

2-Methylbutyryl-CoA Dehydrogenase Deficiency in Hmong Infants Identified by Expanded Newborn Screen

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ABSTRACT

In April 2000, the Wisconsin Newborn Screening Program implemented tandem mass spectrometry (MS/MS) technology to expand the newborn screening panel from 13 to 48 disorders, the majority of which are inborn errors of metabolism. Among other tests, this technology measures the acylcarnitine profile from blood spots collected from infants at 24 to 48 hours of age. During the first 5.75 years of expanded screening, 27 infants were identified with elevated C5-acylcarnitine concentrations, an unexpectedly high number for any inborn error of metabolism. For these infants, elevated C5-acylcarnitines suggested a diagnosis of isovaleric acidemia (IVA), a metabolic defect of leucine metabolism. Subsequent testing showed that the infants did not have isovaleric acidemia, but did have 2-methylbutyryl-CoA dehydrogenase deficiency or 2-MBAD deficiency, a newly described defect of isoleucine metabolism. (An official abbreviation has not been established for this disorder. Other abbreviations include SBCADD, 2-MBG, and 2-MBCD deficiency.) All but 1 of the 27 infants identified with 2-MBAD deficiency are offspring of Hmong parents. To date, those diagnosed with the disorder in the Hmong community have been largely asymptomatic, though further re-

search is needed to determine whether newborns with 2-MBAD deficiency are at risk for neurodevelopmental disorders.

INTRODUCTION

2-Methylbutyryl-CoA Dehydrogenase Deficiency (2-MBADD, OMIM #600301) is an autosomal-recessive disorder of isoleucine metabolism associated with severe developmental delay, seizures, muscular atrophy, hypotonia, and/or cerebral palsy.¹⁻⁴ This dehydrogenase deficiency causes an error in the third step of l-isoleucine metabolism (Figure 1), and a subsequent accumulation of 2-methylbutyryl-CoA-related metabolites, chiefly 2-methylbutyrylglycine and 2-methylbutyrylcarnitine (a C5-acylcarnitine species). These abnormal metabolites can be detected via urine organic acid and acylglycine analyses, and blood acylcarnitine analysis using tandem mass spectrometry (MS/MS).

Before the widespread use of MS/MS technology in newborn screening (NBS), there were only 5 individuals reported in the world literature with this enzyme deficiency. The first patient, described in 1999 was a 3-day-old male infant of European/Eritrean heritage presenting with lethargy, hypoglycemia, and apnea.¹ Urine organic acid analysis revealed isolated 2-methylbutyrylglycinuria, and elevated plasma C5-acylcarnitine was found by tandem mass spectrometry. Isoleucine degradation studies in intact fibroblasts were consistent with a deficiency of 2-methylbutyryl-CoA dehydrogenase. The diagnosis was further supported by the finding of compound heterozygosity for C778T and intron 3 splice mutations in the 2-MBAD gene.^{2,3} The child's methylbutyrylglycine excretion was normalized following treatment with a low-protein diet and carnitine supplementation. However, at last report the child continues to be challenged with developmental delay and seizures.³ The second patient with 2-MBAD deficiency is the younger sister of the first patient; she was diagnosed prenatally, treated since birth, and reported

