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A patient with mitochondrial trifunctional protein deficiency due to the mutations in the *HADHB* gene showed recurrent myalgia since early childhood and was diagnosed in adolescence ☆

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ABSTRACT

Mitochondrial trifunctional protein (MTP) is a multienzyme complex involved in the metabolism of long-chain hydroxyacyl-CoA, a product of the fatty acid β-oxidation cycle. MTP is an α4β4 hetero-octomer encoded by two different genes: *HADHA* (OMIM 600890) and *HADHB* (OMIM 143450). MTP deficiency induces three different types of presentation: (1) a lethal phenotype with neonatal onset (severe); (2) a hepatic phenotype with infant onset (intermediate); and (3) a neuromyopathic phenotype with late-adolescent onset (mild). While acylcarnitine analysis has revealed increased levels of long-chain hydroxyacylcarnitine in blood when an MTP deficiency exists, the neuromyopathic type is usually asymptomatic and does not always result in an abnormality in acylcarnitine analysis results. We report here the case of a 13-year-old girl with recurrences of intermittent myalgia since her early childhood, for whom the disorder had not been definitely diagnosed. Since she was referred to our hospital because of rhabdomyolysis, we have repeatedly performed blood acylcarnitine analysis and found slight increases in long-chain 3-OH-acylcarnitine levels, on the basis of which we made a chemical diagnosis of MTP deficiency. Immunoblot analysis of skin fibroblasts revealed loss of α- and β-subunits of MTP. In addition, analysis of the *HADHB* gene, which encodes long-chain 3-ketoacyl-CoA thiolase, one of the enzymes constituting MTP, identified compound heterozygous mutations of c.520 C>T (p.R141C) and c.1331 G>A (p.R411K).
MTP deficiency is considered an extremely rare disorder, as only five cases (lethal phenotype, two patients; hepatic phenotype, two patients; and neuromyopathic phenotype, one patient) have thus far been reported in Japan. However, it is likely that the neuromyopathic phenotype of MTP deficiency has not yet been diagnosed among patients with recurrences of intermittent myalgia and rhabdomyolysis, as in our patient reported here.

1. Introduction

Mitochondrial trifunctional protein (MTP) is a multienzyme complex involved in the metabolism of long-chain hydroxyacyl-CoA, a product of the fatty acid β-oxidation cycle [1]. When an abnormality exists in this complex, the fatty acid β-oxidation cycle fails to supply an adequate amount of energy, resulting in three different types of presentation: a lethal phenotype with neonatal onset, in which hepatic and/or cardiac muscular disturbance occurs early in infancy, causing sudden death; a hepatic phenotype with infant onset, in which non- or low-ketotic hypoglycemia occurs; and a neuromyopathic phenotype with late-adolescent onset, in which muscular symptoms such as intermittent myalgia or rhabdomyolysis occur [2,3]. The diagnosis is based on increased levels of long-chain 3-OH-acylcarnitine, as demonstrated by blood acylcarnitine analysis using ESI-MS/MS. However, the neuromyopathic phenotype is usually asymptomatic and frequently shows no abnormal test results; therefore, its definite diagnosis may require an extended length of time.

A 13-year-old girl with repeated myalgia since early childhood, in whom MTP deficiency had not been diagnosed, was referred to our hospital because of rhabdomyolysis. We repeatedly performed blood acylcarnitine analysis, found slight increases in long-chain 3-OH-acylcarnitine levels, and finally diagnosed MTP deficiency by Western blot and genetic analysis.

☆ Databases: HADHA OMIM:600890, GDB:434026, GenBank:NM_000182HADHB OMIM:143450, GDB:344953, GenBank:NM_000183

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2. Materials and methods

2.1. Case

The 13-year-old girl was the first child of non-consanguineous parents, and was born at 42 weeks of gestation after an uneventful pregnancy. Her birth weight was 3032 g. One month after birth, an apparent life-threatening event (ALTE) developed; however, she showed normal growth and development. From the age of 3 years, she complained of pain in her leg muscles after walking over a long distance. From the age of 9 years, the frequency of intermittent acute muscle pain increased and even mild exercise occasionally caused severe discomfort in her leg muscles. The symptom was often triggered by infection and menstruation. She experienced bouts of muscle pain after hard exercise such as running on a school field day or hiking on a school excursion. After hard exercise, she felt difficulty in moving because of unusual severe pain in her generalized muscles. Serum acylcarnitine analysis performed at 10 years of age revealed slightly increased levels of long-chain acylcarnitine, on the basis of which very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency was suspected. However, her VLCAD activity was normal and the disorder had not been definitely diagnosed.

At the age of 13 years, she was admitted to Kobe University Hospital with severe myalgia over the whole body. On admission, hematological and biochemical investigations revealed markedly elevated serum creatine kinase (22,885 IU/L), aldolase (62.6 IU/L), and myoglobin (2960 ng/mL). Plasma free and total carnitine and total keton bodies concentration were normal (Table 1). Echocardiography and electocardiography did not reveal any evidence of a cardiomyopathy.

