Balkan Med J 2022;39:290-296

Characterization of 31 Patients with Riboflavin-Responsive Multiple acyl-CoA Dehydrogenase Deficiency

Jinru Zhang[1#], Jingzhe Han[2#], Yaye Wang[1], Yue Wu[1], Lixia Ma[3], Xueqin Song[1], Guang Ji[1]

[1]Department of Neurology, The Second Hospital of Hebei Medical University, Shijiazhuang, China
[2]Department of Neurology, Harrison International Peace Hospital, Hengshui, China
[3]Department of Geriatrics, The Third Hospital of Shijiazhuang, Shijiazhuang, China
[#]Contributed equally.

Aims: To evaluate the clinical, pathological, and genetic features of patients with riboflavin-responsive multiple acyl-CoA dehydrogenase deficiency (RR-MADD).

Methods: Thirty-one patients with RR-MADD admitted to our hospital from January 2005 to November 2020 were enrolled, and their clinical data were collected. Pathological characteristics of the muscle tissue and possible pathogenic gene mutations were analyzed.

Results: The most common clinical features in all patients were symmetrical proximal muscle weakness. Laboratory examination revealed elevated levels of creatine kinase, homocysteine, and uric acid, acylcarnitines, and organic acid. The muscle biopsy revealed typical pathological changes like lipid deposition. Genetic analysis identified ETFDH mutations in 29 patients, among which one had homozygotes, 19 had compound heterozygotes, 7 had heterozygous mutations, and 2 had heterozygous mutations of both ETFDH and ETFA. Two patients had no pathogenic gene mutations. All patients were treated with riboflavin, and their symptoms improved, which was consistent with the diagnosis of RR-MADD.

Conclusion: The clinical manifestations and genetic test results of patients with RR-MADD are heterogeneous. Therefore, a comprehensive analysis of clinical, pathological, and genetic testing is essential for the early diagnosis of RR-MADD.

INTRODUCTION

Lipid storage myopathies (LSMs) are considered a heterogeneous group of genetic disorders characterized by pathological lipid deposition in muscle fibers. At present, the pathological diagnosis of LSMs accounted for approximately 3% of muscle specimens in our center, in which multiple acyl-CoA dehydrogenase deficiency (MADD) is the most common type. MADD is a rare autosomal recessive metabolic disorder of fatty acid, amino acid, and choline, which is caused by the deficiency of electron transfer flavoprotein (ETF) encoded by *ETFA* and *ETFB* or electron transfer flavoprotein ubiquinone oxidoreductase (ETF: QO) encoded by *ETFDH*.[1] The severity of MADD varied from a neonatal-onset lethal form to a variable milder late-onset form presenting with myopathy. Riboflavin-responsive MADD (RR-MADD) is a special type of MADD that can be dramatically resolved by riboflavin treatment. Olsen et al.[2] revealed that *ETFDH* deficiency is a major cause of RR-MADD. Early recognition of RR-MADD is crucial to improve the prognosis of patients.This study aimed to investigate the clinical, pathological, and genetic characteristics and prognosis of RR-MADD to improve the understanding of clinicians and provide a theoretical basis for the early diagnosis and treatment of RR-MADD.

MATERIAL AND METHODS

Patients

From January 2005 to November 2020, 31 patients were admitted to the Department of Neurology due to muscle diseases and suspected of LSMs pathologically, which were diagnosed as RR-MADD based on gene results and riboflavin treatment effect. The improvement

Corresponding author: Xueqin Song, Department of Neurology, The Second Hospital of Hebei Medical University, Shijiazhuang, China
e-mail: shenjnksxq@163.com

Received: February 10, 2022 Accepted: June 02, 2022 Available Online Date: July 22 • DOI: 10.4274/balkanmedj.galenos.2022.2022-1-127

Available at www.balkanmedicaljournal.org

ORCID iDs of the authors: J.Z. 0000-0001-8866-8053; J.H. 0000-0002-6086-7533; Y.W. /0000-0003-0505-3243; Y.Wu. 0000-0001-7847-6752; X.S. 0000-0003-0463-8450; G.J. 0000-0002-6349-5114

Cite this article as:
Zhang J, Han J, Wang Y, Wu Y, Ma L, Song X, Ji G. Characterization of 31 Patients with Riboflavin-Responsive Multiple acyl-CoA Dehydrogenase Deficiency. *Balkan Med J.*; 2022; 39(4):290-296.

of effect was made based on clinical recovery including the disappearance of muscle weakness, exercise intolerance, and pathological recovery including the disappearance of abnormal fat deposition in muscle fibers after riboflavin treatment. This study was approved by the ethical committee of the local hospital, and informed consent was obtained from each patient. Muscle and/or blood samples were collected from all patients.

