esponsive epilepsy appears not to be caused by mutations in *GAD2* genes. D.-F. Bu[1], J. Christodoulou[2], M.J. Murrell[2], L. ibson[1], A. J. Tobin[3] and R. R. McInnes[1]. [1]Dept. Genetics, Hosp. for Toronto,Ontario, Canada: [2]Dept. Peds. & Child Health, Univ. Sydney, alia; [3]Dept. Biology, UCLA, Los Angeles, CA.

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Another Tay-Sachs pseudodeficiency mutation in the same codon as PD II in an Ashkenazi Jewish 'carrier': Dilemmas of prenatal diagnosis. J.W. Callahan, J. Warren, M. A. Skomorowski, R. Babul, J. T. R. Clarke, P. Strasberg. Hospital for Sick Children and Univ. of Toronto, Ontario, Canada

Tay-Sachs disease (TSD) is due to mutations in the HEXA gene (chr 15), encoding the α subunit of ß-hexosaminidase. Of the 50 different mutations that have been identified, 98% of carriers in the Ashkenazi Jewish (AJ) population have one of 3 mutations. Only 2% of AJ carriers (based on residual HEX A activity) have rare mutations. Healthy individuals lacking serum HEX A activity, ie., pseudodeficient (PD), have been described with a 'benign' C739T (R247W) mutation in exon 7 (named PD I) plus one of the infantile TSD alleles; prognosis remains uncertain. This PD I allele accounts for 32-42% of non-Jewish and 3% of AJ carriers. A second pseudodeficiency allele, PD II, C745T (R249W), found in 6% of non-Jewish enzyme-defined carriers, was originally identified in a non-Jewish healthy 42-year-old, in compound heterozygosity with the known G805A exon 7 "adult-onset" mutation.

Our patient is an AJ man who had 47% serum HEX A (normal 58 to 65%), and was normal for the 3 AJ mutations. Analysis for the exon 7 adult onset TSD mutation revealed an extra band indicative of a new *EcoRII* site at nt 746 within the same codon as PD II. Hydrolysis with *GsuI* and sequencing confirmed a G746A, R249Q mutation. His wife (41% Hex A) was a carrier of the infantile exon 11 TSD mutation. CV sampling from their first pregnancy showed 10-18 % HEX A activity, the paternal G746A, R249Q mutation and the maternal exon 11 mutation. Previous prenatals involving PD and infantile alleles in fetuses with less than 25% HEX A in amniocytes were considered to be at risk. Here it was unknown if G746A, R249Q would give the same PD state as C745T (R249W) in the presence of a known infantile allele and we could not guarantee that the fetus would be unaffected with TSD. Expression studies should clarify this. Assigning risk to compound heterozygous fetuses with PD and 'true' TSD alleles is a difficult counselling problem, requiring one to err on the side of caution.

enetics of propionic acidemia: a proposal for deficient oxylation of the α-subunit of propionyl-CoA carboxy- npeau, * D. Leclerc, L. Dupuis and R. A. Gravel. McGill Univer- Children's Hospital. Quebec, Canada.

cidemia is a rare autosomal recessive disorder caused by a deficiency lependent enzyme propionyl-CoA carboxylase (PCC, $\alpha_4\beta_4$ where α n). The mechanism of PCC is divided in two half-reactions: first, transfered onto the biotin upon hydrolysis of ATP (biotin carboxy- second, the carboxyl group is transfered from carboxybiotin to the acceptor converting it into methylmalonyl-CoA. The proposed ac- ie biotin carboxylase is well conserved in evolution and spans about uino acids (residue 244 to 344 in PCCα). Propionic acidemia pa- ; mutations in the PCCA gene encoding the α-subunit were identified lementation experiments after somatic fusion of patient fibroblasts. fferent mutations found, two are localized to the vicinity of the bi- se site, D343G and G354V. These mutations may result in defective of biotin. To investigate the first half reaction by detection of the intermediate, we have subcloned the entire PCCα into a bacterial ex- r and stably expressed it in *E.coli* where it was efficiently biotinylated. his α-subunit-biotin substrate for the ATP-dependent carboxylation noiety in the analysis of patient mutations.

1013

A novel point mutation in α-galactosidase A gene causing Fabry disease in Chinese. C.-H. Chen[1,2], P.-W. Shyu[3], S.-J. Wu[4], S.-S. Sheu[5], K.-J. Hsiao.[3,4] [1]Cheng Hsin Rehabilitation and Medical Center, [2]School of Medicine, [3]Institute of Genetics, National Yang-Ming University, and [4]Dept. of Med. Res., Veterans Gen. Hosp.-Taipei; [5]Dept. of Med., Veterans Gen. Hosp.-Taichung, Taiwan.

