Mitochondrial 3-Hydroxy-3-Methylglutaryl-CoA Synthase Deficiency: Unique Presenting Laboratory Values and a Review of Biochemical and Clinical Features

Erin Conboy • Filippo Vairo • Matthew Schultz •
Katherine Agre • Ross Ridsdale • David Deyle •
Devin Oglesbee • Dimitar Gavrilov • Eric W. Klee •
Brendan Lanpher

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Abstract We report an 8-month-old infant with decreased consciousness after a febrile episode and reduced oral intake. He was profoundly acidotic but his lactate was normal. Serum triglycerides were markedly elevated and HDL cholesterol was very low. The urine organic acid analysis during the acute episode revealed a complex pattern of relative hypoketotic dicarboxylic aciduria, suggestive of a potential fatty acid oxidation disorder. MRI showed extensive brain abnormalities concerning for a primary energy deficiency. Whole exome sequencing revealed heterozygotic *HMGCS2* variants. *HMGCS2* encodes mitochondrial 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase-2 (*HMGCS2*), which catalyzes the irreversible and rate-limiting reaction of ketogenesis in the mitochondrial matrix. Autosomal recessive HMG-CoA synthase deficiency (HMGCS2D) is characterized by hypoketotic hypoglycemia, vomiting, lethargy, and hepatomegaly after periods of prolonged fasting or illness. A retrospective analysis of the urine organic acid analysis identified 4-hydrox-6-methyl-2-pyrone, a recently reported putative biomarker of

HMGCS2D. There was also a relative elevation of plasma acetylcarnitine as previously reported in one case. Our patient highlights a unique presentation of HMGCS2D caused by novel variants in *HMGCS2*. This is the first report of HMGCS2D with a significantly elevated triglyceride level and decreased HDL cholesterol level at presentation. Given this, we suggest that HMGCS2D should be considered in the differential diagnosis when hypertriglyceridemia, or low HDL cholesterol levels are seen in a child who presents with acidosis, mild ketosis, and mental status changes after illness or prolonged fasting. Although HMGCS2D is a rare disorder with nonspecific symptoms, with the advent of next-generation sequencing, and the recognition of novel biochemical biomarkers, the incidence of this condition may become better understood.

Introduction

Mitochondrial 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase is a 471-residue, 51,350-Da peptide that catalyzes the irreversible and rate-limiting reaction of ketogenesis within the mitochondrial matrix. *HMGCS2* maps to chromosome 1p12–13. *HMGCS2* encodes for the mitochondrial HMG-CoA synthase which is highly abundant in the liver in contrast to a cytoplasmic form of HMG-CoA synthase, which is encoded by a homologous *HMGCS1*, and is targeted to the cytoplasm. Through a proposed three-step reaction, HMG-CoA synthase mediates the formation of HMG-CoA, a required intermediate of ketone bodies and a precursor of mevalonate and cholesterol (Mitchell et al. 2014). Ketone bodies are necessary for

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Erin Conboy and Filippo Vairo contributed equally to this work.

E. Conboy • K. Agre • D. Deyle • D. Oglesbee • D. Gavrilov •
B. Lanpher

Department of Clinical Genomics, Mayo Clinic, Rochester, MN, USA

E. Conboy • F. Vairo • K. Agre • D. Deyle • D. Gavrilov • E.W. Klee •
B. Lanpher (✉)

Center for Individualized Medicine, Mayo Clinic, Rochester, MN,
USA

e-mail: Lanpher.Brendan@mayo.edu

M. Schultz • R. Ridsdale • D. Oglesbee • D. Gavrilov
Department of Laboratory Medicine, Mayo Clinic, Rochester, MN,
USA

energy transfer, particularly to the brain, during periods of fasting.

HMG-CoA synthase deficiency (MIM: 605911; HMGCS2D) is a rare autosomal recessive inborn error of metabolism that presents in the first year of life with hypoketotic hypoglycemia, vomiting, lethargy, and hepatomegaly after a period of prolonged fasting or intercurrent illness (Morris et al. 1998; Thompson et al. 1997; Ramos et al. 2013). Some cases have reported acidosis in addition to these symptoms (Morris et al. 1998). Diagnosis of HMGCS2D is problematic, but it can be made using molecular genetic testing or by enzyme activity measurement (Ramos et al. 2013). More recently, the phenotypic spectrum has expanded as children have been identified with HMGCS2D after periods of hypoglycemia during early childhood (Pitt et al. 2015).

