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Case Report

Twin brothers, one of whom first presented with gastrointestinal perforation, with long chain-3-hydroxyacyl-CoA dehydrogenase deficiency and novel compound heterozygous mutations in the HADHA gene: case report and literature review

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Abstract: Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD) is an autosomal recessive disease, characterized by mitochondrial fatty acid β-oxidation disorder. Clinical symptoms such as hypoketotic hypoglycaemia, hepatopathy, cardiomyopathy and myopathy are mainly induced by activated fatty acid oxidation, as occurs during illnesses, intensive exercise, or prolonged fasting. LCHADD is associated with the HADHA gene mutation. Here, twin brothers diagnosed with LCHADD who harboured the same novel compound heterozygous mutations in the HADHA gene were examined. Despite a similar genetic background, their clinical presentations differed greatly, and one of them presented with gastrointestinal perforation, a rarely reported manifestation. Additionally, tandem mass spectrometry combined with gene analysis is important in making a diagnosis of a suspected patient.

Keywords: Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency, HADHA gene mutation, gastrointestinal perforation, medical exome sequencing

Introduction

Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD) is an autosomal recessive disorder associated with HADHA gene mutations, and it leads to mitochondrial fatty acid β-oxidation disorder. The typical features of LCHADD include the accumulation of toxic β-oxidation intermediates, which cause immediate symptoms such as hypoketotic hypoglycaemia, hepatopathy, cardiomyopathy, and myopathy, as well as long-term complications such as progressive peripheral neuropathy and pigmentary retinopathy [1-5]. The HADHA gene contains 20 exons spanning more than 52 kb. More than 70 variants in the HADHA gene have been described. The most common variant in the HADHA gene is a c.1528G>C substitution in exon 15. In the majority of LCHADD patients, at least one allele of the predominant mutation is detected [6]. Here, twin brothers with LCHAD deficiency are reported. Without the c.1528G>C substitution these twins had novel compound heterozygous HADHA mutations. Additionally, one of the twins first presented with gastrointestinal perforation.

Case report

Clinical data

The younger brother, aged 11 months and 9 days, was admitted to the hospital because of vomiting and diarrhoea for four days, and he experienced cardiopulmonary resuscitation half an hour before admission. The patient showed vomiting and watery stools 4 days after coming in contact with the elder brother, who was infected with rotavirus enteritis. One day before admission, the patient had one occurrence of bloody stool and vomited coffee-like substances twice. Routine fecal tests showed a negative white blood cell, occult blood 3+, and a positive rotavirus test, and the patient was on outpa-

tient service of our hospital. Half an hour before admission, the patient had sudden cardiac arrest. His blood glucose was measured as 0.7 mmol/L. After cardiopulmonary resuscitation, rehydration, and glucose administration, his heart rate recovered, and he was hospitalized in a paediatric intensive care unit immediately. Upon admission, his body weight was 8 kg and height was 71 cm. There were no rales or heart murmurs detected upon auscultation of the chest. The abdomen was soft and without obvious hepatosplenomegaly. Laboratory investigations showed a white blood cell count of 22,250/mL with 59.5% neutrophils, 9.8 g/dL haemoglobin, 401×10^9/L platelets, 27 mg/L C-reactive protein concentration, 1687 IU/L serum lactate dehydrogenase, 472 IU/L alanine aminotransferase, 839 IU/L aspartate aminotransferase, 10641 IU/L creatine kinase, 588 IU/L creatine kinase-MB activity, and normal bilirubin and renal function.

Upon admission, he was given ventilator-assisted ventilation, nutritional support, and anti-infective treatment. The child had abdominal distention on the second day of hospitalization, and an X-ray examination prompted pneumoperitoneum. The patient underwent an exploratory laparotomy, a duodenal descending perforation was found, and perforation repair was carried out.

Tandem mass spectrometry (TMS) screening of inherited metabolic diseases revealed an elevated 3-hydroxyhexadecanoylcarnitine (C16-OH) (0.26 μmmol/L), 3-hydroxyoctadecanoylcarnitine (C18-OH) (0.08 μmmol/L), 3-hydroxyoctadecenoylcarnitine (C18:1-OH) (0.19 μmmol/L), and a high ratio of C16-OH/C16 (0.23). Further examination included an abdominal B-ultrasound that revealed hepatomegaly. No abnormalities were seen on cardiac ultrasound, but the brain MRI scan showed atrophic changes in the brain, abnormal left basal ganglia signals, and possible cerebral infarction. The brain magnetic resonance angiography (MRA) suggested that both posterior communicating arteries were not present.

