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Novel *ETFDH* mutations in four cases of riboflavin responsive multiple acyl-CoA dehydrogenase deficiency

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ABSTRACT

Multiple acyl-CoA dehydrogenase deficiency (MADD) is an autosomal recessive disorder of fatty acid, amino acid, and choline metabolism caused by mutations in *EFTA, EFTB,* or *ETFDH*. Many MADD patients are responsive to treatment with riboflavin, termed riboflavin-responsive MADD (RR-MADD). Here, we report three novel mutations and one previously reported mutation in *ETFDH* in four RR-MADD patients who presented at various ages, and characterize the corresponding changes in ETF-QO protein structure. Clinicians should consider MADD in the differential diagnosis when patients present with muscle weakness and biochemical abnormalities. Gene testing plays a critical role in confirming the diagnosis of MADD, and may not only prevent patients from invasive testing, but also allow timely initiation of riboflavin treatment. The novel variants in *ETFDH* and the corresponding clinical features reported here enrich the allelic heterogeneity of RR-MADD and provide insight into genotype-phenotype relationships.

1. Introduction

Multiple acyl-CoA dehydrogenase deficiency (MADD), also known as glutaric aciduria II (GAII, OMIM #231680), is an autosomal recessive disorder of fatty acid, amino acid, and choline metabolism. MADD is highly clinically heterogeneous, and has been categorized into three types: neonatal onset with congenital abnormalities (Type I), neonatal onset without congenital abnormalities (Type II), and late-onset (Type III) [1–3]. MADD results from a defect in either electron transfer flavoprotein (ETF, encoded by the alpha ETF (*ETFA*) and beta ETF (*ETFB*) genes) or ETF-ubiquinone oxidoreductase (ETF-QO, encoded by the ETF dehydrogenase (*ETFDH*) gene) [4, 5].

Types I and II are severe and typically fatal, characterized by non-ketotic hypoglycemia, metabolic acidosis, and accumulation and excretion of metabolites, while Type III is milder and more variable, characterized by recurrent episodes of hypoglycemia, metabolic acidosis, vomiting, and muscle weakness during catabolic stress [6, 7]. Many MADD patients, especially late-onset MADD patients, can be effectively treated with riboflavin, termed riboflavin-responsive MADD (RR-MADD). Previously, it has been reported that *ETFDH* mutations are the major cause of RR-MADD [8, 9]. Here, we report four additional RR-MADD patients from three unrelated families who presented at various ages and carry three novel mutations and one previously reported mutation in *ETFDH*. In addition, we predict changes in the ETF-QO protein structure due to these mutations.

2. Materials and methods

2.1. Patients

The four patients are from southern China, and were clinically diagnosed with MADD. Informed consent was obtained, and this study

Abbreviations: AST, aspartate aminotransferase; CK, creatine kinase; ETF, electron transfer flavoprotein; ETF-QO, ETF-ubiquinone oxidoreductase; GAII, glutaric aciduria II; LDH, lactate dehydrogenase; MADD, multiple acyl-CoA dehydrogenase deficiency; RR-MADD, riboflavin-responsive MADD; WES, whole exome sequencing

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was approved by the local ethics committee.

2.2. Mutation analysis

Genomic DNA was extracted from peripheral blood using standard protocols (QIAGEN, Hilden, Germany). Direct sequencing of *ETFA*, *ETFB*, and *ETFDH* was performed for two patients, and no mutations were identified in *ETFA* or *ETFB*. Whole exome sequencing (WES) was performed for the other two patients. Sequence capture, enrichment, and elution were performed using an Illumina TruSeq Exome Enrichment kit (Illumina, San Diego, CA, USA) according to the manufacturer's protocols, and sequencing was performed on an Illumina HiSeq2000 (Illumina). Sequence alignment and variant calling against the reference human genome (GRCh37) were performed using BWA and the Genome Analysis Toolkit. Variant interpretation of WES data was performed using Ingenuity Variant Analysis pipeline (Ingenuity, Redwood City, CA, USA). SIFT, Polyphen2, and MutationTaster were used for in silico analysis of candidate variants. Deleterious variants were confirmed by Sanger sequencing.

2.3. Structure prediction of ETF:QO

Human and porcine ETF-QO proteins share high amino acid sequence identity (95%), thus the porcine ETF-QO is an excellent structural template. The structural model of the human ETF-QO was made based on the crystallographic structure of porcine ETF-QO [10] (PDB: 2GMH) by homology modeling (Swiss-Model).

3. Results

3.1. Clinical presentations

All four patients were clinically diagnosed with MADD, and changes in blood acylcarnitines, urine organic acids, and muscle enzymes are listed in Table 1.

