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Original article

Expanding phenotypic and mutational spectra of mitochondrial HMG-CoA synthase deficiency

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ABSTRACT

Mitochondrial 3-hydroxy-3 methylglutaryl-CoA synthase-2 deficiency (HMGCS2D) is a rare autosomal recessive inborn error of hepatic ketogenesis, caused by mutations in *HMGCS2*. As its clinical and laboratory manifestations resemble many other metabolic disorders, HMGCS2D definite diagnosis presents a challenge, frequently requiring molecular tests. Only 26 patients with *HMGCS2* mutations have been previously described, and this study reports the first two unrelated Thai patients, a 9-month-old male and an 8-month-old female, with HMGCS2D. During acute episodes, steatorrhea and dyslipidemia occurred, both previously unreported. Increased serum levels of triglycerides, very low density lipoproteins (VLDL), and low density lipoproteins (LDL), along with a decreased serum level of HDL were found. Both patients had hypophosphatemic encephalopathy, and the female had metabolic acidosis without hypoglycemia. Trio whole-exome sequencing (WES) revealed that the male harbored two *HMGCS2* mutations, a novel c.1480C>T (p.Arg494*) and a previously reported c.1502G>C (p.Arg501Pro), while the female was compound heterozygous for the c.1502G>C (p.Arg501Pro) and a previously reported mutation, c.520T>C (p.Phe174Leu). Interestingly, c.1502G>C (p.Arg501Pro) was not only found in both of our patients but also detected heterozygously in 9 out of 1081 unrelated individuals (allele frequency of 9/2162; 0.42%) in our in-house Thai exome database. Discovery of this common mutation suggests there could be about 14 babies with HMGCS2D within 800,000 newborns in Thailand annually. Therefore, awareness of HMGCS2D among medical personnel in Thailand should be raised.

1. Introduction

Mitochondrial 3-hydroxy-3 methylglutaryl-CoA synthase-2 deficiency (mitochondrial HMG-CoA synthase deficiency; HMGCS2D; MIM#605911) is a rare autosomal recessive metabolic disorder. The disease is caused by mutations in the *HMGCS2* gene (MIM*600234), which encodes mitochondrial HMG-CoA synthase (HMG-CoA synthase 2). The enzyme catalyzes the reactions of acetyl-CoA and acetoacetyl-CoA into HMG-CoA during ketone body synthesis (Hegardt, 1999). The lack of this enzyme leads to an underproduction of ketone bodies, which are an important energy supply for brain, heart, kidneys and metabolic systems during fasting and carbohydrate deprivation.

Clinical symptoms include intermittent hypoglycemia, vomiting, lethargy, hepatomegaly, respiratory distress, and encephalopathy, precipitated by prolonged fasting or infections (Thompson et al., 1997; Wolf et al., 2003). Hypoketosis, dicarboxylic aciduria, transaminitis, and metabolic acidosis are common laboratory findings (Pitt et al., 2015; Puisac et al., 2018; Wolf et al., 2003). Without proper treatment, this deficiency can progress to coma.

The presentation of hypoketotic hypoglycemia with an elevation of non-specific dicarboxylic aciduria in HMGCS2D resembles that of other fatty acid oxidation disorders. However, normal acyl-carnitine profile

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and elevation of specific biomarkers, such as 3-hydroxydicarboxylic acids, 5- hydroxyhexanoic acid, trans-5-hydroxyhex-2-enoic, and trans-3-hydroxyhex-4-enoic may be suggestive of the disease (Pitt et al., 2015). Definite diagnosis is obtained by detecting decreased activity of mitochondrial HMG-CoA synthase in liver cells is invasive, required controls, and complicated by cytosol HMG-CoA synthase. Genetic analysis of *HMGCS2* is currently considered to be the best diagnostic tool (Ramos et al., 2013), and to date, less than 40 mutations in *HMGCS2* have been reported. Most are missense variations located in exons 1–9, with the exception of exon 8 (Pitt et al., 2015; Puisac et al., 2018; Ramos et al., 2013).

Here, we describe two unrelated Thai infant patients with unique presentations which add to the phenotypic spectrum of HMGCS2D. Whole exome sequencing identified one novel mutation to expand the mutational spectrum of *HMGCS2*. Notably, allele frequency for the c.1502G>C (p.Arg501Pro) mutation is 0.42% in our Thai exome database, suggesting that Thai patients with HMGCS2D have been underdiagnosed.

2. Clinical report

Our study was approved by the Human Research Ethics Committee of the Faculty of Medicine, Thammasat University No. 1 (MTU-EC-PE-0-061/62). Written informed consent was obtained from each participant.

