Original Article

Skeletal Muscle Magnetic Resonance Imaging of the Lower Limbs in Late-onset Lipid Storage Myopathy with Electron Transfer Flavoprotein Dehydrogenase Gene Mutations

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Abstract

Background: Lipid storage myopathy (LSM) is a genetically heterogeneous group with variable clinical phenotypes. Late-onset multiple acyl-coenzyme A dehydrogenation deficiency (MADD) is a rather common form of LSM in China. Diagnosis and clinical management of it remain challenging, especially without robust muscle biopsy result and genetic detection. As the noninvasion and convenience, muscle magnetic resonance imaging (MRI) is a helpful assistant, diagnostic tool for neuromuscular disorders. However, the disease-specific MRI patterns of muscle involved and its diagnostic value in late-onset MADD have not been systematic analyzed.

Methods: We assessed the MRI pattern and fat infiltration degree of the lower limb muscles in 28 late-onset MADD patients, combined with detailed clinical features and gene spectrum. Fat infiltration degree of the thigh muscle was scored while that of gluteus was described as obvious or not. Associated muscular atrophy was defined as obvious muscle bulk reduction.

Results: The mean scores were significantly different among the anterior, medial, and posterior thigh muscle groups. The mean of fat infiltration scores on posterior thigh muscle group was significantly higher than either anterior or medial thigh muscle group ($P < 0.001$). Moreover, the mean score on medial thigh muscle group was significantly higher than that of anterior thigh muscle group ($P < 0.01$). About half of the patients displayed fat infiltration and atrophy in gluteus muscles. Of 28 patients, 12 exhibited atrophy in medial and/or posterior thigh muscle groups, especially in posterior thigh muscle group. Muscle edema pattern was not found in all the patients.

Conclusions: Late-onset MADD patients show a typical muscular imaging pattern of fat infiltration and atrophy on anterior, posterior, and medial thigh muscle groups, with major involvement of posterior thigh muscle group and gluteus muscles and a sparing involvement of anterior thigh compartment. Our findings also suggest that muscle MRI of lower limbs is a helpful tool in guiding clinical evaluation on late-onset MADD.

Key words: Electron Transfer Flavoprotein Dehydrogenase; Fat Infiltration Atrophy; Late-onset Lipid Storage Myopathy; Magnetic Resonance Imaging; Multiple Acyl-coenzyme A Dehydrogenation Deficiency

Introduction

Late-onset lipid storage myopathy (LSM) is an autosomal recessive muscular disorder associated with errors of fatty acid, amino acid, and choline metabolism. The four main genetic causes of LSM are multiple acyl-coenzyme A dehydrogenase deficiency (MADD, OMIM 231680), primary carnitine deficiency (OMIM 212140), neutral lipid storage disease with myopathy (OMIM 610717), and neutral lipid storage disease with ichthyosis (OMIM 25630).[1] The mutations of electron transfer flavoprotein dehydrogenase (*ETFDH*) gene have been described in a considerable number of patients[2,3] and MADD appears to be a rather main form of LSM in Chinese population.[4] Previous data indicated that the *ETFDH*-c.250G>A had a high mutation frequency in Southern China whereas c.770A>G and c.1227A>C were more widespread hot spot mutations in both Southern and

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Northern China.[4-6] The clinical symptoms of late-onset MADD are heterogeneous, varying from pure muscle weakness to serious metabolism crisis. Patients predominantly present in adolescence or adulthood with episodes of proximal myopathy, exercise intolerance, vomiting, hypoglycemia, and encephalopathy usually triggered by metabolic stress.[7]

