Brief Report

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A novel ETFDH mutation identified in a patient with riboflavin-responsive multiple acyl-CoA dehydrogenase deficiency

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SUMMARY: Lipid storage myopathies (LSM) are a group of inherited metabolic muscle disorders characterized by abnormal lipid metabolism and the deposition of lipids within muscle fibers. Multiple acyl-coenzyme A dehydrogenase deficiency (MADD) is the most common type of LSM in China, caused by mutations in the gene expressing electron transfer flavoprotein dehydrogenase (*ETFDH*). Here, we report a 14-year-old girl presenting with exercise intolerance, followed by muscle weakness and pain. Initially, the patient showed rhabdomyolysis (RML) and was misdiagnosed with polymyositis (PM). However, muscle biopsy and genetic analysis led to a diagnosis of MADD. After the initiation of vitamin B2 administration, her symptoms were rapidly ameliorated. Genetic testing revealed compound heterozygous mutations in the *ETFDH* gene, specifically c.250G>A and c.929A>G, the second of which has not previously been reported. In conclusion, we report a novel mutation of *ETFDH* in a patient with riboflavin-responsive MADD, which expands our knowledge of MADD-related gene variants in the Chinese population.

Keywords: MADD, *ETFDH* gene, magnetic resonance imaging, RML, PM

1. Introduction

Multiple acyl-coenzyme A dehydrogenase deficiency (MADD) is an autosomal recessive genetic disorder caused by mutations in the *EFTA*, *EFTB*, or *ETFDH* (expressing electron transfer flavoprotein dehydrogenase) genes. The most common symptoms of MADD are muscle weakness, exercise intolerance, and muscle pain (*1*), and the signs of MADD include poor muscle strength and weak head lifting (*2*). Patients with late-onset MADD typically exhibit imaging findings of fatty infiltration and atrophy in the anterior, posterior, and medial muscle groups of the thigh on muscle magnetic resonance imaging (MRI) (*3*). Blood acylcarnitine analysis typically reveals high concentrations of medium- and long-chain acylcarnitines (*4*), and serum creatine kinase (CK) activity is often high, although this is not specific to MADD (*5*). The key pathologic feature of MADD is the deposition of fat within muscle fibers (*6*). MADD can easily be misdiagnosed as polymyositis (PM) (*7*), and patients with MADD rarely present with Rhabdomyolysis (RML) (*8*). Some patients show a significant amelioration of their symptoms after treatment with riboflavin, and these are referred to as having riboflavin-responsive (RR)-MADD (*9*).

This study retrospectively analyzed a case of a patient with MADD, attempting to elucidate the differentiation of MADD from PM and RML based on early clinical manifestations and serum CK activity. We followed up with the patient's muscle MRI and observed the resolution process of muscle edema-signals in this MADD patient. Meanwhile, this study analyzed the *ETFDH* gene in a MADD patient using Sanger sequencing, and the results revealed a novel *ETFDH* mutation, further expanding the genetic data associated with this disease.

2. Patient and Methods

2.1. Patient

This was an opportunistic study, but the patient and her parents were informed about the possible use of the data for research and the purpose of the study, the methods used, and gave their consent. The patient was able to understand the study, regardless of her age, was informed and gave her consent. The study conformed to the provisions of the latest version of the Declaration of Helsinki.

2.2. Clinical data collation and analysis

We collected information from the patient and her parents about the symptoms of the disease, and the history of diagnosis and treatment, past medical history, genetic history, *etc*. After sorting out the clinical data and analysis, the next step of the diagnosis and treatment plan was launched.

2.3. Muscle biopsy and pathology

We first took a total of about 0.3 × 0.3 × 0.6 cm of skeletal muscle tissue, performed frozen sections, and completed histochemical and immunohistochemical staining. In addition, two pieces of glutaraldehyde fixed gray and yellow tissue, about 0.2 × 0.3cm long, were taken and a box was used for electron microscopy.

2.4. Sanger sequencing

Sanger sequencing was applied to validate the mutation of the *ETFDH* gene for the patient and her parents. Gene mutation analysis was performed on the DNA sequences of exons 3 and 8 of *ETFDH* gene of the patient.

