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Clinical Observation

Determinants of Riboflavin Responsiveness in Multiple Acyl-CoA Dehydrogenase Deficiency

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ABSTRACT

Background: Multiple acyl-CoA dehydrogenase (MADD) deficiency, which is a rare metabolic disorder involving electron transport flavoproteins, has a wide array of clinical phenotypes. In this article, we describe 25 patients with MADD deficiency and present the clinical and laboratory characteristics and diagnostic challenges associated with riboflavin-responsive MADD deficiency.
Methods: Hospital records of patients with biallelic mutations in *ETFA*, *ETFB*, or *ETFDH* genes diagnosed in a single center were analyzed retrospectively. Demographic, clinical, and laboratory characteristics of patients with riboflavin-responsive and riboflavin-unresponsive MADD deficiency were compared using Mann-Whitney U and Fisher's exact tests.
Results: Respiratory distress and depressed consciousness were significantly more common in patients with riboflavin-unresponsive MADD deficiency ($P = 0.015$ and $P < 0.001$), who presented at a younger age ($P < 0.001$). Patients with riboflavin-responsive MADD deficiency had favorable outcomes but also had life-threatening complications, longer diagnostic delay (median of two years versus 30 days; $P < 0.001$), and multiple differential diagnoses, resulting in unnecessary investigations and maltreatment. Biopsies showed lipid storage, and complete autopsy was performed in one newborn with riboflavin-unresponsive MADD deficiency, revealing multiple abnormalities. Metabolic profiles were not distinguishable between riboflavin-responsive and riboflavin-unresponsive MADD deficiency ($P > 0.05$). Four novel variants were detected in *ETFDH*, one of which (c.1790C>T) may confer riboflavin responsiveness. Siblings with the common myopathic *ETFDH* c.1130T>C mutation presented with a new phenotype dominated by chronic fatigue without apparent myopathy.
Conclusions: Symptoms and outcomes significantly differed between riboflavin-responsive and unresponsive MADD deficiency, but metabolic profiles did not. Functional studies are needed to better characterize the novel *ETFDH* variants. As treatment is available for riboflavin-responsive MADD deficiency, physicians should maintain a high index of suspicion for MADD deficiency in all age groups.

Introduction

Electron transfer flavoprotein (ETF, coded by *ETFA* and *ETFB* genes) and ETF-ubiquinone oxidoreductase (ETFQO, coded by *ETFDH* gene) are flavoproteins required for transfer of electrons from numerous dehydrogenases to the electron transport chain.[1] Inherited defects of ETF or ETFQO cause multiple acyl-CoA dehydrogenase deficiency (MADD), also known as glutaric aciduria type II. Failure of ETF or ETFQO to relay electrons causes functional abnormalities in the flavoenzymes degrading different lengths of fatty acyl-CoA, resulting in the classical metabolic signature of MADD: increased plasma concentrations of both medium- and long-chain acylcarnitines and urinary excretion of corresponding dicarboxylic acids, ethylmalonic acid, acylglycines, glutaric acid, and its derivatives.[2,3] Defects in other genes involved in the metabolism and transport of

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riboflavin (vitamin B_2) can also cause a MADD-like biochemical profile.[4-8]

MADD due to ETF or ETFQO deficiency is a heterogeneous disease classified into three phenotypes. Newborns may develop hypoglycemia, hypotonia, poor feeding, hepatomegaly, respiratory distress, hyperammonemia, and metabolic acidosis. Those with congenital anomalies (MADD type I) die in the neonatal period, whereas those without (MADD type II) may die in infancy or survive to experience recurrent episodes of decompensation. Patients with MADD type III, sometimes known as late-onset MADD, may develop episodic vomiting, hypoglycemia, and acidosis in infancy or progressive lipid storage myopathy in adolescence or adulthood.[3] MADD may also be classified as mild or severe MADD.[9] Relationships between genotype, residual ETF/ETFQO activity, and clinical phenotype have been extensively studied.[3,10] Most patients with MADD type III have milder missense mutations in *ETFDH* and respond favorably to riboflavin, whereas severely disruptive mutations in *ETFA, ETFB,* or *ETFDH* give rise to type I or type II disease.[10-12]

