INTERNAL MEDICINE

□ CASE REPORT □

A Case of Late Onset Riboflavin-responsive Multiple Acyl-CoA Dehydrogenase Deficiency Manifesting as Recurrent Rhabdomyolysis and Acute Renal Failure

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Abstract

We report an adult case of late-onset riboflavin-responsive multiple acyl-CoA dehydrogenase deficiency (MADD) characterized by episodic recurrent rhabdomyolysis and acute renal failure after the age of 46. Muscle biopsy revealed lipid storage myopathy and the finding of serum acylcarnitine and urine organic acid analyses were consistent with MADD. A compound heterozygous mutation was identified in the electron-transferring-flavoprotein dehydrogenase (*ETFDH*) gene, including a novel missense mutation, which confirmed the diagnosis of MADD. After administration of riboflavin and L-carnitine, the muscle weakness and fatigability gradually improved. Acylcarnitine and urine organic acid were also normalized after supplementation. Thus, MADD should be included in one of the differential diagnoses for adult recurrent rhabdomyolysis. Gene analysis is useful to confirm the diagnosis, and early diagnosis is important because riboflavin treatment has been effective in a significant number of patients with MADD.

Key words: multiple acyl-CoA dehydrogenase deficiency (MADD), lipid storage myopathy, recurrent rhabdomyolysis, acute renal failure, riboflavin, electron-transferring-flavoprotein dehydrogenase (*ETFDH*)

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Introduction

Multiple acyl-CoA dehydrogenase deficiency (MADD), also known as glutamic aciduria type 2, is an autosomal recessive inherited organic acid disorder. MADD is caused by defects in electron transfer flavoprotein (ETF) or ETF-ubiquinone oxidoreductase (ETF-QO), which are indispensable in the final process of fatty acid oxidation, leading to impaired adenosine triphosphate (ATP) biosynthesis from fatty acid, excessive lipid accumulation and insufficient gluconeogenesis.

The clinical phenotypes of MADD have been classified into three groups, namely, neonatal onset form with (type 1) or without (type 2) congenital anomalies, and mild and/or late onset form (type 3) (1). Mild and/or late onset cases manifest their first symptoms till early adulthood, such as intermittent vomiting, abdominal pain, hypoketotic hypoglycemia, hepato/cardiomegaly, metabolic acidosis, and/or hyperammonemia/lactatemia, which are often preceded by general infection or a catabolic condition.

Here, we present a case of extremely late onset MADD characterized by episodic recurrent rhabdomyolysis followed by acute renal failure, in whom a novel compound heterozygous mutation within the *ETFDH* gene was identified. The patient showed a favorable response to riboflavin replacement therapy.

Case Report

A 56-year-old man recognized fatigability and weakness in his neck and lower limbs since he was 46. He had lost 8

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Table 1. Laboratory Examination Data on Admission

Blood Gas Analysis (room air)

pH	7.182	
pCO_2	16.9	mmHg
pO_2	121.7	mmHg
HCO_3^-	6.1	mmol/L
BE	-21.4	mmol/L
Anion gap	35.9	

Urinalysis

Protein	(±)
Glucose	(−)
Occult blood	(1+)
Ketone body	(3+)

Blood Cell Count

WBC	11900	/μL
RBC	482 × 10^4	/μL
Hb	15.7	g/dL
Ht	47.0	%
Plt	30.9	× 10^4/μL

Blood Chemistry

T-Bil	0.8	mg/dL	Glucose	54	mg/dL
AST	76	IU/L	HbA1c	5.1	%
ALT	63	IU/L	HDL	61	mg/dL
LDH	543	IU/L	LDL	205	mg/dL
TP	8.5	g/dL	TG	48	mg/dL
Alb	4.8	g/dL			
BUN	43	mg/dL	CK	907	IU/L
Cr	1.2	mg/dL	- BB	1	%
Na	144	mEq/L	- MB	6	%
K	5.7	mEq/L	- MM	93	%
Cl	101	mEq/L	myoglobin	4138	ng/mL
Ca	10.5	mg/dL	aldorase	8.6	IU/L
P	8.1	mg/dL	lactate	44.1	mg/dL
Mg	2.8	mg/dL	pyruvate	3.23	mg/dL
CRP	0.2	mg/dL	NH_3	112	μg/dL

