

Mini Review

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Clinical, biochemical, and molecular spectrum of short/branched-chain acyl-CoA dehydrogenase deficiency: two new cases and review of literature

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Abstract

Background: Short/branched-chain acyl-CoA dehydrogenase (SBCAD) deficiency is a rare inborn error of metabolism with uncertain clinical significance. As it leads to C5-carnitine (i.e. isovalerylcarnitine, 2methylbutyrylcarnitine, or pivaloylcarnitine) elevation, SBCAD deficiency is detectable at newborn screening, requiring differential diagnosis from isovaleric acidemia and pivalic acid administration. Increased urinary excretion of 2-methylbutyrylglycine (2MBG) is the hallmark of SBCAD deficiency.

Methods: We report two cases of SBCAD deficiency and provide a review of the available literature on this condition.

Results: Two siblings newly diagnosed with SBCAD deficiency are reported. Newborn screening allowed the early diagnosis in the second-born (C5=0.5 $\mu\text{mol/L}$, normal 0.05–0.3 $\mu\text{mol/L}$) and addressed selective screening in the 5-year asymptomatic brother (C5=1.9 $\mu\text{mol/L}$). Both patients showed increased urinary excretion of 2MBG and two mutations in the *ACADSB* gene (c.443C>T/c.1145C>T). Currently, both the patients are asymptomatic. Longitudinal biochemical monitoring of the two patients while on treatment with carnitine (100 mg/kg/day) was provided. Based on our experience and the literature review (162 patients), SBCAD deficiency is symptomatic in about 10% of reported patients. Clinical onset occurs in newborns or later in life with seizures, developmental delay, hypotonia, and failure to thrive. On longitudinal follow-up, epilepsy, developmental delay, microcephaly, and autism can develop. Acute metabolic decompensation due to catabolic stressors can occur, as observed in one newly reported patient. Fifteen mutations in the *ACADSB*

gene are known, including the newly identified variant c.1145C>T (p.Thr382Met), variably associated to the phenotype. In the Hmong population, SBCAD deficiency is highly prevalent, mostly due to the founder mutation c.1165A>G, and is largely asymptomatic.

Conclusions: Although mostly asymptomatic, considering SBCAD deficiency as a non-disease in non-Hmong subjects appears unsafe. Catabolic situations can precipitate acute metabolic decompensation. Carnitine supplementation and valproate avoidance appear to be indicated. Providing an emergency protocol for the management of acute catabolic episodes seems reasonable in asymptomatic patients with SBCAD deficiency. Longitudinal follow-up is recommended.

Keywords: 2-methylbutyrylglycinuria; *ACADSB* gene; newborn screening; short/branched-chain acyl-CoA dehydrogenase deficiency.

Introduction

Short/branched-chain acyl-CoA dehydrogenase (SBCAD, also known as 2-methylbutyryl-CoA dehydrogenase, EC 1.3.99.12) is a homotetrameric mitochondrial enzyme catalyzing the third step of the isoleucine S-pathway – the conversion of (S)-2-methylbutyryl-CoA into tiglyl-CoA – and the first oxidative step of L-2-methylated short acyl-CoA compounds, being likely implicated in valproate metabolism [1, 2]. SBCAD deficiency (OMIM 600301/610006) was first described in 2000 [3, 4]. Biochemically, increased urinary excretion of 2-methylbutyrylglycine (2MBG) and elevated blood concentrations of 2-methylbutyrylcarnitine are the hallmarks of this disorder. The latter can be detected at newborn screening by acylcarnitine analysis as C5-carnitine (i.e. isovalerylcarnitine, 2methylbutyrylcarnitine, or pivaloylcarnitine) elevation, requiring differential diagnosis from isovalerylcarnitine (a marker of isovaleric acidemia) and pivaloylcarnitine (a component of several antibiotics).

The clinical significance of SBCAD deficiency is unclear. The first patients identified with 2-methylbutyrylglycinuria showed severe neurologic pictures at onset and developmental delay on long-term follow-up [3, 4]. On the other hand, SBCAD deficiency was diagnosed in

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asymptomatic adults as well, and neonatal screening studies revealed high prevalence of asymptomatic newborns, especially in the Hmong population [5–7].