3. Results and Discussion

3.1. Review and analysis of medical history

A 14-year-old girl presented with generalized muscle weakness and pain, along with a 3-year history of exercise intolerance. Her condition gradually worsened, weakness in lifting her head and chewing developed, and this was accompanied by respiratory distress, necessitating non-invasive ventilation. Her CK activity was as high as 88,703 U/L (reference range: 40-200 U/L). She was initially diagnosed with PM and RML at another hospital, underwent treatments including blood dialysis and immunoglobulin and methylprednisolone administration. However, her symptoms were not significantly ameliorated.

After the patient was admitted to our hospital, we completed muscle biopsy and genetic screening. Soon, the patient was definitively diagnosed with MADD. Then she was prescribed oral vitamin B2. Two months later, she was able to walk normally and perform simple physical activities, such as squatting and standing up repeatedly.

When we retrospectively analyzed this case, we considered whether it was possible to differentiate MADD from PM and RML through early clinical manifestations. The early clinical manifestations of this patient were similar to those of MADD and PM, along with extremely high CK activity. We could not rule out whether she had RML at the onset of the disease. Given the limitations of our case, we reviewed the literature. Torres *et al*. stated that a combination of MADD and RML is extremely rare, and RML is more commonly associated with viral myositis, polymyositis, and other muscle diseases (*8*). In addition, there was a scholar who attempted to distinguish between MADD and PM based on clinical manifestations. Wang *et al*. showed that the symptoms of this disease have more variability in muscle weakness, significant involvement of the muscles of mastication, fewer extra-muscular manifestations, and lower CK activity, in the absence of RML. Under these circumstances, MADD should be considered the primary diagnosis, rather than PM (*10*).

However, due to the extremely high CK level in the early stage of this patient, we were unable to determine whether the abnormal increase in CK was caused by the patient's own condition or was due to concurrent RML. Moreover, during the diagnostic process of a single patient, it is difficult to distinguish between PM and MADD solely based on clinical manifestations. Therefore, we believe that in the early stage, it is challenging to differentiate muscle-related diseases with similar symptoms through clinical manifestations and test results alone. Muscle biopsy and genetic screening should be completed as early as possible.

3.2. Analysis of muscle MRI

Our patient's muscle MRI of both lower extremities showed edema - like changes in multiple muscle groups of the lower limbs before treatment (Figure 1, A and B), but did not show typical fatty infiltration imaging findings of MADD (*3*). 1 month later, the symptoms of this patient were significantly improved, but the MRI did not change significantly (Figure 1, C and D). One year later, we re-examined the MRI of both ankles and found that the edema of the bilateral soleus muscles, the right tibialis anterior muscle, and the right posterior tibialis muscle was less than before (Figure 1, E and F).

By contrast, Hong *et al.* found that some patients showed edematous changes and fatty infiltration in the soleus and biceps femoris muscles on muscle MRI before treatment. After 1 month of treatment, these edematous changes rapidly resolved and the symptoms were alleviated (*11*). Therefore, based on the results of our patient, not all MADD patients will show fatty infiltration on muscle MRI. Moreover, the resolution of abnormal signals on muscle MRI may not be consistent with the improvement of clinical manifestations. In the future, whether the abnormal muscle manifestations can actually serve as an indicator for the prognosis of the disease requires reference and research from more clinical cases.

3.3. Muscle pathological results

Muscle biopsy revealed the presence of vacuoles and lipid droplets in muscle fibers, predominantly in type I muscle fibers, which is consistent with the pathologic characteristics of MADD (Figure 2).

