Original Article

Clinical, Neuroimaging, and Pathological Analyses of 13 Chinese Leigh Syndrome Patients with Mitochondrial DNA Mutations

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Abstract

Background: Leigh syndrome (LS) is a rare disease caused by mitochondrial defects and has high phenotypic and genotypic heterogeneity. We analyzed the clinical symptoms, neuroimaging, muscular histopathology, and genotypes of 13 Chinese LS patients with mitochondrial DNA (mtDNA) mutations.

Methods: Mutations in mtDNA were identified by targeted sequencing. The brain imaging features on magnetic resonance imaging (MRI) were analyzed. The levels of lactate in fasting blood and cerebrospinal fluid (CSF) were routinely tested. The levels of urinary organic acids, plasma amino acids, and acylcarnitines were examined with gas chromatography–mass spectrometry and tandem mass spectrometry. The histopathological traits of skeletal muscles were analyzed under microscope.

Results: Among 13 patients, mutations of *MT-NDs* (n = 8) and *MT-ATP6* (n = 4) genes were most common. Strabismus (8/13), muscle weakness (8/13), and ataxia (5/13) were also common, especially for the patients with late-onset age after 2 years old. However, respiratory distress was common in patients with early-onset age before 2 years old. The most frequently affected brain area in these patients was the brain stem (12/13), particularly the dorsal part of midbrain, followed by basal ganglia (6/13), thalamus (6/13), cerebellum (5/13), and supratentorial white matter (2/13). Besides, the elevated lactate levels in CSF (6/6) were more common than those in serum (7/13). However, the analysis of abnormal plasma amino acid and urinary organic acid showed limited results (0/3 and 1/4, respectively). Muscular histopathology showed mitochondrial myopathy in the three late-onset patients but not in the early-onset ones.

Conclusions: Noninvasive genetic screening is recommended for mtDNA mutations in *MT-NDs* and *MT-ATP6* genes in patients with ophthalmoplegia, muscle weakness, ataxia, and respiratory disorder. Furthermore, the lactate detection in CSF and the brain MRI scanning are suggested as the diagnosis methods for LS patients with mtDNA mutations.

Key words: Clinical Features; Leigh Syndrome; Mitochondrial DNA; Neuroimaging; Pathology

Introduction

In 1951, Archibald Denis Leigh first described a boy with an aggravated clinical manifestation of subacute necrotizing encephalomyelopathy, which was later named as Leigh syndrome (LS).[1] LS is a progressive neurodegenerative disorder with an incidence of approximately 1/40,000.[2] The lesion is usually located in the basal ganglia and the brain stem of the patient, showing cavernous degeneration and necrosis.[3] LS occurs mainly in the 1st year of birth,

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especially between 3 months and 12 months. About 80% of patients have LS symptoms within 24 months after birth, while some patients have symptoms in late childhood, puberty, or adulthood, which is known as the late-onset LS.[4-7]

In 1996, Rahman *et al*.[2] proposed the criteria for LS diagnosis, including progressive nervous system disorders with motor and mental retardation, signs and symptoms of brain stem and/or basal ganglia disease, high lactate levels in blood and/or cerebrospinal fluid (CSF), and characteristic neuroimaging and/or typical neuropathology at postmortem or in a similarly affected sibling. However, the blood and/or CSF lactate levels are normal in some patients, and thus, the requirement for lactate levels has been eliminated from the recent diagnostic criteria. Moreover, with the development of molecular genetics, multiple mitochondrial diseases involving the brain, muscle, and/or other organs have been characterized. In the guideline proposed by Baertling *et al*.[4] in 2014, LS was defined as a neurodegenerative disease with variable symptoms due to mitochondrial dysfunction, which is caused by a hereditary genetic defect accompanied by bilateral central nervous system lesions that can be associated with further abnormalities in diagnostic imaging. Now, LS is considered as one of the most common mitochondrial diseases. However, as far as we know, the LS epidemiological data of the Chinese population are missing.