2.1. Patient data

2.1.1. Patient 1

A nine-month-old male, was the second child of healthy and non-consanguineous Thai parents. He was delivered at term by cesarean section, without complications, with a birth weight of 2830 g. He had been in good health until nine months of age, when he got a cold and presented with drowsiness. Admitted to a local hospital, he was found to have hypoglycemia (glucose 1.28 mmol/L) and 2+ urine ketones. He recovered after receiving intravenous glucose infusion, intravenous ampicillin, and supportive treatment. Two weeks later, he had poor appetite, low grade fever, and oily-stool diarrhea. Investigations revealed milky serum (Fig. 1A) and a blood glucose level of 6.55 mmol/L.

He was then referred to our tertiary care hospital. His weight was 7.5 kg (10th percentile), length 68 cm (10th-25th percentile), and head circumference 44 cm (50th percentile). He had hepatomegaly (liver span of 13 cm), mild anemia (hemoglobin 6.52 mmol/L and hematocrit 0.30 L/L), high triglycerides (31.31 mmol/L; reference 0.68–1.69), high cholesterol (9.9 mmol/L; < 5.17) with high LDL (3.65 mmol/L; < 2.59) and low HDL (0.23 mmol/L; >1), cholestasis with total bilirubin of 61.05 μmol/L, direct bilirubin 45.66 μmol/L, AST 6.81 μmol/L, ALT 4.5 μmol/L, and ALP 3.48 μmol/L. Electrolytes showed hyponatremia and metabolic acidosis with high anion gap (corrected Na 134 mmol/L, HCO_3 14 mmol/L with a gap of 22; reference 8–16); serum phosphate was low (1.03 mmol/L). The levels of lipase (0.4 μmol/L; 0.35–0.85) and amylase (0.4 μmol/L; 0.47–1.67) were within normal limits. Serum ketones were 0.2 mmol/L (reference 0.6–1.5). Serum lipoprotein electrophoresis revealed the presence of chylomicron as well as elevated VLDL and LDL. He received supportive care, recovered, and was discharged from the hospital.

A week later, he was again drowsy, had fatty diarrhea, and was admitted to our hospital. He was found to have normoglycemia, hyponatremia, metabolic acidosis with a wide anion gap (corrected Na 119, K 3.2, Cl 84, corrected Cl 89, HCO_3 11.8 mmol/L), elevated triglycerides (28 mmol/L), high cholesterol (9.5 mmol/L), high LDL (3.9 mmol/L), low HDL (0.44 mmol/L), fractional excretion of sodium 0.14%, with normal findings from a brain CT scan. His liver ultrasound demonstrated infiltrative liver disease suggesting fatty liver: a core needle liver biopsy revealed marked steatosis (Fig. 1B).

A serum phosphate of 0.36 mmol/L and tubular resorption of PO_4 of 5% (normal range >85%) were detected. Hypophosphatemia was determined to be the cause of his drowsiness. Proximal tubular function was evaluated and revealed evidence of marked hyperphosphaturia and proteinuria (urine protein/creatinine ratio of 1.88). Glucosuria was not detected. After being given a PO_4 supplement, his serum PO_4 was above 0.81 mmol/L, and he became alert and active.

2.1.2. Patient 2

An eight-month-old female, was born at term after an uncomplicated pregnancy from non-consanguineous parents. She had been healthy until the age of 8 months when she had vomiting and diarrhea. Then, she became unresponsive with a Kussmaul breathing pattern. Laboratory investigations showed metabolic acidosis and wide anion gap of 41 (Na 140, K 4.85, Cl 97, HCO_3 2 mmol/L). Besides the multiple dicarboxylic acids and ketone bodies detected in her urine, semi-quantitative urine organic acid analysis found a large amount of lactic acid with a moderate amount of beta-hydroxyisovaleric acid. 4-OH-6-methyl-2-pyrone (4-HMP) was not detected. Serum glucose was 3.39 mmol/L, and serum ketones were negative (0.66 mmol/L). Her AST (6.5 μmol/L; 0.13–1) and ALT (2.95 μmol/L; 0.12–0.91) levels were elevated. Lipid profiles were not measured at the critical period.

New onset seizures began while the patient was hospitalized, and a CT scan revealed mild brain atrophy. Hypophosphatemia was observed (PO_4 0.61 mmol/L) while magnesium, calcium and albumin levels were within normal ranges. Stool culture was positive for Salmonella group C. Intravenous fluids and bicarbonate bolus were given. However, due to persistence acidosis with pH < 7, she eventually underwent hemodialysis. After two weeks of hospitalization, she fully recovered.

