Myoclonic epilepsy with ragged red fibers syndrome associated with mitochondrial 3302A>G mutation in the MT-TL1 gene: A case report

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Received August 2, 2022; Accepted December 19, 2022

DOI: 10.3892/etm.2023.11786

Abstract. A 37-year-old woman presented with proximal limb weakness, an unstable gait, tiredness and paroxysmal jitters. Neurological examination showed decreased deep tendon reflexes and positive signs indicating damage to the cerebellum. The patient's children reported no symptoms but were found to have the mitochondrial 3302A>G mutation in the mitochondrially encoded tRNA-Leu (UUA/G) 1 gene. The patient presented with increased blood lactic acid and lactic acid dehydrogenase levels, myopathy-related limb muscle electromyographic activities, ragged red fibers (RRFs), cytochrome oxidase-negative muscle fibers and mitochondrial 3302A>G mutation. Inverted lactic acid peaks in the basal ganglia, an atrophied cerebellum and multiple electroencephalographic spike waves were also observed. Therefore, myoclonic epilepsy with RRFs syndrome with the 3302A>G mutation was considered.

Introduction

Myoclonic epilepsy with ragged red fibers (MERRF) syndrome is a type of mitochondrial encephalomyopathy characterized by progressive myoclonic epilepsy, ataxia and RRFs, mostly caused by the 8344A>G mitochondrial DNA mutation. Myoclonic epilepsy is only present in 20% of patients and can be either continuous or intermittent, which is usually evoked and aggravated by light and other activities. It was reported that most of the patients with generalized tonic-clonic seizures, and some with atonic and absence seizures and focal seizures (1). Generalized epilepsy might represent a putative negative prognostic factor as it was very common to all deceased patients (2). In addition, ataxia, eyelid ptosis, hearing loss, myopathic signs and symptoms, cognitive impairment, neuropathy, exercise intolerance and multiple lipomatosis are also common clinical features (2).

Most patients with MERRF syndrome (80%) have a positive family history but phenotypes among family members vary significantly, which might partially be explained by the difference in the heteroplasmy of mutated mtDNA (2). MERRF syndrome in adolescents may progress very rapidly, with a fatal outcome, but in early adulthood or childhood, it often progresses slowly. The mean age of onset was reported to be 35 years old approximately (2), and the eldest age of death was reported to be 79 years old and the youngest age of death was 7 years old (3).

The 8344A>G mutation in the mitochondrially encoded tRNA lysine gene has been found in ~80% of patients with MERRF syndrome (4), and the prevalence of this point mutation in the adult population varies from 0.39:100,000 to 1.5:100,000 (5,6). The 3243A>G mutation in the mitochondrially encoded tRNA-Leu (UUA/G) 1 (MT-TL1) gene is even more prevalent (1). However, to the best of our knowledge, there is no report of an association between mitochondrial 3302A>G mutation in the MT-TL1 gene and MERRF syndrome so far. The present report describes a case of MERRF syndrome with the 3302A>G mutation.

Case report

A 37-year-old woman presented to the Jiangxi Provincial People's Hospital (Nanchang, China) in December 2018 complaining of tiredness after doing relatively little housework and weight loss after the birth of a second child at the age of 25 years, since then the symptoms had slowly worsened. Starting at the age of 32 years, the patient had felt proximal limb weakness, noted an unstable gait and occasionally fell. In the 2 weeks before admission to the hospital, paroxysmal jitters had repeatedly (1-5 times/day; ~1 min each time) occurred in the limbs and trunk, with occasional loss of consciousness.

