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

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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

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MS/MS in Wisconsin (2001–2011), 97 infants had elevated C5 values ($> 0.44 \mu\text{mol/L}$), 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 concentrations $> 0.44 \mu\text{mol/L}$, 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 $0.20 \mu\text{mol/L}$ to detect all infants homozygous for the *ACADS* 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 $0.60 \mu\text{mol/L}$ 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.

Keywords

Short/branched chain acyl-CoA dehydrogenase deficiency; 2-Methylbutyryl-CoA dehydrogenase deficiency; Expanded newborn screening; Isovaleric academia; Inborn errors of metabolism

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 neuro-developmental 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 $\mu\text{mol/L}$. 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 $\mu\text{mol/L}$. 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 H₂O 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 isopropanol 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 MgCl₂, 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.

2.2. Statistical analysis

Mean values and standard deviations were computed for C5 acylcarnitine concentrations and C5/C2 and C5/C3 ratios. Proportions, including prevalence, are given with 95% confidence intervals (CI) that were computed with continuity correction [23]. To evaluate the validity of C5 acylcarnitine for detection of the c.1165 A>G mutation and the specificity and sensitivity of the 0.44 $\mu\text{mol/L}$ cut-off, a receiver operating curve (ROC) was calculated [24]. The ROC curve plots the false-positive rate of a screening test against its sensitivity under increasing cut-off values [25]. The level of statistical significance was $P < 0.05$ for all analyses using two-tailed comparisons. All analyses were performed using IBM SPSS Statistics Version 20 software (2011, <http://www.01.ibm.com/software/analytics/spss/products/statistics/>). The Hardy–Weinberg equation was used to predict the homozygosity and heterozygosity of *ACADSB* c. 1165 A>G in the Wisconsin Hmong population [26].

3. Results

In the 10 years following initiation of expanded newborn screening in Wisconsin in April of 2001, 97 infants were detected with elevated C5 concentrations $\geq 0.44 \mu\text{mol/L}$ with subsequent biochemical analyses confirming either an IVA ($n = 5$) or SBCADD ($n = 92$) diagnosis in this cohort. Of the 92 infants diagnosed with SBCADD, 90 were of Hmong descent and two were Caucasian. This represents a prevalence of screening positive for SBCADD of 1 in 131 (95% CI 1 in 162.3 to 107.5) or 7.6 per 1000 (95% CI 6.2–9.3 per 1000) Hmong births. In comparison, the approximate prevalence of SBCADD in non-Hmong births in Wisconsin during the same time period was 1 in 540,780 (95% CI 1 in 1,000,000 to 1 in 76,923).

For the 92 confirmed cases of SBCADD, the initial mean blood spot C5 acylcarnitine was $0.69 \pm 0.03 \mu\text{mol/L}$ (range 0.44 to $2.05 \mu\text{mol/L}$) collected at 47 ± 7 h of age. On repeat, the mean C5 concentration was $1.24 \pm 0.06 \mu\text{mol/L}$ (range 0.52 to $3.56 \mu\text{mol/L}$) collected at 527 ± 66 h of age. Both the C5/C3 and C5/C2 ratios were elevated in 13 and 73 of the 92 infants on the first and second screen, respectively (Table 1). Initial C5 elevations measured in NBS spots from two Caucasian infants with confirmed SBCADD were 0.77 and $0.62 \mu\text{mol/L}$, within the range of values measured in NBS spots from the infants of Hmong descent. In contrast, initial C5 concentrations in five infants with a confirmed diagnosis of IVA averaged $3.18 \pm 0.37 \mu\text{mol/L}$ (range 2.41 to $4.15 \mu\text{mol/L}$, age of collection 32.2 ± 9.5 h) (Table 1). Of the 92 confirmed cases of SBCADD, analysis of the *ACADSB* c.1165 A>G mutation was completed on NBS spots from 74 (80%) of these cases. Of these, 69 were homozygous (93%), three heterozygous (4%) and two were wild-type (3%) for this mutation. The c.1165 A>G mutation was not found in blood spots from the two non-Hmong infants diagnosed with SBCADD.