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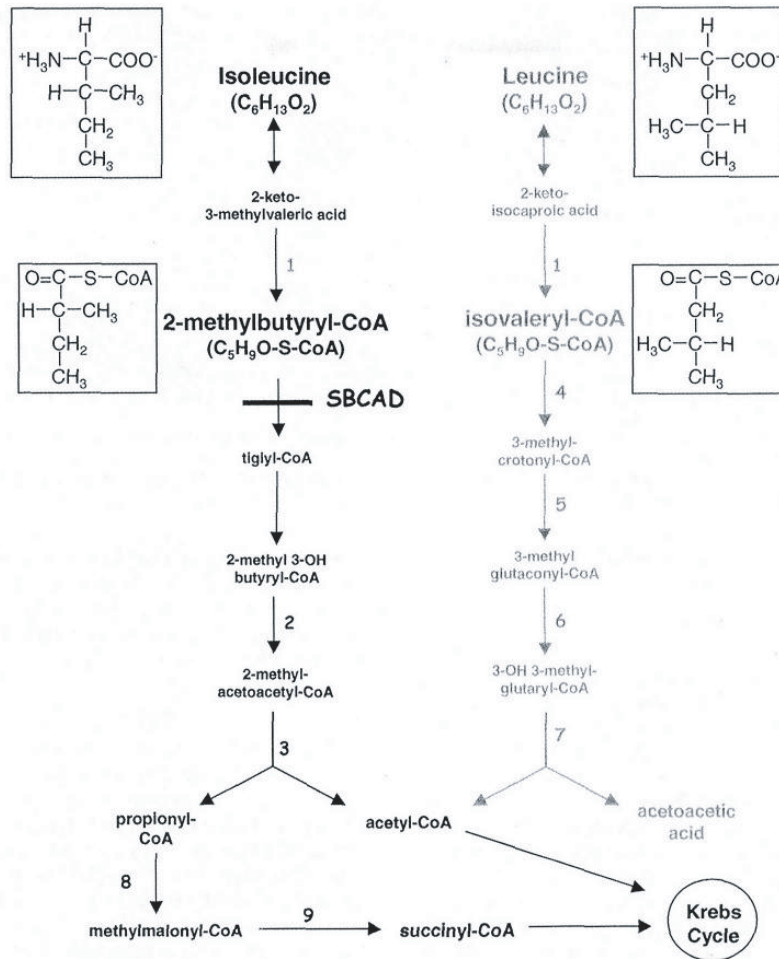


Figure 1. Metabolic pathway of the branched-chain amino acids, isoleucine and leucine, and location of SBCAD. Selected chemical structures and formulas are given to demonstrate the mass equivalence between isoleucine and leucine as well as 2-methylbutyryl-CoA and isovaleryl-CoA, respectively. 1) branched-chain α -ketoacid dehydrogenase complex; 2) 2-methyl 3-hydroxybutyryl-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.

to be asymptomatic.³ The third 2-MBAD patient, of consanguineous Pakistani descent, had a normal neonatal course but was hospitalized at 3 months of age due to poor oral intake during a febrile illness. Progressive hypotonia, delayed motor development, muscular atrophy, and strabismus became apparent in the second year of life. Organic acid analysis revealed methylbutyrylglycinuria and the diagnosis of 2-MBAD deficiency was further supported by the identification of a homozygous G1228A mutation in the 2-MBAD gene. Interestingly, the fourth reported patient is the mother of the third patient, also homozygous for the G1228A mutation with elevated methylbutyrylglycine excretion, but no evidence of a neurodevelopmental disorder.⁴

Before expanded newborn screening was implemented in Wisconsin, a patient of Hmong descent pre-

sented to our clinic at 18 months of age with failure to thrive, hypotonia, and moderate developmental delay. Urine analysis revealed elevated methylbutyrylglycine and the diagnosis of 2-MBAD deficiency was further supported by identification of a homozygous A1165G mutation of the 2-MBAD gene.⁵ Following 2 years of dietary therapy, gross motor skills and muscle tone had improved, while a speech delay remained.⁵

During the first year that MS/MS was incorporated into Wisconsin and Minnesota's newborn screening programs (April 2000-March 2002), 8 additional individuals with 2-MBAD deficiency were detected.⁵ Like the fifth patient discussed above, all were of Hmong heritage. For 3 of these patients, 2-MBAD mutation analysis was carried out, revealing a homozygous A1165G mutation in each. All 8 infants were placed on a low-protein diet and

carnitine supplementation during the newborn period, with varying degrees of compliance. At follow-up (3-14 months of age), 7 patients were asymptomatic, while the eighth patient had mild hypotonia at age 6 months. Three older siblings (ages 3, 4, and 6 years) of 1 of the patients identified by the newborn screening program were found to have elevated methylbutyrylglycine excretion and all 3 were reportedly hypotonic as infants but had no other apparent symptoms of developmental disorder.⁵