Here we review the clinical, biochemical, and molecular data of published cases of SBCAD deficiency. Furthermore, we report two new cases of SBCAD deficiency, expanding the clinical and molecular spectrum of this condition.

Patients and methods

PubMed was searched using the keywords short/branched chain acyl-CoA dehydrogenase, 2-methylbutyryl-CoA dehydrogenase, 2-methylbutyrylglucosuria, and the *ACADSB* gene. Patients whose data had been published were subdivided according to the occurrence of clinical symptoms. Data on diagnostic approach, biochemical characteristics (C5 and 2MBG), family descent, genotype, treatment, and the duration of follow-up were collected for all the patients. As for symptomatic patients, age at onset, clinical symptoms, and imaging studies were recorded. Additionally, two newly diagnosed patients with SBCAD deficiency were described. Informed consent was obtained for inclusion in the study. Newborn screening was performed using the NeoBase™ Non-derivatized MSMS kit (PerkinElmer, Inc., Waltham, MA, USA). Organic acids and molecular analyses were performed by gas chromatography-mass spectrometry and Sanger sequencing, respectively, as described elsewhere [8, 9]. Statistical analysis was performed using R: A Language and Environment for Statistical Computing (R Foundation for Statistical Computing, Vienna, Austria). The Shapiro-Wilk test was used for testing the normality of data distribution. Differences between groups were established using Student's t-test or the Mann-Whitney U test [10]. Statistical significance for all calculations was considered achieved when the two-tailed p-value was less than 0.050. The study was conducted according to the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects.

Results

Case reports

The first diagnosed patient was detected at newborn screening performed at 3 days of life showing slight C5-carnitine elevation (0.5 $\mu\text{mol/L}$, normal range 0.05–0.3 $\mu\text{mol/L}$). Urinary organic acids showed increased excretion of 2MBG. Molecular analysis revealed a compound heterozygosity for the known pathogenic mutation *c.443C>T* (p.Thr148Ile) associated to the new variant *c.1145C>T* (p.Thr382Met). The functional effect of this new variant was predicted using a bioinformatics tool (PolyPhen-2), resulting in its high probability of protein damage (score: 1.000). Its minimal allele frequency resulted in

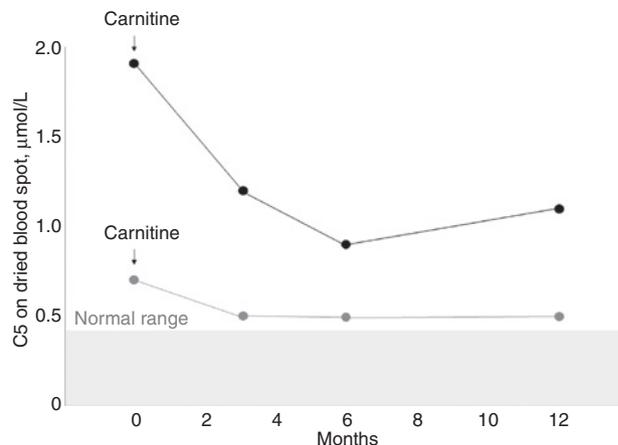


Figure 1: Longitudinal monitoring of blood C5 concentrations in two siblings with short/branched-chain acyl-CoA dehydrogenase deficiency while on treatment with carnitine supplementation (100 mg/kg/day).

C5 concentrations in the asymptomatic younger patient detected at newborn screening (gray) and in her older brother (black) previously suffering an acute metabolic decompensation are shown. The gray area represent the normal C5 range.

~1:50,000 in non-Finnish European individuals (based on GnomAD).

Investigations in her 5-year-old asymptomatic brother (who did not undergo expanded newborn screening) revealed overlapping biochemical (C5=1.9 $\mu\text{mol/L}$, increased urine 2MBG) and molecular features. Medical history of this patient revealed a life-threatening episode characterized by metabolic acidosis and coma during an acute febrile gastroenteritis that occurred at 3 years of age. After urgent hospitalization, glucosaline infusion rapidly corrected the clinical features and the biochemical picture.