3.1. Analysis of a cohort of anonymous newborn screening cards

In the randomly selected sample of 1139 newborn screening cards from Hmong infants, including nine screening positive for SBCADD based on the $0.44 \mu\text{mol/L}$ cut-off value for C5 acylcarnitine, mutation analysis found 876 (76.9%) were wild-type, 248 (21.8%) were heterozygous and 15 (1.3%, including all nine screening positive and six additional infants)

were homozygous for the common c.1165 A>G mutation (Table 2). This corresponds to a prevalence of homozygosity for the mutation of 1 in 77 infants or 1.3% (95% CI 0.8–2.2%) and a carrier frequency of 21.8% (95% CI 19.4–24.3%) in the Hmong ethnic group. Based on the Hardy–Weinberg equation, the predicted homozygosity and heterozygosity is 1.5% and 21.3%, respectively.

The ROC curve showed a highly significant relationship between C5 concentrations and the probability of being homozygous for the c.1165 A>G mutation, with an area under the curve of 99.4% (95% CI: 98.6–1.00%); $P = 0.001$. The sensitivity and specificity of the 0.44 $\mu\text{mol/L}$ cut-off were 60% (95% CI 32.9–80.2%) and 100% (95% CI 99.6–100%), respectively (Table 3). Lowering the cut-off to 0.20 $\mu\text{mol/L}$ would detect all infants homozygous for the c.1165 A>G mutation but would also result in the referral of 74 “false positive” (non-homozygous) screens each year or five for every true positive screen (Table 3). Increasing the cut-off from 0.44 to 0.60 $\mu\text{mol/L}$ decreases the number expected to screen positive each year from nine to seven and lowers the sensitivity for detecting homozygosity for the c.1165 A>G mutation from 60% to 46.7% (Table 3). The number expected to screen positive each year is reduced further (from seven to five) using the 0.60 $\mu\text{mol/L}$ cut-off for C5 along with both C5/C2 and C5/C3 ratios elevated above the established cut-offs (Table 3).

4. Discussion

The aims of this study were to obtain a precise estimate of the prevalence of SBCADD, confirm that SBCADD in the Hmong-American population is associated with the c. 1165 A>G mutation, and evaluate how various newborn screening C5 concentration cut-off values affect the detection rate of this disorder. Clearly, there is a high prevalence of SBCADD in the Hmong ethnic group with an estimated frequency of 13 cases in every 1000 births, if the diagnosis is based on detection of homozygosity of the c. 1165A>G mutation. Our failure to find the c. 1165 A>G mutation in the two white infants with SBCADD provides further evidence of a specific common mutation in the Hmong ethnic group, possibly explained by a founder effect [6,13]. A founder effect is plausible given the isolated nature of Hmong refugees in Southeast Asia and, for many, only recent relocation in the United States. Our observation that the homozygosity and heterozygosity of the c. 1165 A>G mutation are in Hardy–Weinberg equilibrium is further evidence of the population’s stability.

An important strength of this study is that a large, population-based sample of newborn screening specimens allowed for precise estimation of the prevalence of the c. 1165 A>G mutation and the validity of varying C5 concentration cut-off values for detecting it. An important limitation, however, is that analysis was limited to identifying the c. 1165 A>G mutation and did not identify other possible mutations in the *ACADSB* gene in the three infants of Hmong descent with biochemically confirmed SBCADD who were heterozygous for the c. 1165 A>G mutation or in the two Caucasian infants with wild-type mutations. Previous studies have identified other mutations in the *ACADSB* gene in the non-Hmong population [11–13]. Another limitation of this study is that it is restricted to an analysis of anonymous newborn screening specimens and does not include information on clinical outcomes associated with SBCADD or the c. 1165 A>G mutation. Further research is

needed to determine these clinical outcomes and whether newborn screening for SBCADD is merited.

Since isovalerylcarnitine is also a C5 acylcarnitine that is indistinguishable from 2-methylbutyrylcarnitine, detection of elevated C5 concentrations, along with elevated C5/C3 and C5/C2 ratios, can also signal a potential identification of IVA. While the clinical outcome of SBCADD remains unclear, it is known that IVA can cause metabolic acidosis early in infancy and typically requires long-term dietary management [27,28]. Thus, it is important to distinguish between the two disorders. In our sample, there was no overlap in the values of initial C5 concentrations for infants diagnosed with SBCADD and those diagnosed with IVA (Table 1). However, data collected as part of the Region 4 Genetics Collaborative MS/MS Project [29], which includes a large number of IVA cases, does show considerable overlap in C5 acylcarnitine concentrations between those diagnosed with SBCADD and IVA (Fig. 1). For IVA, C5 acylcarnitine concentrations ranged from 0.68 $\mu\text{mol/L}$ to 19.0 $\mu\text{mol/L}$ representing the 1st to 99th percentile, respectively. These data suggest that a C5 acylcarnitine cut-off would need to be set below 0.68 $\mu\text{mol/L}$ to prevent missing a potential positive screen for IVA.