Herein, we retrospectively screened the LS patients diagnosed in our center. The clinical manifestations, neuroimaging signs, muscular pathology, as well as the serological and CSF characteristics of 13 patients positive for mitochondrial DNA (mtDNA) mutations were further analyzed. Our findings may facilitate the diagnosis of LS and provide some epidemiological data for LS in China.

Methods

Ethical statement

This study was approved by the Ethics Committee of Qilu Hospital of Shandong University. Informed consents were obtained from all participants or their guardians.

Patients

A total of 13 LS patients at Qilu Hospital of Shandong University between 2000 and 2017 were enrolled in this study, including 9 males and 4 females. The onset age ranged from 3 months to 10 years, with 4 patients younger than 2 years and 9 late-onset patients older than 2 years. Among these patients, two had positive family history. All the participants were included according to the diagnostic criteria[2,4] of (1) progressive neurological manifestations of LS, (2) symmetrical lesions located in the basal ganglia and/or brain stem on magnetic resonance imaging (MRI), and (3) presence of pathogenic mtDNA mutations. Patients were excluded if they were diagnosed with mitochondrial disease other than LS. Samples of peripheral blood, CSF, and muscle tissues were obtained.

Electroneurophysiological examination

Electroneurophysiological examination, including nerve conduction velocity (NCV) and electromyography (EMG), was performed in 4 patients (patient no. 1, 3, 10, and 12) according to routine procedure.

Laboratory examination

The fasting blood levels of lactic acid and creatine kinase (CK) levels were examined. CSF was collected for routine testing including lactate levels. The levels of urinary organic acids, plasma amino acids, and acylcarnitines were also examined with gas chromatography–mass spectrometry (Shimadzu GC-MS 2010) and tandem mass spectrometry (AB Sciex 3200 MD).

Neuroimaging analysis

The patients' imaging features on MRI were analyzed. Signal abnormalities on diffusion-weighted images (DWI), T1-weighted images (T1WI), and T2WI were recorded. Magnetic resonance spectroscopy (MRS) was available only in one of the 13 patients.

Muscle histological assay

Skeletal muscle biopsies were performed in 9 patients. Briefly, muscle samples were obtained from the musculus biceps brachii or musculus quadriceps femoris by open biopsy under local anesthesia. Muscle cryosections, with 10 μm in thickness, were stained with hematoxylin and eosin, modified Gomori trichrome, succinate dehydrogenase, cytochrome C oxidase (COX), oil red O (ORO), and periodic acid–Schiff (PAS). The histopathological traits of skeletal muscles were then analyzed under microscope.

Mitochondrial DNA mutation screening

The total DNA was extracted from 2-ml anticoagulant blood (n = 6) or muscle biopsy tissue (n = 7). Five most common point mutations (A3243G, A8344G, T8993G, T8993C, and T10191C) in mtDNA were directly screened. The whole mitochondrial genome sequencing was performed alone or when no pathogenic mutation was identified by targeted sequencing. The sequence results were then compared with MITOMAP (http://www.mitomap.org/MITOMAP) and the Human Mitochondrial Genome Database.[8]

Results

Genetic characterization

To identify mutations in mtDNA, DNA sequencing was performed. Five different pathogenic mutations in mtDNA genes of complex I subunits were identified in 8 patients, which were mitochondrial NADH dehydrogenase gene 3 (MT-ND3) T10191C (n = 3), MT-ND5 G13513A (n = 2), MT-ND4 C11777A (n = 1), MT-ND4 G11778A (n = 1), and *MT-ND6* G14459A (n = 1), respectively. In addition, 3 patients had *MT-ATP6* T8993C mutation and 1 patient was with *MT-ATP6* T9185C mutation related to complex V. Mitochondrial tRNA for leucine 1 (MTTL1) A3243G mutation was found in one patient.

