Clinical and muscle magnetic resonance image findings in patients with late-onset multiple acyl-CoA dehydrogenase deficiency

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Abstract

Background: Late-onset multiple acyl-coA dehydrogenase deficiency (MADD) is an autosomal recessive inherited metabolic disorder. It is still unclear about the muscle magnetic resonance image (MRI) pattern of the distal lower limb pre- and post-treatment in patients with late-onset MADD. This study described the clinical and genetic findings in a cohort of patients with late-onset MADD, and aimed to characterize the MRI pattern of the lower limbs.

Methods: Clinical data were retrospectively collected from clinic centers of Peking University People's Hospital between February 2014 and February 2018. Muscle biopsy, blood acylcarnitines, and urine organic acids profiles, and genetic analysis were conducted to establish the diagnosis of MADD in 25 patients. Muscle MRI of the thigh and leg were performed in all patients before treatment. Eight patients received MRI re-examinations after treatment.

Results: All patients presented with muscle weakness or exercise intolerance associated with variants in the electron transfer flavoprotein dehydrogenase gene. Muscle MRI showed a sign of both edema-like change and fat infiltration selectively involving in the soleus (SO) but sparing of the gastrocnemius (GA) in the leg. Similar sign of selective involvement of the biceps femoris longus (BFL) but sparing of the semitendinosus (ST) was observed in the thigh. The sensitivity and specificity of the combination of either "SO+/GA−" sign or "BFL+/ST−" sign for the diagnosis of late-onset MADD were 80.0% and 83.5%, respectively. Logistic regression model supported the findings. The edema-like change in the SO and BFL muscles were quickly recovered at 1 month after treatment, and the clinical symptom was also relieved.

Conclusions: This study expands the clinical and genetic spectrums of late-onset MADD. Muscle MRI shows a distinct pattern in the lower limb of patients with late-onset MADD. The dynamic change of edema-like change in the affected muscles might be a potential biomarker of treatment response.

Keywords: Multiple acyl-coA dehydrogenase deficiency; Electron transfer flavoprotein dehydrogenase; Muscle magnetic resonance imaging; Muscle edema-like change

Introduction

Multiple acyl-coA dehydrogenase deficiency (MADD) is an autosomal recessive inherited metabolic disorder mainly caused by the defect of electron transfer flavoprotein: ubiquinone oxidoreductase (ETF:QO) complex encoded by the ETF dehydrogenase (*ETFDH*) gene, alpha ETF (*ETFA*) gene, and beta ETF (*ETFB*) gene.[1-3] The phenotype of late-onset MADD is highly variable and mainly characterized by fluctuating muscle weakness, vomiting, hypoglycemia, metabolic acidosis, and hepatomegaly usually preceded by metabolic stress.[4] In the recent decade, a lot of patients with late-onset MADD have been reported in Chinese population who mainly presented with proximal muscle weakness, exercise intolerance, myalgia, and dramatic riboflavin responsiveness.[2,3,5-9]

Muscle magnetic resonance imaging (MRI) has been considered a valuable means to distinguish different myopathies and follow up the disease progression.[10] Muscle MRI has been widely applied as a useful diagnostic tool for inflammatory myopathy,[11] limb girdle muscular dystrophy (LGMD),[12] Duchenne muscular dystrophy/ Becker muscular dystrophy (DMD/BMD),[13] congenital muscular dystrophy (CMD),[14] and spinal muscular atrophy.[15] Recently, Liu and colleagues[9] found that muscle MRI was a useful tool for clinical evaluation through the observation of proximal lower limb in a cohort of late-onset patients with MADD. Zhao and colleagues[16] reported that the thigh muscle MRI could

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efficiently differentiate late-onset MADD from immune-mediated necrotizing myopathy. However, muscle MRI patterns of the distal lower limb pre- or post-treatment are still unclear in patients with late-onset MADD. This study described the clinical and genetic findings in a cohort of patients with late-onset MADD, and aimed to characterize the MRI pattern of the lower limbs.

Methods

Ethical approval

The study was conducted in accordance with the *Declaration of Helsinki* and was approved by the Ethics Committee of Peking University People's Hospital. As retrospective study and data analysis were performed anonymously, this study was exempt from the requirement of informed consent from patients.

Patients

A total of 25 patients were retrospectively collected in clinic centers of Peking University People's Hospital between February 2014 and February 2018. Two hundred patients with non-MADD myopathy from an in-house database were as the controls. Basic information such as demographic data, age at onset, and clinical manifestation was collected. Blood biochemistry, serum homocysteine, abdominal ultrasonography, and electromyography (EMG) were examined in some patients. All patients' tissue samples were obtained after a written consent signed by each individual.

Molecular pathologic investigation

Muscle biopsies were performed from the left or right biceps brachii in all patients. The specimen sections were stained according to the standard histologic and enzyme histochemical procedures. Blood acylcarnitines and urine organic acid profiles were analyzed in 16 patients by tandem mass spectrometry and gas chromatography-mass spectrometry, respectively. Variant screening was conducted in all patients and a part of family members. Genomic DNA (gDNA) was extracted from blood samples. Coding exons of the *ETFA*, *ETFB*, *ETFDH*, flavin adenine dinucleotide synthetase 1 (*FLAD1*), and solute carrier family 25 member 32 (*SLC25A32*) genes were amplified using polymerase chain reaction with intronic primers, and sequenced with an ABI 3730 DNA Analyzer (Applied Biosystems, Inc., Foster City, CA, USA).