Since the diagnosis of SBCAD deficiency, both patients were treated with carnitine (100 mg/kg/day). Additionally, instructions to avoid fasting and protein overloads and an emergency protocol for the management of intercurrent febrile illnesses were delivered to the parents. In both patients, uneventful clinical follow-up lasted until present (1 year). Longitudinal biochemical monitoring of blood C5-carnitine in the two patients while on treatment with carnitine is depicted in Figure 1. Both patients showed steadily increasing urinary excretion of 2MBG while on treatment.

Clinical features of SBCAD deficiency

Symptomatic patients

Including this report, 13 symptomatic patients with SBCAD deficiency were reported (Table 1). Age at onset

Table 1: Clinical, biochemical, and molecular characteristics of reported symptomatic patients with short/branched-chain acyl-CoA dehydrogenase (SBCAD) deficiency.

Reference	Patients	Nbs	Age at onset	Clinical onset	MRI	Biochemical data	Descent	Genotype	Long-term follow-up
Gibson et al. [3] Madsen et al. [11]	1	No	3 days	Poor feeding, lethargy, hypothermia, hypoglycemia, abnormal EEG	Bilateral subacute ischemias	C5 = 1.4–2.4 μmol/L 2MBG	European, Eritrean	c.303+3A>G/c.763C>T	6 years: epilepsy, developmental delay, microcephaly
Andresen et al. [4]	1	No	24 months	Hypotonia, motor delay, muscle atrophy, strabismus	Normal	2MBG	Pakistani	c.1228G>A/c.1228G>A	36 months: developmental delay (walk with support)
Yoon et al. [12]	1	–	3 days	Hypotonia, respiratory distress, seizures (sepsis)	–	2MBG	–	–	–
Matern et al. [5]	1	Yes	6 months	Mild hypotonia	–	C5 = 1.4 μmol/L 2MBG = 22.5 μg/mg crea	Hmong	–	6 months: mild hypotonia
Matern et al. [5]	1	No	18 months	Patent ductus arteriosus, failure to thrive, hypotonia, developmental delay	–	2MBG	Hmong	c.1165A>G/c.1165A>G	40 months: speech delay
Korman et al. [13]	1	No	8 days	Seizures, hypothermia, lactic acidosis	–	C5 = 0.97 μmol/L 2MBG = 22.2–36.7 μg/mg crea	–	c.908G>C/c.908G>C	–
Korman et al. [13]	1	No	11 months	Recurrent vomiting, failure to thrive	–	C5 = 1.75 μmol/L 2MBG = 14.5 μg/mg crea	–	c.443C>T/c.443C>T	–
Korman et al. [13]	1	No	6 years	ADHD, developmental delay	–	C5 = 0.5–0.55 μmol/L	–	c.1102T>C/c.1102T>C	–
Madsen et al. [11]	1	No	6 months	Seizures, hypotonia, developmental delay	–	C5 = 0.39 μmol/L	Somali	c.303+3A>G/c.303+3A>G	12 months: hypotonia, sparse eye contact
Kanavin et al. [14] van Calcar et al. [6]	1 1	No Yes	5 months 8 days	Seizures Seizures, developmental delay, failure to thrive	Normal –	2MBG Associated NKH	Somali Caucasian	c.303+3A>G/c.303+3A>G –	5 years: autism –
Alfardan et al. [15]	1	No	8 months	Developmental delay	Brain dysgenesis	C5 = 1.47 μmol/L	Non-Hmong	c.303+3A>G/c.303+3A>G	40 months: microcephaly, developmental delay, speech delay
This study	1	No	36 months	Metabolic coma during gastroenteritis	–	C5 = 1.9 μmol/L 2MBG	Italian	c.443C>T/c.1145C>T	6 years: asymptomatic

Normal values are in bold. 2MBG, 2-methylbutyrylglycinuria; C5, 2-methylbutyrylcarnitine; EEG, electroencephalography; MRI, magnetic resonance imaging; Nbs, newborn screening; NKH, non-ketotic hypoglycinemia.

ranged from 3 days to 6 years. The main clinical symptoms at onset included seizures, developmental delay, hypotonia, and failure to thrive [3–5, 11–15].