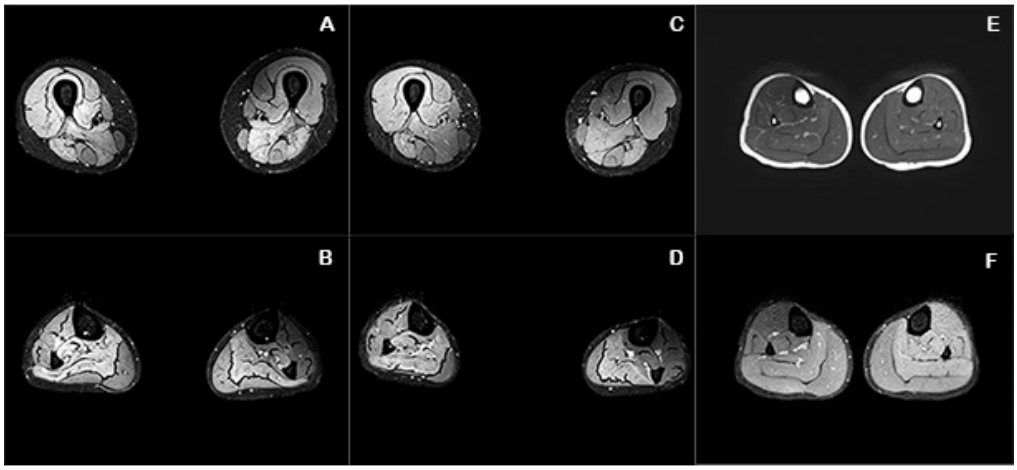

Figure 1. Muscle magnetic resonance imaging findings before and after riboflavin treatment. (A, B) Before treatment, the patient exhibited abnormal edema - like signals in both lower limbs; **(C, D)** After one - month treatment, these signals had not significantly changed; **(E, F)** A year later, the bilateral calf muscle edema became less obvious.

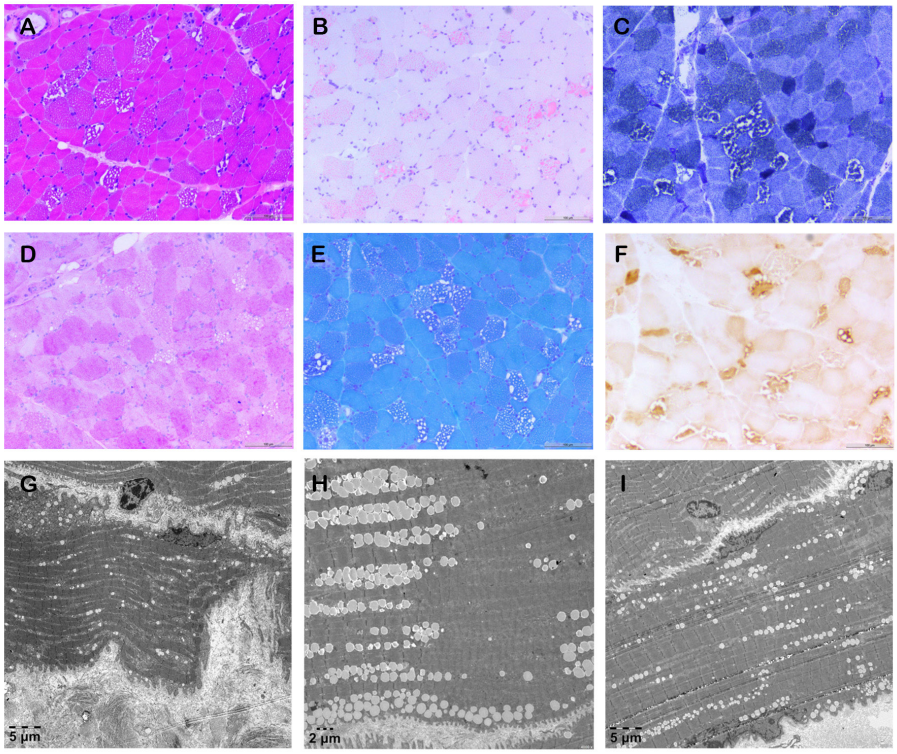

Figure 2. Muscle histopathologic findings. (A) Hematoxylin and eosin staining revealed abundant small vacuoles scattered within muscle fibers, with some showing fusion to form larger vacuoles; **(B)** Oil red O staining revealed diffuse lipid deposition within muscle fibers; **(C)** NADH staining demonstrated a mixed fiber-type distribution, with scattered atrophic fibers appearing darker against a lightly stained background; **(D)** PAS staining did not reveal significant glycogen accumulation within muscle fibers; **(E)** MGT staining did not show fragmentation of red fibers or rimmed vacuoles; **(F)** Deep COX staining was present in scattered atrophic muscle fibers against a lightly stained background, with no COX-negative fibers; **(G, I)** Electron microscopy revealed numerous lipid droplets arranged in a "beads-on-a-string" pattern within muscle fibers.

