

Figure S1. Simplified map of the bacterial expression vector.
IPTG, isopropyl β -D-thiogalactoside.

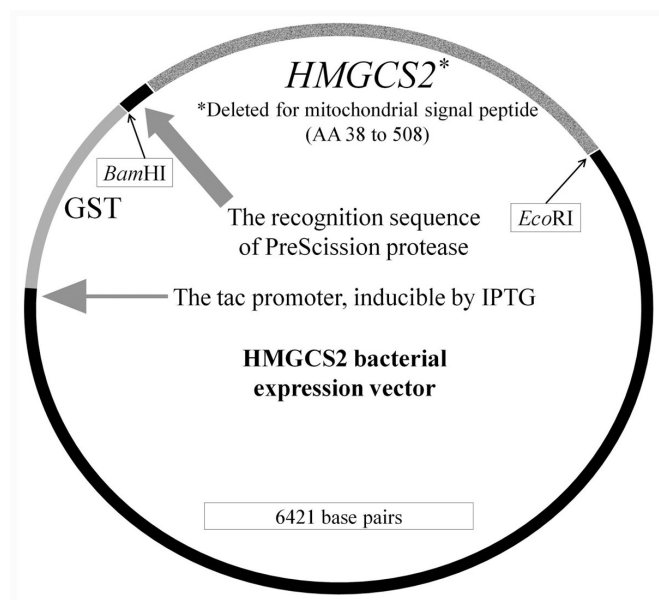


Figure S2. Purification of wild-type HMGCS2 protein from *Escherichia coli* lysates. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Coomassie brilliant blue R250 staining of protein fractions obtained by affinity chromatography of wild-type HMGCS2 expressed in *E. coli*. Lane 1, whole bacterial cell extract before induction with IPTG; lane 2, whole bacterial cell extract after IPTG induction for 16 h; lane 3, cell pellet after sonication; lane 4, supernatant after sonication; lane 5, soluble fraction passed through a glutathione sepharose bead column; lane 6, fraction eluted from the affinity column; lane 7, solution after cleavage of the GST-HMGCS2 fusion protein with PreScission protease; lane 8, solution after removal of GST and PreScission protease. HMGCS2, 3-hydroxy-3-methylglutaryl-CoA; IPTG, isopropyl β -D-thiogalactoside; GST, glutathione s-transferase.

