# poster presentations in biochemical genetics

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2-Methylbutyryl-CoA dehydrogenase (2-MBCDase) deficiency: a new inborn error of L-isoleucine metabolism. K.M. Gibson[1,5], T. Burlingame[1], B. Hogema[1,2], C. Jakobs[2], R.B.H. Schutgens[2], D. Millington[3], C. Roe[4], D. Roe[4], L. Sweetman[4], R.D. Steiner[1,5], L. Linck[5], P. Pohowalla[6], D. Kiss[6], M. Sacks[6], P. Rinaldo[7] and J. Vockley[7]. [1]Dept. Mol. and Med. Genet. and [5]Peds., Oregon Health Sci. Univ., Portland, OR; [2]Metab. Screening Lab., Free Univ., Amsterdam, The Netherlands; [3]Mass Spectrometry Unit, Duke Univ. Med. Ctr., Durham, NC; [4]Inst. of Metab. Dis., Baylor Univ. Med. Ctr., Dallas, TX; [6]Dept. of Peds., Legacy Emanuel Children's Hosp., Portland, OR; [7]Dept. of Med. Genet. and Biochem. Genet. Lab., Mayo Clinic, Rochester, MN

With the exception of beta-ketothiolase (BKT) deficiency, there are no reported inborn errors of human metabolism specific to L-isoleucine degradation. EA presented at three days of age with lethargy, hypoglycemia and apnea. MRI demonstrated ischemic changes bilaterally in parietal and occipital lobes and abnormal EEG. Acylcarnitines in plasma were increased (range 8-22 uM, n=7; nl, 3-10). Tandem mass spectrometry (MS/MS) analysis revealed elevated plasma $C_5$-acylcarnitine (1.4-2.4 μM; nl < 0.4), verified as 2-methylbutyrylcarnitine by gas chromatography-mass spectrometry. Urine 2-methylbutyrylglycine (2-MBG) was consistently elevated (range 8-30 mg/g creatinine, n=6; nl < 8). Intact fibroblast conversion of U-$^{14}$C-isoleucine to $^{14}CO_2$ was decreased to 26% of control, comparable to that of BKT deficient fibroblasts. Conversion of $^{13}C_6$-leucine and $^{13}C_5$-valine in patient's fibroblasts incubated with L-carnitine revealed no abnormal accumulation of acylcarnitines; in the same cells, there was a 10-fold accumulation of $C_5$-acylcarnitine in comparison to control (consistent with impaired 2-MBCDase activity) when incubated with $^{13}C_6$-isoleucine and L-carnitine. Western-blot analysis of fibroblasts from EA revealed greatly reduced 2-MBCDase cross-reactive material. Mutation screening in patient fibroblasts revealed a C778T substitution in the 2-MBCDase gene, substituting leucine for phenylalanine at amino acid 222 (L222F); the patient's mother also carried this mutation. In a subsequent pregnancy, the concentration of 2-MBG in amniotic fluid obtained at 15 weeks gestation was 0.27 umol/L (nl < 0.04, n=5), while total $C_5$-acylcarnitine concentration was 1.9 umol/L (nl < 0.7, n=27), suggesting an affected fetus. Cultured amniocytes accumulated excess (10-fold) $C_5$-acylcarnitine when incubated with $^{13}C_6$-isoleucine and L-carnitine. At 1 year of age EA carries the diagnosis of athetoid cerebral palsy, and continues to manifest impaired visual, motor and cognitive skills. Our studies reveal 1) the first documented case of isolated 2-MBCDase deficiency; 2) a novel mutation in the 2-MBCDase gene; and 3) the first prenatal diagnosis of an affected fetus with 2-MBCDase deficiency, a new inborn error of L-isoleucine metabolism in humans.

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**Neonatal cholestasis: A new presentation of X-linked adrenoleukodystrophy.** W.T. Gibson[1], G. Lepage[2], K. Smith[3], H. Moser[3], A. Moser[3] and G.A. Mitchell[2] [1] U Calgary, AB, Canada, [2] U Montreal, QC, Canada, [3] Kennedy-Krieger Institute, Baltimore, MD.

A French-Canadian boy presented with neonatal cholestasis and failure to thrive, bilateral polar cataracts, anemia, thrombocytopenia and hypotonia. Born at 37 weeks' gestational age, he weighed 2450 g (10%ile), with length 46 cm (10%ile) and head circumference 33 cm (50%ile). Jaundice was present at birth. No dysmorphism was noted. Transaminases were consistently elevated >2-fold. Liver biopsy showed cholestasis and confirmed the presence of catalase-positive structures, with normal peroxisomes and mitochondria. HIDA scintigraphy showed mild hepatocellular dysfunction. Other investigations including endoscopy, plasma and urine amino acids, serum copper, alpha-1-antitrypsin levels, erythrocyte galactose-1-phosphate levels, karyotype (46, XY), and TORCH viral studies were non-diagnostic. Brainstem auditory evoked potentials showed mild peripheral hearing loss at 3 weeks of age, and a central loss when repeated at 8 months of age. He developed cachexia, progressive hepatomegaly, splenomegaly, osteopenia, bruxism and opisthotonic posturing, with a progressive motor delay. CT scan revealed cortical atrophy. He died of liver failure at age 12 months after frequent episodes of gastrointestinal bleeding and continued neurological deterioration. Post-mortem, plasma very long-chain fatty acids (VLCFAs) results became available, showing elevation of the C26:C22 ratio. Phytanic acid, assayed on three stored samples, was normal in two and 3-fold elevated in one. Other peroxisomal function tests including plasma pipecolic acid and rbc plasmalogen ratios were normal, but C18 DMA levels were somewhat lower than normal. Fibroblast C26:0 and C22 levels were elevated 5-fold, and the C26/C22 ratio, 9-fold. Oxidation of C24:0 was 20% of control values. On immunostaining with antibody to the ALD protein, fibroblasts were CRIM negative. The ALD protein gene contained a previously-unreported mutation, a thymine-to-cytosine transition at nucleotide 1654 in exon 7, predicted to change Ser 552 to Pro in the ATP binding domain. Although the patient's mother had normal plasma VLCFA levels, a subsequent pregnancy showed an elevated C26/C22 ratio in amniocytes and was terminated. A similar patient with biochemically-proven ALD and rapidly-progressive cholestasis was recently reported (Corzo, D. *et al., Am J Hum Genet* 65, 4 (Suppl.): A1295) demonstrating that the association of mutations in the ALD protein gene and neonatal cholestasis is not spurious, and expanding the phenotypic spectrum of ALD to include neonatal cholestatic jaundice with liver failure.

