentation will focus on recent basic and applied advances in such therapeutic developments for LSDs, and how they will alter the delivery of medical care to afflicted patients.

Challenges in the diagnosis and management of VLCAD and trifunctional protein deficiencies

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Cardiomyopathy, hypoketotic hypoglycemia, or recurrent rhabdomyolysis are the most common phenotypes associated with inherited disorders of mitochondrial long chain fatty acid oxidation (LCFAO). Deficiency of very long chain acyl-CoA dehydrogenase (VLCAD), the first enzymatic step in the mitochondrial β oxidation cycle, is caused by recessively inherited mutations in the ACADVL gene and is the most common LCFAO defect detected by newborn screening. Although infants with null mutations exhibit severe VLCAD deficiency and present with cardiomyopathy or episodes of hypoketotic hypoglycemia, the spectrum of disease severity is extremely broad, and many infants with VLCAD deficiency are asymptomatic at the time of screening. The prediction of disease phenotype either from biochemical screening data or from genotype requires further study. Also, the appropriate treatment approach for asymptomatic infants with VLCAD deficiency continues to be debated. Infants with deficiency of mitochondrial trifunctional protein (TFP), including infants with isolated long chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency, caused by mutations in either the HADHA or HADHB genes, although occurring more rarely than infants with VLCAD deficiency, are much more likely to be symptomatic and more difficult to treat. In addition to the cellular energy deficit created by TFP deficiency, the accumulation of 3-hydroxyacyl compounds, such as long chain 3-hydroxy-fatty acids or 3-hydroxyacylcarnitines, likely is toxic. Hypoketotic hypoglycemic episodes with lactic acidosis occur frequently in this disorder and may be associated with cardiomyopathy. Recurrent rhabdomyolysis triggered by infection, dehydration, or exercise often develops during childhood. Pigmentary retinopathy and peripheral neuropathy are also frequent long term complications in TFP deficiency that are not prevented by contemporary therapies. The mainstay of
therapy for both VLCAD and TFP deficiencies has been fasting avoidance, restriction of dietary long chain fat intake, and oral supplementation with medium chain triglyceride (MCT) oil to provide a usable fuel substrate that bypasses the LCFAO system. Although this approach is generally very successful in many individuals with VLCAD deficiency, individuals with TFP deficiency and some with VLCAD deficiency continue to experience chronic difficulties such as exercise intolerance and recurrent rhabdomyolysis despite therapy. New treatment modalities are sorely needed. Current therapeutic investigations include optimization of dietary macronutrient content, supplementation with triheptanoin (C7 triglyceride) rather than MCT oil, and treatment with bezafibrate, a PPARα agonist that seems to stimulate the expression of enzymes in the FAO pathway.

Liver cell transplantation (LCT) in urea cycle defects

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With increasing shortage of donor organs and changes in organ allocation systems, there is a considerable need for alternative or supportive techniques in the field of liver transplantation. Liver cell transplantation (LCT) is such an innovative technique that is especially promising for use in children, because it is less invasive than orthotopic liver transplantation (OLT). For the treatment of inborn errors of metabolism, experience mainly exists with Crigler–Najjar syndrome type 1 (CNS1, n = 6) and urea cycle disorders (UCD, n = 10). All but two patients experienced metabolic stabilisation before the majority of children underwent OLT. Whereas transplant success in CNS1 patients can be easily monitored by plasma bilirubin levels, it is much more difficult in UCDs, where many factors can contribute to clinical deterioration. In two UCD patients the clinical situation stabilised over a period of up to two years. In the following the first prospective study on safety and efficacy of liver cell application (SELCIA II) on patients with severe neonatal UCD was initiated with six patients treated so far. In parallel to a broader clinical use of LCT based on clinical studies, research on suitable liver stem cells should be promoted to overcome the limited availability of adult hepatocytes.

Cerebral folate deficiency

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Cerebral folate deficiency (CFD) is becoming increasingly recognized as a cause of neurological disease. The characteristic biochemical finding is a low cerebrospinal fluid (CSF) concentration of 5-methyltetrahydrofolate (5MTHF) in the presence of normal peripheral folate. The condition can occur at any age and there appears to be a multitude of causes/mechanisms that can lead to its development. A fairly well defined CFD syndrome has been described where presentation occurs around 4 months of life with irritability and sleep disturbance followed by development of psychomotor retardation, dyskinesia, cerebellar ataxia, deceleration of head growth, visual disturbances and sensorineural hearing loss. The underlying mechanism causing this syndrome is reportedly to be the development of autoantibodies against the folate transporter that mediate the transfer of 5MTHF across the choroid plexus. There are, however, other situations where CFD has been described where it is unclear if the presence of autoantibodies is involved in the etiology. These include, but are not limited to, the Aicardi–Goutieres and Rett syndromes, some cases of autism, some of the mitochonoiopathies, Alpers disease, hyponymelination with atrophy of the basal ganglia and cerebellum, and some cases of cerebral palsy. Treatment of CFD requires replenishment of the folate that is missing within the central nervous system. It is important to recognize that folic acid should not be used as oxidized folates can compete for the entry of 5MTHF across the choroid plexus. Treatment is with reduced folates, using either Leucovorin (D,L-5-formyltetrahydrofolate-folinic acid), Isovorin (L-5-formyltetrahydrofolate) or Deplin (5MTHF). Currently the outcome of treatment is not well documented. This presentation is designed to overview the background leading to the description and measurement of biomarkers other than 5MTHF that may demonstrate a functional deficiency of 5MTHF within the CNS. Such biomarkers could aid in determining the initial necessity for treatment following a finding of low 5MTHF in CSF and could act as functional markers to assess efficacy and outcome of treatment.

Short chain acyl-CoA dehydrogenase deficiency and other rare disorders of metabolism with elevated C4-carnitine detected by tandem mass spectroscopy newborn screening

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Background: The initiation of tandem mass spectrometry (MS/MS) newborn screening (NBS) has detected many infants with disorders of metabolism based upon elevated butyrylcarnitine/isobutyrylcarnitine (C4-carnitine). Many infants with short chain acyl-CoA dehydrogenase (SCAD) and several with isobutyryl-CoA dehydrogenase (IBDH) deficiency (D) have been detected based upon elevated C4-carnitine concentrations in newborn blood spots analyzed by MS/MS. However, the prognosis for infants with these two disorders and other conditions detected through screening for elevated C4-carnitine remains uncertain, as does the value of early detection of these disorders.

Methods: A review of published papers and abstracts regarding conditions detected by elevated C4-carnitine, focusing upon clinical presentations and detection by MS/MS NBS.

Results: SCAD-D has been reported frequently in association with clinical presentations involving neurological complications. The outcome of patients detected by NBS has usually been normal, which could be attributed to ascertainment bias among patients detected clinically. IBDH-D has been reported less frequently than SCAD-D, and the outcome of patients detected by NBS has been similarly benign. However, other disorders...
with more severe complications were detected by monitoring C4-carnitine with MS/MS NBS, including multiple acyl-CoA dehydrogenase deficiency and ethylmalonic encephalopathy. The latter condition has recently been treated successfully with oral metronidazole and N-acetylcysteine.

**Conclusions:** MS/MS NBS for disorders with elevated C4-carnitine will very likely reveal the natural history of disorders that currently appear to be clinically benign and promote the early treatment of more severe disorders of metabolism with elevated butyryl-carnitine.

**Metabolic outreach service: A comprehensive approach to metabolic education, care, and recruitment**

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The field of metabolic medicine continues to grow with the expansion of newborn screening, availability of testing for a wider array of diseases, and development of new therapies. However, the ongoing shortage of clinicians (medical and otherwise) and trainees threatens the success of these advances and does not bode well for the well-being of both patients and their providers.

Since 2007, the Metabolic Outreach Service (MOS), based at Tufts Medical Center, has traveled every 1–2 months to five teaching hospitals in the northeastern US which do not have an on-site metabolic service. Whereas the SIMD-sponsored North American Metabolic Academy (NAMA) is present providing an excellent educational opportunity for genetic and biochemical genetic trainees, the priorities of the MOS include, 1) raising the level of awareness of non-genetic clinicians and trainees so they may participate more in the diagnosis and management of metabolic patients, and 2) recruiting young, “undifferentiated” trainees by exposing them to clinical cases and stories that highlight the achievements and excitement in this area of medicine.

The MOS has provided interactive workshops about metabolic symptoms (vs. diseases), patient presentations, and consultation support to local clinicians. As of June 2010, the number of participants in all MOS activities exceeded 5100, the number of lectures and workshops = 136, and the number of cases brought for review = 299. Twenty-one patient presentations have highlighted 13 different diseases.

Attendance at MOS activities has been consistent since 2007. Evaluations by participants and local program directors have been very favorable, short-term retention of information is documented, and there is some evidence to suggest a change in clinical practice. There are some instances of very long term retention following patient presentations. Finally, during the MOS so far, 2 residents have requested electives in the metabolic clinic, 1 genetic counseling student did a project about a metabolic topic, and 8 first-year medical students have done selective in metabolic disease, of which 3 (so far) have completed summer projects in the field. This compares with 1 resident who did an elective during the previous 7 years.

The MOS agenda remains an ambitious one, including the new development of a 1- and 2-day metabolic lecture series for pediatric teaching programs, expansion of the venues for patient speakers to include the four medical schools associated with the participating medical centers, development of day-long conferences focusing on clinical approaches to metabolic symptoms of interest to pediatric neurologists, pediatric gastroenterologists, and neonatal intensivists, and piloting a “apprenticeship model of collaboration” in which established metabolic clinics are paired with clinics in need of clinical support in order to provide more patients with options for metabolic care.

**Disorders of homocysteine transsulfuration and remethylation: Introduction and classic homocystinuria**

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There are both required and inherited causes for elevated homocysteine (Hcy) in plasma and urine. Decreased intake or absorption of folate, vitamins B12 and B6 can result in elevated Hcy. The inherited disorders of methionine metabolism can be divided into disorders of coenzyme cobalamin absorption or delivery to the cytoplasmic methionine synthase (cblC, D, E, F, G) and disorders involving mutations in the enzymes in the remethylation and transsulfuration of Hcy (MTHFR, MS, MTRR, and CBS).

By far the most common cause of homocystinemia is the deficiency of CBS estimated at ~1 per 10,000. Cystathionine beta-synthase (CBS) deficient homocystinuria (CBSHD) is an inherited metabolic defect that if untreated typically results in mental retardation, thromboembolism and a range of connective tissue disturbances such as dislocated eye lenses and a number of skeletal problems. Patients with homocystinuria are at high risk for vascular problems with deep vein thrombosis being the most serious one. CBS is a PLP dependent hemoprotein whose activity is regulated by the availability of S-adenosylmethionine (SAM). We have crystallized a truncated form of the enzyme (45 kDa) missing the C-terminal SAM binding regulatory domain and we reported its X-ray structure. This form of the enzyme is constitutively activated by about 3-fold and no longer responds to SAM activation.

We have also prepared a mouse model of the disease. These mice have the mouse CBS gene inactivated and are transgenic for the human gene (HO mice). The human gene expresses at a very low level allowing these mice to survive and have a near normal life span. The mice are biochemically very similar to human CBSHD patients with homocysteine levels between 200–400 μM (normal level is ~8 μM), significantly increased methionine and low cysteine. In terms of treatment there are at least three classes of patients. One group of patients is classified as “pyridoxine-responsive” as CBS enzyme function can be restored by a pharmacological dose of pyridoxine (vitamin B6 therapy). Such treatment effectively mitigates the biochemical and clinical findings in these individuals. The second group of functional mutations is represented by the “C-terminal CBS mutants” that are defective in their ability to respond to post-translational up-regulation by SAM. Patients with this class of mutations lack mental retardation and connective tissue aspects of the phenotype and are detected after investigation of plasma Hcy levels following an idiopathic thrombotic event before the age of 40. The final group of CBSHD mutations are the pyridoxine non-responsive ones (~60% of the patients) which represent the severest form of the disease. For these latter two groups of patients, the standard treatment is a methionine restricted diet and supplementation with betaine (trimethylglycine) which serves as a methyl donor for the remethylation of Hcy.
Carnitine and carnitine palmitoyl transferase II deficiency

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Carnitine plays an essential role in the transfer of long-chain fatty acids across the inner mitochondrial membrane. This transfer requires enzymes and transporters that accumulate carnitine within the cell (OCTN2 carnitine transporter), conjugate it with long chain fatty acids (carnitine palmitoyl transferase 1, CPT1), transfer the acylcarnitine across the inner mitochondrial membrane (carnitine–acylcarnitine translocase, CACT), and conjugate the fatty acid back to Coenzyme A for subsequent beta oxidation (carnitine palmitoyl transferase 2, CPT2). Deficiency of the OCTN2 carnitine transporter encoded by the SLC22A5 gene causes primary carnitine deficiency, characterized by increased losses of carnitine in the urine and decreased carnitine accumulation in tissues. Patients can present with hypoketotic hypoglycemia and hepatic encephalopathy, or with skeletal and cardiac myopathy. Sudden death from arrhythmia can occur at any age and can be the only presentation in adults. This disease is diagnosed by finding low carnitine levels in plasma and confirmed by measuring decreased carnitine transport in fibroblasts or by DNA testing. It responds to life long carnitine supplementation. Adult patients with primary carnitine deficiency might have mutations leaving minimal function sufficient to prevent symptoms at a young age. Deficiency of CPT2 presents more frequently in adults with rhabdomyolysis triggered by exercise or prolonged fasting. More severe variants of CPT2 deficiency present in the neonatal period similarly to CACT deficiency associated or not with multiple congenital anomalies. Intermediate forms present with fasting-induced hypoketotic hypoglycemia and muscle symptoms. Patients with the most severe presentation usually have two mutations that completely abolish CPT2 enzyme activity. The other patients have at least one mutation with residual activity, such as p.S113L. Diagnosis of this condition relies on measurement of plasma acylcarnitine profile, with a characteristic pattern of elevated long-chain acylcarnitines, and it is confirmed by enzyme assay in fibroblasts and/or DNA testing. Treatment for CPT2 deficiency consists in exercise and fasting avoidance. Some patients benefit from a low-fat diet supplemented with medium chain triglycerides that can be metabolized by mitochondria independently from carnitine, and low-dose carnitine supplements (when indicated).

The genetic hypermethioninemia

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This presentation will cover the six known genetically determined deficiencies that may be accompanied by severe elevations of plasma methionine. Four involve enzymes that participate in the transsulfuration pathway from methionine to cysteine. (a) methionine adenosyltransferase, isoforms I and III (MAT I/III); (b) glycine N-methyltransferase (GNMT); (c) S-adenosylhomocysteine hydrolase (AHCY); and (d) cystathionine β-synthase (CBS). The other two are deficiencies of fumarylacetoacetate hydrolase (FAH) and citrin.

MAT I/III deficiency. Three humans have been reported to present findings of the conversion of methionine and ATP to S-adenosylmethionine (AdoMet), pyrophosphate and inorganic phosphate, occur in mammals: MAT I and MAT III are tetrameric and dimeric holoenzymes of a subunit encoded by MAT1A, a gene expressed chiefly in adult liver, but also elsewhere. MAT II contains an active subunit with, in humans, 84% amino acid identity with that of MAT I and III. It is encoded by MAT2A, a gene expressed in fetal (and to a slight extent adult) liver and all non-hepatic tissues. At least 80 MAT I/III-deficient humans have been identified with 37 MAT1A mutations reported. Methionine accumulates prior to the deficient activity, but AdoMet is not elevated as it would be with elevated methionine and normal MAT I/III activity. tHcy is, at most, only slightly elevated. Most known patients are young, having been detected in newborn screening programs because of elevated blood methionine, so there is relatively little experience on which to base long-term prognoses. A few patients have survived in good health in spite of truncating mutations that leave no residual MAT I/III activity, almost certainly because they possess MAT II activity. However, at least 10 known MAT I/III-deficient patients have manifested CNS problems, including abnormalities of brain myelination.

GNMT deficiency. Only three humans are known with this deficiency, detected during attempts to find causes for their slightly elevated liver transaminases and severe hypermethioninemia. Key metabolic findings are hypermethioninemia, with elevation of AdoMet without elevation of S-adenosylhomocysteine (AdoHcy), tHcy, or sarcosine (N-methylglycine). AdoMet elevation is due to deficiency of the pathway that normally disposes of excesses of that compound; hypermethioninemia, due to feedback down-regulation of conversion of methionine to AdoMet. Three GNMT mutations have been identified. At last report, at ages from 12 to 19½ years, except for mildly raised transaminases, the patients were clinically well without treatment for their condition. However mice with GNMT knocked-out have been reported to develop liver abnormalities, including excessive glycogen storage and hepatocellular carcinoma.

AHCY deficiency. This condition has been reported in six humans and among them five AHCY mutations identified. Key metabolic findings are elevations of methionine, AdoMet, and, especially, AdoHcy, but not tHcy. AdoHcy accumulates because in mammals AHCY catalyzes the only means to catabolize that compound; AdoMet, because AdoHcy is a general inhibitor of the many AdoMet-dependent methyltransferases; methionine, because of feedback down-regulation by AdoMet (as occurs in GNMT deficiency). Severe muscular weakness has been present in all untreated patients, usually accompanied by elevated creatine kinase, hypoalbuminemia, prolonged prothrombin time, and retarded brain myelination. Two patients died within weeks of birth due to these abnormalities. In the initial patient, treatment by dietary methionine restriction and supplementation with phosphatidylcholine and creatine was accompanied by gain of muscle strength and improved brain
outcomes, whereas delay can result in irreversible neurological abnormalities. A nutritional deficiency of folate or cobalamin (vitamin B_{12}), due to abnormal intake or absorption, can result in elevated total homocysteine (tHcy) levels in the blood. In both folate and cobalamin deficiency, there may be megaloblastic anemia; in the case of cobalamin deficiency there will also be methylmalonic acidemia/uria (MMA).

Inherited disorders of intracellular metabolism with elevated levels of homocysteine can be divided into those with high methionine (“classical” homocystinuria due to cystathionine synthase deficiency) or those without high methionine and either without (severe MTHFR deficiency) or with deficiency of folate or cobalamin (vitamin B_{12}), due to enhanced remethylation of homocysteine, the primary abnormally accumulated metabolite, back to methionine. tHcy, AdoMet, and AdoHcy are also elevated in untreated individuals.

**FAH deficiency** (also known as tyrosinemia type I). This defect in the pathway for tyrosine catabolism has been known to be due to loss of FAH activity for more than 30 years and at least 48 FAH mutations have been identified. Methionine elevations vary widely in untreated patients and may be absent or only slight in newborns. The elevations may be due to the liver disease that accompanies FAH deficiency and/or to inhibition of MAT III by fumarylacetoacetate. If the latter is the case, severe elevations of AdoMet would be expected and would account for the hypermethioninemia, but, to the best of the knowledge of the present author, plasma AdoMet has not been assayed in an untreated hypermethioninemic FAH-deficient patient. In recent years detection of this condition by screening of newborns for succinylacetone has been successful and treatment with nitisinone has been shown to limit the accumulation of homogentisate and the toxic metabolites formed from it (including fumarylacetoacetate), and to mitigate most of the clinically serious consequences of this deficiency, although concern about hepatocellular carcinoma remains.

**Citrin deficiency.** Citrin is a mitochondrial protein that catalyzes the exchange of a mitochondrial aspartate for a glutamate plus a proton from the cytoplasm. Citrin deficiency was shown in 1999 to be the cause of adult-onset type II citrullinemia (CTNL2), and, shortly thereafter, to cause also a form of neonatal intrahepatic cholestasis now termed NICCD (neonatal intrahepatic cholestasis due to citrin deficiency). More than 50 mutations in SLC25A13, the gene that encodes citrin, are known. Hypermethioninemia is frequently, but not always, among the several metabolic abnormalities that may be found in NICCD, including elevations of galactose, citrulline, threonine, tyrosine, phenylalanine, lysine, and arginine. The hypermethioninemia is usually transient and disappears by age one year. The specific cause of the accumulation of methionine has not been established. Citrin deficiency is especially prevalent in eastern Asia countries: for example in Japan the carrier rate for SLC25A13 mutations is about 1:65, and the frequency of homozygotes and compound heterozygotes estimated to be 1:17000, about the same as the incidence of NICCD (1:17,000–34,000). A subset of these individuals go on to manifest a decade or more later the sudden disturbances of consciousness, behavioral aberrations, disorientation, loss of memory and possibly coma, of CTNL2, although present knowledge does not permit prediction of which ones will do so. Citrin deficiency is not restricted to East Asia: recently patients have been identified in persons of Arabic, Pakistani, French-Canadian and Northern European origins.

Clinical characterization of patients with various forms of homocyst(e)ine elevation in blood or urine

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Elevated homocysteine levels can have both acquired and inherited causes. A nutritional deficiency of folate or cobalamin (vitamin B_{12}), due to abnormal intake or absorption, can result in elevated total homocysteine (tHcy) levels in the blood. In both folate and cobalamin deficiency, there may be megaloblastic anemia; in the case of cobalamin deficiency there will also be methylmalonic acidemia/uria (MMA).

Inherited disorders of intracellular metabolism with elevated levels of homocysteine can be divided into those with high methionine (“classical” homocystinuria due to cystathionine synthase deficiency) or those without high methionine and either without (severe MTHFR deficiency, cb{E}, cb{L}, cb{D} variant 1), or with (cb{C}, “classical” cb{D}, cb{F}) MMA. Of clinical importance, of all the above disorders, only severe MTHFR deficiency should not be associated with megaloblastic anemia.

Although there is far less documentation of tHcy and MMA levels in patients with inherited disorders of cobalamin transport, elevations of both can be expected and found in inherited intrinsic factor deficiency, Imerslund–Gräsbeck syndrome, transcobalamin (TC) deficiency, and TC receptor deficiency. The elevation of metabolites seen in these disorders of absorption or transport is not thought to be as high as those seen in the intracellular disorders.

One of the commonest causes of elevations of tHcy and MMA in the newborn is breast feeding by mothers who are either vegan, or who have subclinical pernicious anemia. Early recognition of this diagnosis is essential as prompt supplementation with cobalamin prevents all deleterious outcomes, whereas delay can result in irreversible neurological abnormalities.

Most of the inherited disorders with the exception of Imerslund–Gräsbeck syndrome and cb{C} have been seen in fewer than one hundred individuals. In newborn screening programs, the frequency of cb{C} may be similar to that of classical methylmalonic aciduria. In the inherited forms of elevated homocysteine, early treatment can also be associated with a better outcome. For the defects of intracellular cobalamin metabolism, hydroxycobalamin (OHCbl) is the cobalamin of choice for treatment, and studies suggest that it should be given systemically. In all forms of elevated tHcy, betaine can be a useful, if not the primary treatment.

Folate metabolism and MTHFR deficiency

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Methylenetetrahydrofolate reductase (MTHFR) is an important enzyme in folate and homocysteine metabolism since it catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the primary circulatory form of folate and the major methyl donor for homocysteine remethylation to methionine. Severe MTHFR deficiency is associated with elevated homocysteine in plasma and urine, and low to normal plasma methionine levels. Clinical features include developmental delay, motor and gait abnormalities, neurological impairment and thrombotic episodes. Mice with 2 null alleles in Mthfr serve as good models for severe MTHFR deficiency. Depending on the genetic background of
the murine strain, Mthfr−/− mice may have reduced survival; decreased body, brain and spleen weights; abnormal cerebellar histology and altered haematological profiles. Biochemical disturbances include marked hyperhomocysteinemia, decreased methionine, decreased S-adenosylmethionine or increased S-adenosylhomocysteine levels, and decreased global DNA methylation in some tissues. The availability of these mice has allowed us to identify other biochemical disturbances which may influence the phenotype. Mthfr−/− deficient mice have decreased levels of betaine and some choline metabolites in an attempt to use the alternate homocysteine remethylation pathway catalyzed by betaine-homocysteine methyltransferase. These mice also have abnormalities in apolipoprotein synthesis, which influence lipid metabolism and the inflammatory response. Recent studies on behaviour, motor function and brain morphology and biochemistry will also be discussed.

Clinical trials in urea cycle disorders

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The urea cycle is composed of five catalytic enzymes, a cofactor producer, and at least two transport proteins. In addition to being the principal mechanism for clearance of waste nitrogen from the body, the enzymes of the urea cycle are the sole source for endogenous synthesis of L-arginine, the precursor for nitric oxide (NO) production. While the hyperammonemia associated with proximal urea cycle disorders (UCD) largely determines the outcome and natural course of the condition, recent data show that some phenotypic manifestations in the UCD arginosuccinic aciduria is independent of hyperammonemia and provide a link between the urea cycle enzymes and NO synthesis. Various clinical trials exploring the pathogenesis, natural history, and efficacy of different modalities of treatment have been conducted. These trials can be broadly categorized as 1) Observational and descriptive studies evaluating the natural history, disease progression, treatment, and outcome; 2) Metabolic studies studying the urea and metabolite flux; 3) Exploratory studies evaluating anaplerotic functions of the urea cycle (such as nitric oxide biology); 4) Intervention trials evaluating efficacy of metabolites and alternate nitrogen scavenging medications in prevention and treatment of hyperammonemia; 5) Liver cell transplantation; and 6) Evaluating the role of urea cycle intermediates in common clinical conditions.

We review the data available from the longitudinal study pertaining to natural history or various UCD in general and argininosuccinic aciduria in particular; the recent studies evaluating alternate pathways of nitrogen disposal in UCD and our translational studies exploring the link between urea cycle and nitric oxide biology.

Why are there no proven therapies for genetic mitochondrial diseases?

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At first glance, the answer to this question seems anything but obvious. After all, the mitochondrion is arguably the most scientifically scrutinized component of the eukaryotic cell. Moreover, the last 10–15 years has witnessed such a steady march of publications implicating mitochondrial misbehavior in the pathobiology of human ills as to make such an association almost axiomatic. However, a distillation of those, 28,902 (0.2%) were classiﬁed as pertaining to “mitochondrial disease” and 102 reported results of clinical trials (7 × 10^4%). Forty-three (42%) of the published trials were randomized and double-blinded and most compared active treatment to placebo. However, only 10 of those (26%) were conducted in patients with congenital causes of mitochondrial disease due to proven enzymatic and/or molecular genetic defects in the pyruvate dehydrogenase complex (PDC) or in one or more respiratory chain complexes or in subjects with histological or immunohistochemical criteria consistent with “mitochondrial cytopathy.” All studies were single-center trials (3 from the U.S., 4 from Canada) that evaluated dichloroacetate (4), a naturally occurring vitamin or cofactor (5) or a mixture of neutraceuticals (1). Six trials reported a positive effect of treatment on 1 or more clinical or biochemical primary endpoints. When speciﬁed, funding for these trials came from peer-reviewed, extramural grants (3), and/or private foundations (6). No publication listed support for the trial from pharmaceutical or biotechnology sources except for one trial in which a nutritional supplement was provided by the manufacturer. None of the 4 U.S. trials involving dichloroacetate was preceded by meetings with the FDA and none resulted in the ﬁling of a New Drug Application. Not included among these studies is an ongoing multicenter, randomized, placebo-controlled trial of coenzyme Q10 supported by the Orphan Products Division of the FDA and by the product manufacturer (Tishcon).

The recent emergence of the North American Mitochondrial Disease Consortium (NAMDC), which is part of the NIH Rare Disease Consortium, represents a growing network of mitochondrial disease clinical investigators dedicated to fostering controlled trials for genetic mitochondrial diseases. NAMDC is facilitating the organizational development of two multicenter, multinational, randomized controlled trials in genetic mitochondrial diseases involving cell-based treatment of mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) and dichloroacetate for PDC deﬁciency. The DCA trial is also supported by a Planning Grant award from the NICHD/NIH and is designed as potentially the ﬁrst phase 3 trial of any therapy for PDC deﬁciency.

What lessons have been learned from the “ﬁrst generation” of clinical trials for genetic mitochondrial diseases? Those to be discussed relate to the inherent heterogeneity in the deﬁnition, expression and clinical course of mitochondrial disease patients; the historical over-reliance of mitochondrial disease practitioners and affected families on anecdote, rather than on rigorously controlled trials; competition among treatment sites for an extremely small patient pool; logistical difﬁculties in recruitment and retention of patients; challenges in obtaining extramural funding; and the dearth of interest from the pharmaceutical and biotechnology industries.
Hypoglycemia in children: Interactions between the endocrine system, fatty acid oxidation and ketone bodies (schad, cact, cpt-1 and hmg-coa synthase deficiency)

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**Background:** Hypoglycemia disorders can be categorized by the major metabolic system affected (glycogenolysis, gluconeogenesis, lipolysis, ketogenesis) and the consequent disturbance in major fasting fuels (glucose, ketones, FFA, lactate). The hypoketotic disorders include genetic defects in enzymes of fatty acid oxidation and ketone synthesis, as well as, genetic disorders of hyperinsulinism. This review touches on hypoketotic disorders from beginning (CPT1 and CACT) to end (HMG-CoA synthase) of fatty acid oxidation, but also in-between where, quite unexpectedly, SCHAD deficiency causes hyperinsulinism.

**Disorders:** CPT1 deficiency affects the liver–kidney specific form of the enzyme: CPT1a. Affecteds present with episodes of hypoketotic hypoglycemia provoked by fasting, but without muscle or cardiac involvement seen on other FODs. Episodes may include liver and renal tubular dysfunction, more severe than in other FODs, and which may be slow to resolve. CACT deficiency may have a mild presentation resembling MCAD deficiency, but may be more severe with progressive cardiomyopathy and weakness resembling the severe form of vLCAD deficiency. Secondary changes in carnitine transport lead to elevated plasma levels in CPT1, but secondary deficiency in CACT. Acyl-carnitine profiles may identify cases in newborn screening. Mitochondrial HMG-CoA synthase is the penultimate step in ketone synthesis; the few reported cases have presented with episodes of fasting hypoketotic hypoglycemia, but without specific abnormalities in usual metabolic screening assays. Since the enzyme is not expressed in fibroblasts, diagnostic testing is most conveniently done by mutation analysis. SCHAD deficiency blocks oxidation of short and medium-chain 3-OH fatty acyl-CoAs; however, the clinical phenotype of complete SCHAD deficiency differs from other FODs and presents with episodes of hypoglycemia due to hyperinsulinism. Affected patients also have protein-sensitive hypoglycemia and some, but not all, have increased plasma 3-OH-butyryl-carnitine. Based on studies of SCHAD-KO mice, the mechanism involves activation of beta-cell glutamate dehydrogenase due to loss of an inhibitory protein–protein interaction between SCHAD and GDH. Treatment with diazoxide to suppress insulin secretion provides good control of hypoglycemia.

**Conclusions:** These less common disorders of ketogenesis may not be detected by standard acyl-carnitine profiles, but can often be suspected based on clinical features.

Glycomacropeptide (GMP): A new option for PKU diet management

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Phenylketonuria (PKU), an inborn error in phenylalanine hydroxylase, requires life-long treatment with a low-phenylalanine (phe) diet. Compliance with the required amino acid (AA) medical formulas is poor in older individuals with PKU and new dietary strategies are needed. Pure glycomacropeptide (GMP), a whey protein produced during cheese manufacturing, contains no phe or other aromatic amino acids, yet is naturally enriched with the large neutral amino acids threonine (thr) and isoleucine (ile). Various foods and beverages with minimal phe content can be made with GMP and blind sensory evaluations show that GMP products are well accepted by those with this disorder.

Growth and feed efficiency was not different in weaning wild-type mice fed casein or GMP supplemented with limiting AA. PKU mice fed GMP compared with AA showed a significant 11% and 20% decrease in the concentrations of phe in plasma and brain, respectively, suggesting competitive inhibition of phe transport from elevated concentrations of thr and ile with the GMP diet.

The safety and efficacy of replacing all AA formula with GMP products was evaluated in 11 PKU subjects (11.5 to 31 years) in an 8-day inpatient metabolic study. There were no medical concerns when subjects consumed the GMP diet and postprandial plasma phe concentrations measured on the GMP diet was not different than AA diet. As an intact protein, GMP improved phe and protein utilization as shown by reduced daily variation of phe concentrations in fasting and postprandial plasma and significantly lower postprandial blood urea nitrogen and higher insulin concentrations with the GMP diet compared with the AA diet.

The concentration of the appetite-stimulating hormone ghrelin measured 180 min after breakfast was significantly lower on the GMP compared to the AA diet. Using a visual analog scale, subjects noted greater feelings of fullness in the postprandial state on the GMP diet compared with the AA diet. These results suggest greater satiety with ingestion of a meal containing GMP compared with AA.

In an outpatient trial, an adult with PKU successfully substituted GMP products for AA formula for 10 weeks. Mean blood phe concentrations were 14% lower on the GMP diet suggesting improved phe utilization with increased protein distribution throughout the day and/or reduced phe absorption in association with increased plasma thr and ile concentrations.

Thus, GMP, supplemented with limiting AA, is a new alternative to replace some or all of the traditional AA formulas currently required in the PKU diet. Our research group plans further studies to investigate the efficacy of GMP in the nutritional management of PKU.
The NAMA experience
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The North American Metabolic Academy (NAMA) is an educational program developed by the SIMD to augment training in inborn errors of metabolism. Goals of the program include providing course attendees a strong knowledge base in inborn errors of metabolism, encourage them to pursue a career as clinical biochemical geneticists, and increase membership in the SIMD. The course runs for a full calendar week and provides in excess of 50 contact hours between faculty and trainees. Almost 100 trainees have attended the course in its first three years. The curriculum, designed and implemented by almost 20 SIMD members, provides a broad introduction to inborn errors of metabolism aimed at first year medical genetics residents in the United States and equivalent medical genetics trainees from Canada. Multiple teaching methods are utilized including didactic lectures, interactive patient case-based workshops, and interpretation of clinical laboratory results. A companion web site currently under design will allow continued interaction via the Internet between NAMA faculty and prior attendees, providing additional learning opportunities. Extensive fund raising efforts have allowed course planning and delivery to occur at modest cost to trainees and the Society plans to endow the course for future years to ensure its continuity and financial stability.

Biotin holocarboxylase synthetase, biotinidase deficiency and biotin transporter disorders: What do we know and what is still left to learn?

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Biotin, a water-soluble B-complex vitamin, is the coenzyme for four carboxylases in man. The discovery of inherited, biotin-responsive disorders has heightened interest in the known and potential functions of biotin and the enzymes and proteins involved in its metabolism. Clinically, both untreated individuals with HCS (holocarboxylase synthetase deficiency) and biotinidase deficiency (BD) have many neurological symptoms in common, but only sensorineural hearing loss is a consistent feature of BD. Individuals with both disorders respond to pharmacological doses of biotin. Both untreated HCS and BD result in multiple carboxylase deficiency characterized by lactic acidosis, elevations of organic acids, and hyperammononemia, but only BD is associated with low plasma biotin concentrations and the accumulation of biocytin. Do these differences in biochemical features provide clues to the mechanism(s) that cause the differences in clinical symptoms?

In untreated individuals with BD, the neurological symptoms may occur in the absence of organic acidemia(uria), whereas the metabolic abnormalities are essentially always present in symptomatic children with HCS. It is known that organic acid elevations occur in the CSF of children with BD before they are evident in the peripheral tissues. There is lower biotinidase activity in the brain than in most peripheral tissues. This appears to be due to the localization of biotinidase to specific regions of the CNS. Does the brain poorly recycle biotin, if at all? Is the brain dependent on the transport of biotin from the periphery across the blood–brain barrier? If so, is the transport of biotin into the brain an active process?

Do B and HCS each have other functions? Are both enzymes involved in biotinylation of proteins, in general, or are specific proteins biotinylated, such as histones? Why is there such extensive endogenous, non-carboxylase-related staining of the brain with avidin even when there is no exogenously administered biotin or biocytin?

The inherited disorders of biotin metabolism, which at first seemed so straight-forward and simple to understand, have provided “food for thought” for novel and specific functions. Using a knock-out mouse with BD we have begun to address some of these intriguing questions.

Competitive inhibition of large neutral amino acids as an alternate treatment modality in inborn errors

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Background: New treatment strategies for treating phenylketonuria (PKU) and other inborn errors have emerged. One novel treatment is use of LNAAs which share a competitive transport system at the blood brain barrier (BBB). The LAT1 transporter, an L-type transporter, regulates uptake of phenylalanine (PHE) and other LNAAs. A low Km and high affinity for PHE potentiates influx of PHE over other LNAAs into the brain. In PKU, use of LNAAs may offset neurotoxicity associated with high blood and brain concentrations.

As early as 1953, it was postulated that high PHE can interfere with uptake of other LNAAs into the brain. The concept of using competing amino acids to block substrate uptake in PKU was first tested in chemically-induced PKU rodents in 1976. Since then, a number of clinical studies and have been reported an effect of LNAAs supplementation on decreased blood and brain PHE concentrations, increased brain protein synthesis and increased neurochemical production. Neurocognitive measures have also shown improved function. Research has shown that LNAAs may have increased benefit at the BBB in conjunction with lower blood PHE concentrations, which is in line with our knowledge of the tight binding of PHE at the LAT1 transporter.

Understanding the role of BBB amino acid transport mechanisms can lead to potential new therapies in other IEM, including maple syrup urine disease and glutaric aciduria type 1 (GA1). In the mouse model of GA1, studies have shown that reducing lysine uptake, via the CAT1 transporter, reduced brain glutaric acid concentrations and brain injury. Use of homoarginine and glucose together provided increased survival rates through competitive inhibition of lysine. Clinical trials have not yet been initiated in patients with GA1.