2.2. Mutation analyses

Trio whole-exome sequencing was performed as previously described (Intarak et al., 2019; Porntaveetus et al., 2018). SNVs and indels were filtered by the following criteria: located in exons or flanking introns, not synonymous, and minor allele frequency less than 1% in both the 1000 Genomes Project and our in-house database of 1081 Thai exomes (2162 alleles). Compound heterozygous mutations in *HMGCS2* (NM_005518.3) were identified in both patients. Patient 1 harbored the *HMGCS2* mutations, c.1480C>T, p.Arg494* (ClinVar accession SCV001169710) inherited from his father and c.1502G>C, p.Arg501Pro from his mother (ClinVar accession SCV001189983) (Fig. 1D). The c.1480C>T, p.Arg494* variant was observed in two out of 282,752 alleles in gnomAD (https://gnomad.broadinstitute.org). Patient 2 also possessed the c.1502G>C, p.Arg501Pro mutation in *HMGCS2* inherited from her father and the known mutation c.520T>C, p.Phe174Leu from her mother (Fig. 1E). The known c.520T>C variant was previously reported in patients with HMGCS2D (Bouchard et al., 2001; Pitt et al., 2015) and predicted to be possibly damaging (PolyPhen: 0.855), possibly pathogenic (M-CAP: 0.09), tolerated (SIFT: 0.07), and highly conserved among several species. Interestingly, both patients shared the c.1502G>C, p.Arg501Pro mutation. This variant was found in three out of 251,332 alleles in genomAD, whereas it was present in nine out of 2162 alleles (0.42%) of 1081 unrelated Thai individuals. It was predicted to be probably damaging (PolyPhen: 1.0), possibly pathogenic (M-CAP: 0.110), deleterious (SIFT: 0.0), and highly conserved among species (Fig. 1E).

3. Discussion

Clinical and molecular findings of cases with *HMGCS2* mutations are summarized in Table 1. Patient 1 had three notable laboratory findings. The first was severe hypertriglyceridemia and low HDL, previously reported in only one patient (Conboy et al., 2018). Marked elevation of serum triglycerides typically results from increased plasma concentration of chylomicron and VLDL. This may be due to abnormal ketogenesis and hypoglycemia, leading to an increase of lipolysis, and markedly

Table 1
Clinical and molecular findings of cases with *HMGCS2* mutations.

Cases	This study		Liu et al. (2019)	Lee et al. (2019)	Zhang et al. (2019)	Ma and Yu (2018)	Puisac et al. (2018)		
	Patient 1	Patient 2	Patient 1	Patient 1	Patient 1	Patient 1	Patient 1	Patient 2	Patient 3
Ethnicity	Thai	Thai	Chinese	Japanese	Chinese	NA	Romanian	South-Asian	French/ Canadian
Gender	M	F	M	M	F	F	M	M	M
Age of the first symptoms (month)	9	8	9	12	11	8	3	11	36
Trigger by vomiting/diarrhea/ poor intake	+	+	+	+	–	+	+	+	–
Coma	–	+	NA	+	+	–	–	–	–
Kussmual breathing	–	+	+	–	NA	NA	–	–	–
Hepatomegaly	+	–	NA	+	+	NA	–	–	–
Fatty liver	+	NA	NA	+	+	NA	NA	NA	NA
Seizure	–	+	+	+	NA	+	NA	NA	+
Encephalopathy	+	+	+	+	+	NA	–	+	–
Hypoglycemia	+	–	+	–	NA	+	+	+	+
Metabolic acidosis	+	+	+	+	+	+	–	–	–
Renal replacement therapy	–	+	NA	–	+	NA	–	–	–
Steatorrhea	+	–	NA	NA	NA	NA	NA	NA	NA
Transaminitis	+	+	+	+	+	+	–	+	+
Dyslipidemia (hypertriglyceridemia, low HDL)	+	NA	NA	NA	NA	NA	–	–	–
Elevation of free fatty acids	NA	NA	NA	+	NA	NA	NA	NA	NA
Hypophosphatemia	+	+	NA	NA	NA	NA	NA	NA	NA
Ketonuria	++	+	NA	+	NA	NA	+	+	+
Urine organic acid									
- Multiple dicarboxylic acids	NA	+	+	+	+	+	+	+	+
- Ketone bodies (3-hydroxy-n-butyric acid, acetone)	NA	moderate	moderate	mildly elevated	increased level	NA	low	low	NA
Mutation (allele 1)	c.1480C>T (p. Arg494*)	c.1502G>C (p. Arg501Pro)	c.100C>T (p.Gln34*)	c.130_131insC (p. Leu44Profs*29)	c.1347_1351del (p. Ala450Profs*7)	c.1502G>A (p. Arg501Gln)	c.334C>T (p. Arg112Trp)	c.430G>T (p. Val144Leu)	c.1514G>A (p. Arg505Gln)
Mutation (allele 2)	c.1502G>C (p. Arg501Pro)	c.520T>C (p. Phe174Leu)	c.1465delA (p. Thr489Leufs*55)	c.1156_1157insC (p. Leu386Profs*73)	c.1201G>T (p. Glu401*)	c.1502G>A (p. Arg501Gln)	NA	c.430G>T (p. Val144Leu)	c.1514G>A (p. Arg505Gln)