Muscle magnetic resonance image

Muscle MRI scans of the thigh and leg were performed in all patients before riboflavin formula treatment. Spinal muscle MRI scans were conducted in six patients. Eight patients received thigh and leg MRI re-examinations after treatment with riboflavin formula. Conventional T1 weighted image (T1WI) sequence was used to evaluate the extent of fatty infiltration according to the modified Mercuri scale (0–5 scores).[17] An fatty score was adopted as follows: normal (score 0); punctate hyperintense (score 1); fatty streaks detected below 30% muscle volume (score

2); hyperintense detected among 30% to 60% muscle volume (score 3); hyperintense detected above 60% muscle volume (score 4); and hyperintense in the whole muscle (score 5). Short tau inversion recovery (STIR) sequence was used to assess the extent of edema-like change (0–5 score).[18] The scope of edema-like change was defined as segmented if they affected less than half of each muscle section or as global if they were distributed in more than half the section. An edema-like change score was adopted as follows: absent (score 0); mild interfascicular (score 1); mild intrafascicular segmented (score 2); mild intra-fascicular global (score 3); moderate intrafascicular segmented (score 4); and moderate intrafascicular global (score 5). Two doctors, who were blinded to all clinical data including the diagnosis and clinical characteristics, evaluated abnormal edema-like change and fatty infiltration in the different muscles using MRI scans. If differences were happened between them, the scores would be discussed by all co-authors together.

Statistical analysis

Data were analyzed using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA). The normality of variables distribution was evaluated by Kolmogorov-Smirnov test. Dichotomous variables were expressed as percentages and absolute frequencies, and continuous features with non-normal distribution were shown as median (Q1, Q3). Comparisons of categorical variables between groups were conducted by the Chi-squared test or Fisher's exact test, as appropriate. The Mann-Whitney U-test was used to compare continuous variables among groups. Correlations between MRI changes and various clinical parameters were evaluated by the Spearman's rank test. Forward multiple logistic regression was used to adjust the MRI features that were most informative for clinical diagnosis. A likelihood ratio test significance of 0.01 was selected to include variables in the model and maintain a manageable number of items in each formula. Sensitivity and specificity were calculated to evaluate the diagnostic values of the MRI signs based on the muscle patterns in 200 patients with non-MADD myopathy. Differences were considered statistical significance if $P < 0.05$.

Results

Clinical data

The cohort of patients with MADD included 15 males and 10 females. The median age at onset was 34.0 (22.3–44.5) years. Proximal muscle weakness was observed in 22 patients. The detailed muscle and extramuscular symptoms are shown in the Table 1. The median level of serum creatine kinase (CK) was 773.0 (413.5, 1538.5) U/L (normal 48.0–172.0 U/L). The median level of serum homocysteine was 76 (48, 105) µmol/L (normal 0–15 µmol/L) in 15 examined patients. Blood acylcarnitine profiles in 16 examined patients revealed a combined elevation of short-, medium-, and long-chain acylcarnitines in all patients. Urine organic acids analysis showed increase of multiple metabolic acids in seven patients, but the others were normal. EMG was conducted in nine patients, and there were myogenic pattern in three cases and normal pattern in the

Table 1: Clinical characteristics of the 25 patients with MADD in this study

Patient no.	Age (years)/ gender	Disease duration	Muscular symptom	Extramuscular symptoms	CK (U/L)	Hcy (μmol/L)	Gene mutation
1	36/female	1 year	LW, NW	DS, EA,SD	328.0	ND	c.1586A>G
2	59/female	5 years	LW, NW, myalgia, dysmasesia	DS, FL	148.0	ND	c.1827_1828insCAC*
3	36/male	6 months	NW, EI, dysmasesia	None	1305.0	ND	c.524G>T
4	19/female	10 days	LW, EI, dysmasesia	SD	3057.0	ND	c.250G>A, c.542G>A*
5	23/male	15 days	LW, NW, EI	EA	2973.0	99	c.1211T>C, c.265_266delCA*
6	55/male	1 month	LW, NW, EI, dysphagia	FL	334.0	ND	c.736G>A
7	46/male	4 months	LW, NW, EI, dysmasesia	DS,SD	798.0	42	c.1227A>C, c.176–1G>T*
8	35/female	2 months	LW, EI, myalgia	FL	1074.0	ND	c.389A>T, c.1586A>G
9	39/male	3 months	EI, myalgia	None	983.0	ND	c.295C>T, c.1227A>C
10	64/male	4 months	LWNW, EI, dysmasesia	OCD	1887.0	41	c.34G>C*, c.1454C>G
11	19/female	10 months	LW, NW, dysmasesia	DS	4653.0	ND	c.389A>T, c.740G>T*
12	35/male	3 years	LW, NW, EI	FL	627.0	ND	c.920C>G, c.1691–3C>G
13	37/male	10 days	EI, dysmasesia, dysphagia	None	1772.0	48	c.770A>G
14	11/male	11 years	LW, NE	SS	425.0	55	c.250G>A, c.736G>A
15	43/female	6 months	LW, NW, myalgia	DS, FL	773.0	ND	c.389A>T, c.1399G>C
16	46/male	3 months	LW, NW	SD, FL	568.0	108	c.770A>G, c.920C>G
17	24/male	1 month	LW, NW, myalgia, dysmasesia, rhabdomyolysis	DS, OCD	27090.0	45	c.389A>T, c.1227A>C
18	22/female	14 days	LW, NW, EI	None	163.0	62	c.303T>A, c.1450T>C
19	36/male	2 months	LW, NW, dysmasesia, dysphagia	DS, EA	1038.0	119	c.528G>C, c.1211T>C
20	17/female	1 month	LW, NW, EI	none	315.0	105	c.1586A>G
21	45/female	4 months	LW, NW, dysmasesia	DS, FL	2509.0	89	c.389A>T, c.1468+2T>G*
22	33male	10 days	LW, NW, myalgia, dysmasesia	DS	402.0	76	c.250G>A, c.1395T>G
23	19/female	15 days	LW, EI, dysmasesia	None	852.0	126	c.1027T>C
24	31/male	1 year	LW, NW, EI	SS, FL	663.0	58	c.250G>A, c.1828G>A
25	27/male	1 month	LW, NW, EI, dysmasesia	DS, FL	425.0	77	c.35–2A>C*