We describe an 8-month-old infant who presented with encephalopathy, hepatomegaly, Kussmaul breathing, and high anion gap metabolic acidosis. Uniquely, this child had a markedly elevated triglyceride level with very low high-density lipoproteins (HDL) cholesterol, a finding not yet observed in previously published cases of HMGCS2D.

Materials and Methods

IRB and Patient Consent

We evaluated the proband at the Mayo Clinic in Rochester, MN, USA, with parental consent. The study protocol was approved by the Mayo Clinic Institutional Review Board.

Biochemical Testing

Biochemical testing was performed at the Mayo Clinic Biochemical Genetics Laboratory. Urine organic acids were analyzed by gas chromatography-mass spectrometric analysis of trimethylsilyl ethers of urinary organic acids as previously described (Rinaldo 2008). Plasma acylcarnitines were analyzed by tandem mass spectrometry of butylated carnitine esters as previously published (Rinaldo et al. 2008). Figures and reference ranges for acetylcarnitine analysis were established from 24,671 normal profiles with the aid of Collaborative Laboratory Integrated Reports (CLIR) software.

Genetic Analysis

Whole exome sequencing (WES) of the proband was performed at the Baylor Miraca Genetics Laboratory (Houston, Texas, USA). In summary, for the paired-end pre-capture library procedure, genome DNA is fragmented by sonicating genomic DNA and ligating to the Illumina

multiplexing PE adapters. The adapter-ligated DNA is then PCR amplified using primers with sequencing barcodes. For target enrichment/exome capture procedure, the pre-capture library is enriched by hybridizing to biotin labeled VCRome 2.1 in-solution exome probes at 47°C for 64–72 h. Additional probes for over 3,600 Mendelian disease genes were also included in the capture in order to improve the exome coverage. For massively parallel sequencing, the post-capture library DNA is subjected to sequence analysis on Illumina HiSeq platform for 100 bp paired-end reads. The following quality control metrics of the sequencing data are generally achieved: >70% of reads, aligned to target, >95% target base covered at 20×, >85% target base covered of target bases >100×. SNP concordance to genotype array: >99%. The individual's DNA was also analyzed by an SNP array (Illumina HumanExome-12v1 array). The output data from Illumina HiSeq were converted from blc file to FASTQ file by Illumina CASAVA 1.8 software and mapped by BWA program to the Genome Reference Consortium human genome build 37. The variant calls and annotations were performed using algorithms developed in-house by the laboratory. Sanger sequencing was performed in the proband's and parents' DNA for variant confirmation. The *HMGCS2* cDNA reference sequence used was NM_005518.3.

Results

Patient Case

At presentation, the patient was 8-month-old male born at term after an uncomplicated pregnancy and delivery to non-consanguineous parents. There were no perinatal concerns. State newborn screening was normal. His growth, development, and health were normal prior to presentation. He presented after several days of upper respiratory illness symptoms and decreased oral intake. He became unresponsive after a febrile episode and emesis. A rapid Kussmaul breathing pattern was noted. Laboratory investigations in the emergency department revealed an initial metabolic acidosis with pH of 6.8, and subsequently a pH of 7.0 with an anion gap of −28. Urinary dipstick showed ketones at 40 mg/dL (reference: negative), a point of care lactate was normal at 0.79 (reference: 0.6–2.3 mmol/L), and glucose was normal at 3.89 mmol/L (reference range: 3.89–5.55 mmol/L). He received intravenous fluids prior to labs being obtained. His AST and ALT was elevated at 686 and 161 U/L (reference range: AST 8–60 U/L, ALT: 7–55 U/L). Total cholesterol was within normal limits at 4.06 mmol/L, but his triglycerides were significantly elevated at 18.84 mmol/L (reference range: cholesterol: normal <4.4 mmol/L, triglycerides: normal <1.7 mmol/L) and returned to normal ranges

when checked 1 week after presentation. Non-HDL cholesterol was 3.98 mmol/L; HDL cholesterol was 0.07 mmol/L (reference range for non-HDL cholesterol is not well established in children, HDL cholesterol normal >0.9 mmol/L). He also had significant thrombocytopenia with platelet counts as low as $10 \times 10^9/L$ (reference range: $150\text{--}450 \times 10^9/L$). No other similarly affected family members, including 3-year-old sister were reported.

