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ARTICLE

Mitochondrial 3-hydroxy-3-methylglutaryl-coenzyme A synthase deficiency: From metabolism to clinical implications

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ABSTRACT

Purpose: Ketone bodies represent an important energy source and can contribute much to the energy supply of the brain. Mitochondrial 3-hydroxy-3-methylglutaryl-coenzyme A synthase deficiency (HMGCS2D) is an autosomal recessive disorder of ketogenesis caused by biallelic variants in *HMGCS2*. Only 59 patients with this disorder have been reported so far.

Methods: We performed a comprehensive literature search to identify all published cases of HMGCS2D (n = 59). Additionally, the data of 16 patients with this disorder who are yet undescribed were collected. Clinical course, biochemical findings, and mutation data are highlighted and discussed. An overview on all *HMGCS2* variants reported in patients is provided.

Results: Sixty-eight patients (91%) presented with an acute metabolic decompensation, mostly within the first year of life but beyond the neonatal period. Asymptomatic individuals were identified in several families. Six patients (8%) had died, mainly during the initial metabolic crisis. The neurologic long-term outcome of surviving patients was favorable with almost all patients (98%) showing normal development. Only 1 variant was identified to be common, (*HMGCS2*) NM_005518.4:c.634G>A p.(Gly212Arg), and found in 6 families. No genotype-phenotype correlation can be established.

Conclusion: This comprehensive data analysis provides an overview on all published patients reported with HMGCS2D, including a list of *HMGCS2* variants identified in affected individuals.

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Introduction

Mitochondrial 3-hydroxy-3-methylglutaryl-coenzyme A synthase 2 deficiency (HMGCS2D; OMIM 605911) is an ultrarare inherited disorder of ketogenesis caused by biallelic pathogenic variants in the *HMGCS2* gene (HGNC:5008). The mitochondrial enzyme 3-hydroxy-3-methylglutaryl-coenzyme synthase 2 (HMGCS2) catalyzes the rate-limiting step of ketogenesis, the conversion of acetyl-coenzyme A (acetyl-CoA) and acetoacetyl-CoA derived from mitochondrial fatty acid oxidation into 3-hydroxy-3-methylglutaryl-CoA and free coenzyme A.[1,2] Because leucine enters the pathway at the subsequent step, deficiency of HMGCS2 affects ketogenesis from fatty acids but not from leucine, whereas ketogenesis from both types of substrates is impaired in patients affected by 3-hydroxy-3-methylglutaryl-CoA lyase (HMGCL) deficiency.[1]

Ketone bodies play a key role as energy source for extrahepatic tissues, especially during catabolism. During periods of prolonged fasting and starvation, ketone bodies provide up to two-thirds of the brain's energy requirements.[3,4] Patients with HMGCS2D usually present with metabolic decompensation characterized by hypoketotic hypoglycemia, metabolic acidosis, lethargy, encephalopathy, and hepatomegaly, typically triggered by catabolic stress.

HMGCS2D deficiency was first described in 1997.[5] Since then, only 59 cases of HMGCS2D have been reported in the literature, many as single case reports or small case series.

We herein give an overview on the natural course and clinical and biochemical data, as well as the spectrum of *HMGCS2* variants of all HMGCS2D cases published to date.

Materials and Methods

We performed a comprehensive literature search in PubMed using the terms "mitochondrial 3-hydroxy-3-methylglutaryl-coenzyme A synthase deficiency," "mitochondrial 3-hydroxy-3-methylglutaryl-coA synthase deficiency," "mHMGCS deficiency," "mitochondrial 3-HMG-coenzyme A synthase deficiency," and "HMGCS2 deficiency" to obtain information on the clinical course of all patients reported in the literature. The search was performed in June 2024 and was supplemented by searches in the public part of Human Gene Mutation Database http://www.hgmd.cf.ac.uk/ . Additional unpublished cases known to the authors or identified via the European Reference Network for Hereditary Metabolic Disorders MetabERN were added. All patients with metabolically and genetically proven HMGCS2D on whom relevant clinical information was available were included in this study, as well as a single patient with a heterozygous *HMGCS2* nonsense variant in whom HMGCS2D (absence of the normal protein) was confirmed by Western blot.[6,7] With this approach we identified a total of 75 HMGCS2D patients, of whom 59 have been previously published in case reports or small case series.

Abbreviations

HMG-CoA – 3-hydroxy-3-methylglutaryl-CoA
HMGCLD – 3-hydroxy-3-methylglutaryl-CoA lyase deficiency
HMGCS2 – mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase 2
HMGCS2D – mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase 2 deficiency

The analysis of clinical and biochemical data was focused on the patients' age at onset, number of metabolic decompensations, neurologic outcome, treatment, and variants in the *HMGCS2* gene. A list of all publications included in the data analysis is given in Supplemental Table 1. Reference sequences NM_005518.4 and NP_005509.1 were used for coding DNA and protein of mHMGCS. Variant descriptions have been validated using the VariantValidator Batch Tool.[8]

Assessment of sequence variants in the *HMGCS2* gene was done utilizing the tools GeneBe (https://genebe.net/),[9] MutationTaster (https://www.mutationtaster.org/),[10] SIFT (https://sift.bii.a-star.edu.sg/),[11] PolyPhen-2 (genetics.bwh.harvard.edu/pph2/),[12] and PROVEAN (http://provean.jcvi.org/index.php),[13] REVEL,[14] and AlphaMissense[15] were accessed via https://franklin.genoox.com.