Clinical Data

Patients' clinical data were collected, including sex, age at onset, first symptoms, disease course, characteristics of weakness, muscle strength (modified Medical Research Council; 0-5 point manual muscle testing score), other systems involved, laboratory tests, and electromyography. Blood samples from 13 patients and urine samples from 12 patients before riboflavin treatment were collected. Appropriate amounts of peripheral blood and fresh morning urine were analyzed for acylcarnitine and organic acid, respectively, by high-performance liquid chromatography-mass spectrometry and gas chromatography-mass spectrometry. Patients were followed up either by telephone or outpatient visits.

Muscle Biopsy and Muscle Pathology

Muscle biopsy was performed on all patients under local anesthesia. The deltoid muscle, biceps brachii, gastrocnemius muscle, or quadriceps femoris muscle was selected as the surgical site according to the patient's condition. The removed muscle specimens were embedded and placed in isopentane that was precooled in liquid nitrogen. Frozen muscle specimens were placed in a frozen slicer at approximately -22 °C. Frozen sections with a thickness of 8 μm were stained with hematoxylin-eosin (HE), modified Gomori trichrome (MGT), oil red O (ORO), succinate dehydrogenase (SDH), nicotinamide adenine dinucleotidetetrazolium reductase (NADH-TR), ATPase (pH 4.5, pH 10.2), nerve specific enolase (NSE), and periodic acid Schiff (PAS) The pathological characteristics of each patient were observed and recorded in detail.

Genetic Analysis

Genomic DNA of 1-3 μg was extracted, interrupted, and amplified to establish standard DNA libraries.

The amplified DNA libraries were captured using GenCap skeletal system capture kit (My Genostics, China). The gene panel for skeletal system disease includes 239 genes such as *ETFDH, ETFA, ETFB*, *PNPLA2*. High-throughput sequencing was performed using the sequencer HiSeq 2000 (Illumina, CA, USA). Single nucleotide polymorphism or insertion/deletion (Indel) can be analyzed by bioinformatics to identify the mutation information of related genes. According to the results of exome capture and sequencing, Sanger sequencing was performed to verify the possible pathogenic gene mutations.

RESULTS

Clinical Data Results

In this study, 18 male and 13 female patients were enrolled. The age at onset ranged from 9 to 59 years. This disease was mainly characterized by a chronic course and fluctuating symptoms. The most common clinical features in all patients were symmetrical muscle weakness, mainly proximal muscle weakness. Eight patients developed neck muscle weakness. Fourteen patients had fluctuating symptoms during the disease course. Some environmental factors, such as cold, infection, and fatigue, can make the disease deteriorate. Nine patients had gastrointestinal symptoms, including nausea, vomiting, and diarrhea (Table 1). Laboratory examination revealed an elevated plasma creatine kinase level ranging from several to 194 times the normal value in 24 patients. Twelve patients had elevated homocysteine levels, and 12 patients had elevated uric acid levels. Electromyography showed diversiform changes including myogenic (15 of 26), neurogenic and myogenic (5 of 26), neurogenic (2 of 26), and normal (4 of 26) patterns (Table 1). Acylcarnitine analysis of 13 samples showed a combined elevation of medium-chain (C6, C8, C10, and C12) and long-chain (C14 and C16) acylcarnitines in nine patients. Organic acid analysis of 12 urine samples revealed an increase in various dicarboxylic acids (glutarate, adipate, etc.), ethylmalonic acid, and pyruvic acid.

Histopathological Results

Fine vacuoles were observed in muscle fibers among the 31 patients, and some of them fused to form cracks. Atypical ragged red fibers were observed in six patients. The ORO staining showed that lipid droplets were prominently increased in all patients. ATPase staining showed a mosaic distribution of two types of fibers in 31 patients, with vacuolar muscle fibers predominantly in type I fibers (Figure 1).