An abdominal ultrasound revealed hepatomegaly with increased echogenicity and coarse echotexture. New onset seizures began while the patient was hospitalized. A brain MRI showed extensive abnormalities of signal, diffusion, and perfusion throughout the gray and white matter (Fig. 1a). MR spectroscopy of the right basal ganglia, parietal white matter, and cerebellum reveals diffusely decreased NAA:choline ratios with large lactate peaks at 1.35 ppm, and amino acid/macromolecular peaks at 0.9 ppm. Increased glutamate-glutamine peaks at 2.1–2.5 and 3.75 ppm, and mildly depleted myoinositol peaks at 3.56 ppm, suggesting a primary energy failure with neurotoxic by-products and disrupted cerebral autoregulation. There was also evidence of cerebral venous congestion and soft tissue edema. Electroencephalography showed multiple and multifocal discharges consistent with status epilepticus. Five-month follow-up MRI showed significant volume loss as well as extensive white matter encephalomalacia (Fig. 1b).

At 15 months of age, the proband continued to be profoundly delayed, without the ability to sit or stand independently, with significant head lag, lack of consistent visual tracking, and intractable seizures. He is gastrostomy-tube-dependent. Despite the resolution of his acute metabolic decompensation, we expect that the severity of brain

injury will have a significant impact on his cognitive and physical development.

Biochemical Testing

Urine organic acid analysis during an acute episode revealed a complex pattern of metabolites. Ketone bodies (3-hydroxy-*n*-butyric acid and acetoacetic acid) as well as lactic acid were detected at moderate amounts. The excretion of adipic acid was markedly elevated (539 mmol/mol creatinine; reference: <15), while other dicarboxylic acids were increased to a lesser extent or in the normal range, including suberic (24 mmol/mol; reference: <8) and sebacic acid (1 mmol/mol; reference: <8). Glutaric acid excretion was markedly elevated (557 mmol/mol creatinine; reference: <13) with a moderate amount of 3-OH glutaric acid. Acylcarnitine testing did not detect an elevation of glutaryl carnitine (C5-DC) in the plasma or urine, a result inconsistent with glutaric acidemia type I. The origin of glutaric acid in his urine sample is unclear and possibly related to impaired ketogenesis. 4-hydroxyphenyl lactate and 4-hydroxyphenyl pyruvate were also elevated in a typical tyrosyluria pattern consistent with liver injury. Several unusual trans-hydroxyhexenoic acids were present at moderate concentrations and not components of a typical organic acid profile (Fig. 2a). Two days after presentation, the glutaric acid remained mildly elevated (23 $\mu\text{mol}/\text{mmol}$ creatinine) as well as a minimal lactic aciduria and resolving tyrosyluria. These abnormalities normalized in urine over a number of days with dextrose infusion and nutritional support.

Initial plasma acylcarnitine analysis did not demonstrate abnormalities consistent with a recognized disorder of fatty

Fig. 1 T2/FLAIR axial view MRI showing (a) multifocal hyperintense signal abnormality involving the cerebral cortex, subcortical white matter, and basal ganglia at presentation. (b) Five months later, images showing development of moderate bihemispheric cerebral

volume loss with involvement of both gray and white matter. Extensive white matter encephalomalacia with progressive, now-confluent hypointense signal throughout the subcortical white matter, preferentially involving arcuate fibers

