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Prospective Diagnosis of 2-Methylbutyryl-CoA Dehydrogenase Deficiency in the Hmong Population by Newborn Screening Using Tandem Mass Spectrometry

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ABSTRACT. *Objective.* 2-Methylbutyryl-CoA dehydrogenase deficiency, also known as short/branched-chain acyl-CoA dehydrogenase (SBCAD) deficiency, is a recently described autosomal recessive disorder of L-isoleucine metabolism. Only 4 affected individuals in 2 families have been described. One patient developed athetoid cerebral palsy, and another had severe motor developmental delay with muscle atrophy. A sibling of the first patient is asymptomatic after prenatal diagnosis and early treatment. Family investigations in the second family revealed that the patient's mother was also affected but asymptomatic.

Methods. We report 8 additional patients identified by prospective newborn screening using tandem mass spectrometry.

Results. Molecular genetic analysis performed for 3 of these patients revealed that all are homozygous for an 1165A>G mutation that causes skipping of exon 10 of the SBCAD gene. Although there was no obvious consanguinity, all patients belong to the Hmong, an ancient ethnic group that originated in China and constitutes only 0.8% and 0.6% of the Minnesota and Wisconsin population, respectively. Dietary treatment was initiated in the neonatal period. Except for 1 patient who developed mild muscle hypotonia, all patients remain asymptomatic at ages ranging from 3 to 14 months of age.

Conclusions. These cases suggest that SBCAD deficiency is another inborn error of metabolism detectable by newborn screening using tandem mass spectrometry. The continued efficacy of long-term dietary therapy instituted presymptomatically remains to be established. *Pediatrics* 2003;112:74–78; 2-methylbutyryl-CoA dehydrogenase deficiency, short/branched-chain acyl-CoA dehydrogenase deficiency, SBCAD, ACADSB, L-isoleucine, newborn screening, tandem mass spectrometry, Hmong, founder effect.

ABBREVIATIONS. SBCAD, short/branched-chain acyl-CoA dehydrogenase; MS/MS, tandem mass spectrometry.

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2-Methylbutyryl-CoA dehydrogenase, also known as short/branched-chain acyl-CoA dehydrogenase (SBCAD, OMIM No. 600301), catalyzes the third step in the metabolic pathway of the branched-chain amino acid L-isoleucine (Fig 1). The gene for SBCAD (ACADSB) consists of 11 exons distributed over >20 kb, is located on chromosome 10q25–26, and encodes a precursor protein of 431 amino acids, which yields a mature protein of 399 amino acids.^{1–3}

Three SBCAD-deficient patients in 2 families were recently described.^{3,4} One patient was born at term after an uneventful pregnancy to healthy, nonconsanguineous parents of European and Eritrean ancestry.⁴ He was admitted to the hospital on his third day of life because of poor feeding, lethargy, hypothermia, hypoglycemia, and a metabolic acidosis. Electroencephalogram and brain magnetic resonance imaging studies were suggestive of global hypoxia. Biochemical investigations revealed 2-methylbutyrylglucosuria indicative of a defect in L-isoleucine metabolism. The diagnosis of SBCAD deficiency was confirmed by demonstration of compound heterozygosity for a missense mutation in ACADSB (L255F in the precursor protein, L222F in the mature protein) and a splice mutation in intron 3 at the +3 position of the 5' splice site causing the loss of SBCAD activity.⁵ The patient was treated with a protein-restricted diet and L-carnitine supplements. He is now 4 years old and has developmental delay and a seizure disorder. A sibling who was diagnosed prenatally and treated from birth is asymptomatic at 2 years of age.

A third patient was born after a normal pregnancy to consanguineous parents of Pakistani origin and presented with progressive muscle weakness at 2 years of age.³ A brain magnetic resonance imaging scan was normal, but urine organic acid analysis revealed 2-methylbutyrylglucosuria and SBCAD deficiency was confirmed by the demonstration of homozygosity for a mutation in the ACADSB gene (1228G>A), causing skipping of exon 10 and leading to complete deficiency of SBCAD activity. Unexpectedly, his healthy mother had the same genotype, as well as 2-methylbutyrylglucosuria. We report 8 patients of Hmong ancestry who were diagnosed prospectively through expanded newborn screening using tandem mass spectrometry.