kg of weight during the first year. At the age of 47, he was referred to a local hospital. The first physical examination revealed mild muscle weakness in the neck, trunk and proximal limbs. Muscle CT demonstrated striking atrophy in the thighs. Serum CK, lactate, pyruvate were elevated to 517 IU/L, 16.9 mg/dL, 44.0 mg/dL, respectively. An aerobic exercise test showed excessive production of both lactate (45.3 →90.4 mg/dL) and pyruvate (2.28→2.59 mg/dL). Muscle biopsy from the quadriceps femoris revealed non-specific myogenic changes including mildly increased central nuclei (3.8%) with a few infiltrating cells around the vessels. There was no ragged-red fibers nor cytochrome c oxidase deficiency suggesting mitochondrial myopathy. Immunohistochemical analyses using specific antibodies for dystrophin, dysferlin, α-sarcoglycan showed no abnormal findings. Oil Red O staining was not examined at that time. Mitochondrial DNA analyses of MELAS (3243A→G) and MERRF (8344A→G) were both negative.

At the age of 48, he first experienced rhabdomyolysis followed by acute renal failure and lactic acidosis after gastroenteritis and poor diet. Such episodes occurred 5 times during next 7 years and were usually elicited by physical exercise, fasting, irregular diet or infection. However, renal function, acidosis and the serum CK level were immediately normalized after short term dialysis. Since the age of 55 years, sustained hyperCKemia (above 500 IU/L) was observed together with weakness in the neck and proximal limbs. At the age of 56, he was transferred to our hospital due to difficulty in breathing and severe muscle weakness after fasting for a whole day.

At admission, the patient revealed that, apart from a difficulty in heavy aerobic exercise since adolescence, his developmental milestones and dietary life were normal. His past medical history was notable for fatty liver, hyperlipidemia revealed by a health check at the age of 47, and thalamic hemorrhage resulting in mild dysarthria at the age of 54. His parents were not consanguineous and had no myopathic symptoms. He had no other affected siblings.

Physical examination on admission showed a normally developed, well nourished man (height 182 cm, weight 64.8 kg, and body mass index 19.6 kg/m^2). Vital signs were BP 118/59 mmHg, HR 120 bpm, BT 36.6, RR 18/min, SpO_2 99%. Neurological examination revealed dysarthria, muscle weakness and myalgia. The manual muscle test results were 2 in neck flexor and extensor and 3 in the proximal limbs with muscle atrophy observed overall in the proximal limbs. Routine biochemical analyses showed remarkable increments in myogenic proteins such as CK, which rose from 907 IU/L to a maximum of 5,955 IU/L one day after the admission, myoglobin at 4,138 ng/mL, and aldorase at 8.6 IU/L, accompanied by lactic acidosis, hypoglycemia and impaired renal function (Table 1). However, all these findings were immediately normalized after short term dialysis and bed rest. Cardiac and abdominal echography did not reveal any visceral abnormalities. To further elucidate the pathogenesis of the recurrent rhabdomyolysis and progressive muscle weakness, we restained the stock muscle specimen taken at the age of 47. HE staining revealed increased numbers of fibers with cytoplasmic or subsarcolemmal vacuoles (Fig. 1A). These vacuoles, mainly distributed in type 1 muscle fibers, were positively stained with Oil red O, suggesting lipid storage myopathy (Fig. 1B). Modified Gomori trichrome, NADH, PAS and CCO staining did not show any other specific abnormalities. Additional biochemical analyses revealed decreased serum free carnitine (16.0 μmol/L, normal range: 36-74), and elevated acyl-carnitine (59.2 μmol/L, normal range: 6-23), which was compatible with MADD. Likewise, increased glutarate and 2-OH-glutarate in the urine organic acid analysis also supported the diagnosis. After receiving informed consent, genetic analysis was carried out and compound heterozygous missense *ETFDH* gene mutations were identified: c.1211T > C in exon 10 and