Clinical manifestations

Patients' clinical presentations were summarized in Table 1. The clinical manifestations included short stature, psychomotor retardation or regression, muscle weakness, hypotonia, ptosis, ophthalmoplegia, optic atrophy, hearing impairment, ataxia, tremor, dystonia, and respiratory dysfunction. Ophthalmological manifestations (9/13) included strabismus ($n = 8$), ptosis ($n = 4$), nystagmus ($n = 2$), and optic atrophy ($n = 1$), which were mostly present in the late-onset patients. In addition to hypotonia (6/13), symptoms of muscle weakness (8/13) and ataxia (5/13) were also common, particularly in the late-onset patients. Dyskinesia mainly manifested as tremor (4/13) and dystonia (4/13). Respiratory disturbance was the most

Table 1: Clinical characteristics and mtDNA mutations of the study cohort

Characteristics	Patient number						
	1	2	3	4	5	6	7
Gender	Male	Female	Male	Male	Male	Male	Male
Age at onset	7 years	2.5 years	10 years	14 months	4 years	2.8 years	7 years
Family history	–	–	–	–	–	–	–
Short stature	+	+	–	+	–	+	–
Mental retardation	–	–	–	+	+	–	–
Weakness	–	+	–	–	+	+	–
Hypotonia	–	+	–	–	–	+	–
Abnormal reflexes	–	+	+		+	–	–
Strabismus	–	+	+	–	+	+	+
Ptosis	–	+	–	–	–	–	–
Nystagmus	–	–	+	–	+	–	–
Optic atrophy	–	–	–		–	–	–
Hearing impairment	+	–	–	–	–	–	–
Tremor	+	+	–	+	–	+	–
Ataxia	+	+	–	–	+	+	+
Dystonia/spasticity	–	–	+	+	–	–	–
Respiratory distress	–	–	–	–	–	–	–
Others	GI trouble and RT	T1DM	PN				LVNC and WPW
Gene	MT-TL	MT-ND3	MT-ND3	MT-ND3	MT-ND4	MT-ND4	MT-ND5
Mutation and heteroplasmy	A3243G (67%, B)	T10191C (NA, B)	T10191C (90%, M)	T10191C (89%, M)	C11777A (74%, M)	G11778A (92%, M)	G13513A (46%, B)

Characteristics	Patient number					
	8	9	10	11	12	13
Gender	Female	Female	Male	Male	Male	Female
Age at onset	8 months	1 year	7 years	2.3 years	4 years	3 months
Family history	+	–	–	–	–	+
Short stature	–	–	–	–	–	–
Mental retardation	–	+	–	–	–	+
Weakness	+	–	+	+	+	+
Hypotonia	–	+	–	+	+	+
Abnormal reflexes	–	–	–	+	+	
Strabismus	+	–	+	+	–	–
Ptosis	–	–	+	+	+	–
Nystagmus	–	–	–	–	–	–
Optic atrophy		–	+	–	–	–
Hearing impairment	–	–	–	–	–	–
Tremor	–	–	–	–	–	–
Ataxia	–	–	–	–	–	–
Dystonia/spasticity	–	+	–	+	–	–
Respiratory distress	+	–	+	–	–	+
Others			AV block		PN	
Gene	MT-ND5	MT-ND6	*MT-ATP6*	*MT-ATP6*	*MT-ATP6*	*MT-ATP6*
Mutation and heteroplasmy	G13513A (81%, M)	G14459A (96%, B)	T8993C (100%, M)	T8993C (99%, B)	T8993C (95%, B)	T9185C (100%, B)

GI: Gastrointestinal; RT: Renal tubulopathy; T1DM: Type 1 diabetes mellitus; PN: Peripheral neuropathy; LVNC: Left ventricular noncompaction; WPW: Wolff-Parkinson-White syndrome; AV: Atrioventricular; B: Blood; M: Muscle; NA: Not available; +: Positive; –: Negative; mtDNA: Mitochondrial DNA.