Since the clinical manifestations of late-onset MADD are highly heterogeneous and relatively unspecific, the ultimate diagnosis is based on a standard muscle biopsy and genetic analysis, none of which is widely available on different levels of hospitals. Moreover, patients refuse the invasive muscle biopsy occasionally. In such situation, we need a helpful and practical tool to formulate a reasonable diagnosis or limit the differential diagnosis. In the last decade, as a noninvasive tool in the diagnosis, various muscle magnetic resonance imaging (MRI) patterns of neuromuscular diseases have been found. MRI allows detection and characterization of much muscular pathology, such as inflammation, atrophy, edema, and fat infiltration. For diagnostic purposes, T1- and T2-weighted axial images of lower limbs may be sufficient for muscular dystrophy.[8] Hence, muscle MRI has been widely used in accessory diagnosis of spinal muscular atrophy,[9] limb girdle muscular dystrophy (LGMD),[10] Duchenne muscular dystrophy/Becker muscular dystrophy (DMD/BMD),[11] and congenital muscular dystrophy (CMD),[12] especially in the absence of robust muscle biopsy results. Recently, a case report highlighted the diagnostic clues and body fat MRI in metabolic disorders.[13] Indeed, muscle MRI pattern in late-onset MADD due to *ETFDH* gene mutations has not been systematic analyzed and concluded. Based on our initial clinical, genetic, and treatment studies in a large cohort of Chinese patients with late-onset MADD,[5,14] we were interested to characterize the disease-specific patterns of involved muscle by the pelvic girdle and thigh muscles MRI to help in diagnostic work-up, exploring the diagnostic value of muscle MRI on late-onset MADD.

Methods

Patients

In total, 28 patients (18 males and 10 females) visited the First Affiliated Hospital of Fujian Medical University from January 2012 to December 2015, and these patients were pathologically and genetically diagnosed as late-onset MADD and recruited to perform the muscle MRI scan of pelvis and thigh muscles. In addition, cases involving lipid storage abnormalities secondary to steroids and mitochondrial diseases were excluded. This study was approved by the Ethical Committees of the First Affiliated Hospital of Fujian Medical University.

Data collection

Basic information, including gender, age, onset age, and family history, was first collected. Before riboflavin treatment, manual muscle testing (MMT), blood biochemistry, muscle biopsy, abdominal ultrasonography, electromyography (EMG), and acylcarnitine and urine organic acids profile were performed. Muscle strength grade in MMT scoring was counted and referenced to Kendall 0–10 point scale as previously described.[15,16] The strength of 14 muscles distributed in neck, proximal limb, and distal limb was examined. The neck muscles were neck flexion and neck extensor. The proximal limb muscles contained deltoid, biceps, triceps, iliopsoas, gluteus medius, gluteus maximus, posterior group, and quadriceps femoris. The muscles in distal limb were wrist flexion, wrist extension, ankle dorsiflexion, and ankle plantar flexion.

Blood biochemistry test items were creatinine kinase (CK), MB isoenzyme of CK, aspartate amino transferase (AST), lactate dehydrogenase (LDH), and blood lactic acid deficiency (LAC). Blood acylcarnitine and urine organic acid profiles were analyzed by tandem mass spectrometry (MS/MS) and gas chromatography-mass spectrometry, respectively. Hematoxylin-eosin (HE) and oil red O (ORO) staining were carried out.

Mutation detection

After informed consent, peripheral blood samples were collected from these patients and their available immediate relatives. DNA was abstracted using DNA extraction kit (Qiagene, Hilden, Germany). Polymerase chain reaction (PCR) was performed to amplify the exons of *ETFA*, *ETFB*, and *ETFDH* genes. The PCR products were purified for Sanger sequencing. The primers and reaction condition referred to the published literature.[5]

