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Mitochondrial trifunctional protein deficiency due to *HADHB* gene mutation in a Chinese family☆

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ABSTRACT

We report an 8-year-old girl with lower limb weakness since birth in whom mitochondrial trifunctional protein (MTP) deficiency, an autosomal recessive fatty acid oxidation disorder caused by *HADHA* or *HADHB* mutations, had not been definitively diagnosed before she was referred to our hospital. Repeated blood acylcarnitine analysis revealed slightly increased long-chain 3-OH-acylcarnitine levels; electromyography (EMG) suggested peripheral nerve injury; muscle biopsy confirmed a neurogenic lesion in muscle fibers, as shown by EMG. Analysis of the *HADHB*, which encodes long-chain 3-ketoacyl-CoA thiolase, one of the enzymes constituting mitochondrial trifunctional protein, identified homozygous missense mutation c.739C > T (p.R247C). Mitochondrial trifunctional protein deficiency is an extremely rare disorder and has not been reported in Chinese people to date. It is likely that neonatal onset, as seen in our patient, has not been reported for the neuromyopathic phenotype of mitochondrial trifunctional protein deficiency.

1. Introduction

Mitochondrial trifunctional protein (MTP), an enzymatic complex that catalyzes the last three steps of long-chain fatty acid oxidation, is an inner mitochondrial membrane-bound protein consisting four α-subunits with long-chain 2,3-enoyl-CoA hydratase (LCEH) and long-chain 3-hydroxyacyl CoA dehydrogenase (LCHAD) activity, and four β-subunits with long-chain 3-ketoacyl-CoA thiolase (LCKT) activity [1]. The α- and β-subunits are encoded by two nuclear genes: *HADHA* and *HADHB*, respectively [2]. MTP deficiency caused by *HADHA* or *HADHB* mutations is a rare autosomal recessive fatty acid oxidation disorder, and has been classified into three clinical phenotypes: lethal (neonatal onset, severe form), hepatic (infantile onset, intermediate form), and neuromyopathic(late adolescent onset, mild form) [3]. The recent research demonstrated that mutation in *HADHB* causes a systemic disorder with cardiomyopathy originating already in fetal life [4].

To date, MTP deficiency has not been reported in Chinese people. We report a Chinese girl with *HADHB* mutation classified as the neonatal-onset neuromyopathic phenotype, manifesting lower limb weakness as the initial symptom and a slowly progressive course; genetic analysis finally diagnosed MTP deficiency.

2. Materials and methods

2.1. Case

The 8-year 5-month-old girl was the first child of non-consanguineous Chinese parents, and was born at 38 weeks of gestation after an uneventful pregnancy. Her birth weight was 2600 g. Her parents complained that she had less lower limbs movement compared to normal newborns when having tub bath from birth. She could lift her head and just hold it up for several minutes at 5 months, sit without support for a while at 6 months, and walk at 13 months. Her walking duration was shorter than that of other children in the same age, and she seldom walked independently before the age of 3 years. From the age of 3 years onwards, she presented obvious lower limb weakness and exercise intolerance, where she was unable to walk more than 500 m consecutively. From the age of 6 years onwards, she had trouble climbing stairs, walking more than 400 m consecutively, and fell frequently. She could not attend normal gym classes. From the age of 8 years onwards, she complained monthly of pain in her leg muscles after mild exercise. There was concomitant lethargy and weakness each time she had a fever. At the age of 5 years onwards, she was referred to hospitals when she developed fever, lethargy, and weakness, and was diagnosed with viral encephalitis. Her cognitive development was intact. Physical examination revealed reduced muscle strength of the extremities (the distal extremities muscle strength was grade 4; the proximal extremities was grade 4^+), reduced lower extremity muscle volume, loss of knee jerk reflex and Achilles tendon reflex, dragging gait, and high-arched feet.

☆ Databases: *HADHB* OMIM: 143450, GDB: 344953, GenBank: NM_000183.