In riboflavin-responsive MADD (RR-MADD), riboflavin is the cornerstone of therapy. Levocarnitine may be supplemented to correct carnitine deficiency. Coenzyme Q_{10} supplementation is recommended to correct its deficiency in skeletal muscle.[13,14] In patients with riboflavin-unresponsive MADD (RU-MADD), fat restriction, avoidance of fasting, and a diet rich in carbohydrates are necessary.[15]

Except for a few larger series, clinical information on MADD is derived from case reports and small case series.[11,14,16] Here, we present a large retrospective cohort of 25 patients with ETF or ETFQO deficiency diagnosed in a single center. We have primarily aimed to assess the presenting features that are associated with riboflavin responsiveness. In addition, we describe the clinical, biochemical, histopathological, and molecular characteristics of these patients including four novel *ETFDH* mutations and a complete autopsy.

Methods

Patients of all ages diagnosed at Hacettepe University Hospitals with MADD due to ETF or ETFQO deficiency, as confirmed by biallelic mutations in *ETFA*, *ETFB,* or *ETFDH* genes, were included. Clinical and laboratory data were retrieved retrospectively from medical records. Data were collected using a checklist addressing patient demographics, clinical information (including age at onset, age at diagnosis, presence or absence of symptoms and signs such as fatigue, myalgia, muscle weakness, respiratory distress, poor feeding, vomiting, hypotonia, altered consciousness, hepatomegaly, hypoglycemia, metabolic acidosis, hyperammonemia, elevated liver and muscle enzymes), and laboratory investigations including metabolic (blood carnitine and acylcarnitines, urine organic acid analysis for increased excretion of glutaric acid derivatives, ethylmalonic acid, and acylglycine species such as hexanoylglycine and other short-chain glycine conjugates), histopathological (muscle and liver biopsies, autopsy if available), enzymatic, and genetic evaluation. Age at diagnosis was accepted as the age when the diagnosis was confirmed or when specific therapy was started, whichever is earlier. MADD type was ascertained as explained above.[3] Riboflavin responsiveness was defined as marked improvement in signs and symptoms (such as improvement in consciousness or muscle tone, strength or pain, cessation or significant amelioration of fatigue, feeding difficulties, vomiting or respiratory distress, normalization of muscle or liver enzymes, based on which signs or symptoms were initially present) or as exacerbation after discontinuation of riboflavin. Statistical significance of the differences between riboflavin-responsive and riboflavin-unresponsive patients regarding the clinical and laboratory characteristics detailed above was determined by Mann-Whitney U and Fisher's exact tests, using SPSS v22.0; $P < 0.05$ was accepted to be statistically significant. The study was approved by the local Ethics Board for Non-Interventional Clinical Studies, which waived the need for informed consent.

Results

Twenty-five patients (13 female) from 19 unrelated families were diagnosed with MADD due to ETF or ETFQO deficiency, and 19 of these individuals were responsive to riboflavin. Clinical and laboratory characteristics of the patients classified according to riboflavin responsiveness are individually depicted in Supplementary Material 1. An overview of the data is summarized in Table 1.

Clinical characteristics and types of MADD

All 19 patients with RR-MADD had type III disease. These patients had a wide range of age at onset (four months to 21 years with a median of 10 years), long diagnostic delay (up to 15 years), and a median follow-up of approximately four years (range: six months to 15 years). In RR-MADD, the most common symptom was fatigue, followed by muscle weakness and myalgia. Elevated creatine kinase was the most common basic laboratory abnormality, although creatine kinase was normal in four patients with RR-MADD, including two asymptomatic cases detected by family screening, who have since remained symptom free. All 19 patients with RR-MADD had a favorable clinical outcome, with 12 being completely free from disease symptoms (Table 1). One patient (Patient 7) discontinued riboflavin treatment despite medical advice and had an episode of severe rhabdomyolysis, which caused acute renal failure requiring hemodialysis and invasive mechanical ventilation. She completely recovered from this episode by reintroduction of riboflavin.