The patient's mother had a history of fatigue and weight loss and died of a lung infection. The patient's son (15 years old)

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Key words: myoclonic epilepsy with ragged red fibers syndrome, mitochondrial DNA, gene mutation, MT-TL1 gene, mitochondrial encephalomyopathy

and daughter (13 years old) reported no symptoms, but whole-exome sequencing with next-generation sequencing (NGS) method on the Illumina HiSeq 3000 Sequencing Systems in the Laboratory of the Wuhan Kindstar Diagnostics Co., Ltd. with the use of Clinvar (accession number: VCV000689871.2) and the Revised Cambridge Reference Sequence (GenBank accession number: NC_012920.1) in the Mitomap databases for the validated NGS assay (7-9) showed the presence of a mitochondrial 3302A>G mutation. HiSeq 3000 Sequencing Systems in the Laboratory of the Wuhan Kindstar Diagnostics Co., Ltd. with the use of Clinvar (accession number: VCV000689871.2) and the Revised Cambridge Reference Sequence (GenBank accession number: NC_012920.1) in the Mitomap databases for the validated NGS assay (7-9) showed the presence of a 3302A>G mutation.

The patient was short (height, 150cm) and slender (body weight, 28.5kg) with normal limb muscle tone. Neurological examination showed decreased deep tendon reflexes, a positive Romberg test and positive finger-to-nose and heel-knee-shin tests. Assessment of the cranial nerves was unremarkable. Biochemical tests showed increased serum lactate dehydrogenase (305 IU/l; normal range: 114-240 IU/l), normal level of creatine kinase and increased serum lactate (3.34 mmol/l; normal range: 0.5 to 2.2 mmol/l) and cerebrospinal fluid (CSF) lactate (2.55 mmol/l; normal range: 0-2.0 mmol/l). Myopathy-related electromyographic activities in bilateral quadriceps, right deltoid and sternocleidomastoid muscles, and right thoracic paraspinal muscles at T10 and T11 levels were found.

For comparison purposes, corresponding magnetic resonance imaging (MRI)/magnetic resonance spectroscopy (MRS) and histopathological pictures from a 'healthy' control subject are also shown (Fig. 1). The control subject was a 47-year-old woman who was admitted to the Jiangxi Provincial People's Hospital owing to repeated convulsions of all four limbs for 3 months with aggravation and limb weakness for 20 days. The control subject started to have limb convulsions without any stimulation 3 months before admission, which happened 3 times/week for 10 min each time and with no symptoms between episodes. In the 20 days before admission, the convulsion attack frequency increased to 1-2 times/day, with an attack duration of 10-20 min. Following admission, a series of tests were performed on the control subject and normal electroencephalographic examinations, hypocalcemia (blood calcium, 1.71 mmol/l; normal range: 2.0-2.6 mmol/l), hyperphosphatemia (blood phosphate, 2.16 mmol/l; normal range: 0.73-1.35 mmol/l) and a low blood parathyroid hormone level (12.34 pg/l; normal range: 15-65 pg/l), were recorded. A diagnosis of hypoparathyroidism was considered. Head MRI examinations were performed to determine if the hypoparathyroidism was caused by pituitary lesions, but normal findings were shown. In addition, the blood creatine kinase level was found to be elevated (creatine kinase, 860 IU/l; normal range, 40-200 IU/l) and an electromyographic examination of the limbs suggested suspicious myogenic myopathy. However, no abnormality was found in the histopathological examination of the right quadriceps femoris muscle. Therefore, hypoparathyroidism myopathy was excluded from the diagnosis and the increased blood creatine kinase in the patient was considered as a result of limb convulsions.