Medical exome sequencing

The following characteristics were reported from the patient's medical history: the pathogenic condition changed rapidly, multiple systems were involved, growth and development were poor, and a genetic abnormality was suggested by the abnormal inherited metabolic diseases screening. Therefore, the baby was recommended for medical exome sequencing, which targeted 4450 known disease-causing genes. Genetic analysis showed that the patient carried compound heterozygous mutations in the HADHA gene. The first mutation was c.2230T>C in exon 15, which alters phenylalanine into leucine (p.Phe744Leu) and was inherited from his mother (**Figure 1A**). The second mutation was c.1521delG in exon 20, which causes amino acid changes (p.Gln507HisfsX20) and was inherited from his father (**Figure 1B**); both are novel mutations. The c.1521delG in exon 20 causes a frame shift that creates a premature stop codon, and conservation analysis showed that these amino acids were highly conserved among various species (**Figure 2**). These mutations can lead to LCHADD and, combined with the phenotype of the child, the mutations are considered to be pathogenic. His elder twin brother had been verified to have the same genetic mutations in the HADHA gene. The elder brother was also found to have poor growth and development as well as mild liver damage, but he did not show episodes of hypoglycaemia or nervous system abnormalities.

Further treatment and follow-up

On the sixth day after the operation, the child's condition improved, the ventilator was removed, and anti-infection and nutritional support were continued. Finally, the patient was discharged on the 26th day of hospitalization. When he was discharged, the muscle strength of the limbs had decreased on the right side more significantly than the left.

In this study, the twin brothers were followed for more than four months. The younger brother was placed on a strict low long-chain fat diet, with an average daily weight gain of 8.8 g, a total height increase of 4 cm, and a head circumference increase of 1.2 cm. A follow-up brain MRI revealed atrophic changes in the left basal ganglia of the brain, and the range of anomalous signals in the left basal ganglia had decreased. With rehabilitation training, the left limb function returned to normal, but the muscle tone of the right limbs remained low. The