frequent clinical manifestation in early-onset LS (2/4). No patient had an epileptic seizure. It was worth to mention that vomiting and bellyache with recurrent hypokalemia were the initial clinical complaints in patient no. 1 at age 7, and he was later diagnosed with Fanconi syndrome. Patient no. 2 had type I diabetes and required insulin injection. Patient no. 7 suffered from left ventricular noncompaction and preexcitation syndrome, and patient no. 10 had atrioventricular (AV) block. Patient no. 3 had lower motor NCV in the left common peroneal nerve. The sensory NCV was slow in the left ulnar nerve, left median nerve, bilateral superficial peroneal nerves, and bilateral sural nerves in patient no. 12. The NCV of motor and sensory nerves was normal in patient no. 1 and patient no. 10. The EMG revealed no significant change in all 4 patients (patient no. 1, 3, 10, and 12).

Neuroimaging analysis

All the participants received cranial MRI [Table 2]. We found that brain stem abnormalities including those in the medulla oblongata, pons, and midbrain were found in 12 patients. Furthermore, midbrain, especially the dorsal part, was the most frequently affected in brain stem [Figure 1]. Lesions were located in the basal ganglia in 3 patients with late-onset age and 3 patients with early onset. In addition, lesions were also found in the thalamus (6/13), cerebellum (5/13), and cerebral white matter (2/13) [Figure 2]. The lesions were symmetrically located in the above areas in general. Cortical atrophy was observed in 1 patient (patient no. 8) as well. Typically, the lesions were hypointense on T1WI, hyperintense on T2WI, and isointense or hyperintense on DWI. The peak of lactate in the brain lesions was present in patient no. 13 on MRS scanning [Figure 2].

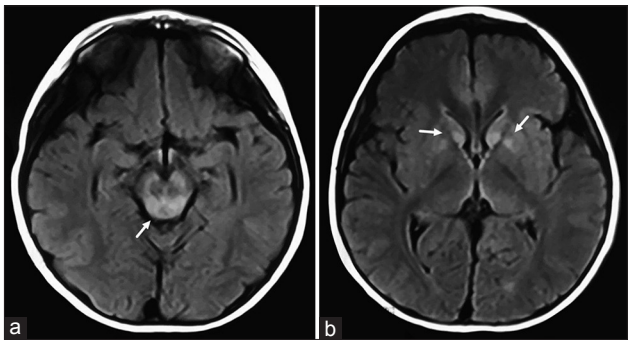

Figure 1: Brain MRI of patient no. 11. T2WI-FLAIR (a and b) showed hyperintensity in the midbrain and basal ganglia. MRI: Magnetic resonance imaging; T2WI: T2-weighted images.

Laboratory tests and histological features

The laboratory and histological findings were summarized in Table 3. The fasting plasma levels of lactate were increased in 7 patients. Serum CK was increased in patient no. 10 (1/13), with a value of 294 U/L (normal value: 38–174 U/L). Eight patients received lumbar puncture and CSF protein levels were mildly increased in 1 patient. All the six recorded patients had elevated lactic acid levels in CSF (6/6). Blood amino acid analysis was performed in 3 patients and no abnormality was found. Urine organic acid test was performed in 4 patients. Of them, 2 patients were with normal result, 1 was with nonspecific change of increased 3-hydroxybutyric acid indicating ketonuria, and another was with increased levels of lactate, 3-methylglutaconic acid, and 3-hydroxypropionic acid (3HPA).

A total of 9 patients underwent muscle biopsy. The skeletal muscle pathology showed no muscle fiber necrosis, regeneration, or obvious infiltration of inflammatory cells around muscle fibers and vessels. Ragged-red fibers (RRFs) and COX deficiency were found in 2 patients. The subsarcolemmal mitochondrial proliferation was detected in 1 patient. All the above 3 patients were late onset. ORO staining showed lipid accumulation in 7 patients including the 3 mitochondrial myopathy patients. Type II muscle fiber selective atrophy was detected in 1 patient (patient no. 5). All the components of muscle fiber glycogen were normal on PAS staining.