In a 2004 international report, 4 additional cases of 2-MBAD deficiency were described.⁶ Three of these patients presented with clinical symptoms, while the fourth was an infant identified through NBS and described as asymptomatic thus far. In personal communication with the authors of this report, the presenting symptoms and findings for the 3 clinically affected patients (age range 8 days to 6 years) included severe failure to thrive, recurrent vomiting, attention deficit and hyperactivity disorder, epilepsy, hypothermia, lactic acidosis, and developmental delay.⁷ These 3 patients were of Middle Eastern descent and their genotypes were variable. The fourth patient was an infant of Vietnamese descent who was heterozygous for the same mutation (A1165G) that is found in the asymptomatic Hmong patients.⁶

METHODS

In 2000, Wisconsin expanded the newborn screening panel from 13 to 48 disorders, most of which are inborn errors of metabolism. Infants in Wisconsin are typically screened within 48 hours after birth. An abnormal acylcarnitine profile consisting of an elevated isovalerylcarnitine (C5) level, elevated isovalerylcarnitine/propionylcarnitine (C5/C3) ratio, and elevated isovalerylcarnitine/acetylcarnitine (C5/C2) ratio is reported as "definite" abnormal and prompts a referral to a metabolic center for diagnostic testing and treatment, if necessary, for IVA or 2-MBAD deficiency. Newborn screening results with only an elevated C5 or elevated C5 and 1 abnormal ratio are reported as "possible" abnormal for an organic acidemia and a repeat screen is requested. A newborn with abnormal C5 results on repeat screen is then referred to a metabolic clinic for diagnostic urine testing for IVA or 2-MBAD. Metabolic referral centers in Wisconsin include the Biochemical Genetics Program, Waisman Center, University of Wisconsin-Madison and the Metabolic Genetics Clinic at Children's Hospital of Wisconsin, Medical College of Wisconsin in Milwaukee.

This paper reviews the NBS acylcarnitine levels, confirmatory laboratory values, and subsequent clinical history of these infants.

RESULTS

Through 2005, the Wisconsin Newborn Screening Program has detected 27 confirmed cases of 2-MBAD deficiency. The C5 levels on the newborn screen ranged from 0.4 to 1.02 micromoles/L. (The screening cutoff for C5 acylcarnitine is ≥ 0.44 micromole/L). The diagnosis was confirmed by elevations of 2-methylbutyrylglycine in urine organic acid and acylglycine profiles. Twenty-six of the confirmed cases were infants of Hmong descent and 1 case was classified as white. Based on identification of 2-MBAD deficiency since the expansion of newborn screening, the prevalence of 2-MBAD deficiency is 1:12,285 (95% confidence interval 1:17,385, 1:8425) in the Wisconsin population of newborns overall, 1:325,593 (95% confidence interval 0, 1:109,998) in white newborns, and 1:223 (95% confidence interval 1:362, 1:161) in newborns of Hmong descent.

At the time of their initial evaluation, all of the Hmong infants with 2-MBAD were growing and developing normally. In light of the reports in the literature regarding the possible course of 2-MBAD deficiency, a conservative approach to treatment was adopted. All of the infants with a confirmed diagnosis were placed on a protein-controlled formula (1.5-1.8 grams protein/kg weight) and supplemented with l-carnitine (50 mg/kg weight). Compliance to the treatment regimen has been variable.

Follow-up examinations have found hypotonia of short duration in 2 of the 26 Hmong infants. One of these patients had 3 siblings who also had some degree of hypotonia in infancy, but were otherwise healthy. At last follow-up, all of those infants (current age range to 5 years) diagnosed with 2-MBAD deficiency by newborn screening are reported to be healthy with normal growth and have not had any episodes suggesting metabolic decompensation with hypoglycemia and ketoacidosis.

For 3 of the Hmong patients, 2-MBAD mutation analysis has been carried out, revealing a homozygous A1165G mutation in each child.⁵ This genetic mutation is different from mutations reported in white and other ethnic groups.^{1-4,6}

Interestingly, the white infant diagnosed with 2-MBAD deficiency presented at 8 days of age with severe seizures, developmental delay, and failure to thrive. Subsequent testing showed that this infant also has nonketotic hyperglycinemia (NKH), an inborn error of metabolism known to cause early-onset, severe seizures. NKH is the likely primary cause of this infant's clinical sequelae; the contribution of 2-MBAD deficiency to the clinical picture is unclear.