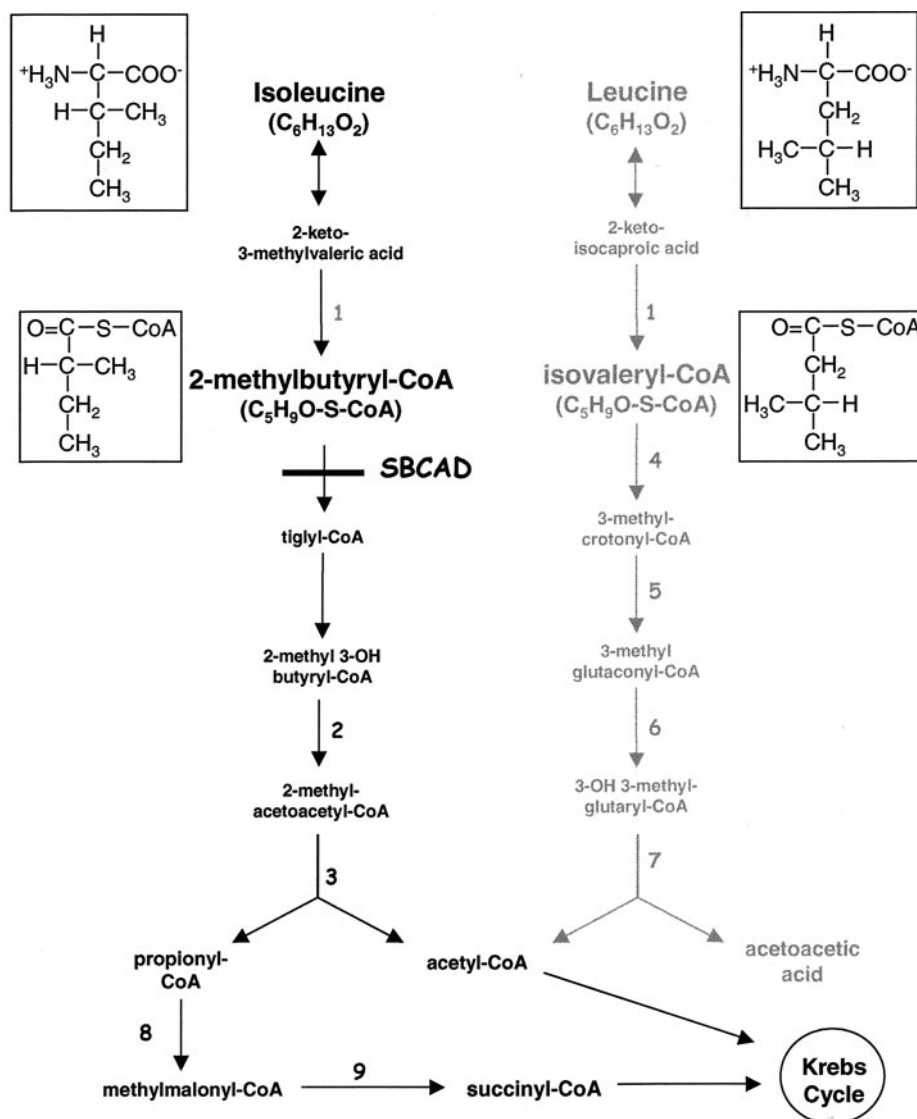


Fig 1. Metabolic pathway of the branched-chain amino acids, isoleucine and leucine, and location of SBCAD. Selected chemical structures and formulas are given to demonstrate the mass equivalence between isoleucine and leucine as well as 2-methylbutyryl-CoA and isovaleryl-CoA, respectively. 1, branched-chain α -ketoacid dehydrogenase complex; 2, 2-methyl 3-hydroxy-butyryl-CoA dehydrogenase; 3, short-chain 3-ketoacyl-CoA thiolase; 4, isovaleryl-CoA dehydrogenase; 5, 3-methylcrotonyl-CoA carboxylase; 6, 3-methylglutaconyl-CoA hydratase; 7, 3-hydroxy 3-methylglutaryl-CoA (HMG-CoA) lyase; 8, propionyl-CoA carboxylase; 9, methylmalonyl-CoA mutase.