Muscle magnetic resonance imaging

MRI scans of the lower limbs were performed on a 3.0-T whole-body scanner (Magnetom Verio; Siemens AG, Erlangen, Germany) before riboflavin treatment. Coronal, horizontal, and anteroposterior axial planes of the pelvis and bilateral thighs were obtained by the conventional T1- and T2-weighted spin-echo sequences. Short tau inversion recovery (STIR) sequences for fat suppression were also available for all the individuals. Sections were generally analyzed with pelvis and mid-upper thighs, for the reason that muscle bulk is greatest at this level and that muscle abnormality could be more clearly visualized.[17] The combination of T1-/T2-weighted imaging with STIR sequences was used to assess the degree of muscle fat infiltration and atrophy. Individual thigh muscles were scored for fat infiltration, including anterior (sartorius, rectus femoris, vastus lateralis, vastus intermedius, and vastus medialis), medial (gracilis, adductor longus, adductor brevis, and adductor magnus), and posterior thigh muscle groups (biceps femoris, semitendinosus, and semimembranosus). The degree of fat infiltration in single muscle is described as follows: Grade 0, normal; Grade 1, punctate hyperintense; Grade 2, mildly abnormal (fatty streaks detected below 30% muscle volume); Grade 3, moderately abnormal (hyperintense detected among 30–60% muscle volume); Grade 4, severely abnormal (hyperintense detected above 60% muscle volume); and Grade 5, terminal stage (hyperintense in the whole muscle).[18-20] As gluteus with rich fascia and connective tissue, the accurate score was unavailable. The frequent of

gluteus involvement was calculated to represent the degree of fat infiltration in gluteus. Muscular atrophy was defined as obvious muscle bulk reduction.

Statistical analysis

The scores were averaged to produce mean fat infiltration scores for each muscle group. The mean scores were compared among muscle groups using one-way analysis of variance. The comparison between two muscle groups was conducted by Dunnett's T3 test due to the heterogeneity of variance. $P < 0.01$ indicated statistical significance. Analysis was performed using SPSS 19.0 software (SPSS Inc., Chicago, IL, USA).

Results

Clinical manifestations

Among the 28 patients, the mean onset age was 21.0 ± 11.1 years, ranged from 3 to 55 years. The age of initial clinical assessment ranged from 10 to 58 years. Proximal muscle weakness was found as the most frequent initial symptom in 24 patients (85.7%). All patients exhibited exercise intolerance and weakness of proximal limbs as well as neck muscle. Sixteen (57.1%) patients displayed gastrointestinal symptoms, such as nausea, vomiting, or diarrhea. Dysmasesis and dysphagia were observed in 17 and six patients, respectively. Two patients had myalgia. In physical examination, two patients were found to have muscle atrophy in bilateral thighs. When analyzed the muscle strength, the most severely involved muscles were neck muscle, deltoid, iliopsoas, gluteus maximus, and gluteus medius. The means of lower limbs muscle strength are summarized in Figure 1. All the distal limbs and quadriceps femoris were not clinically affected. Clinical manifestations of the patients are summarized in Table 1.

Mutation

Among the 28 patients, five missense mutations in *ETFDH* were detected, including c.250G>A, c.380T>A, c.770A>G, c.1601C>T, and c.524G>A. Twenty-one patients (75.0%) were c.250G>A homozygote, as well as five heterozygous patients also carried c.250G>A. The remaining two patients showed compound heterozygous mutations without hot spot. Three patients complained a suspect family history of this disease and six patients had heterozygous parents. Neither *ETFA* nor *ETFB* mutation was discovered.

Blood biochemistry

The median and range of CK, AST, LDH, and LAC are summarized in Table 1. For most patients, the muscle enzyme values ranged from normal to 29 times of normal upper limits [Table 1]. Twenty-two patients (78.6%) showed a high LAC level. In the blood acylcarnitine profile test, 21 patients (21/28, 75.0%) represented increased multiple acylcarnitines while three patients showed decreased multiple acylcarnitines and free carnitine. The remaining patients were normal. In urine organic acids test of 26 patients, nine patients (34.6%) showed ketonuria and the remaining 17 patients were normal.