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We confirmed that her parents are asymptomatic, while her younger brother had increased serum creatine kinase (CK) when he developed fever and diarrhea at 4 months of age, and had died suddenly when he developed fever at 5 months of age with the reason unknown.

2.2. Methods

2.2.1. Routine examination, urine organic acid and blood acylcarnitine analyses

Routine examination contains blood biochemical indexes, electrocardiogram, echocardiography, and eye examination.

Urine organic acid and blood acylcarnitine from dried blood spots were analyzed using gas chromatography–mass spectrometry and liquid chromatography–tandem mass spectrometry, respectively.

2.2.2. Nerve conduction velocity and electromyography

Peripheral nerve conductive velocity (NCV) and needle electromyography (EMG) were performed routinely using a Keypoint electromyography instrument (Denmark). The sensory nerve conductive velocity of the right sural nerve and left median nerve was tested. The motor conductive velocity of the right tibial nerve, left common peroneal nerve, left median nerve, and left ulnar nerve was tested. Needle EMG was used to examine the left anterior tibial muscle, left quadriceps femoris muscle, and left biceps brachii muscle.

2.2.3. Skeletal muscle biopsy and histochemical staining

Skeletal muscle biopsy of the left gastrocnemius muscle was performed; the sample was precooled using isopentane and cryofixed in liquid nitrogen. Sections (8-μm thick) were obtained for routine and enzyme histochemical staining.

2.2.4. Genomic DNA extraction and mutation analysis

EDTA-anticoagulated peripheral blood (5 mL) was collected from the patient and her parents, and genomic DNA was extracted from peripheral blood lymphocytes by salt fractionation. The DNA of the patient's brother was not available because he had already deceased.

The Ethical Committee of Peking University First Hospital approved the DNA study protocol. Informed consent for the DNA analysis was obtained from the patient's parents. We next performed gene mutation analysis using an inherited metabolic disease gene panel consisting of 153 genes by next-generation sequencing (Illumina Genome Analyzer, USA). The mutations detected by next-generation sequencing were verified using Sanger sequencing. Nucleotide and amino acid numbering was performed according to the *HADHB* chromosomal DNA sequence deposited in GenBank.

3. Results

3.1. Routine examination, urine organic acid and blood acylcarnitine analyses

Intermittently increased serum CK levels (111–2735 U/L, CK MB fraction/CK 2.6–5.5%) was repeatedly confirmed (Table 1). Electrocardiogram revealed sinus arrhythmia and uncertain incidental atrial extrasystole. Echocardiography revealed left ventricular false tendons. Eye examination showed no abnormalities.

Blood acylcarnitine analysis at first admission revealed obviously decreased free carnitine (C0, 4.15 μmol/L); a presumed diagnosis of primary carnitine deficiency was made. However, there was no muscle strength improvement following 3-week L-carnitine (2 g/d) treatment. Repeated blood acylcarnitine analysis revealed normal C0 levels and slightly increased C14:1, C14:2, C16-OH, and C18:1-OH. Urine organic acid analysis performed at the same time revealed normal levels. Increased long-chain 3-OH-acylcarnitines (C16-OH, C18:1-OH) indicated MTP deficiency. Conversely, increased long-chain acylcarnitines (C14:1, C14:2) and decreased C0 suggested very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency (Table 1). These findings suggested VLCAD or MTP deficiency.

3.2. Electromyography and skeletal muscle biopsy pathologic analysis

The left anterior tibial muscle, left quadriceps femoris muscle, and left biceps brachii muscle motor unit potential (MUP) amplitude was increased to 14%, 41%, and 10% respectively. The left quadriceps femoris muscle MUP duration was obviously increased to 12.7 ms. The sensory nerve action potential (SNAP) of the right sural nerve was not elicited. Left median nerve SNAP amplitude and sensory conduction velocity were decreased to 2.3 mV. There was slightly increased breadth of the right tibial nerve compound muscle action potential waveform, and

Table 1
Laboratory biochemistry findings.