Cases	Conboy et al., 2017	Pitt et al., 2015						
	Patient 1	A1-1	A1-2	A1-3	A1-4	A2-1	A3-1	A4-1
Ethnicity	NA	Lebanese	Lebanese	Lebanese	Lebanese	Greek	Egyptian	Indian
Gender	M	NA	NA	NA	NA	NA	NA	NA
Age of the first symptoms (month)	8	8	9	6	10	37	6	29
Trigger by vomiting/diarrhea/poor intake	+	NA	NA	NA	NA	NA	NA	NA
Coma	+	NA	NA	NA	NA	NA	NA	NA
Kussmual breathing	+	NA	NA	NA	NA	NA	NA	NA
Hepatomegaly	+	+	+	+	+	NA	+	NA
Fatty liver	NA	NA	NA	NA	NA	NA	NA	NA
Seizure	+	NA	NA	NA	NA	NA	NA	NA
Encephalopathy	NA	NA	NA	NA	NA	NA	NA	NA
Hypoglycemia	–	+	+	+	+	+	+	+
Metabolic acidosis	+	+	+	+	+	NA	+	NA
Renal replacement therapy	NA	+	+	+	+	–	–	–
Steatorrhea	NA	NA	NA	NA	NA	NA	NA	NA
Transaminitis	+	NA	NA	NA	NA	NA	NA	NA

(*continued on next page*)

Table 1 (*continued*)

Cases	Conboy et al., 2017	Pitt et al., 2015						
	Patient 1	A1-1	A1-2	A1-3	A1-4	A2-1	A3-1	A4-1
Dyslipidemia (hypertriglyceridemia, low HDL)	+	NA	NA	NA	NA	NA	NA	NA
Elevation of free fatty acids	NA	+	+	+	+	+	+	NA
Hypophosphatemia	NA	NA	NA	NA	NA	NA	NA	NA
Ketonuria	++	NA	NA	NA	NA	NA	NA	NA
Urine organic acid								
- Multiple dicarboxylic acids	+	+	+	+	+	+	+	+
- Ketone bodies (3-hydroxy-n- butyric acid, acetone)	moderate	low	low	high	low	low	low	low
Mutation (allele 1)	c.409A>T (p. Lys137*)	c.1-?_104+?del (exon 1 deletion)	c.1-?_104+?del (exon 1 deletion)	c.1-?_104+?del (exon 1 deletion)	c.1-?_104+?del (exon 1 deletion)	c.797T>C (p. Leu266Ser)	c.1162G>A (p. Gly388Arg)	c.506G>A (p. Gly169Asp)
Mutation (allele 2)	c.1141A>G (p. Met381Val)	c.1-?_104+?del (exon 1 deletion)	c.1-?_104+?del (exon 1 deletion)	c.1-?_104+?del (exon 1 deletion)	c.1-?_104+?del (exon 1 deletion)	c.1220T>C (p. Ile407Thr)	c.1162G>A (p. Gly388Arg)	c.1514G>A (p. Arg505Gln)