3.4. Analysis of gene screening results

Genetic sequencing identified compound heterozygous mutations in the *ETFDH* gene of the patient, with c.250G>A having been inherited from her father and c.929A>G from her mother (Figure 3).

In China, most patients with LSM have late - onset MADD caused by mutations in the *ETFDH* gene (*12*). The most frequent site of mutation in Southern China is c.250G>A (*13*). Our patient had both c.250G>A (p.A84T) and c.929A>G (p.Y310C) compound heterozygous mutations, and c.250G>A (p.A84T) has previously been reported to be a pathogenic mutation (*14*). In addition, we entered the following search terms

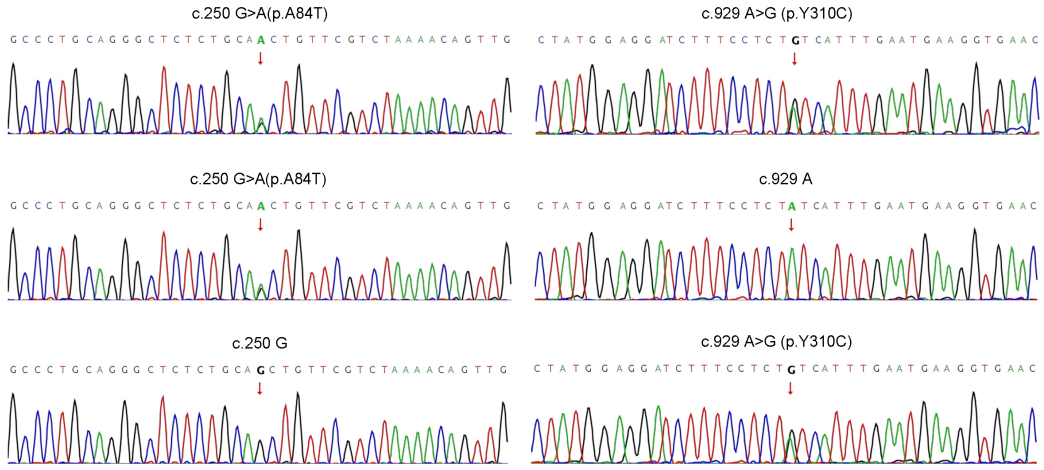

Figure 3. Genetic analysis of the *ETFDH* gene. (FIRST LINE) The genetic analysis suggested the presence of compound heterozygous mutations (c.250G>A and c.929A>G) in the *ETFDH* gene of the patient. **(SECOND LINE)** The c.250G>A mutation had been inherited from her father; **(THIRD LINE)** the c.929A>G mutation had been inherited from her mother.

in the Advanced function of PubMed: (c.929A>G) AND (Multiple acyl - coenzyme A dehydrogenase deficiency); (c.929A>G) AND (MADD). There are no relevant reports in the literature indicating that it is a pathogenic mutation. To our knowledge, nor has it been reported as a benign polymorphism.

We attempted to interpret the newly discovered mutation and identified the following consequence: The amino acid change is semi-conservative as both Tyrosine and Cysteine are uncharged, polar amino acids, but the introduction of a Cysteine could affect the disulfide bonds of the *ETFDH* protein. This change is at a highly conserved position in the ETFDH protein, and multiple *in-silico* analysis programs predicted that Y310C would be damaging to the ETFDH protein. Therefore, Y310C can be interpreted as a pathogenic mutation.

4. Conclusion

We have discussed the rarity of MADD in clinical practice and the complexity of making a clinical diagnosis. Clinical diagnosis should involve early muscle biopsy and genetic sequencing. We reported a novel *ETFDH* mutation in a patient with RR - MADD, expanding knowledge of the genetic spectrum of MADD in the Chinese population.

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