# 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?

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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 (rr < 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).

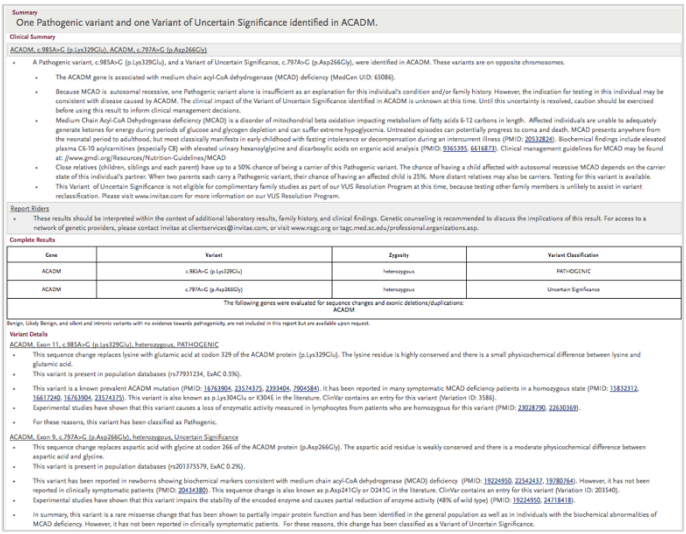
Enrichment and analysis focused on the coding sequence of the indicated transcripts, 10 bp of flanking intronic sequence, and other specific genomic regions demonstrated to be causative of disease at the time of assay design.

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 identified was confirmed with Sanger sequencing.

## Results and Discussion



Summary

One Pathogenic variant and one Variant of Uncertain Significance identified in ACADM.

Clinical Summary

ACADM, c.985A>G (p.Lys329Glu), ACADM, c.797A>G (p.Asp266Gly)

- A Pathogenic variant, c.985A>G (p.Lys329Glu), and a Variant of Uncertain Significance, c.797A>G (p.Asp266Gly), were identified in ACADM. These variants are on opposite chromosomes.
  - The ACADM gene is associated with medium chain acyl-CoA dehydrogenase (MCAD) deficiency (MedGen UID: 65086).
  - Because MCAD is autosomal recessive, one Pathogenic variant alone is insufficient as an explanation for this individual's condition and/or family history. However, the indication for testing in this individual may be consistent with disease caused by ACADM. The clinical impact of the Variant of Uncertain Significance identified in ACADM is unknown at this time. Until this uncertainty is resolved, caution should be exercised before using this result to inform clinical management decisions.
  - Medium Chain Acyl-CoA Dehydrogenase deficiency (MCAD) is a disorder of mitochondrial beta oxidation impacting metabolism of fatty acids 6-12 carbons in length. Affected individuals are unable to adequately generate ketones for energy during periods of glucose and glycogen depletion and can suffer extreme hypoglycemia. Untreated episodes can potentially progress to coma and death. MCAD presents anywhere from the neonatal period to adulthood, but most classically manifests in early childhood with fasting intolerance or decompensation during an intercurrent illness (PMID: 20532824). Biochemical findings include elevated plasma C6-10 acylcarnitines (especially C8) with elevated urinary hexanoylglycine and dicarboxylic acids on organic acid analysis (PMID: 9161395, 6616873). Clinical management guidelines for MCAD may be found at: www.gmdi.org/Resources/Nutrition-Guidelines/MCAD
  - Close relatives (children, siblings and each parent) have up to a 50% chance of being a carrier of this Pathogenic variant. The chance of having a child affected with autosomal recessive MCAD depends on the carrier state of this individual's partner. When two parents each carry a Pathogenic variant, their chance of having an affected child is 25%. More distant relatives may also be carriers. Testing for this variant is available.
  - This Variant of Uncertain Significance is not eligible for complimentary family studies as part of our VUS Resolution Program at this time, because testing other family members is unlikely to assist in variant reclassification. Please visit www.invitae.com for more information on our VUS Resolution Program.

Report Riders

- These results should be interpreted within the context of additional laboratory results, family history, and clinical findings. Genetic counseling is recommended to discuss the implications of this result. For access to a network of genetic providers, please contact Invitae at clientservices@invitae.com, or visit www.nsgc.org or tagc.med.sc.edu/professional.organizations.asp.

Complete Results

| Gene | Variant | Zygosity | Variant Classification |
|---|---|---|---|
| ACADM | c.985A>G (p.Lys329Glu) | heterozygous | PATHOGENIC |
| ACADM | c.797A>G (p.Asp266Gly) | heterozygous | Uncertain Significance |

The following genes were evaluated for sequence changes and exonic deletions/duplications: ACADM

Benign, Likely Benign, and silent and intronic variants with no evidence towards pathogenicity, are not included in this report but are available upon request.

Variant Details

ACADM, Exon 11, c.985A>G (p.Lys329Glu), heterozygous, PATHOGENIC

- This sequence change replaces lysine with glutamic acid at codon 329 of the ACADM protein (p.Lys329Glu). The lysine residue is highly conserved and there is a small physicochemical difference between lysine and glutamic acid.
- This variant is present in population databases (rs77931234, ExAC 0.5%).
- This variant is a known prevalent ACADM mutation (PMID: 16763904, 23574375, 2393404, 7904584). It has been reported in many symptomatic MCAD deficiency patients in a homozygous state (PMID: 15832312, 16617240, 16763904, 23574375). This variant is also known as p.Lys304Glu or K304E in the literature. ClinVar contains an entry for this variant (Variation ID: 3586).
- Experimental studies have shown that this variant causes a loss of enzymatic activity measured in lymphocytes from patients who are homozygous for this variant (PMID: 23028790, 22630369).
- For these reasons, this variant has been classified as Pathogenic.

ACADM, Exon 9, c.797A>G (p.Asp266Gly), heterozygous, Uncertain Significance

- This sequence change replaces aspartic acid with glycine at codon 266 of the ACADM protein (p.Asp266Gly). The aspartic acid residue is weakly conserved and there is a moderate physicochemical difference between aspartic acid and glycine.
- This variant is present in population databases (rs201375579, ExAC 0.2%).
- This variant has been reported in newborns showing biochemical markers consistent with medium chain acyl-CoA dehydrogenase (MCAD) deficiency (PMID: 19224950, 22542437, 19780764). However, it has not been reported in clinically symptomatic patients (PMID: 20434380). This sequence change is also known as p.Asp241Gly or D241G in the literature. ClinVar contains an entry for this variant (Variation ID: 203540).
- Experimental studies have shown that this variant impairs the stability of the encoded enzyme and causes partial reduction of enzyme activity (48% of wild type) (PMID: 19224950, 24718418).
- In summary, this variant is a rare missense change that has been shown to partially impair protein function and has been identified in the general population as well as in individuals with the biochemical abnormalities of MCAD deficiency. However, it has not been reported in clinically symptomatic patients. For these reasons, this change has been classified as a Variant of Uncertain Significance.

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

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