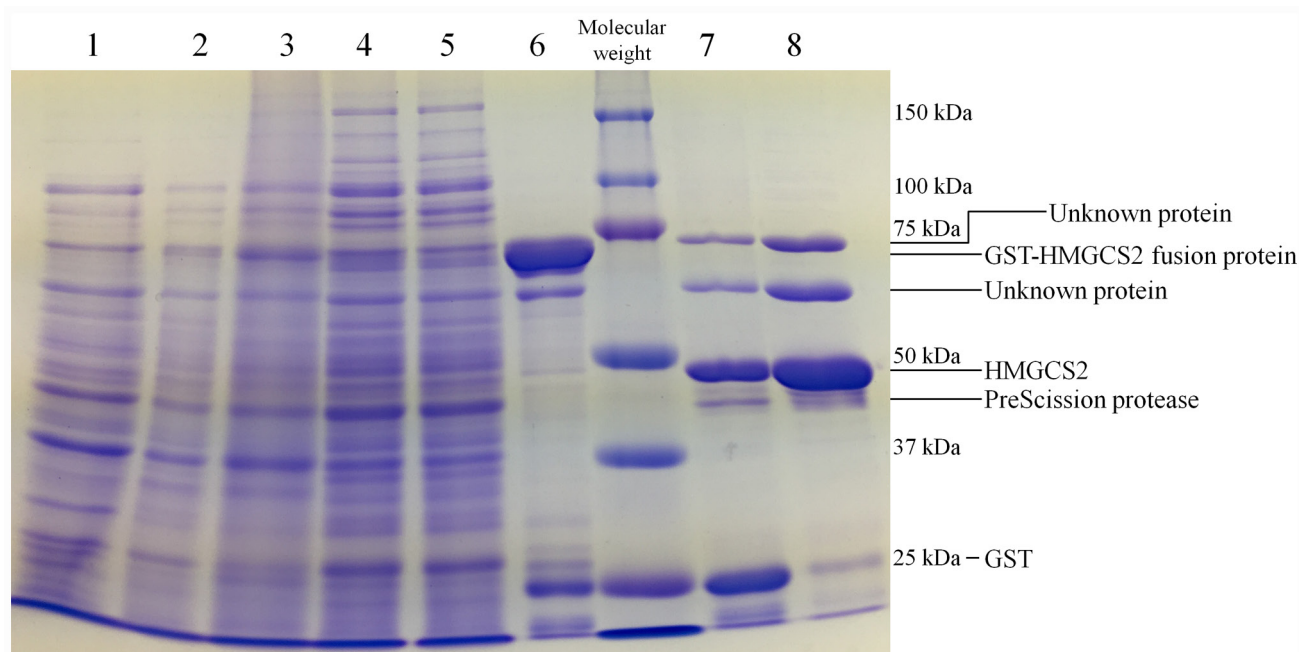


Figure S3. DNA sequencing data of the four patients using the Sanger method.

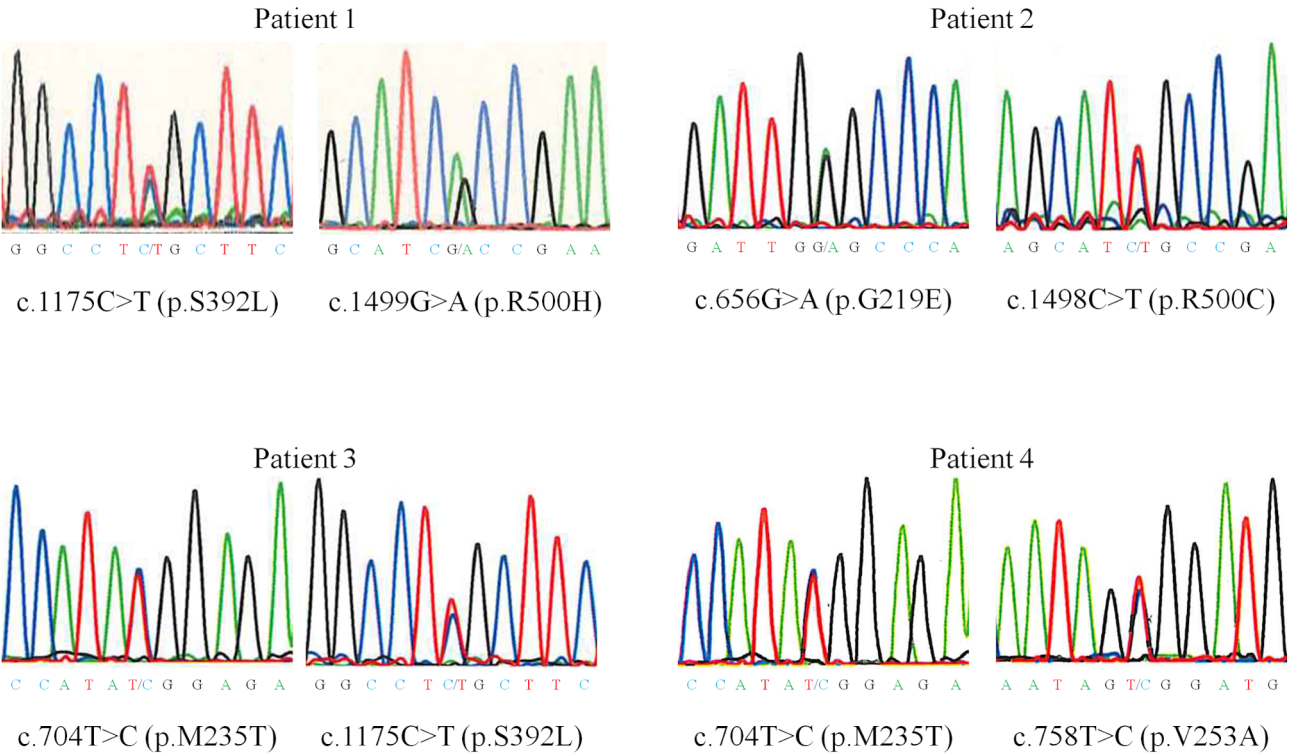


Table SI. The details of blood acylcarnitine analyses.