Fabry disease is an X-linked inborn error of sphingolipid catabolism resulting from deficient enzyme activity of α-galactosidase A. The deposition of sphingolipid results in various clinical manifestations, including angiokeratoma of skin, corneal opacity, acroparesthesias, cardiovascular defects and renal dysfunction. The molecular defects of human α-galactosidase A gene causing Fabry disease have been characterized, including gene rearrangement and point mutations, which show the genetic heterogeneity in Fabry disease. The prevalence of Fabry disease in Chinese is rare, however, several Chinese patients with Fabry disease were confirmed by determination of the α-galactosidase A activity in plasma form 3 unrelated families.

To characterize the molecular defects of these patients, each exon of α-galactosidase A gene including intron-exon junctions were PCR amplified using biotin-labelled primer and sequenced using magnetic beads solid-phase sequencing. A G to C transversion was identified in the last nucleotide of exon 1 in two unrelated Chinese patients. This mutation obliterates an EcoN1 restriction site. Family studies show close linkage with the affected family members. Screening of 100 alleles (22 males, 39 females) of unrelated normal Chinese can not find this mutation. This mutation not only changes the amino acid from serine to threonine, but also likely cause splicing defects. To our knowledge, this is the first report of mutation in Chinese patients with Fabry disease, and a novel mutation causing Fabry disease not reported in literature previously.

Poster Presentations

se A pseudodeficiency in families at risk for metachromatic ohy. M.B.Coulter-Mackie[1], D.A. Applegarth[2], J. Toone[2], H. [3], Rip[3], M. Ludman[3], and J. Beis[3]. [1]B.C. Children's Hospital B.C., Vancouver; [2]CPRI and Univ. of Western Ontario, London; esearch Centre and Dalhousie Univ., Halifax, Nova Scotia;

tase A pseudodeficiency (Apd) occurs with a carrier frequency . Enzyme activity in Apd carriers may be reduced to below the ange. While the carrier status confers no clinical problems vidual, it can be a cause for concern in families with a LD. Routine enzyme assay alone may not distinguish between Apd and carriers of metachromatic leukodystrophy (MLD). The plicated in Apd have been identified and can be detected in h a combination of PCR and restriction enzyme digestion. We DNA analysis to clarify ambiguous results from the e A assays alone in two separate families where an individual s a "possible MLD carrier" married into a known MLD family. es, DNA testing showed that the "possible MLD carrier" was Apd carrier. In one case, a prenatal test was avoided. In the amniocentesis had already been done but future tests can be a third case, a family had a child with MLD. Enzymatically had carrier levels of arylsulfatase A. The child's MLD was to a paternal severe MLD allele and a de novo chromosome 22

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Correction of neutropenia in Barth syndrome by G-CSF.

G.F. Cox[1,2], M. Pulsipher[3], M. Rothenberg[2,3] M. Korson[1], and R.I. Kelley[4]. [1]Division of Genetics, [2]Howard Hughes Medical Institute, and [3]Department of Hematology, Children's Hospital and Harvard Medical School, Boston, MA; [4]Kennedy-Krieger Institute and Department of Pediatrics, Johns Hopkins Medical School, Baltimore, MD

Barth syndrome (MIM# 302060; 3-methylglutaconic aciduria, Type II; endocardial fibroelastosis, Type II) is an X-linked disorder that is being increasingly recognized as a cause of severe neutropenia and cardiomyopathy in young boys. Additional features include skeletal myopathy, growth retardation, hypocholesterolemia, 3-methylglutaconic aciduria, and mitochondrial abnormalities. Although a gene for Barth syndrome has been mapped to Xq28, neither the genetic defect nor the pathophysiology of this disease is understood. Original reports considered Barth syndrome to have a poor prognosis with death due to congestive heart failure or sepsis by three years of age. More recently, the outlook has improved with early diagnosis and aggressive medical and nutritional management. The neutropenia of Barth syndrome may be chronic or cyclic, is lifelong, and is characterized by an arrest in granulopoeisis at the myelocyte stage in bone marrow. Since granulocyte colony stimulating factor (G-CSF) promotes the growth and differentiation of granulocyte progenitors, we asked whether pharmacologic doses of exogenous G-CSF could correct the neutropenia of Barth syndrome. Two unrelated male infants with Barth syndrome, whose baseline absolute neutrophil counts (ANC) were 100-300/ul, were administered G-CSF subcutaneously at 5 μg/kg/d and 10 μg/kg/d over

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Pyridoxine-responsive epilepsy appears not to be caused by mutations in the *GAD1* or *GAD2* genes. D.-F. Bu[1], J. Christodoulou[2], M.J. Murrell[2], L. Ploder[1], W. Gibson[1], A. J. Tobin[3] and R. R. McInnes[1]. [1]Dept. Genetics, Hosp. for Sick Children, Toronto,Ontario, Canada; [2]Dept. Peds. & Child Health, Univ. Sydney, Sydney, Australia; [3]Dept. Biology, UCLA, Los Angeles, CA.