*Means novel mutation. CK: Creatine kinase; DS: Digestive symptoms; EA: Exertional angina; EI: Exercise intolerance; FL: Fatty liver; Hcy: Homocysteine; LW: Limb weakness; MADD: Multiple acyl-coA dehydrogenase deficiency; ND: Not done; NW: Neck weakness; OCD: Obsessive-compulsive disorder; SD: Sensory disorder; SS: Short stature.

others. The nerve conduction studies indicated axonal sensory neuropathy in four patients with sensory disturbance. The muscle biopsies of all patients showed numerous small vacuoles in the type 1 fibers filled with lipid droplets deposition on oil red O staining.

Genetic variants

All patients were confirmed to have variants in the *ETFDH* gene including compound heterozygous variants in 17 patients and single heterozygous variant in eight patients [Table 1]. No causative variants were found in the *ETFA*, *ETFB*, *FLAD1*, and *SLC25A32* genes. The variants were cosegregated in the family members available. A total of 29 different variants were found in this study, including 21 previously reported and 8 novel variants (c.34G>C, c.35-2A>C, c.176-1G>T, c.265_266delCA, c.542G>A, c.740G>T, c.1468+2T>G, c.1827_1828insCAC). The pathogenicity of novel variants was evaluated according to the American College of Medical Genetics and Genomics (ACMG) criteria [Table 2].

Muscle MRI characteristics

The comprehensive muscle MRI of the lower limb showed a relatively mild involvement, but had variability in the extent of edema-like change and fat infiltration [Figure 1]. Among the 25 patients, 22 patients showed edema-like change with STIR high signal in their leg muscles, and 16 patients displayed edema-like change in their thigh muscles. Two patients were absent of visible fat infiltration in muscles, whereas the other 23 patients symmetrically developed muscle fat infiltration. As for paraspinal muscles, all six patients had fat infiltration in the cervical segment [Figure 2A]; and five patients showed fat infiltration in the lumber segment [Figure 2B].

At the leg level, the edema-like change in the soleus (SO), tibialis posterior (TP), and tibialis anterior (TA) muscle were obvious, but the peroneus longus and brevis (PLB), flexor digitorum longus (FDL), extensor hallucis longus (EHL), and gastrocnemius (GA) muscles were relatively intact (Spplementary Table 1, http://links.lww.com/CM9/A3). The fat infiltration in the leg muscles showed a similar pattern to that of edema-like change (Figure 2C; Supplementary Table 2, http://links.lww.com/CM9/A3). The scores of edema-like change and fat infiltration in the SO were significantly higher than those in the GA [Table 2]. There was an underlying correlation between edema-like change and fat infiltration in the SO muscle (coefficient: 0.896, $P < 0.001$). However, the edema-like change or fatty infiltration in the SO muscle had no correlation with the age, gender, duration of illness, proximal weakness, and CK level.

Table 2: Pathogenic analysis of novel variants in the *ETFDH* gene of this study

Novel variants	Protein change	Controls (frequency)	ExAC (frequency)	GnomAD database (frequency)	ACMG criteria
c.34G>C	p.A12P	0	0	0.000004061	Likely pathogenic
c.35−2A>C	p.A12_59delinsG	0	0	0	Pathogenic
c.176−1G>T	p.G58fs*3	0	0	0	Pathogenic
c.265_266delCA	p.Q89Vfs*5	0	0	0	Pathogenic
c.542G>A	p.S181N	0	0	0	Likely pathogenic
c.740G>T	p.G247V	0	0	0.000004065	Likely pathogenic
c.1468+2T>G	p.G490fs*15	0	0	0	Pathogenic
c.1827_1828insCAC	p.610insH	0	0	0	Pathogenic

