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Biochemical, molecular, and clinical characteristics of children with short chain acyl-CoA dehydrogenase deficiency detected by newborn screening in California

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ABSTRACT

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 frequencies have been shown between patients diagnosed clinically versus those diagnosed by newborn screening. Moreover, while patients diagnosed clinically have a variable clinical presentation including developmental delay, ketotic hypoglycemia, epilepsy and behavioral disorders, studies suggest patients diagnosed by newborn screening are largely asymptomatic. Scant information is published about the biochemical, genetic and clinical outcome of SCADD patients diagnosed by newborn screening.

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

Results: During the study period, 2,632,058 newborns were screened and 76 confirmed SCADD cases were identified. No correlations between initial C4 value and follow-up biochemical markers (C4, EMA or MS levels) were found in the 76 cases studied. We found significant correlation between urine EMA versus MS, and correlation between follow-up C4 versus urine EMA. Of 22 cases where ACADS gene sequencing was performed: 7 had two or more deleterious mutations; 8 were compound heterozygotes for a deleterious mutation and common variant; 7 were homozygous for the common variant c.625 G > A; and 1 was heterozygous for c.625 G > A. Significant increases in mean urine EMA and MS levels were noted in patients with two or more deleterious mutations versus mutation heterozygotes or common polymorphism homozygotes. Clinical outcome data was available in 31 patients with follow-up extending from 0.5 to 60 months. None developed epilepsy or behavioral disorders, and three patients had isolated speech delay. Hypoglycemia occurred in two patients, both in the neonatal period. The first patient had concomitant meconium aspiration; the other presented with central apnea, poor feeding, and hypotonia. The latter, a c.625 G > A homozygote, has had persistent elevations in both short- and medium-chain acylcarnitines; diagnostic workup in this case is extensive and ongoing.

Conclusions: This study examines the largest series to date of SCADD patients identified by newborn screening. Our results suggest that confirmatory tests may be useful to differentiate patients with common variants from those with deleterious mutations. This study also provides evidence to suggest that, even when associated with deleterious mutations, SCADD diagnosed by newborn screening presents largely as a benign condition.

1. Introduction

Short-chain acyl-CoA dehydrogenase (SCAD, MIM 606885) catalyzes the first step in mitochondrial short-chain B-oxidation. SCAD deficiency (SCADD, MIM 201470) is an autosomal recessive inborn

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error of mitochondrial fatty acid oxidation with highly variable biochemical, genetic, and clinical characteristics [1]. SCAD is in the family of acyl-CoA dehydrogenases (ACADs), which are encoded in the nuclear genome but function in the mitochondria to catalyze alpha, beta-dehydrogenation of acyl-CoA esters, passing electrons to electron transferring flavoprotein (ETF) [2]. As butyryl-CoA (C4-CoA) is the primary substrate of SCAD, a deficiency of this enzyme is associated with accumulation of C4-CoA byproducts including butyrylcarnitine (C4), butyrylglycine, ethylmalonic acid (EMA), and methylsuccinic acid (MS) in blood, urine and cells [3].

To date, more than 35 inactivating mutations and two missense variants,c.511 C>T (R147W) and c.625 G>A (G185S) have been reported in the SCAD gene [4–6]. In a United States population study of 694 samples, the allele frequency of the c.625 G>A variant was found to be 22% and that of the c.511 C>T variant was 3% [7]. Additionally, 7% of the population was found to be either homozygous for one of these variants or compound heterozygous for each. Both the common *ACADS* variants and most reported deleterious mutations cause single amino acid substitutions, leading to protein misfolding and aggregation [6,8]. Studies in a prokaryotic system showed the common variant proteins R147W and G185S to be stable, with catalytic properties similar to wild-type enzyme [8]. Genotype–phenotype correlation is weak and poorly understood [1].