Neonatal-onset SBCAD deficiency was reported in four patients (31%), all sharing seizures as the common feature (one newborn was concomitantly affected by non-ketotic hyperglycinemia). Infantile-onset (within the first year) was reported in five patients (38%) with SBCAD deficiency, characterized by variable association of seizures, developmental delay, hypotonia, and failure to thrive. Four patients (31%) presented clinical symptoms after the first year, generally showing hypotonia and neuromotor delay, with the exception of one patient presenting with acute metabolic decompensation during febrile gastroenteritis (newly described case) followed by normal clinical outcome.

On longitudinal follow-up, epilepsy, developmental delay, and microcephaly were the most common features; autism was reported in one patient.

Asymptomatic patients

One-hundred and forty-nine asymptomatic patients with SBCAD deficiency were reported (Table 2). All but seven patients (95%) were detected at newborn screening [5–7, 15, 13, 16]. One-hundred and twenty-six patients were of Hmong descent (85%), sharing homozygosity for the c.1165A>G mutation. Treatment with carnitine with or without diet was reported in 38 asymptomatic patients (26%).

Biochemical features of SBCAD deficiency

Available C5 concentrations at diagnosis were generally higher in symptomatic patients compared to asymptomatic patients, although not significantly different (median 1.2 and 0.76 $\mu\text{mol/L}$, respectively; $p=0.98$). Two out of 162 patients (one symptomatic and one asymptomatic) were reported with C5 within the normal range (Tables 1 and 2). Overall, newborn screening allowed the diagnosis of 144 patients (89%). Data on 2MBG concentrations were scantily available; two asymptomatic subjects were reported with normal urinary excretion of 2MBG (Table 2).

Longitudinal biochemical monitoring is available for the two newly reported patients with SBCAD deficiency (Figure 1). In both patients, carnitine supplementation was followed by the reduction of blood C5 concentration; in particular, the second-born asymptomatic patient showed borderline blood C5 concentrations, whereas the

patient previously suffering an acute metabolic decompensation showed steadily higher C5 concentrations while on treatment. Urinary excretion of 2MBG was elevated while on treatment.

Molecular features of SBCAD deficiency

Fifteen variations in the *ACADSB* gene are known. Of them, 13 mutations were reported in 97 genotyped patients with SBCAD deficiency (c.303+3A>G, c.763C>T, c.1228G>A, c.1165A>G, c.908G>C, c.443C>T, c.1102T>C, c.1145C>T, c.38G>A, c.1159G>A, c.295C>T, c.50G>A, c.621G>A). Genotype-phenotype correlation based on available clinical and molecular data is presented in Figure 2. Asymptomatic Hmong patients are characteristically homozygous for the c.1165A>G mutation, the most prevalent allele in the SBCAD population. Other common alleles were c.443C>T and c.303+3A>G, collectively found in 14 symptomatic or asymptomatic patients, mostly in a heterozygous state (Figure 2). Five alleles were exclusively observed in asymptomatic patients, namely c.38G>A, c.1159G>A, c.295C>T, c.50G>A, and c.621G>A.

Mutations found in symptomatic SBCAD patients were c.1165A>G, c.443C>T, c.303+3A>G, c.763C>T, c.1228G>A, c.1145C>T, c.908G>C, and c.1102T>C. The two latter alleles were associated with clinical symptoms at the homozygous state (two patients).

The two known variations c.512A>G and c.254G>A were not associated with SBCAD deficiency.

Discussion

SBCAD deficiency is an inborn error of isoleucine metabolism with uncertain clinical significance [15]. As this condition can be detected at expanded newborn screening through C5-carnitine elevation, having a picture of its potential clinical implications is essential to effectively inform parents of newly detected newborns.