ACMG: American College of Medical Genetics and Genomics criteria; ETFDH: Electron transfer flavoprotein dehydrogenase; ExAC: Exome aggregation consortium; GnomAD: Genome aggregation database.
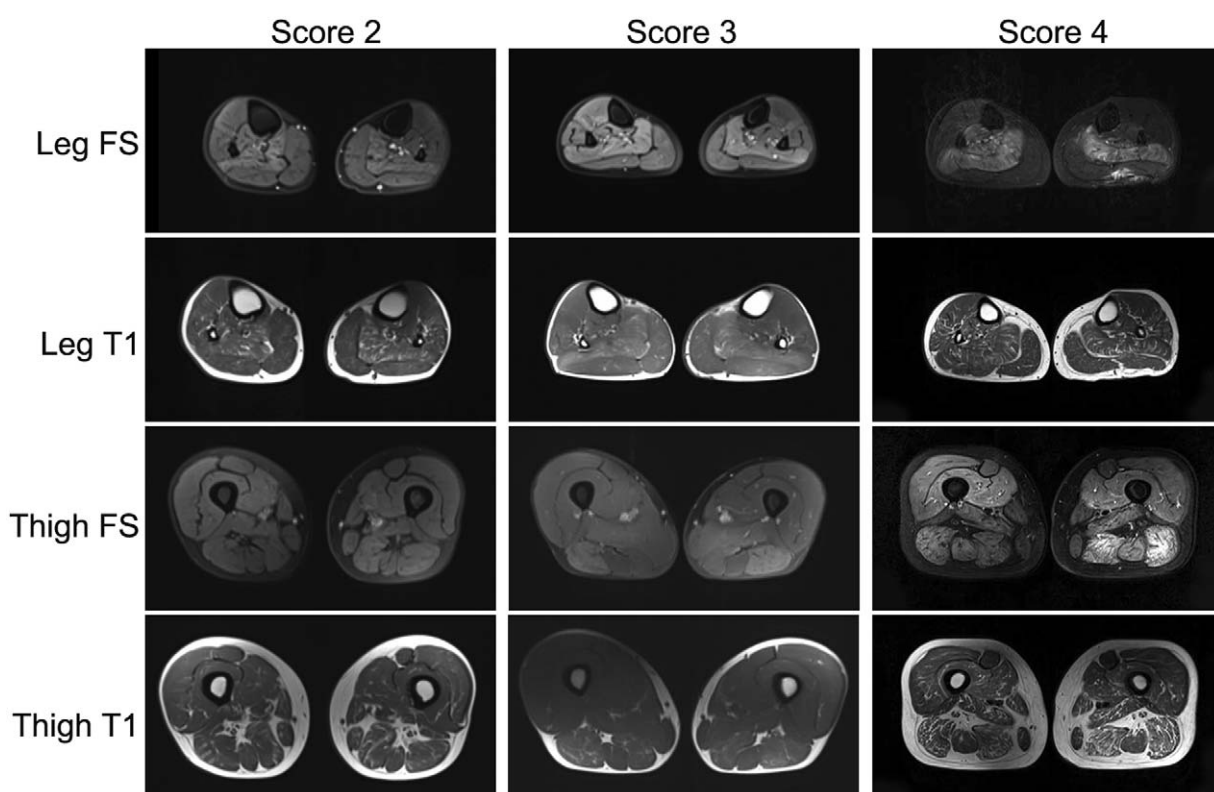

Figure 1: Muscle MRI revealed variability in the extent of edema-like change and fat infiltration in the patients with late-onset MADD. Edema score 2: patient 3; edema score 3: patient 19; edema score 4: patient 10. FS: Fat suppression; MADD: Multiple acyl-coA dehydrogenase deficiency; MRI: Magnetic resonance imaging.

At the thigh level, the edema-like change in the biceps femoris longus (BFL), semimembranosus (SM), and adductor magnus (AM) muscles were mildly detected, but the semitendinosus (ST), quadriceps femoris (QF), adductor longus (AL), biceps femoris brevis (BFB), sartorius (SA), and gracilis (GR) muscles were relatively not affected (Supplementary Table 3, http://links.lww.com/CM9/A3). The fat infiltration in the thigh muscles also had a similar pattern to that of edema-like change (Figure 2D; Supplementary Table 4, http://links.lww.com/CM9/A3). The scores of edema-like change and fat infiltration in the BFL were higher than those in the SM [Table 3]. There was an underlying correlation between edema-like change and fat infiltration in the BFL muscle (coefficient: 0.774, $P < 0.001$). However, the edema-like

change or fatty infiltration in the BFL muscle had no correlation with the age, gender, duration of illness, proximal weakness, and CK level.

In the eight patients with follow-up MRI, the STIR high signal in the SO and BFL muscles were quickly recovered at about 1 month after riboflavin treatment, but the signal of fat infiltration was not significantly improved on the T1WI at that time [Figure 3]. The fat infiltration completely disappeared at least 1 year after riboflavin treatment.