Blood biochemistry	The day after first visit						Reference range
	1	5	6	28	48	56	
ALT (IU/L)		109	92	22	120	39	7–40
AST (IU/L)		85	46	20	68	23	13–35
LDH (IU/L)	422	865	685	318	865	458	100–240
HBDH (IU/L)	404	907	720	295	897	446	90–220
CK (IU/L)	2735	1468	569	111	2090	115	25–170
CK-MB (ng/mL)	150.9	51.2	25.9	3.8	53.4	4.1	<5
Lactic acid (mmol/L)		1.7	0.8		2.0		0.5–2
Pyruvic acid		127	81		114		30–100
β-hydroxybutylate		0.03	0.02		0.02		0.03–0.3
Ammonia (μmol/L)		32	20				<60
Homocysteine (μmol/L)		7.53			7.44		5–15
bicarbonate (mmol/L)			34.6		23		22–30
Glu (mmol/L)			4.64		5.07		3.61–6.11
T.chol			2.78		3.43		3.4–5.2
TG			0.73		0.49		0.56–1.7
Blood spot							
C0 (μmol/L)			4.15		27.25		10–52
C14:1 (μmol/L)			0.14		0.38		0–0.20
C14:2 (μmol/L)					0.19		0–0.10
C16OH (μmol/L)			0.2		0.14		0–0.10
C18:1OH (μmol/L)			0.25		0.16		0–0.10

AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; HBDH, hydroxybutyrate dehydrogenase; CK, creatine kinase; CK-MB, CK MB fraction; Glu, glucose; T.chol, total cholesterol; TG, triglyceride; C0, free carnitine; C14:1 and C14:2, long-chain acylcarnitines; C16-OH and C18:1OH, long-chain 3-OH-acylcarnitines.

slightly decreased right tibial nerve motor conduction velocity. Thus EMG suggested peripheral nerve abnormality.

Muscle biopsy revealed the muscle fiber type grouping distribution; type 1 fibers predominated, which conformed to the pathological changes of neurogenic injury. There was no obviously fat storage (oil red O, ORO), and modified Gomori trichrome (MGT) staining revealed no typical ragged red fibers (Fig. 1).

3.3. Mutation analysis

A homozygous missense mutation c.739C > T, p.Arg247Cys was detected in exon 9 of the *HADHB* gene (NM_000183) in the patient. Sanger sequencing confirmed this mutation and detected the heterozygous missense mutation c.739C > T, p.Arg247Cys in her parents. Since the DNA of her brother was unavailable, he did not have genetic proven diagnosis of MTP deficiency (Fig. 2). This mutation has been reported as the pathogenic mutation of MTP deficiency in the Human Gene Mutation Database [5]. The missense mutation had been expressed to demonstrate conclusively that it destroys enzyme activity. They performed transient expression analysis of the mutant cDNAs, and mitochondrial LCKT activity in lysates of transiently transfected cells was under the detection limit [5]. We have submitted the variant to LOVD database, and the URL is http://databases.lovd.nl/shared/phenotypes/0000021245.

4. Discussion

The patient had insidious-onset slowly progressive lower limb weakness with exercise intolerance, which was aggravated after fever with concomitant lethargy and weakness; we confirmed intermittently increased CK levels and peripheral nerve injury, which suggested a neuropathy and likely episodes of rhabdomyolysis (episodic hyperCKemia), and supported the diagnosis of metabolic neuromyopathy. According to the family history, it was possibly an autosomal recessive inherited disorder. The first metabolic screen suggested primary carnitine deficiency; however, this disease mostly affects muscle, and neuropathy is rarely present. Additionally, acylcarnitine analysis did not disclose typical primary carnitine deficiency. Importantly, she did not respond to carnitine therapy. Repeated acylcarnitine analysis revealed slightly increased C14:1, C16-OH, and C18:1-OH, which are present in some fatty acid oxidation deficiencies. We rechecked the initial results and found that these components were slightly increased as well. We next performed gene mutation analysis by next-generation sequencing, eventually discovering a known *HADHB* homozygous mutation. She was genetically diagnosed with MTP deficiency.