Genetic Results

Of the 31 patients with RR-MADD, 29 were confirmed to have *ETFDH* mutations, including homozygous mutations, 19 compound heterozygous mutations, 7 heterozygous mutations, and 2 heterozygous mutations in both *ETFDH* and *ETFA*. Two patients had no skeletal system disease panel gene mutation. In this study, a total of 26 *ETFDH* mutations (NM_004453.4), including 10 novel mutations [c.1534G>A (p.G512R), c. 1552 C>G (p.L518V), c.1285+2T>C, exon 1-5 deletion, c.470C>T (p.P157L), c.511A>G (p.N171D), c.1819dupG (p. E607Gfs*16), exon 1-6 deletion, exon 7 deletion, c.1085_1107del (p. A363Lfs*18)] and a *ETFA* novel mutation (NM_000126.4: c.796A>G (p.T266A)] were found. There are two frequent mutations: c.770A>G (p.Y257C) mutation found in nine different chromosomes and c.1227A>C (p.L409F) mutation found in six different chromosomes. The mutation types include missense, splicing, and frameshift mutations, and most of them are missense mutations. The mutation sites of *ETFDH* and *ETFA* are summarized in Table 2.

Treatment and Prognosis

All patients received riboflavin, energy support, and other drug therapy, and the response was good. The clinical symptoms began to improve after 1-2 weeks of treatment. Patient 17 regained nearly normal muscle strength after riboflavin treatment. Re-examination of muscle pathology showed that the amount of fat drops

TABLE 1. Clinical Data Results of Patients with RR-MADD

							Additional findings				Limb weakness								
N	Sex	Onset age (year)	Course (year)	Initial symptom	Exercise intolerance	Gastrointestinal symptom	Neck muscle weakness	Chewing weakness	Dysphagia	Respiratory muscle weakness	Symmetry (yes/no)	Upper limb P	Upper limb D	Lower limb P	Lower limb D	CK	HCY	UA	EMG
1	M	16	0.58	B-WLL	+						Y	5	5	5-	5	1192	-	523	MD
2	M	21	1	B-WLL							Y	4	5	4	5	2250	-	587	MD
3	F	39	13	WL	+						Y	4	5	4	4	226	-	178.8	NCAG
4	F	-	-	-							-	-	-	-	-	-	-	-	-
5	M	10	4	WL						+	Y	5	5	5-	5	10520	-	-	-
6	M	13	1	B-WLL	+	+					Y	4	5	3	5	15050	-	1516.2	MD + ND
7	F	47	1	B-WUL							-	-	-	-	-	-	-	-	-
8	F	59	0.08	WL	+			+	+		Y	3	4	3	4	319	28.6	-	MD + ND
9	M	25	1	WL	+						Y	5	5	5	5	620	33.7	537	ND
10	M	25	0.33	WL	+			+			Y	4	5	4	5	108	-	554.2	NCAG
11	F	9	6	WL	+	+	+		+		Y	3	4	3	4	410	15.1	436.3	MD
12	F	30	0.5	B-WLL				+			Y	4-	5	4	5	375	25.9	489	MD + ND
13	M	-	-	-							-	-	-	-	-	-	-	-	-
14	M	-	-	-							-	-	-	-	-	1838	-	-	MD
15	M	37	1	WL	+					+	Y	5	5	5-	5	394	-	-	NCAG
16	F	30	0.5	WL							Y	5	5	5	4	1971	158.7	135	ND
17	F	17	0.5	WL			+				Y	5	5	5	5	8234	-	-	MD
18	M	19	0.33	B-WLL	+	+					Y	5	5	5-	5	22842	58	361	NCAG
19	F	47	0.08	B-WLL							Y	5-	5	5-	5-	89.1	11.1	167.2	MD
20	M	57	0.08	B-WLL							Y	5	5	5	5	1043	-	-	MD
21	M	29	0.5	WL		+					Y	4	5	5-	5	1429	-	386	MD
22	M	29	0.02	WL			+				Y	4	5	4	5	2649	-	755	MD
23	M	34	0.83	WL							Y	5	5	5	5	571	10.8	406	MD
24	M	23	6	B-WLL		+					Y	5	5	5	5	537	7.7	761.5	MD + N D
25	M	28	0.08	B-WLL							Y	5	5	5-	5	147	83.1	381	-
26	M	58	4	B-WLL			+				Y	5	5	5-	5-	2419	29.1	371	MD
27	F	40	1	WL		+			+		Y	4-	5	3	5	1581.1	29.1	707	MD
28	F	43	3	WL	+		+	+			Y	5	5	3	5	881	8.5	278	MD + ND
29	F	20	5	WL	+	+	+	+			Y	4	5	4	5	425	22.6	313	MD
30	F	34	3	WL	+	+	+	+			Y	3	4	3	4	4302	33	501	MD
31	M	35	0.42	WL	+	+	+	+	+		Y	4-	4-	3-	3	60352	61.8	554	MD