Diagnostic signs for MADD

The above observations indicated that edema-like change and fat infiltration significantly involved in the SO but

Figure 2: Muscle MRI features in late-onset MADD. (A) Cervical muscle MRI showed an involvement of fat infiltration in the paraspinal muscles. (B) Lumber muscle MRI showed an involvement of fat infiltration in the paraspinal muscles. (C) Coronal scan showed the fat infiltration obviously involved in the biceps femoris longus muscle but sparing of the semitendinosus muscle on the thigh level. (D) Similarly, a selective involvement in the soleus muscle but sparing of the gastrocnemius muscle on the leg level. MADD: Multiple acyl-coA dehydrogenase deficiency; MRI: Magnetic resonance imaging.

sparing of the GA (SO+/GA– sign) on the leg level, and significantly involved in the BFL but sparing of the ST (BFL+/ST– sign) on the thigh level [Figure 4]. To calculate the sensitivity and specificity of the diagnostic signs, 200 patients with non-MADD myopathy proven by muscle biopsy and/or genetic tests, including 67 patients with dystrophinopathy, 60 patients with LGMD2, 60 patients with inflammatory myopathy, and 13 patients mitochondrial myopathy, were adopted as disease controls (Supplementary Table 5, http://links.lww.com/CM9/A3). There were some differences in the demographics, clinical manifestations, and relevant laboratory data between patients with MADD and patients with non-MADD [Table 4]. After evaluating the SO+/GA– sign and BFL+/ST– sign under different combinations of edema-like change and fat infiltration [Table 5], a combination of

either SO+/GA– sign or BFL+/ST– sign was suboptimal. The diagnostic sensitivity and specificity of either SO +/GA– sign or BFL+/ST– sign for late-onset MADD were 80.0% and 83.5%, respectively. Multivariate analysis revealed that the combination exhibited a higher specificity in the patients with MADD than that in the patient with non-MADD myopathies ($P < 0.010$) independent of age, gender, duration of illness, and CK level [Table 6].

Discussion

This study reported a cohort of the patients with MADD, in which some clinical features and genetic mutations were expanded. The muscle MRI of lower limb revealed that edema-like change and fatty infiltration exhibited distinct

Table 3: Distribution of edema-like change and fat infiltration scores in the lower limb muscles of patients with late-onset MADD

Muscle	Edema scores	Z^*	P	Fat infiltration scores	Z^*	P
Thigh						
BFL	2.0 (0, 3.0)	−3.640	<0.001	1.0 (1.0, 1.5)	−4.456	<0.001
SM	2.0 (0, 2.0)	−3.900	<0.001	1.0 (1.0, 1.0)	−4.707	<0.001
AM	2.0 (0, 2.0)	−3.753	<0.001	1.0 (1.0, 1.0)	−4.600	<0.001
AL	0 (0, 1.0)	−1.947	0.052	0 (0, 1.0)	−2.236	0.025
QF	0 (0, 0)	−1.633	0.102	0 (0, 1.0)	−2.000	0.046
BFB	0 (0, 0)	0	1.000	0 (0, 0)	−1.000	0.317
SA	0 (0, 0)	−1.000	0.317	0 (0, 0)	−1.000	0.317
GR	0 (0, 0)	−1.000	0.317	0 (0, 0)	−1.414	0.157
ST	0 (0, 0)	−	−	0 (0, 0)	−	−
Leg						
SO	3.0 (2.0, 3.0)	−4.242	<0.001	2.0 (1.0, 2.0)	−4.332	<0.001
TP	2.0 (2.0, 3.0)	−3.923	<0.001	1.0 (1.0, 2.0)	−4.179	<0.001
PLB	0 (0, 0)	−1.633	0.102	0 (0, 1.0)	−2.928	0.005
FDL	0 (0, 0)	0	1.000	0 (0, 1.0)	−2.121	0.034
EHL	0 (0, 0)	−1.000	0.317	0 (0, 1.0)	−1.890	0.059
TA	0 (0, 2.0)	−2.449	0.014	0 (0, 0)	−1.414	0.157
GA	0 (0, 0)	−	−	0 (0, 0)	−	−