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Riboflavin responsive ethylmalonic encephalopathy in two Korean sibs. S.H. Hahn[1], E.H. Lee[1], B.L. Eun[2], P. Rinaldo[3]. [1]Univ. of Ajou, Suwon, Korea, [2]Univ. of Korea, Seoul, Korea, [3]Mayo Clinic, Rochester, MN, USA.

Ethylmalonic encephalopathy is a complex organic aciduria characterized by ethylmalonic aciduria, lactic acidemia with developmental delay, acrocyanosis, petechia and chronic mucoid diarrhea. Since 1994, less than 30 patients have been reported and the underlying metabolic defect is unknown.

We report 2 affected sibs with typical clinical and biochemical phenotypes, and brain MRI findings. Patient 1 is a 5 years old girl who presents with developmental delay, spastic quadriplegia, and chronic diarrhea. She was hospitalized several times due to chronic diarrhea and pneumonia in the early infancy. She developed frequent skin petechiae on compression sites and cyanosis on the lower extremities. Brain MRI taken at the age of 25 months was suggestive of hypoxic ischemic encepahlopathy and no further evaluation was undertaken. Patient 2, her younger sister, is a 34 months old girl who presents with a similar clinical course but had no evaluation until recently when she developed respiratory difficulty due to pneumonia which required hospitalization. She was able to control her head at 4 months of age and crawl at 9 months of age but never sit, stand or talk. Her spastic quadriplegia became worse after this episode and she is almost wheel chair bound. Brain MRI showed multifocal nodular T2 high signals in both basal ganglia with enhancement, patchy T2 high signals in both periventricular white matter, centrum semiovale, and cerebellum. Muscle biopsy showed diffuse atrophy but no evidence of mitochondrial disorder. Organic acid and acylglycine profiles showed markedly elevated excretion of ethylmalonic acid (134 mmol/mol creatinine: controls: <18), isobutyrylglycine, butyrylglycine, methylbutyrylglycine, and isovalerylglycine were also elevated. Serum lactate was 3.2 mmol/L (control: 0.7-2.0). 100 mg/kg/day oral carnitine supplementation for 2 months had no visible effect. However, they became significantly more active and alert on riboflavin treatment (100 mg/day). Patient 1 rolled over and was socially more active with laughing and sounds. In patient 2, motor improvement was not significant but she became more alert and socially active. Notably, the chronic mucoid diarrhea subsided considerably in both children, but petechiae were unaffected.

Our observations suggest that some cases with ethylmalonic encephalopathy could be responsive to riboflavin. In vitro evaluation of fatty acid and branched chain amino acid metabolism is in progress.

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Severe Conradi-Hünermann Syndrome (CDPX2) is a phenocopy of peroxisomal Rhizomelic Chondrodysplasia Punctata (RCDP).
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RCDP was initially diagnosed in a newborn female who had typical symmetric rhizomelic shortening of the extremities, metaphyseal changes, multiple punctate skeletal calcifications, icthyosis, bilateral cataracts and Koala bear facies. The patient succumbed at 12 weeks of age. Diagnostic evaluations were unrevealing, including normal peroxisomal very long chain fatty acids, phytanic acid hydrolase and plasmalogen biosynthesis. Fibroblasts where reanalyzed for defects in 3-beta-hydroxysteroid-Δ(8), Δ(7)-isomerase, which was recently identified to cause X-linked Conradi-Hünermann Syndrome. GC/MS analysis revealed a pattern of metabolites consistent with this enzymatic defect in cholesterol biosynthesis, specifically showing increased levels of 8-dehydrocholesterol and 8(9)-cholesterol. Genomic DNA was isolated from the patient's fibroblasts and the gene for 3-beta-hydroxysteroid-Δ(8), Δ(7)-isomerase (EBP, located at Xp11.2) was sequenced (all exons and intronic junctions). Results showed 3 mutations in cis in exon 2 : L42X (125T>A), W47X (140G>A) and L49L (147G>A)(a synonymous change). The other allele showed a wild type sequence. The EBP gene were sequenced in blood lymphocytes from both parents and were normal.

This case highlights the heterogeneity of CDPX2. The range of phenotypic severity seen in this disorder is presumably due to variable lyonization. We show that severe CDPX2 is a phenocopy of peroxisomal RCDP. We have also identified a novel mutation that could have originated from a pseudogene or another unknown mechanism.