Conclusions: As of today, LNAAs can offer an alternative to strict dietary treatment in non-compliant or off-diet patients. Moreover, increasing LNA to standard medical foods may have a potentiating effect on lowering blood and brain PHE concentrations. More studies are needed to identify dose responsiveness, actual response on brain PHE using more precise MRS. Identifying other amino acid transporter genes and polymorphisms, and neuropahtophysiology of IEM will further improve our understanding of the full potential of LNAAs and other competitive-inhibition therapies.
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3) Known-disease diagnoses in the NIH Undiagnosed Diseases Program

Adams D1, Tifft C1, Wolfe L1, Golas G1, Markello T1, Prierson T1,2, Toro C1, Gropman A1,3, Gahl W1

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The NIH Undiagnosed Diseases Program (UDP) was started in 2008 as a pilot program with stated goals of finding accurate diagnoses, and discovering new diseases that have the potential to provide insight into human physiology and genetics. To date, ~4000 inquiries have been received, ~1300 medical records have been reviewed, ~250 people have been accepted to the program and ~100 have been referred to other ongoing studies. So far, ~160 accepted individuals have been brought to the NIH for in-depth medical evaluation. Participants are selected in part by the completeness of their prior medical workup, thus enriching for new conditions. So far, approximately 15% of evaluated patients have received a diagnosis, verified by such objective means as enzyme assay, specific analyte measurement, identification of pathognomic histological findings, identification of well-characterized disease-associated mutations and/or identification of new mutations with subsequent functional-assay verification. Most diagnoses have been inborn errors of metabolism and/or neurological disorders. Diagnoses have included amyloid myopathy, spinocerebellar ataxia type 28, congenital disorder of glycosylation Iib, adenosuccinate lyase deficiency, GM1 gangliosidosis and other conditions. The list of UDP diagnoses provides some perspective as to the nature of conditions that may elude diagnosis despite extensive workup. In our cohort, diagnoses were missed for a variety of reasons including false-negative diagnostic testing, lack of available clinical testing for rare conditions and atypical disease presentations. We present a list of UDP diagnoses, a subset of which are discussed in detail to illustrate the means by which a final diagnosis was made.

4) Clinical outcomes of patients diagnosed with isobutyryl-CoA dehydrogenase (IBD) deficiency after positive newborn screening

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**Background:** IBD deficiency is a disorder of valine metabolism and a secondary condition listed on ACMG's recommended expanded newborn screening (NBS) panel. The index case was a 12 month-old who presented with anemia, dilated cardiomyopathy and carnitine deficiency (1). While guidelines do not exist for treating IBD deficiency identified by NBS, various medical interventions are done based on the index case's presentation (1). These include an echocardiogram, carnitine levels, and its supplementation if plasma levels are low. In 2007, an algorithm for the diagnosis of IBD deficiency after a positive NBS was published and recommended measuring urine C4-acylcarnitine levels to confirm the diagnosis (2). In order to understand the natural history of this organic acidemia, we conducted a survey of physicians caring for IBD deficient patients that were identified through NBS and who obtained a urine C4-acylcarnitine level.

**Methods:** Urine acylcarnitine results from Mayo Clinic's Biochemical Genetics Laboratory's database were reviewed over the last 5 years for patients with elevated C4-acylcarnitine levels consistent with IBD deficiency. Ordering physicians were contacted and a phone survey was conducted. Questions were asked about: echocardiogram results, carnitine levels before supplementation, carnitine dose, if any, and current carnitine levels, developmental delay, speech delay, consanguinity, mutation analysis and clinical follow up.

**Results:** A total of 30 patient samples were identified with urine C4-acylcarnitine levels indicative of IBD deficiency. Follow up information was obtained for 11 patients. All 11 were diagnosed from a positive NBS, 3 were lost to follow up, and 7 had echocardiograms, which were normal. Only one patient is still on carnitine supplementation but developmental or speech delay has not been reported for a single case to date. One child was reported to have consanguineous parents. Mutation analysis was not completed for most cases following the positive biochemical laboratory findings.

**Conclusions:** Our preliminary survey of 11 additional IBD deficiency patients suggests that NBS positive cases do not demonstrate a clinical phenotype that matches the index case. This inference is based on the following salient points: 1) echocardiograms are consistently normal; 2) most NBS positive cases are not carnitine supplemented; 3) NBS positive cases reach developmental milestones despite a lack of active treatment; and 4) a significant number of cases have been lost to follow up, likely indicating a lack of concern by parents and genetic professionals. While our data is preliminary, additional retrospective evidence will allow for the complete characterization of the natural history of this organic acidemia.


5) The clinical spectrum of pancreatitis in organic acidemias

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**Background:** Pancreatitis has been described as a rare complication attributed to organic acidemias. No known pathophysiologic mechanism has been identified and no clear therapeutic recommendations exist. Usual treatment of pancreatitis involving avoidance of enteral nutrition complicates management of organic acidemias in which avoidance of catabolism is paramount. We describe three examples of pancreatitis in patients with organic acidemias that illustrate the wide clinical spectrum of pancreatic abnormalities.

**Case A** was a 12-year old boy with recurrent pancreatitis but no other problems noted who was transferred to tertiary care from an outpatient hospital with severe metabolic acidosis and loss of consciousness. He had been fasted for several days for pancreatitis, receiving only intravenous dextrose for nutrition. Organic acid analysis revealed significantly elevated propionic acid and 3-OH-propionic acid. Within 36 h of admission he expired of brainstem herniation. His mother, who was pregnant at the time of his death, subsequently delivered a healthy male who also has an attenuated form of propionic acidemia (asymptomatic with diagnostic organic acids).

**Case B** is a 6-year old Vietnamese boy with methylmalonic acidemia diagnosed in infancy after presenting with lethargy and metabolic acidosis. He was managed with protein restriction, metabolic formula, oral carnitine and IM OH-cobalamin without recurrent exacerbations until age 6 years when he presented with intractable abdominal pain and elevated lipase/amylase. Abdominal imaging revealed splenic vein thrombosis and pseudocyst in the tail of the pancreas measuring 1.4 cm x 1.4 cm. At age 8 years, following two recurrent bouts of pancreatitis, the pseudocyst size had increased in size to 4.8 cm x 6 cm.

**Case C** is a 5-year old girl diagnosed with methylmalonic acidemia in infancy with severe metabolic acidosis. After a history of yearly severe metabolic exacerbations she then presented with abdominal pain and nausea with elevated lipase and amylase but no metabolic exacerbation. Conservative care with gut rest and optimization of parenteral calories (15% dextrose) including amino acids was successful in abating two episodes of pancreatitis without inducing catastrophic crisis.

**Conclusions:** Pancreatitis is a rarely reported complication of many organic acidemias and can be the presenting symptom in mild/attenuated forms. Early detection is important and avoidance of catabolism in management is critical to successful outcomes. Clinical severity and exacerbations do not seem to correlate with incidence of pancreatitis which may be recurrent.

6) Homocystinuria in Qatar: the challenge

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Homocystinuria is an autosomal recessive disorder of methionine and homocysteine metabolism caused by cystathionine β-synthase (CBS) deficiency. It is characterized by developmental delay/mental retardation, ectopia lentis and/or severe myopia, a Marfan-like appearance,
epilepsy and thromboembolism. The best results occur in those individuals identified by newborn screening and treated shortly after birth with combined dietary and pharmacological therapy. Methionine analysis is unreliable for the detection of homocystinuria in a neonatal screening program, however, it may be reliably detected in routine neonatal screening with a novel tandem mass spectrometry method; this method has a higher sensitivity than DNA testing for the detection of homocystinuria in Qatar. A very high incidence of homocystinuria in Qatar reaching up to 1:1000, caused primarily by homozygosity for the mutation R336C in the CBS gene as a result of strong founder effect in a highly consanguineous population. We will present follow up data on affected children detected by this novel neonatal screening method.

7) Cholesterol esterification and Filipin staining in non-classical forms of Niemann–Pick Type C

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**Background:** Niemann–Pick Type C (NPC) is characterized by a defect in intracellular lipid trafficking of cholesterol and excessive accumulation of unesterified cholesterol in the lysosomes leading to progressive neurodegeneration. Age of onset and rate of progression vary between individuals and range from a rapidly progressive infantile presentation to an adult chronic form of the disease.

**Methods:** Cholesterol esterification and Filipin staining on cultured fibroblasts are standard biochemical tests used to confirm a diagnosis of NPC in suspected patients. In most cases, a definitive diagnosis is made using these tests.

**Results:** We report two siblings (#1 — 24 year old male, and #2 — his 20 year old sister) with clinical features suggestive of NPC. Each patient presented in childhood with ataxia, gait disturbance, slurred speech, and poor school performance. Sibling #1 was initially evaluated for NPC; however, staining (mild) and esterification (26% of normal control cells) were different from those usually seen in typical NPC patients and interpreted as not consistent with classic NPC. Subsequently, an extensive work-up for ataxia and neuromuscular disorders was normal. Repeat staining and esterification showed results similar to the first. Since the clinical picture strongly suggested NPC, DNA was sent for analysis. A known pathological mutation (p.R404Q) was identified along with a mutation previously reported in a specific ethnic group (p.N968S), different from our cases. Subsequent work up of sibling #2 whose clinical features were also suggestive of NPC, revealed the same mutations.

**Conclusions:** These cases suggest that results of cholesterol esterification and Filipin staining in some individuals with clinical features consistent with NPC may have a broader variability than previously thought. Accordingly, even when esterification is not reduced and Filipin staining not as abnormal as in classic NPC, molecular genetic analysis should be considered when the clinical presentation is suspicious or the family history positive for NPC.

8) Inborn errors of metabolism information system (IBEM-IS)

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**Background:** While long-term follow-up is critical for monitoring health outcomes and evaluating the effectiveness of newborn screening, standards of clinical care for most screened conditions have never been subjected to evidence-based study. More information about outcomes for these disorders is essential to a better understanding of the natural history of the conditions and development of best practice models for treatment. The Region 4 Collaborative includes Illinois, Indiana, Kentucky, Michigan, Minnesota, Ohio, and Wisconsin and screens approximately 740,000 babies per year. Clinicians and representatives from state departments of health in each of these states and parents have participated in a project to create a dynamic database to collect longitudinal information on individuals affected with IBEM. Our objectives were to create data collection elements for all core metabolic conditions subjected to newborn screening, identifying a core set of information to be ascertained at enrollment and at each interval visit and to identify condition-specific initial and interval elements, and to increase the number of centers entering information over time. Our ultimate goal is to create long-term follow-up data sets for each condition and to collect prospective outcomes information to direct research and clinical care.

**Methods:** Subjects are asked to provide prospective informed consent for inclusion in the data set. Each center planning to enroll has sought IRB approval. Definitions for data elements are defined by literature review, collection of care plans from participating centers, and adopted by consensus of the group. The intent is to enroll subjects upon confirmation of their newborn screening diagnosis and to complete a short data collection survey at each clinic visit. A web-based entry system is employed; the system allows printing of paper-based data entry forms as needed. Data entry occurs at each site using the password-protected website.

**Results:** Data collection began in January 2007 with a single center. As of October 2010, 13 centers have received IRB approval in 10 states (IN, IL (2), MI (2), MN, MO, OH (2), OK, SD, WI (2)) and three additional centers in three states (NY, PA, KY) are seeking approval. Data entry has occurred for 254 persons with 19 separate disorders. Data collection is proceeding for 164 individuals with fatty acid oxidation disorders, 35 individuals with organic acidemias, 17 individuals with biotinidase or holocarboxylase synthase deficiency, 21 individuals with galactosemia, and 17 individuals with maple syrup urine disease. Data elements have been created for an additional 9 disorders that are now pending data collection.

**Conclusions:** This project demonstrates substantial progress in a large-scale pilot effort to initiate data collection regarding long-term follow-up outcomes for individuals with inborn errors of metabolism. Challenges remain: sustaining data collection and unifying the data set with other data collection efforts will be essential for the ultimate success of this endeavor.
9) Planning for long-term follow-up data collection after newborn screening to advance research and improve service delivery and health outcomes

Susan A Berry and Amy M Brower

for the Joint Committee of the NBSTRN Clinical Centers Workgroup1 and the NCC/RC Long-Term Follow-Up Workgroup2

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Background: Newborn screening as a public health imperative has emerged over the last half-century. With the advent of tandem mass spectrometry, a uniform panel for screening was defined and subsequently adopted by most states. However, despite this major achievement, long-term care and treatment strategies for the individually rare conditions detected by screening are lacking. In many cases, disorders are sufficiently rare that only data collected nationally or internationally will yield sufficient numbers to determine patient outcomes, quality of service delivery, appropriate practices and interventions, and deployment of direct care and resources. Only this ascertainment will identify a population robust enough for research trials needed to generate novel treatments. To provide the clinical history necessary for translational research and program enhancements leading to improving health outcomes, a uniform minimum data set with accompanying information collection, management and analysis tools is needed.

Methods: A broadly constituted workgroup comprised of content experts from laboratory-based specialties, departments of health, and clinical activities was convened under the aegis of the Joint Committee, described above. A series of national meetings was held to define a uniform data set common to all screened conditions. Additional meetings were held with content experts to define condition-specific collection elements.

Results: A uniform minimum data set that comprises approximately 80% of desired immediate and longitudinal data collection elements in common across all screened disorders has been defined by consensus among the stakeholders on the Joint Committee. Additional workgroups for metabolic and endocrine conditions, and hemoglobinopathies detected by newborn screening have undertaken definition of condition-specific elements for initial encounter and follow-up for each screened condition. In addition, new groups have been convened to define elements for follow-up of lysosomal storage diseases, severe combined immunodeficiency, hearing loss, and cystic fibrosis.

Conclusions: A national community of specialty providers residing in public health, clinical centers and academic research centers can reach consensus regarding priorities for data collection for long-term follow-up. This effort lays an effective foundation for a uniform minimum data set to ascertain the clinical history of screened disorders and for both public health and research-related activities.

10) Prevalence of Fabry disease in a French cohort of 900 young patients with ischemic stroke: The FIND study

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Background: Fabry disease (FD) is a rare X-linked lysosomal storage disorder leading to multi-systemic life-threatening complications including an increased risk of stroke in young adults. Because of its low prevalence (about 1/80000 for the classical phenotype) and its clinical variability, FD is usually diagnosed with an average delay of about 10 years or even missed. However, Fabry prevalence may not be as rare as previously thought, especially in some high-risk populations as patients with chronic kidney disease or hypertrophic cardiomyopathy. Several epidemiologic studies suggest that FD should also be considered in young patients with cryptogenic stroke.

Methods: The FIND (“Fabry: Initiative Nationale de Dépistage”) study was supported by the French society for neurovascular diseases. It was a national population-based cross-sectional study initiated to investigate FD prevalence in a population of young patients in France. Study recruitment was conducted between 2007 and 2009. Cases were men, aged 28 days to 55 years, hospitalized with a first or recurrent ischemic stroke and identified from hospitals throughout France (50 adult neurology centers and 8 neuropediatric departments). Enzymatic activity of α-galactosidase A was measured by a validated method from dried blood spots (DBS) using a filter-paper test. When activity was below a specific threshold a second visit was scheduled to: 1) answer a questionnaire oriented to characterize signs or symptoms of FD and 2) confirm FD diagnosis using the gold standard leucocyte enzyme activity assay.

Results: The study sample consisted of 902 men with ischemic stroke with a mean age of 43 years old. Low plasma α-galactosidase A activity was detected in 3 patients by DBS but leucocyte enzyme activity didn’t confirm FD diagnosis. A 59-year-old man, who was wrongly included in the study as his age was an exclusion criterion, also showed a reduced activity of α-Gal A by the DBS assay. FD diagnosis was confirmed for this man by a reduced leucocyte enzyme activity; his GLA gene analysis revealed a new heterozygote mutation (c.593T>C, p.Ile 198 Thr; Exon 4). Cerebral CT-Scan showed multiple vertebro-basilar ischemic strokes and he also had a history of cardiac diseases. His renal function was normal. Enzymotherapy was initiated with a good safety profile. Genetic testing undertaken for his at-risk family members revealed 3 other undiagnosed pre-symptomatic patients.
Conclusions: Our results show that specific populations may be screened systematically for FD with a simple method using DBS on filter-paper test. To date, five other studies have investigated the prevalence of FD among various populations with different type of stroke. Reported prevalence in men ranged from 0% to 4.9%. The results of the FIND study suggest that the yield for FD screening in young patients with ischemic stroke is low and that the decision to screen for FD in such patients should be made on an individual basis. Despite this low prevalence, we detected one patient with FD, excluded from the study, and his diagnosis allowed us to identify 3 pre-symptomatic cases in the family who will benefit from a close monitoring and early therapeutic intervention if needed. Thus, French national guidelines recommend screening for FD in young patients with ischemic stroke when extensive investigations did not identify the stroke etiology.

11) Clinical correlations in nutrition and metabolism: Outcomes of a practical training course for genetic metabolic clinicians

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Background: With the nationwide expansion of newborn screening (NBS), and more international attention being paid to inherited metabolic disorders (IMD), medical and dietetic clinicians are called on to know more about IMD, to develop the skills needed to address immediate and life-long interventions, and to integrate a developmental approach with patients as they transition from birth to adulthood. There are few professional development opportunities in the U.S. or elsewhere that offer education in the areas of expanded NBS and genetic metabolic nutrition. To address this gap, in 2007 the Southeast NBS & Genetics Collaborative (SERC) at Emory University began offering the annual Genetic Metabolic Nutrition Symposium for U.S. and international genetic metabolic dietitians, nurse practitioners, and medical fellows.

Methods: The interactive symposium covers biochemistry, metabolic pathways, pathophysiology, and management strategies for the short- and long-term follow-up of patients with an IMD, with a focus on the four major categories of disorders: amino acid disorders, organic acidemias, fatty acid oxidation disorders, and disorders of carbohydrate metabolism. Instructors are national leaders in the fields of NBS, genetics, and IMD and hail from prominent academic institutions and treatment centers around the U.S. The training is typically portrayed as a combination of educational materials on flash drive, face-to-face presentations, live videoconference, and an optional teleconference. Attendees complete assigned pre-course reading, case study for discussion, knowledge assessments at the beginning (day 1) and end (day 5) of the symposium, and event evaluation (day 5). Results from the 2010 attendees are presented here.

Results: In 2010, 23 clinicians from the U.S., Australia, Mexico, and the Netherlands were accepted to the symposium. Average experience in IMD was 2 years, with a range of 2 months to 9 years. The average pre-test score was 76.7 ± 9.6%. The average post-test score was 84.0 ± 8.3%. Scores on the post-test went up significantly (P < 0.0001). In the symposium evaluation, all attendees indicated that the course provided new and useful information and an appropriate curriculum overall. Participants were also positive about the choice of instructors and their expertise, in terms of effective sharing of their knowledge, and the opportunity to network with other clinicians in the field.

Conclusions: The day 1 and day 5 test results and the evaluation by participants illustrate that the symposium gave these early-career practitioners more knowledge of the content presented, helping improve the management and outcomes of those living with IMD.

12) Brown-Vialetto-Van Laere and Fazio Londe syndrome is caused by a riboflavin transporter defect: A new inborn error of metabolism with potential treatment

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Background: Brown-Vialetto-van Laere syndrome (BVVL) and Fazio Londe syndrome (FZ) are one disease entity: a rare neurological disorder which may present in infancy with a devastating neurological deterioration with hypotonia, respiratory insufficiency and early death, or later in life with deafness (only BVVL) and progressive ponto-bulbar palsy. The multiple acyl-CoA dehydrogenation defect (MADD) is an inherited defect of mitochondrial fatty acid beta-oxidation and amino acid catabolism in which the flavin adenine dinucleotide (FAD) is an important co-factor.

Methods: Two siblings and one unrelated patient presented in infancy with progressive muscle weakness and paralysis of the diaphragm. Metabolic studies were suggestive of a mild form of the MADD. Subsequently, a profound flavin deficiency in spite of a normal dietary riboflavin intake was established in the plasma of all three children, suggesting a riboflavin transporter defect. Mutation analysis for a possible riboflavin transporter defect was performed by sequencing all exons plus flanking intronic sequences amplified from genomic DNA extracted from the patients’ cells.
Results: Genetic analysis of these patients demonstrated mutations in the C20orf54 gene which encodes the human homolog of a rat riboflavin transporter. This gene was recently implicated in the BVVL syndrome. Supplementation of riboflavin rapidly improved the clinical symptoms as well as the biochemical abnormalities in our patients.

Conclusion: At least part of the clinical signs and symptoms observed in BVVL and FZ are caused by a deficiency of flavins caused by a transporter defect. Riboflavin is a potential treatment for the BVVL and FL syndrome.

13) Cognitive tests sensitive to the neurological impairment in ornithine transcarbamylase deficiency (OTCD)

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Ornithine transcarbamylase deficiency (OTCD) is an X-linked inborn error of metabolism and the most common of the urea cycle disorders. It is characterized by hyperammonemia leading to neurologic injury and downstream effects on executive function (EF). Severity ranges from subclinical neurocognitive deficits (non-symptomatic OTCD) to protein intolerance, hyperammonemic episodes and marked cognitive impairment (symptomatic OTCD). Here, we used the Comprehensive Trail-Making Task (CTMT) and the Stroop task to investigate EF differences between symptomatic, non-symptomatic, and control subjects. The CTMT requires subjects to integrate numerical and verbal information to connect points on a visual array. The Stroop task includes “congruent” trials (words correspond to the color in which they are printed), and “incongruent” trials (the word and color are mis-matched), which require subjects to resolve conflicting cognitive signals. We found significant performance differences between the three groups at each level of the CTMT and in the Trails Composite Index \((F(2,41)=8.78, p = .001)\). On the Stroop task, incongruent trials, but not congruent trials, differentiated the three groups. Symptomatic patients performed slowest and control subjects performed fastest \((F(2,43)=5.75, p = .006)\). This pattern was also detected in the difference score between incongruent and congruent trials \((F(2,43)=4.36, p = .019)\). Overall, the CTMT and Stroop task appear to be sensitive to the neurocognitive deficits in OTCD and differentiate symptomatic and non-symptomatic patients with respect to EF.

14) Confirmation of an attenuated disease causing splice site mutation, c1398 + 2delT in propionic acidemia

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Background: We present a patient with classic biochemical findings suggestive of propionic acidemia. She has been identified to have two mutations in PCCB, one of which, to our knowledge, has not been previously reported. This report confirms this as a disease causing mutation; however, suggests it may result in attenuated disease, with more relaxed dietary limitations and normal psychomotor development.

Patient: Our patient, now 32 months old, was identified by newborn screening in the state of Missouri, to have an elevated C3, 18.40 μmol/L, and C3/C2 ratio, 1.14 at 1 day 18 h of life placing her at high risk. She was referred to our center, and on day of life 6, repeat screen confirmed this risk, with a C3 of 20.35 μmol/L and a C3/C2 ratio of 1.33. Urine organic acids revealed hydroxyl-propionate and methylcitrate with an absence of methylmalonic acid. She had an elevated ammonia, 242 μmol/L, and propionate, 224 μmol/L. Amino acids revealed an elevated glycine, 423 μmol/L, and lysine, 307 μmol/L, along with a low Valine 59μmol/L. She was hospitalized and transitioned to Propimex-1 without difficulty. PCCB gene sequencing (Prevention Genetics) was performed and demonstrated heterozygosity for a known missense mutation in exon 15, c1606A>G resulting in AsnN536AspD, as well as a novel mutation in a splice site near exon 13, c1398+2delT. Her family has been very compliant with dietary treatment since birth, and she has exhibited uniquely high isoleucine tolerance. She is currently transitioning from Propimex-1 to Propimex-2, which along with a protein restricted diet provides 2.3 g/kg of protein per day, and 1200 mg of isoleucine per day. Developmentally, she has excelled. At 32 months of age, she is able to count to 13, say her ABCs. She started walking independently at 12 months of age and running at 18 months, at which time she had 30+ words. She has had two hospitalizations since diagnosis. The first was within the first week of diagnosis. She experienced feeding intolerance along with elevated ammonia and propionate. This resolved with simple IV fluid hydration and reintroduction of metabolic formula. Her second hospitalization was at two years of age. She had a prolonged viral gastroenteritis and multiple ER visits. She was admitted for 24 h observation and discharged following fluid rehydration without difficulty. She did not exhibit hyperammonia or elevation of her propionate above baseline during this hospitalization.

Conclusion: Based on our experience with this patient, a c1398 + 2delT splice site mutation in PCCB is pathogenic and results in an attenuated disease phenotype.

15) Utilization of the newborn screening translational research network to advance research and clinical applications in lysosomal storage disorders

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Newborn screening programs in all U.S. states and territories screen over four million infants each year for at least thirty heritable disorders with the goal of early detection and treatment to reduce morbidity and mortality. The decision to add new disorders to screening programs occurs on a state-by-state basis and is based on several factors including an improved understanding of the disorder, the development of novel technologies to screen for the disorder and new therapeutic strategies for treatment. Expanded screening may also be the result of advocacy, legislation or recommendation by local, state and federal advisory boards. The Secretary's Advisory Committee on Heritable Conditions in Newborns and Children (SACHDNC) advises the Secretary of Health and Human Services on a broad range of issues including newborn screening, and in 2007 SACHDNC established a system for nomination, external evidence-based review and recommendation/rejection of nominated disorders for screening in the newborn period. Successful implementation of screening for new disorders requires system-wide changes and encompasses not only screening but the diagnosis, short- and long-term follow-up of screen positive infants and, ideally, should advance understanding of the disorder and track patient outcomes based on early diagnosis and treatment.

To support this systems approach and facilitate the translation of new technologies and treatments while fostering research, the Eunice Kennedy Shriver National Institute on Child Health and Human Development, National Institutes of Health established the Newborn Screening Translational Research Network (NBSTRN). NBSTRN work focuses on creating content and infrastructure, and establishes an analytical, clinical and research framework for use by the research, clinical and public health care communities. We present an overview of the NBSTRN and demonstrate its tools and resources as they are applied to advance newborn screening and research for a new group of conditions, lysosomal storage disorders (LSDs). Recent advances in the detection and treatment of some LSDs as well as legislative actions have led several state-based public health programs to begin screening for LSDs. The implementation of newborn screening for these six LSDs (Gaucher, Pompe, Mucopolysaccharidoses I/II, Fabry and Krabbe disease) provides a useful test case for the NBSTRN. The NBSTRN is involved in coordinating comparative evaluation of technologies for screening for LSDs and the development of diagnostic, treatment, and evaluation protocols and related tools to be used in point-of-care data collection.

16) Cardiomyopathy in methylmalonic aciduria. A case report

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Introduction: Methylmalonic aciduria (MMA) is an autosomal recessive inborn errors of metabolism caused by deficient methylmalonyl-CoA mutase (MUT) activity (most common) or impaired transport/synthesis of its cofactor, cobalamin. The disorder is characterized by accumulation of methylmalonic acid and other metabolites in body fluids. It is a heterogeneous disorder, with onset from infancy to adulthood and varying degrees organ involvement and severity.

Case Report: The patient is a 22 year old female who was diagnosed with MMA (Type Cbl B by complementation) at age 4 months. Her past medical history was significant for a history of numerous hospitalizations (related to metabolic crises), developmental delay, treated primary hypothyroidism, and living-donor renal transplant (secondary to MMA related nephropathy). Over the past several years, however, she was generally in good health and had not experienced a metabolic crisis, despite a liberalized protein restricted diet. She presented to the hospital with a ~1 week history of nausea and non-bloody/non-bilious emesis and was admitted for hydration and acute management of a metabolic crisis. At admission, she had a metabolic alkalosis (chronically treated with bicitra) with lactic acidosis (3.1 mmol/L). Urine organic acids demonstrated significant amounts of MMA and methylcitrate. A chest X-ray showed cardiomegaly, prompting a further cardiac evaluation. Electrocardiogram demonstrated persistent T-wave inversion, which was a change from her baseline. An echocardiogram demonstrated moderate pericardial effusion, but normal chamber sizes and left ventricular systolic function (left ventricular fractional shortening, 35%). Follow up echocardiograms demonstrated worsening pericardial effusions and left ventricular systolic function (left ventricular fractional shortening, 18%). This was associated with development of severe lactic acidosis (7.1 mmol/L). Despite careful monitoring/treatment, the patient developed a ventricular arrhythmia and went into cardiopulmonary arrest from which she died. Autopsy findings confirmed cardiomegaly with increased epicardial adipose tissue, myocardial edema, and a pericardial effusion. On histological examination, myocardial fiber hypertrophy was present, as well as focal areas of myocardial cell degeneration (e.g., atrophy and vacuolar changes). Viral studies (PCR/in situ) were performed and did not show evidence of a viral etiology for her cardiac dysfunction.

Conclusion: Cardiomyopathy has been identified in a number of other organic acidurias, i.e., propionic aciduria (1), but is usually discovered during screening studies, rather than an acute event. To our knowledge, this represents the first published case of cardiomyopathy in MMA. Sudden death has been reported in individuals with MMA (2), therefore it is possible that cardiac dysfunction is under-recognized, particularly during acute metabolic events. The mechanism for cardiomyopathy in organic acidurias is unclear, but may be related to metabolic acidosis/decompensation and/or accumulation of toxic metabolites within the myocardium. In conclusion, cardiac dysfunction in is a potential complication of methylmalonic aciduria, particularly during acute illness, and may be responsible for rapid deterioration and/or sudden death in this patient population. Further studies are needed to further elucidate the association of cardiomyopathy and MMA. Additional monitoring for this potential complication should be considered, particularly during episodes of acute illness.

17) Baseline characteristics of PKU patients enrolled in the PKUDOS registry

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**Background:** The PKUDOS registry was designed to provide 15 years of data on PKU patients of all ages who are currently or previously treated with sapropterin dihydrochloride (sapropterin, Kuvan®) or who plan to initiate treatment with sapropterin. Baseline data were provided by participating centers.

**Methods:** Baseline characteristics are presented for the 589 patients enrolled at 45 centers across the United States during the first 2 years after launch of the registry. This PKUDOS population was aged 0–55 years (median = 14 years) at enrollment, evenly distributed between males and females, and 89% white. Age at PKU diagnosis ranged from 0 to 49 years (median = 3 days). Overall median height (n = 552) was slightly below and weight (n = 562) was slightly above the 50th percentile for both current and prior sapropterin exposure patients (n = 553) of +0.7 SD above the 50th CDC growth curve. Prescribed phenylalanine (Phe) free medical foods and formulas, large neutral amino acids, and nutrient supplements (tyrosine [Tyr], vitamins, minerals, energy, and dietary Phe) were recorded. At enrollment, 315 (53%) patients were taking sapropterin, 188 (32%) had prior sapropterin exposure (not currently taking sapropterin), and 86 (15%) were to begin treatment with sapropterin per registry enrollment criteria. Median duration of exposure for both current and prior sapropterin users (n = 457) was 15.5 months with a median dose level of 20 mg/kg/day. For patients with daily Phe intake reported, median daily prescribed Phe and actual Phe intake were approximately 30–50% higher in patients taking sapropterin than in patients with prior sapropterin exposure and patients that were to begin sapropterin treatment. Median blood Phe levels at enrollment were 333, 666, and 598 μmol/L among patients currently taking sapropterin, patients with prior sapropterin exposure and patients that were to begin sapropterin treatment, respectively. Phe/Tyr ratios showed a similar trend, with values of 6.7, 11.6, and 10.3 among patients taking sapropterin, patients with prior sapropterin exposure and patients that were to begin sapropterin treatment, respectively.

**Conclusions:** The PKUDOS registry allows the longitudinal follow up of patients with PKU. Patients had mildly increased BMI compared with CDC growth curves. Patients taking sapropterin had higher prescribed and actual dietary Phe intake while maintaining lower Phe levels and Phe/Tyr ratios. The PKUDOS registry is an opportunity for healthcare providers to engage in active research regarding management and long-term outcomes of PKU patients who have had, or will have, exposure to sapropterin.

18) Nutritional therapies for inherited metabolic diseases: Building an infrastructure to guide clinical practice through evidence-based research

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**Background:** Improving health and developmental outcomes for persons with inborn errors of metabolism (IEM) has driven clinical practice and research in this field for over half a century. Many treatment strategies to date are based on clinical trial and error and theoretical constructs. The rarity of these diseases and lack of clinical therapeutic trials often result in provision of unproven therapies that may adversely affect therapeutic advancement (Vockley J. 2010. Molecular Genetics and Medicine. 99:244). With direct and ongoing input from clinical, research, government, and non-profit organizations, industry, and policy stakeholders, the Office of Dietary Supplements and the Office of Rare Diseases Research are developing an infrastructure to conduct evidence-based research on the safety, efficacy, and effectiveness of nutritional interventions, including dietary supplements, used to treat IEM. The ultimate goal is to support collaborative, multicenter clinical trials to derive outcome data that can be used to inform practice guidelines with the intention of broad adoption in clinical practice. This work may also be the foundation to: inform the development of models that would be suitable for all rare disorders; assess the therapeutic efficacy of dietary interventions and nutritional supplements in the broader context of public health; and develop solutions that would afford access to evidence-based treatments for all patients who would benefit from them.

**Methods:** To inform the development of this infrastructure, a white paper is being written that will include the history and evolution of dietary treatment for IEM; current clinical practices and the range and depth of published research on dietary therapies; gaps in knowledge, infrastructure, and funding deficiencies that hinder evidence-based research; and recommendations to address and fill the knowledge gaps and define and develop a framework for future research. In tandem with development of this background report, core government, professional
association representatives, and stakeholders from the private sector and industry will be invited to serve on a planning group to further develop the infrastructure and achieve the goals and objectives of the project.

**Expected Impact of the Project:** This project is inclusive in its scope by engaging stakeholders across government and in the private sector who are involved in research, clinical care, advocacy, and policy related to IEM. The innovative strategies developed to conduct evidence based research for IEM will ultimately improve patient care and lead to optimal patient outcomes and may serve as a model for other rare diseases.

**19) Effective gene therapy for propionic acidemia**

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Propionic Acidemia (PA) is a severe disorder of intermediary metabolism caused by deficiency of propionyl-CoA carboxylase. PA patients suffer from lethal metabolic instability and can develop multisystemic complications despite precursor restrictive dietary therapy. A murine model of PA (Pcca−/−) that perishes within the first two days of life was used to examine the efficacy of adeno-associated viral (AAV) gene transfer as a potential therapy for PA. An AAV serotype 8 vector designed to express the human PCCA cDNA was delivered to newborn mice and effectively rescued Pcca−/− mice for as long as 7 months after treatment. Propionyl-CoA carboxylase activity was partially restored after gene therapy, circulating metabolites were lowered and viral transgene expression was present in multiple tissues. Treated Pcca−/− mice appear healthy and male mice are fertile. In summary, AAV gene delivery of PCCA effectively rescues Pcca−/− mice from neonatal lethality, reduces disease related metabolites and provides persistent transgene expression. These experiments are the first to demonstrate that a gene transfer approach might be used as a treatment for PA, a devastating and often lethal disorder desperately in need of new therapeutic options.

**20) Cells from individuals with propionic acidemia have abnormal Krebs cycle gene expression**

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**Introduction:** Propionic acidemia (PA, OMIM #60654) is an organic acidemia which is characterized by dysfunction of the propionyl CoA carboxylase enzyme. Deficient energy metabolism is an emerging complication that affects patient’s heart, muscles, and brain. Hypothesis: Cells from patients with PA have different expression response (RNA levels) from controls in energy pathways in response to cellular stress (hypoglycemia). Methodology: RNA from lymphoblastoid cell lines from PA patients and gender/age-matched controls grown at normal conditions and low-glucose conditions was collected and hybridized to Affymetrix Human Gene 1.0 ST microarrays and expression for 28,869 genes was determined. Gene expression differences between the two conditions (normal growth conditions and hypoglycemia) for all assayed genes were calculated and comparisons were made. Results: In our entire screen, 1% of all signals were different between the two treatments. We found that genes for most of the Krebs cycle enzymes were significantly decreased compared to controls in the presence of hypoglycemia. We also found a decrease in ASL, CPT1 and SLC25A11 (aspartate/malate shuttle). In the Krebs cycle, expression was statistically different in 9 of 17 of the Krebs cycle genes in PA patients compared to controls suggesting specific effectors of the Krebs cycle in PA patients (p<0.05). Discussion: Adequate energy metabolism is required for a number of cellular processes and patients with PA have signs of energy deficiency. These studies suggest specific resultant blocks in energy production at the level of Krebs cycle may be at the heart of the observed decreased energy. In addition, specific targeting of these Krebs cycle intermediates suggests novel approaches to increased energy metabolism.

**21) Efficacy or inefficacy of replacement enzyme therapy on central nervous system in Fabry's disease**

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Fabry’s disease (FD) is an X-linked inherited lysosomal sphingolipidosis that leads to a multisystem disease. The efficacy of replacement enzyme therapy on the central nervous system has not been clearly evaluated.

A 42 year old in-patient suffering from Fabry’s disease was diagnosed at the age of 10. Since the age of 26, he suffered from repeated ischemic cerebral vascular accidents. Since the age of 34, he was treated by enzyme replacement therapy. He had a cerebral MRI evaluation 1 year and 4 years after the beginning of the enzyme therapy. No new clinical event was noticed and the imaging did not show new lesion. However, the neurological status worsened since the age of 38. A pseudobulbar palsy occurred progressively and a gastric tube was needed. The renal function also progressively worsened. A MRI was performed after 8 years. No new lesion appeared.

We report the MRI evolution under enzyme replacement therapy of a patient with Fabry’s disease with central nervous system involvement. No new lesion appeared.