The data were shown as median (Q1, Q3). *Compared with ST muscles in thigh or GA muscles in leg. AL: Adductor longus; AM: Adductor magnus; BFB: Biceps femoris brevis; BFL: Biceps femoris longus; EHL: Extensor hallucis longus; FLD: Flexor digitorum longus; GA: Gastrocnemius; GR: Gracilis; MADD: Multiple acyl-coA dehydrogenase deficiency; PLB: Peroneus longus and brevis; QF: Quadriceps femoris; SA: Sartorius; SM: Semimembranosus; SO: Soleus; ST: Semitendinosus; TA: Tibialis anterior; TP: Tibialis posterior; −: Not applicable.
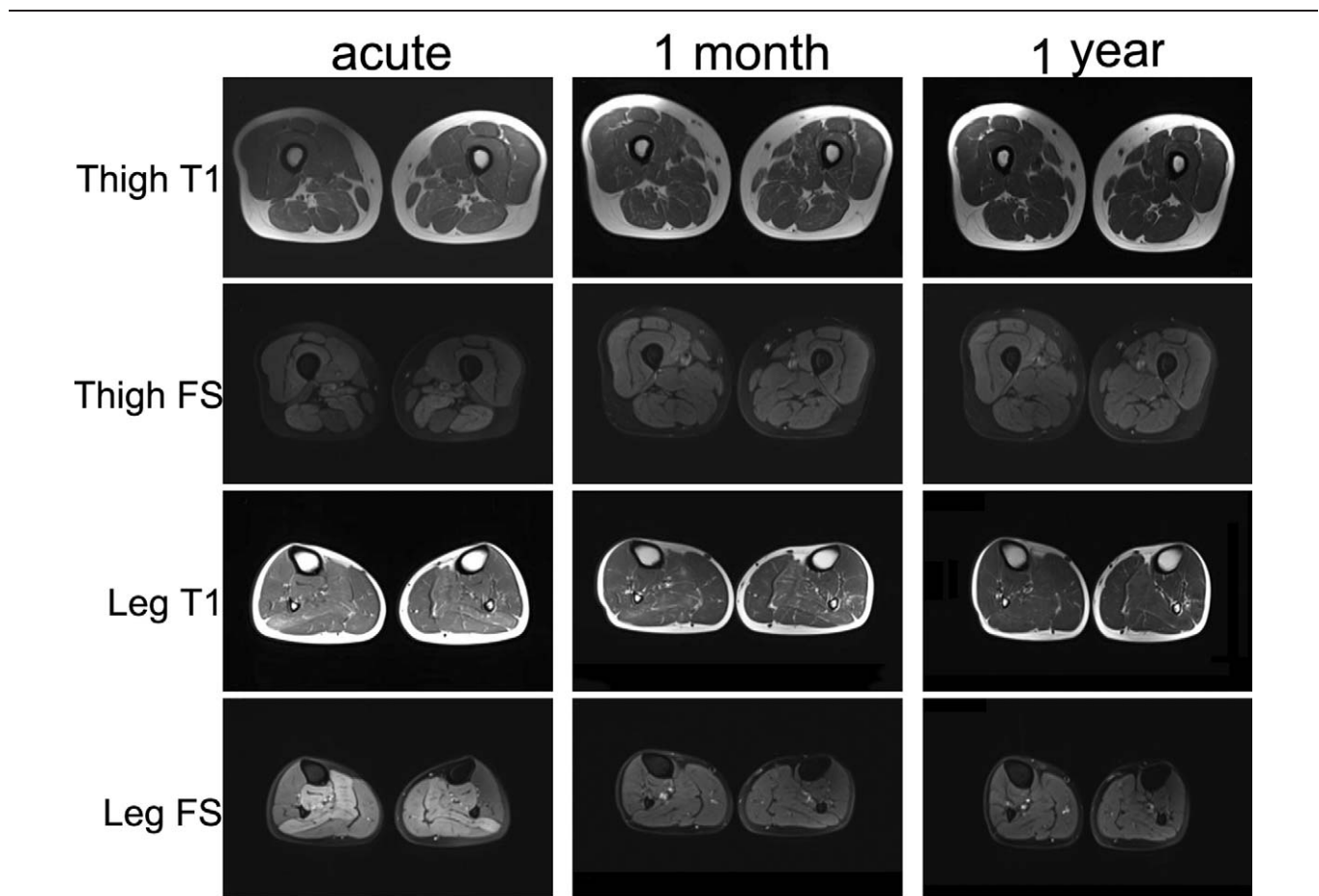

Figure 3: Dynamic changes of muscle MRI after riboflavin treatment. The 1st column showed fat infiltration and edema-like change before treatment. The 2nd column exhibited edema-like change was faded after a month. The last column indicated fat infiltration completely disappeared after a year. MRI: Magnetic resonance imaging.
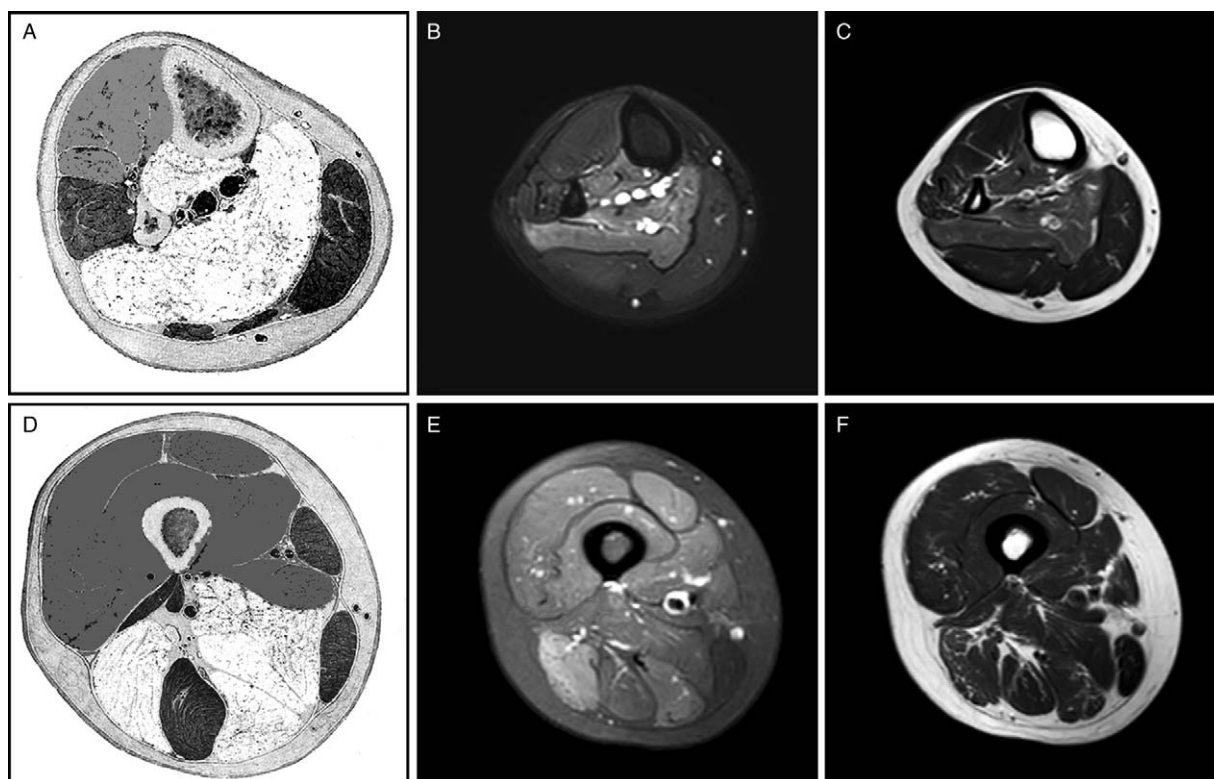
Figure 4: The schemes of the "SO+/GA−" sign (A) and "BFL+/ST−" sign (B). SO+/GA− sign indicated that both edema-like change (C) and fat infiltration (D) selectively involved in the SO but sparing of the GA. BFL+/ST− sign indicated that both edema-like change (E) and fat infiltration (F) selectively involved in the BFL but sparing of the ST. BFL: Biceps femoris longus; GA: Gastrocnemius; SO: Soleus; ST: Semitendinosus.