Fatty acid metabolism, in which more than 20 enzymes and transporters are involved, plays an important role in energy supply in the body. MTP is a multi-enzyme complex involved in long-chain hydroxyl acyl CoA metabolism. In MTP deficiency, β-oxidation of long-chain fatty acids is impaired; the deficiency is asymptomatic when energy supply and demand are in balance; however, if there is inadequate energy supply when infection, disease, exercise, or extended intervals between meals increase energy demand, the body cannot compensate for the energy shortage. MTP deficiency can present with a wide array of clinical manifestations, including nonketotic hypoglycemia, cardiomyopathy, myopathy, neuropathy, retinopathy, and liver disease. This patient had lower limb weakness and exercise intolerance since birth; the symptoms became prominent at around the age of 3 years. Her condition followed a slowly progressive course over the next five years. With serum CK levels, NCV and muscle biopsy revealing episodic hyperCKemia and a neurogenic lesion affecting the peripheral sensorimotor system, we concluded that her clinical manifestations stemmed from the neuromyopathic phenotype of MTP deficiency.

MTP deficiency is a very rare disorder, and to our knowledge, no case has been reported in mainland Chinese people to date. About 20 patients were reported thus far in Europe and North America classified as having the neuromyopathic phenotype. Spiekerkoetter et al. [6] reported a series of 11 patients with the neuromyopathic phenotype of MTP deficiency. The age of onset ranged 1–13 years, whereas our patient had onset at birth. Spiekerkoetter et al. [6] reported that seven of 10 patients developed neuropathic weakness before the onset of rhabdomyolysis, and indicated that the neuromyopathic phenotype is the major phenotype of MTP deficiency. Though we did not examine the blood and urine myoglobin of the patient, therefore we cannot definitely confirm whether she has rhabdomyolysis or not when she has prominent lethargy and weakness, muscle pain, and very high CK levels, she likely to have episodes of rhabdomyolysis based on the episodic symptoms and hyperCKemia.