M, Male; F, female; WL, weakness of limbs; B-WLL, bilateral weakness of lower limbs; B-WUL, bilateral weakness of upper limbs; P, proximal; D, distal; CK, creatine kinase (reference values, 50-310 U/L); HCY, homocysteine (reference values, 0-15 μmol/L); UA, uric acid (reference values, 208-428 μmol/L); EMG, electromyography; MD, myogenic damage; ND, neurogenic damage; NCAG, nothing abnormal detected; -, not provided. Muscle strength was assessed according to a modified Medical Research Council muscle grading system.

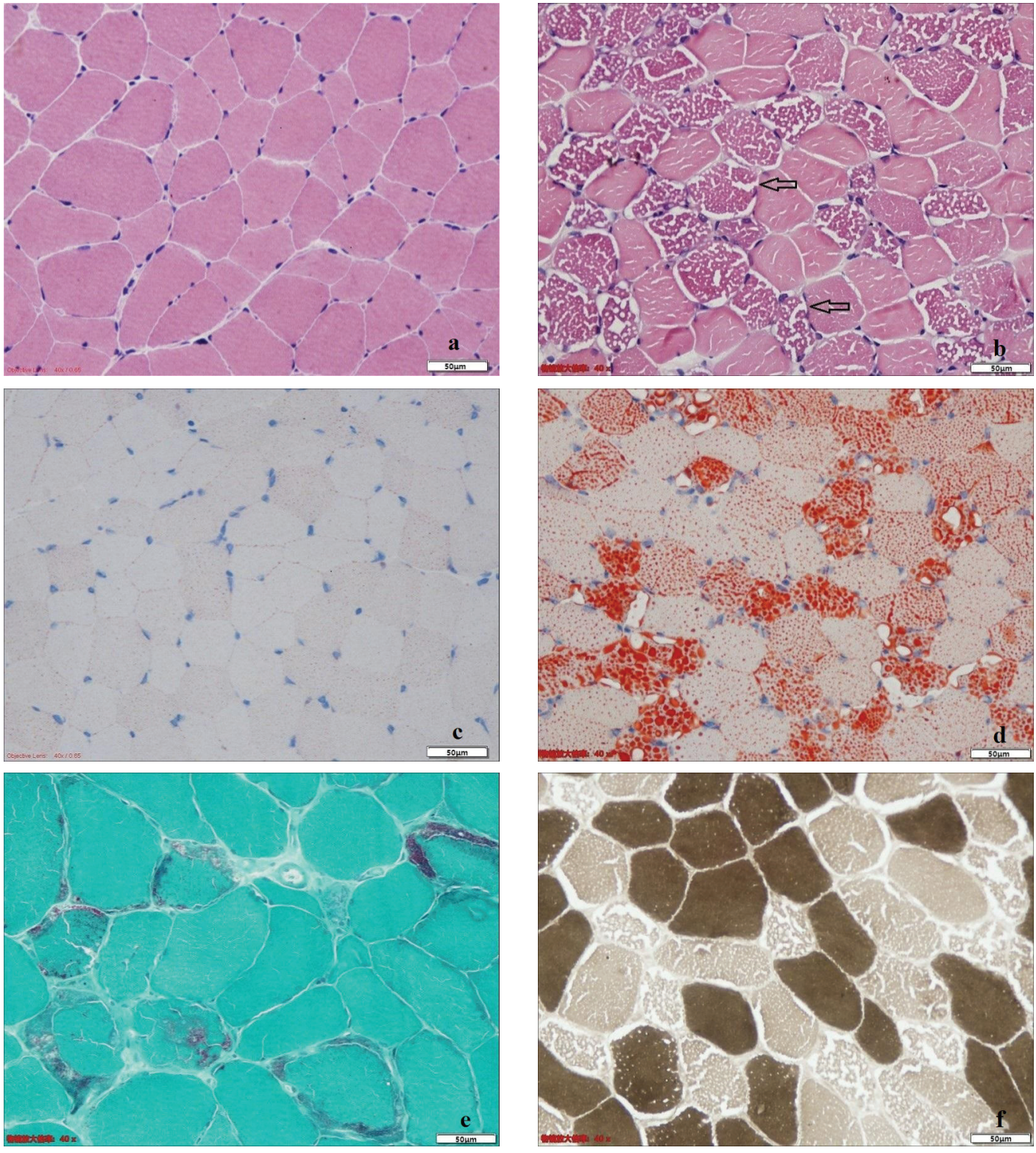

FIG. 1. Pathological characteristics of the muscle tissue.
a) Hematoxylin and eosin (HE) staining (×400) of normal controls; b) HE staining (×400) showed vacuolar muscle fibers and atrophic muscle fibers (patient 27); c) oil red O (ORO) staining (×400) of normal control; d) ORO staining (×400) showed lipid deposition between muscle fibers (patient 27); e) modified Gomori trichrome staining (×400) showed ragged red fibers (patient 15); f) ATPase (PH 10.2) staining (×400) showed that the vacuolar muscle fibers were mainly type I fibers (patient 31).

TABLE 2. Summary of Mutations in RR-MADD Related Genes

N	Gene	Exon	Nucleotide	Amino acid	Homozygous/heterozygous
1	ETFDH	Exon3	c.250G>A	p. A84T	Het
	ETFDH	Exon12	c.1531G>A	p. D511N	Het