Table 4: Clinical characteristics of patients with MADD and non-MADD myopathy

Characteristics	Patients with MADD ($n=25$)	Patients with non-MADD ($n=200$)	Statistical values	P
Age (years)	35.0 (22.5, 44.0)	20.0 (7.0, 42.8)	−2. 901[*]	0.002
Age at onset (years)	35.0 (22.5, 44.0)	16.5 (4.0, 41.5)	−3.748[*]	<0.001
Disease duration (months)	3.0 (0.8, 8.0)	36.0 (1.0, 52.0)	−7.168[*]	<0.001
Male	15 (60.0)	118 (59.0)	0.012[†]	0.987
Proximal weakness	22 (88.0)	183 (91.5)	0.020[†]	0.887
CK (U/L)	773.0 (413.5, 1538.5)	4533.5 (2307.5, 7826.0)	−5.993[*]	<0.001

Data were shown as median (Q1, Q3), or n (%). [*]Z value. [†]χ^2 value. CK: Creatine kinase; MADD: Multiple acyl-CoA dehydrogenase deficiency.

patterns of muscle involvement in the patients with MADD. The combination of either SO+/GA− sign in the leg or BFL+/ST− sign in the thigh had a good sensitivity and specificity for the diagnosis of late-onset MADD.

Different from the scarcity of late-onset MADD in Western countries, a large number of patients with MADD with late-onset muscle type were identified by general neurologists in China recently.[3,5,19] The age at onset of muscle-type MADD usually varied from adolescent to adulthood, only a few number of young children with late-onset muscle-type MADD were reported.[2,7] No newborn-onset cases with pure muscle type were described up to now, because newborn-form patients usually died of severe metabolic disturbance or encephalopathy in the neonatal period.[20] Most intriguingly, this study found a newborn-onset case in this cohort of patients with MADD. The patient presented with isolated myopathy phenotype, and gradually got better with age. The disease progression mimicked that of congenital myopathy.[21]

Abnormalities of peripheral neuropathy were recently reported in patients with late-onset MADD.[8] This study confirmed the existence of the variant phenotype of late-onset MADD in four patients presenting with proximal limb weakness and loss of sensations in the distal limbs. Most patients only complained of hypoesthesia, but some patients presented with severe sensory ataxia and poor prognosis. In the first 15 cases of this cohort of patients, hyperhomocysteinemia was found in some patients by

Table 5: Evaluation of SO+/GA– and BFL+/ST– signs under different combinations of edema-like change and fat infiltration

Signs	Patients with MADD (N=25), n	Patients with non-MADD (N=200), n	Sensitivity (%)	Specificity (%)	Patients with non-MADD, n			
					DMD/BMD (n=67)	LGMD2 (n=60)	IM (n=60)	MM (n=13)
SO+/GA–(E only)	21	18	84.0	91.0	2	10	3	5
SO+/GA–(F only)	21	20	84.0	90.0	3	9	6	5
SO+/GA–(both E and F)*	17	19	68.0	90.5	3	8	3	5
SO+/GA–(either E or F)	17	22	68.0	89.0	3	11	6	5
BFL+/ST–(E only)	14	29	56.0	85.5	8	13	4	4
BFL+/ST–(F only)	19	69	76.0	65.5	45	14	4	6
BFL+/ST–(both E and F)*	14	25	56.0	87.5	8	9	4	4
BFL+/ST–(either E or F)	20	77	80.0	61.5	48	18	5	6
SO+/GA–(both E and F) or BFL+/ST–(both E and F)*	20	33	80.0	83.5	9	14	4	6

*The signs of both edema-like change and fat infiltration were suboptimal. BFL: Biceps femoris longus; DMD/BMD: Duchene/Becker muscular dystrophy; E: Edema-like change; F: Fat infiltration; GA: Gastrocnemius; IM: Inflammatory myopathy; LGMD: Limb-girdle muscular dystrophy; MADD: Multiple acyl-coA dehydrogenase deficiency; MM: Mitochondrial myopathy; SO: Soleus; ST: Semitendinosus; +: Involved; –: Non-involved.