CASE REPORTS

All 8 patients were unrelated and born to nonconsanguineous parents. Patient 1 was delivered vaginally in breech position at 33 weeks of gestation as a result of preterm labor unresponsive to magnesium sulfate and terbutaline therapy. Birth weight, head circumference, and length were appropriate for gestational age. Postnatally he required intensive care management for respiratory distress and pneumonia. Expanded newborn screening by tandem mass spectrometry (MS/MS) revealed an abnormally elevated 5-carbon saturated acylcarnitine species (C₅-acylcarnitine). Additional biochemical genetic workup by plasma acylcarnitine, urine organic acid, and urine acylglycine analyses were initiated (Table 1).

Patients 2, 3, 4, 5, and 6 were also born after normal pregnancies, whereas the mother of patient 7 experienced hypertension and the mother of patient 8 had iron deficiency as well as low platelet counts during pregnancy. The postnatal course was normal except for the finding of moderately elevated C₅-acylcarnitine concentrations by expanded newborn screening using MS/MS. Follow-up was initiated as for patient 1 (Table 1).

All patients were initially treated with L-carnitine supplementation (50–100 mg/kg/d) and a low-protein diet. Patient 1 was discharged in good condition at 4 weeks of age and is developing

appropriately at 12 months of age; 6 older children in this family are reported to be “healthy” but have not been formally evaluated. Patients 2, 3, 4, 5, 7, and 8 are between 3 and 14 months of age and also remain clinically asymptomatic. An older sibling of patient 2 was found to have a normal plasma acylcarnitine profile, whereas urine acylglycine analyses of 3 older and healthy siblings of patient 4 also demonstrated 2-methylbutyrylglucururia.

In all cases, the parents have attended follow-up clinic appointments. However, compliance with treatment was poor and eventually discontinued in patients 1, 2, and 8 by 4 months of age but without obvious negative clinical consequences. Patient 6, whose treatment seems to be ongoing, was noticed to have mild muscle hypotonia at the most recent examination when 6 months of age.

METHODS

Plasma acylcarnitine, urine organic acid, and urine acylglycine analyses were performed as previously reported.^{6–8} The presence of SBCAD cross-reactive material was determined by Western blot analysis of crude fibroblast extracts.⁴ Polymerase chain reaction analysis of fibroblast or lymphocyte ACADSB cDNA from patients 1, 2, and 3, a control subject and the previously described Danish patient of Pakistani origin was performed as previously

TABLE 1. Comparison of Plasma C₅-Acylcarnitine and Urine 2-Methylbutyryl- and Isovaleryl-glycine Concentrations in 8 Patients With SBCAD Deficiency and a Patient With IVA

	Plasma C ₅ -Acylcarnitine (μmol/L)	Urine Acylglycines	
		2-MBG (μg/mg creatinine)	IVG (μg/mg creatinine)
Patient 1	0.7; 3.4	1.8; 10.2; 13.4; 28.7	0.1; 0.3; 0.4; 1.1
Patient 2	3.0; 3.2	2.9; 15.5	0.1; 0.6
Patient 3	-	55.7†	1.2†
Patient 4	1.7*	103.1†	1.4†
Patient 5	2.2; 1.0	0.7; 10.0	0.1; 0.2
Patient 6	1.4*	22.5†	1.1†
Patient 7	0.8; 1.2*	21.4†	0.3†
Patient 8	1.4*	67.4†	1.5†
Patient with IVA	27.3; 48.2	0.4	6390.0
Control range	<0.6	0.3–7.5	0.3–14.3

Analyses for patients performed by the Biochemical Genetics Laboratory at Mayo Clinic, Rochester, MN, unless specified otherwise. IVA indicates isovaleric acidemia; IVG, isovalerylglycine. Abnormal results are in **boldface**.

* Analysis performed by the Biochemical Genetics Laboratory at Duke University Medical Center, Durham, NC.

† Analysis performed by the Biochemical Disease Detection Laboratory at Yale University Medical Center, New Haven, CT.

described using primers located in exon 9 (sense) and exon 11 (antisense) of the SBCAD gene.³

RESULTS

Plasma acylcarnitine analysis revealed a moderately elevated C₅-acylcarnitine in all patients. Urine organic acid and particularly acylglycine analyses were significant for 2-methylbutyrylglycinuria in the absence of an abnormal isovalerylglycine excretion (Table 1).