Enzyme replacement therapy seems beneficial in Fabry’s disease with central nervous system involvement on MRI. However, the clinical status progressively worsened without any explanation.
22) First French case of NAGS deficiency. 20 years of follow up

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NAGS deficiency is the rarest deficit of the first enzyme of the urea cycle. It leads to hyperammonemia and is clinically undistinguishable from the other urea cycle disorders.

Case report:
We present a 20 year-old man with a NAGS deficiency diagnosed when he was 6 month-old. Since the third day of life, he suffered from vomiting, feeding intolerance and episodic confusion. Ammoniemia fluctuated between 300 and 500 μM but there was no obvious evidence of a urea cycle disorder, without urinary orotic acid. Plasma citrulline, argininosuccinate and arginine were normal. Carglumic acid test (350 mg/kg/d in 3 equal amounts) evoked a NAGS deficiency, confirmed by enzymatic activity and molecular biology: chromosome 17q21.31, exon 1: c.278delC heterozygous; exon 2: c.499A>C heterozygous.

He was initially treated with Carglumic acid (140 to 23 mg/kg/d). Then, the dose of 200 mg/d was kept unchanged for about 4 years. Today, carglumic acid is 800 mg/d, with a normal diet. He's now 20 year-old and has a normal school life without great delay. Postprandial ammoniemia remains always in a normal range. No clinical abnormality was noticed.

Conclusion:
Our case show that Carglumic treatment is effective in NAGS deficiency. Long term prognosis is excellent.

23) Dihydropteridine reductase deficiency and treatment with tetrahydrobiopterin: A case report

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Dihydropteridine reductase (DHPR) deficiency is a genetic disorder of tetrahydrobiopterin (BH4) regeneration and may present with hyperphenylalaninemia, microcephaly, hypotonia, mental retardation and convulsions. BH4 is an essential cofactor for the hydroxylation of aromatic amino acids and a deficiency of BH4 results in decreased synthesis of dopamine and serotonin. We present a 27-month-old patient with DHPR deficiency who was treated with 1-L-dopa/carbidopa (2 mg/kg/day, four times per day), L-tryptophan (2 mg/kg/day, four times per day), Folic acid (10 mg/day), and BH4 supplementation (20 mg/kg/day, twice a day). Although remarkable clinical improvement with normal plasma phenylalanine (Phe) levels was noted one month after the treatment, CSF neurotransmitters, and BH4 did not improve. BH4 supplementation was increased to 40 mg/kg/day, and the CSF study was repeated one month later. There was no significant improvement of CSF neurotransmitters or BH4 levels but Phe level was within normal range. Surprisingly, she had developmental improvement following a 3-month-treatment. She was able to pull herself to the standing position and sit down on her own. She was noted to be more alert and responsive. Her expressive language did not improve although her receptive language was markedly improved. The aforementioned treatment improved patient’s clinical findings, normalized blood Phe levels and increased Phe tolerance in the diet, but neither 20 nor 40 mg/kg/day BH4 supplementation corrected neurotransmitter or BH4 levels in CSF. Further studies are needed to find the optimal management plan for patients with DHPR deficiency.

24) Pregnancy and Cobalamin C deficiency: A patient presenting with elevated homocysteine and MMA levels following two spontaneous abortions

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Background: Cobalamin C (Cbl C) deficiency (Methylmalonic aciduria and Homocystinuria; OMIM# 277400) is the most common inherited form of vitamin B12 metabolism. Individuals with Cbl C deficiency may present with visual, cardiac, renal and central nervous system involvement and there is variable response to treatment with vitamin B12 supplementation. Cbl C deficiency is a clinically heterogeneous disorder with symptomatic presentation ranging from the first year of life and attenuated disease presenting in adulthood.

Case report: We present a 27-year old woman who was evaluated for an inherited thrombosis disorder following two first trimester spontaneous abortions. A single methylenetetrahydrofolate reductase (MTHFR) mutation (c.C677T) was identified and elevated plasma total homocysteine (Hcy) level and an elevated serum Methylmalonic acid (MMA) level were subsequently noted. Familial studies revealed elevated total Hcy and MMA levels in a full sibling without an MTHFR variant present. As a result of the biochemical findings the patient
was referred for a metabolic evaluation. At the time she presented to care, there was no evidence of protein aversion, ophthalmologic findings, or neurologic involvement. The patient was pregnant with a fetus measuring at 7 weeks of gestation at the time of her initial metabolic evaluation.

**Results:** At presentation, an acylcarnitine profile revealed an elevated Propionyl carnitine (C3) level of 1.84 (reference range 0–1.60), total Hcy level of 56.18 (reference range of 10.50–16.7 μmol/L) and 205 mg/g creatinine of MMA measured in urine organic acid analysis. Molecular genetic sequencing of the MMACHC gene revealed two missense mutations [c.388T> C (p.Y130H), c.643T> C (p.Y215H)] consistent with the biochemical diagnosis of Cbl C deficiency.

The patient was treated with 1 mg of intramuscular hydroxocobalamin per week. After one dose of hydroxocobalamin, her total Hcy level decreased from a level of 56.18 μmol/L to a level of 37.71 μmol/L and her MMA level decreased from a level of 205 mg/g creatinine to a level of 150 mg/g creatinine. She remained on a weekly regimen of hydroxocobalamin and her metabolites quickly normalized and remained within normal limits throughout the pregnancy. The patient delivered a healthy child following a full-term pregnancy without complications.

**Conclusion:** The attenuated phenotype of Cbl C deficiency may not be apparent in early adulthood. The late onset form of Cbl C deficiency may respond well to therapeutic treatment with hydroxocobalamin and treatment is able to normalize metabolites throughout pregnancy. The association between elevated total Hcy levels and increased risk for spontaneous abortions is not well delineated and should be studied further.

**25** Phase 3 blinded, randomized, crossover comparison of sodium phenylbutyrate (NaPBA) and glycerol phenylbutyrate (GPB): Ammonia (NH₃) control in adults with urea cycle disorders (UCDS)


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Glycerol phenylbutyrate (GPB, also referred to as GT4P or HPN-100) is being developed as a treatment alternative to sodium phenylbutyrate (NaPBA) for UCDS patients. Both drugs mediate waste nitrogen scavenging through conjugation of phenylacetic acid (PAA), derived from phenylbutyric acid (PBA) with glutamine to form phenylacetylglutamine (PAGN) which is excreted in the urine. GPB is a short chain triglyceride (liquid) and 5.8 mL TID is anticipated to be the equivalent of 40 tablets (20 g) of NaPBA.

**Study design:** Protocol HPN-100-006 was a 4-week randomized, blinded, cross-over comparison of the NaPBA with a PBA-equimolar GPB dose to establish non-inferiority of GPB to NaPBA in NH₃ control. Subjects underwent 24-h blood sampling for NH₃ and PK after each two week treatment period.

**Results:** 46 subjects (41 OTC, 3 ASS, and 2 CPS) were randomized; 45 received at least one dose of study drug and constituted the ITT population; 44 completed the study. Subjects were evenly distributed between treatment arms, had been taking NaPBA for an average of ~13 yr and received an average dose of 13.95 g/day (7.8 g/m²/day) of NaPBA and 13.49 g/day (7.55 g/m²/day) of GPB while on study. NH₃ values on both drugs were lowest after overnight fasting and peaked postprandially. 95% confidence intervals for NH₃ (24 h AUC) on GPB relative to NaPBA in the ITT population (0.79, 1.03) were below the predefined non-inferiority margin of 1.25; mean NH₃ levels on GPB were ~10% lower than on NaPBA (34.7 vs. 38.4 μmol/L; not significant). Glutamine was lower on GPB than NaPBA (758 vs. 809 μmol/L; ULN=746; p<0.05) by post-hoc analysis. At baseline, 91% of patients reported ≥ 1 organ system complaint with GI (56%) most common. AEs on study were reported by 61% and 51% of subjects during GPB and NaPBA treatment, respectively, with most being GI and generally mild. No clinically significant lab or ECG changes were observed. One subject experienced a hyperammonemic crisis and one withdrew early because of high NH₃ and headache; both were on
NaPBA. One subject had an SAE of gastroenteritis on GPB judged unrelated. As compared with NaPBA, 24-hour AUC and peak plasma PAA levels were significantly lower on GPB, trough values higher, and overall 24 h urinary PAGN output identical but with more even day/night distribution.

Conclusions: This pivotal trial demonstrated non-inferiority of GPB to NaPBA for NH3 control. The authors theorize that the PK findings and trend toward lower NH3 and glutamine observed in the pivotal study may be explained by slower GI absorption of PBA when delivered as a triglyceride (GPB) rather than a salt (NaPBA), leading to higher trough levels of the active moiety, PAA. The data also support the utility of urinary PAGN as a clinically useful biomarker.

26) Natural history study of very-long-chain Acyl-CoA dehydrogenase deficiency in The Netherlands

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Background: Since 2007 Very Long Chain Acyl-CoA Dehydrogenase Deficiency (VLCADD) is included in the Dutch neonatal screening program (NSP). Evidence based guidelines for treatment and follow-up of patients detected by the NSP are crucial to sustain proper care after neonatal diagnosis. For VLCADD, however, these guidelines have remained ill-defined.

VLCADD is a rare disorder affecting the mitochondrial beta-oxidation of long-chain fatty acids. Clinical symptoms arise, or are exacerbated during catabolic situations e.g. during illness or fasting. Organs most frequently involved are those using long-chain fatty acids as primary energy source, such as the heart and skeletal muscles. Age at onset, manifestation patterns and clinical severity differ between patients.

Currently in the Netherlands dietary measures aimed at preventing catabolism are the only available treatment option for patients with VLCADD. Long term outcome of these measures is unknown. There is a serious lack of historical controls. In order to be able to evaluate the true yield of the NSP, those historical controls are, however, of the utmost importance.

Goal: To gain insight into incidence, clinical presentation and treatment of VLCADD in the Netherlands.

Methods: Retrospective study. Patients were searched for by contacting clinical metabolic centers; metabolic laboratories specialized in VLCADD diagnostics, patient organizations and the Dutch Diagnosis Registration Metabolic Diseases (www.ddrmd.nl). Patient data were collected by patient file analysis using a standardized protocol.

Results: Thirty patients were identified including 21 males and 9 females, born between 1963 and 2010. Seven patients were identified by newborn screening; including one severely affected, two mildly affected and four are asymptomatic, at least until now. A broad spectrum of mutations was found of which c.848T>C (p.V283A) was found most frequently. Generally no complications were reported during pregnancy or birth. Symptoms most frequently reported in childhood were hypoglycemia, muscular pains and cramps accompanied by increased plasma creatine kinase (CK) levels, and hepatomegaly. In adulthood muscular complaints and severely impaired daily functioning were most prominent. All patients are treated with dietary measures varying from LCT restriction, MCT enrichment, fatty acid supplementation and tube feeding, to carbohydrate or MCT intake before exercise. Treatment with a LCT restricted, MCT enriched diet appears to be effective in short-term disease control. In some adult patients severe impairment of daily functioning despite dietary treatment was reported.

Conclusions: Since the introduction of the NSP, more patients with VLCADD have been identified as previously (est. incidence before screening 1:300,000, after 1:80,000). It is not yet clear whether asymptomatic VLCADD is relatively common but under-diagnosed, or whether a significant portion of infants detected by NSP might never become symptomatic unless experiencing significant catabolic stress. Factors predicting disease course (genotype, enzyme activity, CK, e.o.) and the effects of screening and (early) treatment on long-term outcome have to be determined to gain more insight. We will perform structured follow-up of all identified patients in an expertise centre for metabolic diseases.

27) Metabolic predictors of coagulation abnormalities in glycogen storage disease type 1

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Background: Glycogen storage disease type 1(GSD1) is an inborn error of metabolism caused by defective glucose-6-phosphatase complex. Enzymatic deficiency leads to fasting hypoglycemia, hyperlactatemia, hyperuricemia, and hyperlipidemia. Acquired platelet dysfunction and reduced von Willebrand factor (VWF) have been described in patients with poorly controlled GSD1. The coagulation defects manifest as epistaxis, easy bruising and bleeding during surgical procedures. While, dietary intervention reportedly abrogates the bleeding diathesis, it is unknown if there is a specific metabolic factor which accounts for the laboratory abnormalities and clinical symptoms.

Objectives: To evaluate the relationship between predictors of metabolic control and platelet aggregation and VWF in patients with GSD1.
Methods: Patients with GSD1 (a,b) who were followed at Duke Metabolic Clinic were included. Patients on antiplatelet medications were excluded. Bleeding history and laboratory data were abstracted from the medical records. Metabolic control predictors studied were lactic acid (LA), total cholesterol, triglycerides, and uric acid. Platelet aggregation was measured in response to ADP, collagen, ristocetin, arachidonic acid and thromboxane. VWF testing included factor VIII, VWF Ag, VWF activity (VWF:RCo), and ABO blood group. Pearson correlations were used to test the association of measures of coagulation with individual metabolic parameters. Forward stepwise multiple regression analysis was used to identify a subset of metabolic predictors for each coagulation parameter studied as an end point. Data are reported as mean ± SEM, and (normal values). A value of p < 0.05 was considered significant for correlations.

Results: Twenty-seven patients with GSD1 (12F, 15M), age range (2.7–45 years) were included. 70% of the patients gave a history of episaxis, easy bruising, menorrhagia, or bleeding following minor wounds or dental extraction. There was no evidence of uremia or acidosis, and hepatic transaminases were mildly elevated. Mean glucose and uric acid levels were within normal. The mean LA was 4 ± 0.4 (0.5–2.2 mmol/L); total cholesterol was 239 ± 18 (< 200 mg/dl); and the mean triglyceride level was 699 ± 12 (> 150 mg/dl). The only significant platelet aggregation abnormality was in response to ADP. The mean ADP induced platelet aggregation (ADP agg) was 59 ± 4% (normal–60%) with 14/27 (52%) having levels below normal. Mean VWF:RCo and VWF:Ag were normal. Thirteen patients (48%) had VWF:RCo levels lower than normal, including 7 patients (26%) who also had decreased VWF:Ag levels and platelet aggregation. Univariate analysis showed a negative correlation between LA and VWF:Ag and VWF:RCo (n = 27, p = 0.005, p = 0.03, respectively), and between cholesterol and VWF:Ag and VWF:RCo (p = 0.02, both). In patients with low ADP agg, we found a negative correlation between platelet aggregation and cholesterol (n = 14, p = 0.02). Forward stepwise multivariate regression showed that elevated LA and triglycerides predicted low VWF:Ag (p = 0.005) and VWF:RCo (p = 0.02). In patients with low ADP agg, high LA, and triglycerides and low glucose (p = 0.04) predicted decreased platelet aggregation.

Conclusions: Seventy percent of the GSD1 patients in this cohort had bleeding symptoms, and 52% had defects in platelet aggregation and/or VWF. This study demonstrates for the first time a relationship between ADP-induced platelet aggregation and elevated lactic acid, hyperlipidemia and hypoglycemia and between VWF abnormalities and elevated lactic acid and hyperlipidemia. Larger scale studies are warranted to determine the mechanism by which these metabolic factors influence coagulation.

28) Why 5-methyltetrahydrofolate may be preferred to folinic acid in severe MTHFR deficiency complicated by cerebral folate deficiency. Results of an “n-1-clinical trial”

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Background: Methylene tetrahydrofolate reductase (MTHFR) deficiency is the most common inborn error of folate metabolism. However, the severe form is rare, leading to elevated homocysteine, and low/normal methionine and S-adenosylmethionine (SAM). Clinical severity varies from neonatal demise to apnea, seizures, microcephaly, and global developmental delay presenting in infancy and childhood. Brain atrophy, hydrocephalus and cerebral folate deficiency (CFD) may occur. The mainstay of therapy is oral betaine, acting as a methyl donor to reduce homocysteine and replenish methionine, thereby preventing SAM deficiency. Folinic acid (5-formyl methyltetrahydrofolate) has been recommended to replenish cerebral folate.

Objective: Folinic acid is the immediate precursor of 5,10 methylentetrahydrofolate which undergoes irreversible enzymatic reduction to 5-methyltetrahydrofolate (5-MTHF) via the action of MTHFR. 5-MTHF is the active form of folate that crosses the blood brain barrier. We hypothesized that 5-MTHF (l-methylfolate, Deplin®), could be more effective than folinic acid to treat a child with undetectable MTHFR activity and profound CFD secondary to severe MTHFR deficiency.

Patient/methods: A 26 month old female, born the 5th child to consanguineous parents was evaluated for severe MTHFR deficiency and CFD. Family history was significant for non-ketotic hyperglycinemia, cardiomyopathy and early deaths. Developmental delay at 6 months preceded clinical seizures. MRI of the brain revealed diffuse white matter loss and global cerebral atrophy. At 11 months, she developed hypertonia, hydrocephalus was detected on imaging, and a ventriculoperitoneal shunt was placed. Initial treatment was with betaine, folinic acid, methionine, riboflavin and vitamin B12. Betaine was gradually increased up to 9 g/day, with minimal decrease in homocysteine levels. Folinic acid (2.5 mg, bid) was discontinued after 10 months of therapy and replaced by l-methylfolate, 7.5 mg/d.

Results: The diagnosis of severe MTHFR deficiency was confirmed by absent MTHFR activity in fibroblasts. The patient is also homozygous for the thermolabile G677T polymorphism. Elevated plasma total homocysteine (tHcy) levels (highest 194 μmol/L, normal up to 13) were detected without methylnalonic aciduria or megaloblastic anemia. CSF studies initially showed undetectable levels of 5-MTHF (~5 nmol/L, reference range 40–187) and neopterin = 5, (7–75 nmol/L), and BH4 was < 5 (18–58 nmol/L). Neurotransmitter metabolites, 5-HIAA (115, 129–520 nmol/L) and HVA were also low (225, 294–1115 nmol/L). CSF tHcy level was 5028 nmol/L (reference range 20–114). Plasma methionine (lowest level 3.8 μM, reference range 9–42) normalized after increasing betaine, and adding methionine supplementation. On 5-MTHF, neopterin, and the neurotransmitters normalized and BH4 increased to 15 nmol/L, while CSF 5-MTHF remained undetectable and tHcy decreased to 3150 nmol/L (37%). Plasma tHcy levels decreased by 44%. Clinically, she became more alert, socially interactive, cooing and making sounds, and gained motor milestones.

Conclusions: This “n-1-clinical trial” in a patient with severe MTHFR deficiency, shows that 5-MTHF supplementation resulted in better clinical effects than folinic acid, when combined with betaine. CSF levels of 5-MTHF remained undetectable, warranting dose modification.
and follow up evaluations. Future studies on more patients, including patients treated early in the course of the disease may yield important information about long term outcomes of treated MTHFR deficiency.

29) Mitochondrial respiratory chain dysfunction has a common expression signature in human muscle and fibroblasts at the level of multiple biochemical and transcription factor target pathways

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Background: Mitochondrial respiratory chain (RC) disease is a highly heterogeneous group of multi-systemic disorders diagnosed through biochemical demonstration of deficient electron transport chain (ETC) enzyme activities most commonly in muscle. To determine whether ETC enzyme defects cause a unique and consistent pattern of secondary cellular adaptations which might provide insight into pathophysiologic mechanisms as well as novel therapeutic opportunities in mitochondrial RC disease, we evaluated global transcriptional profiles in skeletal muscle and fibroblasts from unrelated human RC disease subjects.

Methods: Studies were performed following informed consent with IRB approval. Global genome microarray expression profile was performed using the human Affymetrix human exon array platform in total RNA isolated from a total of 22 skeletal muscle specimens and 24 fibroblast cell lines. Samples from subjects with definite ETC enzyme deficiencies (“ETC abnormal”) were organized into data sets for comparison to age- and gender-matched groups of (1) healthy controls, (2) suspected mitochondrial disease subjects in whom ETC enzyme analysis was normal, and (3) PDH deficient subjects. The primary discovery data set comprised 8 matched muscle samples from 4 ETC abnormal and 4 ETC normal patients in whom muscle biopsies were performed at CHOP. A validation data set in muscle included 6 matched muscle samples from 3 ETC abnormal and 3 healthy controls who underwent muscle biopsy at UCSD. Findings in the primary discovery muscle expression data set were compared to that in fibroblasts from the same 8 individuals. Results were also compared in the complete fibroblast dataset (10 ETC abnormal and 14 ETC normal subjects). Subgroup analyses were subsequently performed to assess effects of specimen handling (i.e., isopentane vs liquid nitrogen collection), mtDNA mutations, and mtDNA depletion. Unsupervised sample clustering analysis, such as PCA, was used to distinguish global gene expression patterns by group. In addition to identification of differentially expressed genes between, dysregulation of KEGG-defined biochemical pathways and transcription factor targets was analyzed by a variety of tools including Gene Set Enrichment Analysis.

Results: Genome-wide expression profiling in human muscle and fibroblasts suggests that consistent cellular expression alterations occur at the level of key biochemical genes in primary mitochondrial RC dysfunction. For example, expression of the glutamate receptor and glutathione-s-transferase were significantly decreased and increased, respectively, across all ETC abnormal data sets. Importantly, the entire cohort of ETC deficient subjects could be uniquely distinguished by principle component analysis relative to all other groups. A wide range of biochemical pathways including proteasome, branched chain amino acid catabolism, and immune/defense pathways was significantly altered in ETC deficient muscle and fibroblasts. While very few transcription factor target (TFT) pathways were upregulated in ETC deficient tissues, dozens of TFT families were significantly downregulated in both muscle and fibroblasts from ETC deficient subjects relative to all other groups. In particular, several transcription factor target families including LHX3, FOXO1, FOXO4, and SOX9 were consistently downregulated in all muscle and fibroblast data sets. qRT-PCR validation of key transcriptional alterations is underway.

Conclusion: Human subjects with mitochondrial ETC enzyme deficiencies show a common pattern of expression alterations that is detectable in both muscle and fibroblasts. This is most notably characterized by downregulated expression of a host of cellular pathways involved in biochemical processes such as amino acid catabolism, immune pathways, and cell defenses. These data further suggest that downregulation of a common subset of transcription factor signaling pathways may coordinate the secondary biochemical alterations that occur in response to primary RC dysfunction. Expression profiling thus offers unique insight into the pathophysiologic mechanisms of mitochondrial RC dysfunction, which may guide future development of targeted therapies for the common metabolic sequelae of this devastating class of multi-systemic diseases.

30) Long-term outcomes in children with selected fatty acid oxidation disorders through age three

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Background: Fatty acid oxidation disorders (FODs) are a group of metabolic deficiencies where the body is unable to breakdown fatty acids to make energy because an enzyme is either missing or not working correctly. It is generally believed that with early diagnosis and ongoing management, children identified at birth through newborn screening (NBS) will have better long term outcomes than those diagnosed in the absence of screening. However, this evidence is lacking and no systematic approach to collecting long term follow-up (LTFU) data describing the post-screening natural history of these disorders has been available.

Methods: California began newborn screening for FODS in the summer of 2005, which coincided with the initiation of tandem mass spectrometry screening. In July 2007, a LTFU data collection system was implemented using a web-based Screening Information System (SIS) that contains an annual patient summary (APS) module. This system prompts state-contracted metabolic specialty care centers to submit electronic reports describing the health, clinical and service utilization status of children they are following at the completion of each year of life through age five. We present aggregated one, two, and three-year profiles of children with SCADD, MCADD, VLCADD and CTD collected after each year milestone.

Results: Based on the first 5 years of screening (n = 2,712,684), the overall incidence of the targeted FODs was 1 in 9585 births (1:33,489 for SCADD, 1:21,359 for MCADD, 1:66,163 VLCADD, and 1:79,784 for CTD). The median age at diagnosis was 44 days (64 days for SCADD, 25 days for MCADD, 37 days for VLCADD, and 88 days for CTD), after which all cases were referred for LTFU to one of 14 California metabolic follow-up centers. As of 10/26/2010, 401 APS were entered into SIS: 175 year-one reports, 139 year-two reports and 87 year-three reports. By the end of year one, 70%–90% of cases were referred to be in active care, but by the end of year three, treatment was deemed not necessary for 15% of SCADD reports, 4% of MCADD reports, 0% of VLCADD reports, and 31% of CTD reports, respectively. The lost to follow up rate was highest among the SCADD cases in each of the three years. Between 5% and 10% of children were asymptomatic at the time of diagnosis. Among those that were asymptomatic at diagnosis, 20%, 41% and 42% of MCADD reports went on to develop one or more disorder-related symptoms by the end of the first, second and third years of life, respectively. The parallel percentages for SCADD were 0%, 8% and 15%; for VLCADD: 20%, 53% and 73%; and for CTD: 0% in each year, respectively. At the end of the 2nd year of life, developmental delay was reported in 15% of SCADD, 9% of MCADD, 18% of VLCADD and 6% of CTD reports. Among the different treatment regimens reported, VLCAD cases had the highest percent on medical foods/supplements/formulas each year (80%, 89%, and 83%). The median number (n = 4) and range of metabolic center visits was highest for MCADD (0–10) and VLCADD (0–14) in the first year of life and by year three the median clinic visits were reduced for all disorders to 1–2/year (range 0–4). For all four types of FODs, there was a reported decline in overall health status in year two, followed by an improvement in year three.

Conclusions: This is the first report of a systematic effort to characterize the natural history of SCADD, MCADD, VLCADD and CTD cases following NBS diagnosis in a cross-section of children through age three. Over time, as more APS are entered in the California LTFU data system, the full spectrum of health and clinical outcomes for these rare FODs will emerge.

31) Newborn screening (NBS) results reporting: A national snapshot in 2010

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Background: Concerns exist about how NBS results are accessed, especially during an emergency. Information about how physicians and patients can access NBS results is not currently available. Each state/territory chooses reporting methodologies independently and no current guidelines on NBS results reporting exist.

Methods: A web-based survey was developed and distributed to the NBS lab and NBS follow-up representative in each state/territory in April, 2010. State/territory representatives were identified through the NNSGRC website and emailed with a survey weblink.

Results: Out of the 106 possible responses, there were a total of 79 replies, for an individual response rate of 75%, and responses were received from all 53 states and territories. Of the 79, 26 (33%) identified themselves as a representative of the NBS lab, 34 (43%) a representative of NBS follow-up, and 19 (24%) both representatives of the NBS lab and follow-up divisions. Various methods were queried for reporting normal, borderline and abnormal NBS results to each of the following: 1. submitting entity, 2. non-submitting physician (primary care MD), 3. non-submitting physician (specialist), 4. NBS follow-up coordinator, 5. family of child, and 6. other. Of the 53 states/territories, for normal results, the most frequent methods used were: 74% mailed the result, 43% faxed the result, and 38% posted result on the web. For borderline results: 58% mailed the result, 49% faxed the result and 38% used non-automated telephone call. For abnormal results: 60% mailed the result, 58% faxed and 57% used a non-automated telephone call. Online access to NBS results was available in 38% of states/territories for normal results, 32% for borderline and 30% for abnormals. Families were rarely directly contacted, but most frequently by mailed result (11% for normal results, 9% for borderline result, and 19% for abnormal results). When asked what the preferred method of reporting for abnormal results would be, the most common response was non-automated telephone call.

Conclusions: These results offer the first national snapshot of NBS result reporting methods and have important implications for emergency preparedness. The current methods of results reporting do not account for: 1. displaced families and/or MDs in an emergency requiring evacuation, sometimes to other states, or 2. a NBS lab or follow-up staff who are unavailable due to an emergency. A method of timely retrieval of NBS results by family and/or MD from remote locations is necessary to allow positive screening infants to be urgently evaluated and appropriately treated. A delay of days or weeks may occur under the current circumstances of non-automated results retrieval. A recommended set of results reporting guidelines is necessary to harmonize reporting across states/territories.
32) Nutritional management guidelines for MSUD: Delphi survey

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Background: Although various clinic-specific protocols and guidelines for the nutritional management of rare inborn errors of metabolism (IEM) exist, these have not been subjected to rigorous evidence- or consensus-based evaluation. Case studies and expert opinion populate most of the published literature containing information about management. A multi-year project was undertaken to develop nutritional management guidelines for IEM. Five workgroups, representing major categories of IEM were formed. Workgroups have had training in the evidence-based analysis process through the American Dietetic Association, and further instruction in consensus analysis and survey design. The endpoint of the project will be guidelines based on a combination of: evidence-based analysis of peer-reviewed publications; evaluation of gray literature (materials that are unpublished or published in a non-peer-reviewed format); Delphi survey (1 and 2), nominal group consensus, and field-testing. The aminoacidopathies workgroup is completing the evidence-based analyses and has completed the first round of the Delphi survey in preparation for writing the guidelines for maple syrup urine disease (MSUD). The results of this first round will populate the questions for the nominal group process and the second round of the Delphi, and are presented here.

Methods: The Delphi survey 1 had 84 questions/statements: 21 questions on the background and clinical expertise of the respondents, 39 statements on MSUD general management and 24 on MSUD management under special circumstances, i.e. pregnancy, acute illness and liver transplantation. Invitations were sent to 28 metabolic physicians and dietitians representing each of the HRSA Genetic Collaboratives. The survey was in electronic format, and required 45–60 min to complete. Respondents rated the extent to which they agreed or disagreed (Likert scale) with the management/practice statements. They were asked to give a “best practice” answer even in areas where they had no direct experience. Information in the comment boxes for each statement provided a means of determining if the survey statement was being interpreted as the survey authors had intended.

Results: Of the 28 who were invited to participate, 17 completed the survey (61%). The majority (76%) of respondents had more than 12 years of experience with IEM, and all had treated patients with MSUD. Most (82%) had treated patients with IEM through pregnancies and liver transplantations, but only 45% had treated patients with both MSUD and either of these conditions. The categories that had the best agreement in reported best practice were: the prescription of protein and energy when patients were well, and treatment during pregnancy and acute illness. The most disagreement was in laboratory monitoring guidelines; respondents commented that both cost and noncompliance would prevent implementation of optimal monitoring guidelines. Thirty-two (52%) of the management statements had greater than 50% of respondents choosing to “agree” or “strongly agree”.

Conclusion: This survey demonstrated consensus on nutritional management in MSUD during health, pregnancy and acute illness, but various viewpoints on best practice for monitoring. These results identified areas that will be addressed in the nominal group consensus and Delphi survey 2. In the absence of adequate peer-reviewed literature, surveys provide an additional tool to gather information about usual and/or best practices in the nutritional management of IEM.

33) Hepatorenal tyrosinemia: The devastating natural history of the disease in Mexico

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Background: Hepatorenal tyrosinemia (HTI) is an inherited metabolic disease of tyrosine (Tyr) metabolism due to fumarylacetoacetate hydrolase deficiency. If untreated, it causes severe and progressive hepatic damage, renal failure, neurological crises and a high mortality rate in the first months of life. Early detection by newborn screening (NBS) and pharmacologic therapy with 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC) has changed the natural history of HTI and patients from developed countries are identified and treated before appearance of symptoms. In countries which do not include HTI in their NBS programs and do not have NTBC’s availability for treatment it is difficult to ensure a good prognosis for these patients. This work aims to report the clinical and biochemical characteristics of an HTI Mexican cohort treated at a reference center for inborn errors of metabolism.

Methods: Retrospective study of clinical records from HTI patients who were treated in our center from 1990 to 2010. The diagnosis was based on the presence in urine and/or blood of succinylacetone which was measured by gas chromatography/mass spectrometry and tandem mass spectrometry respectively, and liver damage was determined with prothrombin time, serum transaminases (ALT, AST) and quantification of plasmatic amino acid profiles by high performance liquid chromatography. All patients studied were diagnosed lately by their clinical signs.

Results: Fifteen patients with HTI were found in 15 families, 8 female and 7 male, consanguinity was reported in 2/15 families, and 4/15 patients had one infant sibling who died because of an unspecified hepatic complication. All patients had elevated levels of succinylacetone. Six patients presented prolonged prothrombin time, 4/15 had elevated serum transaminases and mean tyrosine, phenylalanine and methionine concentrations, and all were increased (380 μM, 124 μM and 211 μM respectively). Mean age at the beginning of symptoms was 10 mo (9 d–24 mo) and diagnosis was
made at 20 mo on average (2–60 mo); mean time between the beginning of symptoms and diagnosis was 10 mo (2 mo–5 y). All patients presented hepatic abnormalities (hepatomegaly, cirrhosis); renal insufficiency was presented by 64% of patients, followed by neurological crises (29%). All patients had nutritional management with Tyr restricted diets after diagnosis but none had NTBC therapy. Sixty percent of the patients (9/15) underwent liver biopsy which showed 7 patients with cirrhosis. Three patients had liver transplant. Total mortality was 73% (11/15), mean death age was 2 y 9 mo, and main causes were sepsis, multi-organ failure and hepatocellular carcinoma. Four years was the mean age of surviving patients and 2 of them are actually waiting for liver transplant.

Conclusions: The devastating natural history of HTI is still observed in Mexican patients' because it is not timely diagnosed and correctly treated. Our results are consistent with literature; all our patients had progressive liver damage as the main clinical sign associated to a high mortality rate. The need of NBS programs for early detection of HTI in combination with opportune treatment, availability of orphan drugs like NTBC, adequate follow-up, genetic counseling and practice guidelines for the physicians who have the first contact with these patients in order to suspect the disease is evident.

34) Use and benefit of a subcutaneous indwelling catheter for cobalamin administration in B12 responsive methylmalonic acidemia

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Background: Vitamin B12 responsiveness is a variable finding in methylmalonic aciduria (MMA). Hydroxocobalamin (OHCbl) is the preferred form and parenteral administration is generally required to achieve effect. To assess responsiveness, an empiric trial of 1 mg per day is suggested. Regimen for long term therapy is best determined by titration of both dose and frequency based on biochemical and clinical control. Reported dosing has ranged from 1 mg per week to 20 mg daily. Adherence to the prescribed regimen is necessary in order to assess responsiveness and for sustained effect. Compliance may be compromised by practical issues when therapy necessitates frequent injections. Iatrogenic injection pain is an important consideration in the setting of frequent injections. There is evidence that even apparently minor procedures such as injections result in significant pain and lasting negative effects. Here we report on the use of an indwelling subcutaneous catheter for OHCbl administration in a newborn with MMA, with— to reduce iatrogenic pain and facilitate adherence.

Case report: The patient was identified on newborn screen and found to have MMA due to methylmalonyl-CoA mutase deficiency, caused by mutations R108C and G630A. He was hospitalized at 9 days of age, and started treatment with parenteral OHCbl 1 mg/day of a 1 mg/ml solution. At 14 days of age the MMA level decreased from 42.4 to 6.3 μmol/l and the infant was discharged on home therapy. The patient returned for follow-up one week later. Parenteral report of injection related stress prompted placement of an indwelling subcutaneous catheter (Insulfon™). A 5 mg/ml (1 mg = 0.2 ml) OHCbl solution was secured and daily OHCbl administration continued, now with use of the catheter. The catheter was replaced weekly. Use of the subcutaneous catheter for medication administration continued until five months of age at which time the patient began playing with the catheter making its use problematic. While used, the indwelling catheter was well tolerated without findings suggestive of tissue damage or infection. The parents reported that the catheter was easy to use on a daily basis and that replacement of the catheter was not difficult. MMA levels were stable; values obtained during time of catheter use were comparable to values obtained when medication was administered via standard single injection. Parents and medical professionals felt that the catheter was useful, both emotionally and practically. All appreciated that daily medication administration occurred without skin puncture and with perceived decreased pain to the patient. Parents reported that its use greatly reduced injection related stress.

Discussion: Use of a subcutaneous indwelling catheter is well established for pediatric and/or neonatal delivery of insulin, enoxoparin, and other medications. We propose that it also be considered for delivery of parental OHCbl in MMA patients being assessed for responsiveness, or requiring therapeutic daily administration. Use may decrease medication injection pain in the patient and pre-injection anxiety in the patient and parent. This may lead to enhance acceptance of therapy by the patient, parent, and health care providers, key issues in chronic care management and treatment compliance.

35) Consequences of an R463C mutation in Gaucher disease

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Gaucher disease (GD) is conventionally divided into subtypes based on the presence and rate of progression of neurologic manifestations. Classification of individuals with GD is important for prognosis and management decisions, but such classifications may not be straightforward. For example, individuals diagnosed as type 1 (non-neuronopathic) GD may subsequently develop neurological symptoms and be re-classified as type 3. Furthermore, those with “non-neuronopathic” type 1 GD are at risk for neurologic complications, though distinct from those found in stereotypic type 3. While the subtypes may be distinguishable in their classical presentations, the overlap between them is more substantial than previously anticipated. In this context, we describe a patient with a combination of GBA mutations of lesser frequency, and who cannot be readily classified into a distinct GD subtype. We hypothesize that her disease severity is determined, in part, by the presence of a second missense mutation in cis with a known pathogenic mutation.
The patient is a 21 y woman with GD and developmental delay. Diagnosis at age 14 mo followed presentation with hepatosplenomegaly, anemia and purpura. Glucocerebrosidase activity [leukocytes] was 1 nmol/mg ptn/h (N18–20). Chromosomal analyses were normal. ERT was initiated at age 2 y and continued through adolescence. She showed developmental delays, especially in language, since early childhood, and was slower in development than her three unaffected siblings. Family history includes poor academic achievement in the parents and siblings, but the proband’s developmental delays were more pronounced. She required special education, failed high school, and never held steady employment. Brain MRI [age 2 y] showed patchy myelin abnormalities in the posterior periventricular regions. Eye movements were normal, save for left intermittent esotropia, since age 1.5 y. Slow and hypometric horizontal saccades were first observed at age 20 y.