Results

We herein report data on 75 patients with HMGCS2D from 61 unrelated families. An overview on the clinical data is provided in Table 1. A detailed overview with clinical and biochemical data of the 16 patients that were not previously

Table 1 Clinical information on 75 patients with mitochondrial HMGCS2 deficiency

Parameter	Result
Sex	Female $n = 34$ Male $n = 33$ Not reported $n = 8$
Age at last clinical follow-up	Median of 4.8 years ($n = 66$; range 3 months to 26 years)
Parental consanguinity	20 % (12/61 families)
Deceased patients	8 % (6/74 patients)
Median age at disease onset	9 months ($n = 66$; range 3 months to 6 years)
Patients with at least 1 metabolic decompensation	91% (68/75 patients)
Patients with normal psychomotor development	98% (62/63 surviving patients)
Patients with developmental delay or distinct neurologic abnormalities	2% (1/63 surviving patients)

The total number of patients varies as not all information was available from all patients.

Table 2 Clinical, biochemical, and genetic data of 16 patients who were not previously reported as HMGCS deficient

		HMGCS2 Genotype[b]					Findings During Initial Decompensation							
Patient	Sex	Allele 1	Allele 2	Age at Report (y)	Age at Presentation (y)	Number of Metabolic Decompensations	pH	Base Excess (mmol/L)	Blood Glucose (mmol/L)	Ammonia (μmol/L)	Elevated Transaminases	Hepatomegaly	Dialysis	Outcome
1[a]	m	HMGCS2 NM_005518.4:c.634G>A p.(Gly212Arg) NC_000001.11:g.119759915C>T	HMGCS2 NM_005518.4:c.634G>A p.(Gly212Arg) NC_000001.11:g.119759915C>T	0.8	0.8	1	7.17	−226	2.0	88	X	X	X	deceased
2[a]	f	HMGCS2 NM_005518.4:c.634G>A p.(Gly212Arg) NC_000001.11:g.119759915C>T	HMGCS2 NM_005518.4:c.634G>A p.(Gly212Arg) NC_000001.11:g.119759915C>T	n.a.	1	1	7.36	−6,7	3.7	11	X	X	X	ND
3	f	HMGCS2 NM_005518.4:c.1141A>G p.(Met381Val) NC_000001.11:g.119755473T>C	HMGCS2 NM_005518.4:c.1141A>G p.(Met381Val) NC_000001.11:g.119755473T>C	n.a.	0.6	1	7.17	−20	2.4	108	X	X	-	ND
4	m	HMGCS2 NM_005518.4:c.252T>G p.(Y84Ter) NC_000001.11:g.119764479A>-G	HMGCS2 NM_005518.4:c.252T>G p.(Y84Ter) NC_000001.11:g.119764479A>-G	20	1.4	[c]	n.a.	n.a.	0.6	n.a.	n.a.	n.a.	n.a.	ND
5	f	HMGCS2 NM_005518.4:c.185A>T p.(Tyr62Phe) NC_000001.11:g.119764546T>A	HMGCS2 NM_005518.4:c.636_643 del p. (Ala213TyrfsTer25) NC_000001.11:19759905GCCACAGCT>G	7	0.5	2	7.08	−19	0.5	91	X	X	-	ND
6	f	HMGCS2 NM_005518.4:c.167T>A p.(Ille 56Asn) NC_000001.11:g.119764564A>T	HMGCS2 NM_005518.4:c.167T>A p.(Ille 56Asn) NC_000001.11:g.119764564A>T	18	0.6	2	7.31	−9,3	0.67	154	-	-	-	ND
7	f	HMGCS2 NM_005518.4:c.697A>G p.(Thr233Ala) NC_000001.11:g.119759271T>C	HMGCS2 NM_005518.4:c.727A>G p.(Lys243Glu) NC_000001.11:g.119759241T>C	5	0.9	2	7.12	−7.5	0.33	127	X	X	-	ND
8	f	HMGCS2 NM_005518.4:c.346del p.(Arg116AlafsTer14) NC_000001.11:g.119764384CG>C	HMGCS2 NM_005518.4:c.306C>G p.(Ile102Met) NC_000001.11:g.119764425G>C	7	0.75	2	7.33	−8	1.33	15	X	-	-	ND
9[a]	f	HMGCS2 NM_005518.4:c.520T>C p.(Phe174Leu) NC_000001.11:g.119764211A>G	HMGCS2 NM_005518.4:c.1175C>T p.(Ser392Leu) NC_000001.11:g.119755439G>A	5.7	0.7	1	n.a.	n.a.	0	41	X	X	-	ND
10[a]	f	HMGCS2 NM_005518.4:c.520T>C (p.Phe174Leu) NC_000001.11:g.119764211A>G	HMGCS2 NM_005518.4:c.1175C>T (p.Ser392Leu) NC_000001.11:g.119755439G>A	1.9	3 days[d]	0	n.a.	n.a.	n.a.	n.a.	n.a.	-	-	ND
11	f	HMGCS2 NM_005518.4:c.626G>A p.(Gly209Asp) NC_000001.11:g.119759923C>T	HMGCS2 NM_005518.4:c.626G>A p.(Gly209Asp) NC_000001.11:g.119759923C>T	4.8	2.9	1	7.39	n.a.	2.3	38	-	-	-	ND
12	m	HMGCS2 NM_005518.4:c.634G>A p.(Gly212Arg) NC_000001.11:g.119759915C>T	HMGCS2 NM_005518.4:c.682C>T p.(Arg228Ter) NC_000001.11:g.119759867G>A	7.5	1.2	5	7.37	−5.2	0.4	33	-	-	-	ND
13	f	HMGCS2 NM_005518.4:c.306C>G p.(Ile102 Met) NC_000001.11:g.	HMGCS2 NM_005518.4:c.306C>G p.(Ile102 Met) NC_000001.11:g.	5.75	1.1	1	7.33	−6.8	2.77	n.a.	-	-	-	ND
14	f	HMGCS2 NM_005518.4:c.1017-2del NC_000001.11:g.119755598CT>C	HMGCS2 NM_005518.4:c.772delT p.(Ser258ProfsTer41) NC_000001.11:g.119759195GA>G	4.8	0.4	2	6.86	−29.1	1.0	n.a.	X	X	-	ND
15	m	HMGCS2 NM_005518.4:c.634G>A p.(Gly212Arg) NC_000001.11:g. NC_000001.11:g.119759915C>T	HMGCS2 NM_005518.4: Exon 1 deletion	8.9	n.a.	5	7.46	−2.4	9.7	n.a.	-	-	-	ND
16	f	HMGCS2 NM_005518.4:c.821G>A p.(Arg274His) NC_000001.11:g.119759147C>T	HMGCS2 NM_005518.4:c.1270C>T p.(Arg424Ter) NC_000001.11:g.119753304G>A	2.5	1.5	1	7.28	−8.6	1.0	26	X	-	-	ND