Table 2: The imaging results of patients

Patient number	Medulla oblongata	Pons	Midbrain	Basal ganglia	Thalamus	Cerebellum	Cerebral white matter	Cerebral atrophy
1			+		+	+	+	
2		+	+	+	+	+		
3			+	+				
4			+	+	+			
5			+		+	+		
6	+	+	+			+		
7			+		+			
8			+		+	+		+
9				+				
10			+					
11	+	+	+	+				
12		+	+					
13			+	+			+	

+: Positive.

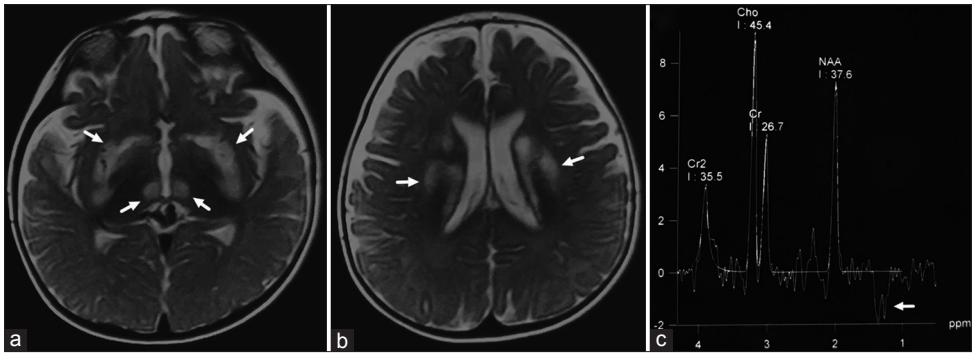

Figure 2: Brain MRI of patient no. 13. T2WI (a and b) showed symmetrical lesions in the bilateral basal ganglia, thalamus, and periventricular white matter; MRS (c) showed raised lactate peak in lesion of the basal ganglia. MRI: Magnetic resonance imaging; T2WI: T2-weighted images; MRS: Magnetic resonance spectroscopy.

Table 3: Laboratory and histological analysis of the clinical samples

Patient number	Age at biopsy	Serum lactate (mmol/L)	CSF lactate (mmol/L)	CSF protein (g/L)	RRFs or MP	COX deficiency	Lipid accumulation
1	12.3 years	2.33	ND	–	+	+	+
2	10 years	3.4	ND	0.8	+	+	+
3	16 years	0.9	2.7	0.19	–	–	–
4	16 months	1.08	ND	ND	–	–	+
5	9.7 years	2.18	3.7	0.33	+	–	+
6	3 years	6.0	3.1	0.11	–	–	+
7	ND	2.67	6.2	0.13	ND	ND	ND
8	8 months	1.7	ND	ND	–	–	+
9	2.8 years	1.2	ND	ND	–	–	+
10	7 years	2.6	4.4	0.19	–	–	–
11	ND	2.12	3.8	0.34	ND	ND	ND
12	ND	3.68	ND	ND	ND	ND	ND
13	ND	3.7	ND	ND	ND	ND	ND

RRF: Ragged-red fiber; MP: Mitochondrial proliferation; COX: Cytochrome C oxidase; ND: Not done; Normal value of lactate in blood and CSF was <2.2 mmol/L; Normal value of protein in CSF was <0.41 g/L; +: Positive; –: Negative; CSF: Cerebrospinal fluid.