Pyridoxine-responsive epilepsy is an autosomal recessive disorder manifest in newborns or infants by convulsions that are partly or completely controlled by pharmacologic doses of pyridoxine. Several indirect lines of evidence have suggested that the disease is due to reduced activity of glutamic acid decarboxylase (GAD), a pyridoxal phosphate (PLP)-dependent enzyme that converts glutamic acid to the inhibitory neurotransmitter γ-aminobutyric acid (GABA). Two GAD isoforms of 67 & 65 kDa are encoded by related genes, *GAD1* and *GAD2*. The ratio of apo- to holo-GAD regulates GAD activity. A mutant GAD with reduced PLP affinity has been suggested to be the primary defect, particularly since some patients have reduced levels of CSF GABA. To find mutations in the ORF of either gene, we performed SSCP analyses on a cohort of 22 patients with classic (7) or atypical (15) forms of pyridoxine-responsive epilepsy; all 16 coding exons of both genes were examined. Three patients had 1 bp substitutions in introns of *GAD1*, changes well outside of splicing domains. One patient had a 2 bp insertion in the 5'UTR of *GAD1*. Two common *GAD2* polymorphisms were identified, a 1 bp substitution at the 8th position of intron 1, and a silent substitution in Gly326. In one patient but not in 50 controls, a nonconservative *GAD2* substitution, S527L, was identified in one allele. Expression studies in COS-1 cells showed that S527L had a normal *Km* and *Vmax* for PLP, although this mutant protein had mild thermolability corrected by preincubation with PLP. However, these properties are not consistent with the pyridoxine-responsive phenotype, and an unaffected sib also carried the S527L allele. No other substitutions were found by direct sequencing of all coding exons of both *GAD1* & *GAD2* of the proband, and of two other patients with unambiguous histories of pyridoxine-responsive seizures. We conclude 1) that most patients with pyridoxine-responsive epilepsy do not have mutations in *GAD1* or *GAD2* genes and 2) that a defect of another PLP-dependent enzyme involved in neurotransmitter metabolism causes this disease.

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Another Tay-Sachs pseudodeficiency mutation in the same codon as PD II in an Ashkenazi Jewish 'carrier': Dilemmas of prenatal diagnosis. J. W. Callahan, J. Warren, M. A. Skomorowski, R. Babul, J. T. R. Clarke, P. Strasberg. Hospital for Sick Children and Univ. of Toronto, Ontario, Canada

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Molecular genetics of propionic acidemia: a proposal for deficient biotin carboxylation of the α-subunit of propionyl-CoA carboxylase. E. Campeau, D. Leclerc, L. Dupuis and R. A. Gravel. McGill University/Montreal Children's Hospital, Quebec, Canada.

Propionic acidemia is a rare autosomal recessive disorder caused by a deficiency of the biotin-dependent enzyme propionyl-CoA carboxylase (PCC, $\alpha_4\beta_4$ where α contains biotin). The mechanism of PCC is divided in two half-reactions: first, bicarbonate is transfered onto the biotin upon hydrolysis of ATP (biotin carboxylase reaction); second, the carboxyl group is transfered from carboxybiotin to the propionyl-CoA acceptor converting it into methylmalonyl-CoA. The proposed active site for the biotin carboxylase is well conserved in evolution and spans about a hundred amino acids (residue 244 to 344 in PCCα). Propionic acidemia patients carrying mutations in the PCCA gene encoding the α-subunit were identified through complementation experiments after somatic fusion of patient fibroblasts. Among the different mutations found, two are localized to the vicinity of the biotin carboxylase site, D343G and G354V. These mutations may result in defective carboxylation of biotin.. To investigate the first half reaction by detection of the carboxybiotin intermediate, we have subcloned the entire PCCα into a bacterial expression vector and stably expressed it in *E.coli* where it was efficiently biotinylated. We will use this α-subunit-biotin substrate for the ATP-dependent carboxylation of the biotin moiety in the analysis of patient mutations.

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A novel point mutation in α-galactosidase A gene causing Fabry disease in Chinese. C.-H. Chen[1,2], P.-W. Shyu[3], S.-J. Wu[4], S.-S. Sheu[5], K.-J. Hsiao.[3,4] [1]Cheng Hsin Rehabilitation and Medical Center, [2]School of Medicine, [3]Institute of Genetics, National Yang-Ming University, and [4]Dept. of Med. Res., Veterans Gen. Hosp.-Taipei; [5]Dept. of Med., Veterans Gen. Hosp.-Taichung, Taiwan.