ND, normal development.

[a]Patients 1 and 2 and patients 9 and 10 are siblings.

[b]In brackets: (predicted change in amino acid level).

[c]Several hypoketotic hypoglycemias.

[d]Diagnosis as newborn without metabolic decompensation because of older affected sister.

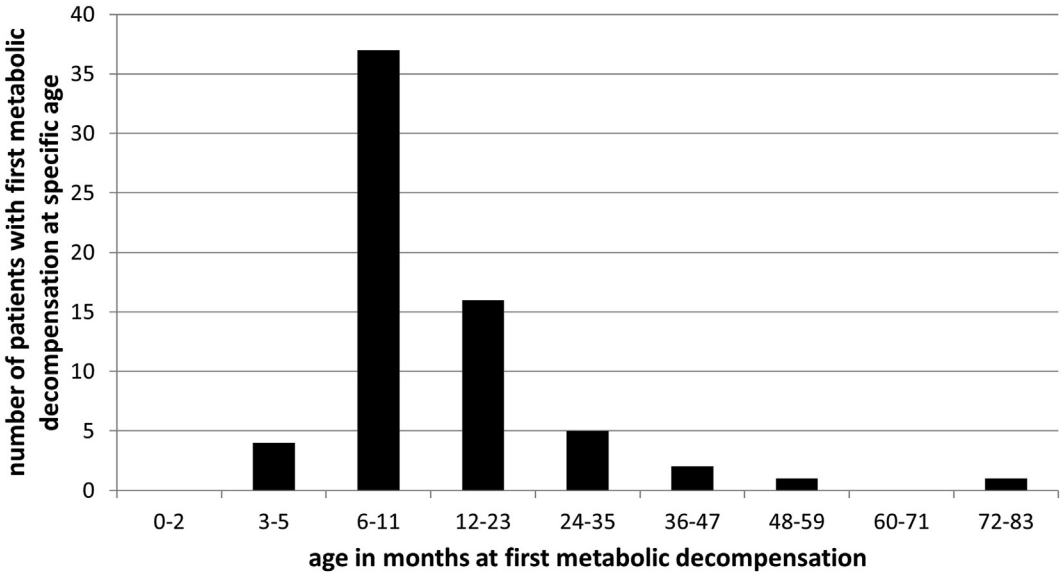

Figure 1 Age at first metabolic decompensation in 66 HMGCS2 patients with acute symptoms. The majority of patients presented within the first year of life. The latest manifestation was observed at 6 years. HMGCS2, 3-hydroxy-3-methylglutaryl-coenzyme A synthase 2.

published is given in Table 2. Thirty-three patients were male, 34 female, and the sex of the remaining 8 patients was not reported. The data set included 6 pairs of siblings. In 2 families, 4 children were affected, and in 1 of them, the father also had HMGCS2D.

The age at last reported follow-up was provided for 66 patients and ranged from 3 months to 26 years (median of 4.8 years). Six patients had already reached adulthood (>18 years).