Symptomatic SBCAD deficiency was reported in about 10% of identified patients so far. Neonatal-onset SBCAD deficiency is very rare and is commonly characterized by seizures and electroencephalography (EEG) abnormalities besides other non-specific clinical manifestations [1, 3, 6]. Epilepsy and developmental delay characterize the long-term follow-up of early-onset SBCAD deficiency. As valproyl-CoA can be a substrate of SBCAD, it is reasonable to avoid valproic acid for treating epilepsy in patients with SBCAD deficiency [2]. Later-onset clinical variants of

Table 2: Biochemical and molecular characteristics of reported asymptomatic patients with short/branched-chain acyl-CoA dehydrogenase (SBCAD) deficiency.

Reference	Patients		Diagnosis		Treatment since diagnosis	Biochemical data (ranges)	Origin	Genotype	Long-term follow-up (age)
	Nbs	Other	Nbs	Other					
Gibson et al. [3]	1	No	Prenatal diagnosis		Carnitine, low protein diet	2MBG	European, Eritrean	c.303+3A>G/c.763C>T	56 months
Andresen et al. [4]	1	No	Mother of patient		No	2MBG	Pakistani	c.1228G>A/c.1228G>A	Adult
Matern et al. [5]	3	Yes	-		Carnitine, low protein diet	C5 = 0.7–3.4 µmol/L 2MBG = 1.8–28.7 µg/mg crea	Hmong	c.1165A>G/c.1165A>G	3–14 months
Matern et al. [5]	4	Yes	-		Carnitine, low protein diet	C5 = 0.8–2.2 µmol/L 2MBG = 0.7–103.1 µg/mg crea	Hmong	-	3–14 months
Matern et al. [5]	3	No	Siblings of patient		-	2MBG	Hmong	-	3–6 years
Korman et al. [13]	1	Yes	-		-	C5 = 1.1–2.5 µmol/L 2MBG = 29.3–36.3 µg/mg crea	-	c.1165A>G/c.848A>G	-
van Calcar et al. [6]	26	Yes	-		Carnitine, low protein diet	C5 = 0.4–1.0 µmol/L 2MBG	Hmong	c.1165A>G/c.1165A>G (3 patients)	5 years
Sass et al. [16]	1	No	Sibling of patient		-	-	Turkish	c.443C>T; c.38G>A/c.443C>T; c.38G>A	7 years
Sass et al. [16]	1	No	Sibling of patient		No	C5 = 1.0 µmol/L 2MBG = 47.2 µg/mg crea	Arab	c.443C>T; c.38G>A/c.1159G>A	36 months
Sass et al. [16]	1	Yes	-		Carnitine since 9 months of age	C5 = 0.57 µmol/L 2MBG	German	c.38G>A/c.38G>A	6 years
Sass et al. [16]	1	Yes	-		No	C5 = 0.75 µmol/L 2MBG	Turkish	c.443C>T; c.38G>A/c.443C>T; c.38G>A	6 years
Sass et al. [16]	1	Yes	-		Carnitine	C5 = 0.68–2.08 µmol/L 2MBG	Turkish	c.1159G>A/c.1159G>A	36 months
Sass et al. [16]	1	Yes	-		Carnitine	C5 = 0.51–0.72 µmol/L 2MBG	Arab	c.443C>T; c.38G>A/c.1159G>A	10 months
Alfardan et al. [15]	4	Yes	-		-	C5: 1.1–19 fold increase 2MBG	Non-Hmong	c.295C>T/c.295C>T c.443C>T; c.1159G>A; c.50G>A c.621G>A/c.621G>A c.303+3A>G/c.303+3A>G	48 months
Alfardan et al. [15]	7	Yes	-		-	C5: 1.1–19 fold increase 2MBG	Non-Hmong	-	48 months
van Calcar et al. [7]	90	Yes	-		-	C5 = 0.44–2.05 µmol/L 2MBG	Hmong	c.1165A>G/c.1165A>G (69 patients)	-
van Calcar et al. [7]	2	Yes	-		-	C5 = 0.62–0.77 µmol/L 2MBG	Caucasian	-	-
This study	1	Yes	-		Carnitine	C5 = 0.5 µmol/L 2MBG	Italian	c.443C>T/c.1145C>T	10 months

Normal values are in bold. 2MBG, 2-methylbutyrylglycinuria; C5, 2-methylbutyrylcarnitine; Nbs, newborn screening.