For the aforementioned histopathological examination, a 0.5x0.5x1-cm piece of muscle was taken from the right quadriceps femoris (15 cm above the knee) of the patient and the control subject with informed consent; the muscle tissue was frozen with isopentane pre-chilled in liquid nitrogen and attached on the cryostat chuck. The tissue block was allowed to equilibrate to the cryostat temperature (-25˚C) before cutting 7-μm sections from it and placing them onto slides. The sections of skeletal muscle tissue were processed and stained according to standard protocols with hematoxylin and eosin (H&E), modified Gomori trichrome (MGT), succinate dehydrogenase (SDH) and cytochrome *c* oxidase (COX), respectively, as previously published (10). Briefly, the sections were stained at room temperature with Harris' hematoxylin for 3 min and with 1% eosin for ≥20 sec after rinsing in running distilled water for H&E staining, or with Harris' hematoxylin for 5 min and with Gomori trichrome mixture for 10 min (until green) after rinsing in distilled water for MGT staining. In addition, the sections were incubated flat in a damp atmosphere at 37˚C for 90 min for SDH staining or in a cytochrome oxidase incubating medium at 37˚C for 3 h for COX staining. All chemical reagents were purchased from Sinopharm Chemical Regent Co., Ltd. Compared with the histopathological findings in the control subject (Fig. 1), the histopathological examination of the skeletal muscle from the patient demonstrated RRFs containing aggregates of abnormal mitochondria that appeared subsarcolemmally and scattered through some muscle fibers as inhomogeneous purple-red granular areas in H&E staining, and abnormal aggregates of mitochondria appeared as reddish blotches, mostly at the periphery of muscle fibers in MGT staining (Fig. 2). In addition, muscle fibers with excessive SDH staining and negative COX staining were found in the patient (Fig. 2), but not in the control subject (Fig. 1).

Compared with the results of the head MRI in the control, the cerebellar sulci of the patient were significantly widened and the fourth ventricle was significantly enlarged (Fig. 2). At the same time, the inverted lactate peaks could be seen at 1.33 ppm in the MRS of the patient (Fig. 2), but not in the MRS of the control (Fig. 1); the inverted lactate peak in the left cerebellum was larger and deeper than that at the anterior horn of the right lateral ventricle in the patient (Fig. 2). In addition, compared with that in the control subject, the left cerebellum, but not the anterior horn of the right lateral ventricle, was atrophied in the patient, and the anterior horn of the right lateral ventricle had no lesions in the patient (Fig. 2). Moreover, a mitochondrial 3302A>G mutation in the MT-TL1 gene was identified in the blood sample from the patient (Fig. 2) and multiple electroencephalographic spike waves were found. Chest computed tomography showed bilateral bronchiectasis and pulmonary infection, as well as atelectasis in the right middle lobe. Therefore, the patient was diagnosed with MERRF syndrome with the 3302A>G mutation.

The patient was treated with antiepileptic drug levetiracetam (500 mg, bid), idebenone (30 mg, tid), coenzyme Q10 (10 mg, tid), vitamin B2 (20 mg, tid), and levocarnitine (1 g, tid), and the patient's myoclonic seizure were controlled, and fatigue after activity was improved at discharge. The