Patients 1 and 2 are siblings and carry the same mutations but presented at different ages. Patient 2 was diagnosed with newborn screening and presented ill at 10 days old with elevated levels of acylcarnitines, consistent with MADD. Gas chromatography-mass spectrometry revealed a significant increase of 3-hydroxyglutaric acid. In addition, she had elevated levels of creatine kinase (CK), lactate dehydrogenase (LDH), and aspartate aminotransferase (AST). Patient 2 was initially treated with 195 mg riboflavin daily, and her symptoms largely resolved after taking the medication for one month. Then, her parents stopped giving her the medication, and she presented with severe acidosis and convulsions. She was then treated with 300 mg riboflavin daily, which she has continued, and she remains symptom-free. Patient 1 was tested at 7 years old following her sibling's diagnosis. She has had intermittent mild symptoms, which her parents believed were viral upper respiratory infections. Levels of acylcarnitines, ethylmalonic acid, and decanedioic acid were elevated, although her muscle enzymes were within the normal range. Patient 1 was initially treated with 150 mg riboflavin daily. Once improved and lab values normalized, Patient 1 was intermittently treated (her parents stop giving her the medication), and she is now 9 years old and remains symptom-free.

Patient 3, a 27-year old male, was previously healthy with no family history of neuromuscular disease. He presented with pneumonia and reported muscle weakness for 8 months, and was found to have elevated levels of acylcarnitines, urine organic acids, and serum ammonia, as well as fatty liver detected by ultrasound. He was treated with 2 tablets of vitamin B composite (B1: 3.0 mg; riboflavin: 1.5 mg; B6: 0.2 mg) three times a day, and his muscle strength and lab values gradually recovered to normal levels.

Patient 4, a 30-day old male infant, presented with non-ketotic hypoglycemia, metabolic acidosis, electrolyte disturbances, and increased muscle enzymes. His clinical symptoms improved for 20 days

Table 1
Blood acylarnitine, urine organic acids, and muscle enzymes in the four MADD patients.

No.	Sex	Onset	Blood acylcarnitine spectrum (μmol/L)	Urine organic acids spectrum	CK (U/L)	LDH (U/L)	AST (U/L)	Genotype
1	F	7 yo	↓: C2 (6.16), C3 (0.47) ↑: C4 (1.18), C5 (0.56), C6 (1.06), C8 (1.47), C10 (1.86), C10:1 (0.55), C12 (0.60), C14:1 (0.57)	↑: Ethylmalonic acid (8.48), decanedioic acid (54.59)	193	234	34	c.524G > A (p.R175H), c.229G > A (p.G77S)
2	F	10 do	↑: C4 (1.03), C5 (0.58), C5DC (0.33), C6 (0.96), C8 (1.50), C8DC (0.23), C10 (2.11), C10:1 (0.34), C12 (2.43), C12:1 (0.45), C14 (2.49), C14:1 (1.53), C16 (6.39), C16:1 (1.47)	↑: 3-Hydroxyglutaric acid (29.22)	235	331	82	c.524G > A (p.R175H), c.229G > A (p.G77S)
3	M	27 yo	↓: C0(8.38) ↑: C6 (0.41), C8 (0.63), C10 (1.06)	↑: Lactate-2 (34.29), Glycolic acid-2 (14.44), Oxalic acid-2 (4.22), 2-Hydroxybutyric acid-2 (4.58), 3-Hydroxypropionic acid-2 (4.43), Pyruvate-OX-2 (58.29), 2-Hydroxyisovalerate-2 (17.98)	1318	1024	557	c.524G > A (p.R175H), c.1450T > C (p.W484R)
4	M	30 do	↓: C0 (5.37), C3 (0.42) ↑: C4 (1.16), C8DC (0.11), C12(0.51), C14 (1.13), C14:1 (1.18), C16 (4.16), C14:1/ C8:1 (32.10), C16/C3 (9.92)	↑: Ethylmalonic acid (10.33), 3-Hydroxyglutaric acid (7.03), 4-Hydroxyphenyllactate-2 (28.34) Hypervanillic acid-2 (80.4), Vanillic acid-2 (25.45), Palmitic acid-1 (131.72), Glycerate-3 (14.3), 4-hydroxyphenylacetic acid (303.27)	976	853	323	c.1157G > A (p.G286D), c.1450T > C (p.W484R)

↑ above normal level; ↓ below normal level; yo: years old; do: days old.
Upper limit of normal: CK 200 U/L, LDH 245 U/L, and AST 40 U/L.