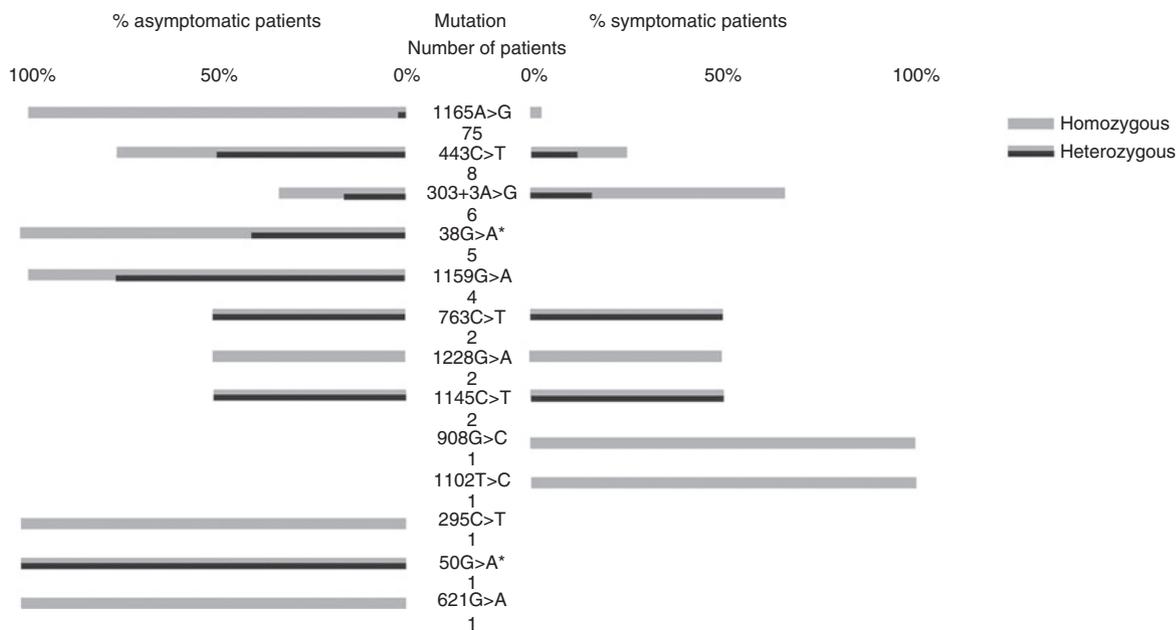


Figure 2: Genotype-phenotype correlation based on 97 patients with short/branched-chain acyl-CoA dehydrogenase deficiency. Some variants (*) were only found in patients harboring two other pathogenic mutations in the *ACADSB* gene.

SBCAD deficiency can manifest in infancy or childhood mostly with neurologic symptoms, including developmental delay, hypotonia, or autism. An additional potential clinical onset of SBCAD deficiency is represented by metabolic decompensation due to an intercurrent febrile illness, as observed in a newly diagnosed SBCAD-deficient patient identified at our center. Interestingly, uncomplicated clinical course followed the resolution of the acute clinical symptoms. Although the actual impact of SBCAD deficiency in determining metabolic decompensation cannot be elucidated in the presented case (as gastroenteritis and severe dehydration can lead to metabolic acidosis and coma), these observations suggest that environmental stressors may trigger acute clinical manifestations of SBCAD deficiency, making it unsafe to consider this condition a “non-disease”. Actually, a number of metabolic diseases share this behavior, including fatty acid oxidation disorders and organic acidemias [17, 18]. This supports a prudential management of asymptomatic patients with SBCAD deficiency, essentially based on carnitine supplementation besides the recommendation to avoid both fasting and protein overload. Furthermore, providing an emergency protocol for the management of intercurrent illnesses can be indicated in SBCAD deficiency. Hmong subjects represent a possible exception to these suggestions. In this population, indeed, SBCAD deficiency was shown to be highly prevalent (13.2/1000 births), almost invariably asymptomatic even on long-term follow-up, and due to homozygosity for the founder