Six patients were unresponsive to riboflavin. Four had MADD type II and developed symptoms within the first weeks of life. Three of them died in infancy, and the other (Patient 20) is eight years old with normal growth and development but is frequently hospitalized due to recurrent episodes of nausea, vomiting, depressed consciousness, hypoglycemia, and metabolic acidosis. Patient 22 developed vomiting, poor feeding, and respiratory distress during the second month of life; did not have acute exacerbations; and developed persistent skeletal myopathy and was therefore classified as MADD type III. This patient's myopathy showed a favorable response to increasing coenzyme Q_{10} dose from 25 to 100 mg/day. The only patient with MADD type I, Patient 24, had anhydramniosis, bilateral renomegaly, renal cysts, and cardiomegaly in prenatal ultrasonography. He was intubated right after delivery owing to the lack of respiratory effort. He had depressed nasal bridge, low-set ears, wide cranial sutures, palpable kidneys, coronal hypospadias, rocker-bottom left foot, and sweaty-feet odor, prompting the diagnosis of MADD. The infant died 84 hours after birth despite therapy (Supplementary Material 1).

All patients with RU-MADD had respiratory distress, poor feeding, and depressed consciousness. Compared with patients with RR-MADD, patients with RU-MADD had a significantly higher prevalence of respiratory distress and depressed consciousness, but there were no significant differences regarding nausea and vomiting, poor feeding, hypotonia, or muscle weakness. In RR-MADD, respiratory distress was due to involvement of axial and respiratory muscles resulting in hypoventilation, whereas in RU-MADD, it was secondary to metabolic acidosis. Hypoglycemia, hyperammonemia, and metabolic acidosis were the basic laboratory

TABLE 1.
Clinical and Laboratory Features of Patients With Multiple Acyl-CoA Dehydrogenase Deficiency in Relation to Riboflavin Responsiveness

Patient Characteristics	Riboflavin Responsive (n = 19)	Riboflavin Unresponsive (n = 6)	*P* value*
Median age at symptom onset (range)	10 y (4 mo-21 y)	1 d (0 d-50 d)	**<0.001**
Median age at diagnosis (range)	12 y (4 mo-23 y)	31 d (3 d-2 y)	**<0.001**
Median diagnostic delay (range)	2 y (7 d-15 y)	30 d (3 d-22 mo)	**0.016**
	N (%)	N (%)	
Symptoms			
Fatigue†	14 (82)	- (-)	-
Muscle weakness	12 (63)	3 (50)	0.653
Myalgia†	10 (59)	- (-)	-
Respiratory distress	7 (37)	6 (100)	**0.015**
Poor feeding	9 (47)	6 (100)	0.051
Nausea/vomiting	7 (37)	3 (50)	0.653
Hypotonia	4 (21)	3 (50)	0.299
Depressed consciousness	3 (16)	6 (100)	**<0.001**
Asymptomatic	2 (11)	0 (0)	0.999
Physical examination and basic laboratory characteristics			
Elevated CK	15 (79)	3 (50)	0.299
Elevated ALT	10 (53)	5 (83)	0.345
Hepatomegaly	6 (32)	4 (67)	0.175
Hypoglycemia	2 (11)	5 (83)	**0.002**
Metabolic acidosis	1 (5)	6 (100)	**<0.001**
Hyperammonemia	1 (5)	3 (50)	**0.031**
Metabolic profile at presentation‡			
Low free carnitine (<10 μmol/L)	8 (44)	4 (67)	0.640
Elevated medium-chain acylcarnitines§	15 (83)	5 (83)	0.999
Elevated long-chain acylcarnitines§	16 (89)	5 (83)	0.999
Simultaneous elevation of medium- and long-chain acylcarnitines§	13 (72)	5 (83)	0.999
Increased glutaric acid or its derivatives in urine	15 (83)	6 (100)	0.546
Elevated acylglycine species in urine	13 (72)	4 (67)	0.999
Ethylmalonic aciduria	11 (61)	5 (83)	0.621
Dicarboxylic aciduria	10 (56)	4 (67)	0.999
Outcome at most recent follow-up			
Favorable	19 (100)	0 (0)	**<0.001**
Free of disease symptoms	12 (63)	0 (0)	
Mild residual disease	7 (37)	0 (0)	
Unfavorable	0 (0)	6 (100)	**<0.001**
Moderate to severe residual disease	0 (0)	1 (17)	
Frequent acute episodes	0 (0)	1 (17)	
Death	0 (0)	4 (67)	