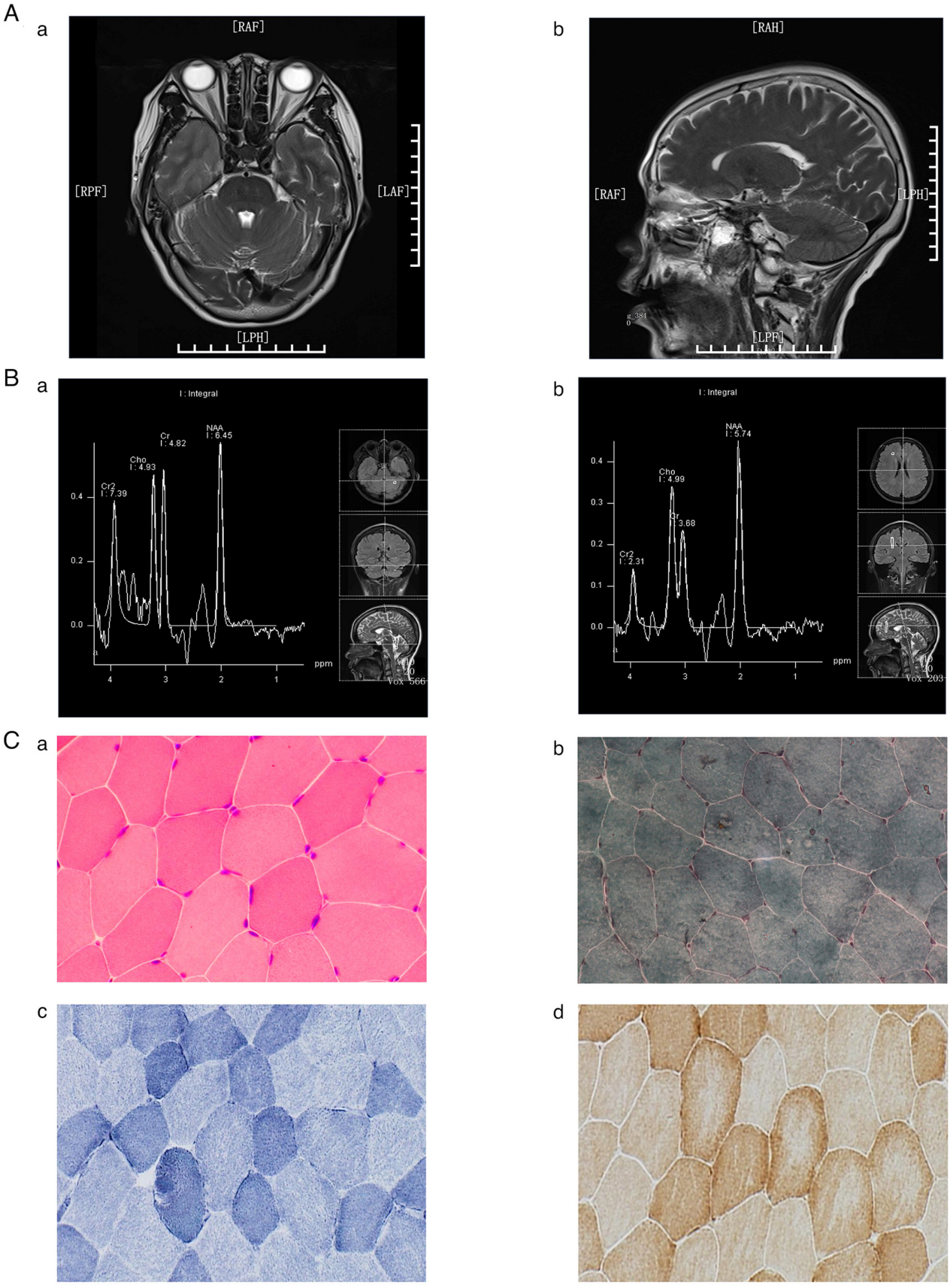

Figure 1. MRI, MRS and histopathological examinations in the control subject. (Aa) Transverse and (Ab) sagittal views of the head MRI T2-weighted image. MRS of (Ba) the left cerebellum and (Bb) the anterior horn of the right lateral ventricle. Histopathological examinations through (Ca) H&E, (Cb) MGT, (Cc) SDH and (Cd) COX staining. Normal MRI and MRS results were recorded. In addition, no ragged red fibers were found on H&E and MGT staining, and no muscle fibers with excessive SDH or absent COX staining were found. (Ca and Cb, x400 magnification; Cc and Cd, x200 magnification). MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; MGT, modified Gomori trichrome; SDH, succinate dehydrogenase; COX, cytochrome *c* oxidase; RAF, Right anterior foot; LAF, Left anterior foot; RPF, Right posterior foot; LPH, Left posterior head; Cr2, creatine 2; Cho, Choline; Cr, Creatine; NAA, N-acetylaspartate.

