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Case Report

From Screening to Diagnosis: *ACADSB* Variant Co-Inheritance Causing 2-methylbutyrylglycinuria in an Iranian Neonate

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ABSTRACT

Background: Short/branched-chain acyl-CoA dehydrogenase deficiency, also known as 2-methylbutyrylglycinuria, is a rare autosomal recessive metabolic disorder affecting the catabolism of L-isoleucine. Early detection through newborn screening programs using tandem mass spectrometry enables timely diagnosis and intervention, potentially preventing severe clinical outcomes.

Case Presentation: In this study, we report a case identified through Iran's national newborn screening program, presenting with elevated C5-acylcarnitine levels. Whole exome sequencing revealed compound heterozygosity for two *ACADSB* gene variants: c.908G>C and c.1159G>A. While c.1159G>A is classified as pathogenic, c.908G>C was previously considered a variant of uncertain significance. However, the observed biochemical phenotype and in silico analysis support reclassification of c.908G>C as likely pathogenic.

Conclusion: This case highlights the importance of integrating genotype–phenotype correlation and advanced genomic tools to refine variant interpretation and improve diagnostic accuracy in rare metabolic disorders.

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Introduction

Screening programs for the detection of inborn errors of metabolism (IEMs) could lead to early detection of the metabolic disorder and prevention of severe clinical after effects [1]. Newborn screening of IEMs began in the 1960s when Guthrie and Susi developed a method for the estimation of phenylalanine level in dried blood spot (DBS) samples [2]. Currently, tandem mass spectrometry (MS/MS) is introduced as a robust method for newborn screening of several (>40) inherited metabolic disorders with just 2 minutes run time [3].

Short/branched chain acyl-CoA dehydrogenase (SBCAD) deficiency, also known as 2-methylbutyryglycinuria (2MBG), is an autosomal recessive inherited metabolic disorder related to homotetrameric mitochondrial enzymes that play an important role in β-oxidation of the unsaturated fatty acids. SBCAD catalyzes the third step in the pathway of L-isoleucine (branched-chain amino acids) metabolism [4]. Elevation of C5-carnitine (isovalerylcarnitine, 2MBG, or pivaloylcarnitine) is an indicator of 2MBG at newborn screening of IEMs by analysis of acylcarnitine profile in DBS [5]. Patients with 2MBG are characterized by urinary secretion of 2-methylbutyrylglycine, the main biomarker of 2MBG, which could differentiate the 2MBG diagnosis from other deficiencies [6]. The initial clinical manifestations of patients with 2MBG could include poor feeding, vomiting, lack of energy, and irritability. However, based on current data, 90% of cases are asymptomatic, but a small percentage that have symptoms lead to serious medical problems, such as seizures, developmental delay, dyspnea, and even coma [7, 8]. SBCAD deficiency (OMIM 600301/610006) was first described in 2000 [9]. 2MBG is a rare inherited metabolic disease with an incidence rate of 1:17,780 live births in the European population, but in the Hmong society, its prevalence is approximately 1:132 live births [8, 10]. Dietary restriction of protein intake and L-carnitine supplementation are prescribed for the treatment of the disease [7, 11].

Newborn screening for IEMs for the detection of metabolic disorders has been implemented in Iran since 2018 using mass spectrometry technology, which has led to the identification of several rare metabolic conditions [12]. Next-generation sequencing (NGS) technologies have also facilitated the identification of the pathogenic variants in the genes responsible for the metabolic disorders as a confirmatory test [13]. The present study aimed to introduce a novel pathogenic variant in the *ACADSB* gene in a case with 2MBG diagnosed in the NBS program in Mazandaran Province, North of Iran.

Case Presentation

This study was reviewed and approved by the Ethics Committee of Mazandaran University of Medical Sciences. All participants provided written informed consent prior to enrollment and voluntarily agreed to participate in the study. As part of the newborn screening program for IEMs, DBS samples were referred to Fajr Medical Laboratory in Sari City, Iran. Initial analysis of acylcarnitines and amino acid profiles was performed using tandem MS/MS on a Shimadzu MS/MS-8030 system (Japan). The results revealed an elevated level of C5-acylcarnitine in a newborn from consanguineous parents.

To confirm the preliminary suspicion of Isovaleric Acidemia or 2MBG, a follow-up biochemical evaluation was conducted using plasma acylcarnitine profiling. This analysis, performed via liquid chromatography (LC)- MS/MS on a Shimadzu LC-MS/MS 8045 system (Japan), confirmed a significant elevation of C5-acylcarnitine, specifically isovalerylcarnitine (Table 1).

WES was performed using the Illumina platform to identify the genetic cause of the suspected metabolic disorder. The analysis revealed that the patient is a compound heterozygote for two variants in the *ACADSB* gene (NM_001609.4): c.908G>C and c.1159G>A.

The c.1159G>A variant has been previously classified as pathogenic in the ClinVar database, while the c.908G>C variant was initially categorized as a variant of uncertain significance (VUS). To further evaluate their clinical relevance, in silico analysis was conducted using Franklin Genomics online tools in accordance with American College of Medical Genetics and Genomics (ACMG) guidelines. This assessment reclassified the c.908G>C variant as likely pathogenic.

In silico predictions using the PP3 criterion indicated that this variant was pathogenic (strong), with an aggregated score of 0.994. Furthermore, population data (PM2) supported classification as pathogenic (moderate), given its very low allele frequency in the gnomAD database, for the c.1159G>A variant, evaluation based on allelic data (PM3), functional studies (PS3), population data (PM2), and in silico analyses (PP3/BP4) classified the variant as pathogenic (very strong), pathogenic (supporting), pathogenic (moderate), and pathogenic (moderate), respectively (Table 2).