Abbreviations:
ALT = Alanine transaminase
CK = Creatine kinase
n = Number

* Statistically significant values ($P < 0.05$) are printed in bold.

† As they are subjective symptoms, fatigue and myalgia at presentation could only be assessed in 17 of 19 riboflavin-responsive patients. Other patients were not old enough to be able to describe these symptoms at presentation.

‡ Available in all six riboflavin-unresponsive and in 18 of 19 riboflavin-responsive patients.

§ Including after L-carnitine supplementation.

abnormalities, which were significantly more common in RU-MADD. All patients with RR-MADD had type III disease and a favorable outcome, whereas most patients with RU-MADD had type II disease and all had an unfavorable outcome (Table 1).

Metabolic profiles

Twelve of 23 patients had a low (<10 μmol/L) serum-free carnitine level at presentation. In two of these patients (Patients 4 and 10), the acylcarnitine profile was normal, which necessitated supplementation with low-dose L-carnitine to elicit a diagnostic profile. The same intervention was used in four more patients with low or low-normal free carnitine (Patients 2, 6, 12, and 13), whose initial acylcarnitine profiles were abnormal, but not diagnostic (Supplementary Material 1). At initial presentation or after L-carnitine supplementation, elevated long-chain acylcarnitines were the most common abnormality in the acylcarnitine profile. However, five patients with RR-MADD (28%) and one with RU-MADD (17%) lacked the classical acylcarnitine profile, i.e., the simultaneous elevation of both medium- and long-chain acylcarnitines. In urinary organic acid analyses, increase of glutaric acid or its derivatives was the most common finding, followed by acylglycines, ethylmalonic acid, and dicarboxylic acids. Metabolic profiles of RR- and RU-MADD did not differ significantly (Table 1).

Palmitate loading test was performed in cultured skin fibroblasts of five patients to study the mitochondrial fatty acid oxidation flux. Three patients with RR-MADD (Patients 2, 6, and 14) had normal results, whereas Patient 20 had elevated C12, C14, and C16 acylcarnitines and Patient 22 had mild elevations of C8, C10, C12, and C14 acylcarnitines, both of whom were riboflavin unresponsive.

Previous working diagnoses

Mostly other inborn errors of metabolism were considered in patients with RU-MADD, whereas patients with RR-MADD had a different spectrum of differential diagnoses. Inflammatory myopathies were listed as a possible diagnosis in six of these 19 patients. Of note, although Patient 8 has been nearly cured of MADD symptoms, he suffers from cataracts and osteonecrosis, which were complications of myositis treatment with corticosteroids. Patient 12 had been investigated for cyclic vomiting

TABLE 2.
Working Diagnoses of Patients Before Confirmation of Multiple Acyl-CoA Dehydrogenase Deficiency

	Diagnosis	Number of Patients
Differential diagnosis of RR-MADD	Inflammatory myopathy (e.g., polymyositis)	6
	Muscle glycogenoses (e.g., Pompe disease)	3
	Cyclic vomiting syndrome	2
	Rheumatological disease, unspecified	2
	Brucellosis	1
	Chronic fatigue syndrome	1
	Carnitine palmitoyl transferase II deficiency	1
	Fructose-1,6-bisphosphatase deficiency	1
	Familial Mediterranean fever	1
	Guillain-Barré syndrome	1
	Hereditary fructose intolerance	1
	Malignancy	1
	Migraine	1
	Pseudotumor cerebri	1
	Psychogenic vomiting	1
	Very-long-chain acyl-CoA dehydrogenase deficiency	1
Differential diagnosis of RU-MADD	Glycogen storage disease type I	2
	Very-long-chain acyl-CoA dehydrogenase deficiency	2
	Autosomal recessive polycystic kidney disease	1
	Chromosomal abnormality	1
	Familial hemophagocytic lymphohistiocytosis	1
	Isovaleric academia	1

