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A case of late-onset riboflavin responsive multiple acyl-CoA dehydrogenase deficiency (MADD) with a novel mutation in ETFDH gene

Zhihong Zhuo, Peina Jin, Fengyan Li, Haiying Li, Xiaoxin Chen, Huaili Wang *

Department of Pediatrics, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, PR China

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ABSTRACT

We report a novel mutation in the electron transfer flavoprotein dehydrogenase (EFTDH) gene in an adolescent Chinese patient with late-onset riboflavin-responsive multiple acyl-CoA dehydrogenase deficiency (MADD) characterized by muscle weakness as early symptom. At the age of 9 years, the patient experienced progressive muscle weakness. Blood creatine kinase level and aminotransferase were higher than normal. The muscle biopsy revealed lipid storage myopathy. Serum acylcarnitine and urine organic acid analyses were consistent with MADD. Genetic mutation analysis revealed a compound heterozygous mutation in EFTDH gene. The patients showed good response to riboflavin and L-carnitine treatment.

1. Introduction

Multiple acyl-CoA dehydrogenase deficiency (MADD) is a rare autosomal recessive disorder causing defects in mitochondrial electron transfer and metabolism of fatty acid, amino acid, and choline [1]. MADD is roughly classified into two forms according to the onset age: an early-onset form and a mild and/or late-onset form. The early-onset form usually occurs in the neonatal or infantile period with a poor prognosis and is characterized by severe hypotonia, nonketotic hypoglycemia, and metabolic acidosis. In contrast, the late-onset is typically characterized by progressive proximal muscle weakness with heterogeneous symptoms such as intermittent episodes of vomiting, hypoglycemia, or metabolic acidosis. Most patients with late-onset MADD can be totally or partly cured on treatment with riboflavin; hence, this clinical phenotype was called riboflavin-responsive MADD (RR-MADD) [2,3].

Here, we report a case of late onset MADD characterized by progressive muscle weakness in which a novel compound heterozygous mutation within the ETFDH gene was identified. The patient showed a dramatic response to riboflavin replacement therapy.

2. Case report

A 9-year-old girl was admitted to our hospital because of muscle weakness with intermittent vomiting. One month before admission, she experienced lower extremity muscle weakness. She had difficulty walking long distances and climbing stairs, and gradually she had difficulty standing up. Her perinatal history was uneventful, and her early development was normal. Her life history was unremarkable except her disgust with meat. Her family history was also uneventful.

General physical examinations revealed hepatosplenomegaly. On neurological examinations, there was moderate weakness of proximal muscles of the extremities (grade 3/5 for proximal muscle). Blood chemistry results were the followings: alanine aminotransferase 964 U/l (normal < 40 U/l), aspartate aminotransferase 7500 U/l (normal < 40 U/l) and creatine kinase 15,587 U/l (normal < 173 U/l). The free carnitine level in plasma was 9 μmol/l (normal 10–60μmol/l). Her proximal muscle electromyography demonstrated myopathic changes. Brain magnetic resonance imaging (MRI) revealed normal. Muscle biopsy revealed increased numbers of fibers with cytoplasmic vacuoles on H–E staining. These vacuoles mainly distributed in type 1 muscle fibers (Fig. 1A). These vacuoles were positively stained with Oil red O (Fig. 1B) suggesting lipid storage myopathy. MGT, PAS, NADH, SDH, and NSE staining did not show any abnormal. Before riboflavin therapy, urine and blood samples were collected for tandem mass spectrometry. Organic acid analysis in urine showed elevated ethylmalonate, glutarate, 2-hydroxyglutaric acid (2-HG), adipic acid,

* Corresponding author at: Department of Pediatrics, the First Affiliated Hospital of Zhengzhou University, Number one, Constructive East Road, Zhengzhou 450052, P.R.China. Tel.: +86 371 66295572.
E-mail address: 672262895@qq.com (H. Wang).