Table 1. Plasma acylcarnitine values in the patient with 2MBG by LC-MS/MS

Acylcarnitines	Patient's Results	Reference Range (micromole/L)
C0 (free carnitine)	39.82	4.39-41.7
C2 (acetylcarnitine)	7.08	2.43-18.29
C3 (propionylcarnitine)	0.45	<0.88
C3DC (malonylcarnitine)	0.01	<0.15
C4 (butyrylcarnitine)	0.26	<0.47
C4OH (hydroxybutyrylcarnitine)	0.02	<0.16
C4DC (succinyl+methylmalonylcarnitine)	0.01	<0.11
C5 (isovalerylcarnitine)	2.18 H	<0.39
C5:1 (tiglylcarnitine)	0.01	<0.07
C5OH (hydroxyisovalerylcarnitine)	0.2	<0.21
C5DC (glutarylcarnitine)	0.01	<0.16
C6 (hexanoylcarnitine)	0.12	<0.2
C6DC (adipoylcarnitine)	0.01	<0.26
C8 (octanoylcarnitine)	0.11	<0.19
C8:1 (octenoylcarnitine)	0.02	<0.48
C10 (decanoylcarnitine)	0.18	<0.3
C10:1 (decenoylcarnitine)	0.01	<0.33
C12 (dodecanoylcarnitine)	0.09	<0.2
C12:1 (dodecenoylcarnitine)	0.13	<0.17
C14 (tetradecanoylcarnitine)	0.01	<0.19
C14:1 (tetradecenoylcarnitine)	0.2	<0.2
C14:2 (tetradecadienoylcarnitine)	0.04	<0.13
C14OH (hydroxytetradecanoylcarnitine)	0.08	<0.09
C16 (hexadecanoylcarnitine)	0.13	<0.35
C16OH (hydroxyhexadecanoylcarnitine)	0.01	<0.1
C16:1OH (hedroxyhexadecenoylcarnitine)	0.01	<0.14
C16:1 (hexadecenoylcarnitine)	0.01	<0.12
C18 (octadecanoylcarnitine)	0.02	<0.1
C18:1 (octadecenoylcarnitine)	0.01	<0.19
C18:2 (octadecadienoylcarnitine)	0.01	<0.17
C18OH (hydroxyoctadecanoylcarnitine)	0.01	<0.05
C18:1OH (hydroxyoctadecenoylcarnitine)	0.01	<0.04

2MBG: 2-methylbutyrylglycinuria; LC-MS/MS: Liquid chromatography-mass spectrometry.

Table 2. Characteristics of two *ACADSB* gene variants identified in a patient with 2MBG

Variant Name	Protein Change	rs Number	ACMG Classification	ClinVar ID/Classification	GnomAD Frequency (Non-founder Subpopulations)	In-silico Prediction	Type of Mutation
c.908G>C	p.Gly303Ala	rs1316417761	Likely pathogenic	RCV003317811.1/ VUS	0.001%	Pathogenic strong	Missense variant
c.1159G>A	p.Glu387Lys	rs188094280	Pathogenic	RCV000009781.34/ pathogenic 5, likely pathogenic 2, VUS 1	0.055%	Pathogenic moderate	Missense variant

Abbreviations: VUS: Variant of uncertain significance; ACMG: American college of medical genetics and genomics; 2MBG: 2-methylbutyrylglycinuria.

It should be noted that an enzyme assay was not performed, which represents a limitation of the present study

Discussion

2MBG deficiency is a rare, typically asymptomatic metabolic disorder caused by impaired isoleucine metabolism due to reduced activity of the 2-methylbutyryl-CoA dehydrogenase enzyme. Most affected infants are identified through neonatal screening programs based on elevated levels of C5 acylcarnitine [14]. Therapeutic interventions for individuals with 2MBG primarily include dietary protein restriction, L-carnitine supplementation, and avoidance of prolonged fasting. Although most cases are asymptomatic, long-term monitoring and follow-up are recommended to ensure metabolic stability and early detection of potential complications [4].

In this case, we identified a compound heterozygote for the *ACADSB* gene variants c.908G>C and c.1159G>A. The c.908G>C variant is currently classified as a VUS in the ClinVar database. However, its co-inheritance with the c.1159G>A variant in the present case was associated with elevated levels of C5 (isovalerylcarnitine), suggesting a functional impact. These findings support reclassification of the c.908G>C variant as likely pathogenic.

Phenotype–genotype investigation plays a critical role in elucidating the clinical significance of genetic variants, particularly in rare metabolic disorders such as SBCAD deficiency. In this case, the co-occurrence of elevated C5 (isovalerylcarnitine) levels with compound heterozygosity for the *ACADSB* variants c.908G>C and c.1159G>A provides compelling evidence of a functional impact. Although c.908G>C is currently classified as a VUS, its association with a biochemical phenotype suggests pathogenicity. Integrating molecular findings with metabolic profiles not only strengthens variant interpretation but also guides accurate diagnosis, risk assessment, and personalized management. This underscores the necessity of combining genetic data with clinical and biochemical evidence to refine variant classification and improve patient care.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Research Ethics Committee of Mazandaran University of Medical Sciences, Sari, Iran (Code: IR.MAZUMS.REC.1404.481). All participants provided written informed consent prior to enrollment and voluntarily agreed to participate in the study.

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Authors contribution's

All authors contributed equally to the conception and design of the study, data collection and analysis, interception of the results and drafting of the manuscript. Each author approved the final version of the manuscript for submission.

Conflict of interest

The authors declared no conflict of interest.

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