Molecular Genetics and Metabolism 110 (2013) 111–115

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

Prevalence and mutation analysis of short/branched chain acyl-CoA dehydrogenase deficiency (SBCADD) detected on newborn screening in Wisconsin

Sandra C. Van Calcar [a,b,1], Mei W. Baker [b,c,d,1], Phillip Williams [c], Susan A. Jones [c], Blia Xiong [a], Mai Choua Thao [a], Sheng Lee [a], Mai Khou Yang [a], Greg M. Rice [a,b,c], William Rhead [e], Jerry Vockley [f], Gary Hoffman [d], Maureen S. Durkin [a,g,*]

[a] Biochemical Genetics Program, Waisman Center, University of Wisconsin–Madison, Madison, WI, USA
[b] Department of Pediatrics, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA
[c] Biochemical Genetics Laboratory, Wisconsin State Laboratory of Hygiene, University of Wisconsin–Madison, Madison, WI, USA
[d] Newborn Screening Program, Wisconsin State Laboratory of Hygiene, University of Wisconsin–Madison, Madison, WI, USA
[e] Genetics Clinic, Children's Hospital of Wisconsin, Medical College of Wisconsin, Milwaukee, WI, USA
[f] Department of Pediatrics, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA
[g] Department of Population Health Sciences, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA

ARTICLE INFO

Article history:
Received 10 February 2013
Received in revised form 29 March 2013
Accepted 29 March 2013
Available online 15 April 2013

Keywords:
Short/branched chain acyl-CoA dehydrogenase deficiency
2-Methylbutyryl-CoA dehydrogenase deficiency
Expanded newborn screening
Isovaleric acidemia
Inborn errors of metabolism

ABSTRACT

Short/branched chain acyl-CoA dehydrogenase deficiency (SBCADD), also called 2-methylbutyryl CoA dehydrogenase deficiency (2-MBCDD), is a disorder of L-isoleucine metabolism of uncertain clinical significance. SBCADD is inadvertently detected on expanded newborn screening by elevated 2-methylbutyrylcarnitine (C5), which has the same mass to charge (m/s) on tandem mass spectrometry (MS/MS) as isovalerylcarnitine (C5), an analyte that is elevated in isovaleric acidemia (IVA), a disorder in leucine metabolism. SBCADD cases identified in the Hmong-American population have been found in association with the c.1165 A>G mutation in the *ACADSB* gene. The purposes of this study were to: (a) estimate the prevalence of SBCADD and carrier frequency of the c.1165 A>G mutation in the Hmong ethnic group; (b) determine whether the c.1165 A>G mutation is common to all Hmong newborns screening positive for SBCADD; and (c) evaluate C5 acylcarnitine cut-off values to detect and distinguish between SBCADD and IVA diagnoses. During the first 10 years of expanded newborn screening using MS/MS in Wisconsin (2001–2011), 97 infants had elevated C5 values (≥0.44 μmol/L), of whom five were Caucasian infants confirmed to have IVA. Of the remaining 92 confirmed SBCADD cases, 90 were of Hmong descent. Mutation analysis was completed on an anonymous, random sample of newborn screening cards (n = 1139) from Hmong infants. Fifteen infants, including nine who had screened positive for SBCADD based on a C5 acylcarnitine concentration ≥0.44 μmol/L, were homozygous for the c.1165 A>G mutation. This corresponds to a prevalence in this ethnic group of being homozygous for the mutation of 1.3% (95% confidence interval 0.8–2.2%) and of being heterozygous for the mutation of 21.8% (95% confidence interval 19.4–24.3%), which is consistent with the Hardy–Weinberg equilibrium. Detection of homozygous individuals who were not identified on newborn screening suggests that the C5 screening cut-off would need to be as low as 0.20 μmol/L to detect all infants homozygous for the *ACADSB* c.1165 A>G mutation. However, lowering the screening cut-off to 0.20 would also result in five "false positive" (non-homozygous) screening results in the Hmong population for every c.1165 A>G homozygote detected. Increasing the cut-off to 0.60 μmol/L and requiring elevated C5/C2 (acetylcarnitine) and C5/C3 (propionylcarnitine) ratios to flag a screen as abnormal would reduce the number of infants screening positive, but would still result in an estimated 5 infants with SBCADD per year who would require follow-up and additional biochemical testing to distinguish between SBCADD and IVA diagnoses. Further research is needed to determine the clinical outcomes of SBCADD detected on newborn screening and the c.1165 A>G mutation before knowing whether the optimal screening cut-off would minimize true positives or false negatives for SBCADD associated with this mutation.