With a cut-off for C5 acylcarnitine of 0.44 $\mu\text{mol/L}$, all cases of IVA should be detected as shown by data from both Wisconsin and the Region IV Collaborative MS/MS Project. However, this cut-off does not ascertain all infants with SBCADD. Detection of 6 infants homozygous for the c.1165A>G mutation who were not identified at the 0.44 $\mu\text{mol/L}$ cut-off suggests a greater range of C5 values in Hmong infants with SBCADD. Indeed, to detect all infants homozygous for this mutation, the C5 cut-off would need to be lowered to 0.20 $\mu\text{mol/L}$. At this cut-off, specificity would decrease to 93.4% (95% CI 91.8–94.8%) and the screening program would generate five false positive screening results for every true positive detected (Table 3).

Given the absence of clear evidence of adverse clinical outcomes in infants diagnosed with SBCADD on newborn screening, Wisconsin increased its C5 acylcarnitine cut-off to 0.60 $\mu\text{mol/L}$ in late 2011 to reduce detection of infants with SBCADD. Applying this cut-off to the previous 10 years of data, 38 of 92 (41%) initial screens would still flag with elevated C5 acylcarnitine concentrations (0.60 $\mu\text{mol/L}$) and would require biochemical testing to distinguish between a potential diagnosis of SBCADD and IVA.

Data from this study suggests an additional strategy to help distinguish between these two disorders. Since the C5/C2 and C5/C3 ratios were above the established cut-offs of 0.05 $\mu\text{mol/L}$ and 0.50 $\mu\text{mol/L}$, respectively, in only 13 of 92 initial screens in those with SBCADD, but always elevated in the initial screen of those with IVA, the absence of elevated ratios on the initial screen could suggest that SBCADD is the more likely diagnosis. This strategy has now been adopted by the Wisconsin Newborn Screening Program. If a screen is 0.60 $\mu\text{mol/L}$, but <1.0 $\mu\text{mol/L}$, and both the C5/C2 and C5/C3 ratios are not elevated above the established cut-offs, then the screen is considered negative. This strategy has reduced the number of reported elevations of C5 acylcarnitine by 80%. However, to prevent missing identification of IVA using these stricter criteria, each year we can expect

that an average of five infants homozygous for the c.1165 A>G mutation will still screen positive and require further diagnostic testing to rule out IVA.

In a state such as Wisconsin with a large Hmong population, establishing mutation analysis as a second tier test for those with an unclear screen might be justified. Homozygosity of the c.1165 A>G mutation would confirm the diagnosis of SBCADD without requiring further biochemical testing. This could be helpful if SBCADD is confirmed to be a benign condition in this population and one not requiring referral or treatment. However, before such a strategy could be pursued, consideration would have to be given to what information from the second tier mutation analysis would be communicated to parents and included in medical records. Experience to date suggests a mild or benign clinical course for SBCADD, at least in those detected by newborn screening. However, a formal analysis of developmental outcomes is needed to verify this conclusion and to determine whether the optimal newborn screening policy with respect to SBCADD is one that minimizes “true positive” or “false negative” screening results.

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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 academia
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

References

1. Gibson KM, Sacks M, Kiss D, Pohowalla P, Linck L, Steiner RD, Burlingame T. 2-methylbutyrylglycinuria in a neonate with CNS dysfunction: evidence for isolated 2-methylbutyryl-CoA dehydrogenase deficiency, an inborn error of L-isoleucine metabolism. *J Inherit Metab Dis.* 1999; 22(Suppl. 1):16.