Prior to expanded newborn screening, patients were identified by clinical symptoms and diagnosed by selective screening. These patients suffered most frequently from developmental delay, ketotic hypoglycemia, epilepsy and behavioral disorders [1,9–11]. However, almost all relatives subsequently diagnosed with SCADD have remained asymptomatic [1,11]. The introduction of tandem mass spectrometry allowed for great expansion in the number of disorders simultaneously and inexpensively detectable by screening [12,13]. Far more cases of certain inborn errors, such as SCADD and medium-chain acyl Co-A dehydrogenase deficiency (MCADD), have been identified through newborn screening than were previously identified by clinical symptoms and selective screening. Evidence for utility of newborn screening for SCADD is lacking. Distinct differences in medical and neurodevelopmental outcome have been demonstrated between SCADD patients diagnosed by selective screening versus expanded newborn screening, with the latter remaining largely asymptomatic after the immediate newborn period [14]. There are numerous hypotheses for this phenomenon: early detection and medical intervention prevent adverse consequences; SCADD confers a disease susceptibility state but requires multiple genetic and/or environmental interactions to manifest clinically; SCADD is benign with diagnosis of cases prior to newborn screening reflecting ascertainment bias. The inclusion of SCADD in the newborn screening program remains controversial. Therefore, there is a critical need for long-term follow-up data on SCADD patients diagnosed via newborn screening.

In this study, we examine California newborn screening data for SCADD from the inception of the program, September 2005, through April 2010. We analyze retrospectively 31 cases of SCADD diagnosed by newborn screening. We are reporting the initial C4 values, follow-up testing, medical and developmental outcome of these 31 patients.

2. Materials and methods

2.1. Patients

SCADD was defined by the presence of: increased C4 or C4 and another marker above the cutoff value on newborn screen dried blood spot followed by increased confirmatory C4 and/or EMA, with or without increased MS. Cases of increased C4 and additional markers were included in the study, with the exception of isolated C4 plus C5 (isovaleryl- or methylbutyrylcarnitine) elevation, which were excluded. The cutoff value for positive C4 was established by the California State Newborn Screening laboratory. To calculate overall prevalence, individuals were included who had been identified by the State of California's Newborn Screening Program as having a positive result for SCADD from September, 2005 through April, 2010.

Three metabolic centers in California, CHOC Children's, University of California at Los Angeles, and University of California at San Diego, participated in this study by providing detailed biochemical, molecular and clinical data.

2.2. Biochemistry

Newborn screening was performed by the California Newborn Screening Program using tandem mass spectrometry. Additional testing was performed at the following accredited laboratories using standard methodology: Quest Diagnostics, Inc., Nichols Institute (plasma acylcarnitines, quantitative and qualitative urine organic acids), CHOC Children's (dried blood spot acylcarnitines), University of California at San Diego (plasma acylcarnitines, quantitative urine organic acids), and Mayo Clinic Biochemical Genetics Laboratory (urine acylglycines). Confirmatory C4 levels were obtained either by plasma or dried blood spot acylcarnitines. EMA and MS were evaluated either by urine organic acid, or urine acylglycine analysis.

2.3. DNA analysis

Molecular sequencing of *ACADS* was performed either at Mayo Clinic Molecular Genetics Laboratory or at Prevention Genetics using their proprietary methods. Some patients had targeted mutation analysis (for c.625 G>A, c.511 C>T, and c.529 C>T) only, performed at Quest Diagnostics, Inc., Nichols Institute. Novel missense mutations were evaluated for likelihood to be deleterious using three separate software programs which have been previously described: Polyphen [15], Polyphen-2 [16], and SIFT [17,18].

2.4. Clinical outcome and treatment

Patient information was obtained by retrospective chart review, following each institution's guidelines. Number of hospitalizations, episodes of hypoglycemia as defined by serum glucose less than 40 mg/dL in a neonate (age less than one month) or 60 mg/dL in older children, episodes of acidosis as defined by serum bicarbonate less than 17 mmol/L, developmental status, age at last evaluation, and treatment were summarized. Developmental delay was assigned if it was mentioned by the physician in the patient's medical chart. Other abnormalities such as seizures, apnea, and behavioral disorders, were noted if present in the patient's chart.

2.5. Statistical analysis

Confirmatory C4, EMA and MS were included as percentages of upper normal limits to control for various upper normal limits of different biochemical laboratories. All DNA changes in *ACADS* except for the common variants c.625 G>A and c.511 C>T were considered to be mutations for the purpose of statistical analysis. Associations between continuous variables were assessed by first fitting a smoothing spline to assess linearity on the log scale. The Pearson correlation was computed on the appropriate log scale. Means were compared using analysis of variance methods on the appropriate log scale. The post hoc p values under this model were computed using the Fisher criterion for pairwise comparisons. Geometric means and their standard errors are reported on the original scale. All analyses were done using JMP® Statistical Discovery Software.