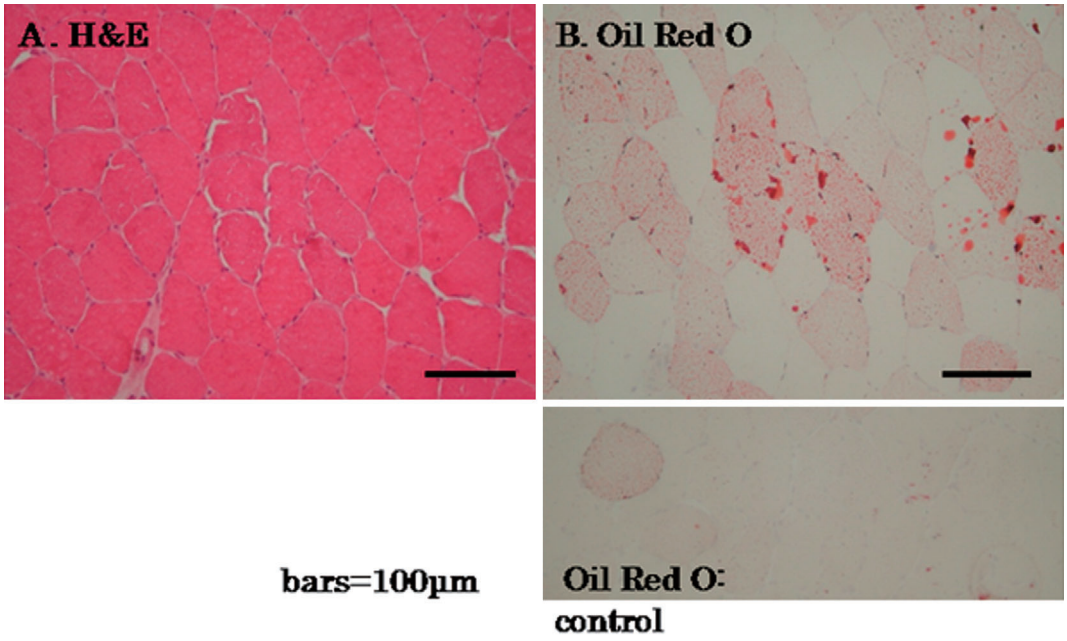

Figure 1. **(A) Hematoxylin and Eosin staining showed a large number of fibers with cytoplasmic and subsarcolemmal vacuoles. (B) Oil red O staining revealed positive staining in the cytoplasm suggesting abnormal lipid storage (bars=100 μm).**

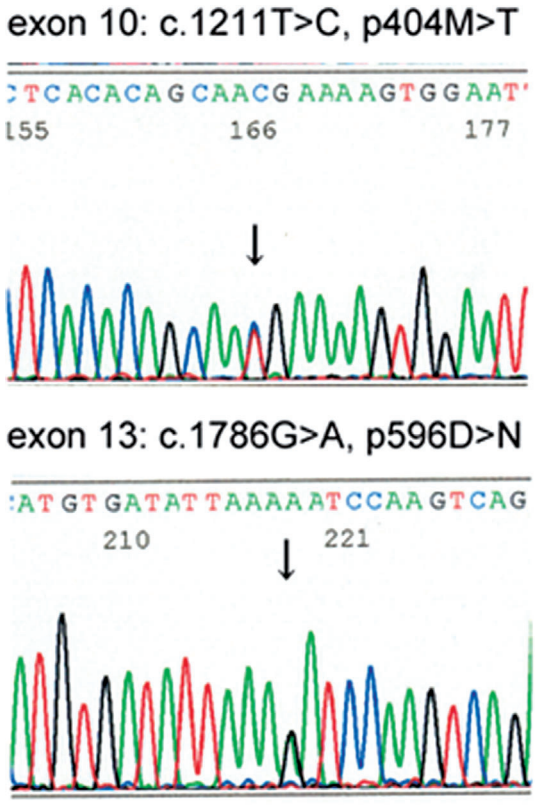

Figure 2. **Genetic analysis of *ETFDH* gene.**

c.1786G > A in exon 13, which resulted in p.M404T and p.D596N, respectively (Fig. 2). Genetic analysis of his parents has not been done, and we examined the patient's frozen muscle sample by reverse transcriptase-PCR and confirmed that each of the mutations was located in separate alleles. We also found that both mutations were absent in 100 chromosomes from Japanese healthy controls.