Among the 61 families, at least 17 were of Chinese origin, 5 were Japanese, and 4 were Turkish. In 12 of 61 families (20 %), parental consanguinity was reported. In several families, siblings or relatives had died of unknown reason. Sixty-eight patients (68/74; 92%) were alive at the time of report, 6 patients (6/74, 8 %) were deceased, and for 1 patient, this information is not available. Five patients died during the first metabolic decompensation at an age between 6 months and 2 years,[16-18] whereas 1 boy who survived the initial metabolic crisis contracted a respiratory infection 2 days after leaving hospital and died of multiple organ failure soon after readmission.[19]

Sixty-eight patients (68/75, 91%) presented with at least 1 metabolic decompensation. Asymptomatic siblings and 1 affected father ($n = 7$, age 23 months to adult) were identified in 4 families by genetic family screening after diagnosis of the index patient. In 2 of these patients, hepatic steatosis was reported.[16] The oldest patient that never experienced acute symptoms was a father who was found homozygous for a splicing variant in the *HMGCS2* gene.[16] He had frequent infections in childhood and was followed up for fatty liver. His analyses of acylcarnitines, blood amino acids, and urinary organic acids yielded normal results.[16]

The number of metabolic decompensations ranged between 1 and 5; however, for most patients, only 1 acute decompensation was reported. This may be partly due to publication shortly after the diagnosis and the lack of long-term follow-up data.

For 66 symptomatic individuals, the exact age at initial metabolic decompensation is available. The median age at presentation was 9 months (range 3 months to 6 years, Figure 1). For 1 additional patient, presentation within the first 5 months of life is reported, but no exact information is available.[20] Four patients presented before the age of 6 months, whereas 56% (37/66) presented in the second half of the first year of life and 16 of 66 (24 %) in the second year of life. Interestingly, no neonatal and no adult acute onset has been described so far.

Typical symptoms of acute decompensations were impaired consciousness (drowsiness/lethargy/coma), vomiting, tachypnea/Kussmaul breathing, seizures, and hepatomegaly (Table 3). One patient developed myocardial injury.[21] The most common laboratory findings were hypoglycemia, severe metabolic acidosis with an elevated anion gap, elevated activities of transaminases, and hyperammonemia (Table 3). If present, hyperammonemia was usually mild; however, 3 patients had ammonium concentrations of more than 300 µmol/L with a maximum of 733 µmol/L. Elevated triglyceride levels were reported in 10 patients. Three patients presented with very severe hypertriglyceridemia of 18.8, 31.3, and 34 mmol/L, respectively.[22-24] Transaminase activities were often increased, but distinct elevations of > 1000 U/L were the exception and only reported in 2 patients.[17] The patients of whose concentrations of serum free fatty acids and beta-hydroxybutyrate were available, most showed a metabolite profile with elevated concentrations of free fatty acids, normal or only slightly elevated 3-hydroxybutyrate levels, and an increased ratio of free fatty acids/beta-hydroxybutyrate. Concentrations of C2 acylcarnitine, compatible with accumulation of the HMGCS2 substrate

Table 3 Clinical symptoms and laboratory findings during first metabolic decompensation

Clinical Findings	Percentage (Number) of Patients Presenting the Symptom
Impaired consciousness (drowsiness/lethargy/coma)	78% of patients (47/60)
Seizures	25% of patients (15/60)
Tachypnoe, respiratory distress, Kussmaul breathing	52% of patients (31/60)
Vomiting	57% of patients (34/60)
Hepatomegaly	67% of patients (40/60)
Laboratory findings (blood)	
Hypoglycemia (< 3.9 mmol/L)	92% of patients (60/65) median blood glucose 1.40 mmol/L (n = 48; range 0-9.39 mmol/L)
Metabolic acidosis (pH < 7.37)	88% of patients (50/57) median pH 7.08 (n = 44; range 6.70-7.46) median BE -22,6 mmol/L (n = 37, range -2.4 mmol/L to -31 mmol/L)
Elevated anion gap (>16 mmol/L)	elevated anion gap reported in 23 patients median anion gap 35.0 mmol/L (n = 23; range 17.5-67 mmol/L)
Elevated transaminases (according to reference values of respective laboratories)	75% of patients (42/56) median ALT 125 U/L (n = 42; range 12-3216 U/L) median AST 213 U/L (n = 34; range 34-6608 U/L)
Hyperammonemia[a]	55% of patients (22/40) median ammonium concentration 99.5 µmol/L (n = 30; range 8-733 µmol/L) severity of hyperammonemia: 100-200 µmol/L: n = 10 200-300 µmol/L: n = 2 300-500 µmol/L: n = 2 >700 µmol/L: n = 1
Elevated lactate (>2 mmol/L)	6% of patients (3/50) median lactate 0.8 mmol/L (n = 34; range 0.4-5.0 mmol/L)
Elevated triglycerides (>1.7 mmol/L)	reported elevated in 10 patients; median triglyceride concentration 3.39 mmol/L (n = 15; maximum 34 mmol/L)

[a]Reference values as stated in the respective publications. In cases without reference data, ammonium levels of >60 µmol/L were considered hyperammonemia.

acetyl-CoA, were elevated in some but not all patients. Urinary organic acid analysis usually showed dicarboxylic aciduria with only mild or missing ketonuria. Detection of 4-hydroxy-6-methyl-2-pyrone in urine was reported in 21 patients. In 2 individuals, this compound was detectable during decompensation and returned to normal after treatment.[25] Hypophosphatemia was observed in 3 patients.[22] Pancytopenia,[16] bicytopenia,[26] hepatosplenomegaly,[26] coagulation dysfunction,[21] and thrombocytopenia[23] were reported in single patients during the initial metabolic crisis.