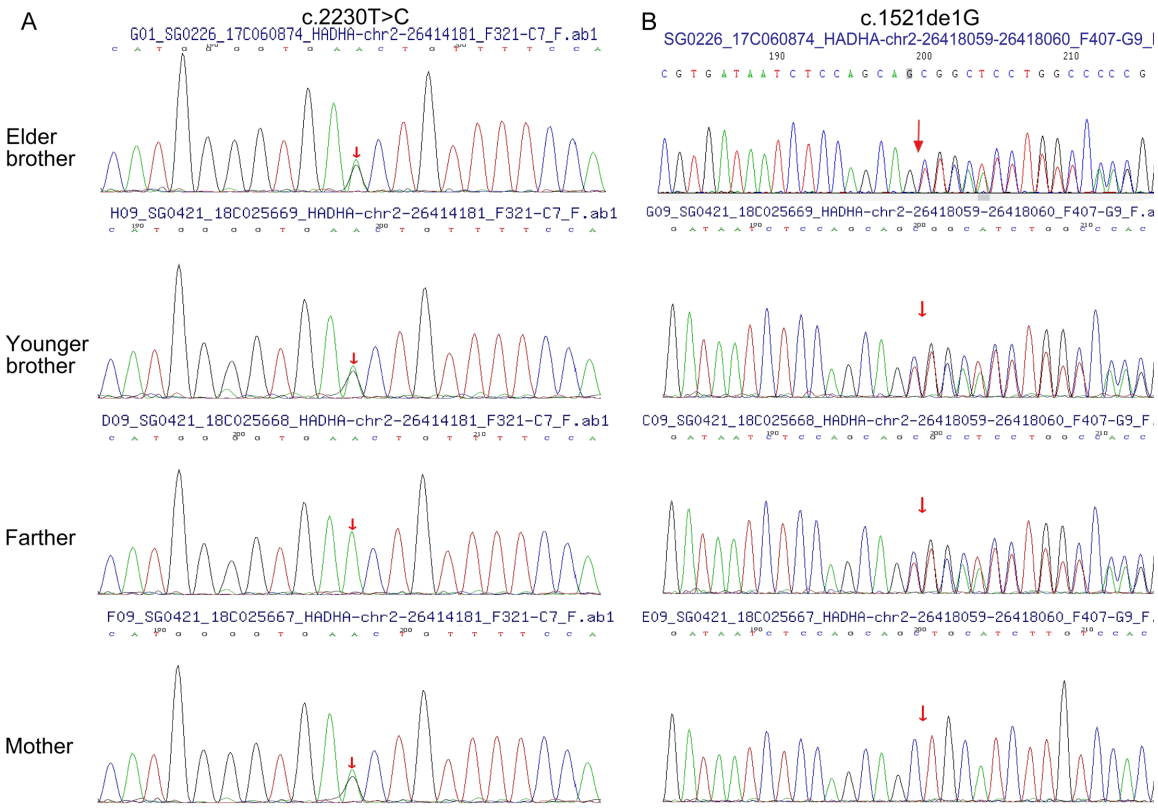

Figure 1. Identification of mutations in the HADHA gene. Electropherogram analysis of HADHA gene showing heterozygous c.2230T>C (p.F744L) in exon 15 inherited from his mother (A), and c.1521delG (p.Q507 Hfs*20) in exon 20 inherited from his father (B).

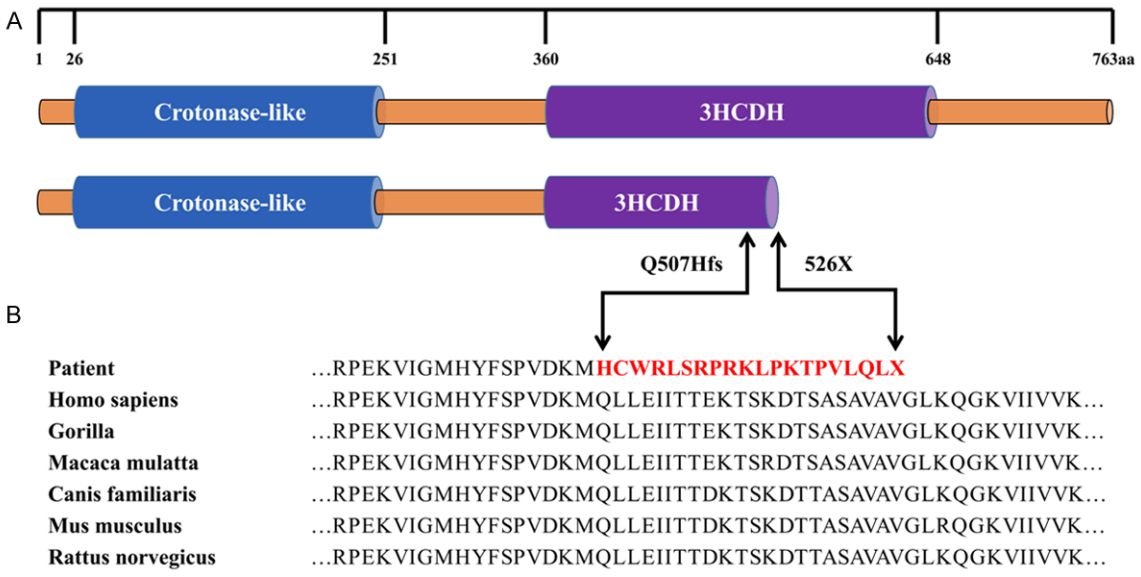

Figure 2. Mutation analysis of the c.1521delG (p.Q507 Hfs*20) in HADHA gene. Diagram of the protein molecule with the locations of the protein sequence changes predicted by the mutations (A). Conservative analysis of amino acids showed they were highly conserved among various species (B).

elder brother followed a normal diet. However, since he was fed by the grandparents, the compliance was poor, and the feeding amount was insufficient. His weight increased by an average