The diagnosis of SBCAD deficiency was confirmed for patients 1, 2, and 3 by Western blot analysis, which was negative for cross-reactive material to SBCAD (data not shown). Sequencing of these patients' ACADSB gene revealed an A to G mutation in exon 10 corresponding to cDNA position 1165A>G, causing a methionine to valine change at position 356 (M356V) in the mature SBCAD protein (corresponding to M389V in the precursor protein). Polymerase chain reaction analysis of cDNA from these patients using primers located in exon 9 (sense primer) and exon 11 (antisense primer) showed that in all 3 patients investigated, exon 10 sequences were missing. Because sequence analysis of the intron exon junctions flanking exon 10 from the patients did not reveal any changes of the splice consensus se-

quences, we believe that the 1165A>G mutation may be the direct cause for the observed exon 10 skipping by a yet unknown mechanism (Fig 2). This mutation has not been observed in 100 alleles of healthy control subjects.

DISCUSSION

SBCAD deficiency is a recently described autosomal-recessive disorder of L-isoleucine catabolism. Two patients in unrelated families have been described to date, both with different phenotypes.^{3,4} A sibling of 1 patient remained asymptomatic after prenatal diagnosis and initiation of treatment at birth. The mother of the other patient is also affected on the basis of biochemical and molecular genetic evaluation; however, she is clinically healthy.³

Seven of the 8 patients described in this report are doing well. Only patient 6 was noted to have mild muscle hypotonia at the last clinic visit when 6 months of age. Although treatment was initiated early in life, it has often been limited as a result of parental noncompliance with diet and medication, and its effect on clinical outcome remains uncertain. Patient 4 has 3 seemingly healthy siblings (3, 4, and 6 years of age) that also seem to be affected with

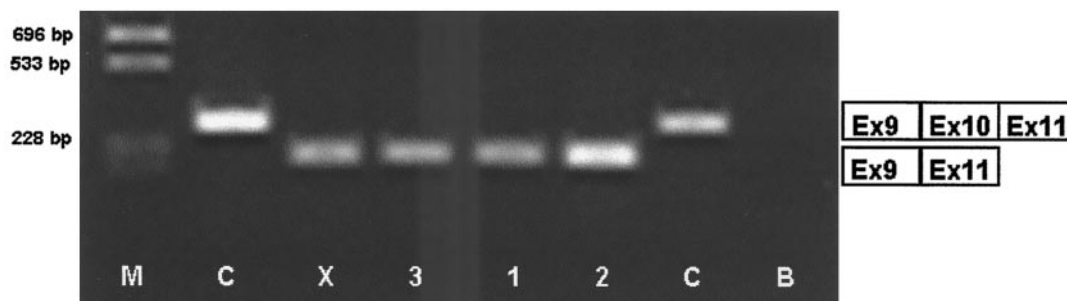


Fig 2. Polymerase chain reaction analysis of fibroblast or lymphocyte cDNA from patients 1, 2, and 3, a control subject and the previously described Danish patient³ using primers located in exon 9 (sense) and exon 11 (antisense) of the SBCAD gene reveals that the 1165A>G (M356V) mutation leads to complete skipping of exon 10. 1, patient 1 (fibroblasts); 2, patient 2 (lymphocytes); 3, patient 3 (fibroblasts); B, blank; C, normal control cDNA (fibroblasts); M, base pair (bp) marker; X, cDNA from fibroblasts of a previously described SBCAD-deficient patient.³