Cases	Pitt et al., 2015						Ramos et al., 2013	Aledo et al. (2006)
	B1-1	B2-1	B3-1	B4-1	B5-1	B6-1	Proband	Proband
Ethnicity	Mediterranean	English	Australian	Northern Irish	Arabian	Australian (Chinese/ Caucasian descent)	Caucasian	NA
Gender	NA	NA	NA	NA	NA	NA	M	M
Age of the first symptoms (month)	12	<24	<24	<24	<24	<24	15	7
Trigger by vomiting/diarrhea/poor intake	NA	NA	NA	NA	NA	NA	+	+
Coma	+	+	+	+	+	+	NA	+
Kussmual breathing	NA	NA	NA	NA	NA	NA	NA	+
Hepatomegaly	NA	NA	NA	NA	NA	NA	+	+
Fatty liver	NA	NA	NA	NA	NA	NA	NA	NA
Seizure	+	+	+	+	+	+	NA	NA
Encephalopathy	NA	NA	NA	NA	NA	NA	NA	+
Hypoglycemia	+	+	+	+	+	+	+	+
Metabolic acidosis	+	+	+	+	+	+	NA	+
Renal replacement therapy	NA	NA	NA	NA	NA	NA	NA	–
Steatorrhea	NA	NA	NA	NA	NA	NA	NA	NA
Transaminitis	NA	NA	NA	NA	NA	NA	+	–
Dyslipidemia (hypertriglyceridemia, low HDL)	NA	NA	NA	NA	NA	NA	NA	NA
Elevation of free fatty acids	NA	NA	NA	NA	NA	NA	+	NA
Hypophosphatemia	NA	NA	NA	NA	NA	NA	NA	NA
Ketonuria	NA	NA	NA	NA	NA	NA	++	–
Urine organic acid								
- Multiple dicarboxylic acids	NA	NA	NA	NA	NA	NA	+	+
- Ketone bodies (3-hydroxy-n-butyric acid, acetone)	NA	NA	NA	NA	NA	NA	trace	trace
Mutation (allele 1)	c.1-?_104+?del (exon 1 deletion)	c.634G>A (p. Gly212RArg)	c.847C>T (p. Gln283*)	c.553T>C (p. Trp185Arg)	c.1078T (p. Ser360Pro)	c.502G>A (p.Gly168Ser)	c.1162G>A (p. Gly388Arg)	c.563G>A (p. Arg188His)
Mutation (allele 2)	c.1-?_104+?del (exon 1 deletion)	c.431_432del (p. Val144Serfs*11)	c.695G>T (p. Gly232Val)	c.1508A>G (p. Tyr503Cys)	c.1078T (p. Ser360Pro)	c.520T>C (p.Phe174Leu)	c.1270C>T (p. Arg424*)	c.920 T>C (p. Met307Thr)

Cases	Wolf et al., 2003	Zschocke et al., 2002	Aledo et al., 2001	Bouchard et al. (2001)[a]	
	Proband	Proband	Proband	Patient 1	Patient 2
Ethnicity	Caucasian	German	NA	Chinese	NA
Gender	F	F	M	M	M
Age of the first symptoms (month)	53	9	11	72	16

(*continued on next page*)

Table 1 (*continued*)

Cases	Wolf et al., 2003		Zschocke et al., 2002		Aledo et al., 2001		Bouchard et al. (2001)[a]	
	Proband		Proband		Proband		Patient 1	Patient 2
Trigger by vomiting/diarrhea/poor intake	+	+	+		+		+	+
Coma	+	+	+		+		+	+
Kussmual breathing	NA		NA		NA		NA	NA
Hepatomegaly	+	+	+		+		NA	
Fatty liver	+	+	NA		NA		NA	+
Seizure	NA		NA		NA		+	-
Encephalopathy	NA		NA		+	+		NA
Hypoglycemia	+	+	+		+	+	+	+
Metabolic acidosis	+	+	NA		NA		NA	+
Renal replacement therapy			NA		NA		NA	NA
Steatorrhea	NA		NA		NA		NA	NA
Transaminitis	+		-		+		-	NA
Dyslipidemia (hypertriglyceridemia, low HDL)	NA		NA		NA		NA	+
Elevation of free fatty acids	+	+	+		+		+	NA
Hypophosphatemia	+	+	NA		NA		NA	-
Ketonuria	NA		-		NA		-	
Urine organic acid								
- Multiple dicarboxylic acids	+		+		+		-	+
- Ketone bodies (3-hydroxy-n- butyric acid, acetone)	low		low		low		-	low
Mutation (allele 1)	c.160G>A (p.Val54Met)		c.634G>A (p.Gly212Arg)		c.634G>A (p.Gly212Arg)		c.520 T>C (p.Phe174Leu)	c.1270C>T (p.Arg424*)
Mutation (allele 2)	c.500A>G (p.Tyr167Cys)		c.1016+1G>A (IVS5+1G>A)		c.1499G>A (p.Arg500His)		c.520 T>C (p.Phe174Leu)	c.1270C>T (p.Arg424*)

M, male; F, female; NA, not available; +, present; -, not present.

[a] Clinical reports by Morris et al. (1998), Thompson et al. (1997).

elevating free fatty acids along with triglycerides (Fukao et al., 2014). The reuptake of free fatty acids into hepatic cells can lead to the synthesis of hepatic VLDL and overproduction of triglycerides, spurring on fatty liver. In addition, metabolic illnesses or stressful conditions potentially lead to a decrease in endothelial lipoprotein lipase activity for the hydrolysis of triglyceride carried in chylomicron and VLDL (Kersten, 2014; Olivecrona, 2016). Hepatocytic inflammation decreases hepatic lipase synthesis and function; therefore, it is possibly all these reasons lead to hypertriglyceridemia (Chatterjee and Sparks, 2011; Olivecrona, 2016). It is possible that dyslipidemia may have occurred in previously studied HMGCS2D patients, as suggested by the findings of fatty liver and elevated free fatty acids (Fukao et al., 2014).

