

# platform presentations in biochemical/ molecular genetics

## 1

Potential for clinical misdiagnosis of combined methylmalonic aciduria/homocysteinemia (MMA/HcYS) due to absence of acute metabolic derangement. K.M. Gibson<sup>1,2</sup>, R.D. Steiner<sup>1,2</sup>, M. Grompe<sup>1,2</sup>, T. Burlingame<sup>1</sup>, H. Senephansiri<sup>1</sup>, T. Bottiglieri<sup>3</sup>, J. Debley<sup>4</sup>, P. Campbell<sup>2</sup>. Depts. of <sup>1</sup>Molec. and Med. Genetics and <sup>2</sup>Peds., Oregon Health Sci. Univ., Portland, OR; <sup>3</sup>Inst. of Metab. Dis., Baylor Univ. Med. Ctr., Dallas, TX; <sup>4</sup>Legacy Emanuel Children's Hosp., Portland, OR.

The cobalamin C (cbl C) complementation group of combined MMA/HcYS is the most common of the inborn errors of vitamin B12 metabolism, affecting both methylmalonyl-CoA and homocysteine (Hcys) degradation. We have recently diagnosed 2 patients with this disorder. AH, a 3 week old male, presented with lethargy, decreased oral intake, dehydration and pancytopenia. There had been a preceding upper respiratory infection. Parents were first-cousin Saudi Arabians. VM, a Hispanic female, presented at 19 days of life with lethargy, hypotonicity, dehydration, cough, decreased oral intake, significant thrombocytopenia, and mild pancytopenia. For both, worsening neurologic status was accompanied by seizures, and sepsis was suspected followed by appropriate intervention. Subsequent metabolic work-up revealed MMA/HcYS; fibroblast analysis for AH verified the cbl C complementation subgroup (Dr. D. Rosenblatt, Montreal, Canada; studies in VM are pending, although she likely falls within the same subgroup). Plasma total Hcys remains persistently elevated in AH (26-39 uM, n=8; nl < 10) and was 216 uM for VM (n=1). CSF total Hcys was 35 uM for VM (nl < 0.08). We conclude that 1) diagnosis of patients with MMA/HcYS of the cbl C complementation subgroup may be hampered by absence of acute metabolic derangement (acidosis, hyperammonemia, hypoglycemia, ketosis) combined with features (lethargy, obtundation) suggesting sepsis; and 2) significantly increased CSF Hcys may be the underlying cause of altered neurologic status in these patients.

## 3

Evaluation and management of urea cycle disorders using stable isotope infusions. F. Scaglia<sup>1</sup>, W. O'Brien<sup>1</sup>, Judy Rosenberger<sup>2,3</sup>, P. Reeds<sup>2,3</sup> and B. Lee<sup>1</sup>. <sup>1</sup>Department of Molecular and Human Genetics, <sup>2</sup>Department of Pediatrics, and <sup>3</sup>Children's Nutrition Research Center, Baylor College of Medicine, Houston, TX.

Diagnosing partial urea cycle deficiencies based on clinical and laboratory data is difficult and there is often poor correlation between genotype, in vitro enzyme activity, and phenotypic expression. We have used stable isotope infusions to measure total body glutamine and urea flux for the evaluation of patients with urea cycle disorders. We have previously determined the rate of total body urea production by measuring blood <sup>13</sup>C-urea isotopic enrichment during an infusion with the stable isotope <sup>13</sup>C-urea, and the proportional contribution of peripheral nitrogen to urea synthesis by measuring the incorporation of <sup>15</sup>N from <sup>15</sup>N glutamine into <sup>15</sup>N urea. The ratio of the isotopic enrichments of <sup>15</sup>N urea/<sup>15</sup>N-glutamine (<sup>15</sup>N-U/G) allowed us to distinguish carriers for ornithine transcarbamylase deficiency (OTCD) both from their affected offspring and from normal control subjects (Lee et al., submitted). Collectively these data suggest that the flux from <sup>15</sup>N glutamine to <sup>15</sup>N urea is a sensitive measure of urea cycle activity and is capable of differentiating female carriers for OTCD from normal controls.

We have now applied this method to the 1) diagnosis of asymptomatic at risk partial OTCD females, 2) titration of dietary protein tolerance for managing a severe OTCD male patient, and 3) comparison of differences in the bioavailability of enteral vs. systemic sources of nitrogen for total body urea production. By determining the <sup>15</sup>N-U/G ratio in 5 at-risk females in two OTCD sibships in which mutations were not found in the index case, we were able to measure normal and abnormal urea production and determine carrier status. In the second case, we determined total body glutamine flux at 1.2 gm/kg/day and at 0.8 gm/kg/day protein intake in a 7 month null activity OTCD male to titrate protein tolerance and guide dietary therapy. In the third case, we tested the hypothesis that intestinally generated ammonia and alanine are more effective immediate precursors for urea synthesis than peripherally generated glutamine and as a consequence individuals who have a compromised urea cycle activity would metabolize excessive dietary protein more effectively than peripherally generated protein. By comparing the transfer of <sup>15</sup>N from oral and intravenous <sup>15</sup>NH<sub>4</sub>Cl to urea, we found that individuals who have partial urea cycle activity metabolize excessive dietary protein more efficiently, underlying the observation that these patients are particularly susceptible to stress and fasting. In conclusion this approach may also lead to improvements in the nutritional management of patients with urea cycle defects and those who are severely catabolic during critical illness.