Abbreviations:
RR-MADD = Riboflavin-responsive multiple acyl-CoA dehydrogenase deficiency
RU-MADD = Riboflavin-unresponsive multiple acyl-CoA dehydrogenase deficiency

syndrome and gastroesophageal reflux disease, misdiagnosed with psychogenic vomiting and chronic fatigue syndrome and treated with selective serotonin reuptake inhibitors. Other differential diagnoses in this group included a myriad of diseases (Table 2).

Histopathology and autopsy

Skeletal muscle biopsy was done in 11 patients with RR-MADD (Patients 1-3, 6-9, 16-19) before initiation of therapy, all of which revealed various degrees of vacuolar changes and increased lipid accumulation in muscle fibers, compatible with lipid-storage myopathy (Fig A and B). Patient 14 underwent muscle biopsy eight years after initiation of riboflavin treatment (after genetic confirmation of the disease). She was minimally symptomatic at the time of biopsy (fatigue and mild cognitive impairment), and there were no specific histopathological findings (Fig C and D). Patient 7 also underwent liver biopsy, which showed macrovesicular steatosis. Similarly, liver biopsies from two patients with RU-MADD (Patients 22 and 23) also revealed diffuse macrovesicular steatosis and mild hemosiderosis (Fig E and F).

Complete autopsy was done on Patient 24 (MADD type I). In addition to the dysmorphic features mentioned above, cardiomegaly with biventricular hypertrophy and renomegaly with diffuse cysts of diameter 0.1 to 0.3 cm were detected. Microscopic examination was compatible with diffuse cystic renal dysplasia (Supplementary Material 2, A and B). Vacuolar changes were seen in the epithelium of some renal tubules, which showed lipid accumulation (Supplementary Material 2, C and D). Mild increase in lipid content of skeletal and heart muscle and mild macrovesicular steatosis were other features of MADD (Supplementary Material 2, E-H). Periventricular, intra-alveolar, and focal adrenal hemorrhage, pneumonia, and pulmonary hypertension were also present.

Molecular genetics

Individual genotypes of the patients are presented in Supplementary Material 1, and the detected variants are summarized in Table 3. Twenty-three patients had variants in *ETFDH*, two patients (Patients 20 and 25) in *ETFA*, and none in *ETFB*. Both patients with *ETFA* mutations had RU-MADD (type II). All patients with RR- or type III MADD had variants in *ETFDH*. Twenty-four patients had one homozygous variant, and one patient (Patient 23) had two compound heterozygous variants. Eight different pathogenic variants (two small deletions and six missense substitutions) were detected. The most common variant was c.1130T>C with 28 alleles in 14 patients, followed by c.1448C>T in *ETFDH*, both of which were detected only in patients with RR-MADD type III. Four variants were novel, which are not previously reported in the literature, or in ClinVar, HGMD, ExAc, or gnomAD databases. Both of the novel small deletions (*ETFDH*; c.1524delA and c.1198_1201delACTC) are frameshift mutations. The novel missense variants c.1165C>A and c.1790C>T in *ETFDH* are predicted to be disease causing by MutationTaster, PolyPhen-2, PROVEAN, and SIFT softwares.