Table 1. Mutational spectrum in HADHA gene

No.	Region	Nucleotide change	Protein change	References
1	Exon 16	c.1678C>T	p.Arg560Term	[9]
2	*Intron 16*	c.1690-2A>G	Unknow	[10]
3	Exon 20	c.2198T>C	p.Leu733Pro	[11]
4	Exon 9	c.871C>T	p.Arg291Term	[12]
5	Exon 8	c.703C>T	p.Arg235Trp	[13]
6	Exon 4	c.274_278del	p.Ser92LysfsX10	[14]
7	*Intron 16*	c.1689+2T>G	Unknow	[15]
8	Exon 11	c.1059del	p.Lys353AsnfsX19	[16]
9	Exon 17	c.1795G>A	p.Val599Met	[17]
10	Exon 12	c.1132C>T	p.Gln378Term	[18]
11	*Intron 3*	c.180+1G>A	p.Thr37SerfsX6	[19]
12	Exon 5	c.361C>T	p.Gln121Term	[20]
13	Exon 4	c.266T>G	p.Val89Gly	[21]
14	Exon 3	c.162del	p.Asn55ThrfsX7	[22]
15	Exon 14	c.1433C>T	p.Ala478Val	[6]
16	Exon 15	c.1493A>G	p.His498Arg	[23]
17	Exon 14	c.1432G>C	p.Ala478Pro	[6]
18	*Intron 4*	c.315-1G>A	Unknow	[24]
19	*Intron 7*	c.676+2T>C	p.Val192AspfsX4	[6]
20	Exon 6	c.479_482delinsAATA	p.Ala161X	[14]
21	*Intron 13*	c.1392+1G>A	Unknow	[25]
22	Exon 1-5	Del ex. 1-5 + ex. 1 of HADHB	Unknow	[20]
23	Exon 10	c.982G>A	p.Gly328Arg	[26]
24	*Intron 9*	c.918+1G>A	p.Lys267SerfsX7	[6]
25	Exon 13	c.1381del	p.Glu461LysfsX2	[6]
26	Exon 19	c.2107G>A	p.Gly703Arg	[27]
27	Exon 13	c.1234G>T	p.Val412Leu	[17]
28	*Intron 18*	c.2000+1G>A	Unknow	[17]
29	*Intron 12*	c.1220+2T>C	Unknow	[28]
30	Exon 8	c.731C>T	p.Ala244Val	[29]
31	Exon 17	c.1793_1794del	p.His598ArgfsX33	[30]
32	Exon 11	c.1072C>A	p.Gln358Lys	[31]
33	Exon 2	c.72del	p.Tyr24X	[17]
34	Exon 20	c.2102A>G	p.Asp701Gly	[17]
35	*Intron 9*	c.919-2A>G	Unknow	[29]
36	Exon 16	c.1663_1665del	p.Met555del	[6]
37	Exon 12	c.1195C>T	p.Arg399Term	[6]
38	*Intron 19*	c.2146+6_2146+18del	p.Pro717TrpfsX7	[6]
39	Exon 18	c.1981_1999del	p.Leu661SerfsX12	[6]
40	*Intron 3*	c.180+3A>G	p.Thr37SerfsX6	[19]
41	*Intron 6*	c.574-2A>G	Unknow	[14]
42	Exon 19	c.2132dup	p.Pro712AlafsX26	[32]
43	*Intron 9*	c.918+6T>G	p.Lys267SerfsX7	[6]
44	Exon 11	c.1025T>C	p.Leu342Pro	[32]
45	*Intron 14*	c.1393_1479del-Del exon	p.Pro467_Ile495del	[26]
46	Exon 9	c.914T>A	p.Ile305Asn	[12]
47	Exon 9	c.845T>A	p.Val282Asp	[12]
48	*Intron 15*	c.1620+2_1620+6del	Unknow	[14]

of 3.2 g per day, the height increased by 2.5 cm, and the head circumference increased by 0.9 cm.

Discussion

In these twin patients, genetic analysis of the HADHA gene identified novel compound heterozygous mutations. Combined with their clinical performance and tandem mass spectrometry results, the twins were diagnosed with LCHADD. Previous reports have indicated that discovering the correlation between the genotype and phenotype of this disease is difficult [7]. The determination of enzymatic activity is important for the characterization of isolated LCHAD deficiency. To reveal the genotype-phenotype correlation between genetic variants and LCHADD, additional patients with the same genotype must be accumulated and analysed.