The second important feature was the intermittent steatorrhea during acute episode, which has not yet been reported in any patients with *HMGCS2* mutations. One possibility is that inflammation of fatty liver stimulates some nuclear receptors, which, in turn, regulate hepatic lipogenesis and reduce bile flow. This decrease of bile in the small bowel interferes with fat absorption and causes steatorrhea in the patient (Mulder et al., 2009).

The third pertinent finding was severe hypophosphatemia leading to encephalopathy. Hypophosphatemia was also detected in Patient 2 and previously reported in one patient (Wolf et al., 2003). Renal phosphate loss could be due to the abnormal absorption of the proximal tubules. Hyponatremia was noted in Patient 1, but his fractional excretion of sodium was less than 1%. It is, therefore, likely that the hyponatremia is pseudohyponatremia due to hypertriglyceridemia and inadequate intake, not renal sodium loss. Although the presence of 4-HMP in urine has been reported as a biomarker of HMGCS2D (Pitt et al., 2015), the substance is not always present (Conboy et al., 2018; Puisac et al., 2018). It is possible that 4-HMP may only appear during acute episodes of hypoglycemia and metabolic decompensation (Pitt et al., 2015).

In HMGCS2D, hypoglycemia, as a result of prolonged fasting or starvation, is usually a trigger for an acute episode. Patient 2 did not have hypoglycemia but suffered from severe metabolic acidosis requiring hemodialysis. There have been few reports of HMGCS2D cases without hypoglycemia (Conboy et al., 2018; Lee et al., 2019) and those needing dialysis (Pitt et al., 2015; Zhang et al., 2019). Here, we show that metabolic acidosis without hypoglycemia can be a metabolic feature of HMGCS2D.

WES successfully identified two mutant alleles in each patient. Three HMGCS2 mutations in total were identified in two patients. A novel mutation for HMGCS2D was a nonsense mutation, c.1480C>T (p.Arg494*), found in Patient 1. Although the c.1480C>T (p.Arg494*) variant has been registered in gnomAD, it had not previously been found in any patients with any diseases. As we have found it to be a causative variant for HMGCS2, we, thus, classify it as a "novel" mutation. The missense mutation, c.1502G>C (p.Arg501Pro), shared by the two patients was recently described in a South Asian patient with HMGCS2D (Bagheri-Fam et al., 2020). This c.1502G>C variant was predicted to be pathogenic withthe p.Arg501Pro protein completely abolishing HMGCS2 enzymatic activity (Bagheri-Fam et al., 2020). The identification of c.1502G>C (p.Arg501Pro) in our study confirms that it is a causative mutation for HMGCS2D. In addition, an infant affected with HMGCS2D was reported as homozygous for the mutation c.1502G>A, p.Arg501Gln (Ma and Yu, 2018). These cases suggest that arginine at the position 501 is critical for enzymatic function.

Notably, the allele frequency of the c.1502G>C (p.Arg501Pro) in our in-house exome database is 0.42% (9/2162); with our database having around 800,000 newborns in Thailand, there could be 14 babies affected with HMGCS2D. Currently, the newborn screening program in Thailand does not include HMGCS2, the manifestation of the disease is rather nonspecific, and there are only a handful of clinical biochemical geneticists and medical centers having facilities to diagnose it in Thailand. If a well-established health care system providing adequate resources for rapid diagnosis and prompt treatment was developed, the prognosis of HMGCS2D in Thai patients would be excellent.