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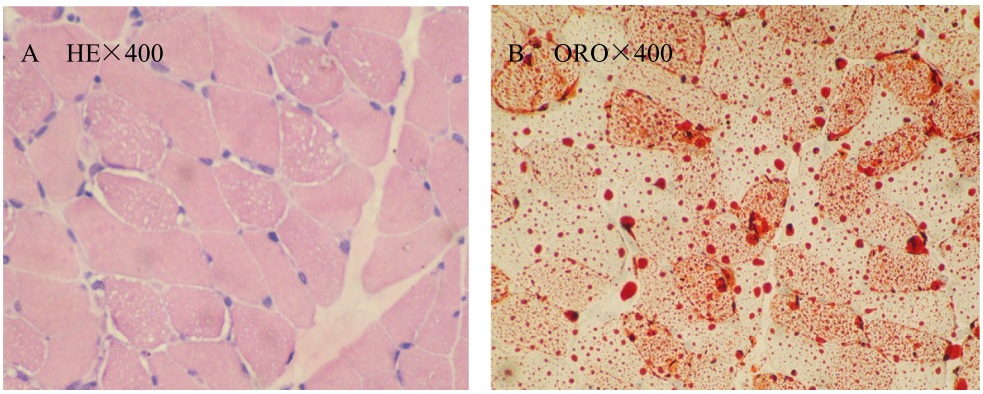

Fig. 1. H–E staining revealed increased numbers of fibers with cytoplasmic vacuoles (Fig. 1A). These vacuoles were most prominent in type-I fibers and positively stained with Oil red O (Fig. 1B), suggesting lipid storage myopathy.

and suberic aid. Acylcarnitine analysis of plasma showed increased acylcarnitines (C4–C18:1), both consistent with the diagnosis of MADD.

Suspecting MADD, we sequenced the ETFA, ETFB and ETFDH genes from the patient and her parents and identified a compound heterozygous ETFDH gene mutation of c.389A > T (p. D130V) in exon3 and c.736G > A (p. E246K) in exon7 (Fig. 2), respectively, from the patient. Molecular diagnostic of the mother showed the heterozygote mutation in exon 3 c.389 A > T (p. D130V) and of the father a heterozygote mutation in exon 7 c.736G > A (p. E246K) compatible with asymptomatic carrier states. No mutation was identified in ETFA or ETFB gene. No carrier of these two mutations was identified from 100 Chinese control subjects. The Polyphen scores were 0.965 for c.389A > T (p. D130V) mutation in exon3 and 1 for c.736G > A (p. E246K) mutation in exon7 (The Polyphen scores are calculated used by the PolyPhen-2 version 2.2.2, represents the probability that a substitution is damaging, so values nearer 1 are more confidently predicted to be deleterious), indicating both mutations were pernicious to the function of the ETFDH protein. To our knowledge, the former mutation has been reported with late onset riboflavin-responsive MADD in China [4]. The latter has not been previously reported.