Different clinical phenotypes of variable severity and age of onset are common in LCHADD [8]. Currently there are 72 HADHA different mutations reported including the mutations described in this paper (**Table 1**). Although the underlying causes are metabolic abnormalities in the long-chain fatty acidβ-oxidation cycle, their clinical manifestations and age of onset are different. In addition to the residual en-

49	Exon 18	c.1828C>G	p.Arg610Gly	[21]
50	Exon 15	c.1528G>C	p.Glu510Gln	[33]
51	Exon 19	c.2114T>A	p.Val705Asp	[26]
52	Exon 20	c.2225_2228dupAACA	p.Phe744ThrfsX10	[22]
53	Exon 15	c.1533dup	p.Ile512Tyrfsx29	[6]
54	Exon 5	c.442G>A	p.Gly148Arg	[6]
55	Exon 3	c.157C>T	p.Arg53Term	[34]
56	*Intron 4*	c.315-2A>T	p.IVS4 as A-T -2	[14]
57	Exon 5	c.389T>C	p.Leu130Pro	[35]
58	Exon 20	c.2220T>A	p.Tyr740Term	[36]
59	Exon 19	c.2063G>A	p.Cys688Tyr	[6]
60	*Intron 3*	c.180_180+5delinsAT	p.Val61CysfsX6	[37]
61	Exon 19	c.2026C>T	p.Arg676Cys	[14]
62	Exon 18	c.1990_1991del	p.Lys664ValfsX2	[26]
63	Exon 11	c.1058_1059delinsT	p.Lys353IlefsX19	[6]
64	Exon 19	c.2027G>A	p.Arg676His	[14]
65	Exon 18	c.1967del	p.Leu656X	[14]
66	Exon 3	c.138dup	p.Gly47ArgfsX9	[6]
67	Exon 13	c.1336G>A	p.Glu446Lys	[27]
68	Exon 11	c.1017G>T	p.Leu339Phe	[38]
69	*Intron 11*	c.1086-3_1092del	Unknow	[14]
70	Exon 17	c.1712T>C	p.Leu571Pro	[26]
71	Exon 15	c.1521del	p.Phe744Leu	This study
72	Exon 20	c.2230T>C	p.Gln507HisfsX20	This study

zyme activity, other genetic and environmental factors are thought to play an important role in the disease manifestation.

Here, twin brothers with the same HADHA gene variants who suffered similar infections are reported. However, while one of them almost died, the other had much less severe disease. This phenomenon supports the theory that environmental factors may play a role in heterogeneous clinical manifestations. The exact mechanism responsible for these heterogeneous presentations is still unknown. However, as is the case with many other enzymatic defects, the amount of residual enzymatic activity is considered important for the development of the clinical manifestation [8, 17, 29].

Clinical symptoms of LCHADD are mainly induced by activated fatty acid oxidation, as occurs during illnesses, intensive exercise, or prolonged fasting [3]. In children with LCHADD, infections, particularly gastroenteritis, constitute an emergency and often require admission for rehydration and carbohydrate supplementation [2-5]. In these patients, the younger brother contracted rotavirus enteritis, as did his brother, but he alone presented with severe hypoketotic hypoglycaemia and gastrointestinal perforation. Many patients first present with hepatic encephalopathy or life-threatening cardiomyopathy during severe metabolic derangement. Patients even die immediately after clinical onset, sometimes too quickly to establish a final diagnosis, not to mention instituting proper therapy [21].

Clinical treatment of LCHADD is mainly based on the introduction of a fat-limited diet with the restriction of long-chain fats [3-5]. Therapeutic intervention also includes the addition of middle chain triglycerides (MCT) to the diet. There is a report of a good response to heptanoate (C7) treatment, but it is still controversial [13]. In these patients, after reasonable treatment and diet control, the younger brother improved, and his growth and development improved. Due to poor compliance, the elder brother's growth and development were not ideal.

LCHADD often presents as severe multiple organ failure with high mortality, and early diagnosis is important. Detection of LCHADD has increased since the application of TMS in expanded newborn screening (NBS), and this has allowed dietary therapy to be implemented at an early stage, thus improving the disease prognosis [37].

In conclusion, twin brothers diagnosed with LCHADD who harboured the same novel compound heterozygous mutations in the HADHA gene are reported here. Despite a similar genetic background, their clinical presentations differed greatly, and one of them presented with a rarely reported manifestation. How-

ever, NBS allows early diagnosis and implementation of dietary therapy at an early stage to improve prognosis. TMS combined with gene analysis is important in making a diagnosis of a suspected patient.

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Disclosure of conflict of interest

None.

