Novel HMGCS2 pathogenic variants in a Chinese family with mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase deficiency

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ABSTRACT

Importance: Mitochondrial 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase deficiency is a rare and underdiagnosed disorder with fewer than 30 patients reported worldwide. The application of whole-exome sequencing in patients could improve our understanding of this disorder.

Objective: To identify the genetic causes and evaluate the phenotype of mitochondrial HMG-CoA synthase deficiency in a pediatric patient with uncommon features that included ketosis and elevated lactate and ammonia.

Methods: The proband was referred to the pediatric intensive care unit of Beijing Children’s Hospital and selected for molecular testing with whole-exome sequencing. Her parents and sibling also underwent sequencing for segregation information.

Results: We identified two novel mutations (c.1347_1351delAGCCT/p.Ala450Profs*7 and c.1201G>T/p.Glu401*) in the HMG-CoA synthase-2 gene (HMGCS2, NM_005518.3) in the proband and her brother. Both variants were classified as pathogenic variants according to the American College of Medical Genetics and Genomics/Association for Molecular Pathology guidelines. Metabolic acidosis in the proband was corrected with continuous renal replacement therapy and she left hospital after 21 days of treatment.

Interpretation: Our results extend the genotypic and phenotypic spectrum of HMGCS2 mutation in mitochondrial HMG-CoA synthase deficiency patients and serve as a reminder for physicians to consider mitochondrial HMG-CoA synthase deficiency in newborns and children with coma and hypoketotic hypoglycemia after fasting.

KEYWORDS
HMGCS2 mutation, Hypoketotic hypoglycemia, Mitochondrial HMG-CoA synthase deficiency, Pediatric intensive care unit, Whole-exome sequencing
INTRODUCTION

Mitochondrial 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase deficiency (OMIM #605911) is a rare and underdiagnosed disorder, with fewer than 30 patients reported worldwide. It is characterized by hypoketotic hypoglycemia, encephalopathy, and hepatomegaly. However, patients are usually asymptomatic prior to a crisis caused by an intercurrent infection or prolonged fasting. The course of disease develops rapidly and affected patients with fasting intolerance may die without a diagnosis.

Mitochondrial HMG-CoA synthase deficiency is caused by mutations in the HMG-CoA synthase-2 gene (HMGCS2) whose protein product mediates the first and rate-limiting step in ketogenesis, the condensation reaction of acetyl-CoA and acetoacetyl-CoA. Ketone bodies are an essential alternative source of energy when glucose is scarce because fatty acids cannot be utilized directly by the brain. Fewer than 40 HMGCS2 variants that cause mitochondrial HMG-CoA synthase deficiency have been reported in the literature, and most (24/31) were missense variants.

In this study, we describe two novel pathogenic HMGCS2 variants, c.1347_1351delAGCCT (p.Ala450Profs*7) and c.1201G>T (p.Glu401*), in a Chinese family with three children with mitochondrial HMG-CoA synthase deficiency, and provide detailed clinical information. Our results extend the phenotype spectrum and expand upon the genotype-phenotype correlation evidence.

METHODS

Patient

The proband was an 11-month-old girl who was admitted to the pediatric intensive care unit (PICU) of Beijing Children’s Hospital for severe metabolic acidosis and shock. Informed consent was obtained from her parents for study participation, and the study was approved by the Institutional Medical Ethics Committee of Beijing Children’s Hospital, Capital Medical University.

Sequencing

DNA was isolated from peripheral blood samples obtained from the proband and her parents using a Gentra Puregene Blood Kit (QIAGEN, Hilden, Germany). The SureSelect Human All Exon Kit (Agilent Technologies, Santa Clara, CA, USA) was used to screen whole exome capture. Target regions were sequenced on NovaSeq (Illumina, San Diego, CA, USA) and aligned to the GRCh37/hg19 human reference genome sequence. Variants were annotated and filtered by TGex (tgex-app.geneCards.cn) and classified following the American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) interpretation standards and guidelines. Putative pathogenic variants detected by next-generation sequencing were confirmed by Sanger sequencing. Primers were designed online using PrimerZ. The proband’s brother was tested by Sanger sequencing for segregation information. Her deceased sister was not sequenced because no samples had been preserved.