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Poster Presentations

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Arylsulfatase A pseudodeficiency in families at risk for metachromatic leukodystrophy. M.B.Coulter-Mackie[1], D.A. Applegarth[2], J. Toone[2], H. Wallance[2], J. Rip[2], M. Ludman[3], and J. Beis[2]. [1]B.C. Children's Hospital and Univ. of B.C., Vancouver; [2]CPRI and Univ. of Western Ontario, London; [3]Atlantic Research Centre and Dalhousie Univ., Halifax, Nova Scotia; Canada

Arylsulfatase A pseudodeficiency (Apd) occurs with a carrier frequency of up to 20%. Enzyme activity in Apd carriers may be reduced to below the low normal range. While the carrier status confers no clinical problems on the individual, it can be a cause for concern in families with a history of MLD. Routine enzyme assay alone may not distinguish between carriers of Apd and carriers of metachromatic leukodystrophy (MLD). The mutations implicated in Apd have been identified and can be detected in the DNA with a combination of PCR and restriction enzyme digestion. We have used DNA analysis to clarify ambiguous results from the arylsulfatase A assays alone in two separate families where an individual designated as a "possible MLD carrier" married into a known MLD family. In both cases, DNA testing showed that the "possible MLD carrier" was actually an Apd carrier. In one case, a prenatal test was avoided. In the second case, amniocentesis had already been done but future tests can be avoided. In a third case, a family had a child with MLD. Enzymatically both parents had carrier levels of arylsulfatase A. The child's MLD was attributed to a paternal severe MLD allele and a de novo chromosome 22 rearrangement. DNA testing showed that the mother carried an Apd allele accounting for her reduced enzyme level and suggesting that this family's risk of recurrence of MLD would depend on the chance of second chromosome event not on the inheritance of parental alleles. These situations are not unique to MLD and can arise in a variety of lysosomal enzyme disorders where pseudodeficiencies occur. Where possible DNA analysis as a supplement to enzyme assay should be done whenever there is any question about carrier status because it can prevent unnecessary expen: , needless medical procedures and parental anxiety.

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Correction of neutropenia in Barth syndrome by G-CSF.

G.F. Cox[1,2], M. Pulsipher[3], M. Rothenberg[2,3] M. Korson[1], and R.I. Kelley[4]. [1]Division of Genetics, [2]Howard Hughes Medical Institute, and [3]Department of Hematology, Children's Hospital and Harvard Medical School, Boston, MA; [4]Kennedy-Krieger Institute and Department of Pediatrics, Johns Hopkins Medical School, Baltimore, MD

Barth syndrome (MIM# 302060; 3-methylglutaconic aciduria, Type II; endocardial fibroelastosis, Type II) is an X-linked disorder that is being increasingly recognized as a cause of severe neutropenia and cardiomyopathy in young boys. Additional features include skeletal myopathy, growth retardation, hypocholesterolemia, 3-methylglutaconic aciduria, and mitochondrial abnormalities. Although a gene for Barth syndrome has been mapped to Xq28, neither the genetic defect nor the pathophysiology of this disease is understood. Original reports considered Barth syndrome to have a poor prognosis with death due to congestive heart failure or sepsis by three years of age. More recently, the outlook has improved with early diagnosis and aggressive medical and nutritional management. The neutropenia of Barth syndrome may be chronic or cyclic, is lifelong, and is characterized by an arrest in granulopoeisis at the myelocyte stage in bone marrow. Since granulocyte colony stimulating factor (G-CSF) promotes the growth and differentiation of granulocyte progenitors, we asked whether pharmacologic doses of exogenous G-CSF could correct the neutropenia of Barth syndrome. Two unrelated male infants with Barth syndrome, whose baseline absolute neutrophil counts (ANC) were 100-300/μl, were administered G-CSF subcutaneously at 5 μg/kg/d and 10 μg/kg/d over several days. At the lower dose of G-CSF, the ANC rose only slightly, whereas at the higher dose the ANC increased to >10,000 by three days in both patients. Granulocytes appeared as band forms or mature cells in blood, indicating effective granulopoeisis. Cessation of G-CSF led to a return to the neutropenic state. We conclude that exogenous G-CSF is capable of transiently correcting the neutropenia of Barth syndrome in a dose-dependent manner. The administration of G-CSF during times of suspected or known bacterial infection may be effective in reducing the incidence of serious bacterial infections and death due to sepsis to patients with Barth syndrome.

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