Fig. 2 Biochemical testing. (a) Organic acid profile during acute episode. Metabolites identified in Pitt et al. (2015) are numbered (1 Trans-3-OH-Hex-4-enoic acid, 2 Trans-5-OH-Hex-2-enoic acid, 3 3,5 adipic lactone, 4 4-hydroxy-6-methyl-2-pyrone). Relevant metabolites are identified (L lactic, 3HB 3-hydroxy-*n*-butyric, 3HVA 3-hydroxyisovaleric, G glutaric, A adipic, Oct octenendiol, Sub

suberic, 4HPLac 4-hydroxyphenyllactate, 4HPyr 4-hydroxyphenylpyruvic, 3HSeb 3-hydroxysebacic). (b) Patient's C0, C2, and C2/C0 values after carnitine supplementation during his acute episode. (c) His C2/C0 ratio before and after carnitine supplementation during his acute episode compared to reference samples

acid oxidation or organic acidemia. He was found to be carnitine deficient with a decreased free carnitine fraction of 4 nmol/mL and acylcarnitine fraction of 23 nmol/mL. He was subsequently supplemented with L-carnitine at a dose of 100 mg/kg three times daily. A repeat sample taken after supplementation revealed an acetylcarnitine (C2) concentration (37 nmol/mL; reference percentiles: 1st percentile = 2.14, 50th percentile = 6.25, 99th percentile = 21.87), which was relatively elevated compared to other acylcarnitine species in the profile (Fig. 2b). His acylcarnitine testing beyond this acute episode was unremarkable. Initial plasma amino acid analysis showed a nonspecific pattern, most notably with elevations in branched chain amino acids consistent with prolonged fasting.

Molecular Genetic Testing

Given the strong suspicion that this child had an inborn error of metabolism, rapid whole-exome sequencing with concurrent mitochondrial DNA analysis revealed compound heterozygous variants in *HMGCS2*. A maternally inherited, pathogenic variant leading to a stop codon at c.409A>T (p.Lys137*) was observed alongside another variant of uncertain significance, c.1141A>G (p. Met381Val), which was paternally inherited. The proband's sister harbored only the maternally inherited variant c.409A>T (p.K137*). The variant, c.409A>T (p. Lys137*), was not observed in approximately 6,500 individuals of European or African American ancestry as cataloged by the NHLBI Exome Sequencing Project (ESP), or from over 60,000 participants of the Exome Aggregation

Consortium (ExAC). The other missense variant, c.1141A>G (p.Met381Val), was not seen in ESP or ExAC. In silico prediction algorithms resulted in conflicting predictions and that estimated the variant as either tolerable, possibly damaging, disease causing, or possibly pathogenic (SIFT (Kumar et al. 2009), PolyPhen-2 (Adzhubei et al. 2010), Mutation-Taster2 (Schwarz et al. 2014), and M-CAP (Jagadeesh et al. 2016), respectively). Based on the very low allele frequency, compound heterozygosity with a pathogenic variant, residue evolutionary conservation, and biochemical results, this novel variant was classified as likely pathogenic via American College of Medical Genetics and Genomics (ACMG) variant classification guidelines (Richards et al. 2015). Sanger sequencing confirmed both variants in the proband and his parents. Other family members beyond his sister, who was a carrier for the c.409A>T variation, were untested at present time. Mitochondrial DNA analysis was insignificant. Worth noting, *GCDH* which mutations cause glutaric aciduria type 1 had a good coverage on WES and no variants were found.

Discussion

HMGCS2D is an autosomal recessive inborn error of metabolism typically presenting within the first year of life after a period of prolonged fasting and illness. Symptoms include hypoketotic hypoglycemia, vomiting, lethargy, and hepatomegaly (Pitt et al. 2015). Prior reports note increased liver echogenicity suggestive of fatty infiltrate as well as abnormal liver function, as seen in our patient (Wolf et al. 2003). Free fatty acid elevations were previously reported

in patients with HMGCS2D during a metabolic crisis (Zschocke et al. 2002). Although this laboratory value was unchecked in our patient while ill, he did present with significantly elevated triglycerides which normalized after 3 days of intensive care. HMGCS2D impairs ketogenesis through an inefficient conversion of fatty acids to ketones. This ketogenesis deficiency, as well as hypoglycemia, may lead to increased lipolysis and marked elevation of free fatty acids as well as, likely, triglycerides (Fukao et al. 2014). A clinical and biochemical comparison between all reported patients with HMGCS2D is seen in Table 1.