Discussion

Clinical characteristics, outcomes, and disease classification

In this article, we present the characteristics and outcomes of 25 patients with confirmed MADD due to ETF or ETFQO deficiency, focusing on presenting features and outcomes of RR- and RU-MADD. In line with previous studies, patients RR-MADD had type III disease and *ETFDH* mutations,[12] but the age of onset was extremely variable, even as early as four months (Supplementary Material 1, Patients 14 and 15). One should note that patients with late onset also developed life-threatening complications such as depressed consciousness, hypoventilation, respiratory distress, and acute renal failure (see Patients 7, 9, and 16 in the section "Clinical Characteristics and Types of MADD" and in Supplementary Material 1). Therefore, it may be misleading to classify MADD as early versus late onset or mild versus severe, as the borders are blurry. Response to riboflavin may be a better parameter for classification of MADD instead of age at onset or severity. In this study, life-threatening symptoms such as respiratory distress and depressed consciousness and laboratory abnormalities such as hypoglycemia, metabolic acidosis, and hyperammonemia were significantly more common in RU-MADD. All patients with RR-MADD had favorable and all patients with RU-MADD had unfavorable outcomes.

As type III disease, late disease onset, and mild *ETFDH* mutations are so closely linked, it is hard to differentiate which of these parameters determines the clinical and laboratory characteristics by relying only on statistics. Most likely, the underlying determinant is the genotype, which encodes the functional properties of the mutant protein, although other modifying genetic, epigenetic, or environmental factors may be involved in the clinical outcome.

Diagnosis

As evident from the previous presumptive diagnoses of our patients, a wide variety of metabolic, inflammatory, autoimmune, infectious, and psychogenic conditions should include MADD in differential diagnosis. In RR-MADD, where prognosis