Discussion

Mitochondrial energy production depends on the activity of the pyruvate dehydrogenase complex (PDHc) and mitochondrial respiratory chain (MRC) encoded by nuclear DNA (nDNA) and mtDNA. The correct assembly of mtDNA- and nDNA-encoded subunits promotes biogenesis of PDHc and oxidative phosphorylation complexes whereas wrong assembly may lead to mitochondrial diseases.[4,9] LS is a rare, devastating disease with phenotypic and genetic heterogeneity that often leads to disability and death. It usually happens before 2 years old.[2,3,5,10,11] However, in our current study, the onset ages ranged from early infancy to later childhood, with onset age of more than 2 years predominant. One possible reason is that we considered the onset of LS as the emergence of brain stem or basal ganglia signs or psychomotor regression with loss of acquired skills. Another possible reason is that our study was a single-center study, and the sample size was relatively small.

To date, more than 75 pathogenic gene mutations have been identified in LS.[12] In general, patients with mtDNA mutations accounted for about 20% of LS.[5,9] Complex I is the largest complex of the MRC, which contains 7 structural subunits (ND1, ND2, ND3, ND4, ND4L, ND5 and ND6) encoded by the mitochondrial genome, at least 38 core subunits encoded by nuclear genome and a few assembly factors.[13,14] Isolated complex I deficiency is the most common biochemical cause of LS.[2,5,8,12,15,16] In our study, of the 13 LS patients, 8 (62%) had mtDNA mutations associated to complex I deficiency (T10191C, G13513A, C11777A, G11778A, and G14459A). We found that MT-ND3 T10191C mutation and MT-ND5 G13513A mutation were the most frequent, consistent with previous findings.[12,15] The m.10191T>C mutation was the first pathogenic mtDNA mutation in the ND3 gene.[17] The T10191C mutation results in substitution of serine to proline (S45P) in a hydrophilic portion of the protein, which appears to affect catalysis much more than complex I assembly or stability.[18,19] The m.13513G>A mutation in ND5 gene was first described in an adult with MELAS phenotype.[20] ND5 is not essential for assembly of the other mtDNA-encoded subunits of complex I but essential for its activity.[21] *MT-ATP6* gene was the second common involvement in our patients, including 3 patients with m.8993 T>C mutation and 1 patient with m.9185T>C mutation, higher than previous reports from China.[15,22] Patients with the m.8993T>C mutation are

thought to have a milder clinical manifestations and slower progression compared with patients with the m.8993T>G mutation.[2,23] In addition, G11778A mutation in MT-ND4 is the most common mutation associated with Leber's hereditary optic neuropathy. We have confirmed that the new mutation C15620A could affect the pathogenicity of the G11778A mutation, which may in turn trigger LS.[24] All of these genotypes are not related to onset ages.

In general, patients with LS can present developmental regression, psychomotor retardation, muscular hypotonia or spasticity, dystonia, seizures, ataxia, dysphagia, nystagmus, ophthalmoparesis, etc. In addition, there were nonneurologic abnormalities as well.[4,7,23] In our study, the clinical features included developmental delay or regression, mental retardation, weakness, hearing impairment, visual deterioration, ophthalmoplegia, respiratory dysfunction, hypotonic, ataxia, and dyskinesia such as tremor and dystonia. We distinguished the early- versus late-onset LS by the age of 2 years according to the descriptions by Sofou *et al*. and Gerards *et al*.[5,25] Our data emphasized the clinical variability of LS onset. The most common syndromes were ophthalmological abnormalities and muscle weakness in our patients, especially in patients with late onset, and ataxia was also common in the late onset. Whereas, respiratory disorder was more frequent in the early onset. Strabismus was the most frequent ophthalmological manifestation, and ptosis, nystagmus, and optic atrophy were less common, which were all consistent with previous reports.[26,27] Epilepsy was not found in our patients, which is inconsistent with the 40–79% proportion in previous studies.[5,10,15] In the 10 LS patients with mtDNA mutations from China, 6 had epilepsy, and no one had ataxia or ophthalmoplegia.[15] In a previous study of 13 patients with onset of LS between 17 and 74 years, the most prominent clinical features were ataxia, spasticity, dysarthria, and abnormal ocular findings.[6] However, Ogawa *et al*.[16] found that besides regression and developmental delay, seizure and respiratory distress were the two major clinical symptoms in Japanese LS despite Asians. Gastrointestinal tract or renal tubular dysfunction is also reported in LS patients.[5,28] It is worth mentioning that patient no. 1 had a previously reported mutation at m.3243A>G in the tRNA Leu. Notably, we first report a LS patient genetically with A3243G mutation but showing gastrointestinal tract trouble as initial manifestation, who was later diagnosed with Fanconi syndrome. In addition, we found extra-nervous system changes in LS patients, including AV block, left ventricular noncompaction, preexcitation syndrome, and diabetes in our study. These findings indicate that we should make comprehensive assessment of systemic organs such as heart, kidney, liver, and endocrine in LS patients.