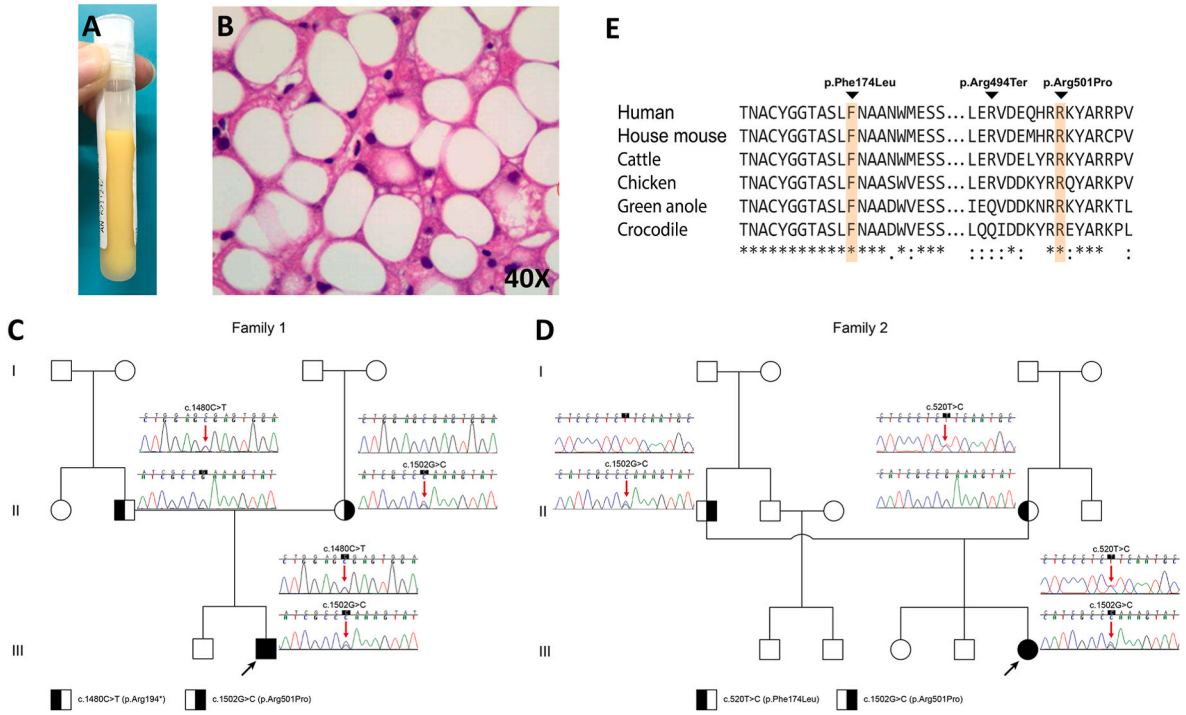

Fig. 1. Phenotype and genotype of the probands. (A) Milky white serum of Patient 1. (B) Histological findings revealed fat accumulation in the hepatocytes (liver steatosis) of Patient 1. (C, D) Family pedigrees and chromatograms of Patient 1 and 2. A black-filled symbol indicates the proband. (E) Amino acid alignment showed the conservation of mutations across species.

In summary, we propose that steatorrhea may be a manifestation of HMGCS2D. We substantiate that hypertriglyceridemia, hypophosphatemic encephalopathy, and metabolic acidosis without hypoketotic hypoglycemia can be its metabolic features. One mutation, c.1502G>C (p.Arg501Pro), is novel as well as common in the Thai population. This justifies raising awareness with medical personnel regarding HMGCS2D in Thailand toward improving patient prognosis.

CRediT authorship contribution statement

Kitiwan Rojnueangnit: Conceptualization, Investigation, Writing - original draft, Writing - review & editing. **Parisa Maneechai:** Investigation, Writing - review & editing. **Patcharapa Thaweekul:** Investigation, Writing - review & editing. **Punnapat Piriyanon:** Investigation, Writing - review & editing. **Sookkasem Khositseth:** Investigation, Writing - review & editing. **Chupong Ittiwut:** Methodology, Investigation, Writing - review & editing. **Wanna Chetruengchai:** Methodology, Investigation, Writing - review & editing. **Wuttichart Kamolvisit:** Methodology, Investigation, Writing - review & editing. **Thanakorn Theerapanon:** Methodology, Investigation, Writing - review & editing. **Kanya Suphapeetiporn:** Methodology, Investigation, Writing - review & editing. **Thantrira Porntaveetus:** Conceptualization, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Vorasuk Shotelersuk:** Conceptualization, Supervision.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmg.2020.104086.

References

Aledo, R., Mir, C., Dalton, R.N., et al., 2006. Refining the diagnosis of mitochondrial HMG-CoA synthase deficiency. J. Inherit. Metab. Dis. 29 (1), 207–211. https://doi:10.1007/s10545-006-0214-2.

Aledo, R., Zschocke, J., Pié, J., et al., 2001. Genetic basis of mitochondrial HMG-CoA synthase deficiency. Hum. Genet. 109 (1), 19–23. https://doi: 10.1007/s004390100554.

Bagheri-Fam, S., Chen, H., Wilson, S., et al., 2020. The gene encoding the ketogenic enzyme HMGCS2 displays a unique expression during gonad development in mice. PloS One 15 (1), e0227411. https://doi:10.1371/journal.pone.0227411.

Bouchard, L., Robert, M.F., Vinarov, D., et al., 2001. Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase deficiency: clinical course and description of causal mutations in two patients. Pediatr. Res. 49 (3), 326–331. https://doi:10.1203/00006450-200103000-00005.

Chatterjee, C., Sparks, D.L., 2011. Hepatic lipase, high density lipoproteins, and hypertriglyceridemia. Am. J. Pathol. 178 (4), 1429–1433. https://doi:10.1016/j.ajpath.2010.12.050.