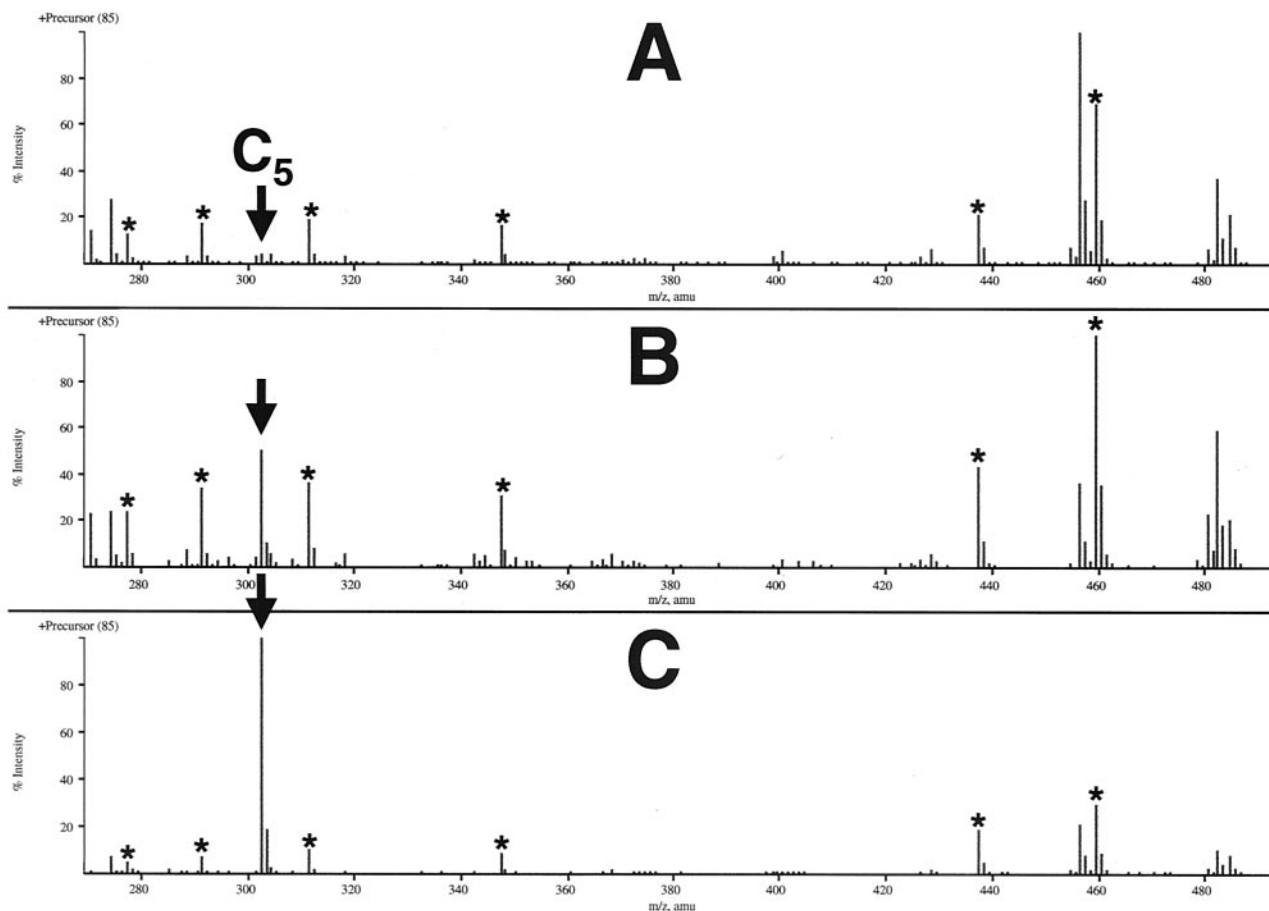


Fig 3. Acylcarnitine profiles obtained by MS/MS analysis of newborn screening blood spots from patient 1 (B), a patient with Isovaleric acidemia (C) and a healthy control (A). Note relatively moderate C_5 -acylcarnitine peak in patient 1 with SBCAD deficiency. *Internal standards (from left to right): [2H_3]propionylcarnitine (C_3); [2H_3]butyrylcarnitine (C_4); [2H_6]isovalerylcarnitine; [2H_3]octanoylcarnitine (C_8); [2H_9]myristoylcarnitine (C_{14}); [2H_3]palmitoylcarnitine (C_{16}).

SBCAD deficiency on the basis of urine acylglycine analysis. However, their parents reported that these children have never fasted for long periods of time or experienced any illness causing inadequate caloric intake. It has been shown for other genetic diseases such as medium-chain acyl-CoA dehydrogenase deficiency (OMIM No. 201450) that the clinical phenotype often depends on environmental factors, such as prolonged fasting.⁹ We therefore believe that our patients' uneventful clinical course and that the affected mother of 1 previously reported patient has apparently remained asymptomatic should not be interpreted to suggest that SBCAD deficiency is a benign condition. This assumption is underscored by the fact that another patient of Hmong descent and with the same molecular defect causing SBCAD deficiency was not diagnosed until 18 months of age when evaluated for a patent ductus arteriosus, failure to thrive, muscle hypotonia, and moderate developmental delay. Twenty-two months after treatment with a low-protein diet and L-carnitine supplementation was started, muscle tone and gross motor function were improved, whereas speech delay persisted. At the current time, with the range of severity of this deficiency still being unknown, a protein-restricted diet and L-carnitine supplementation seem to be a judicious approach, in particular

when this treatment is initiated before the onset of symptoms. Ultimately, it becomes critical to identify more cases either by prospective newborn screening or selective screening of symptomatic patients to understand this disorder better.