Brain stem and basal ganglia are considered as the most frequently impaired sites in LS. Some researches show that basal ganglia are the most common lesion area, with brain stem as the subsequent one.[8,23,29] In our study, MRI findings revealed an opposite result, showing that basal ganglia were the less common lesion area than brain stem. Moreover, basal ganglia were more frequently involved in the early-onset patients than the late-onset ones. We also found that some thalamus lesions were present without striatal lesions, different from the result of previous study.[30] The brain MRI showed symmetric lesions located in the cerebellum in 5 patients (5/13) in our study, with a higher ratio than other studies.[8,29] There was a case report of LS about a boy with primary presentation of cerebellum involvement.[31] Two of our patients had lesions in supratentorial white matter, consistent with the study by Sofou *et al*.,[23] which found that 9 of 31 mtDNA mutation patients had supratentorial white matter lesions. A study reported a case of LS with mtDNA mutation, in whom there was pathologically confirmed spongiotic-cystic necrosis throughout the white matter.[32] Arii and Tanabe reported a LS patient with initial lesions in the cerebral white matter and with A3243G mutation.[33] On the contrary, there are cohort studies indicating no supratentorial white matter lesion or leukoencephalopathy in LS patients with causative mtDNA mutations.[29,30] Lesions in LS may progress or regress over time, and repetitive imaging should be performed.[5,29] Severe ATP depletion could lead to increased lactate production, excessive production of reactive oxygen species, excitotoxicity, and gliosis contributing to lesion formation.[3] The difference in affected lesions may be related to the tolerance to oxidative damage. On MRS, Bonfante *et al*. described abnormal lactate peaks in 3 of the 7 patients (43%) and decreased N-acetylaspartate in the areas of encephalomalacia in 1 patient (14%).[29] Lee *et al*.[10] reported that all the 3 cases had a lactate peak on MRS (100%).[10] The sensitivity of increased lactate peak in the brain by MRS is higher than that of increased lactate levels in blood and/or CSF in some literature.[8,34] We found increased lactate peak of brain lesions in only one recorded patient who underwent MRS, and the sensitivity could not be counted due to the small sample.

A study showed that 25% of LS patients had a normal lactate level in serum, and patients with early onset before 6 months had an elevated lactate level in CSF.[5] However, we found that hyperlactacidemia was present in about 54% of our patients, but the lactate levels of CSF were increased in 100% of the patients, indicating the diagnosis limitation of blood lactate due to its low sensitivity. In addition, protein levels of CSF in 7 patients were normal. The concentration of amino acid and acylcarnitine in blood was normal in 2 recorded patients, who had normal lactate value in blood with increased lactate in CSF. Only 1 of the 4 recorded patients had abnormal organic acid in urine, such as lactate, 3-methylglutaconic acid, and 3HPA. Due to the reason that LS mainly involves the brain and that the increase of lactic acid in CSF suggests mitochondrial energy metabolism disorder in the brain parenchyma, we recommend an examination of lactate and protein levels in CSF to diagnose LS or identify other diseases.