Genotyping revealed a D409H mutation on one allele, and an E326K polymorphism/missense mutation in cis with an R463C mutation on the other allele. The genotype D409H/R463C has been described in one individual diagnosed in adulthood with severe neurologic involvement. The D409H and R463C mutations have each, individually, been observed in trans with other mutations in both mild and severe GD cases. It has been hypothesized that disease severity in individuals with a D409H or an R463C mutation is determined by the mutation in trans. However, we hypothesize that an increase in disease severity in GD observed in those with the R463C mutation may, in fact, be largely determined by the presence and effects of the E326K missense mutation/polymorphism in cis, rather than by the disease-causing mutation in trans.

In sum, we consider that neurologic manifestations in GD are more accurately described along a continuum, rather than in distinct subtypes. For example, the late and subtle presentation of slowed saccades — taken together with the developmental history — made a firm classification as type 1 or type 3 problematic in the patient of this report. This case therefore supports the notion that subdivision of patients into type 1 or type 3 disease is an inaccurate reflection of the natural history of GD, and serves only to confound investigations into the underlying pathogenic mechanisms of the neurologic manifestations of GD.

36) A new approach for normalization of cystine in cystinosis using tandem mass spectrometry

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Cystinosis is a genetic lysosomal storage disease that leads to build up of intracellular cystine and a variety of debilitating symptoms, including kidney failure. Cystine crystals build up at high concentrations in lysosomes due to either a dysfunctional or completely absent transporter for the disulfide form of cysteine. In recent years, the drug cysteamine has been effective in forming mixed disulfide bonds with cysteine and relieving most symptoms, but renal failure, though delayed, still generally does occur progressively. For both diagnosis and monitoring of therapy, it is crucial to have accurate measures of intracellular cystine. Currently, our laboratory quantifies cystine from mixed leukocytes using tandem mass spectrometry, and reports the value of total cystine normalized per milligram of protein. The amount of protein is currently quantified by a Lowry assay of the cell extract. Though clinically useful and technically valid, there are various drawbacks to this method, which include variations in total protein that we find are due in large part to pre-analytical factors which are complex and difficult to control. The preparation of leukocytes is quite critical, and an undetermined portion of the protein measured may arise from cells other than granulocytes, non-cellular material, or other sources not specifically related to the lysosomal storage of cystine. To minimize this source of variation, the preparation must be exceedingly exacting in terms of anticoagulant and technique, but it is still not possible to completely control for elements of biologic variation. It would be preferable to measure single or multiple proteins that are specific to lysosomes in cells that store cystine. We have done proteomic analysis of crude lysates of mixed white cell, following trypsin digestion and cleanup, and have found three lysosome specific proteins found in neutrophils that may serve as much more appropriate protein biomarkers for normalizing intracellular cystine. The three target proteins are elastase 2, proteinase 3, and azurocidin 1. In the proteomic analysis we have found a highly abundant peptide from each protein that we are using for quantification. A method was developed to measure the parent/daughter ion transitions of the peptides using tandem mass spectrometry, and isotopic versions of each peptide were designed for use as internal, quantitative standards. We are currently collecting a series of normal and cystinotic patient blood samples from which we will extract both cystine and protein, and measure the ratios by both the current method (including Lowry assay), as well as the newly developed mass spec method that quantifies peptides from lysosomal protein. We will evaluate the utility of the new approach in the clinical testing of cystinosis.

37) Mutation within the anticodon-stem of MTTV causes MELAS syndrome and impairs mitochondrial translation

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Background: The A1630G mutation in the mitochondrial valine tRNA has been reported in association with the mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) syndrome. We identified a patient with this mutation after she presented with stroke-like episodes. She was also noted to have deafness and growth and intellectual impairment. MRI showed subclinical infarcts in the basal ganglia of varying ages.

Methods: Mutation loads in urine, fibroblasts and blood were evaluated in the proband and her mother using both restriction-fragment length polymorphism analysis and quantitative PCR. We created cybrid lines that were wild type, G1630 homoplasmic or heteroplasmic using the proband’s fibroblasts as mtDNA donors and 143BpΔ recipients. Oxygen consumption was evaluated in the cybrids. The levels of tRNAs were evaluated using Northern blotting and mitochondrial protein levels were determined by Western blotting.
Results: The patient had a broad distribution of heteroplasmy in renal epithelial cells, blood and fibroblast-derived DNA. Her mother, who is clinically unaffected, also had high loads of mutation. Homoplasmic G1630 cybrids showed markedly decreased oxygen consumption in intact cells. We observed reduced levels of the mitochondrial valine tRNA and reduced levels of mitochondrial proteins.

Conclusions: We identified a patient with classic features of the mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS) who carries a heteroplasmic A1630G mutation. The mutation has been previously associated with MNGIE syndrome, however distinctions between the two clinical presentations are quite subtle. As with the previous case, we identified high levels of the mutation in the proband’s mother, who is clinically unaffected. To confirm the pathogenicity of the mutation we studied it in cybrid lines. Our results suggest that the A1630G mutation impairs the stability of the valine tRNA leading to an impairment in protein synthesis or stability. Genetic modifiers or tissue-specific heteroplasmy may explain the difference in clinical outcomes between affected and unaffected individuals carrying this mutation.

38) Energy expenditure and body composition in long chain fatty acid oxidation disorders

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Background: Inherited disorders of long-chain fatty acid oxidation (FAO) inhibit the ability to oxidize long-chain fatty acids (LCFAs) for energy production. Patients with long-chain FAO disorders frequently complain of lethargy, and experience bouts of rhabdomyolysis associated with increased activity. Decreased activity and/or the inability to oxidize fatty acids may effect regional and tissue lipid deposition. Thus, impaired FAO may lower energy expenditure and predispose to increased adiposity.

Objectives: To determine body composition, tissue lipid deposition, total (TEE) and resting energy expenditure (REE) among participants with Carnitine Palmitoyl-transferase 2 (CPT2), Very Long-Chain Acyl-CoA Dehydrogenase (VLCAD) and Long-Chain 3-Hydroxyacyl CoA Dehydrogenase (LCHAD) deficiencies compared to age, sex and body mass index (BMI) matched controls.

Design and methods: Body composition was measured by DEXA, abdominal fat depots by MRI, and tissue lipid deposition by proton MRS. TEE was measured by doubly labeled water and REE by indirect calorimetry in 9 subjects with a long-chain FAO disorder and 9 controls. The difference between groups was determined with a paired t-test. The energy expenditure was compared by using fat free mass as a covariate (Fig. 1).

Results: Total lean and fat mass did not differ between groups. Subjects with a long-chain FAO disorder had toward lower intra-myocellular lipid compared to controls (p = 0.09). There was no significant difference in extra-myocellular or intrahepatic lipid content, or in visceral or subcutaneous adipose stores. Total energy expenditure was 86% of estimated energy requirement but resting energy expenditure was not different than estimated basal energy expenditure in subjects with a long-chain FAO disorder (Fig. 1).

Conclusions: Subjects with a long-chain FAO disorder have lower intra-myocellular lipid stores compared to control subjects but no difference in total body lean or fat mass. In addition, subjects have a lower TEE but REE was within normal expected limits. The decreased energy expenditure is most likely due to decreased daily activity compared to matched controls and is not associated with abnormal accumulation in total, regional, or intra-organ fat.

Fig. 1:

39) Two siblings diagnosed with adenylosuccinate lyase deficiency in late adolescence

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Background: Adenylosuccinate lyase deficiency (ADSL) is a devastating neurometabolic autosomal recessive disease of purine metabolism with yet unknown effective treatment. First described in 1984, there have been fewer than 60 cases (Caucasian and Asian) to date reported in the
Course of chemotherapy concurrent with ERT dramatically improves hematological profile in male with Gaucher disease Type 1 and secondary high-grade B-cell lymphoma

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Background: Gaucher disease (GD), first described in 1882, is one of 40 lysosomal storage disorders, a family of inborn errors recently the focus of intensive therapeutic innovations. The lysosome organellae fails in the normal processes that break down unwanted cellular debris, resulting in the accumulation of storage material. Gaucher disease is caused by a deficiency of the lysosomal enzyme glucocerebrosidase inherited in an autosomal recessive pattern that leads to a complex continuum of multisystem involvement, from a perinatal-lethal form to asymptomatic individuals.

Here, we describe a 59 year-old Ashkenazi Jewish male with Type 1 Gaucher disease. He presented initially with bone pain and left upper quadrant pain and demonstrated hepatosplenomegaly on physical examination. A bone marrow biopsy showed 70% scattered lipid-laden histiocytes, which stained positive with PAS, consistent with Gaucher disease. He was well compensated for many years under the management of his hematologist.

Initial evaluation for ERT revealed a low beta-glucocerebrosidase enzyme activity, and we identified two known pathogenic alleles in Glucosidase, Beta, Acid (GBA), the only gene associated with Gaucher disease. He has the N370S and L444P alleles, seen in 16% of affected individuals as reported by the International Collaborative Gaucher Group (1999). He suffered from anemia, leucopenia and thrombocytopenia in the normal range (140–285 thousand/µL). After completion of the three rounds of chemotherapy, platelets were 169 thousand/µL, in the normal range (41–80 thousand/µL) for the first time and remain in the normal range.

Conclusions: The combination of the chemotherapy and the ERT greatly improved our patient’s platelet count and other blood cell lineages beyond that achieved on ERT alone. We believe that the course of chemotherapy, perhaps by clearing the marrow, may well have established a new state in the marrow that allowed our patient who was concurrently receiving ERT to repopulate his bone marrow with more healthy...
precursor cells that have been able to sustain themselves for over 17 months. We are certainly not suggesting chemotherapy as a part of routine ERT treatment for Gaucher disease, but we conclude that a more comprehensive study of the combined treatments is required. In the two patients receiving both therapies, there have been no adverse effects. We feel that this study does indicate that should a malignancy occur in a patient with Gaucher, ERT should continue with staggered chemotherapy. There may well be unanticipated long-lasting advantages to having undertaken the course of chemotherapy.

**41) Improvement in neurologic outcome in a patient with UMP synthase deficiency with oral uridine supplementation**

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**Introduction:** Uridine Monophosphate Synthase deficiency (OMIM 258900) is an autosomal recessive disorder that leads to orotic aciduria and a clinical phenotype of megaloblastic anemia, decreased cellular immunity and susceptibility to infection. Variable neurologic symptoms have been described, including “cerebral palsy”. Replacement therapy with uridine has been shown to cause clinical remission and decreased urinary orotic acid excretion.

**Case report:** The patient is a 16 year old female born after an uncomplicated pregnancy and delivery. Her older sister was healthy and developmentally appropriate. Global developmental delay was noted in early childhood. She developed recurrent cyclic vomiting, ADHD, learning difficulties, tics, anxiety, and staring spells, but no history of developmental regression. Her EEGs have shown abnormal epileptiform activity with both generalized and focal features. Her staring spells resolved with anticonvulsant (topiramate) treatment. Neuroimaging studies have remained normal, including MRI and MRS.

An extensive genetic and metabolic evaluation revealed elevated urinary orotic acid of 11.6 (range 8–13) with an elevation on allopurinol loading to 54 without hyperammonemia. Liver biopsy showed OTC at less than 2SD below the mean but subsequent gene testing for OTC deficiency was negative. Urine pyrimidine level was below the mean (53.9 μmol/g liver/min: 62.4% control). Fibroblasts showed UMP synthase activity of 5 nmol/mg/h with normal range of 32+/−15 nmol/mg/h. She was originally treated with a protein-restricted diet with supplemental citrulline and Alimentum formula for suspected OTC deficiency but subsequently transitioned to EO28 formula for treatment of her pyrimidine metabolism disorder. Once diagnosed with UMP synthase deficiency, she was placed on uridine supplementation. She was able to stop her citrulline, liberalize her dietary protein, and stopped topiramate without return of her clinical staring spells.

**Discussion:** Previous reports of treatment of UMPS with uridine have shown a clinical and hematologic remission. This patient never had a hematologic disorder, but her neurologic and cognitive symptoms improved on uridine supplementation. Though most of these patients have been described as having “cerebral palsy” our patient shows no focality on neurologic exam and her MRI/MRS remains normal despite her Tourette’s syndrome and epilepsy. Pursual of an underlying etiology allowed for a specific corrective treatment with uridine and discontinuation of the anticonvulsant medication.

**References:**


**42) Treatment of NAGS deficiency: Retrospective data on 23 patients treated with carglumic acid over 16 years**

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N-acetylglutamate synthase (NAGS) deficiency is a rare Urea Cycle Disorder. If untreated, it leads to cerebral edema, coma and eventually death. Psychomotor retardation is also frequent. We present the clinical/biological responses of NAGS deficiency (NAGSD) patients to carglumic acid (CGA, Carbaglu®), an oral analogue of N-acetylglutamate. Carglumic acid (CGA) reactivates the urea cycle by activating Carbamoyl Phosphate Synthetase I.
**Methods:** We analyzed retrospective, non-comparative data on NAGSD patients treated with CGA (at least one dose) between 1991 and 2007 in European centers. Short (within 7 days of treatment) and long-term responses were assessed. The biological short and long term responses were based on plasma levels of ammonia, glutamine and citrulline. Secondary objectives included patients’ clinical development, neurological/psychomotor status, protein restrictive diet, growth, concomitant treatment and adverse events (AEs).

**Results:** Twenty-three confirmed NAGSD patients (14 males) treated with CGA were analyzed. Eighteen patients were on continuous treatment with CGA at data cut-off (December 2007). DNA confirmation tests were available for 19/23 patients, and 14/19 were homozygous. Eighteen started CGA within first year of life (9 of them neonates).

The mean initial daily dose of CGA was 172 mg/kg, matching mostly with the recommended daily dose (100–250 mg/kg). The total exposure to treatment was >2300 months (187.4 patient-year), with a mean treatment duration of 97.8 months. In the longterm, the mean daily dose of CGA was 31 mg/kg (range 6–100 mg/kg). Prior to treatment, mean and median ammonia levels were 218.9 μmol/L (SD = 299.0) and 142.0 μmol/L respectively (normal value 50 μmol/L).

On Day 3, mean and median plasma ammonia level decreased to 43.3 μmol/L (95%CI 0.5, 86.2) and 29.5 μmol/L respectively (Fig. 1). Glutamine levels showed similar decrease, reaching normal values within 24–72 h. In the long-term, CGA maintained plasma ammonia and glutamine within the normal range.

Prior to CGA, 70% of patients were on protein restricted diet. At last follow-up, 84% were on free protein intake, allowing growth improvement over time. At treatment start, 7/10 patients presented altered neurological development, but at follow up 5 of those 7 had normal cognitive and neurological outcome.

At baseline, 4/7 patients presented psychomotor retardation. There was recovery of psychomotor performance in 2 patients. None of patients with normal neurological/psychomotor status deteriorated since CGA treatment. Two patients treated with extremely low doses of CGA died (multi-organ failure with encephalopathy and severe episode of hyper-ammonemia), both unrelated to CGA. 17/23 patients experienced an AE, mostly due to the underlying disease. Two related-AEs were found (bitter taste and hyperhidrosis). Concomitant treatments were used, but assessment of timing of co-administration was difficult.

**Conclusions:** These retrospective data show the benefits of carglumic acid as a specific pharmacological therapy in NAGSD patients. Ammonia and glutamine levels normalized quickly after introduction of carglumic acid. Long term benefits included continuous control of metabolic parameters, discontinuation of protein restricted diet, growth improvement and stable neurological/psychomotor development. No serious safety issue has been identified.

The administration of carglumic acid in NAGSD patients ensures control of metabolic parameters, preventing from metabolic decompensation and neurological sequelae.

**Fig. 1:** Ammonemia prior to the first dose of Carglumic acid, ≤7 days and at last follow-up.

43) **Natural clinical course of glutamine synthetase deficiency in a 3 year old**

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The cytosolic enzyme glutamine synthetase (GS) is expressed in almost all body cells and catalyzes the ATP dependent conversion of glutamate and ammonia to glutamine. GS is the only known enzyme for endogenous glutamine synthesis and plays an important role for many pathways in the human organism. Until now, a defect in GLUL, encoding for GS, was reported in only two patients of Turkish origin who both were affected by neonatal onset and early lethal multi-system disease. Here, we describe the natural clinical course in yet another patient with GS deficiency who is still alive at 3 years of age.

The patient is an offspring of healthy first cousin Arab parents from Sudan. He presented during the neonatal period with seizures and developed chronic encephalopathy and severe developmental delay. Levels of plasma glutamine were low normal at the beginning but decreased...
during the course. Also, moderate hyperammonemia (values ranging from 70 to 400 μmol/l) was present in all investigations. GS deficiency was suspected based on the combination of low plasma glutamine and hyperammonemia.

Genetic analysis identified a homozygous mutation c.970C→A (p.R324S) in the GLUL gene. In cultured fibroblasts, a strong GS upregulation was seen in accordance with previous findings in GS deficiency. MRI revealed only moderate changes with mild brain atrophy but a normal sized cerebellum.

GS deficiency is a rare neonatal-onset disorder but patients might survive beyond infancy. The main symptoms are neurological with seizures and chronic encephalopathy.

44) Novel therapies in treatment of presumptive multiple acyl-CoA dehydrogenase deficiency

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Multiple acyl-CoA dehydrogenase deficiency (MADD, or glutaric acidemia type 2) is an autosomal recessive inherited disorder of fatty acid, amino acid, and choline metabolism. Here we describe an atypical case detected by NBS with rapidly progressing cardiac disease ultimately requiring cardiac transplantation.

The child was born after an uncomplicated pregnancy at full-term. California expanded newborn screening was consistent with MADD demonstrating elevated acylcarnitines: C16 15.95 (normal range 0–9 μmol/L), C14:1 11.11 (0–8.8), C8:1 0.81 (0–0.5), C5 2.12 (0–1.2), and C5DC 0.47 (0–0.35). She had a low blood sugar at 1 day old with a bicarbonate of 8 and elevated lactate and pyruvate. She was stabilized and discharged at 10 days old. Her parents then moved to Seattle and treatment continued with l-carnitine, riboflavin and dietary fat and protein restriction. She otherwise was developing normally without episodes of illness, hypoglycemia, or liver dysfunction until 6 months of age. Routine screening ECG showed right atrial enlargement and nonspecific ST and T wave abnormalities and subsequent echocardiogram showed a shortening fraction of 25%. Follow up plasma acylcarnitine analysis was consistent with biochemical diagnosis of GAII. At 7 months, she was hospitalized with transient tachypnea, mild subternal retractions and vomiting. She rapidly developed symptoms from a progressive cardiomyopathy with her shortening fraction falling to 11% within 3 weeks of the prior exam. An emergency FDA IND was obtained for the use of sodium DL-3-hydroxybutyrate (Special Products Limited, UK) at an increasing dose from 300 to 1200mg/kg/day with no obvious advantages. During her nearly 6-month admission, she also required high-frequency ventilation, inotropic support, extracorporeal membrane oxygenation, then advancing to a left ventricular assist device. She suffered a small embolic cerebral vascular accident in right parietal occipital region with left-sided weakness and seizures, two cardiac arrests, and successfully received an orthotopic heart transplant 5 months after admission. Throughout her hospitalization she had no episodes of liver dysfunction or hypoglycemia.

Her initial diagnostic sequencing of the ETFDH, ETFA, and ETFB genes reported that no mutations were detected. Enzyme activities of electron transfer flavoprotein (ETF) and ETF:ubiquinone oxidoreductase (ETF-QO) in cultured fibroblasts were normal and not consistent with MADD. Of note, no other heterozygous positions suggestive of polymorphisms that would rule out gross lesions or gene deletions were detected. Left ventricular pathology demonstrated myocyte vacuolization with regional cytoplasmic fat droplets. Electron Transport Chain (ETC) testing on her heart post-transplant showed normal activity of complexes I, II, III, and IV. Comparison fibroblast ETC assay was also normal but showed increased citrate synthase activity suggesting mitochondrial proliferation, possibly an adaptive response to mitochondrial dysfunction. Fibroblast fatty acid oxidation probe assay demonstrated elevations of C16 but no other consistent markers of MADD.

Conclusions: This once presumed GAII patient is now post-cardiac transplant with some continuing renal and neurologic issues, but appears to be making some developmental progress. She remains on l-carnitine, riboflavin, Coenzyme Q10, glycine and vitamin E as well as transplantation support medications. She continues to show similar profile in plasma acylcarnitine analysis. This case highlights the challenge in managing, as well as diagnosing, this disorder. Future studies of fatty acid metabolism, mitochondrial function and next generation sequencing are planned.

45) MethyImalonic acidemia and optic nerve atrophy: Reversal of sub-acute loss of visual function with anti-oxidant therapy

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Background: Isolated methyImalonic acidemia (MMA) results primarily from a defect in the mitochondrial enzyme methylmalonyl-CoA mutase (MUT). Patients present with recurrent metabolic crises and multisystemic complications including growth retardation, chronic renal failure, pancreatitis and developmental delay despite optimal management. Studies in MMA knock out mice and patient tissues have suggested that mitochondrial dysfunction plays a significant role in the pathophysiology of MMA. Optic nerve atrophy (ONA) has been described as a rare complication of the disorder; the pathophysiology is unknown.

Methods: Patients with isolated MMA evaluated through NIH study 04-HG-0127 (clinicaltrials.gov identifier: NCT00078078) “Clinical and Basic Investigations of Methylmalonic Acidemia and Related Disorders” underwent comprehensive ophthalmologic evaluation. We report five mut1 patients with ONA out of the 58 with isolated MMA followed in our protocol. All five patients were males, and presented with a decreased visual acuity at the ages of 7, 7, 22, 23 and 24 years. Vision loss was bilateral but asymmetric and the progression was variable. Best-corrected vision ranged from 20/40 to 20/800 and in two patients visual evoked potentials were severely diminished and delayed. There were no apparent biochemical or environmental triggers shared by the patients and in only one the diagnosis followed an episode of severe acute metabolic decompensation. Each carried two mutations in the MUT gene. Two patients were negative for the Leber Hereditary Optic Neuropathy
mitochondrial mutations. Patient 5 was a 24 year-old male, 4 year status-post cadaveric kidney transplantation, whose vision progressively worsened from 20/20 to 20/125 OD and 20/40 OS over a 4-week period. Daily oral coenzyme Q10 (ubiquinol), vitamin E, ascorbic acid, thiamine, and intravenous infusions followed by oral N-acetylcysteine were employed for the first 3 months, and he remained on maintenance doses of coenzyme Q10 and vitamin E thereafter.

**Results:** The patient’s visual acuity improved to 20/25 OD and 20/20 OS over one month, although the nerve fiber layer thickness remained decreased at 8 month follow up.

**Conclusions:** Our experience suggests that 1) ONA is a late onset complication of isolated MMA and therefore all patients should have periodic ophthalmologic evaluation and counseling about the symptoms of ONA. 2) Our data and a recent report from the literature indicate that antioxidant therapy and improved metabolic control might help in partially reversing the acute visual changes of optic neuropathy in MMA patients.

46) **Remembering the zebras in genetics — Uncovering I-cell disease**
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Mucolipidosis II (I-cell disease) is a lysosomal storage disease caused by a dysfunction of N-acetylglucosamine-1-phosphotransferase. This enzyme phosphorylates the terminal mannose residue of the N-linked oligosaccharide. This creates the necessary biochemical “zipcode” for proper targeting of newly synthesized acid hydrolyases to the lysosome. Affected infants often present at birth with cardiac defects, skeletal dysplasia, coarse features, and failure to thrive.

We were asked to evaluate a female infant at 12 months of age who as a neonate had micrognathia, a high palate, and difficulty feeding, ultimately necessitating placement of a gastrostomy tube. She also had mild mitral valve regurgitation (MVR), thickened mitral leaflets, and a diaphragmatic hernia (Morgani type). A very narrow thoracic cage was a profound finding. A skeletal survey identified healing rickets and she was treated for vitamin D deficiency. Additional skeletal abnormalities were identified but were not consistent with any syndrome. At five months of age a repeat skeletal survey showed progression of the chest wall deformity with significant narrowing of the thorax.

She had a very comprehensive molecular and biochemical evaluation primarily focusing on possible etiologies of skeletal dysplasia and connective tissue disorders. Sequencing of the IFT80 and DYN2CH1 genes (Asphyxiating thoracic dysplasia of Jeune), FB1N and TGFBR1/2 genes (Marfan and Loey–Deitz syndrome) did not demonstrate any pathogenic mutations. Biochemical testing including liver function tests, CK, routine comprehensive biochemical testing including, urine organic acids; plasma very long chain fatty acids, amino acids, acylcarnitines, and carbohydrate deficient glycosylation testing were within normal range. A skeletal survey sent out for second opinion identified “soft findings of dysostosis multiplex”.

Referral to the metabolism clinic led us to be concerned for a storage disorder given the infants’ coarse facial features, gingival hyperplasia, and skeletal dysplasia with dysostosis multiplex, worsening MVR, hepatomegaly, and a recent finding of corneal clouding. Due to her hypoplastic thoracic cage she has required continuous supplemental oxygen and gastrostomy feedings. Although she has delays in motor milestones, she is highly socially interactive, cognitively aware and vocalizes. Biochemical testing of leukocyte and plasma lysosomal enzyme activities then led to a diagnosis of Mucolipidosis II or III. Follow up testing of Arylsulfatase-A activity demonstrated plasma level elevated 150 times above normal, and normal leukocyte level consistent with Mucolipidosis II. A peripheral blood smear revealed abnormal lymphocytic vacuolar inclusions. Molecular confirmation of Mucolipidosis II is pending.

Metabolic diseases present in varying severity where classic findings may be masked by a more (or less) serious disease presentation than is expected. Review of this case and its complexities serves to highlight the importance of a comprehensive differential diagnosis when evaluating children who present with multiple congenital anomalies and progressive symptomatology. Lysosomal storage disorders should be considered in such cases, as therapy is available for some disorders, and a molecular and biochemical diagnosis can be important for family genetic counseling.

47) **N-carbamylglutamate enhancement of ureagenesis leads to discovery of a mutation in the enhancer of the NAGS gene**
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N-acetylglutamate synthase (NAGS) deficiency is the rarest but highly treatable among the urea cycle disorders and its diagnosis relies exclusively on DNA sequencing. A patient presented with hyperammonemia, a biochemical profile consistent with a proximal urea cycle disorder, and no deleterious mutations in the ornithine transcarbamylase, carbamyl phosphate synthase 1 and NAGS genes. While sequencing of the exons and intron/exon boundaries of NAGS failed to detect mutations, stable isotope studies with a 3-day trial of N-carbamylglutamate (NCG) markedly improved incorporation of $^{13}$C isotope into urea (enhanced ureagenesis), increased blood urea and decreased glutamine concentration. This marked response to NCG is characteristic of a NAGS deficiency, and prompted sequencing of introns and the recently identified upstream regulatory regions of the NAGS gene. We identified a mutation in the conserved Hepatic Nuclear Factor 1 binding site of the putative NAGS enhancer which is highly conserved in mammals. Genotyping of the patient’s parents indicated that each was a carrier of the mutation and genotyping a cohort of 500 subjects consisting of Caucasian, Asian, or African descent failed to identify the mutated allele. Functional studies are underway to examine the regulatory role of this binding site in NAGS transcription and the effects of this mutation. With these findings, we demonstrate the fruits of collaborative basic science and clinical research.

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48) Long term follow-up of two siblings with Cobalamin E (MTRR) deficiency, one treated prenatally

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We report the clinical and laboratory features and 11 year follow up of two siblings with Cobalamin E deficiency, one of the rarer forms of cobalamin synthesis defects. Less than 20 cases have been reported to date.

The older brother was presented to us at 7 months of age with repeated admissions for congenital megaloblastic anemia needing red cell transfusions from week 1 of life (Hgb down to 46 g/L) and low vitamin B12 (114 pmol/L). He was born at 42 weeks of gestation and the pregnancy was complicated by maternal thrombocytopenia. Mother’s platelets fell to 50 × 10⁹/L at the time of Cesarean section. His anemia responded partially to cyanocobalamin IM. Plasma homocysteine level was 36.9 (N < 15 μmol/L) but methionine was normal at 18 μmol/L (N: 15–21). He was confirmed on complementation studies to have Cobalamin E deficiency and found to be a compound heterozygote for mutations, c.1637G>A (paternal; novel) and c.1573C>T (maternal; previously reported) in the methionine synthase reductase gene. Homocysteine ranges on treatment with betaine, folate and IM hydroxycobalamin were 36.1–87.9 μmol/L (average 62.7; N 5–12). He had normal early milestones with mild gross motor delays identified at age 4 y. Nerve conduction studies have been normal. At age 12 years he is slightly myopic in both eyes. MRI at age 3 showed mild delay in myelin maturation and prominent ventricles. Neuropsychological assessment at age 11 has confirmed a learning disability in reading, math and writing.

The mother’s second pregnancy was also complicated by thrombocytopenia however appeared to be relatively stable at 86 × 10⁹/L late in gestation. Anniocentesis at 4 months of gestation confirmed that the fetus was affected with Cobalamin E deficiency. Homocysteine measured in amniotic fluid was elevated (3.7 μmol/L; N 0.96–1.99) and complementation studies on cultured amniocytes confirmed the diagnosis. Mother was treated with folic acid plus hydroxycoobalamin (1 mg IM 3×/wk) from 6 weeks of gestation and then daily once the diagnosis was confirmed. The baby was born by Cesarean section at 38 weeks and began treatment with betaine, hydroxycoobalamin and folate on day 1 of life. At 36 days, CBC was normal except for RBC changes on smear suggesting megaloblastic anemia, and MCV (104 fl) was normal and the changes were resolved. Homocysteine was 17.5 μmol/L (N < 15 μmol/L); methionine level was 28 μmol/L (normal 15–21). Plasma homocysteine ranged over time from 17.5 to 90.0 μmol/L (average 50.2). She had normal early milestones. Nerve conduction studies have been normal and at age 9 years she is also slightly myopic. MRI at age 12 months showed only delay in myelin maturation. Repeat MRI at age 3 years showed normal myelination. Neuropsychological assessment at age 8 years confirmed a learning disability in reading, math and spelling.

In conclusion we show that despite prenatal treatment and good metabolic control, the second affected child in this family has similar learning disabilities to her older brother who began treatment at 7 months of age. Another sib pair has been previously reported and long term follow-up to adulthood (unpublished) of the second sibling also shows mild-moderate developmental delay. Further therapeutic modalities are needed and we discuss the theoretical options. Also, transient maternal thrombocytopenia appears to be associated with a fetus affected with Cobalamin E deficiency. Macrocytosis (MCV 127), schistocytosis, and hypersegmented neutrophils persist only in the older sibling despite adequate therapy. The homocysteinemia in these cases was not severe, the methionine was normal and neither child had evidence of encephalopathy, thus the diagnosis may have been overlooked.

49) Inborn errors of metabolism: Not just for kids

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Background: Alkaptonuria, the first inborn error of metabolism (IEM), was identified and classified by Archibald E. Garrod in 1902. Although this disorder is commonly associated with advanced age, the majority of IEMs identified since are associated with pediatric populations, especially with the advances in newborn screening. Accordingly, older patients with delayed onset or intermediate phenotypes of IEM often remain undiagnosed. This study looks at a 10 year period for patients over 60 years old that have been diagnosed biochemically with an IEM by Mayo Clinic’s Biochemical Genetics Laboratory.

Methods: A retrospective survey was performed of all positive results from 2000 to 2009 on patients ≥ 60 years old. Clinical information was available for some of these cases.

Results: A total of 205 patients ≥ 60 years old had positive testing associated with an IEM between 2000 and 2009. The most frequently identified IEM in the older population is alkaptonuria (41/205; 20%). 93 patients were found to have a lysosomal disorder; 35 Gaucher disease, 24 GM-1 gangliosidosis, 21 Fabry disease (19 males and 2 females), 6 metachromatic leukodystrophy, 3 Krabbe disease, 2 San Filippo B disease, 1 Pompe disease, and 1 Tay Sachs disease. 23 patients were affected with peroxisomal disorders; 20 X-ALD and AMN (10 male and 10 female), and 3 Refsum disease. 3 patients had a fatty acid oxidation disorder (1 VLCAD deficiency and 2 CPT2 deficiency), 16 patients had biotinidase deficiency, 16 patients had cystinuria, 3 patients had homocystinuria, 3 patients had PKU, 2 patients had Smith–Lemli–Opitz syndrome, 2 patients had galactokinase deficiency, and another 3 patients had a urea cycle disorder (1 male and 2 female OTC deficiency).

Of particular note, one 72 year old patient was investigated for proximal muscle weakness and abnormal EMG. Her symptoms became obvious x years prior to being diagnosed with Pompe disease (acid α-glucosidase deficiency). Enzyme replacement therapy has been implemented and the patient’s clinical phenotype has improved. An 81-year old patient presented with recurrent rhabdomyolysis and myoglobinuria as of 61 years old leading to renal failure. This patient was found to have Carnitine Palmitoyl-CoA Transferase type II (CPT II) deficiency and passed away as a result of the renal failure when 81 years old.
Conclusions: Inborn errors of metabolism must be considered in patients of all, even advanced ages. Although the more commonly identified severe forms of these disorders have an early onset, the late-onset variants can still represent significant disease burden emphasizing the need to establish a diagnosis and initiate appropriate treatment as soon as possible.

50) Amplification and Comparison of GLB1 sequence from normal and GM1-affected sheep utilizing the cloned bovine GLB1 gene

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Background: GM1 gangliosidosis (GM1) is a fatal lysosomal storage disorder marked by a deficiency in lysosomal β-galactosidase activity, resulting in a corresponding buildup of GM1 ganglioside. The disease is inherited as an autosomal recessive disorder with mutations in the GLB1 gene-coding for structurally altered β-galactosidase. This research focused on a unique ovine model of GM1 that has an additional partial deficiency of α-neuraminidase, which combines with β-galactosidase to form the lysosomal multienzyme complex (LMC). We hypothesized that a novel mutation in the affected sheep GLB1 gene alters the LMC and leads to the dual enzyme deficiencies.

Methods: Because the ovine GLB1 gene has not yet been sequenced, bovine genomic DNA was used to design PCR primers to amplify comparable ovine sequence. To see if this strategy was feasible, the sixth exon of the GLB1 gene was chosen for amplification using normal and GM1-affected sheep. A number of mutations that result in human GM1 occur in the sixth exon of the GLB1 gene; consequently, this region is plausibly a hotspot with a high rate of mutation.

Results: We were able to successfully clone the ovine targeted sequence and to show that it consists of 181 nucleotides, which when compared to the equivalent bovine and human sequences had 94% and 79% base pair identity, respectively. Unfortunately, no base differences were found between the affected and normal ovine exon 6 GLB1 sequences.

Conclusions: Although the sixth exon of GLB1 does not contain the mutation that leads to GM1, we are continuing to use this same strategy to amplify additional GLB1 sequences from normal and GM1 affected sheep in order to identify the disease-causing mutation.

51) Severe infantile presentation of arginase I deficiency

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The deficiency of enzymes of the urea cycle typically present with significant hyperammonemia and its associated toxic signs and symptoms, usually in the first few days to months. However, arginase I deficiency, a rare autosomal recessive disorder (incidence ~1:350,000), has classically been the exception. The enzyme catalyzes the last step of ureagenesis and affected patients usually present later in life with spasticity, seizures, failure to thrive and subsequent developmental regression. Neonatal presentation of arginase I deficiency with severe hyperammonemia remains rare.

We present an affected infant and review the literature of early hyperammonemic presentation in arginase I deficiency. Our patient, a previously healthy 5 week old male presented with a 10 hour history of lethargy and poor feeding, quickly progressing to encephalopathy. Initial plasma ammonia was 736 µmol/L with significantly elevated arginine. Ammonia normalized within 12 h on dialysis but recovery was prolonged due to severe cerebral edema. At age 2 years, he has mild delay and has experienced numerous episodes of hyperammonemia. Elevations in orotic acid have been noted in our patient. We review the reported cases with hyperammonemic neonatal presentation of arginase I deficiency. The age at presentation varies from 3 days to 2 months, initial symptoms vary from hyperammonemia to liver dysfunction and the outcomes range from full recovery to death. We also review the clinical spectrum of eight previously unpublished patients with arginase I deficiency who experienced recurrent hyperammonemia and were diagnosed after infancy. The genotype of the neonatal and late presentation cases is presented, although a correlation with phenotype could not be established.

We discuss the pathophysiology of arginase I and the addition of arginine as a primary target in newborn screening programs (where not already in place) to allow for earlier diagnosis and treatment, while acknowledging that cases can be missed.

52) Complex II deficiency: A case report and review of literature

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Mitochondrial complex II deficiency is a heterogeneous autosomal recessive disorder, comparatively rare to other respiratory chain complex deficiencies. Complex II (CII) is a nuclear encoded protein comprised of four subunits, two of which, a flavoprotein (SdhA) and iron sulfur (SdhB) unit make up the active enzyme succinate dehydrogenase. Decreased activity of this enzyme impedes the function of the respiratory chain, leading to symptoms associated with mitochondrial disorders. The phenotypic spectrum of CII deficiency varies from Leigh syndrome, Kearns Sayre syndrome, primarily neurological manifestations to isolated hypertrophic cardiomyopathy. Outcomes have varied from infantile fatality to mild late onset disease. Although, mutations have been identified in SDHA and SDHAFT (SDH assembly factor 1) genes, overall knowledge of phenotypes and genotypes associated with CII deficiency remains limited.
We present a case with complex II deficiency with a previously undescribed phenotype. This preterm female presented at birth with dilated cardiomyopathy. At 10 months of age, after a mild respiratory illness, acute onset of developmental regression was noted. Other symptoms included failure to thrive, hypotonia and muscle weakness. MRI brain was highly suggestive of mitochondrial disease. Muscle biopsy revealed reduced activity of Succinate Cytochrome c Reductase and Succinate DCIP Reductase, confirming complex II deficiency. Mutation analysis of SDHA and SDHAF1 was negative.