Abbreviations: SBCADD, short/branched chain acyl-CoA dehydrogenase deficiency; 2-MBCD, 2-methylbutyryl CoA dehydrogenase deficiency; C2, acetylcarnitine; C3, propionylcarnitine; C5, 2-methylbutyrylcarnitine or isovalerylcarnitine; IVA, isovaleric acidemia; MS/MS, tandem mass spectrometry; VLCADD, very long chain acyl-CoA dehydrogenase deficiency; 3-MCC, 3-methylcrotonyl-CoA dehydrogenase deficiency; MCADD, medium chain acyl-CoA dehydrogenase deficiency; SCADD, short chain acyl-CoA dehydrogenase deficiency.

* Corresponding author at: Population Heath Sciences, University of Wisconsin School of Medicine and Public Health, 1500 Highland Ave., Madison, WI 53705, USA. Fax: +1 608 263 2820.
E-mail address: mdurkin@wisc.edu (M.S. Durkin).
[1] Sandra Van Calcar and Mei Baker have contributed equally to this article.

1096-7192/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ymgme.2013.03.021

1. Introduction

Short/branched chain acyl-CoA dehydrogenase deficiency (SBCADD, OMIM # 600301), also called 2-methylbutyryl CoA dehydrogenase deficiency (2-MBCDD), is a disorder in isoleucine metabolism detected by expanded newborn screening with an elevated 2-methylbutyrylcarnitine (C5) and elevated C5/C3 (propionylcarnitine) and C5/C2 (acetylcarnitine) ratios. The C5 acylcarnitine and C5/C3 and C5/C2 ratios are also elevated in isovaleric acidemia (IVA, OMIM# 243500) as isovalerylcarnitine and 2-methylbutyrylcarnitine are indistinguishable when measured by tandem mass spectrometry (MS/MS).

SBCADD was first described in 1999 in a child with neurodevelopmental impairments [1]. Prior to expanded screening, the clinical outcome of this disorder was limited to case reports of five individuals from three families, including three children with moderate to severe developmental disabilities, seizures, muscular atrophy, hypotonia and/or cerebral palsy and two asymptomatic relatives [1–4].

Since these initial cases, 33 additional cases of SBCADD have been reported in the literature [5–13]. These represent a wide range of individuals from various countries and ethnic backgrounds. Seven of the 33 cases were ascertained in children with various clinical symptoms who were referred for metabolic evaluation. Symptoms included developmental delay, intellectual disability, seizures, autism and neonatal crisis [5–9,11,13]. The remaining 26 cases were detected by MS/MS during routine newborn screening or were siblings diagnosed as part of family studies. The majority of cases in this latter group are reported to have apparently normal development without significant health problems [5,6,8,10,12,13].

With the expansion of newborn screening using MS/MS, SBCADD is not the only disorder found to have a higher frequency and greater clinical variability than initially believed. Our understanding of the natural history of the protein metabolism disorders IVA [14] and 3-methylcrotonyl-CoA dehydrogenase deficiency (3-MCC) [15], as well as several disorders in fatty acid oxidation including very long chain acyl-CoA dehydrogenase deficiency (VLCADD) [16,17], medium chain acyl-CoA dehydrogenase deficiency (MCADD) [18] and short chain acyl-CoA dehydrogenase deficiency (SCADD) [19], has evolved over the past decade to include greater recognition of milder, and often sub-clinical variants.

With the initiation of MS/MS screening in Wisconsin, a higher than expected number of screens with elevated C5 acylcarnitine concentrations were observed. Biochemical testing confirmed diagnoses of SBCADD rather than IVA in most newborns with positive screens. During the first year of expanded screening, eight infants born in Minnesota and Wisconsin were identified with SBCADD [6]. All of these infants were from the Hmong ethnic group. The Hmong living in the United States arrived primarily from Laos via refugee camps in Thailand where they were forced to emigrate as a result of their pro-American involvement in the war in Indochina in the 1960s. Today, the largest Hmong-American communities are located in California, Minnesota and Wisconsin [20].