Conboy, E., Vairo, F., Schultz, M., et al., 2018. Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase deficiency: unique presenting laboratory values and a review of biochemical and clinical features. JIMD Reports 40, 63–69. https://doi:10.1007/8904_2017_59.

Fukao, T., Mitchell, G., Sass, J.O., et al., 2014. Ketone body metabolism and its defects. J. Inherit. Metab. Dis. 37 (4), 541–551. https://doi:10.1007/s10545-014-9704-9.

Hegardt, F.G., 1999. Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase: a control enzyme in ketogenesis. Biochem. J. 338 (Pt 3), 569–582.

Intarak, N., Theerapanon, T., Thaweesapphithak, S., et al., 2019. Genotype-phenotype correlation and expansion of orodental anomalies in LTBP3-related disorders. Mol. Genet. Genom. 294 (3), 773–787. https://doi:10.1007/s00438-019-01547-x.

Kersten, S., 2014. Physiological regulation of lipoprotein lipase. Biochim. Biophys. Acta 1841 (7), 919–933. https://doi:10.1016/j.bbalip.2014.03.013.

Lee, T., Takami, Y., Yamada, K., et al., 2019. A Japanese case of mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase deficiency who presented with severe metabolic acidosis and fatty liver without hypoglycemia. JIMD Reports 48 (1), 19–25. https://doi:10.1002/jmd2.12051.

Liu, H., Miao, J.-K., Yu, C.-W., et al., 2019. Severe clinical manifestation of mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase deficiency associated with two novel mutations: a case report. BMC Pediatr. 19 (1), 344. http://doi:10.1186/s12887-019-1747-5.

Ma, D., Yu, D., 2018. Mitochondrial 3-hydroxy-3-methylglutaryl CoA synthase deficiency: a case report and literature review. Chin. J. Contemp. Pediatr. 20 (11), 930–933. http://doi:10.7499/j.issn.1008-8830.2018.11.010.

Mulder, J., Karpen, S.J., Tietge, U.J., Kuipers, F., 2009. Nuclear receptors: mediators and modifiers of inflammation-induced cholestasis. Front. Biosci. 14, 2599–2630. https://doi:10.2741/3400.

Olivecrona, G., 2016. Role of lipoprotein lipase in lipid metabolism. Curr. Opin. Lipidol. 27 (3), 233–241. https://doi:10.1097/mol.0000000000000297.

Pitt, J.J., Peters, H., Boneh, A.Y., et al., 2015. Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase deficiency: urinary organic acid profiles and expanded spectrum of mutations. J. Inherit. Metab. Dis. 38 (3), 459–466. https://doi:10.1007/s10545-014-9801-9.

Porntaveetus, T., Abid, M.F., Theerapanon, T., et al., 2018. Expanding the oro-dental and mutational spectra of kabuki syndrome and expression of KMT2D and KDM6A in human tooth germs. Int. J. Biol. Sci. 14, 381–389. http://doi: 10.7150/ijbs.23517.

Puisac, B., Marcos-Alcalde, I., Hernandez-Marcos, M., et al., 2018. Human mitochondrial HMG-CoA synthase deficiency: role of enzyme dimerization surface and characterization of three new patients. Int. J. Mol. Sci. 19 (4). https://doi:10.3390/ijms19041010.

Ramos, M., Menao, S., Arnedo, M., et al., 2013. New case of mitochondrial HMG-CoA synthase deficiency. Functional analysis of eight mutations. Eur. J. Med. Genet. 56 (8), 411–415. https://doi:10.1016/j.ejmg.2013.05.008.

Thompson, G.N., Hsu, B.Y., Pitt, J.J., Treacy, E., Stanley, C.A., 1997. Fasting hypoketotic coma in a child with deficiency of mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase. N. Engl. J. Med. 337 (17), 1203–1207. https://doi:10.1056/nejm199710233371704.

Wolf, N.I., Rahman, S., Clayton, P.T., Zschocke, J., 2003. Mitochondrial HMG-CoA synthase deficiency: identification of two further patients carrying two novel mutations. Eur. J. Pediatr. 162 (4), 279–280. https://doi:10.1007/s00431-002-1110-x.

Zhang, P., Hu, X., Guo, R., et al., 2019. Novel HMGCS2 pathogenic variants in a Chinese family with mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase deficiency. Pediatric Investigation 3 (2), 86–90. https://doi.org/10.1002/ped4.12130.

Zschocke, J., Penzien, J.M., Bielen, R., et al., 2002. The diagnosis of mitochondrial HMG-CoA synthase deficiency. J. Pediatr. 140 (6), 778–780. https://doi.org/10.1067/mpd.2002.123854.