We review and summarize the phenotype of our case along with 22 other published cases in the literature, where available biochemical, imaging and genotypic information is compared to elucidate expected outcome.

53) Nebulous presentation and elusive diagnosis: Congenital disorders of glycosylation type IK

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Congenital disorders of glycosylation (CDG) are a group of more than 40 distinct biochemical disorders due to improper assembly or processing of glycans onto proteins. Presentation of the myriad symptoms that may involve all organs, tissues and cells, can be from the fetal to childhood years.

Our patient was a 3.86 kg, 39 wk product to a 29 year old primigravida mother. Pregnancy was notable for early intrauterine demise of a twin. He was born by C-section because of failure to progress but his Apgar scores were 8 and 9. His newborn course was uneventful. At 4–5 months of age he was seen by ophthalmology because of unusual eye movements. Seizures began at 9 months, initially associated with fever and then progressed to afebrile seizures which were very difficult to control. His developmental milestones were quite delayed. His family history was unremarkable. At 14 months of age his weight was at the 3rd%, length 17th% and head circumference 1st%. He had a slender body habitus without any abnormal fat pads or inverted nipples. He had upslanting eyes with epicanthal folds, wide depressed nasal root and bridge, marked hypotonia with a head lag, inconsistent fixing and following, and had numerous staring episodes. He could not sit, roll over or reach for objects. Brain MRI showed slightly prominent bifrontal extraxial spaces and a small pons. Genetic testing included karyotype, chromosomal microarray, methylation of chromosome #15, VLCFA, leukocyte lysosomal enzyme screen; plasma amino acids, acylcarnitines and carnitine; all of which were normal. DNA methylation array revealed a CG island aberration consistent with the phenotypic presentation, with a di-methylation of chromosome #15, with a reduced activity of Succinate Cytochrome c Reductase and Succinate DCIP Reductase, confirming complex II deficiency.

54) Clinical and biochemical findings in 22 patients with Maroteaux–Lamy syndrome from Central and Eastern Europe

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Background: Mucopolysaccharidosis type VI (MPS VI; Maroteaux–Lamy syndrome) is a rare autosomal recessive disorder caused by a deficiency of N-acetylglactosamine-4-sulfatase (ARSB).

Aim: The aim of the study was to describe the natural clinical course in patients with Maroteaux–Lamy syndrome ever diagnosed in Poland, Baltic States and Belarus.

Patients and methods: Patients with MPS VI (n = 22) were identified by retrieving the data from the registries of the five diagnostic centers for MPS VI in Central and Eastern Europe. Four MPS VI families were diagnosed at The Children’s Memorial Health Institute (Warsaw, Poland), 6 at the Center for Medical Genetics (Vilnius, Lithuania), 8 at Institute for Hereditary Diseases, Centre for Medical Genetic Services (Minsk, Belarus) and 2 families at Department of Genetics, Tartu University Hospital (Tartu, Estonia). So far no patient has been diagnosed in Latvia. In all patients, clinical diagnosis was biochemically confirmed by demonstrating abnormal excretion of DS in urine and deficient activity of ARSB in plasma and/or fibroblasts.

Results: Clinical heterogeneity was observed among our patients and two major clinical phenotypes of the disease can be distinguished: rapidly advancing and relatively attenuated. For patients with rapidly advancing MPS VI disease (n = 14, 63.6%) mean age at diagnosis was 8 years (range 2–14 years, median 9 years). These patients developed symptoms very early in life (mean age at the onset of symptoms was 2.2 years; range 9 months–8 years, median 1 year) and presented with short stature, significant skeletal malformations and other clinical abnormalities. 75% of these patients had onset of symptomatology before the age of 16 months. For patients with slower progression of the disease (n = 8, 36.4%), mean age at diagnosis was 22 years (range 13–37 years, median 20.5 years). In these patients height was only slightly decreased and MPS VI features developed later in the course of the disease (mean age at the onset of symptoms was 8 years; range 1–21 years, median 7 years). Over 60% of these patients showed first signs of the disease after the age of 5 years, in some cases at the age of 8, 12 or even 21
years. All patients had similar characteristics at the time of birth but showed significant differences in body proportions when compared with the healthy population. Children with MPS VI grew considerably slower, and differences between healthy and affected children increased with age and were reflected in phenotypes.

Conclusions. When compared with data published in the literature, patients in our study were diagnosed later and more than 1/3 of them presented with a relatively attenuated phenotype.

55) Molecular analysis of mucopolysaccharidosis type VI in Poland, Baltic states and Belarus: A possible founder effect in Central and Eastern Europe

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Background: Mucopolysaccharidosis type VI (MPS VI; Maroteaux–Lamy syndrome) is a rare autosomal recessive disorder caused by a deficiency of N-acetylgalactosamine-4-sulfatase (ARSB). Approximately 140 ARSB gene mutations have been identified thus far. Allele frequencies of the different mutations are very low, and most mutations are unique to individual families.

Aim: We aimed to analyze the spectrum of mutations responsible for the disorder in Central and Eastern Europe.

Material and methods: We studied 20 unrelated MPS VI families (4 Polish, 6 Lithuanian, 2 Estonian and 8 Belarussian), in whom clinical diagnosis was biochemically confirmed by demonstrating abnormal excretion of dermatan sulphate in urine and deficient activity of ARSB in plasma or fibroblasts.

Results: We identified 97.5% of the ARSB mutant alleles, 6 of them novel, 2 in patients from Poland, 2 in patients from Lithuania, and 2 in patients from Belarus. The novel changes were as follows: c.31091insCCTGAAG+delATACT, Q88X, T92K, W57C, G167R and 161_166insT. All novel mutations, except for c.31091insCCTGAAG+delATACT, were found in heterozygous state: Q88X, W57C, 161_166insT and G167R with R152W and T92K with Y210C. We also report 5 previously described mutations (R152W, Y210C, Y266S, G302R, and Q97X) as well as 2 non-pathogenic polymorphisms (A33V and S384N). R152W attained the high prevalence of 35% for mutated alleles in this group of patients.

Conclusions: 1. Our observation indicates a possible founder effect and suggests that screening of the R152W mutation may be appropriate in MPS VI patients from Central and Eastern Europe. 2. The milder phenotype may be associated with the R152W mutation, which suggests a specific genotype-phenotype correlation.

56) Restricted joint range of motion in MPS II patients: Correlation with age and height

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Background: Hunter syndrome, or mucopolysaccharidosis type II, is an X-linked, progressive lysosomal storage disease in which patients are deficient in the lysosomal enzyme iduronate-2-sulfatase, which results in cellular accumulation of the glycosaminoglycans dermatan and heparan sulphate.

Aims: The aims of the study were to assess joint range of motion (ROM) in patients with mucopolysaccharidosis type II (MPS II) and to correlate joint mobility with patients’ age as well as height.

Material and methods: Passive ROM and SDs of height were followed in 29 patients with MPS II between years 2005 and 2010. Passive ROM was measured by a goniometer and height was measured by a stadiometer.

Results:
1. Restriction in upper extremities ROM in patients with MPS II was observed since the second year of life. These limitations were particularly visible in the elbows (reduced extension), the shoulders (limitation of flexion and abduction) and the wrists (restriction of flexion and extension).
2. ROM limitations intensified and became more severe with the patients’ age, making patients’ self-care more difficult or even impossible. A particularly strong correlation between joint mobility and patient’s age was visible for elbow and wrist extension.
3. A strong correlation was observed between ROM and patients’ height, particularly for elbow and wrist extension.
4. A strong correlation was observed between patients’ age and patients’ height.
**Conclusions:** Documentation of the potential ROM limitations as early as the second year of life supports the need for early treatment as MPS II is a progressive disorder. To achieve holistic benefits of the I2S therapy in patients with MPS II, physical therapy should be added and adjusted to the patient’s efficiency and capabilities.

57) Development of nutrition guidelines for inborn errors of metabolism (IEM): Results of the first Delphi survey on propionic acidemia guidelines

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**Background:** Evidence-based guidelines for diagnosis and management of inherited metabolic disorders (IMD) are lacking. A multi-year project was undertaken with funding from the Southeastern Newborn Screening and Genetics Collaborative and the expertise of metabolic dietitians who are members of Genetic Metabolic Dietitians International (GMDI) to develop medical nutrition therapy (MNT) guidelines for patients living with IEMs. Four workgroups, representing major categories of IEMs, were trained on evidence-based analysis methodology used by the American Dietetic Association, and on consensus based analysis methods. Propionic acidemia (PROP) was one of the first IEMs chosen for guideline development by the Organic Acidemia (OA) workgroup, based on the results of the needs assessment survey of GMDI members. The OA workgroup developed 11 research questions for this disorder to be subjected to an evidence-based analysis, utilizing both peer-reviewed and non-peer-reviewed publications. Since evidence-based PROP published literature is limited, a PROP Delphi survey was developed and initially piloted by the OA workgroup, to determine consensus of ‘best practices’ from clinical experts in the field.

**Methods:** The web-based PROP Delphi survey was sent to expert metabolic physician and dietitian teams representing each of the seven HRSA regional genetics collaboratives. The electronic survey required 45–60 min to complete, and included questions on the clinical experience of respondents in addition to those pertaining to PROP. Respondents rated whether they agreed or disagreed to clinical practice statements using a 7 point Likert scale, and were also able to comment on each question. PROP Delphi surveys were sent out to 28 experts, allowing 4 weeks to respond, resulting in 11 completed surveys (39% response rate). The majority of respondents (73%) had greater than 12 years of experience with IEMs and had taken care of 1–5 PROP patients. Ninety-one % of respondents had treated pregnancy and liver transplant in IEMs, but only 18% in PROP. The majority had taken care of PROP patients with cardiomyopathy (73%) and pancreatitis (82%), but only few (27%) had clinical experience with PROP and diabetes.

**Results/conclusions:** Delphi survey results highlighted agreement in >80% of the respondents in 36% of the management statements. The categories that had best agreement in MNT included; monitoring of anthropometrics, dietary intake data, and development, use of medical foods to enhance anabolism, evaluation for biotin responsiveness, and restarting enteral feedings in critically ill patients as soon as possible. Less agreement was seen in prescriptions for offending amino acids with comments describing how practices change under different circumstances. The most disagreement was in laboratory monitoring guidelines. Areas of less agreement identified by this initial PROP Delphi survey will next be clarified at a Nominal Group meeting; allowing face-to-face expert discussion. A final PROP Delphi survey will serve to confirm and validate PROP nutrition management guideline recommendations. The PROP nutrition guidelines will be field-tested in metabolic clinics; submitted for publication and be updated and revised in the future. It is hoped that the consistent use of evidence and consensus based PROP nutrition guidelines will improve the outcomes for patients living with PROP.

58) Threonine dehydratase deficiency: A case report

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Infantile nonketotic hyperglycinemia (NKH) manifests as myoclonic jerks, hypotonia, and lethargy beginning shortly after birth and progressing to tonic or clonic seizures and respiratory insufficiency, believed to be due to elevations in glycine in the central nervous system. Typically, NKH is due to defects in the glycine cleavage system, a mitochondrial complex of four enzymes. As threonine is a precursor to glycine in the central nervous system, it seems plausible that defects in the threonine degradative pathway may lead to a similar clinical picture in patients with features of NKH and elevations of threonine.

We report a case of threonine dehydratase deficiency. The patient, a Hispanic female, was born to a 35-year-old primigravid mother. There were two episodes of decreased fetal movement during the pregnancy. Birth was by emergency C-section due to late fetal decelerations. Family history was noncontributory and her parents are nonconsanguineous. She was noted to have seizures from her third day of life, initially occurring more than 40 times per day. She had severe feeding difficulties requiring gastrostomy tube placement and developed progressive microcephaly, hypotonia, global developmental delay, and arthrogryposis. At initial evaluation, her plasma threonine was 1530 nmol/mL (normal 86–321 nmol/mL). The threonine decreased after institution of a threonine-restricted diet, but remained mildly elevated between 229 and 544 nmol/mL. Plasma glycine was also elevated at presentation at 1516 nmol/mL (normal 103–424 nmol/mL) and remained elevated with a range of 805–1516 nmol/mL. Threonine and glycine were also elevated in cerebrospinal fluid (CSF) samples (163 nmol/mL, normal 17–80 nmol/mL; 22.4 nmol/mL, normal 0–18 nmol/mL, respectively). Urine organic acid quantitation, plasma and CSF lactate and pyruvate, and plasma acylcarnitine profile were all normal. Despite a
Mouse model of human Barth syndrome, mitochondrial cardiolipin disorder

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Background: Barth syndrome is a rare but serious X-linked genetic disorder caused by mutations in the tafazzin (taz) gene. In its full spectrum of manifestation Barth syndrome is characterized by dilated cardiomyopathy, exercise intolerance, chronic fatigue, growth delay, neutropenia and 3-methylglutaconic aciduria. Tafazzin is a mitochondrial transacylase required for cardiolipin remodeling. Although tafazzin function has been studied in both non-mammalian model organisms, mammalian genetic loss of function approaches have not been explored.

Methods: We examined the consequences of tafazzin sh-RNA silencing on sarcomeric mitochondria and cardiac function in mice.

Results: Tafazzin ablation resulted in dramatic reduction of tetra-linoleoyl cardiolipin levels in cardiac and skeletal muscles and accumulation of monolysocardiolipins. Electron microscopy revealed extensive mitophagy and pathological changes in myofibrils and endoplasmic- reticular membranes in cardiac and skeletal muscles. Echocardiography and cardiac MRI revealed severe cardiac abnormalities, including left ventricular dilation, left ventricular mass reduction and depression of fractional shortening and ejection fraction in tafazzin-deficient mice.

Conclusions: Tafazzin-knockdown mice provide the first mammalian model system for Barth syndrome in which the pathophysiological relationships between altered content of mitochondrial phospholipids, ultrastructural abnormalities, myocardial and mitochondrial dysfunction and clinical outcome can be investigated.

Bone mineral density in a cohort of PKU patients: Comparison between responders and non-responders to Kuvan treatment

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Background: Patients with phenylketonuria (PKU) are at greater risk of fractures, osteopenia, and osteoporosis than those without PKU. Kuvan, a BH4 analog, is an adjunct therapy for PKU, but its effect on bone mineral density has yet to be explored. The objective of this analysis was to determine the effect of Kuvan on total bone mineral density (tBMD) in a group of PKU patients (≥4 years old) after 1 year of treatment, as well as to examine differences between responders and non-responders and differences between gender.

Methods: tBMD was measured with dual-energy X-ray absorptiometry (DXA) at baseline and at 12 months in 35 male and 23 female patients between the ages of 6 and 49. PKU patients were categorized as either responders (≥15% decrease in blood Phe levels) or non-responders at the 4-week follow-up visit after the start of treatment. Responders to Kuvan treatment were kept on drug for the duration of the study, whereas those found to be non-responders were simply asked to continue following diet therapy. Z-score values were used for within- and between-group comparisons; 2-sample t-tests were used for the analysis, and the level of significance was p<0.05.

Results: Baseline and 12-month DXA results are available for 41 patients, with 12-month results pending for 2 patients. Thirty-six percent (36%) of patients were considered responders based on the defined criteria. Average total bone density Z-scores were similar between responders and non-responders at baseline (−0.42±0.9 vs. −0.59±1.0, respectively). At baseline, the prevalence of tBMD z-scores ≤ −1 was 33%, and 67% had Z-scores over −1. When divided by age group, 22% of children (5–11), 54% of adolescents (12–18), and 33% of adults (19+) had Z-scores ≤ −1.

A 2-sample t-test revealed that there was a non-significant difference (p>0.847) in Z-score from baseline to endpoint between responder and non-responder groups. The change in Z-score between males and females, regardless of treatment group, was also not significant (p>0.160). Within the group of responders, females had a greater change in Z-scores than males (mean 0.3000 vs. −0.0455, p>0.0587). Within the group of non-responders, females and males were similar in Z-score change (mean 0.129 vs. 0.125, p>0.987).
61) Management challenges for patients detected by newborn screening: Lessons from very long chain acyl COA dehydrogenase (VLCAD) deficiency

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The correct diagnostic assignment for patients with abnormal metabolites on newborn screening is often challenging, but is vital for determining a plan of action. Management decisions become increasingly difficult as many disorders have great phenotypic variability. Which patients are at greatest risk for decompensation? Here we consider the biochemical and molecular findings in a severe case of VLCAD deficiency. A newborn female was referred by the New York State newborn screening program with abnormal acylcarnitines consistent with VLCAD deficiency. The C14:1 was 4.73 μmol/l (Normal < 0.65). She was born to 25-year-old primigravid at 41 wks by C-section for failure to progress, birth weight was 3.67 kg. Pregnancy history was otherwise uncomplicated. Initial family history revealed that both parents were from Peru, but consanguinity was denied. A repeat acylcarnitine profile revealed C14:1 of 3.99 μmol/l and low total and free carnitine levels. The patient had a normal cardiac evaluation at 13 days of age. The patient was started on low dose i-carnitine (25 mg/kg) and Lipistart (Vitaflo) was supplemented into her diet. Her C14:1 level dropped gradually over the next few months. She grew well on a mixture of breast milk and Lipistart, and maintained growth parameters within normal centiles. Her C14:1 level at 5 months of age was 1.8 μmol/l. At 6 months she was admitted with a picture of gastroenteritis with vomiting and poor PO intake. Hepatomegaly was noted and confirmed by abdominal ultrasound. On the second day of admission the patient went into cardiopulmonary arrest, she was resuscitated and transferred to the pediatric intensive care unit. She expired on day three of the admission. The autopsy revealed severe cardiomegaly, and the admission acylcarnitine profile revealed a C14:1 of 7.36 μmol/l. Enzyme assay on fibroblasts revealed no VLCAD enzyme activity (University of Amsterdam). Sequence analysis of the ACADVL gene (NM_000018.2) (GeneDx) on a peripheral blood sample revealed a single missense change, K299M. Exon level deletion/duplication analysis by array CGH, performed to identify a second mutation, suggested a partial deletion of several exons of the ACADVL gene and was confirmed by quantitative PCR. The deletion appears to be a somatic mutation present at a low-level of mosaicism. In 15% to 20% of patients with VLCAD deficiency sequencing identifies only a single mutation. Presumably the second mutations in these cases are deep within an intron, in the promoter region or they are due to a large deletion of ACADVL that would not be detectable by sequencing. Somatic deletions of the ACADVL gene have not been reported previously, to our knowledge.

62) Krabbe disease newborn screening: Ethical conflicts in a novel screening program

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Objective: To explore the recent New York State (NYS) experience for Krabbe disease newborn screening (KD-NBS) to highlight conflicts between two ethical paradigms that have shaped genetic policies in presymptomatic testing. The first paradigm accepts mandated, nonconsented routine screening for a genetic disorder (e.g., PKU), in some cases without knowledge of parents, to detect disease susceptibility in order to initiate early treatment to prevent life-threatening or life-altering sequelae (such as mental retardation). The second paradigm involves incurable, adult onset disorders such as Huntington disease where presymptomatic testing in children is discouraged to preserve the autonomy of the child to make decisions in adulthood. In such cases, a number of professional organizations, including the American College of Medical Genetics, have considered such presymptomatic testing unethical.

Methods: Review of the outcomes from the KD-NBS program to identify issues that geneticists and other pediatric specialists involved in NBS are likely to face as newborn screening targets more neurologic disorders.

Results: In the absence of evidentiary support from pilot or prior studies, child neurologists and geneticists in NYS developed confirmatory laboratory and clinical follow-up procedures to ensure prompt identification of newborns at risk for infantile KD and enable human stem cell transplantation (HSCT) within 4–6 wks of life. After over one million infants screened, NYS found three cases of infantile KD; one infant died from complications related to HSCT, one survived HSCT with moderate neurological deficits but a milder disease course, and one did not undergo transplant and has infantile KD. However, an additional 20 infants were identified with two gene variants who are at risk for developing KD later in life, when treatment options are not clear, and more than 30 infants with borderline enzyme activity were found and offered entry into a follow-up registry. The overall incidence of detecting two mutations is about 3 times the expected incidence of Krabbe disease, thus the long term implications of the newborn screening result are unclear for the majority of cases.

Discussion: The New York state program appears to successfully identify infantile onset KD and provide potential for risky but disease attenuating treatment by HSCT. As such it is a model for the screening of other lysosomal storage disorders (LSDs) associated with rapid neurologic decline. However, it appears that the majority of screen positive infants will have later onset or possibly even asymptomatic courses.

Although HSCT does not appear curative for infantile KD, infants who survive transplant appear to have a significantly attenuated course. Thus ethically the program relies on traditional newborn screening justifications: to identify presymptomatic serious diseases likely to cause
irreversible effects and provide timely treatment. However, the unexpected high incidence of later onset KD (as defined by presence of two gene variations in an asymptomatic child) means that the vast majority of cases diagnosed by NBS will not have symptom onset until later in life or perhaps may never even become symptomatic. This has inadvertently clashed with the determination that screening for serious and non-treatable adult-onset diseases is unethical. This conflict is likely to become even more serious as screening expands to other storage disorders. Practitioners need to determine how to deal with this conflict. Some possible options:

1) Offer screening as a research protocol until evidentiary support regarding outcomes is available, or require parental consent for screening for these disorders.
2) Re-evaluate ethical principles regarding NBS, particularly balancing the benefits of early treatment for a few vs. the potential harms of early disclosure for many.
3) Do not introduce new screens without evidentiary support in place to separate those likely to have early onset disease, then withhold milder/late onset results until the patient can decide to seek the information.

Though some of these challenges are unique to Krabbe disease, similar issues will likely arise with newborn screening for other lysosomal storage disorders (LSD) associated with profound neurodegeneration. We feel it is important to consider these ethical conflicts now so that medical providers can adequately prepare for the often agonizing challenges of counseling families about a fatal and possibly untreatable condition whose onset cannot be predicted.

63) Comprehensive bioinformatic analysis of urea cycle gene transcription regulation using cis-element over representation (CLOVER) software and patient sequences to identify disease causing mutations

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Background: The conversion of nitrogenous waste into urea through the urea cycle is controlled by regulatory factors that respond to nitrogen load. These regulatory mechanisms help to maintain a nitrogen balance and prevent the toxic buildup of ammonia. While most urea cycle disorders are a result of mutations in coding sequences or splice sites, it is likely that deleterious changes to their regulatory regions may also disrupt ureagenesis. The regulation of the Carbamyl Phosphate Synthase I and Ornithine Transcarbamylase (OTC) have been the topic of much investigation but little is known about the regulation of other urea cycle proteins, including Argininosuccinate Synthetase and Argininosuccinate Lyase.

Methods: To analyze the transcriptional regulation of urea cycle genes we used the Cis-Element Over Representation (CLOVER) software that allows us to identify over-represented transcription factor binding motifs that are likely to play a functional role within highly conserved regions upstream of the urea cycle gene coding sequence. We then compiled the sequences upstream of the OTC gene from individuals with OTC deficiency without mutations in the exons or splice sites of the OTC gene and compared to them to wild type sequences.

Results: We identified three single nucleotide substitutions that are disease-causing candidates in the promoter region of the OTC gene. One of the changes occurred in a known HNF-4 binding site while the other two occurred in a highly conserved region without known or predicted regulatory function. These alterations were absent in 800 alleles from individuals of Caucasian, Hispanic and African decent and were not found in any of the SNP databases. We are currently conducting DNA-Protein Avidin–Agarose pull-down assays using liver nuclear protein extract and expression assays to confirm the deleterious effect of the identified mutations.

Conclusions: Bioinformatic identification and molecular verification of regulatory elements and mutations found in these conserved elements are important for understanding the regulation of the urea cycle genes. Detailed understanding of the DNA sequences that govern expression of urea cycle genes is essential for better diagnosing urea cycle disorders.

64) Case report: 45-year-old female with propionic aciduria, renal failure, and premature ovarian failure; late complications of propionic aciduria?

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We describe a 45 year old patient who was diagnosed with propionic aciduria as a neonate. Diagnosis was suspected because her sister passed away of the same disorder, was made initially based on her biochemical profile, and was later confirmed by sequencing the PCCB gene that revealed the already described mild mutation c.1606A→G (p.N536D) and the novel severe frameshift mutation c.1142dupG (p.C381fs).

The patient initially experienced an unstable course marked by almost constant hospitalization during the first two years of life for ketosis and/or poor feeding, failure to thrive, and developmental delay. Her course improved during mid-childhood, and by late adolescence the number of hospital admissions diminished and her diet was simplified to be only a low protein diet without other supplementation. During her adult life the patient developed a host of other medical problems including migraines, appendicitis, allergic rhinitis, hypothyroidism, irritable bowel...
syndrome, premature ovarian failure at age thirty-seven, and end stage renal failure eventually treated with renal transplantation at age forty-two. Work up for the underlying etiology of her renal deterioration was inconclusive and the possible contribution of long-standing propionic acidaemia to the renal failure could not be addressed.

Renal failure and premature ovarian failure have not yet been associated with propionic acidaemia. The association between renal failure and methylmalonic acidaemia, a closely related disorder, however, has already been established. We hypothesize that propionic acidaemia may have contributed to her renal failure based on this fact and the knowledge that there are several unpublished cases of patients with both propionic acidaemia and adult onset renal failure that have been discussed on Metafl, a metabolist listerv, as well as in our patient’s personal communication with other patients with propionic acidaemia. We also speculate that a possible underlying mechanism for late onset organ failure is a secondary electron transport chain deficiency leading to energy depletion and increased oxidative stress. Of note, in McGuire et al., markers of oxidative stress were found to be more elevated and levels of antioxidant lower in Cobalamin C, a form of methylmalonic acidemia with homocystinuria compared to that in propionic acidemia, which would be consistent with the increased frequency of renal failure in methylmalonic acidemia compared to in propionic acidemia.

In the era of newborn screening, many more mild cases of metabolic disorders such as propionic acidaemia will be ascertained. Patients with these milder forms of the disease may develop later complications, which have not previously been recognized. Although the association of renal failure and propionic acidaemia is far from established, we feel it is unlikely to be coincidental.

65) Transient neonatal hypermethioninemia in maternal MAT I/III deficiency
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We describe a family in which a mother with methionine adenosyltransferase (MAT) I/III deficiency was rediscovered because her offspring had transient neonatal hypermethioninemia identified by tandem mass spectrometry newborn screening.

Background: MAT I/III deficiency, characterized by isolated persistent hypermethioninemia with only slightly elevated homocysteine, is caused by mutations in the MAT1A gene. This gene encodes the protein MATα1, the subunit of the hepatic enzymes MAT I ([α1]4) and MAT III ([α1]5), that synthesize from methionine the main biological methyl donor, S-adenosyl-methionine. More than 60 patients with MAT I/III deficiency have been described, many detected by newborn screening, and the great majority have been symptom free suggesting a benign disorder. Neurological abnormalities and demyelination of the brain have been observed in a few patients, possibly linked to the severity of the enzyme deficiency and insufficient synthesis of S-adenosyl-methionine. Interestingly, four pregnancies in a woman with moderately severe MAT I/III deficiency have been reported, resulting in three normal babies who were healthy on follow-up. Fetal arrest was detected in one embryo at 10–11 weeks of gestation felt to be unrelated to MAT I/III deficiency.

Case report: The newborn in the family we describe had a newborn screen methionine level of 403 μmol/L (6 mg/dL) in blood collected at 42 h of life. On evaluation at age 10 days her plasma methionine and homocysteine levels were normal at 52 μmol/L (0.8 mg/dL) and 6 μmol/L respectively. Amino acid analysis in the mother, however, revealed elevated plasma methionine and homocysteine levels of 556 μmol/L (8.3 mg/dL) and 28 μmol/L respectively. Of importance, although being recommended to adhere to a methionine restricted diet, the mother stayed on it for less than one year, having high homocysteine levels. She was lost to follow-up after childhood. Interestingly, her rediscovery was based upon the finding of hypermethioninemia in her offspring. She has been doing well ever since childhood with normal liver function tests and slightly elevated homocysteine. To the best of our knowledge, this is the first published case in which transient neonatal hypermethioninemia is shown to be present by newborn screening in a neonate from maternal MAT I/III deficiency.

Summary: We describe a transient neonatal hypermethioninemia identified by newborn screening of a newborn born to a diagnosed mother with hypermethioninemia. Therefore, maternal MAT I/III deficiency should be suspected in any newborn with hypermethioninemia and, therefore, in such cases a maternal plasma amino acid analysis and homocysteine level should be performed along with the clinically indicated neonatal metabolic-related work-up.

66) Change in timing of sapropterin dose results in inappropriate liberalization of diet in 10 year old patient with PKU
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Phenylketonuria (PKU), an autosomal recessive disorder due to defects in the enzyme phenylalanine hydroxylase (PAH), results in accumulation of phenylalanine in the body. The mainstay of treatment is dietary intervention to limit the phenylalanine in the diet. Tetrahydrobiopterin (BH4) is a required cofactor for enzymatic activity. Sapropterin dihydrochloride, a synthetic tetrahydrobiopterin (BH4), has been shown to be effective in the treatment of PKU by activating residual PAH activity in responsive patients. The medication is labeled to be effective in the treatment of PKU by activating residual PAH activity in responsive patients. The medication is labeled to be

Case report: We describe a 10 year old, Caucasian male, with historically extremely well-controlled PKU on diet, who had an unexpected response to a dose administration change. Sapropterin (20 mg/kg/day) was initially taken in the morning with food, followed by a regular phe-restricted dietary regimen throughout the day. After ten days, he began taking it in the evenings, with food, and phenylalanine levels were obtained following an overnight fast. Based on these levels, his response to this medication was determined to be an 82% decrease in fasting phe level after 2 weeks on therapy, at which time his diet was significantly liberalized. However, when he again began taking it in the morning, with no additional dietary
changes, his measured phenylalanine level tripled, suggesting that measurement of fasting phenylalanine levels after evening dosage might result in a spuriously low phenylalanine level and erroneous identification of responder status, resulting in inappropriate liberalization of the diet.

Conclusion: The findings in this case suggest that the pharmacokinetics of once-daily sapropterin dosage may be different from previously reported pharmacokinetics, and particularly dependent upon timing of dose and prolonged fasting after dosing.

67) Maternal medium chain acyl CoA dehydrogenase deficiency identified by newborn screening

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Background: Medium chain acyl-CoA dehydrogenase deficiency (MCADD) is an autosomal recessive fatty acid oxidation defect characterized by severe metabolic decompensation during periods of prolonged fasting or illness. Without swift intervention, these episodes can result in hypoketotic hypoglycemia, Reye-like syndrome, coma and death. Through the use of tandem mass spectrometry (MS/MS), newborn screening can detect elevated medium chain acylcarnitines in dried blood spots, allowing for the identification of infants with MCADD. MS/MS has also been shown to diagnose some maternal conditions. For example, the presence of elevated metabolites in newborns has led to the identification of maternal 3-methylcrotonyl-CoA carboxylase deficiency and holocarboxylase synthetase deficiency. Whereas, decreased free carnitine in newborns has led to the diagnosis of maternal asymptomatic glutaric aciduria Type I, carnitine transport defects, and combined homocystinuria and methylmalonic aciduria (cbiC). We now present the case reports of two women, who were diagnosed with MCADD through the abnormal newborn screening results of their infants.

Methods: Our patients were brought to clinical attention after the expanded newborn screening results of their children revealed low free carnitine. Confirmatory testing for the newborns included plasma and urine carnitine. Confirmatory testing for the mothers included plasma and urine carnitine, acylcarnitine profiles, and urine organic acids. Once the abnormal maternal acylcarnitine profiles were identified, acylcarnitine profiles were then ordered on the newborns, as well.

Results: Physical examinations of both the newborns and the mothers were normal. Mother 1 described symptoms of fasting intolerance including headache, irritability, nausea and somnolence. To ameliorate these symptoms, she had already self-imposed a regimen of eating every 3 h during the day with a maximum overnight fast of 10 h. During her second pregnancy she reported increased fatigue and episodes of feeling faint. Apart from these symptoms, Mother 1 had no history of metabolic decompensation. Mother 2 had no reported history of fasting intolerance or metabolic decompensation and neither mother reported a history of serious illness, surgery or hospitalization, apart from hospitalization during childbirth. Results of the newborn screening and confirmatory testing are summarized below in Table 1. Both newborns had low levels of total and free carnitine in plasma, with normal urine carnitine. Plasma levels normalized in both infants without treatment and their acylcarnitine profiles were normal. In contrast, both mothers had low total and free carnitine in plasma. Urine organic acids revealed elevated suberylglycine and hexanoglycine. Acylcarnitines showed elevated C6, C8, C10:1 and C8/C10 ratios consistent with MCADD. Supplementation with carnitine was initiated, along with fasting precautions and a heart healthy diet. Plasma carnitine levels normalized in Mother 1. Carnitine levels trended up in Mother 2 after two days of carnitine supplementation. Sequencing of the ACADM gene revealed that our first patient is homozygous c.985A>G (p.K329E), a mutation, generally, associated with a severe MCADD phenotype. Sequencing for our second patient is pending.

Conclusions: The identification of our adult patients through their children's newborn screening results provides evidence that expanded newborn screening not only identifies infants with MCADD, but may also identify affected mothers who are at risk to experience the life-threatening metabolic decompensations associated with the disease.

| Table 1. Biochemical results in newborns and their mothers. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Newborn 1       | Mother 1        | Newborn 2       | Mother 2        |
| Newborn screening | C0 = 10.9 (12–150) | N/A | C0 = 5.1 (7.1–125) | N/A |
| 1st plasma carnitine | | | | |
| Free | 6 (25–54) | 10 (19–48) | 9 (25–54) | 3 (19–48) |
| Urine carnitine | | | | |
| Total | 173 (125 ± 75) | N/A | 235 (125 ± 75) | 81 (125 ± 75) |
| Free | 26 (51 ± 40) | N/A | 76 (51 ± 40) | 12 (51 ± 40) |
| Acylcarnitines | | | | |
| C6 | 0.08 (<0.10) | 0.34 (<0.09) | <0.12 (<0.34) | 0.17 (<0.09) |
| C8 | 0.11 (<0.35) | 1.57 (<0.65) | <0.15 (<0.21) | 1.50 (<0.65) |
| C10:1 | 0.09 (<0.64) | 0.59 (<0.81) | <0.07 (<0.18) | 0.79 (<0.81) |
| Urine organic acids | N/A | SG = 29 (0) HG = 6 (0) | N/A | SG = 45 (0) HG = 11 (0) |
68) Mutation analysis of 44 children with biotinidase deficiency identified by newborn screening in Michigan

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Background: Biotinidase deficiency is an autosomal recessively inherited disorder in which the vitamin biotin cannot be appropriately recycled. Untreated individuals with biotinidase deficiency can develop neurological and cutaneous symptoms. Biotinidase deficiency screening has been included in newborn screening programs for more than two decades. Enzymatic analysis and/or molecular analysis are used to confirm the diagnosis.

Method: Forty-four children, 5 with profound biotinidase deficiency and 39 with partial biotinidase deficiency, were identified by enzyme activity in the screening program of Michigan. Molecular analysis was performed on these individuals by sequencing all coding and splicing regions of the biotinidase gene (BTD).

Results: Three of the five children with profound biotinidase deficiency were compound heterozygotes and the other two were homozygotes for c.1489C>T (p.P497S), a previously described mutation. 38 out of 39 children with suspected partial biotinidase deficiency had c.1330G>C (p. D444H) as one of their mutations together with a second known or putative mutation for profound deficiency. One child with partial biotinidase deficiency (enzyme activity: 1.8 nmol/min/ml; reference range: 5.2–13 nmol/min/ml) was a compound heterozygote for c.626G>A (p.R209H) and c.310-15delT, which are both novel mutations. Parental studies of this child showed that these mutations were in trans configuration. R209H, predicted to be a deleterious mutation, together with c.1368A>C (p.Q456H) was found in one child with profound deficiency. Two other novel mutations, c.683A>G (p.D228G) and c.898A>C (N300H), were identified in three individuals with partial deficiency together with c.1330G>C (p. D444H), respectively; both of these novel mutations are predicted to be deleterious to enzymatic function. Two children suspected as having partial biotinidase deficiency by enzymatic activity were shown to be homozygous for D444H and, therefore, do not require treatment.

Conclusion: As previously reported, partial biotinidase deficiency is almost always caused by c.1330G>C (p. D444H) in combination with a second mutation causing profound deficiency. This is the first report that c.310-15delT appears to be associated with higher residual biotinidase activity similar to that due to the D444H mutation; further functional studies are needed to confirm this finding. In addition, this study demonstrates the importance of sequencing the BTD gene, because enzymatic analysis alone may lead to the unnecessary treatment of some individuals, such as those homozygous for the D444H mutation.

69) Acute porphyrias: Diagnosis and treatment of a life-threatening and underrecognized neurometabolic disorder

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Background: Acute porphyrias are mostly autosomal dominant inborn metabolic errors, caused by a disturbance in the heme biosynthetic pathway. Clinical manifestations involve central and peripheral nervous system. The diagnosis is based on the elevated urinary excretion of porphyrins precursors δ-aminolevulinic acid and porphobilinogen.

Aims: To analyze the clinical manifestations and the management of patients with acute porphyrias in a single reference Hospital in Brazil.

Material and methods: Retrospective study of medical records from 43 patients with acute porphyrias seen in a reference center in the Southeast region of Brazil, between January 2007 and August 2010.