Table 1
Laboratory findings upon hospitalization.

<Blood biochemistry>			Reference range
AST	529	IU/L	13–31
ALT	205	IU/L	8–34
LDH	909	IU/L	115–217
CK	22,885	IU/L	46–168
CK-MB	434	IU/L	0–25
Aldolase	62.6	IU/L	2.2–5.5
Myoglobin	2960	ng/mL	0–60
BUN	11	mg/dL	9–22
Cre	0.34	mg/dL	0.5–1.3
T.chol	167	mg/dL	146–219
TG	58	mg/dL	28–149
Glu	180	mg/dL	61–92
Lactic acid	19.1	mg/dL	3–17
Pyruvic acid	1.47	mg/dL	0.3–0.94
Total ketone bodies	39	μmol/L	26–122
Acetoacetate	24	μmol/L	13–69
β-hydroxy butylate	15	μmol/L	0–76
Total carnitine	57	μmol/L	45–91
Free carnitine	45.7	μmol/L	36–74
Acylcarnitine	11.3	μmol/L	6–23
<Acylcarnitine analysis>			Reference range
(Blood spot)			
C14:1	0.21	μM	<0.4
C14-OH	0.12	μM	<0.12
C16:1	0.15	μM	<0.785
C16:0-OH	0.18	μM	<0.12
C18-OH	0.066	μM	<0.1
C18:1-OH	0.15	μM	<0.07
(Serum)			
C14:1	0.4	μM	<0.1
C14-OH	0.2	μM	<0.1
C16:1	0.2	μM	<0.1
C16:0-OH	0.16	μM	<0.8
C18-OH	0.077	μM	<0.05
C18:1-OH	0.13	μM	<0.7

2.2. Methods

2.2.1. Urine organic acid and acylcarnitine analysis

Urine organic acid and blood acylcarnitine analysis from dried blood spots and serum were performed by GC/MS and ESI-MS/MS, respectively, as described in detail previously [4].

2.2.2. Cell culture

Fibroblasts from the patient were cultured in Eagle's minimum essential medium containing 10% fetal calf serum and antibodies (100 μg/mL each of penicillin and streptomycin; Nissui Pharmaceutical Co. Ltd., Tokyo, Japan).

2.2.3. Western blot analysis

Western blot analysis was performed following 12.5% SDS/PAGE [5] using rabbit polyclonal antibodies raised against purified MTP protein as the primary antibody. Bound antibodies were visualized using the ImmunoPure NBT/BCIP Substrate Kit (Promega, Madison WI, USA). Protein concentrations were determined using the Bio-Rad protein assay protocol (Bio-Rad Laboratories, Hercules, CA).

2.2.4. Mutation analysis

Genomic DNA was extracted from the patient's fibroblasts using a QIAamp DNA Micro Kit (Qiagen GmbH Hilden, Germany). We designed 20 sets of primers for amplification of *HADHA* (one for each exon, including 5′ and 3′ splice sites), and 16 sets for amplification of *HADHB*. Each exon was amplified by polymerase chain reaction (PCR) and directly sequenced as described previously [6].

3. Results

3.1. Urine organic acid and acylcarnitine analysis

Urine organic acid analysis performed at the same time revealed slight ketosis (as indicated by slightly increased excretion of 3-OH-butyrate), low-ketotic dicarboxylic aciduria (as indicated by increased excretion of adipate and suberate), and 3-OH dicarboxylic aciduria (as indicated by increased excretion of 3-OH-sebacate and 3-OH-dodecanedioate).

Acylcarnitine analysis of blood spots at the time of hospitalization revealed increases in the levels of long-chain 3-OH-acylcarnitines (C14-OH, C16-OH, C18-1-OH). The changes indicated an MTP deficiency. On the other hand, elevation of both long-chain acylcarnitines (C14-1, C16-1) and long-chain 3-OH-acylcarnitines (C14-OH, C18-OH) in serum suggested a VLCAD deficiency (Table 1). These findings strongly suggested long-chain fatty acid metabolism disorder, VLCAD or MTP deficiency, however, it was difficult to distinguish between the two. So acylcarnitine analysis was performed another four times, with inconsistent results: slightly increased levels of long-chain 3-OH-acylcarnitine (C16-OH, C18-1-OH), a feature of MTP deficiency, were noted on two occasions, while no abnormality was noted on the other two occasions. On the basis of these results and her clinical manifestations, we made a chemical diagnosis of a mild form of MTP deficiency, in which neither symptoms nor abnormal laboratory findings are noted during attack-free intervals.

3.2. Western blot analysis

Western blot analysis using samples extracted from the patient's skin fibroblasts detected neither α- nor β-subunits of MTP (Fig. 1), whereas both subunits were detected in control fibroblasts. These findings showed that the patient had an MTP deficiency.