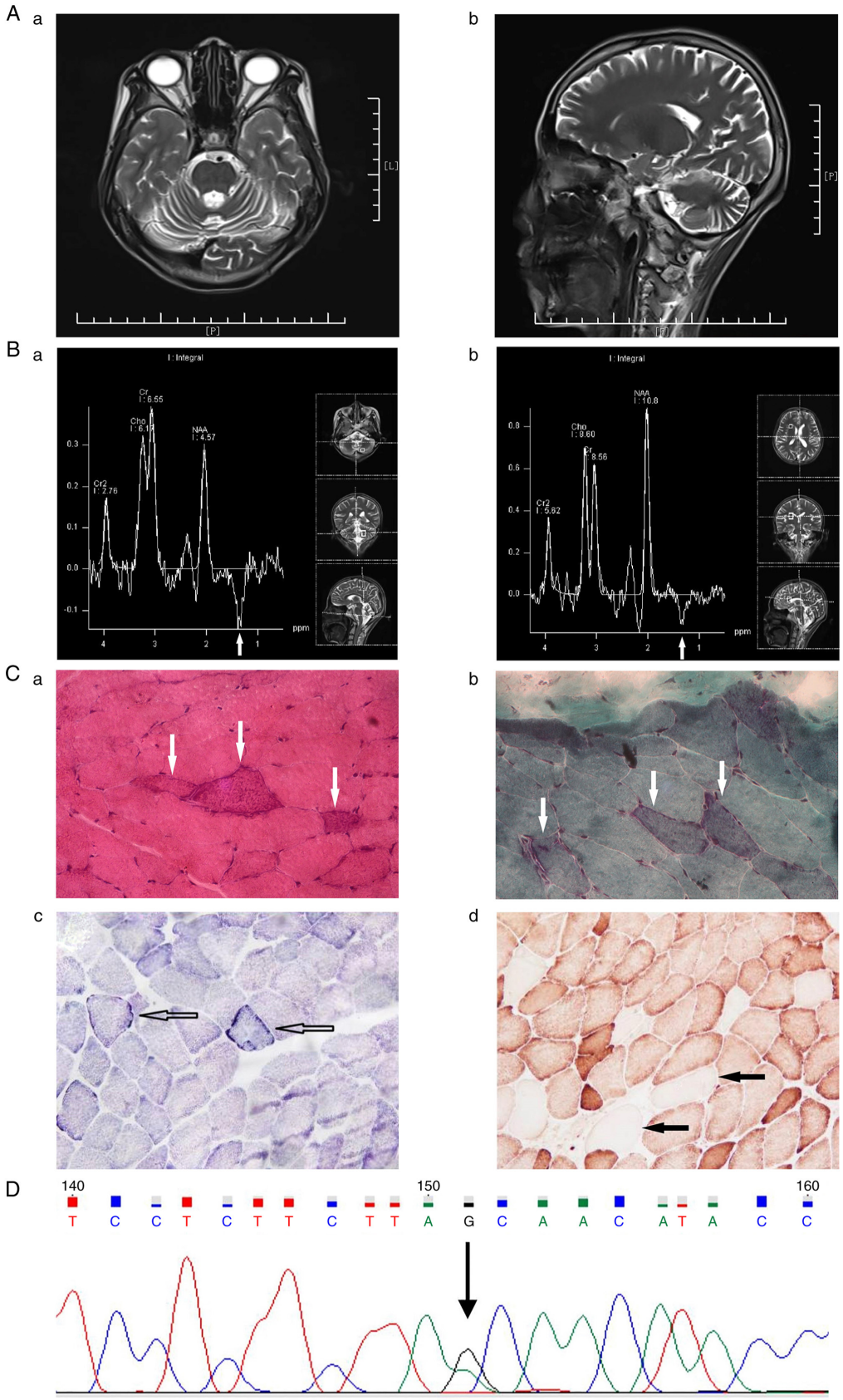

Figure 2. MRI imaging, MRS and histopathological studies in the patient. (Aa) Transverse and (Ab) sagittal views of the head MRI T2-weighted image. MRS of (Ba) the left cerebellum and (Bb) the anterior horn of the right lateral ventricle showed inversed lactate peak. (Ca) H&E staining showing 'ragged red fibers', as indicated by white arrows. (Cb) MGT staining showing abnormal aggregates of mitochondria appearing as a reddish blotch, as indicated by white arrows. (Cc) SDH staining showing muscle fibers with excessive SDH, as indicated by empty arrows. (Cd). COX staining showing muscle fibers with absent COX staining, as indicated by solid arrows. (D) Mitochondrial 3302A>G mutation in the MT-TL1 gene. (Ca and Cb, x400 magnification; Cc and Cd, x200 magnification). MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; MGT, modified Gomori trichrome; SDH, succinate dehydrogenase; COX, cytochrome *c* oxidase; RAF, Right anterior foot; LAF, Left anterior foot; RPF, Right posterior foot; LPH, left posterior head; Cr2, creatine 2; Cho, Choline; Cr, Creatine; NAA, N-acetylaspartate.

patient follow-up was performed every 6 months. After discharge, the patient got respiratory infection several times, and recently the patient passed away at home due to severe respiratory infection. The patient's son and daughter are currently asymptomatic.