In these eight Hmong infants diagnosed with SBCADD, one was noted to have mild hypotonia at 6 months of age; all others were clinically asymptomatic without any developmental delays during infancy. Molecular genetic studies performed on three of these cases revealed all to be homozygous for a single mutation, c.1165A>G, causing skipping of exon 10 of the *ACADSB* gene [6]. This history suggests the possibility of a founder mutation in the *ACADSB* gene in the Hmong population.

Here, we provide updated information on the number of children identified with SBCADD during the first 10 years of MS/MS screening in Wisconsin (April 2001–March 2011), present *ACADSB* c.1165 A>G analysis results from a random sample of newborn screening (NBS) cards from Hmong infants, and evaluate the prevalence of SBCADD and carrier frequency in the population. In addition, we provide an analysis of the detection rate of the homozygous *ACADSB* mutation c.1165 A>G at various C5 cut-off values in the Wisconsin Hmong population.

2. Methods

Using non-derivatized MS/MS technology, infants with SBCADD were initially identified with a C5 acylcarnitine concentration equal to or greater than the established cut-off of 0.44 μmol/L. In these cases, the C5/C2 (cut-off ≥ 0.05) and C5/C3 (cut-off ≥ 0.50) ratios may or may not have been elevated in either the initial or repeat screens. The SBCADD diagnosis was confirmed with detection of 2-methylbutyrylglycine in urine acylglycine analysis and/or elevated 2-methylbutyric acid in quantitative urine organic acid analysis [13,21]. Analysis of the c.1165 A>G mutation was completed on a dried blood spot from each infant's screening card or a buccal swab collected during a home visit if a dried blood NBS specimen was no longer available. All study procedures were approved by the University of Wisconsin Health Science Institutional Review Board prior to initiation of the study.

To determine the prevalence and carrier frequency of the c.1165 A>G mutation in the Hmong ethnic group, mutation analysis was completed on dried blood spots from a randomly selected, anonymous set of newborn screening cards (n = 1130) from infants of Hmong descent born between 2004 and 2007 who did not flag with C5 concentrations ≥ 0.44 μmol/L. This sample size corresponds to the average number of Hmong births in Wisconsin each year [22]. To this cohort, initial C5 concentrations from 9 Hmong infants with biochemically confirmed SBCADD were added to the analysis. This number represents the average number of infants screening positive for SBCADD each year in Wisconsin.

2.1. DNA isolation and ACADSB c.1165 A>G mutation analysis

Genomic DNA from dried blood NBS specimens was isolated using Generation DNA Purification Solution and Generation DNA Elution Solution (Qiagen, Valencia, CA) in a 96-well plate format. A 3.2 mm disk from each NBS card was punched into a MicroAmp™ Optical 96-Well Reaction Plate well (Applied Biosystems, Foster City, CA) using a standard MultiPuncher (PerkinElmer, Waltham, MA), washed twice with 90 μL of Purification Solution, followed by washing once with 90 μL of Elution Solution. 40 μL of autoclaved Milli-Q H_2O was then added to each well, and the plate was heated at 99 °C for 25 min. Genomic DNA from buccal cells was isolated using Gentra Puregene Buccal Cell Kit (Qiagen, Valencia, CA) according to the manufacturer protocol. Briefly, buccal cells were collected by scraping the inside of the mouth with a Buccal Collection Brush. Buccal cell lysis took place after the cells were incubated with Cell Lysis Solution and proteinase K at 65 °C for 25 min. Following protein precipitation, a genomic DNA pellet was obtained by mixing the supernatant with isopropanal and Glycogen Solution. The DNA pellet was washed with 70% ethanol, and dissolved in DNA Hydration Solution.

The PCR reaction was carried out in a total volume of 25 μL, containing 4 μL of genomic DNA, 1 μM of each forward and reverse primer, 200 μM DNTPs, 2.5 mM $MgCl_2$, and 2.5 U of *Taq* polymerase. The reaction mix underwent 1 cycle of 5 min at 95 °C, 32 cycles of 30 s at 95 °C, 30 s at 64 °C, 40 s at 72 °C and 1 cycle of 2 min at 72 °C. The PCR products are 260 bp and flank the *ACADSB* c.1165 A>G site. The sequences of the primers used in the assays are available upon request. 10 μL of the PCR products were then incubated with restriction enzyme BtsCI at 50 °C for 2 h followed by electrophoresis in 3% agarose gel. The wild type fragments were cut into two fragments of 201 bp and 59 bp. The *ACADSB* c.1165 A>G mutation abolishes the BtsCI recognition site, and the PCR products remained 260 bp.