At least 29 patients received intensive care treatment during the initial metabolic decompensation, and in many of them, mechanical ventilation was provided. Hemodialysis or peritoneal dialysis was performed in at least 16 patients, mostly due to severe metabolic acidosis.

Liver imaging results (ultrasound or computer tomography) were reported for 22 patients and were suggestive of steatosis in most of them. In 1 patient who presented with hepatosplenomegaly, lymphadenopathy, and bicytopenia, abdominal ultrasound, computed tomography with intravenous contrast, and magnetic resonance imaging revealed hepatic bilobar hypodense focal lesions in the liver. Liver biopsies were performed in 2 patients, and liver histology confirmed hepatic steatosis.[5,22]

The median follow-up time of symptomatic patients was 39 months (n = 59, range 0 months to 21.5 years after initial presentation). Information on the neurologic outcome was available for 63 patients who survived the first metabolic decompensation. Of these, all but 1 (62/63, 98%) showed normal development without neurologic abnormalities. One 15-month-old boy who had presented with severe metabolic decompensation at the age of 8 months showed profound developmental delay without the ability to sit or stand independently, had intractable seizures, and was gastrostomy tube dependent.[23] Brain imaging results were available for 20 patients and were abnormal in 14 (14/20, 70%). Reported abnormalities included white matter changes, cerebral atrophy with dilatation of the ventricular system, basal ganglia involvement, and abnormal myelination.

Details on long-term management of patients were scarce. Usually, the avoidance of prolonged fasting and glucose supplements during illnesses were recommended. Some patients were recommended to use uncooked cornstarch at bedtime. Few patients received L-carnitine supplementation.

Enzymatic studies were only reported in the 2 patients who were the first reported[5,6]; the analyses were performed in liver sample homogenates and may have been affected by extramitochondrial isozymes of 3-hydroxy-3-methylglutaryl-coenzyme A synthase.

Results of *HMGCS2* genetic analysis were reported for 74 patients. In 1 patient, no genetic analysis was performed because a homozygous *HMGCS2* variant had been identified in several family members before, and homozygosity for this variant can be assumed in this individual because of the typical symptoms of HMGCS2D. Twenty-eight patients (28/74, 38%) were homozygous, 44 patients (44/74, 60%) were compound heterozygous, and in 2 patients, only 1 variant was detected. An overview on all variants identified in the *HMCGS2* gene of patients with HMGCS2D is given in Figure 2[20,27,28] and Supplemental Table 2. This comprises variants of cases reported in the literature, as well as those identified in previously unpublished cases.