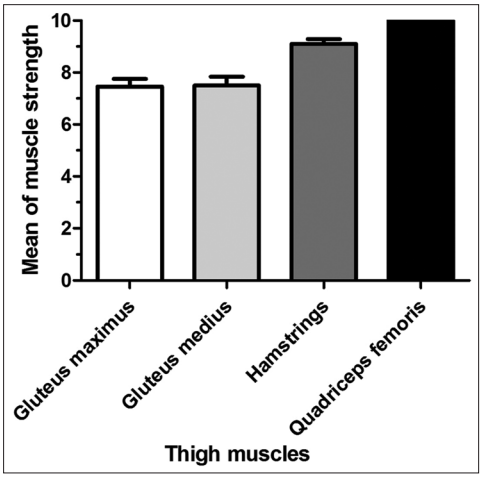

Figure 1: Means of muscle strength of gluteus maximus, gluteus medius, hamstrings, and quadriceps femoris.

Table 1: Clinical manifestation and accessory examinations of 28 late-onset MADD patients

Clinical manifestation	Values
Male	18/28
c.250G >A homozygote	21/28
Muscle	
Proximal limbs weakness	28/28
Distal limbs weakness	0/28
Dysmasesis	17/28
Dysphagia	6/28
Myalgia	2/28
Amyotrophy	2/28
Gastrointestinal symptoms	
Diarrhea	3/28
Vomit	15/28
Blood biochemistry	
CK (U/L)	516 (129–4129)
CKMB (U/L)	33 (4–358)
AST (U/L)	91 (17–407)
LDH (U/L)	757 (189–3507)
LAC (mmol/L)	3.60 (0.96–9.60)
Electromyography	
Myopathic changes	25/28
Neurogenic changes	0/28
No obvious abnormality	3/28
Fatty liver	6/20

Values are presented as *n*/*N* or median (range). Upper limit of normal: CK, 140 U/L; CKMB, 27 U/L; AST, 40 U/L; LDH, 245 U/L; and LAC, 2.10 mmol/L. MADD: Multiple acyl-coenzyme A dehydrogenation deficiency; CK: Creatinine kinase; CKMB: MB isoenzyme of creatinine kinase; AST: Aspartate aminotransferase; LDH: Lactate dehydrogenase; LAC: Lactic acid deficiency.

Electromyography, abdominal ultrasonography, and muscle pathology

For the EMG examination, 25 patients (89.3%) were found myopathic changes and the remaining were neurogenic or no obvious myopathic changes. For twenty patients carried abdominal ultrasonography examination, six patients (30.0%) showed fatty liver in varying degrees.

All the patients showed vacuolus on HE staining and lipid droplets deposition on ORO staining of the muscle fibers.

Muscle magnetic resonance imaging

There was obvious variability in the severity degree of muscle MRI results among the patients included. Two patients were sparing of muscle involvement whereas the other 26 patients symmetrically developed mild to severe muscle involvement, including fat infiltration and atrophy [Figures 2–4]. Muscle involvement could be identified in patients with either homozygous mutation or compound heterozygous mutation [Figures 3 and 4]. The detailed muscle fat infiltration scores of each patient are listed in Supplement Table 1. At thigh level, a major involvement of the posterior thigh muscle group was noticeable. The mean scores for fat infiltration of anterior, medial, and posterior thigh muscle groups were 0.13 ± 0.22, 0.47 ± 0.40, and 1.35 ± 0.75, respectively. The mean scores were significantly different among the three groups of thigh muscles ($P < 0.001$). In the comparison of each two muscle groups, the mean of fat infiltration scores on posterior was significantly higher than either anterior or medial thigh muscle group ($P < 0.001$) [Figure 5]. Moreover, the mean on medial thigh muscle group was significantly higher than that of anterior thigh muscle group ($P < 0.01$) [Figure 5]. Twelve patients (42.9%) exhibited thigh muscle atrophy, especially in medial and posterior thigh muscle groups. Of 24 patients, 15 (62.5%) who conducted gluteus muscle MRI displayed fat infiltration and atrophy in gluteus muscles. Twelve patients showed atrophy especially in medial and/or posterior thigh muscle groups, which had the similar distribution with fat infiltrated muscles, especially in posterior group. It was worth noting that increased STIR signal, suggestive of edema and inflammation, was found in none of pelvic and thigh muscles in our patients [Table 2].