Our patient's urine organic acid profile is similar to those previously reported (Zschocke et al. 2002; Aledo et al. 2001), however the profile differed in several key aspects. The dicarboxylic aciduria was composed of primarily adipic acid, while suberic and sebacic acid were only

minimally or insignificantly elevated. In addition, glutaric acid excretion was markedly elevated and was not a component described in previous organic acid profiles. Ketone bodies were present in moderate amounts as observed by others for HMGCS2D (Fukao et al. 2014). HMG-CoA can be formed through the catabolism of leucine which may account for the observed ketones. Additionally, this analysis could not distinguish the isomers of 3-hydroxy-*n*-butyric acid, and thus, we cannot confirm that the profile consists of only the D-isomer of 3-hydroxybutyrate. Upon retrospective analysis, the proposed disease-specific metabolites reported by Pitt et al. (2015) were detected in this patient's organic acid profile. Trans-3-hydroxyhex-4-enoic and trans-5-hydroxyhex-2-enoic acids were prominent metabolites in the initial profile while 3,5-dihydroxylhexenoic-1,5-lactones and 4-hydroxy-6-methyl-

Table 1 Comparison of the biochemical and clinical features of the reported individuals with *HMGCS2* deficiency

	This report	Thompson et al. (1997)	Morris et al. (1998)	Aledo et al. (2001)	Zschocke et al. (2002)	Wolf et al. (2003)	Aledo et al. (2006)	Ramos et al. (2013)	Pitt et al. (2015)
Number of patients	1	1	1	1	1	2	2	1	8
Youngest presentation of symptoms	8 months	6 years	16 months	11 months	9 months	19 months	7 months	15 months	5 months
<i>Biochemical features (present in at least one patient)</i>									
Elevated ammonia	—	?	?	?	—	—	?	?	+
Elevated lactate	+	?	—	—	—	—	—	?	+
Elevated free fatty acids	?	+	+	+	+	+	+	+	+
Elevated triglycerides	+	?	?	?		?	?	?	?
Low HDL	+	?	?	?		?	?	?	?
Ketosis	+	—	—	—	—	—	—	+	—
Dicarboxylic aciduria	+	—	+	+	+	+	+	+	+
Elevated C2 after carnitine supplementation	+	?	?	?	?	?	+	?	—
<i>Clinical and other laboratory features (present in at least one patient)</i>									
Decompensation after illness	+	+	+	+	+	+	+	+	+
Hypoketotic hypoglycemia	—	+	+	+	+	+	+	+	+
Coma	+	+	+	+	+	+	+	?	+
Hepatomegaly	+	+	+	+	+	+	+	+	+
Elevated liver function testing	+	+	+	+	—	+	+	+	+
Improvement of metabolic disturbance after intravenous glucose administration	+	+	+	—	+	+	+	+	+

+ present, — absent, ? unknown

2-pyrone were detected in minimal amount only (Fig. 2a). While the presence of these metabolites may point towards a putative HMGCS2D diagnosis, in our experience, these metabolites are present in other conditions including long chain hydroxy acyl-CoA dehydrogenase deficiency and severe ketosis (unpublished observations). This case strengthens the recommendation that the presence of these metabolites in the urine during the setting of acute hypoglycemic episode should prompt investigation for HMG-CoA synthase deficiency by molecular or enzymatic methods.

Upon reexamination of the plasma acylcarnitine profiles around our patient's acute episode, we noted a relative elevation of acetylcarnitine (C2) during his episode (Fig. 2b), which may reflect the same biochemical findings reported by Aledo et al. (2001). In that report, a marked increase in acetylcarnitine was observed after supplementing a decompensated HMG-CoA synthase deficient patient with intravenous L-carnitine. They hypothesized that a buildup of acetyl-CoA combined with carnitine deficiency during an episode of decompensation resulted in an elevated acetylcarnitine concentration upon carnitine supplementation. In our patient's testing, acetylcarnitine was in the normal range at presentation but elevated after supplementation. While the value obtained after supplementation is not elevated to the extent seen in Aledo et al., our case represents the second description of an elevated acetylcarnitine value after L-carnitine supplementation during an acute decompensation.