Case no.	Patient 1		Patient 2		Patient 3		Patient 4	
Sample	Serum		Serum		Blood spot		Serum	
Instrument	LCMS-8040		API 3200™ LC-MS/ MS System		API 3200™ LC-MS/ MS System		Quattro Premier MS/MS	
Ionization mode	Positive		Positive		Positive		Positive	
Acylcarnitines	<i>m/z</i> (Q1)	<i>m/z</i> (Q3)	<i>m/z</i> (Q1)	<i>m/z</i> (Q3)	<i>m/z</i> (Q1)	<i>m/z</i> (Q3)	<i>m/z</i> (Q1)	<i>m/z</i> (Q3)
C0	218.2	103	162.1	102.9	162.1	102.9	218.2	103
C0 IS	227.2	103	171.1	102.9	171.1	102.9	227.2	103
C2	260.2	85	204.1	85	204.1	85	260.2	85
C2 IS	263.2	85	207.1	85	207.1	85	263.2	85
C3	274.2	85	218.1	85	218.1	85	274.2	85
C3 IS	277.2	85	221.1	85	221.1	85	277.2	85
C3DC	360.2	85	N/A	N/A	N/A	N/A	N/A	N/A
C3DC IS	347.2	85	N/A	N/A	N/A	N/A	N/A	N/A
C4	288.2	85	N/A	N/A	N/A	N/A	288.2	85
C4 IS	291.2	85	235.1	85	235.1	85	291.2	85
C4DC	374.3	85	N/A	N/A	N/A	N/A	374.3	85
C4DC IS	347.2	85	N/A	N/A	N/A	N/A	N/A	N/A
C5	302.2	85	246.2	85	246.2	85	302.2	85
C5 IS	311.2	85	255.2	85	255.2	85	311.2	85
C5:1	300.2	85	244.2	85	244.2	85	300.2	85
C5:1 IS	311.2	85	N/A	N/A	N/A	N/A	N/A	N/A
C5DC	388.3	85	276.2	85	276.2	85	388.3	85
C5DC IS	391.3	85	279.2	85	279.2	85	391.3	85
C5OH	318.2	85	262.2	85	262.2	85	318.2	85
C5OH IS	321.2	85	265.2	85	265.2	85	321.2	85
C6	316.2	85	N/A	N/A	N/A	N/A	316.2	85
C6 IS	347.2	85	N/A	N/A	N/A	N/A	N/A	N/A
C8	344.2	85	288.2	85	288.2	85	344.2	85
C8 IS	347.2	85	291.2	85	291.2	85	347.2	85
C8:1	342.2	85	N/A	N/A	N/A	N/A	N/A	N/A
C8:1 IS	347.2	85	N/A	N/A	N/A	N/A	N/A	N/A
C8DC	430.4	85	N/A	N/A	N/A	N/A	N/A	N/A
C8DC IS	437.4	85	N/A	N/A	N/A	N/A	N/A	N/A
C10	372.3	85	316.3	85	316.3	85	372.3	85
C10 IS	403.3	85	N/A	N/A	N/A	N/A	N/A	N/A
C10:1	370.3	85	N/A	N/A	N/A	N/A	370.3	85
C10:1 IS	403.3	85	N/A	N/A	N/A	N/A	N/A	N/A
C10DC	458.4	85	N/A	N/A	N/A	N/A	N/A	N/A
C10DC IS	459.4	85	N/A	N/A	N/A	N/A	N/A	N/A
C12	400.3	85	344.3	85	N/A	N/A	400.3	85
C12 IS	403.3	85	347.3	85	347.3	85	403.3	85
C12:1	398.3	85	N/A	N/A	N/A	N/A	N/A	N/A
C12:1 IS	437.4	85	N/A	N/A	N/A	N/A	N/A	N/A
C12DC	486.3	85	N/A	N/A	N/A	N/A	N/A	N/A
C12DC IS	487.4	85	N/A	N/A	N/A	N/A	N/A	N/A
C14	428.4	85	N/A	N/A	N/A	N/A	428.4	85
C14 IS	437.4	85	381.3	85	381.3	85	437.4	85
C14:1	426.4	85	370.3	85	370.3	85	426.4	85
C14:1 IS	437.4	85	N/A	N/A	N/A	N/A	N/A	N/A
C14OH	444.4	85	N/A	N/A	N/A	N/A	444.4	85
C14OH IS	437.4	85	N/A	N/A	N/A	N/A	N/A	N/A
C16	456.4	85	400.3	85	400.3	85	456.4	85
C16 IS	459.4	85	403.4	85	403.4	85	459.4	85
C16:1	454.4	85	N/A	N/A	N/A	N/A	N/A	N/A
C16:1 IS	459.4	85	N/A	N/A	N/A	N/A	N/A	N/A
C16OH	472.4	85	416.4	85	416.4	85	472.4	85

Table SI. Continued.