RESULTS

Clinical information

The proband, G4P3, was born after a full-term cesarean section with a birth weight of 3500 g. She achieved normal psychomotor developmental milestones. At the age of 8 months, she showed poor food intake, vomiting, and hypsometria for 3 days after a fever. Routine blood tests revealed an elevation of C-reactive protein (CRP), procalcitonin, and leucocytes. Abdominal computed tomography showed hepatomegaly and fatty infiltration. Blood amino acid and carnitine profiles revealed an increase in malonylcarnitine/3-hydroxybutyrylcarnitine (C3-DC/C4-OH = 0.57). Her metabolic acidosis was corrected with intravenous renal replacement therapy, and ceftriaxone and mezlocillin were also given as anti-infective therapy. She recovered and left hospital after an 8-day treatment.

At the age of 11 months, she was brought to the PICU of Beijing Children’s Hospital for pneumonia and metabolic acidosis. The main clinical symptoms were cough, dyspnea, disturbed consciousness, and inadequate food intake. No vomiting, diarrhea, or fever were observed. Laboratory tests revealed decompensated metabolic acidosis with a blood pH of 7.01 and a partial pressure of carbon dioxide of 7 mm Hg. Her serum anion gap was greatly elevated, but intravenous sodium bicarbonate therapy was ineffective. She showed liver and myocardial damage: levels of aspartate aminotransferase were 160.9 U/L, alanine aminotransferase was 71.8 U/L, and creatine kinase MB was 61 U/L. She was also diagnosed with mild anemia (hemoglobin: 82 g/L) and neutropenia (neutrophils: 0.67 × 10^9/L). Cranial magnetic resonance imaging (MRI) revealed an obvious enlargement of the sulcus and bilateral ventricles, widening of the bilateral subarachnoid space (particularly on the right side), and a slightly long T2 signal in the bilateral mastoid region (Figure 1).

The proband’s sister, G2P1, had previously demonstrated fever, vomiting, and hypsometria at the age of 12 months. Cranial MRI had revealed symmetrical lesions of the bilateral basal ganglia, and she died after failed acid correction and cardiopulmonary resuscitation. No genetic testing was performed. At the time of writing, the proband’s brother, G3P2, was 4 years of age, with normal psychomotor development. He did not have any phenotype of mitochondrial HMG-CoA synthase deficiency.
Metabolic screening

Blood amino acid and carnitine profiles of the proband revealed increased acetylcarnitine (C2), dodecanoylcarnitine (C12), C4-OH, glutamine, histidine, piperidine, serine, leucine/alanine, and phenylalanine/tyrosine, and decreases in carnitine (C0), C0/C2, and propionylcarnitine (C3)/C2. Urinary organic acid profiles showed increased levels of lactic acid, ketone bodies, dihydroxy acid, glutaric acid (GA), 3-hydroxyglutaric acid, 2-hydroxyisovaleric acid, 2-hydroxyisocaproic acid, creatine (methyl-guanidine acetic acid), trans-3-hydroxyhex-4-enoic acid, trans-5-hydroxyhex-2-enoate, and 4-hydroxy-6-methyl-2-pyrone, indicating mitochondrial HMG-CoA synthase deficiency and secondary carnitine deficiency.

Treatment

After the failure of intravenous sodium bicarbonate therapy to correct metabolic acidosis, continuous renal replacement therapy (CRRT) was performed. Acidosis was corrected with CRRT, vitamins B and C, and L-carnitine treatment. Diazepam was also given because the patient had seizures in the PICU. After treatment in the PICU for 7 days, her condition became stable and she was referred to the endocrinology ward. Levocarnitine was used to prevent carnitine deficiency. Coenzyme Q10 and creatine phosphate sodium were used to treat myocardial damage. Ceftriaxone was used as anti-infective therapy, bicyclol was offered to improve liver function, and leucogen was used to increase leucocyte levels once procalcitonin and CRP were normal. After 21 days of treatment, the proband’s body temperature, venous blood gas, blood biochemical test results, and neutrophil granulocyte levels were within normal ranges.