Discussion

In this study, we have identified and evaluated the muscle MRI finding in a cohort of late-onset LSM patients carried pathological *ETFDH* gene mutations. Our results indicated that lower limbs MRI is an additional and helpful tool in guiding the clinical diagnosis of late-onset MADD.

A previous MRI findings about MADD have described those T2 hyperintense lesions in the bilateral globus pallidus in a young child with neonatal-onset form.[21] While a recent report using whole-body MRI revealed an increase subcutaneous fat but normal visceral fat, despite fatty liver

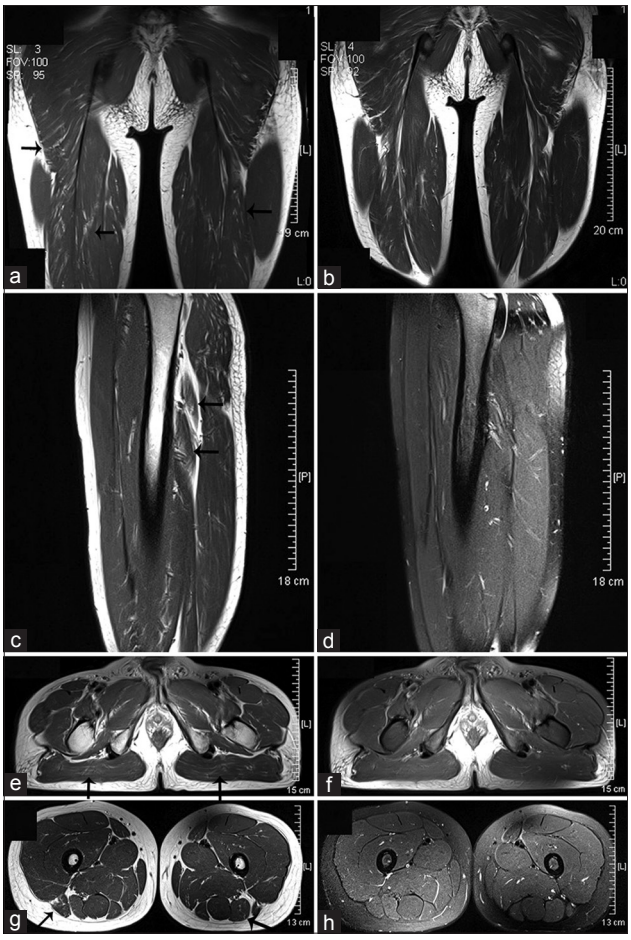

Figure 2: T2-weighted images (a, c, e, and g) and fat suppression images (b, d, f, and h) showed mild fat infiltrated regions (arrows) from a male with c.250G>A homozygous mutation on coronal scanning (a and b), anteroposterior axes of right thigh (c and d), horizontal scanning of pelvic (e and f) and thigh (g and h) level.