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References

[1] Wanders RJ, Vreken P, den Boer ME, Wijburg FA, van Gennip AH and IJlst L. Disorders of mitochondrial fatty acyl-CoA beta-oxidation. J Inherit Metab Dis 1999; 22: 442-487.

[2] den Boer ME, Wanders RJ, Morris AA, IJlst L, Heymans HS and Wijburg FA. Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: clinical presentation and follow-up of 50 patients. Pediatrics 2002; 109: 99-104.

[3] Gillingham MB, Connor WE, Matern D, Rinaldo P, Burlingame T, Meeuws K and Harding CO. Optimal dietary therapy of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. Mol Genet Metab 2003; 79: 114-123.

[4] Gillingham MB, Weleber RG, Neuringer M, Connor WE, Mills M, van Calcar S, Ver Hoeve J, Wolff J and Harding CO. Effect of optimal dietary therapy upon visual function in children with long-chain 3-hydroxyacyl CoA dehydrogenase and trifunctional protein deficiency. Mol Genet Metab 2005; 86: 124-133.

[5] Spiekerkoetter U, Lindner M, Santer R, Grotzke M, Baumgartner MR, Boehles H, Das A, Haase C, Hennermann JB, Karall D, de Klerk H, Knerr I, Koch HG, Plecko B, Röschinger W, Schwab KO, Scheible D, Wijburg FA, Zschocke J, Mayatepek E and Wendel U. Management and outcome in 75 individuals with long-chain fatty acid oxidation defects: results from a workshop. J Inherit Metab Dis 2009; 32: 488-497.

[6] Boutron A, Acquaviva C, Vianey-Saban C, de Lonlay P, de Baulny HO, Guffon N, Dobbelaere D, Feillet F, Labarthe F, Lamireau D, Cano A, de Villemeur TB, Munnich A, Saudubray JM, Rabier D, Rigal O and Brivet M. Comprehensive cDNA study and quantitative analysis of mutant HADHA and HADHB transcripts in a French cohort of 52 patients with mitochondrial trifunctional protein deficiency. Mol Genet Metab 2011; 103: 341-348.

[7] Purevsuren J, Fukao T, Hasegawa Y, Kobayashi H, Li H, Mushimoto Y, Fukuda S and Yamaguchi S. Clinical and molecular aspects of Japanese patients with mitochondrial trifunctional protein deficiency. Mol Genet Metab 2009; 98: 372-377.

[8] Gregersen N, Andresen BS, Corydon MJ, Corydon TJ, Olsen RK, Bolund L and Bross P. Mutation analysis in mitochondrial fatty acid oxidation defects: exemplified by acyl-CoA dehydrogenase deficiencies, with special focus on genotype-phenotype relationship. Hum Mutat 2001; 18: 169-189.

[9] Isaacs JD Jr, Sims HF, Powell CK, Bennett MJ, Hale DE, Treem WR and Strauss AW. Maternal acute fatty liver of pregnancy associated with fetal trifunctional protein deficiency: molecular characterization of a novel maternal mutant allele. Pediatr Res 1996; 40: 393-398.

[10] Joost K, Ounap K, Zordania R, Uudelepp ML, Olsen RK, Kall K, Kilk K, Soomets U and Kahre T. Prevalence of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency in Estonia. JIMD Rep 2012; 2: 79-85.

[11] Ijlst L, Ruiter JP, Vreijling J and Wanders RJ. Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: a new method to identify the G1528C mutation in genomic DNA showing its high frequency (approximately 90%) and identification of a new mutation (T2198C). J Inherit Metab Dis 1996; 19: 165-168.

[12] Ibdah JA, Tein I, Dionisi-Vici C, Bennett MJ, IJlst L, Gibson B, Wanders RJ and Strauss AW. Mild trifunctional protein deficiency is associated with progressive neuropathy and myopathy and suggests a novel genotype-phenotype correlation. J Clin Invest 1998; 102: 1193-1199.

[13] Scheuerman O, Wanders RJ, Waterham HR, Dubnov-Raz G and Garty BZ. Mitochondrial trifunctional protein deficiency with recurrent rhabdomyolysis. Pediatr Neurol 2009; 40: 465-467.