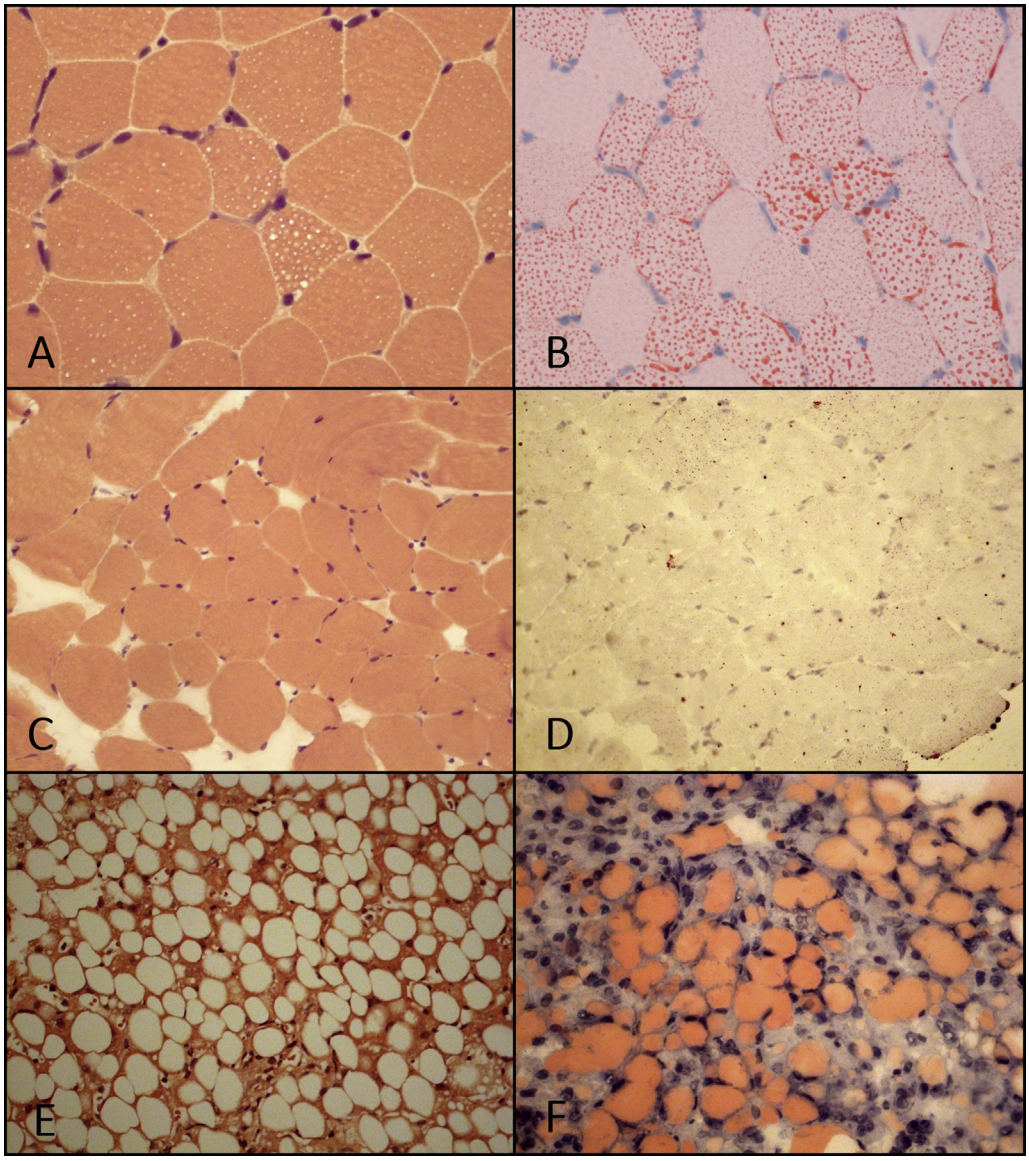

FIGURE. Muscle and liver biopsies. Skeletal muscle biopsy of Patient 9 showing features of lipid storage myopathy: vacuolar changes (A) and increased lipid accumulation (B). Mild variation in fiber size and rounding of fibers are seen in the post-treatment biopsy of Patient 14; neither vacuolar change nor lipid accumulation is present (C and D). Liver biopsy of Patient 23 reveals diffuse macrovesicular steatosis (E and F). (A, C, and E: Hematoxylin and eosin stain; B, D, and F: Oil Red O stain; all original magnification ×20). The color version of this figure is available in the online edition.

is excellent after initiation of riboflavin, perhaps the most important hurdle is diagnostic delay. It is clear that early diagnosis and treatment with riboflavin results in better outcomes. Physicians should maintain a low threshold to consider a treatable myopathy.

To facilitate diagnosis, acylcarnitine profiling and urinary organic acid analysis are recommended in patients with myalgia, exercise intolerance, and rhabdomyolysis to screen for defects of fatty acid oxidation, such as MADD.[19] However, as seen in a quarter of our patients with RR-MADD, acylcarnitine profile may be

TABLE 3.
Variants Detected in *ETFA*, *ETFB*, *ETFDH* Genes*

Gene	Nucleotide Change	Amino Acid Change	Number of Patients	Number of Alleles	Reference
ETFDH	c.1130T>C	p.Leu377Pro	14	28	13
ETFDH	c.1448C>T	p.Pro483Leu	4	8	12
ETFA	c.797C>T	p.Thr266Met	2	4	17
ETFDH	c.1141G>C	p.Gly381Arg	2	3	18
ETFDH	c.1165C>A	p.Pro389Thr	1	2	Novel
ETFDH	c.1524delA	p.Lys509Asnfs*16	1	2	Novel
ETFDH	c.1790C>T	p.Pro597Leu	1	2	Novel
ETFDH	c.1198_1201delACTC	p.His401Glnfs*3	1	1	Novel

* Reference sequences: RefSeq NM_004453.3 and NP_004444.2 for *ETFDH*; NM_000126.4 and NP_000117.1 for *ETFA*.

nondiagnostic. A trial of L-carnitine can be helpful to elicit a diagnostic profile as demonstrated here and in previously published work.[9]

Diagnostic metabolic profiles may obviate muscle or liver biopsies, which can guide further evaluation when metabolic tests are inconclusive. Detection of lipid storage in muscle or liver with compatible clinical symptoms should raise suspicion of MADD. With the widespread availability of genetic testing, fatty acid oxidation flux studies such as palmitate or myristate loading in cultured fibroblasts are now less commonly used for diagnosis. In this study, palmitate loading tests were normal in RR-MADD and abnormal in RU-MADD. This is consistent with previous reports, because cell culture media contain riboflavin that ameliorates the defect in RR-MADD. However, it has been reported that fibroblasts from patients with RR-MADD show very low fatty acid flux if grown in severely riboflavin-depleted medium.[12]