After the definitive diagnosis of MADD, we started therapy with riboflavin (100 mg/day) and L-carnitine supplement (50 mg/kg/day) together with a low-fat diet (fat: 670 mg/kg/day, total calories: 26 kcal/kg/day). His muscle weakness and fatigability gradually improved within 3 weeks concomitant with the normalization of serum acylcarnitine and urinary organic acid (Table 2). After starting the supplementation therapy, his daily life was uneventful and he returned to his normal daily activity. His serum CK level has remained within the normal range and there has been no relapse of rhabdomyolysis for 6 months.

Discussion

MADD is caused by homozygous or compound heterozygous mutations in the *ETFA*, *ETFB*, or *ETFDH* genes, which encodes ETF (electron transfer flavoprotein) α subunit, ETFβ subunit, and ETF-QO (ETF-ubiquinone oxidoreductase), respectively. These mutations reflect functional defects in ETF or ETF-QO (1). In mitochondria, ETF, which is located in the matrix, receives electrons from several FAD-containing acyl-CoA dehydrogenases involved in fatty acid oxidation. ETF transfers electrons to ETF-QO, located in the inner mitochondrial membrane and, subsequently, electrons are passed to ubiquinone in the respiratory chain. Consequently, a dysfunction of ETF or ETF-QO causes the final process of fatty acid oxidation to fail, thereby leading to disturbed ATP biosynthesis from fatty acid, excessive lipid accumulation and disturbed gluconeogenesis.

Table 2. **Serum CK, Acylcarnitine and Urine Organic Acid. Before and after Treatment with Riboflavin and L-carnitine**

	Before Supplement	After Supplement	Reference Range
serum acylcarnitine analysis			
total carnitine	75.2	74.1	45-91 (μmoL/L)
free carnitine	16.0	49.3	36-74 (μmoL/L)
acylcarnitine	59.2	24.8	6.0-23 (μmoL/L)
urine organic acid profile			
Elevation of	glutarate		
	2-OH-glutarate		
	adipate		
	octenedioate		
	suberate	normalized	
	decadienediate		
	pyruvate		
	lactate		
	3-OH-butyrate		
	acetoacetate		
Serum CK	Max 5955	71	50-197 (IU/L)

The neonatal onset forms of MADD, both type 1 and type 2, usually present at 24-48 hours after birth with severe hypoglycemia, metabolic acidosis, hypotonia, hepatomegaly, and most patients show a fatal course within the first week regardless of intensive treatments. The age at the presentation of the late onset form ranges from neonatal to adulthood. Most of the late onset cases manifest their first symptoms till early adulthood, usually showing intermittent vomiting, abdominal pain, hypoketotic hypoglycemia, hepato/cardiomegaly, metabolic acidosis, and/or hyperammonemia/lactatemia, which are preceded by infection or a catabolic condition.

On the other hand, adult onset cases often manifest a pure myopathy form as progressive severe proximal and axial muscle weakness, particularly in the neck flexors and extensors (2), sometimes, dysphagia and respiratory insufficiency (3).

The present case developed the first symptoms in later adulthood showing a pure myopathic form and later suffered episodic recurrent rhabdomyolysis with acute renal failure. Pathophysiologically, defects of fatty acid oxidation can cause rhabdomyolysis by the depletion of ATP within myocytes, which is usually triggered by prolonged exercise, fasting or infection. It should be emphasized that among lipid metabolic diseases, recurrent rhabdomyolysis is common in carnitine palmitoyltransferase 2 (CPT2) deficiency (4, 5) and very-long chain acyl-CoA dehydrogenase deficiency (6, 7). Other defects of fatty acid oxidation have also been reported in long (8), medium (9) and short (10) chain acyl-CoA dehydrogenase deficiency. In contrast, recurrent rhabdomyolysis is extremely rare in MADD throughout all ages, only equine cases have been identified (11), and the precise mechanism by which MADD causes rhabdomyolysis remains unknown.