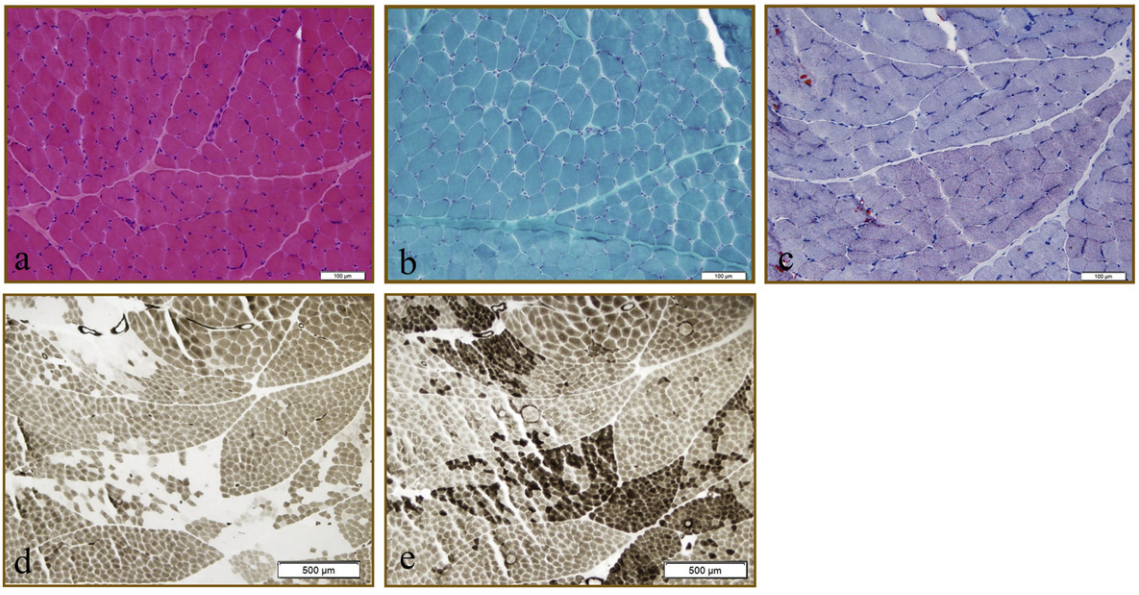

Fig. 1. Skeletal muscle biopsy pathologic analysis. (2-column fitting image, color online only) (a) HE staining of skeletal muscles revealed a few small rounded or small angular fibers in grouping distribution and some hypertrophic fibers. Muscle fiber nucleus internal migration was not found. (b) Typical or atypical ragged red fibers were not found in MGT staining. (c) ORO staining revealed slightly increased lipid droplets in some fibers. (d) ATP 4.3 staining revealed grouped distribution fibers with type 1 fiber predominance. Hypertrophic and atrophic fibers involved both type 1 and type 2 fibers, while type 1 fiber was predominant in hypertrophic fibers. (e) ATP 10.5 staining confirmed the findings of ATP 4.3 staining.