Table 6: Multivariate logistic regression analysis of sensitivity and specificity of the SO+/GA– sign and BFL+/ST– sign between 25 patients with MADD and 200 patients with other myopathies

Signs	MADD patients, n	Patients with other myopathies, n	Sensitivity (%)	Specificity (%)	OR	95% CI	P
SO+/GA–	17	19	68.0	90.5	15.243	3.719–31.089	<0.001
BFL+/ST–	14	25	56.0	87.5	7.909	2.644–19.782	<0.001
SO+/GA– or BFL+/ST–	20	33	80.0	83.5	5.166	1.829–13.056	<0.001

BFL: Biceps femoris longus; CI: Confidence interval; GA: Gastrocnemius; MADD: Multiple acyl-coA dehydrogenase deficiency; OR: Odds ratio; SO: Soleus; ST: Semitendinosus; +: Involved; –: Non-involved.

chance. Hereafter we consecutively measured the level of serum homocysteine in the last ten cases. To our surprise, the level of serum homocysteine was significantly elevated in the patients with late-onset MADD. The hyperhomocysteinemia would be neutralized by supplement of riboflavin that is an important cofactor of methylenetetrahydrofolate reductase in folate metabolism cycle for homocysteine metabolism.[22] However, the underlying mechanism how the defects of ETF:QO cause hyperhomocysteinemia still needs to be investigated.

In the past a few years, there were increasing evidence that distinctive muscle MRI changes were useful to diagnose neuromuscular disorders showing specific patterns of muscle involvement.[10,12] However, the characteristics of muscle MRI about the late-onset muscle type of MADD was insufficient up to now. Rosenbohm and colleagues in 2014 found an increase subcutaneous fat but normal visceral fat in a young patient with ETFDH gene mutation through whole-body MRI.[23] Liu and colleagues[9] in 2016 reported that late-onset patients with MADD showed mild to severe fat infiltration and atrophy in the muscles of medial and posterior thigh compartment, as well as gluteus, but relatively sparing of the anterior thigh compartment. Moreover muscle edema pattern was not found in all those patients. Different from Liu et al's study,[9] our study and Zhao et al's study[16] evaluated each muscle in the thigh level, and identified that the fat infiltration mainly involved in femoris longus, semimem-

branosus, and adductor magnus muscles. Moreover, the edema-like change represented by STIR high signal was also found in the three muscles in more than half of our patients. The differences might be originated from the disputed edema-like change score scale,[18] because no good consensus about muscle edema-like change was currently reached.

The edema-like change in the soleus and tibialis posterior muscles, as well as the BFL and semimembranosus muscles were obviously observed in our patients. However, the detailed mechanism of STIR indicative edema-like change was still elusive. Given the lipid metabolic disorder of MADD happening at mitochondria, the STIR high signal might be associated with alteration in the mitochondrial metabolism that caused cytotoxic edema due to the dysfunction of Na-K-ATPase pump.[24] The STIR high signal faded more quickly than that of fat infiltration after riboflavin treatment. The phenomenon might suggest that muscle edema-like change had great contributions to muscle weakness in the pathologic process of MADD, and STIR signal might be potentially considered a biomarker of treatment response.

Most intriguingly, the muscles with edema-like change were generally identical to those with fat infiltration. Therefore, we suggested that the distinct patterns of selective muscle involvement, that is, a combination of either "SO+/GA–" sign or "BFL+/ST–" sign had a good

sensitivity and specificity for the diagnosis of late-onset MADD. To support the diagnostic values of the signs, muscle MRI characteristics should be differentiated from multiple muscular disorders. Mitochondrial myopathy exhibited a similarity with MADD, and also had a positive SO edema-like change and a negative GA edema-like change, but fat infiltration signal was unusually detected in both muscles.[25] The specificity of "SO+/GA−" sign or "BFL+/ST−" sign was high compared with other neuromuscular disorders, but if only compared with mitochondrial myopathy, the specificity would be decreased. Therefore, the signs should be cautiously differentiated in the metabolic myopathies. Dystrophinopathy showed a positive BFL fat infiltration and a negative ST fat infiltration, but no edema-like change can be simultaneously observed in both muscles.[13] Flaminoapthy displayed a positive SO or BFL fat infiltration and a negative GA or ST fat infiltration, but edema-like change cannot be simultaneously found in these muscles.[26] Therefore, both of edema-like change and fat infiltration signals simultaneously consistent with "SO+/GA−" and "BFL+/ST−" might facilitate a correct decision to molecular screening of late-onset MADD.

This study had some limitations that need to be explicitly acknowledged. First, it was a small-sample retrospective study; thus, some clinical data were incomplete that would lower the statistical power. A large-sample, prospective study of the muscle MRI characteristics should be conducted in the future. Second, the fact that the disease controls were quite heterogeneous undermined the reliability of the sensitivity and specificity of diagnostic signs. Confounders such as the age at onset, duration of illness, and CK level might have produced some bias with respect to results. Overall, the outcomes of this study should be cautiously interpreted. However, it seemed to indicate that the sensitivity and specificity of "SO+/GA−" sign or "BFL+/ST−" sign were higher compared with other neuromuscular disorders, and the regression models supported these results.

In conclusion, this study expands the clinical and genetic spectrums of late-onset MADD. The muscle MRI characteristics in the late-onset MADD shows distinct patterns and highlightes the diagnostic values. The dynamic change of STIR signal in the affected muscles might be potentially considered a biomarker of treatment response.

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Conflicts of interest

None.

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