[14] Ibdah JA, Bennett MJ, Rinaldo P, Zhao Y, Gibson B, Sims HF and Strauss AW. A fetal fatty-acid oxidation disorder as a cause of liver disease in pregnant women. N Engl J Med 1999; 340: 1723-1731.

[15] Choi JH, Yoon HR, Kim GH, Park SJ, Shin YL, Yoo HW. Identification of novel mutations of the HADHA and HADHB genes in patients with mitochondrial trifunctional protein deficiency. Int J Mol Med 2007; 19: 81-87.
[16] Matern D, Schehata BM, Shekhawa P, Strauss AW, Bennett MJ and Rinaldo P. Placental floor infarction complicating the pregnancy of a fetus with long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency. Mol Genet Metab 2001; 72: 265-268.
[17] Spiekerkoetter U, Khuchua Z, Yue Z, Bennett MJ and Strauss AW. General mitochondrial trifunctional protein (TFP) deficiency as a result of either alpha- or beta-subunit mutations exhibits similar phenotypes because mutations in either subunit alter TFP complex expression and subunit turnover. Pediatr Res 2004; 55: 190-196.
[18] Sims HF, Brackett JC, Powell CK, Treem WR, Hale DE, Bennett MJ, Gibson B, Shapiro S and Strauss AW. The molecular basis of pediatric long chain 3-hydroxyacyl-CoA dehydrogenase deficiency associated with maternal acute fatty liver of pregnancy. Proc Natl Acad Sci U S A 1995; 92: 841-845.
[19] Brackett JC, Sims HF, Rinaldo P, Shapiro S, Powell CK, Bennett MJ and Strauss AW. Two alpha subunit donor splice site mutations cause human trifunctional protein deficiency. J Clin Invest 1995; 95: 2076-2082.
[20] Bo R, Yamada K, Kobayashi H, Jamiyan P, Hasegawa Y, Taketani T, Fukuda S, Hata I, Niida Y, Shigematsu Y, Iijima K and Yamaguchi S. Clinical and molecular investigation of 14 Japanese patients with complete TFP deficiency: a comparison with Caucasian cases. J Hum Genet 2017; 62: 809-814.
[21] Sykut-Cegielska J, Gradowska W, Piekutowska-Abramczuk D, Andresen BS, Olsen RK, Ołtarzewski M, Pronicki M, Pajdowska M, Bogdańska A, Jabłońska E, Radomyska B, Kuśmierska K, Krajewska-Walasek M, Gregersen N and Pronicka E. Urgent metabolic service improves survival in long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency detected by symptomatic identification and pilot newborn screening. J Inherit Metab Dis 2011; 34: 185-195.
[22] Yang Z, Zhao Y, Bennett MJ, Strauss AW and Ibdah JA. Fetal genotypes and pregnancy outcomes in 35 families with mitochondrial trifunctional protein mutations. Am J Obstet Gynecol 2002; 187: 715-720.
[23] De Biase I, Viau KS, Liu A, Yuzyuk T, Botto LD, Pasquali M and Longo N. Diagnosis, treatment, and clinical outcome of patients with mitochondrial trifunctional protein/long-chain 3-hydroxy Acyl-CoA dehydrogenase deficiency. JIMD Rep 2017; 31: 63-71.
[24] Vasta V, Ng SB, Turner EH, Shendure J and Hahn SH. Next generation sequence analysis for mitochondrial disorders. Genome Med 2009; 1: 100.
[25] Bo R, Hasegawa Y, Yamada K, Kobayashi H, Taketani T, Fukuda S and Yamaguchi S. A fetus with mitochondrial trifunctional protein deficiency: elevation of 3-OH-acylcarnitines in amniotic fluid functionally assured the genetic diagnosis. Mol Genet Metab Rep 2015; 6: 1-4.
[26] Djouadi F, Habarou F, Le Bachelier C, Ferdinandusse S, Schlemmer D, Benoist JF, Boutron A, Andresen BS, Visser G, de Lonlay P, Olpin S, Fukao T, Yamaguchi S, Strauss AW, Wanders RJ and Bastin J. Mitochondrial trifunctional protein deficiency in human cultured fibroblasts: effects of bezafibrate. J Inherit Metab Dis 2016; 39: 47-58.
[27] IJlst L, Oostheim W, Dionisi-Vici C, Ruiter JPN and Wanders RJA. Molecular basis of MTP deficiency: identification of four new mutations. J Inherit Metab Dis 1998; 21: 65.
[28] Yang Z, Yamada J, Zhao Y, Strauss AW and Ibdah JA. Prospective screening for pediatric mitochondrial trifunctional protein defects in pregnancies complicated by liver disease. JAMA 2002; 288: 2163-2166.
[29] Olpin SE, Clark S, Andresen BS, Bischoff C, Olsen RK, Gregersen N, Chakrapani A, Downing M, Manning NJ, Sharrard M, Bonham JR, Muntoni F, Turnbull DN and Pourfarzam M. Biochemical, clinical and molecular findings in LCHAD and general mitochondrial trifunctional protein deficiency. J Inherit Metab Dis 2005; 28: 533-544.
[30] Hintz SR, Matern D, Strauss A, Bennett MJ, Hoyme HE, Schelley S, Kobori J, Colby C, Lehman NL and Enns GM. Early neonatal diagnosis of long-chain 3-hydroxyacyl coenzyme a dehydrogenase and mitochondrial trifunctional protein deficiencies. Mol Genet Metab 2002; 75: 120-127.
[31] Blish KR and Ibdah JA. Maternal heterozygosity for a mitochondrial trifunctional protein mutation as a cause for liver disease in pregnancy. Med Hypotheses 2005; 64: 96-100.
[32] IJlst L, Oostheim W, Ruiter JP and Wanders RJ. Molecular basis of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: identification of two new mutations. J Inherit Metab Dis 1997; 20: 420-422.
[33] IJlst L, Wanders RJ, Ushikubo S, Kamijo T and Hashimoto T. Molecular basis of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: identification of the major disease-causing mutation in the alpha-subunit of the mitochondrial trifunctional protein. Biochim Biophys Acta 1994; 1215: 347-350.
[34] Sperk A, Mueller M and Spiekerkoetter U. Outcome in six patients with mitochondrial tri-