2. Gibson KM, Burlingame TG, Hogema B, Jakobs C, Schutgens RBH, Millington D, Roe C, Roe D, Sweetman L, Steiner RD, Linck L, Pohowalla P, Sacks M, Kiss D, Rinaldo P, Vockley J. 2-methylbutyryl-coenzyme A dehydrogenase deficiency: a new inborn error of L-isoleucine metabolism. *Pediatr Res*. 2000; 47:830–833. [PubMed: 10832746]
3. Andresen BS, Christensen E, Corydon TJ, Bross P, Pilgaard B, Wanders RJ, Ruiters JN, Simonsen H, Winter V, Knudsen I, Schroeder LD, Gregersen N, Skovby F. Isolated 2-methylbutyrylglycinuria caused by short/branched chain acyl CoA dehydrogenase deficiency: identification of a new enzyme defect, resolution of its molecular basis, and evidence for distinct acyl-CoA dehydrogenase deficiency in isoleucine and valine metabolism. *Am J Hum Genet*. 2000; 67:1095–1103. [PubMed: 11013134]
4. Akaboshi S, Ruiters J, Wanders RJA, Andresen BS, Steiner RD, Gibson KM. Divergent phenotypes in siblings with confirmed 2-methylbutyryl-CoA dehydrogenase (2-MBCD) deficiency. *J Inherit Metab Dis*. 2001; 24(Suppl. 1):58.
5. Korman SH, Zeharia A, Barash V, Corydon TJ, Gregersen N, Gutman A, Kalderon B, Brooks R, Andresen BS. Short/branched acyl-CoA dehydrogenase (SBCAD) deficiency: expanded clinical and molecular spectrum. *J Inherit Metab Dis*. 2001; 24(Suppl. 1):68.
6. Matern D, He M, Berry SA, Rinaldo P, Whitley CB, Madsen PP, van Calcar SC, Lussky RC, Andresen BS, Wolff JA, Vockley J. Prospective diagnosis of 2-methylbutyryl-CoA dehydrogenase deficiency in the Hmong population by newborn screening using tandem mass spectrometry. *Pediatrics*. 2003; 112:74–78. [PubMed: 12837870]
7. Yoon HR, Choeh K, Lee KR, Lee MS, Hahn SH, Rinaldo P, Miao H, Vockley J. 2-methylbutyryl-CoA dehydrogenase deficiency in a 3 day old Korean boy. *J Inherit Metab Dis*. 2003; 26(Suppl. 2): 62.
8. Korman SH. Inborn errors of isoleucine degradation: a review. *Mol Genet Metab*. 2006; 89:289–299. [PubMed: 16950638]
9. Madsen PP, Kibaek M, Roca X, Sachidanandam R, Krainer AR, Christensen E, Steiner RD, Gibson KM, Corydon TJ, Knudsen I, Wanders RJA, Ruiters JPN, Gregersen N, Andresen BS. Short/branched-chain acyl-CoA dehydrogenase deficiency due to an IVS3+3A>G mutation that causes exon skipping. *Hum Genet*. 2006; 118:680–690. [PubMed: 16317551]
10. Van Calcar SC, Gleason LA, Lindh H, Hoffman G, Rhead W, Vockley J, Wolff JA, Durkin MS. 2-methylbutyryl-CoA dehydrogenase deficiency in Hmong infants identified by expanded newborn screen. *Wisc Med J*. 2007; 106:12–15.
11. Kanavin OJ, Woldseth B, Jellum E, Tvedt B, Andresen BS, Stromme P. 2-methylbutyryl-CoA dehydrogenase deficiency associated with autism and mental retardation: a case report. *J Med Case Rep*. 2007; 1:98–104. [PubMed: 17883863]
12. Sass JO, Ensenauer R, Roschinger W, Reich H, Steuerwald U, Schirrmacher O, Engel K, Haberle J, Andresen BS, Megarbane A, Lehnert W, Zschocke J. 2-methylbutyryl-coenzyme A dehydrogenase deficiency: functional and molecular studies on a defect in isoleucine catabolism. *Mol Genet Metab*. 2008; 93:30–35. [PubMed: 17945527]
13. Alfardan J, Mohsen AW, Copeland S, Ellison J, Keppen-Davis L, Rohrbach M, Powell BR, Gillis J, Matern D, Kant J, Vockley J. Characterization of new ACADSB gene sequence mutations and clinical implications in patients with 2-methylbutyrylglycinuria identified by newborn screening. *Mol Genet Metab*. 2010; 100:333–338. [PubMed: 20547083]
14. Vockley J, Ensenauer R. Isovaleric acidemia: new aspects of genetic and phenotypic heterogeneity. *Am J Med Genet Part C (Sem Med Genet)*. 2006; 142C:95–103.
15. Arnold GL, Salazar D, Neidich JA, Suwannarat P, Graham BH, Lichter-Konecki V, Bosch AM, Cusmano-Ozog K, Enns G, Wright EL, Lanpher BC, Owen NN, Lipson NH, Cerone R, Levi P, Wong IJ, Dezrom A. Outcome of infants diagnosed with 3-methylcrotonyl-CoA carboxylase deficiency by newborn screening. *Mol Genet Metab*. 2012; 106:439–441. [PubMed: 22658692]
16. Spiekerkoetter U, Sun B, Zylkovicz T, Wanders R, Strauss AW, Wendel U. MS/MS-based newborn and family screening detects asymptomatic patients with very-long-chain acyl-CoA dehydrogenase deficiency. *J Pediatr*. 2003; 143:335–342. [PubMed: 14517516]
17. Spiekerkoetter U. Mitochondrial fatty acid oxidation disorders: clinical presentation of long-chain fatty acid oxidation defects before and after newborn screening. *J Inherit Metab Dis*. 2010; 33:527–532. [PubMed: 20449660]