DISCUSSION

A surprising outcome of the expanded newborn screening program in Wisconsin is the identification of 26 infants of Hmong descent with 2-MBAD deficiency during the first 5.75 years of screening. Of note is the high prevalence of 2-MBAD deficiency among the Hmong, currently estimated at 1:223 births. This finding suggests that the Hmong cases may share a single genetic trait for this disorder. The history of the Hmong migration from Laos to Thai refugee camps and later migration and expansion of the population in the United States may have created the circumstances for a founder gene.⁷ An alternative explanation for the high prevalence of this disorder in the Hmong is that the genetic trait for 2-MBAD deficiency may confer a selective advantage in the Southeast Asian environment where dietary sources of protein are more limited than they are in settings such as the United States.

From our experience and published reports, it appears that the clinical spectrum observed with 2-MBAD deficiency is quite variable and the severity may depend on the genotype associated with different ethnic groups. In spite of varying degrees of adherence to medical recommendations for diet treatment and carnitine supplementation, symptoms of 2-MBAD deficiency in Wisconsin Hmong infants appear to be mild or absent. This is in contrast to published reports of infants and children who have experienced the severe and life-threatening symptoms common to many of the organic acidemias. Whether there are subgroups of cases at risk for adverse outcomes, or whether exposure to illnesses or nutritional challenges at a critical period of development is necessary to trigger adverse developmental effects is not known.

The implication of these findings is that further study is needed to determine if long-term follow-up is necessary for those who screen positive for 2-MBAD deficiency from the Hmong community. Policies for detection, diagnosis, treatment, and follow-up may be dictated by the results of mutation analysis as a component of newborn screening. It is possible that there are benign mutations of this disorder that do not require treatment and follow-up. Further research is needed.

In a new study funded by the National Institute of Health, we will seek to answer some of the questions that arise from these findings, including: (1) genetic studies to confirm the mutation and incidence of 2-MBAD deficiency in Wisconsin, particularly in the Wisconsin Hmong population; (2) documentation of symptoms or any precipitating events (illness, infection, fasting) resulting in metabolic decompensation in diagnosed in-

fants or affected siblings; (3) developmental outcomes in children diagnosed with 2-MBAD deficiency compared with unaffected siblings; and (4) determine whether a treatment intervention study is needed.

It is hoped that longer term, comprehensive follow-up of 2-MBAD deficiency in the Wisconsin Hmong population will result in clear guidelines for treatment and follow-up. This will bring clarity to the metabolic referral centers in Wisconsin and to other states as they incorporate tandem mass spectrometry into their newborn screening programs.

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REFERENCES

1. Gibson KM, Sacks M, Kiss D, et al. 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, et al. 2-methylbutyryl-coenzyme A dehydrogenase deficiency: a new inborn error of L-isoleucine metabolism. *Pediatr Res.* 2000;47(6):830-833.
3. Akaboshi S, Ruiters J, Wanders RJA, Andresen BS, Steiner RD, Gibson KM. Divergent phenotypes in siblings with confirmed 2-methylbutyryl-CoA dehydrogenase (2-MBAD) deficiency. *J Inherit Metab Dis.* 2001;(suppl 1):58.
4. Andresen BS, Christensen E, Corydon TJ, et al. 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 dehydrogenases in isoleucine and valine metabolism. *Am J Hum Genet.* 2000;67:1095-1103.
5. Matern D, He M, Berry SA, et al. Prospective diagnosis of 2-methylbutyryl-CoA dehydrogenase deficiency in the Hmong population by newborn screening using tandem mass spectrometry. *Pediatrics.* 2003;112(1):74-78.
6. Korman SH, Andresen BS, Zeharia A, Gutman A, Boneh A, Pitt JJ. 2-ethylhydracrylic aciduria in short/branched-chain acyl-CoA dehydrogenase deficiency: application to diagnosis and implications for the R-pathway of isoleucine oxidation. *Clin Chem.* 2005;51(3):1-8.
7. Chan SC (ed). *Hmong Means Free: Life in Laos and America.* Temple University Press; 1995.

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