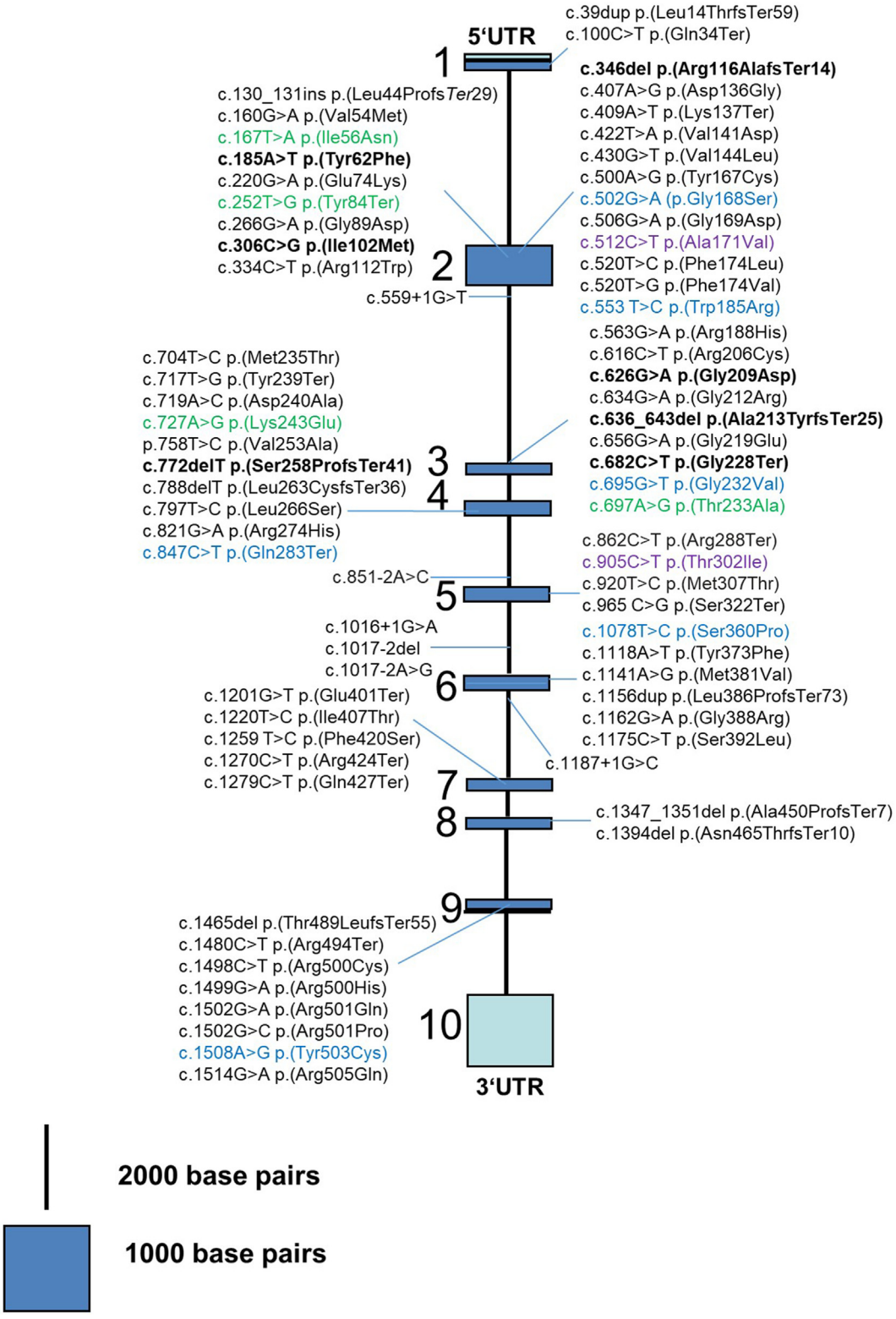

Figure 2 ***HMGCS2*** **pathogenic variants identified in patients with mitochondrial 3-hydroxy-3-methylglutaryl-coenzyme A synthase deficiency.** Variants newly reported in this article are printed in bold. Variants given in green were reported by Shafqat et al,[27] 2010 or Pitt et al,[28] 2015. Variants given in blue were also reported by Shafqat et al[27] or Pitt et al,[28] but the corresponding patients have not been clinically described so far. Similarly, variants printed in violet were reported with very limited clinical data.[20]

The variants are presented using current nomenclature/reference sequences, where required. The careful reassessment of reported variants revealed some cases of incomplete or incorrect information in the literature, which could be clarified, in part via communication with the authors of original reports. For example, variant c.725-2A>C in Kılıç et al,[16] 2020 became c.851-2A>C when using reference sequence NM_005518.4. Variant c.177T>A in Pitt et al,[28] 2015 should be corrected to c.167T>A.

Beyond the variants listed in Figure 2,[20,27,28] patients have been reported with a deletion encompassing exon 1.[28] One of the newly reported patients with compound heterozygosity for *HMGCS2* variants displayed reduced genetic dosage of exon 1, which is in line with a heterozygous deletion of a size between 0.1 and 5.5 kbp.

Although many pathogenic variants in *HMGCS2D* are private, 2 of the novel variants summarized in Table 4 deserve special attention: (*HMGCS2*) NM_005518.4:c.306C>G, p.(Ile102 Met), identified in 2 unrelated families from Italy, and (*HMGCS2*) NM_005518.4:c.634G>A, p.(Gly212Arg), which was found in 7 individuals from 6 families.

Discussion

Since the first description in 1997,[5] a total of 59 patients with genetically proven HMGCS2D have been described. We herein present an overview of the clinical, biochemical, and genetic data of these 59 individuals, as well as 16 previously unreported patients. The vast majority presented with a metabolic decompensation typically characterized by a combination of hypoglycemia and severe metabolic acidosis. However, presentation with isolated acidosis was also observed, and a few cases without hypoglycemia have been reported.[22-24,29]

Additionally, an atypical case with hypertriglyceridemia, hypophosphatemic encephalopathy, and metabolic acidosis without hypoketotic hypoglycemia has been described.[22] HMGCS2D seems not only to be characterized by energy deficiency but also by intoxication, which may require prolonged hemodiafiltration as has been successfully performed in 1 previously unpublished individual. Although HMGCS2 catalyzes a rate-limiting step in the conversion of fatty acids to ketones, and the lack of ketone bodies is considered a hallmark of ketogenesis defects, mild ketonuria/ketonemia has been detected in a substantial number of HMGCS2D patients during metabolic decompensation. An explanation for the latter may be that HMGCS2 is not required for the production of ketone bodies from leucine. In addition, accumulating L-3-hydroxy-*n*-butyric acid produced by fatty acid oxidation will not be distinguished from the ketone body D-3-hydroxy-*n*-butyric acid in typical organic acid analysis.

It seems that the clinical consequences of HMGCS2D are usually less severe than those of the deficiency of the subsequent step in ketogenesis, 3-hydroxy-3-methylglutaryl-coenzyme A lyase deficiency (HMGCLD). This may be explained by the fact that in the latter, not only is ketogenesis from fatty acids impaired but also ketogenesis from leucine. Only HMGCLD, but not HMGCS2D, results in a wide range of potentially toxic leucine derivatives and derangement of coenzyme A metabolism.[30] It can be reasonably hypothesized that the worse outcome in HMGCLD compared with HMGCS2D can be attributed to the accumulation of toxic metabolites of the impaired leucine metabolism in this disorder.