Noting a relative increase in acetylcarnitine in the setting of carnitine deficiency, we investigated whether an acetylcarnitine/free carnitine (C2/C0) ratio could be a possible clue to the biochemical diagnosis of HMGCS2D. The patient's C2/C0 ratio was elevated at presentation (Ratio = 4.2; reference percentiles: 1st percentile = 0.22, 50th percentile = 0.5, and 99th percentile = 1.83), resulting from a low free carnitine value (4.1 nmol/mL; reference percentiles: 1st percentile = 5.46, 50th percentile = 12.85, 99th percentile = 24.21) and an acetylcarnitine value in the normal range (C2 = 17.2 nmol/mL; reference percentiles: 1st percentile = 2.14, 50th percentile = 6.25, 99th percentile = 21.87). In subsequent acylcarnitine testing 8 h post carnitine supplementation the C2/C0 ratio was further elevated (Ratio = 7.5) resulting from an increase in C2 (37.0 nmol/mL) and relatively unchanged C0 (4.9 nmol/mL) (Fig. 2c). In our experience, these C2/C0 ratio values are elevated even compared to known patient profiles of fatty acid oxidation disorders and organic acidemias (not shown). Similar C2/C0 ratio values are observed in physiologic ketosis; however, this

possibility can quickly be excluded clinically by large excretion of ketone bodies. We propose that an elevated C2/C0 ratio in the absence of significant ketosis during an episode of acute hypoglycemia is an additional biochemical signature of HMGCS2D. This observation will need further study to identify whether there is sufficient specificity for clinical utilization.

The MRI changes seen in our patient characterized by "lentiform fork" sign of metabolic acidosis, with branching linear diffusion abnormality surrounding the basal ganglia along bilateral external capsules, external capsules, and medullary laminae could be secondary to undocumented hypoglycemia, since brain abnormalities are not major features of HMGCS2D per se.

Importantly, our case represents a phenotypic expansion on the biochemical profile of cases previously reported. In particular, our case is the first to be reported with a significantly elevated triglyceride level and decreased HDL cholesterol upon presentation. HMGCS2D certainly leads to a relative depletion of HMG-CoA, but the relationship between this and the low HDL cholesterol and high triglyceride level is not apparent. Further delineation of this mechanism will require studying additional affected patients.

Conclusion

This report highlights a unique presentation of HMGCS2D caused by novel compound heterozygous variants in *HMGCS2* identified by whole-exome sequencing. HMGCS2D is a rare disorder that is believed to be underdiagnosed as the symptoms are often nonspecific and may be mistaken for other metabolic conditions (Aledo et al. 2006). However, with the advent of next-generation sequencing, the incidence of this condition may become better understood. Additionally, this diagnosis should be considered when an individual presents with coma induced by fasting, with hypertriglyceridemia, an elevated C2/C0 ratio, or a low HDL cholesterol level from the newborn period through childhood.

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Synopsis

3-Hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase-2 deficiency is a rare disorder with a specific urine organic acid profile, and recently found to have presenting laboratory abnormalities of hypertriglyceridemia and low HDL.

Details of the Contributions of Individual Authors

Erin Conboy, Filippo Vairo, Matthew Schultz: Contributed significantly to the manuscript and critically reviewed manuscript.

Brendan Lanpher, David Deyle, Katherine Agre: Saw patient and critically reviewed, contributed to the manuscript, and edited manuscript.

Ross Ridsdale, Devin Oglesbee, Dimitar Gavrilov: Were essential in the analysis and interpretation of the molecular and biochemical lab results, contributed to the intellectual content of the manuscript and critically reviewed and edited manuscript.

Eric W. Klee: Was essential in the variant interpretation for the Whole-Exome Data, contributed to the intellectual content of the manuscript, and critically reviewed and edited manuscript.

Conflicts of Interest

The authors have no conflicts of interest or competing interests pertaining to the manuscript.

No funding sources were required for this work.

The study protocol was approved by the Mayo Clinic Institutional Review Board.

The patient and family consented to this report.

No laboratory animals were used for this work.

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