Genetically, the present case had compound heterozygous *ETFDH* gene mutations of c.1211T > C (p.M404T) in exon 10 and c.1786G > A (p.D596N) in exon 13. The former mutation was reported in a Chinese female case, whose disease onset was at the age of 23 and only one heterozygous mutation was identified (12). This may be due to mutations in the promoter region, or the nonsense mediated decay pathway resulted from mutations such as exon deletions which cannot be identified by genomic sequencing (12). The latter mutation had not been described previously and the amino acid of this site is conserved among vertebrate, plant, and some insects and fungi. The parents of our case had died in their sixties. Neither of them had any myopathic symptoms. Gene analyses for them had not been done. Accordingly, we cannot exclude the possibility that both mutations occurred *de novo*, however such a possibility should be extremely unlikely. There is no report of MADD with allelic *de novo* mutations in the literature. We presume his parents were healthy carriers of each mutation, which would be consistent with the results of Reverse Transcriptase-PCR.

The severity and age at onset of MADD is speculated to correlate with the genotype and residual enzyme activity. Olsen et al described that the clinical phenotype can be explained by *ETF/ETFDH* genotype that results in different levels of residual enzyme activity (13). That is, null mutations, which would not be expected to result in any residual *ETF/ETFDH* enzyme activity, result in the development of congenital anomalies and thereby type 1, and even small amounts of residual activity is sufficient to give rise to the type 2 phenotype. And the type 3 phenotype is caused by at least one allele with a single missense mutation, which is not directly involved in the active site and has no apparent effect on mRNA processing or stability, and shows significant residual enzyme activity (13). In the Japanese population, though some missense mutations have been reported to be associated with the mild/late onset form (14), no obvious

clustering of mutations has been established.

In the present case, urinary ketone bodies were highly positive. It is speculated that ETF-QO, which is located more down-stream of fatty acid oxidation than ETF, is less influential on this oxidation and its residual activity is expected to remain to some extent in this late-onset case. Therefore the fatty acid oxidation is assumed to be partially working. And here, the failure of electron passage to the respiratory chain may rather cause an acceleration of the fatty acid oxidation, leading to the production of excessive acetyl-CoA and ketone bodies.

ETF-QO is composed of three functional domains: FAD binding domain, 4Fe4S cluster domain and ubiquinone binding domain (15, 16). The p.M404T resides in the FAD-binding domain and p.D596N resides in the 4Fe4S cluster domain. Although the details of the electron transfer machinery within ETF-QO still remain uncertain, FAD and 4Fe4S are thought to work as cofactors and the equilibration between these two cofactors is indispensable for the proper transferring of electrons to ubiquinone. Interestingly, most of the known mutations in the *ETFDH* gene are associated with amino acid substitution in the region of the FAD or ubiquinone domains (2, 12). We speculate that in the present case the former substitution influenced the affinity of FAD to ETF-QO, thereby impairing the electron transfer machinery in mitochondria, and compound heterozygous mutations including 4Fe4S cluster domain may have had some involvement with myocyte vulnerability, which may have led to rhabdomyolysis, but compound heterozygous mutations on such different domains most likely prevented the development of symptoms until the forties though biochemical analysis of the residual enzyme activity should be undertaken.

The therapeutic efficacy of riboflavin replacement for MADD was first reported by Gregersen in 1982 (17). It has been thought that riboflavin supplement may increase the intra-mitochondrial FAD concentration and thereby promote FAD binding to ETF or ETF-QO, stabilizing their catalytic activity and their folding assembly (2). To date, large numbers of cases of riboflavin-responsive MADD associated with *ETFDH* mutations have been reported. In agreement with previous reports, the present case harboring *ETFDH* mutations also showed good responses to riboflavin. Olsen et al suggested that a single amino acid substitution in the region of the protein that forms the interface between the FAD and ubiquinone binding domains would be responsible for the riboflavin responsiveness, whereas most non-responders had a premature termination codon, which leads to nonsense-mediated decay of the transcript (2, 12). Nevertheless, the clear mechanism of the genotype-riboflavin-responsiveness has not yet been established. Indeed, the present case, who had substitutions not only on the FAD binding domain but also on the 4Fe4S domain, showed a favorable response to riboflavin.

MADD should be included in the differential diagnoses for adult onset myopathy and recurrent rhabdomyolysis. Early diagnosis of MADD is important because supplement therapy may improve the symptoms.

The authors state that they have no Conflict of Interest (COI).

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