2	ETFDH	Exon7	c.770A>G	p. Y257C	Het
	ETFDH*	Exon12	c.1534G>A	p. G512R	Het
	ETFDH*	Exon12	c.1552C>G	p. L518	Het
3	ETFDH	Exon7	c.770A>G	p. Y257C	Het
	ETFDH	Exon12	c.1531G>A	p. D511N	Het
4	ETFDH	Exon3	c.380T>G	p. L127R	Het
	ETFDH	Exon10	c.1227A>C	p. L409F	Het
5	ETFDH	Exon3	c.176-1G>A	splicing	Het
	ETFDH	Exon10	c.1211T>C	p. M404T	Het
6	ETFDH	Exon10	c.1227A>C	p. L409F	Het
	ETFDH	Exon11	c.1395T>G	p. Y465X	Het
7	ETFDH	Exon5	c.524G>A	p. R175H	Het
8	ETFDH	Exon3	c.389A>T	p. D130V	Het
	ETFDH	Exon10	c.1123C>A	p. P375T	Het
9	ETFDH	Exon3	c.389A>T	p. D130V	Het
	ETFDH*	Exon10	c.1285+2T>C	splicing	Het
10	ETFDH	Exon10	c.1211T>C	p. M404T	Het
	ETFDH*	Exon1-5	deletion		Het
11	ETFDH	Exon3	c.250G>A	p. A84T	Het
	ETFDH	Exon7	c.770A>G	p. Y257C	Het
	ETFDH*	Exon12	c.1534G>A	p. G512R	Het
12	ETFDH	Exon3	c.250G>A	p. A84T	Het
	ETFDH*	Exon5	c.511A>G	p. N171D	Het
13	ETFA*	Exon9	c.796A>G	p. T266A	Het
	ETFDH	Exon10	c.1285+1G>A	splicing	Het
14	ETFA*	Exon9	c.796A>G	p. T266A	Het
	ETFDH	Exon10	c.1285+1G>A	splicing	Het
15	–				
16	ETFDH	Exon13	c.1691-3C>G	splicing	Het
17	ETFDH*	Exon4	c.470C>T	p. P157L	Het
	ETFDH	Exon7	c.770A>G	p. Y257C	Het
18	ETFDH	Exon7	c.770A>G	p. Y257C	Het
	ETFDH	Exon10	c.1227A>C	p. L409F	Het
19	–				
20	ETFDH*	Exon13	c1819dupG	p. E607Gfs*16	Het
21	ETFDH	Exon12	c.1531G>A	p. D511N	Het
22	ETFDH	Exon10	c.1227A>C	p. L409F	Het