There were a total of 9 patients undergoing a muscle biopsy. The pathological changes of skeletal muscle were relatively

mild. Type II muscle fiber atrophy and lipid accumulation were nonspecific. In a study including 8 patients with pathogenic mtDNA mutations, no RRFs or COX deficiency was found.[11] A previous study indicated that 9 of the 31 mtDNA mutation participants had mitochondrial myopathy pathology.[23] In our study, RRFs or subsarcolemmal mitochondrial proliferation and the muscle fibers with reduced or completely absent COX activity were found in only 3 patients with biopsied ages after 9 years, none in patients with early onset. This was similar to the results reported by Bannwarth *et al*.,[35] showing that the RRF and COX-defect fibers were increased with age in patients with mitochondrial disorders. Furthermore, the 3 cases had different mtDNA mutations. There is no relationship between genotype and skeletal muscular histopathology in our study. The negative changes of muscular histopathology may be attributed to tissue-specific effect or interaction between MRC complexes.

LS is a progressive neurodegenerative disease. In patients with late-onset LS after 2 years, ophthalmologic examination and tests for muscle weakness and ataxia should be routinely performed. And generally, it is better to check the heart, kidney, and endocrine system simultaneously. It should be considered when neuroimaging shows symmetrical lesions located in the brain stem and/or basal ganglia. The lactate level tests of CSF and MRS scan are helpful for LS diagnosis. Muscular biopsy in LS patients with late onset may find mitochondriopathy. However, these changes are rare in infants with early onset. We recommend noninvasive gene detection for those with early onset. *MT-NDs* and *MT-ATP6* are mutation hotspots in Chinese LS patients. This study may provide important clues for the diagnosis of LS.

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Conflicts of interest

There are no conflicts of interest.

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13例具有线粒体DNA突变中国Leigh综合征患者的临床、神经影像及肌肉病理分析

摘要

背景：Leigh综合征（LS）是一种由线粒体缺陷引起的罕见疾病，具有很高的表型异质性和基因型异质性。在本文中，我们分析了13例由线粒体DNA（mitochondrial DNA, mtDNA）突变所致的中国LS患者的基因型，临床表现，神经影像和肌肉组织病理学特点。

方法：通过靶向基因测序确定13例患者的mtDNA突变类型。分析患者的临床和颅脑MRI影像资料。常规测定空腹血乳酸和脑脊液乳酸水平。用气相色谱质谱和串联质谱技术分析患者的尿有机酸，血氨基酸及脂酰肉碱水平。镜下观察9例患者的活检肌肉病理。

结果：在本研究纳入的13名患者中，*MT-NDs*（n = 8）和*MT-ATP6*（n = 4）基因的突变是最常见的。此外，我们发现斜视（8/13），肌肉无力（8/13）和共济失调（5/13）也很常见，特别是对于发病年龄大于2岁的晚发型患者。然而，呼吸窘迫常见于发病年龄小于2岁的早发型患者。这些患者最常受累及的脑区是脑干（12/13），尤其是中脑背侧部，然后是基底节（6/13），丘脑（6/13），小脑（5/13）和幕上白质（2/13）。此外，乳酸水平升高在脑脊液（6/6）中比在血清（7/13）中更常见。然而，血氨基酸和尿有机酸分析得出的异常结果很有限（分别为0/3和1/4）。 9例LS患者的肌肉组织病理显示，有3例发病年龄较晚的患者具有线粒体肌病样改变，而在发病年龄较小的患者中没有发现线粒体肌病。

结论：对于mtDNA突变导致的LS，我们建议对具有眼外肌麻痹，肌肉无力，共济失调及呼吸损害的患者针对*MT-NDs*和*MT-ATP6*基因进行无创性遗传筛查。同时，我们仍建议将脑脊液乳酸测定和颅脑MRI扫描作为mtDNA突变所致LS的诊断方法。