The detection of SBCAD deficiency through acylcarnitine profiling by MS/MS raises important issues because the utilization of this technology as part of newborn screening programs is increasingly considered the standard of care.¹⁰ SBCAD deficiency is characterized by an abnormally elevated concentration of 2-methylbutyrylcarnitine, a 5-carbon saturated acylcarnitine (Fig 1). There are, however, at least 2 other C_5 -acylcarnitine species, pivaloyl- and isovalerylcarnitine. These isomers cannot be routinely differentiated by MS/MS analysis as applied in the newborn screening setting. Pivaloylcarnitine is a metabolite of pivalic acid, which is a component of several antibiotics and therefore of no clinical significance.¹¹ An isolated elevation of the isovalerylcarnitine concentration, however, is indicative of isovaleric acidemia (isovaleryl-CoA dehydrogenase deficiency; OMIM No. 243500), a potentially fatal disorder of L-leucine catabolism that clearly benefits from presymptomatic initiation of treatment (Fig 1). In our experience, isovaleric acidemia is associated with more pronounced C_5 -acylcarnitine elevations

than either SBCAD deficiency or treatment with pivalic acid containing drugs (Table 1; Fig 3). Eventually, it may be possible to provide valid recommendations for result interpretation that are based on disease-specific reference ranges for C₅-acylcarnitine concentrations in newborn blood spots. Currently, however, not enough data are available to prove the assumption that isovaleric acidemia and SBCAD deficiency can be differentiated on the basis of C₅-acylcarnitine levels alone.

These cases highlight an important issue in newborn screening using multi-analyte testing such as acylcarnitine profiling. It is still a common practice for many screening laboratories to request a repeat blood specimen to follow up an abnormal test result. In doing so in response to the detection of an abnormal C₅-acylcarnitine concentration, valuable time will be lost and the precise identity of the elevated metabolite will remain uncertain. Rather, urine organic acid and acylglycine analyses should be pursued, allowing for the unequivocal differentiation among isovalerylglycine, 2-methylbutyrylglycine, and pivalic acid. Furthermore, it is crucial that newborn screening laboratories not only rapidly report abnormal C₅-acylcarnitine levels but also depart from the traditional reporting format that includes only numeric results. Appropriate interpretation of the screening results should include the differential diagnoses and the origin of possible artifacts.

To date, the only patients with SBCAD deficiency that have been detected by newborn screening in Minnesota and Wisconsin are of Hmong descent. The Hmong are an ancient people originally from southwestern China.¹² Although approximately 4 million Hmong still reside there, in the 19th century, some migrated southward into Laos, Burma, Thailand, and Vietnam. The Hmong who live in the United States today have come primarily from Laos after the war in Indochina in the 1960s. Approximately 170 000 Hmong currently live in the United States (0.06% of the population). Relatively large communities are found in California, Minnesota, and Wisconsin, where they constitute 0.2%, 0.8%, and 0.6% of the population, respectively.^{13,14} Because we identified 9 patients (8 by newborn screening) with SBCAD deficiency, all belonging to the Hmong population, it is likely that SBCAD deficiency is prevalent in this ethnic group. On the basis of current evidence, the incidence of SBCAD deficiency among the Hmong could be higher than 1 in 500 live births. A founder effect is also suggested because at least 4 of these 9 Hmong patients are homozygous for the same mutation; molecular genetic studies have not been completed on the other 5 patients with SBCAD deficiency.

CONCLUSIONS

SBCAD deficiency should be added to the disorders that are identifiable by newborn screening using MS/MS. The identification of more patients with this

disorder of L-isoleucine catabolism is likely to aid in the understanding of this enzyme deficiency and will help to delineate better the clinical phenotype. If SBCAD deficiency is a disorder that affects the nervous system as suggested by the previously reported patients, then it seems that early initiation of treatment can significantly improve the patients' prognosis. Finally, screening laboratories must be aware not only of the differential diagnosis of isolated C₅-acylcarnitine elevations but also of the appropriate follow-up of abnormal results that should include urine acylglycine and organic acid analyses.

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