TABLE 2. Continued

N	Gene	Exon	Nucleotide	Amino acid	Homozygous/heterozygous
23	ETFDH	Exon10	c.1227A>C	p. L409F	Het
	ETFDH*	Exon1-6	deletion		Het
24	ETFDH	Exon7	c.770A>G	p. Y257C	Hemi
	ETFDH*	Exon7	deletion		Het
25	ETFDH	Exon10	c.1227A>C	p. L409F	Het
26	ETFDH	Exon7	c.770A>G	p. Y257C	Hom
27	ETFDH	Exon7	c.770A>G	p. Y257C	Het
	ETFDH	Exon10	c.1211T>C	p. M404T	Het
28	ETFDH*	Exon9	c.1085_1107del	p. A363Lfs*18	Het
29	ETFDH	Exon1	c.3G>C	p. M1I	Het
	ETFDH	Exon3	c.389A>T	p. D130V	Het
30	ETFDH	Exon11	c.1395T>G	p. Y465X	Het
	ETFDH	Exon7	c.770A>G	p. Y257C	Het
31	ETFDH	Exon11	c.1450T>C	p. W484R	Het
	ETFDH	Exon2	c.65A>G	p. K22R	Het

Het, heterozygous; hom, homozygous; hemi, hemizygous; *, novel mutations; −, not found.

deposited in muscle fibers was significantly reduced. All patients were followed up for a maximum of 10 years, of which nine patients only had follow-up data during hospitalization due to loss of contact. The muscle strength completely returned to normal in 15 patients, and seven patients were weak compared with healthy ones. Symptoms of limb weakness recurred in four patients, mostly due to fatigue. After treatment with riboflavin, the symptoms were relieved. Eight patients did not take riboflavin after symptom improvement.

DISCUSSION

MADD phenotypes are divided into three categories: a neonatal-onset form with congenital anomalies (type I) or without congenital anomalies (type II) and a mild and/or late-onset form (type III)[3]. Neonatal-onset forms are usually fatal and characterized by metabolic disorders and hypotonia. Late-onset cases are common, with onset age ranging from adolescence to old age. Patients with late-onset forms often have symptoms of muscle involvement, mostly characterized by fluctuating or progressive muscle weakness and exercise intolerance. All patients in this group had late-onset MADD. The clinical symptoms in this group improved after treatment with riboflavin; thus, cases were referred to as RR-MADD. In this study, the incidence rate of patients by onset age and onset characteristics were basically consistent with previous domestic reports on LSMs.[4] Patients mainly manifested symmetrical limb weakness, partly manifested as neck weakness and respiratory muscle weakness, whereas some patients have induced factors, including cold, infection, and fatigue. The clinical phenotype of late-onset MADD may be influenced by environmental factors. Patients can have multiple system involvement[5], and nine patients also demonstrated digestive symptoms, including nausea, vomiting, and diarrhea, which should arouse the warning of clinicians.

Muscle damage can lead to an increase in creatine kinase levels. Mild-to-moderate elevation of creatine kinase occurred in most patients. Electromyography is of great value in the diagnosis of muscle diseases. Although RR-MADD is a muscle disease, its electromyography lacks corresponding specificity. It can be manifested as myogenic damage, neurogenic damage, or normal findings. In this study, electromyography manifestations were mainly myogenic damage, which was consistent with previous reports.[6] Studies have found that the screening of acylcarnitine and organic acid in patients with LSMs can be helpful for the classification and diagnosis[7]. Elevated levels of acylcarnitines and organic acid were observed in the patients. The detection of acylcarnitines and organic acids may be interfered with by several factors, such as drugs, diet, and disease stage. Therefore, the value of screening acylcarnitine and organic acid alone is limited; thus, it should be combined with clinical manifestations and genetic testing for comprehensive analysis. Muscle biopsy and pathology are of great significance in the diagnosis of LSMs. Muscle biopsy of patients presented with typical pathological changes like lipid deposition, mainly involving type I fibers. In this study, several scattered small round vacuoles were observed in the muscle fibers of patients by hematoxylin and eosin (HE) staining. ORO staining showed fatty deposition with type I muscle fibers. In addition, results of MGT, SDH, NADH-TR, ATPase (pH 4.5, pH 10.2), NSE, and PAS staining were consistent with pathological changes in

LSMs.Genetic testing is also of great significance in the diagnosis of RR-MADD. A study reported that *ETFDH* mutations are major causes of RR-MADD.[2] Through genetic testing, most of the cases were related to *ETFDH* mutations. MADD is an autosomal recessive disease that can be caused by compound heterozygous or homozygous mutations. However, in this study, nine patients with RR-MADD were found to carry a heterozygous *ETFDH* mutation, and clinical manifestations were improved with the treatment of riboflavin. However, further investigations are needed to analyze the possible reasons and the mutation sites located in regions outside the exon that cannot be identified by genomic sequencing. In addition, two patients had no pathogenic gene mutations, which were clinically and pathologically diagnosed with RR-MADD. They had symptoms of muscle weakness and presented with typical pathological changes such as lipid deposition. The symptoms were improved after treatment with riboflavin. During the follow-up period, muscle weakness recurred due to fatigue, but improved after treatment with riboflavin again. Further study is needed to determine whether unknown gene mutations are at play.