18. Andresen BS, Dobrowolski SF, O'Reilly L, Muenzer J, McCandless SE, Frazier DM, Udvari S, Bross P, Knudsen I, Banas R, Chace DH, Engel P, Naylor EW, Gregersen N. Medium-chain acyl-CoA dehydrogenase (MCAD) mutations identified by MS/MS-based prospective screening of newborns differ from those observed in patients with clinical symptoms: identification and characterization of a new, prevalent mutation that results in mild MCAD deficiency. *Am J Hum Genet.* 2001; 68:1408–1418. [PubMed: 11349232]
19. Nagan N, Kruckeberg KE, Tauscher AL, Bailey KS, Rinaldo P, Matern D. The frequency of short-chain acyl-CoA dehydrogenase gene variants in the US population and correlation with the C4-acylcarnitine concentration in newborn blood spots. *Mol Genet Metab.* 2003; 78:239–246. [PubMed: 12706374]
20. 2010 Census of Population and Housing. Summary File 1, www.census.gov/prod/cen2010/doc/sf1, (accessed November 2012)
21. Pasquali M, Monsen G, Richardson L, Alston M, Longo N. Biochemical findings in common inborn errors of metabolism. *Am J Med Genet Part C (Sem Med Genet).* 2006; 142C:64–76.
22. Wisconsin Department of Health Services. Wisconsin births and infant deaths. www.dhs.wisconsin.gov/publications/P4/P45364-10.pdf2010, (Accessed November 2012)
23. Newcombe RG. Two-sided confidence intervals for the single proportion: comparison of seven methods. *Stat Med.* 1998; 17:857–872. [PubMed: 9595616]
24. Zweig MH, Campbell G. Receiver Operating Characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clin Chem.* 1993; 39:561–577. [PubMed: 8472349]
25. Metz C. Basic principles of ROC analysis. *Semin Nucl Med.* 1978; 8:283–298. [PubMed: 112681]
26. Guo SW, Thompson EA. Performing the exact test of Hardy–Weinberg proportion for multiple alleles. *Biometrics.* 1992; 48:361–372. [PubMed: 1637966]
27. Tanaka K. Isovaleric acidemia: personal history, clinical survey and study of the molecular basis. *Prog Clin Biol Res.* 1990; 321:273–290. [PubMed: 2326294]
28. Itoh T, Ito R, Ohba S, Sugiyama N, Mizuguchi K, Yamaguchi S, Kidouchi K. Effect of carnitine administration on glycine metabolism in patients with isovaleric acidemia: significance of acylcarnitine determination to estimate the proper carnitine dose. *Tohoku J Exp Med.* 1996; 179:101–109. [PubMed: 8875766]
29. Region 4 Genetics Collaborative MS/MS Project. Laboratory Quality Improvement of Newborn Screening. www.clir4s.org. (Assessed October 2012)

Fig. 1.

