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First contiguous gene deletion causing biotinidase deficiency: The enzyme deficiency in three Sri Lankan children

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ABSTRACT

We report three symptomatic children with profound biotinidase deficiency from Sri Lanka. All three children presented with typical clinical features of the disorder. The first is homozygous for a missense mutation in the *BTD* gene (c.98_104 del7insTCC; p.Cys33PhefsX36) that is commonly seen in the western countries, the second is homozygous for a novel missense mutation (p.Ala439Asp), and the third is the first reported instance of a contiguous gene deletion causing the enzyme deficiency. In addition, this latter finding exemplifies the importance of considering a deletion within the *BTD* gene for reconciling enzymatic activity with genotype, which can occur in asymptomatic children who are identified by newborn screening.

1. Introduction

Biotinidase (EC 3.5.1.12) is the enzyme responsible for cleaving and recycling biotin from biocytin and from dietary protein-bound sources [1,2]. Profound biotinidase deficiency (less than 10% of mean normal serum activity) (OMIM #253260) is an autosomal recessively inherited metabolic disorder [3]. Individuals with profound biotinidase deficiency, if untreated, usually exhibit seizures, hypotonia, skin rash, alopecia, vision problems, hearing loss, and developmental delay with accompanying ketolactic acidosis and organic aciduria [2,3]. Symptoms of the disorder can be successfully improved or prevented with pharmacological doses of oral biotin. However, if untreated, vision or hearing problems and developmental delay occur, they are usually irreversible [3]. Although all states in the United States and many countries screen their newborns for the disorder, some countries do not. Sri Lanka is one of the countries that do not screen for the disorder.

The gene for biotinidase has been isolated and characterized [4,5] and over 150 mutations causing biotinidase deficiency have been identified [6]. We now report the mutations responsible for profound biotinidase deficiency in three symptomatic children from Sri Lanka. Two of these children are from consanguineous parents, whereas one child was placed in foster care and consanguinity could not be established. One child is homozygous for one of the more common mutations found in children in the United States, a second child is homozygous for a novel missense mutation, and the third child is homozygous for a contiguous gene deletion that encompassed three genes, including *BTD*. This latter deletion is instructive in that it exemplifies the importance of reconciling enzymatic activity with the genotype especially for confirming if putative positive asymptomatic children by newborn screening actually have biotinidase deficiency.

2. Materials and methods

2.1. Subjects

Individuals with biotinidase deficiency were identified because they developed symptoms that prompted follow-up by measurement of biotinidase activity in blood spots used for newborn screening in Sri Lanka [7]. Subsequently, profound biotinidase deficiency (less than 10% of mean normal activity) was found in all three children. Family and clinical history were obtained with the consent of the families.

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Fig. 1. The 107 kb contiguous gene deletion (black bar) in Patient 3. The microarray data indicates that Patient 3 is homozygous for the deletion. The deletion includes exon 1 of the *BTD* gene, the entire *HACL1* gene and a small portion of the 5′-end of the *COLQ* gene encompassing exon 1. Each of the parents is heterozygous for the identical deletion (not shown).

2.2. Mutation analysis

Blood for DNA was obtained from these children and from their parents when possible to confirm allelic assignment. Genomic DNA was isolated from peripheral blood lymphocytes using the Gentra Puregene DNA isolation kit (Research Triangle Park, NC) according to the manufacturer's recommendations. The concentration of DNA in each sample was calculated from the optical density at 260 nm and diluted to a concentration of 0.05 μg/μL. DNA sequencing of the biotinidase (BTD) gene was performed by PCR amplification using primers and conditions described previously [8]. All exonic and intron–exon boundaries of the *BTD* gene were sequenced.

2.3. Deletion studies

DNA labeling was carried out with the Enzo CGH labeling kit for oligo arrays (Enzo Life Sciences, Plymouth Meeting, PA). Array CGH was performed with 0.5 μg of DNA according to the manufacturer's protocol (Agilent Technologies, Santa Clara, CA) using probes for each exon and flanking introns of the genes on the array as previously described [6].

2.4. Microarray studies

High density SNP microarray studies were performed using Affymetrix Cytoscan HD SNP Array™. Briefly, whole genome PCR amplification is performed on DNA extracted from peripheral blood followed by hybridization, staining, washing and scanning. Data is then analyzed using Affymetrix Chromosome Analysis Suite™ (ChAS) software (Genome build: hg19). This array contains 2.67 million copy number markers/probes (1.9 million non-polymorphic probes/markers and 750,000 SNP probes/markers) that detect copy number variations (CNVs), loss of heterozygosity (LOH), and segmental or whole chromosome uniparental isodisomy. It has coverage of 12,000 OMIM genes with global resolution of 5–10 kb.

3. Case studies

Patient 1, a male, was the 36 1/2-week product of a normal pregnancy. There were no prenatal complications. The child was given to foster parents at nine days of age. He first developed seizures at seven weeks of age which did not respond to antiepileptic medications. He exhibited screaming episodes, staring gaze, jitteriness and lethargy.

A dried blood spot test revealed elevated 3-hydroxyisovalerylcarnitine and undetectable biotinidase activity (deficient activity is less than 0.029 AU; normal activity is greater than 0.219 AU). Samples were not available from the parents. He improved clinically on biotin therapy and is now asymptomatic at 11 months of age.

Mutation analysis revealed that this boy was homozygous for c.98_104 del7insTCC; p.C33FfsX36 in the *BTD* gene [6]. This mutation is a relative common mutation in Western European countries and results in a frameshift and the synthesis of a truncated, inactive enzyme protein causing profound biotinidase deficiency.

Patient 2, a male, is the product of first cousin parents. The child was well until two months of age when he exhibited seizures which did not respond to antiepileptic medications. He developed global developmental delay. At two years of age he had increased frequency of the seizures, tachypnea, metabolic acidosis, alopecia and a seborrheic skin rash. He was admitted to the intensive care unit of the hospital. A blood sample