Flavin adenine dinucleotide (FAD) is the cofactor of ETF or ETF:QO and has been demonstrated to promote folding and conformational stability and activity of protein.[3] Riboflavin is the precursor of FAD, and riboflavin supplementation can increase cellular FAD content and thus improve clinical symptoms. However, the specific application period and maintenance dose of riboflavin, influencing factors of the fluctuation of RR-MADD symptoms during treatment, and characteristics of clinical outcomes still need to be further studied in a larger sample size. In this study, the riboflavin dosage in the patients ranged from 15 to 210 mg/dl. Riboflavin is not stored in the body, and intakes of riboflavin above tissue requirements are excreted in the urine as riboflavin or other metabolites. Alternatively, an increase in FAD-binding flavoproteins after riboflavin supplementation could release more FAD to the mitochondrial matrix during degradation, keeping a larger circulating FAD pool even after discontinuation of riboflavin treatment.[8] Eight patients in this study did not take riboflavin after symptom improvement. For some patients, short-term riboflavin supplementation brings a long period of symptom resolution. The clinical manifestations and genetic test results of patients with RR-MADD were heterogeneous. Therefore, a comprehensive analysis of clinical, pathological, and genetic testing is essential for the early diagnosis of RR-MADD. In addition, when early diagnosis is difficult, experimental drug therapy can be considered to observe its efficacy.

Ethics Committee Approval: This study was approved by the ethical committee of the local hospital.

Informed Consent: Written informed consent was obtained from each patient.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authorship Contributions: Design- Y.S., X.S.; Data Collection or Processing- Y.W., Y.Wu.; Analysis or Interpretation- G.J.; Literature Search- L.X., L.M.; Writing- J.Z., J.H.;

Conflict of Interest: No conflict of interest was declared by the authors.

Funding: The authors declared that this study received no financial support.

REFERENCES

1. Fu HX, Liu XY, Wang ZQ, et al. Significant clinical heterogeneity with similar ETFDH genotype in three Chinese patients with late-onset multiple acyl-CoA dehydrogenase deficiency. *Neurol Sci.* 2016; 37:1099-1105. [CrossRef]
2. Olsen RK, Olpin SE, Andresen BS, et al. ETFDH mutations as a major cause of riboflavin-responsive multiple acyl-CoA dehydrogenation deficiency. *Brain.* 2007;130:2045-2054. [CrossRef]
3. Chokchaiwong S, Kuo YT, Hsu SP, et al. ETF-QO Mutants Uncoupled Fatty Acid β-Oxidation and Mitochondrial Bioenergetics Leading to Lipid Pathology. *Cells.* 2019;8:106. [CrossRef]
4. Wen B, Dai T, Li W, et al. Riboflavin-responsive lipid-storage myopathy caused by ETFDH gene mutations. *J Neurol Neurosurg Psychiatry.* 2010;81:231-236. [CrossRef]
5. Cwik VA. Disorders of lipid metabolism in skeletal muscle. *Neurol Clin.* 2000;18:167-184. [CrossRef]
6. Cui LY, Tang XF, Yuan J, Li BH, Du H. Clinical and neuroelectrophysiological study of lipid deposition myopathy with peripheral neuropathy. *Chinese Journal of Neuroimmunology and Neurology.* 1998;5:1557-1561. [CrossRef]
7. Gempel K, Topaloglu H, Talim B, et al. The myopathic form of coenzyme Q10 deficiency is caused by mutations in the electron-transferring-flavoprotein dehydrogenase (ETFDH) gene. *Brain.* 2007;130: 2037-2044. [CrossRef]
8. Xu J, Li D, Lv J, et al. ETFDH Mutations and Flavin Adenine Dinucleotide Homeostasis Disturbance Are Essential for Developing Riboflavin-Responsive Multiple Acyl-Coenzyme A Dehydrogenation Deficiency. *Ann Neurol.* 2018;84:659-673. [CrossRef]