Comparison of C5 acylcarnitine concentrations in NBS dried blood spots from infants diagnosed with SBCADD and IVA in Wisconsin and cumulative data from the Region IV Genetics Collaborative MS/MS Project. a. Mean and range of NBS C5 acylcarnitine concentrations (μmol/L) in initial screen for infants with normal screening results (from 2009 only), initial and repeat screen for infants diagnosed with SBCADD (n = 92) and IVA (n = 5) over a 10 year period in Wisconsin. C5 cut-off is 0.44 μmol/L. Boxed values represent the range of C5 concentrations for each disorder; dashed lines represent the mean value. b. Range of NBS C5 acylcarnitine concentrations (μmol/L) measured in NBS spots from infants diagnosed with SBCADD and IVA reported to the Region IV Genetics Collaborative MS/MS Project [29]. Lowest value represents the mean concentration at the 1st percentile; highest value represents the mean concentration at the 99th percentile. For SBCADD, 1st percentile = 0.44 μmol/L, 99th percentile = 2.34 μmol/L. For IVA, 1st percentile = 0.68 μmol/L, 99th percentile = 19.0 μmol/L. (Data accessed May 2012).

Table 1

C5-acylcarnitine concentrations & ratios (C5/C2 & C5/C3) detected by expanded newborn screening in infants with confirmed SBCADD and IVA in Wisconsin.^{a,b}

Acylcarnitine		SBCADD (initial screen)		SBCADD (repeat screen)		IVA (initial screen)	
Analyte	Cut-off (μmol/L)	# elevated/total # ^c	Mean ± SEM (μmol/L)	# elevated/total # ^c	Mean ± SEM (μmol/L)	# elevated/total # ^c	Mean ± SEM (μmol/L)
C5	0.44	92/92	0.69 ± 0.30	92/92	1.24 ± 0.60	5/5	3.18 ± 0.82
C5/C3	0.50	13/92	0.84 ± 0.04	73/89	1.10 ± 0.05	5/5	1.61 ± 0.78
C5/C2	0.05	13/92	0.08 ± 0.01	73/89	0.10 ± 0.01	5/5	0.11 ± 0.04

^aData collection dates: 04/01/2001 to 03/31/2011. For those diagnosed with SBCADD, average age at collection for initial screen = 47 ± 7 h; second screen = 527 ± 66 h. For those diagnosed with IVA, average age at collection = 36.2 ± 9.5 h.

^bNumber of infants diagnosed with SBCADD includes 90 infants of Hmong descent and 2 Caucasian infants.

^cIndicates the total number of screens elevated above the cut-off for each analyte (C5, C5/C3 and C5/C2) out of the total number of positive screens.

Table 2

Analysis of *ACADSB* c. 1165 A>G mutation from 1139 initial newborn screening cards from infants of Hmong descent born in Wisconsin.^a

Mutation	N	Average C5 (μmol/L) (mean ± SEM)	Estimated frequency/1000 births
Homozygous, screening positive	9	0.92 ± 0.148 (95% CI 0.58, 1.26)	7.9 (95% CI 3.9, 15.5)
Homozygous, screening negative	6	0.31 ± 0.026 (95% CI 0.25, 0.38)	5.3 (95% CI 2.1, 12.0)
Homozygous, total	15	0.68 ± 0.118 (95% CI 0.42, 0.93)	13.2 (95% CI 7.7, 22.2)
Heterozygous	248	0.13 ± 0.003 (95% CI 0.12, 0.13)	217.7 (95% CI 194.3, 243.1)
Wild-type	876	0.10 ± 0.002 (95% CI 0.10, 0.11)	769.1 (95% CI 743.3, 793.1)

^a A sample of 1130 cards with C5 concentrations below the screening cut-off (0.44 μmol/L) were randomly selected from NBS cards of infants of Hmong descent born between 2004 and 2007. A random sample of 9 (the average number with elevated C5 values for every 1130 cards from infants of Hmong descent who had C5 values below the screening cut-off) cards with C5 values above the screening cut-off were added to the analysis to allow calculation of sensitivity, specificity and prevalence of the c. 1165 A>G mutation.

Table 3

Evaluation of specificity and sensitivity and the expected number of screens (total, false positive and false negative) for four C5 acylcarnitine cut-off concentrations to detect homozygosity of the c.1165 A>G mutation in infants of Hmong descent in Wisconsin.

C5 cutoff (μmol/L)	Specificity (%)	Sensitivity (%)	Average number screening positive annually	Number of false positive screens expected annually	Number of false negative screens expected annually
0.20	93.4	100	89	74	0
0.44	100	60.0	9	0	6
0.60	100	46.7	7	0	8
0.60 to <1.0 with elevated C5/C2 and C5/C3 ratios	100	33.3	5	0	10