Results: 43 medical records were reviewed and 2 patients were excluded because their biochemical profile was not compatible with acute porphyria; median age of the patients studied ranged from 18 to 47 years old. Usually, female were more severely affected than males. All patients had high levels of delta-aminolevulinic acid and porphobilinogen measured in 24 h urine collection. The age in which most of the crisis occurred was the third decade. 30 patients presented, mainly, in the clinics having their first crisis, but 11 of them presented also with chronic manifestations. The most common clinical presentations were: abdominal pain, change in urine color, change in bowel habits, motor or sensory-motor deficit, vomiting, alteration of consciousness or mental confusion, convulsions, dysautonomic cardiovascular signs and psychiatric disorders. The crisis were classified as mild, moderate and severe, with chronic manifestations in many patients. Peripheral motor or sensory-motor neuropathy was the initial manifestation in two patients. Six patients died after the initial diagnosis as consequence of complications of the disease. The most commonly used treatments were glucose administration, elevation of carbohydrate intake, and phenothiazines use. Although that, heme (Pan-Hematin®/Normosang®) administration was life-saving for many patients with acute decompensation.

Conclusions: Acute porphyrias are severe metabolic disorders with many precipitating factors; they are usually not promptly recognized by internists and general practitioner since they simulate other more common diseases. Correlation was found in our patients between the kind and the number of precipitating factors: absence of peripheral neuropathy was in general related to just one factor, more commonly of endogenous endocrine or metabolic origin, like menstrual period and starvation, while in the crisis with peripheral neuropathy multiple factors might be involved at the same time explaining the high morbimortality in such group of patients.
70) Spino-cerebellar ataxia with hypogonadism: Unraveling an overlooked group of inherited metabolic disorders

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Background: The association between cerebellar ataxia and hypogonadism was first described in four sibs by Holmes in the beginning of the 20th century, and has since become known as Holmes type ataxia. At the time of his description, it was not possible to determine whether hypogonadism was hypergonadotropic or hypogonadotropic hypogonadism. Although the clinical pictures may share similar phenotype, the pathogenic mechanism involved in the two types of hypogonadism is different. Several syndromes with hypo/hypergonadotrophic hypogonadism and ataxia have been published; however there is a remarkable clinical heterogeneity among them. Here, we present the clinical data and molecular/biochemical studies of fifteen Brazilian patients with cerebellar ataxia and hypogonadism. Material and methods: All patients were evaluated in the neurogenetics clinics by geneticists, neurologists and endocrinologists. Brain MRI, ophthalmological examination, EMG/NCV, hormone and biochemical tests, screening for CDG disorders (IEF and MS analysis), karyotype, muscle biopsy with chain respiratory enzyme assays and measurement of coenzyme Q10, molecular tests for Friedreich ataxia and for SCAs (types 1, 2, 3, 6 and 7) were performed in the course of the investigation. Results: All patients had cerebellar ataxia, but the age of the onset was variable; it was worthy to note that ten patients had early onset ataxia (in the first decade of life). Consanguinity of parents was noted in two families; five patients had hypergonadotropic hypogonadism. Mental retardation was seen in two unrelated girls with hypergonadotropic hypogonadism. None of the patients had chromosomal anomalies. Molecular tests for Friedreich and SCAs 1, 2, 3, 6 and 7 were all negative. Optic atrophy and retinocochaloid degeneration were found in five patients; axonal neuropathy was present in four patients. Cerebellar atrophy with spastic or prominent vermis involvement was a constant feature. In two patients with ataxia and hypergonadotropic hypogonadism, coenzyme Q10 deficiency was confirmed in muscle biopsy. Two unrelated adult patients with hypergonadotropic hypogonadism had biochemical features of CDG Ia. One family – with four affected sibs – have features consistent with a rare neurological disorder, Boucher–Neuhauser syndrome. Conclusions: The association between cerebellar ataxia and hypogonadism comprises heterogeneous entities whose clinical investigation can enlighten the pathological basis of these fascinating neuroendocrinological syndromes. Screening for CDG and Coq10 deficiency should be done in such patients as part of the work-up investigation.

71) Antiquitin deficiency: Atypical neonatal presentation with severe hypoglycemia, hyperlacticacidemia, myoclonic epilepsy and hypoxic ischemic encephalopathy

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Background: Pyridoxine dependent epilepsy (PDE) due to antiquitin deficiency (ATQD) is an autosomal recessive disorder characterized by neonatal encephalopathy and intractable epilepsy, ATQD is caused by mutations in the ALDH7A1 gene causing ALDH7A1 deficiency. Pyridoxal phosphate is co-factor for more than 120 enzymes in metabolic pathways. AASA in is dis-equilibrium with L-delta-piperideine-6-carboxylate (PC). PC binds to pyridoxal phosphate which is the active form of pyridoxine and causes functional pyridoxal phosphate deficiency. Pyridoxal phosphate is co-factor for more than 120 enzymes in metabolic pathways.

Patient and results: This 19-month-old girl was born after an uneventful pregnancy at term by normal spontaneous vaginal delivery. Apgars were 4, 9 and 9. She was hyperalert and sleepless in the first 3 days of life. She had myoclonic jerks, vomiting and hematemesis at age 4 days and was presented to emergency room. Her seizures were unresponsive to Phenobarbital and Phenytoin loads. Her initial metabolic investigations showed severe hypoglycemia (0.6 mmol/l; reference range > 2.4), hyperlacticacidemia (11 mmol/l; reference range < 2.2) and metabolic acidosis (pH 7.23; bicarbonate 6) with elevated anion gap (29; reference range < 15). Her hypoglycemia and lactic acidosis resolved with intravenous 4.2 mg/kg/min glucose infusion. Despite Phenobarbital, Midazolam and Phenytoin, EEG showed multiple electrographic subclinical seizures. Because of ongoing seizures, pyridoxine was given 50 mg IV and her seizures stopped on the next day. Her cranial MRI showed intracranial bleeding and basal ganglia involvement and was suggestive of hypoxic-ischemic encephalopathy. Because of severe hypoglycemia and hyperlacticacidemia investigations for glycogenolysis and gluconeogenesis defects were initiated. After clinical improvement, she was discharged on Phenobarbital at age 16 days and pyridoxine was discontinued. One week later she was readmitted for increased myoclonic jerks and irritability. She was unresponsive to more than eight anti-epileptic medications for 3 weeks. Because of ongoing seizures pyridoxine was re-started after 3 weeks. She became seizure free on the next day. Urine alpha-amine-adipic-semialdehyde (21.5 μmol/mmol creatinine; reference range < 1) and plasma picoenic acid (12.3 μmol/l; reference range 0.54–2.46) levels were highly elevated. She had compound heterozygous missense (a novel and a disease causing) mutations in the ALDH7A1 gene confirming ATQD. She is on pyridoxine (200 mg/kg/d) and seizure free since age of 1.5 months. Her EEG normalized during follow-up. She has also lysine-restricted diet to decrease toxic AASA and AASA-PC complex. She has gross motor delay and recently started crawling and pulling herself up to stand.

Conclusions: We presented a 19-month-old girl with ATQD and neonatal encephalopathy. ATQD should be included in the differential diagnosis of neonatal hypoglycaemia, hyperlacticacidemia and intractable myoclonic epilepsy.
72) Six new patients with creatine deficiency syndromes identified by selective screening in British Columbia

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**Background:** Creatine biosynthesis (guanidinoacetate methyltransferase (GAMT) and L-arginine:glycine amidinotransferase (AGAT) deficiencies) and transport (SLC6A8 deficiency) defects are progressive neurodegenerative disorders of creatine metabolism. GAMT and AGAT deficiencies are inherited autosomal recessively, whereas SLC6A8 deficiency has X-linked inheritance. Global developmental delay, intellectual disability, behavioral problems and seizures are common clinical features. Cerebral creatine deficiency is the biochemical hallmark for all three disorders. Guanidinoacetate is elevated in GAMT and low in AGAT deficiencies. Urinary creatine-to-creatinine ratio is elevated in males with SLC6A8 deficiency.

**Methods:** Guanidinoacetate and creatine-to-creatinine ratio were measured in urine by tandem-mass-spectrometer. Intracerebral creatine was measured by MR-spectroscopy. Mutation analysis of GAMT and SLC6A8 genes, GAMT enzyme activity and creatine uptake study were performed according to previously published methods and primers.

**Results:** Two female patients with GAMT deficiency and four patients with SLC6A8 deficiency (2 female siblings and 2 males) were identified within 5 years in British Columbia. All patients presented with global developmental delay. One female patient with SLC6A8 presented with intractable epilepsy. Five patients showed total or partial intracerebral creatine deficiency in cranial-MR-spectroscopy. Two patients with GAMT deficiency had moderately elevated levels of guanidinoacetate in urine. Two males with SLC6A8 deficiency had markedly elevated urine creatine-to-creatinine ratio. However two females with SLC6A8 deficiency had normal urine creatine-to-creatinine ratio. Three previously reported mutations (c.327G>A; splice mutation) and a novel insertion (c.58insT) were identified in the GAMT gene. Both patients had none-detectable GAMT enzyme activity in cultured skin fibroblasts. Both were treated with creatine (400–550 mg/kg/d) and ornithine (400–500 mg/kg/d) supplementation and arginine restricted-diet (250–300 mg/d). Three (2 females and one male) patients had a novel missense (heterozygous and hemizygous respectively) mutation and one patient had a known 3 bp deletion in the SLC6A8 gene. All patients with SLC6A8 deficiency are on creatine, L-arginine and L-glycine supplementation therapy.

**Conclusions:** Creatine deficiency syndromes are treatable cause of intellectual disability and epilepsy. Patients with global developmental delay, intellectual disability, behavioral problems and epilepsy should be investigated for these treatable disorders.

73) Two different clinical phenotypes in two siblings with 3-methylglutaconic aciduria type I

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**Background:** 3-Methylglutaconic aciduria type I (3-MGA-I) is an autosomal recessive organic aciduria in leucine degradation. The disease is caused by mutations in the AUH gene which results in the 3-methylglutaconyl-CoA hydratase (3-MG-CoA-H) enzyme deficiency. Less than 20 patients have been described so far. The clinical phenotype ranges from normal development to severe global developmental delay with progressive neurologic symptoms.

**Patients and results:** A 12-year-old female was identified with elevated 3-methylglutaconate (174.24; reference range <12.42), 3-hydroxysovalerate (88.7; reference range <37.7) and 3-methylglutarate (11.09; reference range <3.9) in urine organic acid analysis for a selective screening of learning difficulty. Her acylcarnitine profile revealed markedly elevated 3-hydroxy-isovalerylcarnitine (C5OH) (1.95; reference range 0.05–0.4). Her parents were first cousins of Pakistani ethnic background. Her family history was remarkable for an 8-year-old brother with severe expressive language delay. Urine organic acid analysis in this brother showed elevated 3-methylglutaconate (261.4), 3-hydroxysovalerate (242.6) and 3-methylglutarate (6.4) as well. His acylcarnitine profile revealed markedly elevated C5OH (2.4). Urine organic acid analysis of the three other siblings and parents was normal excluding 3-MGA-I. General clinical and neurological examinations in both siblings were unremarkable. She had borderline intellectual disability and ADD in the psychological test. Her cranial MRI showed leukodystrophy in subcortical bilateral frontal and parietal lobes. Her cranial MR-spectroscopy showed elevated 3-hydroxysovalerate. The younger brother had severe articulation disorder, oral-motor weakness and severe expressive language delay in the speech-language assessment. 3-MG-CoA-H enzyme activity was not detectable in the cultured skin fibroblasts confirming the diagnosis of 3-MGA-I which was performed only in the younger brother. Both were started on l-carnitine supplementation therapy and sick day management with increased caloric intake and increased dose of carnitine. We also started with protein modified diet. There was no history of metabolic decompensation or encephalopathy in their past medical history.

**Conclusions:** We presented two siblings with 3-MGA-I with 2 different clinical phenotypes. Despite high consanguinity, heterogeneous phenotype could indicate the contribution of other factors, such as alternative genomic loci containing modifier genes.
74) Tissue differences in oxidation of fatty acids in a mouse model of MCAD deficiency

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Background: To better understand the pathophysiology of medium-chain acyl-CoA dehydrogenase (MCAD) deficiency, we undertook a study of the oxidation of fatty acids in different tissues of the acadm knockout mouse. Humans with MCAD deficiency do not typically have myopathy or cardiomyopathy as part of the syndrome, in contrast to long-chain fatty acid oxidation defects; therefore we investigated these tissues, as well as kidney and liver.

Methods: Mitochondria were isolated from fresh samples of liver, heart, skeletal muscle and kidney from acadm+/+ (wild type) and acadm−/− (mcad deficient) mice from a pure strain on a C57B6 background (N=3 for each group). Respiratory function was measured in aliquots of the isolated mitochondria in a Clark oxygen electrode with a variety of substrates to interrogate the individual respiratory complexes and to measure oxidation of pyruvate and fatty acids. Results are presented as mean, ±SEM; statistical significance was determined using uncorrected student’s t-test.

Results: The yield of mitochondrial protein between acadm+/+ and acadm−/− deficient animals was similar in skeletal muscle, increased in kidney (23.7 vs. 36 mg/g wet wt, p=.08) and decreased in liver (25.7 vs. 19.3 mg/g/wet wt, p=.18), although none of the differences attained statistical significance. Substrates donating electrons to the specific respiratory chain complexes I (glutamate and pyruvate + malate oxidation), II, III and IV demonstrated no significant differences in respiratory chain function between acadm+/+ and acadm−/− in any tissue studied. Fatty acid oxidation was significantly reduced only in liver tissue (kidney and skeletal muscle shown for comparison), with p values <.05 for octanoate, and <.03 for octanoylcarnitine and palmitoylcarnitine (p=.055 for palmitoyl-CoA as substrate).

Conclusions: The striking finding of this study is that fatty acid oxidation is impaired only in the liver mitochondria, in spite of the fact that the enzyme defect is observable in all tissues that oxidize fat. This finding is consistent with the clinical observation that muscle and cardiac symptoms are not typically seen in humans with MCAD deficiency. Species-specific differences in chain-length specificity for the FAO dehydrogenases require care in interpreting these data, though. The fact that absence of mcad is rate limiting for fat oxidation in mouse liver, but not in other tissues, raises important questions about the regulation of FAO in different tissues, which may have important implications for treatment. This merits further study.

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75) Viral infection-induced depression of urea cycle function in a murine model of ornithine transcarbamylase deficiency (OTCD)

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Introduction: Intercurrent illnesses, such as viral infections, play a significant role in precipitating acute hyperammonemnic crises in urea cycle disorders (UCD). Respiratory viral infection in rodents has been shown to partially inhibit early urea cycle enzymes. Indeed, the activities of carbamoyl phosphate synthetase 1 and ornithine transcarbamylase are decreased by 12% and 17% respectively in mice infected with PR8 influenza. Decreases in enzyme activity during intercurrent illness due to viral infection may be detrimental in patients with OTCD. The mechanisms by which enzyme activity is decreased remain to be defined.
Purpose: Studies are focusing on profiling the molecular and biochemical events during viral-induced acute metabolic crisis with hyperammonemia using an animal model of ornithine transcarbamylase deficiency, the sparse fur mouse (spf-ash).

Methods: Spf-ash and control mice were matched for decreasing protein intake over 5 days +/− infection with the PR8 influenza virus. All animals were sacrificed at Day 5 and plasma and livers were collected and snap frozen. OTC enzyme activity was determined ex vivo using spectrophotometric determination of citrulline production. Liver free amino acid levels were analyzed by ion exchange chromatography (Biochrom 30). Urea cycle enzymes in the liver were characterized for transcript using qPCR and protein abundance using proteomic analysis (iTRAQ).

Results: Plasma ammonia elevated at baseline in spf-ash rose with infection, while levels in control animals were not elevated above baseline. Infected spf-ash and littermates showed marked decreases (~50%) in OTCase activity when compared to their uninfected protein matched controls. Decreased expression of CPS1 mRNA was seen in both spf-ash and littermate infected animals. Decreased expression of OTC mRNA was limited to littermates. Interestingly, uninfected protein matched spf-ash mice displayed an increase in CPS1, ASL and ARG1 protein levels. PR8 infection in spf-ash dissipated the CPS1 protein increase and also depressed ASS1 protein levels.

Conclusions: Spf-ash mice become increasingly hyperammonemnic with viral infection. This increase in hyperammonemia occurs partly due to further depression of OTCase activity. This decrease in OTCase activity seen in both animals suggests that depression of urea cycle function during infection may be a normal physiologic process not tolerated by spf-ash. One of the mechanisms by which OTCase activity is depressed is due to decreased OTCase mRNA and thus protein level. More importantly, the metabolic and molecular profiling described (i.e. OTCase enzyme activity, mRNA expression and proteomics) demonstrates that urea cycle function is distinctly different during starvation (protein matched) and infected states, two common precipitants of acute hyperammonemia. Defining the molecular events of viral infection-induced hyperammonemia may provide the basis for therapeutic targets to attenuate this process in UCD.

76) LPIN1 gene mutations: A major cause of severe rhabdomyolysis in early childhood

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Background: Autosomal recessive LPIN1 mutations have been recently described as a novel cause of rhabdomyolysis in a few families. The purpose of the study was to evaluate the prevalence of LPIN1 mutations in patients exhibiting severe episodes of rhabdomyolysis in infancy (58 patients) or later in childhood (20 patients) and adulthood (16 patients), or exercise-induced or permanent muscular pain (26 patients), and to investigate two paralogs, LPIN2 and LPIN3 genes.

Methods: After exclusion of primary fatty acid oxidation disorders, LPIN1, LPIN2 and LPIN3 coding sequences were determined in genomic DNA and/or cDNA.

Results: Two recessively-inherited LPIN1 mutations were found in half of the patients presenting with severe episodes of rhabdomyolysis occurring before the age of 6 years. The intragenic deletion, c.2295-866_2410-30del, was identified in 53% of these LPIN1-mutated patients, all Caucasians, and occurred on the background of a common haplotype, suggesting a founder effect. Other mutations were nonsense or frameshift mutations. Several heterozygous variants in LPIN1, LPIN2 or LPIN3 genes were identified in patients presenting with milder muscular phenotype, but their pathogenic role in the disease has not been demonstrated.

Conclusions: In conclusion, only LPIN1 should be regarded as a major cause of severe myoglobinuria in early childhood, but not in milder phenotype.

77) Acylcarnitines: Rapid, accurate, and precise quantification by HPLC–MS/MS

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We present an HPLC–MS/MS method for the rigorous quantification of acylcarnitines in biological samples. Acylcarnitines were quantitatively isolated by strong cation-exchange solid-phase extraction, derivatized with pentfluorophenacyl trifluoromethanesulfonate, and detected by HPLC–MS/MS. Reference standards were synthesized, purified and standardized, and then used to formulate multiple-point calibration curves. Sequential ion-exchange/reversed-phase chromatography, using a strong cation exchange trapping column in series with a fused core silica reversed-phase column, allowed us to chromatographically separate 65 acylcarnitine species of widely different polarities in a 21 min chromatogram, resolving 20 groups of constitutional isomers and diastereomeric pairs. Scheduled MRM efficiently collected data from 77 transitions, generating 65 13-point calibration curves over 200-fold concentration ranges with excellent precision (as shown by very high correlation coefficients and excellent accuracy (%) for the back-calculated calibration curve points). The entire procedure was validated by studies demonstrating the accuracy, precision, stability, reproducibility and specificity of the procedure.

Biological samples were analyzed to generate reference intervals in patient plasma, urine and skeletal muscle for the 65 acylcarnitine species determined and the examples of patient samples with metabolic diseases illustrate the accurate and precise quantification of marker acylcarnitines. Unlike reported tandem MS methods, this procedure accurately and precisely quantifies malonylcarnitine, due to our use of multiple-point calibration curves generated from standardized malonylcarnitine and d3-malonylcarnitine internal standard. We synthesized three isomers of C14:1 acylcarnitine: myristoleoylcarnitine, cis-5-tetradecenoylcarnitine and trans-2-tetradecenoylcarnitine. These were accurately and precisely quantified by multiple point calibration curves generated using d3-myristoleoylcarnitine as the internal standard. In VLCAD samples, only one C14:1 acylcarnitine species, cis-5-tetradecenoylcarnitine, was increased.

78) Acyl-CoAs: Isolation, detection, and quantification by HPLC–MS/MS

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We developed an HPLC–MS/MS method for the analysis of acyl-CoAs in tissue regardless of the acyl-chain length. Acyl-CoAs were isolated using acetonitrile/2-propanol/potassium phosphate buffer followed by solid-phase extraction with 2-(2-pyridyl)ethyl-functionalized silica gel (Minkler PE, Kerner J, Ingalls ST, Hoppel CL Anal Biochem. 2008, 376, 275–276.). This results in quantitative recoveries of all acyl-CoAs. Samples were chromatographed by sequential ion-exchange/reversed-phase chromatography using an anion exchange trapping column in series with a reversed-phase column. This step allows for on-line trapping of all of the acyl-CoAs prior to separating each individual acyl-CoA species by HPLC and their detection by MS/MS.

Multiple-point calibration curves were generated using reference standards and internal standards, and accurate quantification of multiple acyl-CoA species is shown in several tissue types.

79) Evolution of the effect of arginine on thermal stability and oligomerization of n-acetylglutamate synthase

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N-acetylglutamate synthase (NAGS; E.C.2.3.1.1) catalyzes the formation of N-acetylglutamate (NAG) from acetyl coenzyme A and glutamate. In microorganisms and plants, NAG is the first intermediate of arginine biosynthesis pathway, while in animals, NAG acts as an allosteric activator of carbamylphosphate synthetases I and III. NAGS itself is allosterically regulated by arginine. In bacteria, fungi, and plants, arginine acts as an inhibitor, in fish, a partial inhibitor, but in mammals, arginine is an activator. We used Thermofluor methodology to determine if the effect of arginine on the thermal stability of NAGS parallels its effects on NAGS activity. Addition of arginine to bacterial NAGS, which is inhibited by arginine, resulted in a destabilized protein. Addition of arginine to the zebrafish and mouse NAGS stabilized both proteins, despite opposing effects of arginine on their enzymatic activity. We then used analytical gel chromatography to determine if changes in oligomerization state of NAGS could occur upon arginine binding. Our results indicate that bacterial and mammalian NAGS appear to be ensembles of molecules with different oligomerization states that are in rapid exchange with each other. Upon addition of arginine, the partition coefficient of both NAGS increased. The behavior of zebrafish NAGS was different. It eluted as two peaks suggesting two distinct oligomerization states. Upon addition of arginine to zebrafish NAGS the partition coefficients of both peaks decreased. These studies indicate that the effect of arginine on the biophysical properties of NAGS indeed changed during evolution and suggest that the inversion of the allosteric effect and stabilization effects of arginine on NAGS could be linked.

80) In the time of genomics: Genetic disorders and the mistaken diagnoses of child abuse, neglect, or SIDS

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Background

In all populations genetic disorders masquerade under labels like cerebral palsy, autism, mental retardation, epilepsy, and SIDS. Genetic problems are also mistaken for child abuse with tragic medical and legal consequences.
The Clinic for Special Children provides general medical care for approximately 1700 Amish and Mennonite children with 110 different inherited disorders. Even within our small patient group with well-characterized genetic risks, Child Abuse Teams from regional medical centers in Pennsylvania have mistaken genetic syndromes for shaken baby syndrome, medical neglect, and SIDS.

Methods
Case studies, pathophysiology, and population genomics.

Results
More than 90% of the inherited disorders routinely seen at our Clinic can be diagnosed by history, exam, and by targeted mutation-assays using DNA from blood, tissue, or paraffin blocks. For a few conditions diagnosis can also be made by biochemical analysis of filter paper blood spots, plasma, urine, CSF, or bile by MS/MS, GC/MS, or HPLC.

The failure to recognize genetic disorders often arises from a belief that the genetic mimics of child abuse are rare, that specific genetic tests are not available, and that clinical findings like subdural hemorrhages, retinal hemorrhages, and rib fractures are pathognomonic of non-accidental trauma.

Disorders that mimic abuse usually have understandable pathophysiologic causes. Infants with disordered bile salt circulation are at high risk for late-presenting-vitamin-K deficiency, which often presents with intracranial and intra-ocular hemorrhages — 90% of infants with late-presenting-vitamin-K-responsive bleeding have an underlying liver disease. Three different recessive diseases disrupt bile flow in the Amish population of Lancaster County — mutations in TJP2, BAAT, and ATP8B1. In other populations more common causes of the same clinical problem would be cystic fibrosis, alpha-1-antitrypsin deficiency, and biliary atresia. Poorly understood metabolic disturbances of the brain in patients with GA1 cause vascular and connective tissue pathology with disturbances of CSF production and resorption that make affected infants vulnerable to intracranial and ocular hemorrhages after otherwise minor head injuries. Similar CNS pathology is seen in children with defects of CNS collagens, Menkes disease variants, and chronic hypoxia caused by congenital heart disease. SIDS-like, Reye-like, catastrophic deaths from MCADD, VLCAD, PA, MSUD, and galactosemia are well described. Less well known are catastrophic illnesses and deaths in undiagnosed cases of 3-beta-OH steroid deficiency. SCID-of-cartilage hair hypoplasia, ITCH, RAG1, ADA mutations, properdin deficiency, IL7-receptor deficiencies, and a dominantly expressed mutation APOA4. Unexpected infant deaths in our local populations, caused by seizure–apnea–bradycardia, arise from brainstem and cortical malformations associated with mutations in CASPR2, TSPYL, LYK3, ST3GAL5, HARS1, and C7orf27. Several lethal syndromes found in our populations cause chronic-wasting-disease mistaken for medical neglect — TNNT1-troponin-1 myopathy, Cockayne-ERCC6, CYP11B2, TSPYL1, and Yoder Dystonia. The differential diagnosis of pathologic fractures includes mutations in COL1A2, LEPRE1, LRPS, and neuromuscular diseases like SMA and TNNT1. The degree of osteopenia in these disorders is increased by protein malnutrition and vitamin D, K, and C deficiencies, which are surprisingly common in the Plain populations.

Conclusions
95% of the inherited disorders in our practice have distinctive clinical features and should be readily diagnosed by an informed physician and targeted molecular or biochemical tests. Pediatricians, social workers, and coroners who investigate infant and childhood injuries and deaths too often overlook these conditions.

The medical–genetic problems encountered in the children of the Plain People are not unique to them, as is often thought, but arise from gene mutations that were carried from Europe only 300 years ago. The same mutations continue to be expressed in Europe, North and South America, and elsewhere in the world. The Plain People of North America, >900,000 people living in 200 communities, express a small sampling of more than 10,000 known inherited disorders, many of which cause problems that will be mistaken for child abuse.

In this Time of Genomic Medicine testing to uncover genetic disorders that mimic abuse should become a standard part of the investigation of suspected cases of child abuse, neglect, and unexplained death. Samples for genetic testing should be collected routinely. Overt disregard of the need of genetic testing in this setting is negligent and substandard care. Errors in diagnosis that result in the failure to appropriately manage a genetic disorder, contribute to disability or death, and lead to erroneous conviction of a parent of child abuse is medical malpractice.

81) Long-term follow-up of a patient with early onset CBLG disease
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Background: Cobalamin G (cblG) disease is a rare disorder of cobalamin metabolism characterized by failure to thrive, developmental delay, megaloblastic anemia, hyperhomocysteinemia, hypomethionemia, and homocystinuria. Information on the long-term outcome and the natural history of cblG disease is limited. We report the long-term follow-up of an 8-year old boy with cblG disease and review the medical literature.

Case: This male was born to nonconsanguineous parents at term following an uneventful pregnancy and appeared normal at birth. At 2 months of age he had poor feeding, failure to thrive and macrocytic anemia. Vitamin B12 and folate levels in blood were within the reference range. Plasma amino acids revealed a high free homocysteine level of 137 μmol/L (<14) and low methionine of 4 μmol/L (9–42). Plasma methylmalonic acid level was within the reference range. Diagnosis of cblG disease was confirmed by complementation analysis on cultured fibroblasts. He has been treated with intramuscular (IM) injections of hydroxycoabamin, oral betaine, pyridoxine, and folate or folicic acid. He was diagnosed with cortical visual impairment at 6 months of age and developed nystagmus. At age 5 years he developed nephrotic syndrome and a kidney biopsy showed evidence of thrombotic microangiopathy. His cardiac evaluations have been normal without evidence of pulmonary hypertension. Other medical findings include hypothyroidism, eosinophilic gastroenteritis and persistent hypereosinophilia. He has moderate global developmental delay. He speaks in sentences but has articulation difficulties, receives speech
therapy and is on adaptive education. His plasma homocysteine level has remained elevated at 50–100 μmol/L (<14) with methionine levels of 9–20 μmol/L (7–47).

**Conclusions:** Methionine synthase deficiency (cblG) results in deficient conversion of homocysteine to methionine and subsequent homocystinuria, hyperhomocysteinemia and hypomethioninemia. Megaloblastic anemia is common and often provides the initial clue to the presence of a defect in cobalamin metabolism. The most common clinical findings include failure to thrive, anorexia, feeding problems, developmental delay, seizures, episodes of lethargy, and hypotonia. Rare complications include microangiopathic nephropathy, hemolytic uremic syndrome and pulmonary hypertension, which may be related to thrombotic events due to hyperhomocysteinemia. Megaloblastic anemia and biochemical abnormalities generally respond to therapy with IM hydroxycobalamin. However, other findings such as anorexia, ophthalmological complications, and developmental delay may not be preventable despite therapy adequate to improve biochemical parameters. This patient developed significant multisystem disease, including neurological impairment and nephrotic syndrome with microangiopathic thrombosis despite the early institution of therapy. In addition, nephrotic syndrome should be included in the spectrum of findings associated with cblG disease.

**82) The emerging role of Nurse Practitioners in genetics**

**Abstract:**

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**Introduction:** Nurse Practitioners (NPs) are advanced practice nurses who traditionally practiced in primary care roles, but are increasingly found in specialty disciplines such as genetics. Their presence and scope of practice have been expanding steadily and they have become an important source of care for patients with genetic disorders.

**Genetics NP education:** The education of genetics NPs varies, but all are licensed in their respective fields. NPs typically have Master's degrees and have completed at least two years of clinical training as medical providers in their field (e.g., pediatrics, or family practice). Specialty certification in genetics is available through the International Society of Nurses in Genetics (ISONG), and some university programs offer advanced education. Also, since 2005, genetic competencies have been added to all baccalaureate and masters nursing programs in the United States. NPs often gain expertise in genetics through on-the-job type training and self-study efforts. The formal clinical and didactic training offered commonly to physicians in genetics is not yet widely available to NPs.

**Genetics NP practice:** NPs specializing in genetics work in various settings and carry out a variety of roles outside clinical practice. They work in newborn screening programs, private practices, academic medical centers, enzyme replacement infusion centers, and in a variety of specialty clinics, such as metabolism, neuro-genetics, and prenatal care. Within these settings, NPs also function in non-clinical roles, such as administration, program coordination, teaching, and some may be principal or co-investigators. The clinical scope of practice for genetics NPs varies according to their level of training, collaborative agreements, practice settings, and the population they serve. As direct providers of medical care, NPs work in collaboration with physicians to diagnose and manage a variety of acute and chronic conditions across all age ranges. They prescribe approved and investigational treatments, provide medical clearance for surgery, generate referrals to other specialists, and perform a variety of procedures such as veni-puncture, skin biopsies, and lumbar punctures. Because of their expertise, genetics NPs may also serve as consultants, are contributing members of various professional societies, and publish original research. Lastly, true to the philosophy of nursing practice, NPs look beyond patients’ immediate medical needs, and incorporate psychosocial and family concerns, and teaching, into patient management.

**Conclusions:** The role of NPs in genetics has grown substantially as more NPs enter this specialty. Their scope of practice depends upon a variety of factors and reflects a need for standardization through training and legislation. As the need grows for more providers in genetics, NPs can help meet that demand, and should receive the formal didactic and clinical training necessary to optimize their practice. The field of medical genetics is well-suited for NP practice, whose philosophy of care incorporates the family, the psycho-social implications of illness, comprehensive disease management, and patient education.

**83) Trienol a potential new marker of oxidative stress in Smith–Lemli–Opitz syndrome (SLOS)**

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**Background:** Individuals with Smith–Lemli–Opitz syndrome (SLOS) have a defect in the cholesterol biosynthetic pathway at the step of 7-dehydrocholesterol-reductase (EC 1.3.1.21), which converts 7-dehydrocholesterol (7-DHC) to cholesterol enzyme deficiency results in increased concentration of 7-DHC and low or low-normal concentration of cholesterol in plasma and tissues. The pathogenesis of the disease is not fully understood but it is likely that some of the clinical phenotype is caused by the accumulation of 7DHC and its conversion to oxidized derivatives. Several of these oxidized derivatives including choles-ta-5,7,9(11)-trien-3-ol (tri-enol) have been shown to be cytotoxic in vitro (Korade, 2010).

**Methods:** In this study, we developed an LS-MS/MS assay for tri-enol, and measured tri-enol plasma concentration in a cohort of SLOS patients receiving cholesterol supplementation. Some of the patients were also treated with simvastatin. The analysis was performed using a TSQ
Discovery triple-quadrupole instrument equipped with an APCI source operating in the positive mode. Plasma trienol concentrations were measured in SLOS ($n=14$) and control children ($n=33$).

**Results:** The data show a 400 fold increase in trienol concentration in SLOS patients as compared to controls ($45.1 \pm 39.9 \mu g/ml$ vs. $0.12 \pm 0.1 \mu g/ml$ for SLSO and control respectively; $p<0.001$). In SLOS, trienol concentrations were positively correlated with plasma 7-DHC concentrations ($r=0.74, p<0.001$) and negatively with plasma cholesterol ($r=0.58, p<0.001$). The results further show that simvastatin along with dietary cholesterol supplementation reduces plasma trienol concentrations.

**Conclusions:** We conclude that plasma trienol concentration is a biomarker of the disease and could be used, together with 7DHC to monitor disease progression in longitudinal studies or to evaluate treatment efficacy in future clinical trials.

84) National Institutes of Health phenylketonuria scientific conference

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**Background:** Newborn screening for phenylketonuria (PKU) is a successful public health program that has been in place for almost 40 years. Early identification and initiation of treatment in infants with PKU lead to the prevention of mental retardation in children and adults, and severe signs and symptoms of classic PKU are now rarely seen. Technological advances have made DNA testing available for hyperphenylalaninemia, classic and variant PKU, prenatal diagnosis, and carrier status. The National Institutes of Health (NIH) published screening and management guidelines for PKU as the result of a Consensus Conference (NIH Consensus Development Panel. (2000). Pediatrics. 108(4):972) held in October 2000. New screening technologies and treatment strategies, and a larger body of literature on outcomes for persons with PKU warrant revisiting those guidelines to determine their current applicability.

**Methods:** An NIH Scientific Conference will be held in late 2011. Five working groups, composed of 8–12 topical experts, public members, and federal stakeholders, have been established to answer specific questions related to the aims of the conference. In a parallel and collaborative effort, an Evidence-based Practice Center (EPC) of the Agency for Healthcare Research and Quality (AHRQ) will evaluate the role of a new medication in the treatment of PKU. The results of the EPC evidence review will be made available in November 2011. The PKU Scientific Conference, which will be held after the EPC report has been issued, will consider the state of the science, recent research findings, current treatments, the role of a new medication, tetrahydrobiopterin (BH4), and future research needs. The working groups and the specific questions they are addressing are:

- Diet Control and Management
  - Should the dietary recommendations from the 2000 Consensus Statement be changed?
  - Pharmacologic Interventions
    - What is the role of BH4 in individuals with PKU?
  - PKU and Pregnancy
    - Should the recommendations for pregnancy in women with PKU be altered from the 2000 Consensus Statement?
  - Long Term Cognitive Outcomes and Follow-up
    - What should be the current recommendations for adults with PKU in terms of diet and management (excluding pregnancy)?
  - Molecular Testing, New Technologies, and Epidemiologic Considerations
    - Should there be any changes to the 2000 Consensus Statement regarding newborn screening and molecular testing for PKU?

**Timeline:** The EPC report will be finalized in November 2011. The PKU Scientific Conference working groups will complete their work by November 2011 in preparation for the conference to be held shortly thereafter.

**Expected Impact of the PKU Scientific Conference and EPC Report:** Together, the PKU Scientific Conference and the EPC report will provide evidence on important issues, recommend future research, and provide guidance to patients, their families, and health care professionals.

85) Factors influencing adherence to long term sapropterin therapy

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A recent patient survey examined why patients responsive to sapropterin dihydrochloride (sapropterin, Kuvan®) failed to adhere to long-term therapy. In December 2009, 38 English speaking patients were surveyed to determine factors influencing adherence based on the five dimensions of adherence defined by the World Health Organization (2003): social and economic, health care system, condition related, therapy related, and patient related. Twenty patients (52%) had been on sapropterin therapy for one year (active patients) and 18 (48%) who had discontinued therapy (inactive patients) after nine months of treatment. Mean age for inactive patients was 15.9 years and 61% (11) were male. Mean age for active patients was 17.5 years and 61% (12) were female. Marked differences in dietary adherence, support systems, perception of disease on their life, and use of health care services were seen between the two groups. Only 72% (13) of inactive patients used medical foods and formulas to control their phe levels versus 90% (18) of active patients. Only 5% (1) of active patients versus 28% (5) of inactive patients
reported that PKU was a burden and interfered with their ability to attain their full potential. Active patients had a larger support system including parents, teachers, and clinic staff whereas inactive patients relied primarily on their parents. Active patients also had shorter driving distances to clinics and more regular clinic visits. This data provides insight into factors that influence long-term adherence to sapropterin therapy.