Genetic findings

Whole-exome sequencing identified a paternal frameshift variant, c.1347_1351delAGCCT (p.Ala450Profs*7), and a maternal nonsense variant, c.1201G>T (p.Glu401*), in HMGCS2 (NM_005518.3) in both the proband and her brother (Figure 2). Neither variant had been reported in the dbSNP, 1000 Genomes, Exome Sequencing Project (ESP), ExAC, or gnomAD databases, indicating they were very rare in the normal population. These variants were located in exons 7 and 8 of HMGCS2, and would be expected to produce a truncated protein or lead to early degradation of the mRNA transcript through the mechanism of nonsense-mediated decay. Both variants were novel and classified as pathogenic according to ACMG/AMP guidelines. The combination of these two variants was consistent with the patient’s clinical phenotype. The clinical phenotype of the proband’s sister, who died undiagnosed at the age of 12 months without genetic testing, was suggestive of the same genotype as the proband.

FIGURE 1 Cranial magnetic resonance imaging results in the proband showing a widened bilateral frontoparietal and temporal subarachnoid space (A, arrow) and bilateral mastoid region with a slightly long T2 signal (B, arrow).

FIGURE 2 Pedigree of the proband and two novel pathogenic variants verified by Sanger sequencing: c.1347_1351delAGCCT (forward primer) and c.1201G>T (reverse primer).
DISCUSSION

Mitochondrial HMG-CoA synthase deficiency is an underdiagnosed condition, and patients typically remain asymptomatic as long as prolonged fasting and intercurrent infections are avoided. The diagnosis of mitochondrial HMG-CoA synthase deficiency outside of a fasting situation is challenging because biochemical tests results are typically normal and enzymatic activity testing requires an invasive liver biopsy. Increased levels of seven specific components of the urine metabolic profile during the onset period have been identified, which has improved the diagnostic value of mass spectrometry. Moreover, genetic testing has made the diagnosis more effective.

HMGCS2 mutations were first identified in patients with mitochondrial HMG-CoA synthase deficiency in 2001. HMGCS2 is located on chromosome 1 at 1p12.13 and comprises 10 exons. Prior to the current study, 31 mutations had been identified and submitted to the Human Gene Mutation Database. All three children in the Chinese family described in our report either carried, or putatively carried, biallelic variants; however, the wide variation in features among these siblings highlights the importance of molecular diagnosis in such cases.

The phenotypic expression of different mitochondrial HMG-CoA synthase deficiency mutants in patients varies considerably. Of the reported HMGCS2 variants, most (24/31) are missense variants which lead to decreases in gene expression or enzymatic activity levels. Truncating variants usually cause early degradation of the mRNA transcript through nonsense-mediated decay, leading to undetectable enzymatic activity. Our case is the first report of a patient carrying two truncating variants. Our patient had ketosis and elevated lactate and ammonia, which were rarely seen in previously reported cases (in 2/18, 2/13, and 3/9 cases).2,4,10-15 Our proband was also diagnosed with anemia, acrodermatitis, bronchopneumonia, neutropenia, encephalopathy, and acute diarrhea, indicating that patients with truncating variants may have a more severe phenotype. However, the genotype–phenotype correlation will require further study.

Symptoms of this disorder often worsen after prolonged fasting or infection. Treatment in the acute phase includes hypoglycemia correction, metabolic acidosis correction, and organic acid excretion. Long-term treatment includes reducing fasting time, a low-protein diet, L-carnitine supplementation, sufficient caloric intake, and the avoidance of infection. We instructed the parents of the patient to ensure she continued to receive levocarnitine and coenzyme Q10 after leaving hospital to promote the excretion of organic acid metabolites and to prevent myocardial damage. Her growth status, venous blood gas, liver function, myocardial enzymes, and free fatty acids are currently being followed-up.

In conclusion, we have identified the first biallelic truncating variants of HMGCS2 causing mitochondrial HMG-CoA synthase deficiency, and described the clinical presentations in multiple siblings. The continued application of whole-exome sequencing will advance our understanding of this often asymptomatic and misdiagnosed disease. Our results extend the genetic spectrum of HMGCS2 mutations and the phenotypic heterogeneity of mitochondrial HMG-CoA synthase deficiency. This rare disorder should be considered in newborns and children with coma and hypoketotic hypoglycemia after fasting.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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