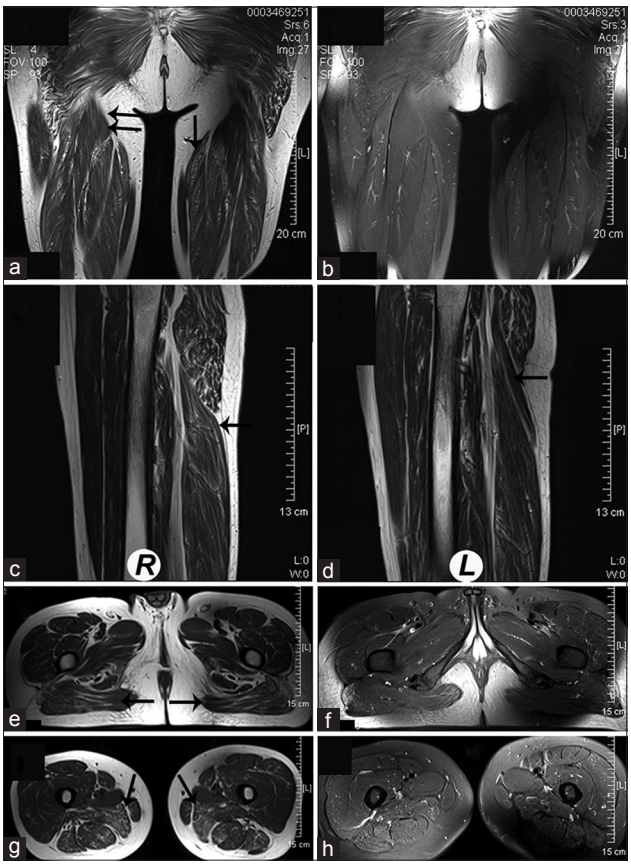

Figure 3: T2-weighted images (a, c, d, e, and g) and fat suppression images (b, f, and h) showed serious fat infiltrated regions (arrows) from a male with c.250G>A homozygous mutation on coronal scanning (a and b), anteroposterior axes of bilateral thighs (c and d), horizontal scanning of pelvic (e and f) and thigh (g and h) level.

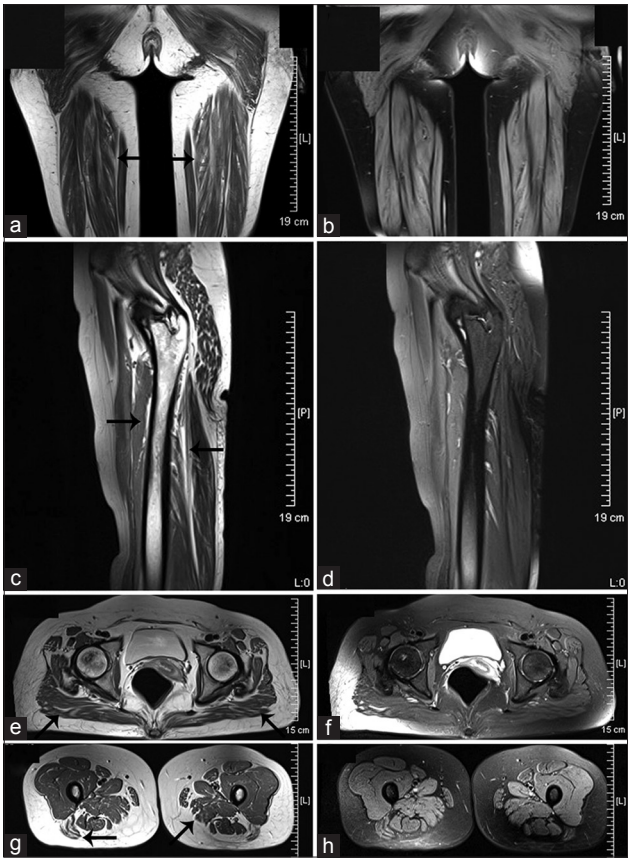

Figure 4: T2-weighted (a, c, e, and g) and fat suppression images (b, d, f, and h) showed serious fat infiltrated regions (arrows) from a female with c.250G>A, c.770A>G compound heterozygous mutation on coronal scanning (a and b), anteroposterior axes of right thigh (c and d), horizontal scanning of pelvic (e and f) and thigh (g and h) level.

Table 2: Lower limbs muscle MRI changes of 28 late-onset MADD patients

Lower limbs muscle MRI changes	Numbers of patients
Thigh	
Fat infiltration	
Anterior thigh muscle group	9/28
Medial thigh muscle group	21/28
Posterior thigh muscle group	26/28
Atrophy	12/28
Anterior thigh muscle group	1/12
Medial thigh muscle group	5/12
Posterior thigh muscle group	12/12
Edema	0/28
Gluteus muscles image available	24/28
Fat infiltration	15/24
Atrophy	15/24
Edema	0/24

Values are presented as *n*/*N*. MADD: Multiple acyl-coenzyme A dehydrogenation deficiency; MRI: Magnetic resonance imaging.