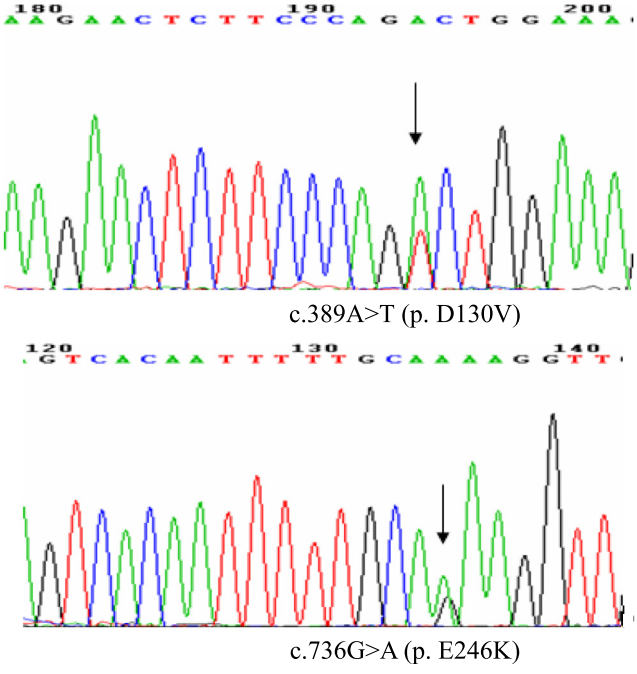

Fig. 2. Analysis of the mutations located in electron transfer flavoprotein dehydrogenase gene, showing a missense a mutation in exon3, c.389A > T (p.D130V), and a missense mutation in exon 7, c.736G > A (p. E246K).

After diagnosis, a high-calorie and reduced-fat diet was started together with oral supplements of riboflavin (100 mg/day) and L-carnitine (50 mg/kg/day). After 1 month, the patient's clinical symptoms improved dramatically. Her muscle weakness disappeared, creatase levels decreased significantly. After 3 months, creatase levels returned to normal, liver and spleen also returned to normal size, the patient has gone to school. Now treatment has stopped for 6 months, further follow-up is ongoing.

3. Discussion

In this paper, we report a case of late-onset riboflavin responsive multiple acyl-CoA dehydrogenase deficiency (RR-MADD) with a novel compound heterozygous mutation in ETFDH gene. We diagnosed RR-MADD in this patient based on her symptom of muscle weakness, relevant biochemical data, muscle biopsy, tandem mass spectroscopy and ETFDH gene mutations. Late-onset MADD is a rare but treatable disorder, and its diagnosis is often difficult when symptoms are nonspecific. Muscle weakness was the main symptom in our patient, it could be commonly misdiagnosed as glycogen storage diseases, polymyositis and progressive muscular dystrophy [5]. In the case we report here, muscle biopsy narrowed the differential diagnosis by revealing lipid accumulation in muscle fibers. The diagnosis of MADD was made based on elevated acylcarnitines and urine organic acids especially 2-hydroxyglutaric acid. The analysis of ETFDH gene confirmed the diagnosis of MADD. The muscle weakness may be the main clinical feature in MADD patients, however the symptom of muscle weakness are also present in almost all types of muscle diseases, such as inflammatory myopathies, metabolic myopathy and progressive muscular dystrophy. MADD begins more often in childhood or in young adulthood featuring proximal muscle weakness and decreased tendon reflexes, and other symptoms of intermittent episodes of vomiting, especially after meals rich in fat or protein, and hypoglycemia, or metabolic acidosis, hepatosplenomegaly. In addition, most MADD patients have fluctuating symptoms during the course of disease; environmental factors, such as cold and infection, can make the disease deteriorate. However, other myopathy has no such clinical manifestations. 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.

To our knowledge, MADD is caused by defects on ETF, ETFB or ETFDH [6]. The phenotype is speculated to correlate with the genotype. Mutations in ETFA and ETFB tend to be early-onset forms, whereas mutations in ETFDH are associated with late-onset MADD, especially the RR-MADD [3,4,7–9]. By now, more than 80 mutations have been reported all over the world, but the same mutant site is rarely found in different

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populations. There were territory differences in the ETFDH mutation spectrum between northern and southern China [10]. c.250G > A was the most common mutation in southern China and c.389A > T was the most common mutation in northern China. Whereas c.770A > G and c.1227A > C were more geographically widespread hot spot mutations in Mainland China, and their prevalence was almost equivalent. According to the spectrum, testing the hot mutation can be a fast and reliable screening method, strongly enhancing genetic counseling and an accurate diagnosis.