Case no.	Patient 1		Patient 2		Patient 3		Patient 4	
Sample	Serum		Serum		Blood spot		Serum	
Instrument	LCMS-8040		API 3200™ LC-MS/ MS System		API 3200™ LC-MS/ MS System		Quattro Premier MS/MS	
Ionization mode	Positive		Positive		Positive		Positive	
Acylcarnitines	<i>m/z</i> (Q1)	<i>m/z</i> (Q3)	<i>m/z</i> (Q1)	<i>m/z</i> (Q3)	<i>m/z</i> (Q1)	<i>m/z</i> (Q3)	<i>m/z</i> (Q1)	<i>m/z</i> (Q3)
C16OH IS	459.4	85	N/A	N/A	N/A	N/A	N/A	N/A
C16:1 OH	N/A	N/A	N/A	N/A	N/A	N/A	470.4	85
C18	484.4	85	428.4	85	428.4	85	484.4	85
C18 IS	487.4	85	431.4	85	431.4	85	487.4	85
C18:1	482.4	85	426.4	85	N/A	N/A	482.4	85
C18:1 IS	487.4	85	N/A	N/A	N/A	N/A	N/A	N/A
C18:2	480.4	85	N/A	N/A	N/A	N/A	N/A	N/A
C18:2 IS	487.4	85	N/A	N/A	N/A	N/A	N/A	N/A
C18OH	500.4	85	N/A	N/A	N/A	N/A	500.4	85
C18OH IS	487.4	85	N/A	N/A	N/A	N/A	N/A	N/A
C18:1 OH	498.4	85	442.4	85	442.4	85	498.4	85
C18:1 OH IS	487.4	85	N/A	N/A	N/A	N/A	N/A	N/A

IS, Internal standard; Q1, the first quadrupole; Q3, the third quadrupole; N/A, Not applicable because the corresponding acylcarnitine was not measured.

Table SII. Sequences of the primers used for PCR amplification of individual exons of the human *HMGCS2* gene.

Primer sequence	Coordinates	Nucleotide length of amplified DNA
5'-TTAGGGAGGCAGGAGTCTTG-3'	8 nucleotides upstream of exon 1	345 base pairs
5'-TGCTTGTCTAAGGCTGCTGT-3'	107 nucleotides downstream of exon 1	
5'-GTAGTCATCTGATAACATAGCT-3'	102 nucleotides upstream of exon 2	715 base pairs
5'-ATGATAGCAGCAGCTGTGTG-3'	118 nucleotides downstream of exon 2	
5'-TAAGCTCCACAGGACTGGGA-3'	120 nucleotides upstream of exon 3	374 base pairs
5'-TCCTCAAACGCATACCTCAG-3'	90 nucleotides downstream of exon 3	
5'-GTAATACCAACAACCTGTCCA-3'	157 nucleotides upstream of exon 4	499 base pairs
5'-GCATAGACCCAGGGAAGAGT-3'	138 nucleotides downstream of exon 4	
5'-GAGATTGCTTAGTAAGTGCA-3'	157 nucleotides upstream of exon 5	504 base pairs
5'-TAACCCACTACCCCGTGGAA-3'	143 nucleotides downstream of exon 5	
5'-ATTAGTGGTATGTTCTCCTC-3'	138 nucleotides upstream of exon 6	480 base pairs
5'-TGAGATTACAGGCATGAGCC-3'	132 nucleotides downstream of exon 6	
5'-TAGTTGCATGAGGTAGACAG-3'	129 nucleotides upstream of exon 7	400 base pairs
5'-TGCTTAAATCCCTTCAGTGA-3'	126 nucleotides downstream of exon 7	
5'-CTTGGTATTCTAAGCCACAC-3'	121 nucleotides upstream of exon 8	420 base pairs
5'-CTTTGGACAACAGATCTTCT-3'	135 nucleotides downstream of exon 8	
5'-TTGGTGACTAAGGGTGACC-3'	127 nucleotides upstream of exon 9	400 base pairs
5'-TTCTCCCATTCTCCTGTCA-3'	123 nucleotides downstream of exon 9	
5'-TCCCTCAGGTGTCCAGGCTT-3'	144 nucleotides upstream of exon 10	570 base pairs
5'-TTGGAGATTCACAGGAACAG-3'	388 nucleotides downstream of intron 9	