and lipid storage in muscle, in a young patient with a novel homozygous missense mutation in *ETFDH* gene.[13] These studies only paid attention on the brain lesion or body fat distribution. Herein, we focused on the lower limbs muscle

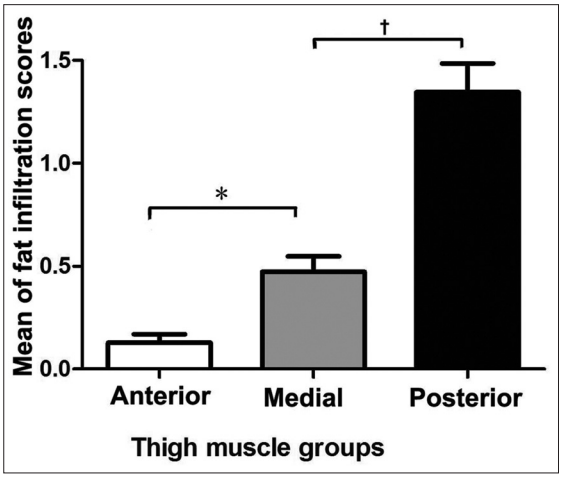

Figure 5: The mean of thigh muscle fat infiltration scores in three thigh muscle groups (*$P < 0.01$, †$P < 0.001$).

MRI findings, including fat infiltration and atrophy, in a group of patients with clinical, pathological, and genetic diagnosed as late-onset MADD. The complete clinical information allowed us to provide a systematic analysis of the muscle MRI pattern and correlate the severity of the MRI finding with the severity of clinical impairments.

MRI plays an important role in detecting alterations in muscle morphology and signal intensity characteristics associated with many neuromuscular disorders although a limited number of nonspecific pathophysiologic changes (e.g., fat infiltration, atrophy, edema, and fibrosis) respond to various muscle damages. MRI shows pathological changes of parenchymal alterations in muscle based on these major patterns of fat infiltration, atrophy, or edema.[21] Our current results suggested that patients with late-onset MADD showed a rather homogenous pattern of muscle pathology on muscle MRI. Indeed, the majority of our patients presented a relatively homogenous involvement of posterior compartment of the lower limbs, mainly in posterior group and gluteus muscles [Figures 3, 4 and Supplement Table 1]. Using fat infiltration scoring, we found that the degree of fat infiltration in posterior group was significantly more severe than those in anterior and medial thigh muscle groups ($P < 0.001$). In addition, associated muscle atrophy was common. Similarly, muscular atrophy was more prominent in posterior group than other parts of thigh muscle groups. In addition to posterior group, fat infiltration and muscular atrophy were also identified in gluteus among most of the late-onset MADD patients. On the contrary, few patients had the quadriceps femoris muscle involvement. Combing with the significant reduction of muscle strength in gluteus maximus, gluteus medius, and posterior group, it could be speculated that the degree of fat infiltration was consistent with the muscle strength. This pattern of muscle involvement may also provide indirect information about the disease process of late-onset MADD. We assume that the most frequently affected muscles, posterior group and gluteus, are affected earlier

in the clinical course. In addition, muscle edema pattern, which is the most common characteristic performance of acute or recent muscle insult, was found in none of our patients. The MRI finding reflects a biologically chronic process in pathogenesis of this metabolic disorder and may be useful for differential diagnosis with other disease. The MRI features of thigh and gluteus muscles existed in patients with both homozygous mutations and compound heterozygous mutations.