Although the molecular mechanism of MADD is still unclear, riboflavin (or vitamin B2) supplementation (100–400 mg/d) has been known to strikingly improve the clinical symptoms and metabolism disorder of MADD patients, particularly those with the late-onset form [11]. The results with combination therapy by carnitine and coenzyme Q10 are still controversial. Our patient showed improvement after treatments with riboflavin combined with carnitine and coenzyme Q10. Consistently, some studies indicate that ETFDH deficiency caused secondary carnitine and coenzyme Q10 insufficiency [12,13] although Liang WC et al. reported the presence of CoQ10 deficiency only in a portion of MADD patients [14].

In summary, we have identified a novel compound heterozygous mutation in ETFDH gene from a patient with late-onset riboflavin responsive multiple acyl-CoA dehydrogenase deficiency which expands the spectrum of mutations found in patients with MADD.

Conflict of interest

The authors declare no conflict of interest.

References

[1] Przyrembel H, Wendel U, Becker K, et al. Glutaric aciduria type II: report on a previously undescribed metabolic disorder. Clin Chim Acta 1976;66(2):227–39.
[2] Gregersen N, Wintzensen H, Christensen SKE, et al. C6–C10-dicarboxylic aciduria: investigations of a patient with riboflavin responsive multiple acyl-CoA dehydrogenation defects. Pediatr Res 1982;16(10):861–8.
[3] Olsen RKJ, Andresen BS, Christensen E, et al. Clear relationship between ETF/ETFDH genotype and phenotype in patients with multiple acyl-CoA dehydrogenation deficiency. Hum Mutat 2003;22(1):12–23.
[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(2):231–6.
[5] Beresford MW, Pourfarzam M, Turnbull DM, et al. So doctor, what exactly is wrong with my muscles?Glutaric aciduria type II presenting in a teenager. Neuromuscul Disord 2006;16(4):269–73.
[6] Goodman SI, Binard RJ, Woontner MR, et al. Glutaric acidemia type II: gene structure and mutations of the electron transfer flavoprotein: ubiquinone oxidoreductase (ETF: QO) gene. Mol Genet Metab 2002;77(1):86–90.
[7] Wang ZQ, Chen XJ, Murong SX, et al. Molecular analysis of 51 unrelated pedigrees with late-onset multiple acyl-CoA dehydrogenation deficiency (MADD) in southern China confirmed the most common ETFDH mutation and high carrier frequency of c.250G > A. J Mol Med 2011;89(6):569–76.
[8] Law LK, Tang NLS, Hui J, et al. Novel mutations in ETFDH gene in Chinese patients with riboflavin-responsive multiple acyl-CoA dehydrogenase deficiency. Clin Chim Acta 2009;404(2):95–9.
[9] Lan MY, Fu MH, Liu YF, et al. High frequency of ETFDH c. 250G > A mutation in Taiwanese patients with late-onset lipid storage myopathy. Clin Genet 2010;78(6):565–9.
[10] Zhu M, Zhu X, Qi X, et al. Riboflavin-responsive multiple Acyl-CoA dehydrogenation deficiency in 13 cases, and a literature review in mainland Chinese patients. J Hum Genet 2014;59(5):256–61.
[11] Liang WC, Nishino I. Lipid storage myopathy. Curr Neurol Neurosci Rep 2011;11:97–103.
[12] Gempel K, Topaloglu H, Talim B, et al. The myopathic form of coenzyme Q10 deficiency is caused by mutations in the electrontransferring-flavoprotein dehydrogenase (ETFDH) gene. Brain 2007;130(Pt8):2037–44.
[13] Di Donato S, Frerman FE, Rimoldi M, et al. Systemic carnitine deficiency due to lack of electron transfer flavoprotein:ubiquinone oxidoreductase. Neurology 1986;36(7):957–63.
[14] Liang WC, Ohkuma A, Hayashi YK, et al. ETFDH mutations, CoQ10 levels, and respiratory chain activities in patients with riboflavin-responsive multiple acyl-CoA dehydrogenase deficiency. Neuromuscul Disord 2009;19(3):212–6.

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