## 2

Maternal complications and sudden infant death in families with mutations in mitochondrial trifunctional protein. J.A. Ibdah<sup>1</sup>, Y. Zhao<sup>1</sup>, J. Viola<sup>1</sup>, and M.J. Bennett<sup>2</sup>. <sup>1</sup>Wake Forest University School of Medicine, Winston-Salem, NC, <sup>2</sup>University of Texas Southwestern Medical Center, Dallas, TX

Mitochondrial trifunctional protein (TFP) catalyzes the final 3 steps of long chain fatty acid oxidation. Patients with TFP mutations have either isolated deficiency of long chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) or complete TFP deficiency of all 3 enzymes. Recently, we characterized the genotypes and phenotypes in pediatric TFP defects and documented an association between fetal LCHAD deficiency and maternal liver disease (Ibdah et al, N. Engl. J. Med. 340:1723-31, 1999). To further characterize the association between TFP mutations, pregnancy outcome, and sudden infant death, we analyzed the pregnancy history and offspring genotypes and phenotypes in all affected and non-affected pregnancies in 31 families with mutations in TFP. 26 of these families had offspring with isolated LCHAD-deficiency and 5 with complete TFP deficiency. In 75 pregnancies, 72% of women who carried affected fetuses with isolated LCHAD deficiency developed acute fatty liver of pregnancy, HELLP syndrome or preeclampsia. 64% and 42% of pregnancies carrying fetuses with isolated LCHAD deficiency or complete TFP deficiency were also associated with premature delivery and birth weight that was small for gestational age, respectively. All pregnancies with heterozygous or wild-type fetal genotypes were uncomplicated. 39% (12/31) of the affected children died. Ten with isolated LCHAD deficiency and 2 with complete TFP deficiency. Four of these children had sudden, unexplained death at few months of age and certified initially as SIDS. Autopsy revealed micro- and macro- vesicular hepatic steatosis. Screening for TFP mutations revealed homozygosity for the prevalent (G1528C, E474Q) mutation in all four children. In five families, there was a history of sudden, unexplained death in a sibling of unknown genotype. These results document that: 1) fetal genotype affects both maternal and fetal outcomes in families with TFP mutations; 2) affected children, if unrecognized and untreated, may suffer sudden death. [Supported by a grant from NIH (DK-02574) and a grant from March of Dimes (#6-FY99-376)]

## 4

Absence of congenital myotonic dystrophy (CDM) in a baby with 1000 DMPK CTG repeats born after a sibling with CDM. Carson NL<sup>1</sup>, Whelan DT<sup>2</sup>, Zeeman S<sup>2</sup>. <sup>1</sup>Children's Hospital of Eastern Ontario, Ottawa, ON, <sup>2</sup>McMaster University Medical Centre, Hamilton, ON.

Myotonic dystrophy has been shown to be caused by an expansion of a CTG repeat in the 3' untranslated region of the myotonin-protein kinase gene (DMPK). Congenital myotonic dystrophy (CDM) is the most severe phenotype and is characterized by marked hypotonia, and facial weakness at birth. Respiratory distress is also common. Individuals with CDM tend to have larger numbers of CTG repeats than individuals with the other forms of DM, with sizes ranging from 700 to >2000 repeats; however, there is considerable overlap and not all individuals with large repeats have CDM. Most cases of CDM are maternally inherited and the likelihood of having a second child affected with CDM has been estimated to be 90-100% if the expanded allele is passed on. We report a baby, born after a sibling with CDM, who does not have symptoms of congenital myotonic dystrophy despite having 1000 CTG repeats.

At delivery the proband was extremely floppy and required intubation due to respiratory distress. He remained ventilator-dependant and died 40 days later. The pregnancy had been complicated by polyhydramnios. Southern blot analysis showed that he had a smear of DMPK CTG repeats ranging in size between 1700 and 2300. The mother had grip myotonia and a typical myotonic facies. Molecular analysis revealed a repeat size of 600. Her then 6 year old daughter who had signs consistent with childhood-onset DM was found to have 1300 CTG repeats. Prenatal diagnosis was offered for a subsequent pregnancy. Analysis of CVS revealed an expansion of 1000 repeats in the fetus. Sizing was repeated on amniocytes with the same result. As an expansion of this size is within the range found in CDM and as the mother had had a previous affected child, the likelihood that this fetus would have CDM was considered high. The mother decided to continue with the pregnancy and was induced at 39 weeks gestation after a normal pregnancy. The baby had no evidence of CDM. The neonatal reflexes were normal and there was no respiratory distress. Analysis of DNA from cord blood confirmed the results obtained for both CVS and amniocytes.

This case illustrates that a fetus with a large CTG expansion in the CDM range, will not necessarily present with CDM despite having had a sibling with CDM.