Discussion

MERRF syndrome is a multisystem disorder characterized by myoclonus, which often starts as the first symptom in childhood after normal early development (11). The classic features of MERRF syndrome include myoclonic epilepsy, ataxia and RRFs. In addition, hearing loss, short stature, optic atrophy and cardiomyopathy, as well as occasionally pigmented retinopathy and lipoma, may also be present (2). The main symptoms in the present patient were consistent with the clinical manifestations of MERRF syndrome.

MRI often reveals cortical atrophy, basal ganglia calcification, white matter abnormalities and brain stem atrophy in patients with MERRF syndrome (11). In the present patient, cerebellar atrophy was shown. Moreover, MRS showed inverted lactic acid peaks in the cerebellum and basal ganglia, which was consistent with the increase in lactic acid level in the serum and CSF. Increased lactic acid levels at rest and after moderate activity indicate mitochondrial dysfunction, and elevated levels of lactic acid in the serum and CSF are important indicators for MERRF syndrome (12).

Although RRF is a marker of MERRF syndrome, it may be missing in 5-10% of cases (11). The presence of RRFs and COX-negative fibers in muscle biopsy, as observed in the present patient, often confirms the diagnosis of MERRF syndrome (12). The manifestations of mitochondrial myopathy in the patient were prominent, but myoclonic epilepsy was not the first symptom, as in patients with the 8344A>G or 3243A>G mutation.

The 3302A>G mutation is related to the lack of respiratory chain complex I, which impairs the processing of RNA19 and leads to the accumulation of RNA precursor RNA19 in the muscles (13). The inefficient cleavage of the precursor RNA19 and defects in aminoacylation due to tRNA structural changes cause a decrease in mitochondria (14). These changes may explain the main symptoms, such as chronically progressive adult myopathy and exercise intolerance, in patients with the 3302A> G mutation. Patients with the 3302A>G mutation may have symptoms of mitochondrial myopathy, encephalopathy, lactic acidosis and stroke (MELAS syndrome) (15). However, it is considered that the 3302A>G mutation can also cause abnormalities in other systems (14). It has been reported a high mutation load of the 3302A>G mutation can lead to fatal cardiorespiratory failure (16). In addition, some patients may develop type II diabetes mellitus and polycystic ovary syndrome (17). Nevertheless, symptoms of the muscular system appear earlier and are more prominent (18), as shown in the present case.

As the phenotype of this gene locus (mitochondrial 3302A>G) is highly heterogeneous, symptoms may vary and the age of onset may not be limited to adulthood. The phenotypic heterogeneity in the patient with MERRF syndrome reported in the present study was demonstrated by chronic progressive adult myopathy, stroke-like episodes, lactic acidosis, ataxia and myoclonic epilepsy.

Acknowledgements

The authors would like to thank Ms. Zhen Li of the Neurological Institute of Jiangxi Province for tissue sectioning and histological staining.

Funding

This case report was supported by Jiangxi Provincial People's Hospital (grant no. 2019-009).

Availability of data and materials

All data generated and/ or analyzed during this study are included in this published article.

Authors' contributions

GH, YW and DY collected, analyzed and interpreted the patient data, and advised on treatment. GH performed the pathological examination. GH, YW and DY confirmed the diagnosis and confirm the authenticity of all the raw data. GH and DY wrote the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Written informed consent was obtained from the patient and control subject.

Patient consent for publication

The patient and control subject gave their consent for the publication of this case report and accompanying images.

Competing interests

The authors declare that they have no competing interests.

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