Interestingly, all symptomatic individuals with acute decompensations presented within early childhood and 61% within the first year of life. The latest manifestation occurred at the age of 6 years. This is different from the disorder of ketogenesis due to HMGCLD, in which an adult presentation has been described.[31,32] The reason for this remains unclear. Considering that HMGCS2D only manifests in situations of prolonged fasting usually in association with an acute illness, such as a gastrointestinal infection in infancy, a substantial proportion of individuals who did not experience such a disease may have stayed asymptomatic. Clinical manifestation of HMGCS2D appears to be determined more by the severity of triggers leading to a catabolic state rather than variable residual HMGCS enzyme activity, although a possible genotype-phenotype correlation cannot be ruled out. Additionally, HMGCS2D deficiency lacks specific clinical symptoms and signs, and the outcome in most children is either complete recovery or death, and it is likely that the diagnosis is easily missed in the acute manifestation. HMGCS2D is difficult to diagnose without genetic analysis, and the presence of non-life-threatening and unspecific symptoms may not trigger further intensive work-up, resulting in underdiagnosis of HMGCS2D. Manifestation is rare after infancy: we are only aware of one adult individual without acute symptoms who is the father of 4 affected children and was diagnosed after the manifestation of his son.[16] Ketogenesis plays a particularly important role for energy homeostasis during early childhood, explaining the early manifestation of most individuals who are HMGCS deficient.

Of the patients reported, 6 patients deceased, often during the initial metabolic decompensation. This highlights that HMGCS2D can manifest as a serious inborn error of metabolism with potentially fatal outcome. However, once the diagnosis has been established and measures are taken to prevent metabolic crises, the outcome is favorable in the great majority of cases. This also includes neurologic development. It is noteworthy that despite manifestation with acute encephalopathy and a broad range of magnetic resonance imaging abnormalities in many individuals, substantial developmental impairment was only reported in 1 patient who in principle could also have had another, independent explanation for this condition. In contrast, neurologic findings are quite common in HMGCLD, in which normal development is only found in 62% of patients.[31] Reported neurologic symptoms in HMGCLD,

Table 4 Newly identified *HMGCS2* genotypes

Case	Allele 1 (Predicted Change in Amino Acid Level)	Allele 2 (Predicted Change in Amino Acid Level)	GeneBe	REVEL Prediction (Score)	AlphaMissense Prediction (Score)	MutationTaster Prediction	SIFT prediction (Score)	Polyphen-2.0 Prediction (Score)	PROVEAN Prediction (Score)
63	c.185A>T p.(Tyr62Phe) NC_000001.11: g.119764546T>A		Uncertain significance, 4 ACMG points (PM2 PP3_moderate) GnomAD: Frequency of 6.8e-7, with no homozygous occurrence. Not reported in ClinVar. Conservation: PhyloP100: 7.52	deleterious, moderate (0.87)	benign, supporting (0.213)	disease causing	tolerated (0.20)	probably damaging (1.000)	deleterious (−7.672)
		c.636_643 del p.(Ala213TyrfsTer25) NC_000001.11: 19759905GCCACAGCT>G	Pathogenic, 10 ACMG points (PVS1, PM2). Not reported in GnomAD or Clin Var. Conservation: PhyloP100: 9.56 Predicted to undergo nonsense mediated mRNA decay.			disease causing			
66	c.346del p.(Arg116AlafsTer14) NC_000001.11: g.119764384CG>C		Pathogenic, 10 ACMG points (PVS1, PM2). GnomAD: Frequency of 6.8e-7, with no homozygous occurrence. Not reported in ClinVar. Predicted to undergo nonsense mediated mRNA decay. Conservation: PhyloP100: 3.19			disease causing			
		c.306C>G p.(Ile102Met) NC_000001.11: g.119764425G>C	Likely pathogenic, 6 ACMG points (PM2, PP3_strong) GnomAD: Frequency of 6.8e-7, with no homozygous occurrence. Not reported in ClinVar. Conservation: PhyloP100: 2.74	deleterious, supporting (0.76)	benign, supporting (0.318)	disease causing (score = 10)	affects protein function (0.02)	probably damaging (1.000)	deleterious (−2.667)

(continued)

Table 4 Continued

Case	Allele 1 (Predicted Change in Amino Acid Level)	Allele 2 (Predicted Change in Amino Acid Level)	GeneBe	REVEL Prediction (Score)	AlphaMissense Prediction (Score)	MutationTaster Prediction	SIFT prediction (Score)	Polyphen-2.0 Prediction (Score)	PROVEAN Prediction (Score)
69	c.626G>A p.(Gly209Asp) NC_000001.11:g.119759923C>T		Likely pathogenic, 6 ACMG points (PM2, PP3_strong). GnomAD: Frequency of 0.0000027, with no homozygous occurrence. Not reported in ClinVar. Conservation: PhyloP100: 5.82	deleterious, moderate (0.86)	deleterious, strong (0.985)	disease causing (score = 94)	affects protein function (0.00)	probably damaging (1.000)	deleterious (−6.504)
70	c.634G>A p.(Gly212Arg) NC_000001.11:g. NC_000001.11: g.119759915C>T	known sequence variant in HMGCSD							
		c.682C>T p.(Arg228Ter) NC_000001.11: g119759867G>A.	Pathogenic, 12 ACMG points (PVS1, PM2, PP5_moderate) GnomAD: Frequency: 0.0000211, with no homozygous occurrence. Single submission to Clin Var (SCV003294642); rs763531478. Conservation: PhyloP100:1.88 Predicted to undergo nonsense mediated mRNA decay.			disease causing (score = 6.0)			
71	c.306C>G p.(Ile102 Met) g.119764425G>C	NC_000001.11:	Likely pathogenic, 6 ACMG points (PM2; PP3_strong) GnomAD: Frequency of 6.8e-7, with no homozygous occurrence. Not reported in ClinVar. Conservation: PhyloP100: 2.74	deleterious, supporting (0.76)	benign, supporting (0.318)	disease causing (score = 10)	affects protein function (0.02)	probably damaging (1.000)	deleterious (−2.667)