Each coordinate indicates the position of the 3'-end nucleotide of each primer in *HMGCS2*.

Table SIII. DNA panel of 193 metabolic disease genes used for mutation analysis in patient 4. The top row refers to classifications of genes in the panel.

Amino acids	Organic acids	Fatty acids	Ketone bodies	Glycogen storage diseases of the liver	Carbohydrates	Riboflavin & monocarboxylate transporters	Mitochondrial respiratory chain disorders & pyruvate metabolism	Congenital disorders of glycosylation	Others
ADK	ABCD4	ACAA2	ACAT1	AGL	ABCC8	SLC52A1	DGUK	ALG3	BSG
BCKDHA	ACSF3	ACAD10	ACAT2	G6PC	ADPGK	SLC52A2	MPV17	MPI	EPM2A
BCKDHB	ALDH6A1	ACAD11	BDH1	GBE1	ALDOA	SLC52A3	POLG	PGM1	GK2
CBS	AUH	ACAD8	HMGCL	GYS1	ALDOB	SLC16A1 ^b	DLD ^c	PMM2	GPD1
DBT	BTD	ACAD9	HMGCS2	GYS2	ENO3	SLC16A10 ^b	PC ^c		GPD2
FAH	ETFA	ACADL	OXCT1	PHKA2	FBP1	SLC16A11 ^b	PCK1 ^c		HIBADH
GCHI	ETFB	ACADM		PHKB	GAA	SLC16A12 ^b	PCK2 ^c		LPIN1
HIBCH	ETFDH	ACADS		PHKG1	GALE	SLC16A3 ^b			MTAP
HPD	GCDH	ACADSB		PHKG2	GALK1	SLC16A4 ^b			NHLRC1
MAT1A	HCFC1	ACADVL		PYGL	GALT	SLC16A5 ^b			PLCH2
MTHFR	HLCS	ACSM1		SLC37A4	GAPDH	SLC16A6 ^b			PPP1R3C
MTR	HSD17B10	ACSS2			GCK	SLC16A7 ^b			PRKAG2
MTRR	IVD	CPT1A			GSK3B	SLC16A8 ^b			PRKAG3
PAH	LMBRD1	CPT1B			GYGI	SLC16A9 ^b			PRKCA
PCBD1	MCCC1	CPT1C			HK1	SLC5A8 ^b			RBCK1
PTS	MCCC2	CPT2			HNF1A				SLC16A13
QDPR	MLYCD	CRAT			HNF4A				SLC16A14
SLC25A13	MMAA	CROT			INS				SLC17A3
TAT	MMAB	DECR1			INSR				SLC2A10
	MMACHC	ECHS1			KCNJ11				SLC2A11
	MMADHC	EC11			LDHA				SLC2A12
ASL ^a	MUT	HADH			LDHB				SLC2A13
ASS1 ^a	PCCA	HADHA			PDX1				SLC2A14
CA5A ^a	PCCB	HADHB			PFKL				SLC2A2
CPS1 ^a	SUGCT	SLC22A5			PFKM				SLC2A3
GLUD1 ^a	TAZ	SLC25A20			PFKP				SLC2A4
NAGS ^a					PGAM2				SLC2A5
OTC ^a					PGK1				SLC2A6
					PHKA1				SLC2A7
					PKLR				SLC2A8
					PKM				SLC2A9
					PPP1R3B				SLC5A12
					PYGB				SLC5A6
					PYGM				SPR
					SLC2A1				UCP2
					SLC5A1				
					ZFP57				

^a , associated with the urea cycle; ^b , monocarboxylate transporter; ^c , associated with pyruvate metabolism.