The highly various clinical symptoms of patients with late-onset MADD easily lead to difficulty for diagnosis and confusion with other diseases, such as polymyositis, BMD, LGMD, and CMD. Even though genetic detection and muscle biopsy are necessary, they are invasive and unavailable in many hospitals. As a noninvasive and universal technique, muscle MRI can evaluate heterogeneous muscle involvement and has been utilized in many neuromuscular disorders for differential diagnosis.[22] Although some neuromuscular disorders have similar MRI appearances, others have distinct patterns of signal intensity abnormality.[21] The MRI characteristics of late-onset MADD summarized in our study provide valuable information for helping diagnosis. Clinical features of patients with polymyositis usually resemble that of late-onset MADD. However, patients with polymyositis present with widespread symmetrical muscle inflammation and prominent edema is the most prominent MRI finding, which is not found in late-onset MADD.[23] In patients with DMD/BMD, progressive muscle hypertrophy and adductor magnus fat infiltrated are the best characterized signs of muscle MRI,[11] unlike the obvious involvement of posterior group and gluteus in late-onset MADD. The muscle MRI of LGMD patients varies from different subtypes with the heterologous gene mutations.[10,24] A part of LGMD patients show that posterior compartment muscles in lower limbs affected while the fat infiltration is not as severe as that in late-onset MADD.[10] The lower limbs muscle MRI of CMD shows diffuse fatty degeneration, especially in gluteus maximus muscle.[12] In this regard, muscle MRI features, when correlated with clinical and laboratory findings, may facilitate a correct diagnosis to molecular screening of late-onset MADD.

In conclusion, late-onset MADD patients harboring *ETFDH* gene mutations presented an overall homogeneous pattern of muscle pathology on muscle MRI appearances, which are among the conditions associated with typical fat infiltration and atrophy. The majority of patients showed a consistent pattern on muscular imaging, with mild to severe involvement of the muscles of medial and posterior thigh compartment, as well as gluteus, and a sparing involvement of the anterior thigh compartment relatively. Our findings highlight diagnostic value of muscle MRI for late-onset MADD.

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Supplementary information is linked to the online version of the paper on the Chinese Medical Journal website.

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Conflicts of interest

There are no conflicts of interest.

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Supplement Table 1: Thigh muscle fat infiltration scores of 28 late-onset MADD patients

Number	Anterior thigh muscle group					Medial thigh muscle group				Posterior thigh muscle group			Total
	Sartorius	Rectus femoris	Vastus lateralis	Vastus intermedius	Vastus medialis	Gracilis	Adductor longus	Adductor brevis	Adductor magnus	Biceps femoris	Semitendinosus	Semimembranosus	
1	0	0	1	0	0	1	1	0	1	3	3	0	10
2	2	0	0	0	0	1	0	0	0	1	3	3	10
3	0	0	0	0	0	0	0	0	0	1	1	0	2
4	0	0	0	0	0	0	0	0	0	1	1	1	3
5	0	0	0	0	0	1	1	0	1	2	1	3	9
6	1	0	1	0	0	2	1	0	2	2	2	2	13
7	1	0	1	1	1	1	0	0	3	2	3	2	15
8	0	0	0	0	0	0	0	0	0	1	2	1	4
9	0	0	0	0	0	0	0	0	1	2	1	1	5
10	0	0	0	0	0	1	0	0	1	0	1	0	2
11	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	1	1	2
13	0	0	0	0	0	0	0	0	1	1	0	0	2
14	1	0	0	0	0	1	0	0	1	2	1	2	8
15	0	0	0	0	0	1	0	0	2	1	1	1	5
16	0	0	0	0	0	1	0	0	1	1	2	0	5
17	0	0	0	0	0	0	0	0	0	0	0	0	0
18	1	0	0	0	0	1	0	0	1	2	2	1	8
19	1	0	0	1	0	1	1	1	2	3	3	3	16
20	0	0	0	0	0	0	0	0	2	2	2	1	7
21	0	0	0	0	0	2	1	0	2	1	2	0	8
22	2	0	1	0	0	1	0	0	0	2	1	3	9
23	0	0	0	0	0	0	0	0	0	1	1	0	2
24	1	0	1	0	0	1	0	0	2	2	2	1	10
25	0	0	0	0	0	1	0	0	2	2	3	1	9
26	0	0	0	0	0	1	0	0	1	1	2	1	6
27	0	0	0	0	0	1	0	0	1	1	1	1	5
28	0	0	0	0	0	0	0	0	1	2	2	1	6

MADD: Multiple acyl-coenzyme A dehydrogenation deficiency.