(continued)

Table 4 Continued

Case	Allele 1 (Predicted Change in Amino Acid Level)	Allele 2 (Predicted Change in Amino Acid Level)	GeneBe	REVEL Prediction (Score)	AlphaMissense Prediction (Score)	MutationTaster Prediction	SIFT prediction (Score)	Polyphen-2.0 Prediction (Score)	PROVEAN Prediction (Score)
72	c.1017-2del; splice_acceptor_variant NC_000001.11: g.119755598CT>C	known sequence variant in HMGCSD							
		c.772delT p.(Ser258ProfsTer41) NC_000001.11: g.119759195GA>G	Pathogenic, 12 ACMG points (PVS1, PM2, PP5_moderate). Not reported in GnomAD. Clin Var submission of this patient: SCV000680260; rs1553240525 Conservation: PhyloP100: 8.90 Predicted to undergo nonsense mediated mRNA decay.			disease causing			
73	c.634G>A p.(Gly212Arg) NC_000001.11:g. NC_000001.11: g.119759915C>T	known sequence variant in HMGCSD							
		Exon1 *HMGCS2* (Deletion)							

ACMG, American College of Medical Genetics and Genomics; *GnomAD*, Genome Aggregation Database; *HMGCSD*, 3-Hydroxy-3-Methylglutaryl-CoA Synthase Deficiency; *mRNA*, messenger ribonucleic acid.

which are typically not found in individuals with HMGCS2D, include seizures, spastic hemiparesis or tetraplegia, distinct muscular hypotonia, impairment of vision and hearing, cerebellar ataxia, movement disorders, tremor, clonic movements, mild dysarthria, exaggerated deep tendon reflexes, and absence of social contact.[31]

Diagnosis of HMGCS2D can be challenging because of unspecific symptoms and the lack of a pathognomonic biochemical profile, although 4-hydroxy-6-methyl-2-pyrone and related metabolites have been suggested as specific biomarkers during periods of decompensations.[28] Although reported for several patients, this metabolite is not reliably detected by all metabolic laboratories, and its specificity for HMGCS2D has been questioned.[23] Enzyme activity testing in patient material would require liver samples and has only been reported for 2 patients.[5,6] The analysis could also be impaired by contributions of cytosolic or peroxisomal isozymes. Therefore, genetic analysis of the *HMGCS2* gene comprising both sequence and structural (deletion) analysis is the gold standard for diagnostic confirmation. In this emerging time of rapid genomic sequencing in the critical care setting, inclusion of HMGCS2D-suggestive symptoms, when testing derives from an acute metabolic decompensation state, will be critical for accurate molecular diagnosis. We herein give an overview on all known and 8 novel *HMGCS2* variants. Most patients are compound heterozygous for 2 detectable variants in the *HMGCS2* gene; identification of a heterozygous pathogenic variant in conjunction with typical clinical presentation does not rule out the diagnosis because pathogenic variants may be missed by standard genetic, tests including exome sequencing. No genotype-phenotype correlation has been established. Besides the genotype, contributions of environmental factors, catabolic episodes, and residual enzyme function to the clinical course and phenotype also deserve consideration.

Conclusion

HMGCS2D remains a rare but potentially severe or even fatal metabolic disease. Its incidence may be underestimated because of the nonspecific symptoms and a potentially severe course leading to death before diagnosis. HMGCS2D must be included in the differential diagnosis of metabolic acidosis with a large anion gap and hypoglycemia even in the presence of mild ketonuria/ketonemia.

Data Availability

Data and materials are available individually upon request.

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Author contributions

Conceptualization: S.C.G., J.O.S.; Data Curation: all authors; Formal Analysis: S.C.G., J.O.S.; Methodology: S.C.G., J.O.S.; Visualization: S.C.G., J.O.S.; Writing-original draft: S.C.G., J.O.S.; Writing-review and editing: all authors

Ethics Declaration

The study was approved by the ethics committee of the University Hospital Freiburg (Nr. 21-1292). For this retrospective study, only data obtained within routine clinical care were collected in a pseudonymized manner. Informed consent was obtained from all novel patients and/or their legal guardians.

Conflict of Interest

Sarah C. Grünert reports no conflicts of interest relevant to this work. She has received honoraria for educational lectures from Vitaflo GmbH and Ultragenyx Pharmaceutical Inc, as well as the creation of patient information material for Danone Deutschland GmbH and received support for attending metabolic expert meetings from Nutricia Metabolics GmbH. Sarah C. Grünert participated in an advisory board for Ultragenyx Pharmaceutical, Inc. Jörn Oliver Sass or his university (for work performed by him) have received honoraria for counselling CoA Therapeutics, Danone Deutschland GmbH and a medical diagnostic laboratory.

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Additional Information

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