functional protein disorders identified by newborn screening. Mol Genet Metab 2010; 101: 205-207.

[35] Spiekerkoetter U, Eeds A, Yue Z, Haines J, Strauss AW and Summar M. Uniparental disomy of chromosome 2 resulting in lethal trifunctional protein deficiency due to homozygous alpha-subunit mutations. Hum Mutat 2002; 20: 447-451.

[36] Tabor HK, Auer PL, Jamal SM, Chong JX, Yu JH, Gordon AS, Graubert TA, O'Donnell CJ, Rich SS, Nickerson DA; NHLBI Exome Sequencing Project and Bamshad MJ. Pathogenic variants for Mendelian and complex traits in exomes of 6,517 European and African Americans: implications for the return of incidental results. Am J Hum Genet 2014; 95: 183-193.

[37] Matern D, Strauss AW, Hillman SL, Mayatepek E, Millington DS and Trefz FK. Diagnosis of mitochondrial trifunctional protein deficiency in a blood spot from the newborn screening card by tandem mass spectrometry and DNA analysis. Pediatr Res 1999; 46: 45-49.

[38] Iossifov I, O'Roak BJ, Sanders SJ, Ronemus M, Krumm N, Levy D, Stessman HA, Witherspoon KT, Vives L, Patterson KE, Smith JD, Paeper B, Nickerson DA, Dea J, Dong S, Gonzalez LE, Mandell JD, Mane SM, Murtha MT, Sullivan CA, Walker MF, Waqar Z, Wei L, Willsey AJ, Yamrom B, Lee YH, Grabowska E, Dalkic E, Wang Z, Marks S, Andrews P, Leotta A, Kendall J, Hakker I, Rosenbaum J, Ma B, Rodgers L, Troge J, Narzisi G, Yoon S, Schatz MC, Ye K, McCombie WR, Shendure J, Eichler EE, State MW and Wigler M. The contribution of de novo coding mutations to autism spectrum disorder. Nature 2014; 515: 216-221.

[39] Karall D, Brunner-Krainz M, Kogelnig K, Konstantopoulou V, Maier EM, Möslinger D, Plecko B, Sperl W, Volkmar B and Scholl-Bürgi S. Clinical outcome, biochemical and therapeutic follow-up in 14 Austrian patients with Long-Chain 3-Hydroxy Acyl CoA Dehydrogenase Deficiency (LCHADD). Orphanet J Rare Dis 2015; 10: 21.