Given the severe phenotype of MADD type I and high possibility of perinatal death, diagnosis is important for genetic counseling. Preferably, a metabolic autopsy should be offered to newborns with dysmorphic features and congenital anomalies or when an inborn error of metabolism is suspected. Metabolic autopsy evaluation may help diagnosis in a variety of diseases with antenatal onset.[20]

ETFDH variants

By far, the most common mutation identified in patients with myopathy and RR-MADD was c.1130T>C in *ETFDH*, which is frequent in Turkish and Kurdish families.[13] A large series of patients with late-onset MADD with *ETFDH* mutations has been reported from China, where this mutation was not identified in any of the 90 Chinese patients.[21] A founder effect in our region may explain the predominance of this mutation in our patient population. Of note, Patients 12 and 13 in our cohort presented with fatigue and intermittent vomiting without evident clinical myopathy (Supplementary Material 1), further expanding the previously reported myopathic phenotype of this mutation and underlining the necessity to consider RR-MADD not only in myopathy but also in chronic fatigue or recurrent vomiting.

Unlike the previous report of a Kurdish neonate from Finland, *ETFDH* c.1141G>C mutation was not associated with riboflavin responsiveness in Patient 21 (Supplementary Material 1). The previously published case was symptomatic at birth and had a lethal disease course.[18] The improvement in muscle strength attributed by the authors to initiation of riboflavin may have resulted from correction of secondary carnitine deficiency or other interventions. Functional studies or more clinical data are needed to assess the riboflavin responsiveness of this mutation.

Four novel variants in *ETFDH* have been identified as shown in Table 3. The frameshift c.1524delA variant identified in homozygous state in Patient 24 with MADD type I may cause complete loss of ETFQO function, explaining the severe phenotype. The other novel frameshift variant (c.1198_1201delACTC) was in compound heterozygous state in a patient with RU type II MADD (Patient 23), whose phenotype may reflect the possibly milder c.1141G>C mutation on the other allele. Patient 22 is homozygous for the novel c.1165C>A variant and presented with hypoglycemia and metabolic acidosis, but evolved into a riboflavin-unresponsive myopathic phenotype characterized by weakness, fatigue, but no acute decompensations. The beneficial effect of increasing coenzyme Q_{10} dose in this patient may have corrected the underlying deficiency in skeletal muscle, underlining the importance of coenzyme Q_{10} replacement in MADD-related myopathy, regardless of riboflavin response. The other novel missense variant, c.1790C>T, has caused a riboflavin-responsive myopathic phenotype in Patient 19 in homozygous state. Although the behavior of these novel variants can be inferred from these patients, functional studies are needed for better characterization.

Conclusions

In this study, we present detailed clinical and laboratory data from 25 patients with MADD due to ETF and ETFQO deficiencies. In these patients, age at presentation; presenting signs and symptoms including respiratory distress, depressed consciousness, hypoglycemia, metabolic acidosis, and hyperammonemia; and clinical outcomes significantly differed between RR- and RU-MADD, but the metabolic profiles were not distinguishable. Fatigue was the most common symptom in RR-MADD. We found that the common local *ETFDH* c.1130T>C mutation may cause a diagnostically challenging clinical picture dominated by recurrent vomiting and fatigue without apparent myopathy. We also identified four novel variants in *ETFDH*, one of which is possibly associated with riboflavin responsiveness. RR-MADD is clinically heterogeneous, and the outcome is dramatically influenced by riboflavin treatment. Early diagnosis and timely treatment play an important role in preventing unnecessary investigations and disease- or maltreatment-related complications. Considering the working diagnoses of these patients at referral and the time lag between symptom onset and diagnosis, it is crucial to maintain a high index of suspicion for MADD spectrum in the differential diagnostic list of multiple clinical phenotypes at any age from the newborn period to adulthood.

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Supplementary data

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