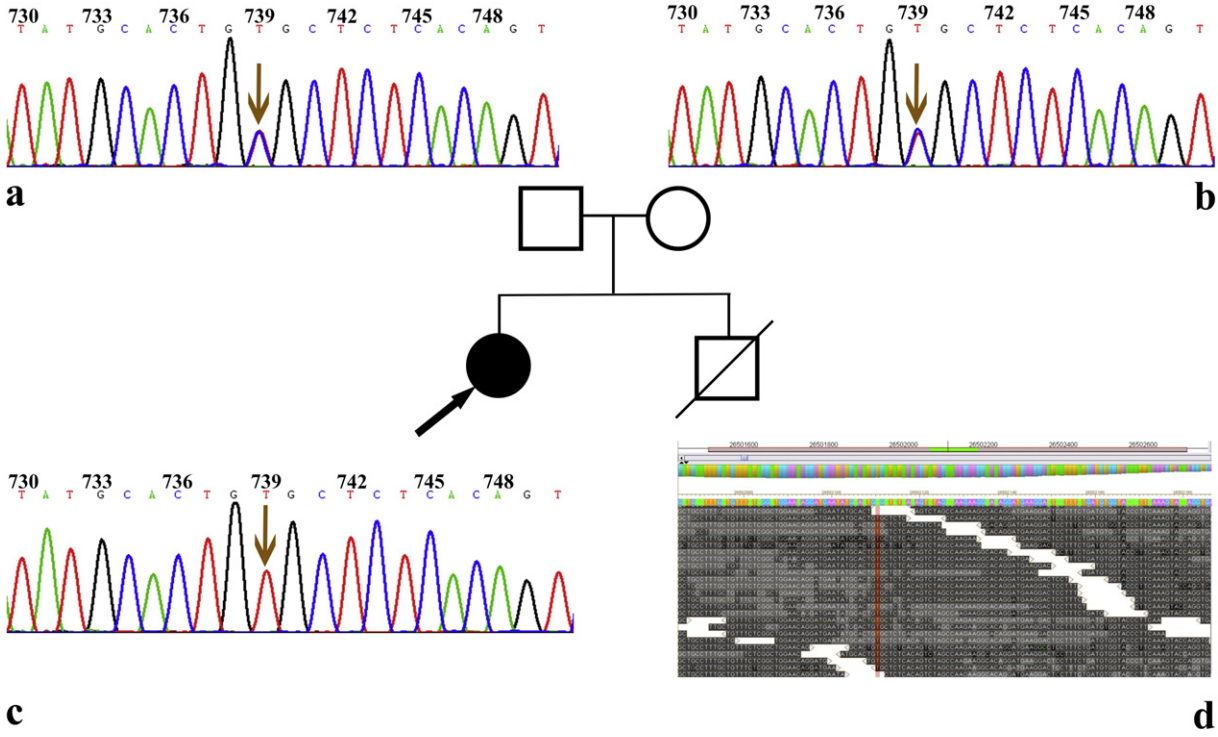

Fig. 2. Sanger sequencing confirming homozygous mutation in the patient and heterozygous mutation in her parents. (2-column fitting image, color online only) (a) The father had heterozygous c.739C > T, p.Arg247Cys mutation. (b) The mother showed heterozygous c.739C > T, p.Arg247Cys mutation. (c) The patient had homozygous missense mutation c.739C > T, p.Arg247Cys. (d) Next generation sequencing showed homozygous missense mutation c.739C > T, p.Arg247Cys in the patient.

Only two of six Japanese cases in the literature presented with the neuromyopathic phenotype [5,7]. As this phenotype has significant features in common with CMT (Charcot–Marie–Tooth disease) and spinal muscular atrophy, this emphasizes the need for clinicians to develop a higher index of suspicion for fatty acid oxidation disorders in patients with neuromyopathic features, given the potential for treatment intervention. Regarding our patient, we believe that the low diagnosis rate is also due to clinicians' lack of knowledge about this disease.

MTP deficiency is characterized by increased serum (or plasma) or blood spot C16-OH, C16:1OH, C18-OH, and C18:1OH, demonstrated by acylcarnitine analysis. However, the analysis does not detect any abnormality when no episodic symptoms occur, and a urine organic acid profile demonstrating 3-OH-dicarboxylic aciduria is also suggestive of this disorder [8]. Our patient had slightly increased blood long-chain 3-OH-acylcarnitine levels, indicating MTP deficiency, and slightly increased blood long-chain acylcarnitine levels and decreased blood C0 levels, which suggested VLCAD deficiency; however, it is difficult to distinguish between VLCAD and MTP deficiency. The acylcarnitine pattern and muscle biopsy are not specific for MTP deficiency, and other fatty acid β-oxidation disorders may have an overlapping pattern of alterations. Muscle biopsy of our patient showed only neurogenic injury. Genetic analysis to detect pathogenic *HADHA* and *HADHB* mutations is required for correct diagnosis, which is crucial given the implications for patients and their families, and for genetic counseling.

MTP deficiency is an autosomal recessive disorder. *HADHA* encodes the LCHAD and LCEH subunits; *HADHB* encodes the LCKT subunits. The two genes are adjacent to each other on human chromosome 2q23 [9] and have 20 and 16 exons, respectively [3,9,10]. Several research groups have attempted to elucidate the underlying *HADHB* genotype–phenotype correlation. Spiekerkoetter et al. analyzed mutations from 15 patients and linked clinical phenotype with the location of the mutation [3]. In an in vitro functional study, Purevsuren et al. demonstrated positive correlations between residual enzymatic activity and phenotypic severity [5]. The *HADHB* homozygous missense mutation c.739C > T (p.R247C) in our patient is the same as that found by Purevsuren et al. in a Japanese patient. However, the Japanese patient presented the hepatic phenotype of MTP deficiency, which might be ascribed to a compound heterozygote of a paternal c.739C > T transition and a maternal single c.817G deletion in his *HADHB* gene. Previously reported patients with this mutation all had the MTP deficiency phenotype but later disease onset than our patient. Even in her family, the severity differed between her and her younger brother, suggesting that environmental predisposition is also an important factor contributing to the disease course. Above all, the neuromyopathic phenotype of MTP deficiency may occur at the neonatal period and progress very slowly. The *HADHB* homozygous missense mutation c.739C > T (p.R247C) may contribute to the neonatal-onset neuromyopathic phenotype of MTP deficiency.

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