mutation c.1165A>G [7]. Mild hypotonia at 6 months of age, indeed, was reported only in one Hmong patient with these molecular findings [5]. This protective genotype was never described in non-Hmong patients.

At newborn screening, SBCAD should be considered as a differential diagnosis of C5-carnitine elevation, besides isovaleric acidemia and pivalic acid administration (present in some antibiotics). Newborn screening outcome in one newly reported patient confirmed the previous observation that SBCAD deficiency can present with near-normal C5-carnitine in newborns [6, 7], subsequently confirmed by increased excretion of 2MBG detected by urine organic acids or acyl-glycine analyses. An oral bolus of 100 mg L-isoleucine/kg body weight may increase the sensitivity of organic acid analysis in detecting 2MBG. 2-ethylhydracrylic aciduria, an intermediate of the minor R-pathway of isoleucine degradation, was suggested as a subsidiary diagnostic marker of SBCAD deficiency [13]. Longitudinal biochemical monitoring of the two siblings with SBCAD deficiency showed consistent reduction of blood C5-carnitine concentration while on treatment with oral carnitine, although not reaching the normal concentration. The clinical significance of monitoring this parameter, however, is unclear due to the lack of published data.

SBCAD deficiency is transmitted as an autosomal recessive trait. Fifteen mutations in the *ACADSB* gene (11 exons, >20 kb) are known, including the new variant identified in the two siblings described in this study. Of them, 13 were associated with SBCAD deficiency. The c.1165A>G founder

mutation in the Hmong population causes exon 10 skipping in the *ACADSB* gene. Exon skipping, indeed, is also implicated in the pathogenesis of other mutations in the *ACADSB* gene, namely c.1228G>A and c.303+3A>G [4, 11]. The former causes skipping of exon 10, whereas the latter induces missplicing through exon 3 skipping. As higher temperatures generally enhance skipping of exons [11, 19, 20], it could be speculated that fever might further reduce SBCAD activity in the presence of such mutations. A tentative genotype-phenotype correlation in SBCAD revealed mutations largely associated with the asymptomatic course (c.1165A>G, c.38G>A, c.1159G>A, c.295C>T, c.50G>A, and c.621G>A), those mostly observed in symptomatic patients (c.303+3A>G, c.908G>C and c.1102T>C), and those non-predictive of the clinical course (c.763C>T, c.1228G>A, c.1145C>T, and c.443C>T). In particular, both the known and the new variants harbored by the two newly reported siblings belong to the latter group, consistently with their clinical course. Furthermore, although never observed in patients with SBCAD deficiency, two additional variations in the *ACADSB* gene (i.e. c.512A>G and c.254G>A) were reported as potential modifiers of systolic blood pressure in adults if associated with polymorphisms in the catecholamine-O-methyltransferase gene [21].

In conclusion, the current data show that about 90% of patients with SBCAD deficiency are asymptomatic. In the Hmong population, SBCAD deficiency is likely benign. In the general population, the clinical course of SBCAD deficiency is poorly predictable. Consequently, clinical monitoring with special attention to situations that could stimulate metabolic decompensation is mandatory. It appears safe that non-Hmong patients identified at newborn screening with SBCAD deficiency receive carnitine supplementation, recommendation of avoiding prolonged fasting and protein overload, and an emergency protocol for the management of acute catabolic episodes. Furthermore, valproate is not indicated for the treatment of epilepsy in SBCAD deficiency. Longitudinal clinical and biochemical follow-up of symptomatic and asymptomatic patients with SBCAD deficiency is recommended.

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