86) Demyelination and axonal degeneration correlates with neurological deficits in biotinidase-deficient, symptomatic mice

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Background: Biotinidase deficiency is a disorder of biotin recycling that, if untreated with biotin, can result in both reversible and irreversible neurological features, such as hypotonia, seizures, ataxia, neuropathy and spastic paraparesis and developmental delay. Biotinidase deficiency is included in many newborn screening programs because biotin treatment can readily prevent the development of symptoms. Universal newborn screening for the disorder is closing the window-of-opportunity to study the natural history of the disorder. We developed a transgenic, null mutation, biotinidase-deficient (BD) mouse to better understand the basis of the neurological features of the disorder.

Methods: Within two weeks of initiating a biotin-deficient diet, these mice develop neurological and cutaneous symptoms similar to those of symptomatic children with profound biotinidase deficiency. Wildtype (WT) and BD mice were fed a biotin-deficient diet for three weeks. At about the 15th day of the diet, all of the BD mice began to exhibit neurological symptoms, such as jitteriness and humped posture, as assessed by abnormal foot fault assay, flexion score, and the clinical neurological deficit score, gross behavioral changes and dermatological changes, whereas all the WT mice remained asymptomatic. On day 21 of the diet, mice from both groups were anesthetized and perfused with paraformaldehyde for brain harvest. Brain slices were stained with Bielschowsky silver and Luxol Fast Blue to examine axons and myelin, respectively. The degree of staining was quantitated in the stratum and corpus callosum regions in a blinded manner and converted to a percentage of the total scanned area.

Results: The degree of demyelination and axonal degeneration correlates with degree of neurological deficits in BD symptomatic mice. When symptomatic BD mice were treated with pharmacological doses for 10 days their neurological, behavioral and dermatological symptoms resolved, whereas they regenerated their axons to normal.

Conclusions: Demyelination may be a major cause of the neurological features of untreated BD. This demyelination is reversible with biotin therapy. The BD mouse appears to be an appropriate animal model to study various aspects of BD.

87) Krabbe disease: Clinical, biochemical and molecular information on six new patients and successful retrospective diagnosis using stored newborn screening cards

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Background: Krabbe disease is a rare, fatal demyelinating disorder resulting from the recessive inheritance of GALC gene mutations and subsequent deficiency of the lysosomal enzyme galactocerebrosidase. Treatment is currently limited to pre-symptomatic hematopoetic stem cell transplantation. In 2006, New York became the first state to implement newborn screening for Krabbe disease. Newborn screening circumvents diagnostic delay, allowing for the possibility of pre-symptomatic treatment and the recognition of at-risk couples who may benefit from reproductive options. As newborn screening diagnosed patients are frequently detected prior to the onset of symptoms, correlations between genotype and phenotype, which may aid in the decision process regarding treatment, are useful.

Purpose: The aim of this report is to describe the clinical, biochemical and molecular characteristics of six clinically diagnosed patients with Krabbe disease and assess the sensitivity of retrospective galactocerebrosidase measurement in newborn dried blood spots.

Methods: The medical records of six patients with Krabbe disease followed by the Division of Metabolic Disorders at CHOC Children’s were reviewed. All patients underwent measurement of galactocerebrosidase activity in leukocytes (Lysosomal Diseases Testing Laboratory, Thomas Jefferson University) and GALC gene mutation analysis (Neurogenetics Laboratory, New York University). Each of the patients’ newborn screening cards (stored for 1.4 to 13.5 years) was sent with 30 age-matched controls to the New York State Newborn Screening Program for retrospective analysis of galactocerebrosidase activity and confirmation of molecular results.

Results: Five patients with Krabbe disease, one of whom also had hydrocephalus, became symptomatic during infancy (median age: 6 months). A sixth patient presented with seizures and developmental regression at two years of age and had a protracted disease course. Galactocerebrosidase activity in leukocytes ranged from 0.00 to 0.20 nmol/h/mg protein. GALC molecular analysis led to the identification of five
previously unreported mutations and two novel sequence variants of unknown significance. Each of the patients was successfully identified as having Krabbe disease by retrospective measurement of galactocerebrosidase activity in newborn dried blood spots (range: 3.17% to 11.09% of the daily mean). None of the control samples were flagged.

**Conclusion:** Our cases represent the clinical variability observed in Krabbe disease and suggest the possibility that hydrocephalus is more common in the disorder than previously reported. While the clinical outcomes associated with a few common GALC mutations are known, our molecular results indicate that the frequency of private mutations in this gene may limit the use of genetic information for making treatment decisions in the newborn period. Finally, measurement of galactocerebrosidase activity in newborn dried blood spots is a highly sensitive test for the diagnosis of Krabbe disease, even when samples have been stored for more than a decade.

**88) Moderate increases of medium chain acylcarnitines: A real MCADD patient or a medication origin?**

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Medium-chain acyl-Co A dehydrogenase deficiency (MCADD) is the most common disorder in fatty acid oxidation defects. The diagnoses used to be made during acute episodes with typical elevations of C6, C8, C10, C10:1 and the related ratios in the acylcarnitine profile and highly elevated excretions of hexanoylglycine and suberylglycine in urine organic acid analysis. However, newborn screened positive babies without clinical symptoms may have normal urine organic acids and moderate elevations of medium chain acylcarnitines, sometimes, indistinguishable from patients taking certain medications. This report compares medium chain acylcarnitine levels from mild MCADD patients with those from a patient who is on tegretol. The levels of medium chain acylcarnitines and the related ratios are in the similar range in both statuses. When the patient was taken off tegretol for one week, the acylcarnitine profile was normalized. However, the mild MCADD patients showed consistent elevations of the medium chain acylcarnitines. Two mutations in the MCAD gene have been identified in these patients, confirming the MCADD disorder. Therefore, moderate elevations of the medium chain acylcarnitine species deserve attention for the workup at the DNA level to identify the real MCADD patients. Differentiation of mild MCADD patients from the patients who are on medications is critical clinically.

**89) Phenotypic variation and evidence for a modifier gene in a mouse model for Sjögren–Larsson syndrome**

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**Background:** Sjögren–Larsson syndrome (SLS) is an inherited neurocutaneous disorder characterized by ichthyosis, mental retardation and spasticity. The disease is caused by mutations in the ALDH3A2 gene encoding fatty aldehyde dehydrogenase (FALDH), an enzyme that catalyzes the oxidation of aliphatic aldehydes derived from metabolism of fatty alcohol and other lipids. To understand the pathogenesis of SLS, we generated FALDH-deficient mice using gene-targeting methods and investigated the effects of genetic background on phenotypic variation.

**Methods:** Using standard transgenic methods, we generated Aldh3a2−/− mice that were homozygous for a mutant allele lacking exon 5 that codes for the catalytic cysteine. FALDH activity was measured using octanecanal as substrate.

**Results:** Aldh3a2−/− mice had no detectable FALDH enzyme activity in liver, but residual aldehyde oxidizing activity was detected in brain (35% of wild-type) and skin (60%), indicating the presence of alternate aldehyde dehydrogenase isozyme(s) in these tissues. The clinical phenotype of the Aldh3a2−/− mice was surprisingly variable on a mixed 129SvJ genetic background with less than one-fourth of the animals exhibiting sparse fur and ichthyosis by 12 months of age. With continued inbreeding, phenotypic abnormalities became less frequent, suggesting the presence of modifier genes. To search for modifier genes that could influence the Aldh3a2−/− phenotype, we bred the mutant gene onto 6 congenic mouse strains (C57BL/6, BALB/C, FVB, 129 SvJ, DBA and Swiss). In striking contrast to all other strains, Aldh3a2−/− mice on a BALB/C background died in utero at 14–18 days of gestation. The lethal phenotype of the Aldh3a2−/− mice on BALB/C background could be rescued by producing F1 hybrid Aldh3a2−/− animals with a mixed BALB/C–C57BL6 genetic background, indicating the presence of a dominant modifier gene(s) in the C57BL6 strain. As an initial step to map the modifier gene(s), we backcrossed the F1 hybrid Aldh3a2−/− mice of mixed strain composition (50% BALB/c + 50% C57BL6) with purebred BALB/c Aldh3a2+/− animals and obtained 129 Aldh3a2+/− mice and 67 Aldh3a2−/− mice, which closely matched the expected 2:1 ratio for a single unlinked dominant modifier gene. Male-to-male transmission of the modifier gene was also seen, indicating that it is not X-linked.

**Conclusions:** This mouse model of SLS provides the first evidence for the existence of an exceptionally strong modifier gene that influences the FALDH-deficient phenotype in mice. Potential modifier candidates include other aldehyde dehydrogenase genes that may account for the enzyme redundancy seen in some tissues from Aldh3a2−/− mice. Identification of the murine modifier gene using positional cloning should provide insight into genetic interactions that contribute to phenotypic variation in SLS and may suggest potential new therapeutic targets for this disease.
90) Genotype–phenotype correlation in primary carnitine deficiency

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Background: Primary carnitine deficiency is an autosomal recessive condition caused by a defect in the OCTN2 carnitine transporter that results in increased losses of carnitine in the urine and decreased carnitine levels in plasma and tissues. It can present early in life with hypoketotic hypoglycemia and hepatic encephalopathy, or with skeletal and cardiac myopathy, usually at a later age. Previous studies have found no correlation between genotype and these two types of presentations or their timing. Recently, asymptomatic mothers with this condition have been identified by expanded newborn screening. They have very low plasma carnitine levels, but it is unclear whether their lack of symptoms can be explained by a milder genotype (with residual activity of the carnitine transporter) or possibly increased activity of other carnitine transporters such as OCTN1. Here we compare functionally and molecularly fibroblasts of patients with primary carnitine deficiency who had symptomatic presentations and mothers identified while asymptomatic.

Methods: Carnitine and ergothioneine (a selective substrate of the OCTN1 transporter) transport were measured in fibroblasts obtained from patients with primary carnitine deficiency and normal controls. DNA sequencing and deletion/duplication analysis were used to identify mutations in the SLC22A5 gene. The missense mutations identified were expressed in CHO cells and their carnitine transport activity was determined.

Results: Carnitine transport was significantly reduced in fibroblasts obtained from all patients with primary carnitine deficiency. However, carnitine transport was significantly higher in fibroblasts obtained from mothers with primary carnitine deficiency as compared to those of symptomatic patients. By contrast, ergothioneine transport (OCTN1 activity) was similar in cells from controls and patients with carnitine deficiency. DNA sequencing indicated an increased frequency of nonsense mutations in symptomatic patients. Expression of the missense mutations in CHO cells indicated that most mutations identified in asymptomatic mothers retained residual carnitine transport activity.

Conclusions: Mutations in the SLC22A5 gene encoding the OCTN2 carnitine transporter cause primary carnitine deficiency. There is no correlation between the type of mutations and time or type of presentation in childhood. However, adult patients with primary carnitine deficiency who remain asymptomatic seem to have at least one missense mutation retaining residual function of the carnitine transporter.

91) Mouse neural stem cells model the Sjögren–Larsson syndrome brain

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Background: Sjögren–Larsson Syndrome (SLS) is a rare disease caused by mutation of ALDH3A2, coding for ER-bound fatty aldehyde dehydrogenase (FALDH). The disease is characterized by severe ichthyosis and mental retardation, and to date there is no cure or treatment. A stable Aldh3a2−/− mouse model was reported, with several SLS phenotypic abnormalities (Lin, 2000). To fully characterize the functional consequences of FALDH deficiency in the SLS brain, we sought to develop a cell model of the SLS brain using NSCs isolated from the Aldh3a2−/− mouse.

Method: Whole brain specimens from Aldh3a2−/− and wild-type mouse neonates (P3) were processed for neurosphere isolation, and adherent NSC cultures were established in poly-α-ornithine coated dishes with serum-free medium supplemented with growth factors.

Results: Mutant NSC growth and replication seemed unaltered. FALDH activity, measured using NSC homogenates was 6713 pmol/min/mg protein in mutant cells vs. 13,767 pmol/min/mg protein in controls (50% reduction), thus confirming enzymatic deficiency. Additional studies showed that the conversion of farnesol, a neurotropic endogenous alcohol with Ca2+ channel blocker activity, into farnesoic acid was reduced by ~60% in mutant NSCs, confirming our previous findings in human SLS fibroblasts. Preliminary experiments with NSCs loaded with fura-2 show preserved glutamate-mediated (Ca2+)1 signaling in mutant cells. Gene expression and differentiation studies are underway.

Conclusion: Aldh3a2−/− NSCs exhibit the typical metabolic phenotype of SLS. The cells should prove useful in the study of the molecular basis of mental retardation in the disease.

92) Defining a semantic web architecture for long term follow up (LTFU) of children positively tested with new born screening (NBS) program

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Background: The development of guidelines for the long term clinical management of children with inherited disorders that are diagnosed through newborn screening (NBS) is hampered by the fact that these disorders are rare and comparatively little evidence-based data exists to
evaluate available treatments. Moreover, the evidence-based long term follow up (LTFU) data that does exist often spans multiple, geographically separated sources (e.g. state labs, primary, secondary, and tertiary hospitals, local practices, group practices and patient support groups), further complicating the development of evidence-based management guidelines. Recently, Semantic Web technologies have been successfully used for the integration of heterogeneous data in the biomedical domain. Particularly, its explicit semantics, the ability to express rich and well-defined models for data aggregation, and using logic to generate new knowledge from the raw data has been quoted as being valuable. Here, we propose to use Semantic Web Technology to address the barriers associated with aggregating LTFU data from disparate sources.

**Methods:** The methods include the development of an ontology for a specific domain Phenylketonuria (PKU) to capture all relevant terms, common data elements by domain experts and proper relationships from available data sources. The possible data sources include paper charts, enterprise Electronic Health Records (EHR), lab databases, billing/claims databases, research data registries, reporting databases, literature reviews and any other relevant documents.

The culled terms will be evaluated for availability using NCBO’s (National Center for Biomedical Ontology) BioPortal (http://bioportal.bioontology.org/). Based on use cases, we will build new application ontology for PKU, incorporating the available ontologies. Protégé 2000 (http://protege.stanford.edu/) will be used as a tool for creating ontologies and knowledge bases. The developed ontology will be evaluated for functionality by testing use cases to answer ad hoc research questions specifically pertaining to PKU.

**Results:** We offer a novel model for developing an application ontology to aggregate data from disparate sources for LTFU.

**Conclusions:** Ultimately, the goal of this project is to develop a model Semantic Web Content Repository populated with aggregate LTFU data for rare disorders identified in newborn screening programs. If successful, the aim would be to apply this same methodology to other inherited metabolic disorders.

93) Altered neural activation in ornithine transcarbamylase deficiency during working memory: An fMRI study

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**Background:** Ornithine transcarbamylase deficiency (OTCD; OMIM #311250) is an X-linked inborn error of metabolism and the most common of the urea cycle disorders. Deficient protein metabolism in OTCD results in episodes of hyperammonemia (HA) with acute elevations of ammonia that confer substantial injury to the brain’s white matter. Additionally, it has been shown that “asymptomatic” OTCD is associated with deficits in an array of cognitive subdomains, despite normal global IQ. These include impaired working memory, executive cognition and reaction speed, and contribute significantly to disability in OTCD.

**Objectives:** To test for differences in BOLD signal activation between OTCD patients and healthy controls during a working memory task.

**Methods:** Nineteen OTCD patients and 21 healthy controls participated in a case–control study at Georgetown University Medical Center. An N-back working memory task was performed in a block design using 3 T functional magnetic resonance imaging.

**Results:** In OTCD patients, we observed increased BOLD signal within the right superior frontal gyrus and decreased signal in the basal ganglia bilaterally, relative to healthy controls.

**Conclusions:** Activation patterns in OTCD patients point to disorganized activation of the working memory network in these patients. Overall, these findings offer preliminary evidence that brain injury conferred by biochemical dysregulation in OTCD may impact the functional neuroanatomy serving working memory processes. OTCD patients show relatively higher DLPFC activity and reduced basal ganglia activity, suggesting a pattern of prefrontal inefficiency and impairment in manipulation and noise-regulation pathways. Further investigation at higher cognitive load is required to further interrogate these neurocognitive differences between OTCD patients and healthy subjects.

94) Improvements in young adult male receiving ERT for Pompe disease

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**Background:** Pompe disease, first described in 1932, is a glycogen storage disorder as well as a lysosomal storage disorder that results in accumulation of glycogen in major organs, particularly the skeletal, cardiac, and smooth muscles, and is represented by a wide spectrum of symptoms with varying age of onset, severity, and rate of progression. The late-onset form, with symptoms presenting in childhood through adulthood, is characterized by proximal muscle weakness, respiratory insufficiency, and no cardiac involvement, which is the cardinal sign of classic or infantile-onset Pompe disease. It is caused by the deficiency of enzyme acid alpha-glucosidase (GAA) and inherited in an autosomal recessive manner.

Here we describe a 15-year-old male diagnosed with Pompe disease at 11 years of age due to elevated urine hexose tetrasaccharide and reduced GAA activity on dried blood spot analysis confirmed via fibroblast analysis in February 2007. He initially presented with fatigue and difficulty breathing secondary to weak diaphragm, scoliosis, and progressive muscle disease of unknown etiology two years prior to diagnosis. He initiated enzyme replacement therapy (ERT) of alglucosidase alfa in May 2007. The infusions are well tolerated for the past three years. One minor setback was experienced following posterior spinal fusion level T4 to sacrum in June 2009. His 6-minute walk test and pulmonary function tests both decreased dramatically, but since have continued an upward trend following recovery.
Results: Continued use of ERT results in observable improvements in 15-year-old male with Pompe disease. His data following ERT demonstrates an increase in pulmonary function (FVC measured pre-ERT 0.72 L to 1.66 L currently), an increase in distance covered in the 6-minute walk test (pre-ERT 1414 ft/6 min to 1830 ft/6 min currently), decrease in his BiPAP IPAP and EPAP settings from 28 to 15 cm H2O and 18 to 6 cm H2O respectively for pre-ERT and currently, an increase in strength by dynamometry. The increase in strength was noted particularly in our patient’s grip strength increasing from 28 lbs pre-ERT to 65 lbs currently. Presently, his improvements following ERT will be paired with a concurrent submaximal aerobic exercise regime and increase in protein ingestion to further slow the progression of the disease.

95) The CPT1A c.1436C–T (p.P479L) variant is common to coastal BC First Nations and is associated with an increased risk of sudden unexpected death

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Background: The carnitine palmitoyltransferase I (CPT1a) c.1436C–T (P479L) variant has been identified at a high frequency in a number of North American Aboriginal groups including the Canadian Inuit, coastal Alaska Natives, and some British Columbia First Nations. Although the variant has been shown to lead to a partial reduction in CPT1 activity and altered fatty acid oxidation flux in fibroblasts and in vitro expression studies, the clinical significance of the variant remains unclear given its very high frequency in these populations.

Methods: Approval for this work was granted through consultation with BC First Nations and Governmental stakeholders and data stewards. All newborn screening cards for BC First Nations’ infants born in 2004 (2331 samples) were identified by linked vital statistics data (including a partial postal code), genotyped for the P479L variant, and linked to the acylcarnitine data produced during routine newborn screening. An identical linkage was also performed for all First Nations sudden death cases (<2 years of age) in BC from 1999 to 2009. Statistically significant regions of high and low P479L homozygosity across the province were established using the SaTSCAN geographical clustering software. Odds ratios for the association of sudden death with P479L homozygosity were then calculated within these geographical clusters. Acylcarnitine test performance was established using ROC analysis.

Results: Province-wide, 9.8% of the First Nation’s births in 2004 were homozygous for the P479L variant. Homozygosity ranged from as high as 25% on southern Vancouver Island to as low as 4.3% in the central interior. Of 48 sudden unexpected death cases in First Nations infants <2 years of age between 1999 and 2009, 40% were homozygous for the P479L variant. Odds ratios for the association of P479L homozygosity with sudden unexpected death were calculated within clusters of uniform underlying genotype frequency. For the 3 overlapping clusters of high P479L frequency covering coastal BC, a significant odds ratio of 3.32 (1.42–7.74, p = 0.005) was calculated, leading to a population attributable risk of 30.4%. Acylcarnitine profiling from the 2004 birth year, stratified by P479L genotype, revealed that the C0/C16 + C18 ratio is an effective measure for prediction of P479L homozygosity with a specificity of 94% and sensitivity of 95% for a cutoff of 20, when measured in blood spots collected <4 weeks of age.

Conclusions: The P479L variant is common to coastal BC First Nations, mirroring the regions with historically elevated rates of sudden infantile death. Homozygosity for the variant leads to a predictable acylcarnitine profile and is associated with a small but significant increased risk of sudden unexpected death. Sudden unexpected death represents an extreme clinical presentation and further study is required to more clearly define the spectrum of clinical phenotypes associated with P479L homozygosity. With carefully established screening cutoffs and a molecular second-tier assay, newborn screening for P479L homozygosity is technically feasible. However, given the small proportional risk of death and corresponding high allele frequency, the best approach to infant mortality reduction must be carefully considered. With respect to newborn screening, the potential health benefits and harms, and effective health care delivery models will need to be fully explored in partnership with the affected communities.

96) Confirmation of newborn screening results for very-long-chain-acyl-CoA dehydrogenase (VLCAD) deficiency by in vitro analysis of palmitic acid oxidation in skin fibroblasts: The Mayo Clinic experience

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Background: VLCAD deficiency is a primary newborn screening (NBS) target due to its high mortality and morbidity when untreated. Confirmation of VLCAD deficiency is possible by plasma acylcarnitine and molecular analyses following an abnormal NBS result. However, VLCAD deficient individuals can have intermittently normal plasma acylcarnitine profiles and ACADVL molecular analysis may not uncover two pathogenic mutations. In the context of a suggestive NBS result, ambiguous plasma acylcarnitine and/or molecular results pose a diagnostic dilemma. Under these circumstances it is recommended to perform in vitro analysis of fatty acid oxidation (FAO) in fibroblast cultures. To evaluate the role of the FAO in vitro probe assay as a conclusive test for confirming VLCAD deficiency, we correlated ACADVL mutation analyses of known patients and carriers with results of FAO in vitro probe assays performed in our laboratory since 2002 to determine precise VLCAD disease ranges. We then reviewed our laboratory’s experience with the FAO in vitro probe assay as a diagnostic test of asymptomatic patients with NBS results suggestive of VLCAD deficiency.

Methods: Mitochondrial fatty acid oxidation in skin fibroblasts was assessed by palmitic acid oxidation in the presence of l-carnitine. The absence of fully functional VLCAD leads to the accumulation of dodecanoyl- (C12) and tetradecanoylcarnitine (C14). ACADVL genotypes were correlated with palmitic acid oxidation rates in skin fibroblasts to determine reference ranges for C12 and C14 levels in fibroblasts from affected
individuals (two pathogenic mutations, n = 23), carriers (one pathogenic mutation, n = 10) and unaffected individuals (normal controls, n = 108). Molecular analysis of 90 fibroblast specimens (33 affected and carrier controls and 57 samples with possible VLCAD deficiency) was performed by direct sequencing all 20 ACADVL exons, and correlated with FAO in vitro probe results.

Results: We found that an elevated C12 and/or C14 greater than 0.04 μmol/g protein was consistent with VLCAD deficiency. Among our retrospective cohort of 57 putative VLCAD deficient patients, 78% were confirmed to have VLCAD deficiency (45/57: 2 with no mutations, 11 heterozygotes, and 32 with 2 alterations) and 21% were negative for VLCAD deficiency (8 homozygotes, 4 with 2 alterations). Mutation analysis of the testing cohort revealed mostly private mutations (6 novel pathogenic mutations: 3 nonsense, 2 splice site, and 1 frameshift and 50 novel variants of unknown significance); however, c.848T>C accounted for 20% of pathogenic alleles in our study with a biochemical phenotype that ranged from mildly abnormal to severe. Interestingly, 20 confirmed cases of VLCAD deficiency had plasma acylcarnitine results that were either consistently normal (10/20), consistently abnormal (6/20), or intermittently abnormal (4/20) in an apparently age-independent fashion.

Conclusions: VLCAD deficiency cannot be excluded by molecular analyses alone and more than half of our retrospective cohort with functional VLCAD deficiency had at least intermittently normal plasma acylcarnitine profiles. In these situations, FAO in vitro probe analysis is instrumental to clarify an abnormal NBS result. These findings support the inclusion of the FAO in vitro probe assay in the ACMG recommendations for follow up of abnormal NBS results for VLCAD deficiency (http://www.acmg.net).

97) Novel missense mutation M185V in exon 7 of the TAZ (G4.5) gene in a patient with atypical Barth syndrome

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Barth Syndrome (BTHS) is an X-linked inherited disorder characterized clinically by cardiac and skeletal myopathy, short stature and neutropenia. While such features generally vary in presentation and severity, they often prove fatal during childhood. Multiple genetic mutations in the TAZ gene, located on Xq28, have been identified in association with BTHS, and we describe a novel missense mutation 553A→G (M185V) in the TAZ gene through bidirectional sequencing of a 4-month-old proband of Irish/German descent. He first presented with respiratory distress, neutropenia, and dilated cardiomyopathy with reduced ejection fraction of 10% by echocardiogram. 3-Methylglutaconic aciduria was not detected from 81 ethnically-matched control subjects (47 males and 34 females). The identification of TAZ gene mutations is important for the diagnosis and genetic counseling in this family with atypical Barth syndrome that is not associated with 3-methylglutaconic aciduria.

98) Measurement of lysophosphatidylcholine and plasmalogens species in cultured cells for rapid diagnosis of patients with peroxisomal disorders

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Background: Peroxisomal disorders can be divided into two main groups, peroxisome biogenesis disorders and single protein defects. The majority of these disorders have defects in fatty acid metabolism and/or plasmalogen synthesis. Measurements of plasma very long chain fatty acids (VLCFA) and erythrocyte plasmalogens are established tests used to diagnose patients with clinical evidence of these disorders. Cultured skin fibroblasts may be used for further biochemical characterization to distinguish Zellweger spectrum disorders (ZSD) and rhizomelic chondrodysplasia (RCDP, type 1, PEX7 defect) from respective single protein defects that cannot always be distinguished by clinical evaluation and body fluid biochemical analysis. Plasma VLCFA are elevated in patients with X-linked adrenoleukodystrophy (X-ALD), the most common peroxisomal disorder. In addition, cultured CVS or amniocytes are often used for prenatal biochemical analysis.

Our laboratory has recently described the measurement of C26:0-lyso-phosphatidylcholine (LPC) in blood spots by liquid chromatography–tandem mass spectrometry (LC–MS/MS). C26:0-LPC is increased in X-ALD and ZSD blood spots as compared to controls. Furthermore, phosphatidylethanolamine (PE) plasmalogens species are reduced in blood from ZSD and RCDP patients.

In cultured cells currently we measure the amounts of total lipid VLCFA by gas chromatography and the activity of plasmalogen synthesis using a radiolabeled assay. However, we wanted to explore the feasibility of measuring specific complex lipid species by LC–MS/MS with the aim of establishing a method that is more comprehensive, requires less sample amount and more rapid. We report here measurement of C26:0-LPC and several PE plasmalogen species in cell lines derived from normal control, X-ALD, ZSD, and RCDP patients.

Methods: A 10 μl aliquot of cell suspension (20–80 μg protein) was extracted with chloroform:methanol containing 1 ng of deuterated C26:0-LPC internal standard. The extract was processed and analyzed by LC–MS/MS similar to the description in Hubbard et al. (Mol Genet Metab 2009; 97:212–220). In addition to C26:0-LPC, four PE plasmalogen species were measured: 16:0p/20:4; 16:0p/18:1; 18:1p/20:4; and, 18:0p/20:4.
Results: Cultured fibroblasts from ZSD patients (*n* = 23) had about a 17-fold increase in C26:0-LPC compared to normal controls (*n* = 19) and a decrease to 23–32% of normal control levels for the PE plasmalogen species. In contrast, fibroblasts derived from males with X-ALD (*n* = 11) had a 5.6-fold increase of C26:0-LPC. RCDP fibroblasts (*n* = 4) had a decrease of PE plasmalogen to 13–20% of normal control levels. These results were comparable to those obtained measuring total lipid VLCFA and PE plasmalogen levels using about 10 times the cellular protein. Cultured CVS yielded similar results when comparing ZSD (*n* = 9) to normal controls (*n* = 14). Similar trends are observed when CVS or amniocyte cultures from normal control, ZSD, RCDP and X-ALD patients are compared.

Conclusions: Our initial studies indicate that measurements of C26:0-LPC and four PE plasmalogen species in cultured fibroblasts, CVS or amniocytes are suitable for the diagnosis of patients with Zellweger spectrum disorder, rhizomelic chondrodysplasia punctata and X-linked adrenoleukodystrophy. This LC–MS/MS method provides a rapid approach to prenatal and postnatal diagnosis of the most common peroxisomal disorders due to the reduced sample requirements, simplicity of sample preparation and fast metabolite analysis.

99) Correlation of plasma and breast milk carnitine levels and the effect of carnitine supplementation in a lactating woman with carnitine uptake deficiency (CUD)

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Introduction: CUD is a disorder of fatty acid oxidation (FAO) with biochemical features including fasting hypoketotic hypoglycemia, decreased plasma carnitine and increased creatine kinase. Patients may present with hypotonia, dilated cardiomyopathy, skeletal myopathy or sudden death. While patients typically present in childhood, the expansion of newborn screening (NBS) to include FAO disorders has led to the identification of previously undiagnosed women with CUD following the birth of an infant with an NBS result indicative of carnitine deficiency. The differential diagnosis includes CUD in the newborn, maternal CUD, and nutritional carnitine deficiency of the mother. Follow up by plasma carnitine determination in both the infant and mother is recommended. Carnitine levels in infants with CUD slowly normalize with carnitine supplementation; whereas, those infants exhibiting the deficiency as a result of a maternal primary or secondary carnitine deficiency respond immediately to supplementation.

Objectives: To determine whether there is a correlation between plasma and human breast milk carnitine levels; and to determine whether there is a sufficient level of carnitine in the breast milk of a woman with CUD to provide adequate carnitine for her breastfed infant.

Methods: Carnitine levels were analyzed pre- and post- oral carnitine supplementation in the plasma and breast milk of a lactating woman with presumed CUD and her breast-fed infant. In addition, the baby's plasma carnitine level was determined after carnitine supplementation was discontinued.

Results: Our patient had an NBS specimen collected at 32 h of life that was indicative of carnitine deficiency (free carnitine: 5.7 nmol/mL; normal >9.0). On follow up, his total plasma carnitine level was low (10 nmol/mL; controls 17–41), and his mother was found to have carnitine deficiency (7 nmol/mL; adult controls 34–78). Carnitine supplementation (50 mg/kg/day) was initiated for the baby on day of life 5 leading to an increased carnitine concentration of 84 nmol/mL by day of life 11. Carnitine supplementation was then discontinued. On day of life 35, our patient’s plasma total carnitine result was at the low end normal (27 nmol/mL), but the maternal level was still low (18 nmol/mL), despite the initiation of maternal oral carnitine supplementation (500 mg, 3 times daily) when the baby was 16 days of age. Measurements of total carnitine in the mother’s breast milk showed low carnitine levels both before (21 nmol/mL compared to 31 and 43 in two control specimens) and 18 days after treatment was started (21 nmol/mL).

Conclusions: Our data suggest that there is a correlation between plasma and breast milk carnitine levels, with low maternal plasma carnitine levels reflective of similarly low levels in breast milk. Carnitine supplementation should be considered in breastfed infants of women confirmed to have low plasma carnitine levels until maternal levels are normalized.

100) Hypodontia of mandibular permanent canines and long with curved tooth roots are associated with mucopolysaccharidosis type VI

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Introduction: Mucopolysaccharidosis VI (MPS VI) is a very rare autosomal recessive lysosomal storage disease caused by deficiency of the enzyme Arylsulfatase B. Lacking or decreasing enzyme activity leads to accumulation of dermatan sulfate. Phenotype is highly variable and involves multigorgan systems. Oral manifestations have been reported including thick lips, high-arched palate, enlarged alveolar process, gingival hyperplasia, opened mouth and enlarged tongue. The previously reported dental anomalies consist of delayed tooth eruption, enamel hypoplasia, malocclusion, multiple dentigerous cysts, and malformed teeth. Here, we report a Thai MPS VI with new dental findings.

Case report: A 17 yr-old girl was referred to a genetics clinic due to coarse facies and short stature. She also had symptoms of left sided heart failure. She is a freshman and can live independently. Her medical problems were first brought to medical attention when she was 9 yr-old. From the medical record at that time, she had short stature, joint stiffness, and cloudy cornea. The time line of each problem was unclear and she had not received any diagnosis or treatment. Family history was significant with a 9 yr-old brother who also had similar clinical phenotypes but with
less stiffness of joints and no heart problems. Both parents were healthy and denied consanguinity. Physical examination showed body weight 20 kg, height 102 cm, macrocephaly, coarse facies and cloudy cornea. Mitral stenosis, aortic stenosis murmurs were detected. She had umbilical hernia, hepatosplenomegaly, limitation of spine-joints movement and claw hands.

Skeletal survey demonstrated dysostosis multiplex. Echocardiography showed severe mitral valve stenosis and mild aortic stenosis. Enzyme analysis indicated Arylsulfatase B deficiency confirming diagnosis with MPS VI.

Oral manifestations consist of congenital missing of the mandibular permanent canines, maxillary constriction, posterior crossbite, anterior openbite, long and curved tooth roots, and widening of alveolar process. Hypodontia and long tooth roots have never been described in patients with MPS VI.

**Conclusion:** This is the first time that hypodontia and long tooth roots are reported in a patient with MPSVI. It is noteworthy that mutation of Arylsulfatase B which led accumulation of dermatan sulfate subsequently caused hypodontia and long tooth root in this patient. A couple immunohistochemistry studies in animal dentinogenesis demonstrated dermatan sulfate and other proteoglycans play important roles in development of mineralization and thickness of teeth. These findings have raised the question of the role of dermatan sulfate in tooth development.

### 101) Short-term follow up for out-of-range MS/MS newborn screening results

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**Background:** Over the last decade, the number of disorders added to state newborn screening (NBS) panels has greatly increased due to the use of tandem mass spectrometry (MS/MS). Little has been documented about the similarities and differences in short-term follow-up procedures employed either within a particular program or among different programs. The contact during the short-term follow-up process marks the first communication between the NBS program and the primary care physician (PCP) or specialist. This communication likely has implications for parents’ future understanding about their child’s NBS result.

**Objective:** To describe the procedure for notification and follow-up across NBS programs for out-of-range MS/MS NBS results.

**Methods:** A written survey was designed and validated. After being piloted, it was modified and mailed to NBS short-term follow up coordinators as identified by [http://genes-r-us.uthscsa.edu/State_contacts](http://genes-r-us.uthscsa.edu/State_contacts). The survey was redirected when the identified individual was not the appropriate contact. Respondents were asked to provide the following information about the notification process for an out-of-range NBS result: who were the individuals notified, how where they notified, and within what time frame. Also, if respondents followed a different notification protocol dependent on the analyte or the degree to which the analyte was out-of-range, they were asked to provide more information. These questions were further broken down into analyte/disease categories and borderline versus strong positive results. Respondents were also asked to report to what extent, if any, parents were notified as part of their short-term follow-up protocol.

**Results:** Surveys were returned for 39 out of 50 state NBS programs (response rate = 78%). Only two state programs had the same notification procedure regardless of identity or magnitude of the out-of-range analyte. As part of their “typical” follow-up notification process, most programs phone (71%) or fax (77%) a physician. 60% send a hard copy report to the physician. 27% recommend evaluation and additional testing of the procedure regardless of identity or magnitude of the out-of-range analyte. As part of their short-term follow-up protocol.

**Conclusions:** These results highlight the differences among state NBS programs’ short term follow up protocols. Further studies should be undertaken to determine the effect of current protocols on infant outcomes, as well as on parent and physician satisfaction.

### 102) Monitoring urinary glucose tetrasaccharide biomarker in patients with infantile and late-onset Pompe disease identified through newborn screening

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**Background:** Newborn screening for Pompe disease in Taiwan has facilitated early treatment of patients by enzyme replacement therapy (ERT) with recombinant human acid alpha glucosidase. The urinary glucose tetrasaccharide, Glcα1–6Glcα1–4Glcα1–4Glc (Glc4) is a biomarker of glycogen accumulation used to monitor the therapeutic response in Pompe disease. In infantile-onset Pompe disease a decrease in Glc4 correlated with a decrease in muscle glycogen and a good motor function response to ERT. We compared Glc4 levels in patients with infantile and late-onset Pompe disease identified by newborn screening, and monitored its trends in patients treated at or before one month of age.

**Methods:** Seven patients with infantile-onset Pompe disease (NBS-IOPD) and 14 patients with late-onset Pompe disease (NBS-LOPD) were identified through newborn screening. For comparison, 6 patients with infantile Pompe disease diagnosed clinically after the onset of symptoms...
(clinical-IOPD group) and subsequently treated with ERT were also studied. Urine on filter paper was collected at regular intervals from consented patients and Glc4 was determined using stable isotope dilution-tandem mass spectrometry.

