Molecular follow-up of a newborn screening positive case of medium-chain acyl–coenzyme A dehydrogenase deficiency identified two ACADM variants: Are they clinically pathogenic?

Yuan-Yuan Ho, Cindy Morgan, Britt Johnson
Metabolic Genetics Clinical Area Team, Clinical Genomics Group, Invitae Corporation, San Francisco, CA

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Introduction

Medium-chain acyl–coenzyme A dehydrogenase deficiency (MCADD) is one of the most common recessively inherited metabolic diseases and has a birth prevalence of 5.3 in 100,000 births. MCADD is a core newborn screening (NBS) condition in all 50 states. MCADD is caused by pathogenic mutations in the ACADM gene that affect the β-oxidation of fatty acids 6–12 carbons long. Impaired hepatic ketogenesis in affected individuals results in hypoketotic hypoglycemia, metabolic acidosis, and liver disease. Lethargy can rapidly progress to coma and death when glycogen stores are depleted during catabolic physiological states. If undiagnosed, MCADD has considerable morbidity and mortality.

Adverse outcomes can be prevented by avoiding metabolic stress and following simple dietary interventions. MCADD can have a significant health impact in early life, but understanding of genotype–phenotype relationship remains limited (1).

Case

The proband is a male infant identified through NBS with a C8 level of 1.710 (t < 0.6). He was 6 weeks old at the time of sample accession. Confirmatory biochemical testing revealed continued elevation of C6, C8, C10, and C10:1 and an elevated C8/C10 ratio.

Acylglycines analysis revealed elevated hexanoylglycine with normal suberylglycine.

The proband has no known family history of MCADD, and no consanguinity was indicated.

Methods

Sequencing of the ACADM gene was the only test ordered and analysis was first performed for the proband. Subsequent analyses were performed on the parents through Invitae’s complementary variant of uncertain significance (VUS) resolution program.

Genomic DNA obtained from whole blood was enriched for targeted regions by using a hybridization-based protocol, and then sequenced with Illumina technology.

Targeted regions were sequenced with ≥50x depth or were supplemented with additional analysis. Reads were aligned to a reference sequence (GRCh37), and sequence changes were identified and interpreted in the context of a single clinically relevant transcript, ACADM (NM_000016.5), in accordance with ACMG guidelines (2).

Enrichment and analysis focused on the coding sequence of the indicated transcripts, 10 bp of flanking intronic sequence, and other specific genomic regions demonstrated to be causative of disease at the time of assay design.

Promoters, untranslated regions, and other non-coding regions were not otherwise interrogated.

Exonic deletions and duplications were called with an in-house algorithm that determines copy number at each target by comparing the read depth for each target in the proband sequence with both mean read depth and read depth distribution obtained from a set of clinical samples (https://www.invitae.com/en/assay/).

The pathogenic variant was confirmed with Sanger sequencing.

Results and Discussion

One pathogenic variant and one variant of uncertain significance identified in ACADM

<table>
<thead>
<tr>
<th>Variant Type</th>
<th>Chromosome</th>
<th>Gene</th>
<th>Protein</th>
<th>DNA Change</th>
<th>RNA Change</th>
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<tbody>
<tr>
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<td>2</td>
<td>ACADM</td>
<td></td>
<td>p.Asp266Gly</td>
<td></td>
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</tr>
<tr>
<td>VUS</td>
<td>2</td>
<td>ACADM</td>
<td></td>
<td>p.Asp266Gly</td>
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</table>

The pathogenic variant p.Lys329Glu (paternal) is the most prevalent mutation in patients with clinically symptomatic MCADD. This variant is reportedly as high as 80% homozygous but is observed considerably less frequently in individuals identified through NBS (63%/47% homozygous) (3). The variant confers high risk for metabolic decompensation, although unaffected homozygous adults have been reported.

The p.Asp266Gly variant (maternal) is currently classified as a VUS. It has been previously reported in newborns with biochemical markers consistent with MCADD and shown to cause partial enzymatic deficiency (4). However, it has not been reported in clinically symptomatic patients.

Although this genotype has been previously described in one NBS-positive MCADD case (5), the long-term outcome is unknown.

This individual showcases the uncertain realities patients and clinicians often face when dealing with a rare disease such as MCADD for which NBS positivity with confirmatory results does not necessarily predict clinical consequences.

Conclusions

We identified two ACADM variants in trans: one pathogenic variant, p.Lys329Glu, and one VUS, p.Asp266Gly, in an MCADD infant identified through NBS. This genotype has not been reported in clinically symptomatic patients, and follow-up is needed to assess the long-term clinical outcome.

Emerging evidence supports an MCADD genotype–phenotype correlation, although further substantiation of this relationship is required.

We share this case report with the goal of contributing to MCADD genotype and phenotype data collection, which is essential for understanding the natural history of MCADD and more accurately projecting its long-term clinical consequences.

References