**Results:** Pretreatment urinary Glc4 levels were close to the upper limit of control or were elevated in NBS-IOPD patients (mean ± SD: 22 ± 6.0; range: 16–32 mmol/mol creatinine (CN), n = 7) compared with age-matched controls (<19 mmol/mol CN). These patients were aged 0.3 to 1.2 months at the time of diagnosis and were started on ERT soon after. All had cardiomyopathy, but no clinical evidence of muscle weakness. In comparison, the clinical-IOPD patients diagnosed between 2.0 and 5.7 months of age, had significantly higher baseline urinary Glc4 levels (mean ± SD: 41 ± 13; range: 24–65 mmol/mol CN, n = 6; p < 0.05). NBS-IOPD patients showed a decrease in Glc4 into the normal range within 1 month of treatment and levels remained significantly lower over the course of treatment for this group compared with the clinical-IOPD group. After one year of ERT, Glc4 was 10 ± 1.9 (n = 5) for the NBS-IOPD group compared with 37 ± 21 (n = 5) for the clinical-IOPD group (p < 0.05). Baseline Glc4 was significantly lower (p < 0.05) for the late-onset compared with the NBS-IOPD group and was within normal limits (mean ± SD: 8.7 ± 4.5; range: 2.8 to 18 mmol/mol CN, n = 14). Four NBS-IOPD patients were started on ERT at 1.5 to 36 months of age. Two of these patients had modest elevations of Glc4 prior to the start of treatment, whereas the other two patients did not. Four untreated patients from this group were monitored for up to two years and Glc4 values remained within normal limits (range: 2.6 to 6.3 mmol/mol CN).

**Discussion:** The current findings indicate that Glc4 levels are helpful in distinguishing IOPD from LOPD in newborns identified through NBS. Additionally, the correlation of Glc4 with the improved outcomes for patients with IOPD treated at or before one month of age, supports a role for Glc4 in monitoring patients identified through newborn screening.

**103) Medication Therapy Management, a new clinical practice for inherited metabolic disorders**

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**Background:** Medication Therapy Management (MTM) is a formal clinical practice that uses a systematic process to identify, resolve and prevent drug therapy problems (DTP). In recent years, MTM has been provided in an increasing number of general practice settings, and has consistently demonstrated improved clinical outcomes and reduced overall healthcare costs in common chronic disease states. Although MTM is globally acknowledged as a valued clinical service in general practice settings, its usefulness in rare diseases has not been explored. The group of orphan diseases known as inherited metabolic disorders (IMD) includes conditions in individual patients commonly require care from a variety of specialty providers and treatment with numerous medications, including orphan drugs, and thereby provides a useful model for studying the value of MTM services in rare diseases.

**Objectives/methods:** We designed a study to explore the usefulness of MTM towards improving clinical outcomes in IMD and initiated an MTM practice for our patients with IMD at the University of Minnesota Pediatric Specialty Clinic, with the initial phase evaluating MTM services in lysosomal diseases (LD) and phenylketonuria (PKU). Outcomes measured included: 1) number and type of DTP identified; 2) number and type of DTP resolved; and 3) patient/caregiver satisfaction with the MTM service (documented through patient/caregiver satisfaction survey).

**Results:** Interim 1-year results of MTM provided for 56 LD patients and 70 PKU patients include identified were: 1) preventative medications needs; and 2) compliance difficulties. Additionally, MTM services provided a systematic method for evaluating, characterizing and improving management of disease specific DTPs, including infusion reactions to enzyme replacement therapy for LD and neuropathic pain management in Fabry patients. Patient satisfaction surveys were overwhelmingly in favor of MTM services.

**Conclusions:** The provision of MTM for IMD is promising as a care model for improving clinical outcomes and disease management for IMD and other rare diseases. Moreover, with increasing legislation to identify LD, as well as other IMD and other rare diseases by newborn screening, the role of MTM in improving and standardizing early and long-term treatment protocols for these rare diseases warrants further study.

**104) Progression and treatment of renal disease in methylmalonic acidemia**

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**Background:** Methylmalonic aciduria (MMA) is a severe inborn of the catabolism of branched-chain amino acids.

**Objective:** To better delineate the natural history with respect to renal outcome of patients with MMA.

**Patients and methods:** Thirty patients with vitamin-B12-unresponsive MMA were managed following standardized guidelines and studied retrospectively with a median follow-up of 8.3 years. All patients were investigated with inulin clearance, biochemical and genetic studies.

**Results:** Fifteen patients had a neonatal onset. Chronic renal disease (CRD) occurred in 14 patients (47%) with a median age of onset of 6.5 years. Renal function further deteriorated in 4 patients within a median period of 5.8 years. Of 25 patients, 17 were classified mut−, 3 mut− and 5 cblA. Mortality, number of acute decompensations, median MMA urinary excretion and neurological impairment were higher in mut− patients compared to mut−/cblA patients. Concerning the CRD, no difference incidence was found although the onset of CRD occurred earlier in mut− patients and was more severe. Four patients underwent successful renal transplantation leading to a correction of the renal function and/or improvement of their metabolic status. One patient died from hepatocarcinoma 2 years after transplantation; all other patients are alive and well.
Conclusions: Patients with mut° phenotype have a more severe phenotype and probably an earlier and more severe CRD than patients with mut−/cBHα phenotype. Renal transplantation is an interesting treatment option for MMA patients with CRD and contributes both to optimal MMA clearance and metabolic stability.

105) Pharmacological chaperones improve the pharmaceutical and pharmacological properties of ERT
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Lysosomal storage disorders (LSDs) are caused by mutations in the genes that encode lysosomal enzymes; to date over 40 different LSDs have been described. The expressed mutant enzymes in these diseases often have reduced stability and trafficking, which leads to substrate accumulation, cellular dysfunction, and disease. Regular infusion of a recombinant form of the deficient enzyme, termed enzyme replacement therapy (ERT), is the primary treatment for several LSDs, including Fabry, Gaucher, and Pompe diseases. However, these recombinant enzymes often have low physical stability (especially at neutral pH and body temperature), short circulating half-lives, variable uptake into different tissues, and can be immunogenic, thus limiting efficacy and tolerability. In contrast, pharmacological chaperones (PCs) are small molecules designed to selectively bind their target endogenous mutant enzymes in patient cells, resulting in increased stability, lysosomal levels, and enzyme activity. We hypothesized that PCs might also stabilize various ERTs to improve their pharmaceutical and/or pharmacological properties. To this end, the orally-available iminosugar PC AT1001 (1-deoxygalactonojirimycin; migalastat hydrochloride) increased the physical stability and minimized denaturation of recombinant human α-galactosidase A (rh-Gal A), the ERT that is used to treat Fabry disease. In Fabry patient-derived fibroblasts, co-incubation of rh-Gal A with AT1001 led to greater cellular levels and activity of the enzyme, as well as larger reductions in the substrate globotriaosylceramide (GL-3) when compared to incubation with enzyme alone. In rats, co-administration with AT1001 increased the circulating half-life of rh-Gal A. Interestingly, in mice that lack endogenous α-Gal A, co-administration with AT1001 increased tissue levels of the exogenous enzyme, and most importantly, reduced tissue GL-3 levels to a greater extent than that seen following administration of rh-Gal A alone. Similar effects on plasma half-life, enzyme uptake, and substrate reduction were observed in mice that lack endogenous acid α-glucosidase (GAA), the enzyme that is deficient in Pompe disease, when co-administered with the PC AT2220 (1-deoxynojirimycin) and the Pompe disease ERT, rhGAA. Lastly, in cell-based assays, co-incubation of the PC AT2101 (isosagomine) and recombinant human β-glucocerebrosidase (rhGCase), the enzyme deficient in Gaucher disease, also resulted in greater uptake of functional enzyme alone. These results indicate that PCs can improve the stability, pharmacokinetic properties, and cellular/tissue uptake of various ERTs, thereby leading to greater substrate reduction both in vitro and in vivo.

106) Evidence-based diagnostic protocol to identify treatable metabolic diseases causing intellectual disability
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Background: Intellectual disability (ID) is a debilitating condition with deficits in cognitive functioning (IQ<70) and adaptive skills, and affects 2.5% of children and adults worldwide. Current management recommendations are based on frequencies of single conditions that lead to ID and on the yield of diagnostic methods and procedures. Therefore cytogenetic techniques, such as karyotyping and array-CGH which yield a causal diagnosis in 20% of ID cases, are considered high priority. Inborn errors of metabolism (IEMs) constitute a subgroup of genetic ID, and causal therapy has become available for many of these. We performed a systematic literature review to identify all treatable IEMs with ID as a major feature, and characterized the specific therapies and evidence for effect on ID and related outcomes.

Methods: All steps in the conduct of this systematic review were performed by two independent reviewers according to Cochrane Collaboration guidelines: formulation of PICO and definitions; literature search in Pubmed (1960–2010) and (online) text books; selection of relevant articles; assessment of evidence (www.cebm.com) for treatment effect.

Results: We identified 72 ‘treatable IEMs’ presenting with ID as a major feature. Of all disorders, 52% (n = 38/72) are potentially identified by routinely available group tests (ammonia, lactate, plasma amino-acids, total homocysteine, acylcarnitine profile; urine organic acids, GAGs, oligosaccharides, purines, pyrimidines, and creatine metabolites). For the remaining disorders a ‘single test for single disease approach’ is necessary. Additional neurologic and systemic manifestations should be evaluated carefully as these may provide essential clues to direct the diagnostic investigations. Reliable biomarkers are detectable by specific biochemical tests for 27 conditions. Direct gene analysis is the most practical diagnostic approach for the remaining 13 IEMs. A pilot study applying high-throughput sequencing for simultaneous screening of these genes in large numbers of DNA samples is currently underway. Therapeutic modalities with proven effect include diet, co-factor/vitamin supplements, substrate inhibition, enzyme replacement, and stem cell transplant. In intervention studies the effect on outcome (defined as effect on IQ, developmental test scores, behaviour, epilepsy, and neuro-imaging) varied from improvement to halting or slowing neurocognitive regression. The levels of evidence for treatment effects range from level 1b,c (n = 4; RCT, ‘all or none’ case series), and level 2a,b,c (n = 13; systematic review of cohort studies; cohort studies, outcomes research) to level 4 (n = 55; case series) and level 5 (n = 5; based on physiology and expert opinion). Finally, 65% of treatments with evidence level 4 or less (which can be very effective), are internationally considered as best clinical practice or ‘Standard of Care’. This number reflects the predicament for rare diseases, with limited patient numbers impeding well-powered RCTs and meta-analyses. International collaborations and patient registries are key in turning the tide.

Conclusion: This approach revisits current paradigms for the diagnostic evaluation of ID. It implies treatability as the premise in the etiologic work-up and applies evidence-based medicine to rare diseases. This knowledge has been translated into a step-wise protocol to effectively identify treatable causes of ID. We will present this state-of-the-art protocol with our web-based applications (App for iPad and website), which will enable instant use in clinical practice.
107) Expanding the clinical phenotype of the mitochondrial M.13513G-A mutation with the first report of a fatal neonatal presentation

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Background and aim: Diagnosis of mitochondrial disease is often a challenge because of the extreme heterogeneity of the clinical phenotype and the variety of underlying gene defects. Insight into the range of clinical phenotypes associated with a particular mitochondrial DNA mutation will facilitate better recognition of patients at risk by focused gene testing.

Case description: We present a family affected by the mitochondrial m.13513G-A (p.D393N, ND5) mutation, illustrating a previously unreported degree of clinical heterogeneity, varying from “MELAS” (Mitochondrial Encephalopathy with Lactic Acidosis and Stroke-like-episodes) in a 10-year old girl, to a fatal neonatal course on the first day of life with metabolic acidosis and hypotonia in a younger sister, to absence of medical problems in the mother. The diagnosis was established in the deceased neonate many years later, based on targeted mutation testing in paraffin embedded tissues. The mutation loads span 66% in the deceased neonate to 30% in the girl with MELAS and 7% in the asymptomatic mother, which correlated with severity of the clinical phenotype.

Conclusions: This is the first report of fatal neonatal acidosis associated with the m.13513G-A mutation, and demonstration of correlation between degree of mutation load and severity of clinical phenotypes within 1 family. The importance of pro-active collection and storage of appropriate samples during the diagnostic work-up of an acutely-ill or deceased neonate, permitting subsequent mitochondrial investigations, is hereby illustrated, along with consideration of mtDNA analysis. Based on the literature and expertise, we will present suggestions for the metabolic autopsy.

108) The adult galactosemic phenotype

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Background: Classic galactosemia is an autosomal recessive disorder due to galactose-1-phosphate uridyltransferase (GALT) deficiency. Newborn screening and early treatment do not completely prevent tremor, speech deficits and diminished IQ in either sexes or premature ovarian insufficiency (POI) in females. Improved treatments for galactosemia depend on a precise delineation of how individuals with galactosemia fare as adults and whether this disorder progresses throughout the adult years.

Methods: We examined health and well-being in adults with classic galactosemia. Examinations were conducted over one weekend by one team of investigators. Subjects (n = 33) were recruited primarily by the national association of Parents of Galactosemic Children. All subjects received the following evaluations: structured physical examination, endocrine assessments, GALT enzyme assay in erythrocytes and GALT gene sequencing, fertility assessment, bone density measurement, psychological assessment, speech/language evaluation, and nutritional history. Eight males were evaluated for sperm count and nine subjects received an electroencephalogram (EEG). Descriptive analyses included means and standard deviations for continuous variables, and frequency and percentages for categorical variables. Multiple logistic regression analyses were used to assess associations among clinical features and laboratory measures.

Results: The sample included 17 males and 16 females; mean age = 32.6 ± 11.7 years, range = 18–59 years. Genotype analyses revealed 15 subjects homozygous for the Q188R mutation, 13 subjects with Q188R/severe mutation, 1 subject with K127E/deletion and 3 subjects with homozygous deletions. Enzyme activity was non-detectable in all subjects. Subjects exhibited cataracts (21%), tremor (46%), ataxia (15%), dysarthria (24%), and apraxia of speech (9%). Electroencephalogram results obtained during a language production task differed from controls. Depression was reported by 39% and anxiety by 66% of subjects. Mean full scale IQ was 88 ± 20, (range=55–122). All subjects followed a dairy-free diet but 75–80% reported low intake of calcium and vitamin D. Mean bone density was below established norms (z = −1.1). Mean height, weight and body mass were within established norms. All female subjects had been diagnosed with POI. Among the 11 male and 9 female subjects who responded to the question about sex, 4 (36%) men and 6 (67%) women had had intercourse. One woman and 2 men had had children. Logistic regression analyses revealed no associations between age, genotype or gender and IQ, anxiety, tremor, ataxia, dysarthria or apraxia of speech. Each 10-year increment of age was associated with a 3-fold increase in odds of depression.
Conclusions: This is the first study to present a comprehensive description of adults with galactosemia. The results demonstrate a consistent pattern of neurological and psychological features, including tremor, motor speech deficits, anxiety and depression. Treatment for galactosemia needs to go beyond diet restrictions to address symptoms that have an impact on later adjustment. Future studies are needed to determine the etiology and timing of abnormalities associated with galactosemia. These studies may create new directions for treatments, but in the meantime appropriate evaluations, medications and psychosocial interventions can do much to alleviate the challenges encountered by adults with galactosemia.

109) Enzyme replacement therapy with agalsidase alfa (replagal) stabilizes renal function in male patients with Fabry disease: Results of an integrated analysis

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Background: Progressive kidney dysfunction is nearly universal in male patients with Fabry disease, an X-linked glycosphingolipid disorder caused by an insufficient activity of the lysosomal enzyme α-galactosidase A (α-Gal A). Evidence from 108 male patients in 3 similarly designed, prospective, randomized, placebo-controlled trials (RCTs) and their open-label extension studies suggests that enzyme replacement therapy with agalsidase alfa (i.e., highly purified human α-Gal A) at a dosage of 0.2 mg/kg every other week (EOW) slows the progression of renal disease (West et al., 2009). To more precisely estimate the effect of agalsidase alfa on the change in estimated glomerular filtration rate (eGFR), we conducted a post hoc analysis of pooled data from these trials, focusing on the double-blind, placebo-controlled treatment period.

Methods: In all 3 RCTs, male patients aged 18–54 years with Fabry disease confirmed by α-Gal A assay received either agalsidase alfa 0.2 mg/kg (n=61) or placebo (n=60) EOW for 6 months (intention-to-treat [ITT] population). In all 3 trials, serum creatinine was assessed at baseline and at least 3 follow-up visits; eGFR was calculated using the Modification of Diet in Renal Disease (MDRD) equation, which incorporates serum creatinine and the patient’s age, gender, and race. Change in eGFR over the 6-month treatment period was calculated using a random slopes and intercepts model with eGFR as the outcome variable and time (years) and treatment by time as the covariates. This longitudinal analysis uses all of the available eGFR data for each patient while allowing for the serial measurements to exhibit correlation within the same patient. All randomized patients who received ≥1 infusion and who had available eGFR measurements were included (eGFR ITT: placebo, n=58; agalsidase alfa 0.2 mg/kg, n=59). In addition, since MDRD reliably estimates renal function in patients with eGFR b80 mL/min/1.73m², the subset of patients with chronic kidney disease (CKD) stages 2 and 3 (eGFR 30–80 mL/min/1.73m²) was also evaluated.

Results: The individual RCTs each demonstrated a numerical improvement in the mean rate of change of eGFR for agalsidase alfa compared with placebo. The overall treatment difference was statistically significant when the pooled data were analyzed (Figure). Kidney function deteriorated in patients receiving placebo (eGFR ITT: mean, −13.26; 95% CI, −21.48 to −5.10 mL/min/1.73 m²/year; CKD Stage 2 or 3: mean, −16.69; 95% CI, −27.51 to −5.88 mL/min/1.73 m²/year) while patients receiving agalsidase alfa did not show deterioration, although confidence intervals crossed zero (eGFR ITT: mean, +0.21; 95% CI, −7.90 to +8.27; CKD Stage 2 or 3: mean, +5.98; 95% CI, −4.78 to +16.69 mL/min/1.73 m²/year). The overall difference in slope for eGFR between the agalsidase alfa and placebo group in the study population was +13.47 mL/min/1.73 m²/year (95% CI, 1.98 to 24.96; p=0.022) and that in the CKD Stage 2 or 3 population was +22.67 mL/min/1.73 m²/year (95% CI, 7.38 to 37.91; p=0.005).

Conclusions: The results of this post hoc analysis of pooled RCT data support the hypothesis that agalsidase alfa at a dosage of 0.2 mg/kg EOW slows the progression of kidney dysfunction in male patients with Fabry disease.

Reference

110) Correction of cystine storage in cystinotic fibroblasts and sialic acid storage in ISSD fibroblasts by vesicles derived from Sf9 cells

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We previously reported conditioned medium from cultured Spodoptera cells infected with baculovirus containing the human cystinosin gene produced 61% cystine depletion in cultured cystinotic fibroblasts in 96 h compared to 21% for controls (p<0.01). Conditioned medium from non-infected Sf9 cells does not produce cystine depletion Passage of conditioned medium through a 0.22 μ filter and ultracentrifugation for 3 h at 140,000 ×g yielded a visible pellet that on TEM showed a dense collection of vesicles of between 60 and 140 nm, the size of exosomes. Resuspension of vesicles in Ham’s F12 and placing on cystinotic fibroblasts caused 52±16.5% cystine depletion in 96 h, compared to 16±4.2% from vesicle-free controls. Initial trials using vesicles from Sf9 cells infected with baculovirus containing the human sialin gene produced 43.3±7.1% depletion in sialic acid of ISSD fibroblasts compared to 11.5±10.6% from vesicle-free controls. Placement of cystinosin medium vesicle fraction on ISSD fibroblasts produced no sialic acid depletion (118±8.3% of To, vesicle free control 108.5±6.3% of To). We conclude that there is a bioactive sedimentable factor present in the baculovirus-infected Sf9 conditioned medium associated with the vesicles. The vesicles may represent exosomes. The bioactive macromolecule formation and/or release may be stimulated by infection with baculovirus. We speculate that fusion of the vesicles with the target cell plasma membrane enables delivery of functional transport molecules to the endo-vascular system and hence to lysosomes where they function to remove the accumulated material.

111) Further characterization of congenital disorder of glycosylation IIB in siblings

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Background: Two siblings born to non-consanguineous, Northern European parents with negative family history, maternal hypothyroidism and gestational diabetes were evaluated. The older sibling was delivered at 40 weeks of gestation by emergency C-section. Birth measurements were normal. Postnatal course was complicated by hypotonia with poor latch and suck requiring bottle feeding. By 8 months of age, he had global developmental delay. Comprehensive molecular and biochemical evaluations were unrevealing. Evaluation at age 11 by the UDP, revealed mild dysmorphism, moderate hypotonia, joint laxity, normal reflexes, and no organomegaly. Weight was 90th centile and height 10th centile. He was non-verbal and able to sit independently and ambulate with assistance. His sister was delivered at 40 weeks of gestation by repeat C-section. Birth measurements were normal. She developed generalized seizures within the first 24 h. The CK was 592 U/L and ammonia 79 μmol/dL. Brain ultrasounds and CT were normal. When evaluated at age 6 by the UDP, she was mildly dysmorphic with nystagmus, severe hypotonia, joint laxity, adducted thumbs bilaterally, normal reflexes, and no organomegaly. Height and weight were both below the 3rd centile, at the 50th centile for a 31.2 year old. She was non-verbal and unable to sit independently.

Methods: Prospective evaluation and clinical biochemical testing.

Results: Evaluations revealed generalized cerebral atrophy, delayed myelination and low NAA by brain MRI/MRS in both sibs, cortical visual impairment with optic nerve atrophy. CSF studies identified cerebral folate deficiency in the younger sibling. Birth measurements were normal. She developed generalized seizures within the first 24 h. The CK was 592 U/L and ammonia 79 μmol/dL. Brain ultrasounds and CT were normal. When evaluated at age 6 by the UDP, she was mildly dysmorphic with nystagmus, severe hypotonia, joint laxity, adducted thumbs bilaterally, normal reflexes, and no organomegaly. Height and weight were both below the 3rd centile, at the 50th centile for a 31.2 year old. She was non-verbal and unable to sit independently.

Conclusions: This case underscores the difficulties of diagnosing complex multi-system disease especially when affected siblings have slightly different phenotypes. It also expands the phenotype of CDGIIb.

112) RRM2B mtDNA depletion syndrome presenting with pyruvate dehydrogenase deficiency

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Background: A 2-week-old male born to consanguineous Hispanic parents after an uneventful pregnancy and delivery, presented at 2 weeks of age with failure to thrive and severe diarrhea was admitted to the hospital for further evaluation. Laboratory evaluation revealed normal acylcarnitine profile, urine organic acids, and CK. Plasma lactic acid (15 mmol/L, normal <2.2), and pyruvate (0.48 mmol/L, normal 0.03-0.10) were elevated with a mild increase of alanine (435.4 μM, normal 142-421). Due to a high L:P ratio (33), a mitochondrial electron transport chain...
(ETC) disorder was suspected. Common mtDNA point mutations and deletions were negative. Sequence analysis of 3 genes (SUCLG1, DGUOK, POLG1, SUCLA2, and TK2) responsible for mtDNA depletion, complex IV assembly genes (SURF1, SCO1, SCO2, and COX10), SDH subunits A–D, and complex I assembly genes (G6ORF66 and NDUF1A) were all negative.

**Methods:** Prospective evaluation and clinical biochemical testing.

**Results:** A novel hemizygous unclassified missense variant, c.677G→A (p. R226H) in the PDHA1 gene was detected. On muscle biopsy, abnormal mitochondria with proliferation were noted. Profound deficiencies in complexes IV and II–III were detected with reduced complex I and I–III activities. Meanwhile, deficiency in pyruvate dehydrogenase complex (PDC) activity was detected in blood lymphocytes. The patient was started on carnitine and ubiquinol, which improved his diaphoretic, and he was discharged home. Subsequently, the patient's clinical condition continued to decline and he became ventilator dependent. MtDNA depletion syndrome was suspected and sequence analysis of the RRM2B gene, encoding a newly discovered p53-inducible ribonucleoside reductase subunit was performed. A homozygous c.109_110delAA (p. K37EfsX14) mutation was revealed.

**Conclusions:** Although an ETC deficiency may be secondary to the PDC defect, it is not known if mtDNA depletion would cause PDC deficiency. Thus, the clinical significance of p. R226H in PDHA1 remains unclear. This case underscores the complications in diagnosing mitochondrial ETC disorders.

113) Biochemical diagnosis of a mucolipidosis type III by urinary screening for a lysosomal storage disorder

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**Background:** Mucolipidosis type III (MLIII) is a heterogeneous disorder, most of which is caused by mutations in the Alpha/Beta or Gamma subunits of N-acetylglucosamine-1-phosphotransferase. It is a slowly progressive disorder that manifests with skeletal abnormalities, cardiac abnormalities and neurodevelopmental delay. In the past, this disorder has been difficult to diagnose through urinary screening since the secretion of abnormal oligosaccharides is often too low to detect by thin layer chromatography. **Methods:** Our laboratory has recently developed a new clinical assay to analyze urinary oligosaccharides by MALDI–TOF. The high sensitivity and specificity of this assay enable us to screen for this disorder in urine among patients with a mild clinical presentation. The patient is a 6 year 1 month old male referred to Genetics for evaluation. He has mildly coarse facial features, a short neck, a mild claw hand deformity, progressive joint contractures, and dysostosis multiplex. He has normal intelligence and there is no hepatosplenomegaly. Cardiac and eye exam are normal. A urinary screening panel for lysosomal storage disorders was ordered. **Results:** Urine oligosaccharide and free glycan profile by MALDI–TOF showed elevations in both sialylated and galactosylated oligosaccharides, a pattern reflecting reductions in multiple lysosomal hydrolyase activities. Quantitative urine mucopolysaccharide levels were normal, while a trace of dermatan sulfate was detected by the fractionation of glycosaminoglycans. A lysosomal storage disorder was suspected and a lysosomal enzyme screening panel in leukocytes was ordered. Consistent with urinary findings, we found mildly reduced activity of β-galactosidase in the patient’s leukocytes at 17 nmol/mg protein/h (normal 45.7–140). A possible MLIII was suspected and serum beta hexosaminidase activity was measured at 12,000 mU/L, 11 times higher than the concurrent controls. The mild reduction of beta-gal activity in leukocytes and marked increases of beta hexosaminidase in serum is consistent with a biochemical diagnosis of mucolipidosis type III. Molecular analysis of GNP T A B and GNPTG is underway.

**Conclusions:** This case presents evidence that urinary screening of lysosomal disorders by MALDI–TOF is an ideal first tier screening for lysosomal disorders, particularly for MLII and MLIII. The unusually mild clinical presentation of our patient highlights the importance of screening for MLIII in all the patients with mild skeletal findings.

114) Differential expression of liver micro-RNAs in long-chain acyl-CoA dehydrogenase deficient mice

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**Background:** MicroRNAs (miRNA) are small noncoding RNAs regarded as important regulators that can inhibit or activate pathways by transcript degradation or translational repression. We have demonstrated previously that long-chain acyl-CoA dehydrogenase deficient (LCAD−/−) mice, a mouse model for mitochondrial fatty acid β-oxidation, present a robust phenotype of cold and fasting intolerance, organic acidemia, hepatic steatosis and insulin resistance. We hypothesized that metabolism related miRNAs contribute to this phenotype by differentially affecting metabolic pathways in LCAD−/− mice when compared to wild-type (WT) mice.

**Methods:** We used C57BL/6NTac (B6) WT and congenic B6 LCAD−/− male mice at six months of age (n = 4 each). The mice were fed a standard rodent diet and were not fasted before the liver tissue harvest. Total RNA was extracted from livers. The cDNAs were generated by reverse transcription using hairpin loop RT primers (Mega Pool) to select for over 600 miRNAs. The miRNA expression was measured by real time PCR Taqman Low Density Array (TLDA). Differentially expressed miRNA targets were selected and validated using individual Taqman assays.

**Results:** MicroRNA 29b was expressed at a significantly higher level (relative quantification [RQ] = 1.3; P = 0.021114) in LCAD−/− mice as compared to WT mice. This miRNA has been implicated in liver fibrosis regulation by transcriptional repression of type I collagen. miRNA 34a (RQ = 0.66; P = 0.011711) and miRNA 107 (RQ = 0.58; P = 0.011593) were expressed at significantly lower levels in LCAD−/− mice as compared to WT mice. As shown by others these miRNAs are involved in lipid metabolism by affecting acyl-CoA synthetase long-chain family members 1 and 4 (Acsl1 and Acsl4). miRNA 375, known to be involved in regulating insulin secretion, preadipocyte differentiation
and inflammatory processes was expressed at significantly lower levels (RQ = 0.32; P = 0.040055) in LCAD−/− mice as compared to WT mice.

**Conclusions:** We first screened LCAD−/− mice with miRNA TLDA using liver tissue total RNA and compared it to WT mice. We found several differentially expressed miRNA targets that were validated using individual assays. As a potential miRNA signature for this phenotype, we found altered expression in metabolism related miRNAs 29b, 34a, 375 and 107.

115) Qualitative and quantitative O-glycan analysis by MALDI–TOF–TOF and LC–QTRAP MSMS for the diagnosis of multiple glycosylation disorders

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**Objective:** The genetic disorders that cause both N-linked and O-linked glycosylation deficiencies are classified as multiple glycosylation disorders. This subgroup of congenital disorder of glycosylation (CDG) includes genetic defects in the conserved oligomeric Golgi complex (COG) and other proteins that affect glyco-transporters, chaperones and Golgi-trafficking complexes. Multiple glycosylation disorders can be diagnosed by the combination of plasma N-linked glycans and O-linked glycan profiles. Our laboratory recently developed clinical testing for plasma N-glycans by MALDI–TOF. Here we describe the qualitative and quantitative analysis of plasma O-glycans by MALDI–TOF/TOF and LC–MS/MS respectively. The relative abundance of all the major plasma O-glycans was obtained by MALDI–TOF profile, including T antigen, mono-sialylated T antigen, di-sialylated T antigen, mono-sialylated core 2 and di-sialylated core 2. The most common deficiency in multiple glycosylation disorders is the generalized deficiency in the sialylation of both N-linked and O-linked glycans. In some patients, the changes in O-glycans can be relatively small. Thus quantification of T antigen (Gal-GalNac) and sialylated T (SA-Gal-GalNac) antigen is important in order to detect such changes. We therefore developed a quantification assay of T and mono-sialylated T antigen using an LC–QTRAP tandem mass spectrometry.

**Methods:** O-linked glycans in 10 μL plasma was released by beta-elimination method and desalted by ion-exchange resin, and then was permethylated by solid phase permethylation. The qualitative profile was obtained by MALDI–TOF scan, and the quantitation of T and Sialyl-T were determined by LC–QTRAP MS/MS.

**Conclusions:** 3 samples from known patients with COG7 or COG4 deficiency were run. Both mono-sialyl-T and di-sialyl-T were markedly reduced with increased levels of T antigen in these patients. The T/Sialyl T ratio is significantly above the reference range that was obtained from 40 control samples. We also tested 3 samples from patients with combined CDG type I and type II. All of these patients have increased carbohydrate deficient transferrin as well as increased multiple truncated glycans, pointing to a mild deficiency in multiple oligo glycosyltransferase activities. Interestingly, we found that the T/Sialyl T ratios in these three patients were all above our reference range. Thus it is likely that the sialylation of O-glycosylation is also mildly affected in these patients which might not have been previously recognized.

116) Nutrient analysis software for inherited metabolic disorders

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**Background:** Nutritional therapy is the primary treatment modality for many inborn errors of metabolism (IEM). An important aspect of nutritional assessment is analysis of the nutrient intake of individuals with IEM. Members of Genetic Metabolic Dietitians International (GMDI) identified the need for a nutrient analysis software program designed specifically for IEM. While several diet analysis software programs are available for the general population, they lack information on foods critical to the management of IEM, specifically medical foods and low protein foods, and much of the nutrient data are incomplete (e.g., do not include amino acid profiles).

**Methods:** GMDI formed a Technology Committee to define the requirements of a diet analysis software program specific to the needs of metabolic dietitians. Both program functionality and database requirements were specified in a Request for Proposal (RFP). The RFP was sent to several software developers and three proposals were considered. Project funding was made possible by unrestricted educational grants from BioMarin Pharmaceutical and Nutricia North America. Abbott Nutrition contributed the database from a formerly used nutrient analysis program for IEM.

**Results:** A novel, web-based diet analysis program, MetabolicPro, was released in July 2010 and is available for an annual license fee through GMDI (www.gmdi.org). MetabolicPro database contains selected foods from the USDA database for which complete data on essential amino acids and conditionally essential amino acids (TYR, CYS, ARG) are available. MetabolicPro also contains the most up-to-date manufacturers’ nutrient data on over 125 medical foods and 150 low protein foods, as well as low protein cookbook recipes used in IEM treatment. To assess nutritional adequacy the program analyzes for 50 nutrients and compares nutrient intake to age and sex-specific Dietary Reference Intakes (DRI). As a web-based software program, new foods and nutrient data can be updated in real time and the program may be used from any computer with Internet access. Protected health information is not stored on the web and the program is compliant with HIPAA regulations. Systems for troubleshooting, responding to user inquiries, and maintaining the foods’ database were
included in the project design. Plans for program enhancements and updating and expanding the database will contribute to maintaining a dynamic clinical tool for users in the future.

Conclusions: A new web-based software program specifically for metabolic dietitians was designed and developed under the guidance of the GMDI Technology Committee. Better ability to analyze the nutrient intakes of patients with IEM may lead to improved nutrition assessment and help optimize nutritional status.

117) Evaluation of endothelial function by endothelial pulse amplitude testing in patients with mucopolysaccharidosis

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Background: Progressive cardiovascular changes have been known as one of the major causes of death in patients with mucopolysaccharidosis (MPS). Based on the MPS-I Registry, more than 75% of MPS-I patients have cardiovascular changes including occlusion of coronary arteries and valvular diseases. All types of MPS have been reported to present with cardiovascular manifestations. Evaluation of endothelial function (EF) by flow-mediated dilation (FMD) of the brachial artery has been studied and endothelial dysfunction (ED) characterized by decreased percent mediated dilation was demonstrated in individuals with risk factors for atherosclerosis. Close relation of EF in coronary and brachial artery has been demonstrated. Studies to evaluate peripheral vascular EF with finger arterial pulse wave amplitude (PWA) with a finger plethysmograph (PAT) have been performed and showed that PAT hyperemia and FMD were significantly correlated. We conducted a study to examine finger arterial PWA with a PAT device i.e., Endo-PAT2000, which is a tool recently approved by the FDA, to evaluate cardiovascular changes frequently seen in patients with MPS.

Methods: Subjects with MPS (total n = 14; type I all females, n = 9, age 9–27 y, type II all males, n = 2, age 30–37 y, III all females, n = 2, age 12–15 y; all subjects with MPS-I and II are treated with enzyme replacement therapy) were enrolled. EndoPAT2000 was used for the study. Subjects were recruited at the Annual National MPS Society Meeting at Nott’s Berry Farm (CA) in Oct. 2010.

Results: The Endo-PAT index of patients with MPS-I was 1.20 ± 0.38 (n = 9, 0.83–1.88). The indices were 0.62 (30 y) and 2.05 (37 y) for patients with MPS-II, and 2.14 (12 y) and 1.46 (15 y) for MPS-III. Past studies showed that the Endo-PAT index cut-off value of 1.67 provides a sensitivity of 82% and a specificity of 77% to diagnose coronary EF for adults. Endo-PAT index for adolescent controls (n = 30, age 13 y to 19 y) was 1.78 ± 0.51 (1). Eight patients with MPS-I showed ED (Endo-PAT index < 1.67). In MPS-II and MPS-III, one of the two patients showed ED.

Conclusions: ED in subjects with MPS was clearly demonstrated with Endo-PAT2000. Eight of 9 patients with MPS-I had ED though they had been treated with enzyme replacement therapy. Follow up studies are indicated for further evaluation on EF in patients with MPS.


118) Elevation of guanidinoacetate in the newborn blood spot of a patient with guanidinoacetate methyltransferase deficiency

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Background: Guanidinoacetate methyltransferase deficiency (GAMT-D) is an autosomal recessive, cerebral creatine deficiency syndrome that results in mental retardation, lack of speech, autistic behavior, movement abnormalities and seizures refractory to standard therapy. Creatine deficiency and guanidinoacetate (GA) accumulation both contribute to the disease process. Replenishment of creatine by oral creatine monohydrate, and reduction of GA using ornithine, benzoate, and arginine restriction are therapeutic goals. Pre-symptomatic treatment was reported to have a favorable outcome in a patient with a positive family history, diagnosed and treated since birth. Limited evidence from two case reports suggests that newborn screening (NBS) for GAMT-D is feasible by measurement of GA in newborn blood spots. We present the case of a 26 month old, male child, who was initially given the diagnosis of athetoid cerebral palsy after presenting with global developmental delay and choreoathetosis. A comprehensive metabolic workup revealed generalized organic aciduria due to low urine creatinine, and markedly elevated GA and reduced creatine in both urine and plasma samples. Molecular analysis confirmed GAMT-D. He was started on treatment at the age of 13 months which resulted in marked improvement in motor skills, cognitive performance and activity level. However, speech remained markedly impaired.

Methods: GA was analyzed by flow injection ESI–MS/MS as the butylated derivative of a methanol extract from the DBS. GA and the [15N,13C]-GA internal standard were detected by monitoring the transitions m/z 174 > 101 and 176 > 103.

Results: The assay was accurate (error ≤ 20%) and precise (intra- and inter-day precision < 15%). GA was stable in the DBS for at least 5 months at room temperature. Retrospective determination of GA in the newborn dried blood spot (DBS) from this patient showed an elevation of GA compared with controls (7.0 μmol/L; control M + 2SD: 2.1 μmol/L, n = 21) after two years in storage. An unaffected younger sibling, who is a carrier for GAMT-D, had a GA level of 2.9 μmol/L in the NBS blood spot.

Conclusions: These results provide further evidence that GAMT-D can be detected using expanded newborn screening methodology already in use in NBS labs. GAMT-D is a good candidate for NBS, as early detection and treatment may prevent severe neurological outcomes.