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CASE REPORTS

# A new phenotype of mitochondrial disease characterized by familial late-onset predominant axial myopathy and encephalopathy

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**Abstract** Axial myopathy is a rare neuromuscular disease that is characterized by paraspinal muscle atrophy and abnormal posture, most notably camptocormia (also known as bent spine). The genetic cause of familial axial myopathy is unknown. Described here are the clinical features and cause of late-onset predominant axial myopathy and encephalopathy. A 73-year-old woman presented with a 10-year history of severe paraspinal muscle atrophy and cerebellar ataxia. Her 84-year-old sister also developed late-onset paraspinal muscle atrophy and generalized seizures with encephalopathy. Computed tomography showed severe atrophy and fatty degeneration of their paraspinal muscles. Their mother and maternal aunt also developed bent spines. The existence of many ragged-red fibers and cytochrome *c* oxidase-negative fibers in the biceps brachii muscle of the proband indicated a mitochondrial abnormality. No significant abnormalities were observed in the respiratory chain enzyme activities; however, the activities of complexes I and IV were relatively low compared with the activities of other complexes. Sequence analysis of the mitochondrial DNA from the muscle revealed a novel heteroplasmic mutation (m.602C>T) in the mitochondrial $tRNA^{Phe}$ gene. This familial case of late-onset predominant axial myopathy and encephalopathy may represent a new clinical phenotype of a mitochondrial disease.

**Keywords** Mitochondrial disease · Predominant axial myopathy · Encephalopathy · Late-onset · Familial case

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## Introduction

Camptocormia, a term coined by Souques and Rosanoff-Saloff from two Greek words (*kamptos* meaning bent and *kormos* meaning trunk), is characterized by involuntary trunk flexion in the erect position that disappears in the supine position. Camptocormia was initially described as a hysterical phenomenon that occurred in male soldiers during World Wars I and II [1, 16]. However, in the last 20 years camptocormia has been reported to be present with various organic diseases, including muscular dystrophies, inflammatory myopathies, dystonia, amyotrophic lateral sclerosis, myasthenia gravis, paraneoplastic syndrome, Parkinson's disease, multiple system atrophy, and spinal deformities, as well as in an idiopathic form. Camptocormia is also referred to as "bent spine syndrome" [1, 32].

Axial myopathy has been described as the selective involvement of the paraspinal muscles in camptocormia or dropped head. Axial myopathy has heterogeneous etiologies, including primary and various other neuromuscular disorders. Primary axial myopathy is characterized by the

insidious and progressive weakness of the extensor muscles of the spine, normal or slightly elevated serum creatine kinase (CK) levels, and a myogenic pattern on electromyography in the elderly. Muscle biopsies show nonspecific myopathic changes with fibrosis, fatty replacement, and variations in fiber size. In addition, some ragged-red fibers and complex I and III deficiencies have been observed; these findings are considered to be the age-related accumulation of various mitochondrial abnormalities [21, 31].

Some cases of autosomal dominant inheritance patterns of familial primary axial myopathy were reported several years ago; however, the genetic analyses that were used have not been described [31]. Recently, a novel heterozygous dominant mutation in the skeletal muscle ryanodine receptor gene was identified in the central cores of muscle biopsy specimens that were excised from sporadic cases of axial myopathy [15]. Furthermore, facioscapulohumeral muscular dystrophy with isolated axial myopathy has also been reported [19]. Five cases of axial myopathy that were associated with mitochondrial dysfunction have been previously reported; however, no familial cases of mitochondrial gene mutation have been reported [8, 11, 28, 30, 32].

In this paper, we have reported about a mitochondrial disease that is characterized by familial late-onset predominant axial myopathy and encephalopathy. In addition, the pathogenicity of a novel, familial, mitochondrial tRNA gene mutation is discussed.

## Methods

### Subjects

#### *Patient 1*

A 73-year-old woman (Fig. 1, III-8) presenting with abnormal posture and gait disturbance. Since the age of 63, the patient had a slight stooping posture and a pushed-out waist. At 68 years of age, she started using a walking stick because of her unstable gait. She was diagnosed with hypothyroidism by her family physician and administrated with 25 μg/day levothyroxine; however, her symptoms did not improve. At 70 years of age, it gradually became more difficult for her to climb the stairs. At 71 years of age, she was admitted to another hospital. Doctors suspected myopathy because of elevated serum CK levels. She visited our hospital presenting with prominent paraspinal muscle atrophy and mild proximal weakness of limbs. Hypothyroidism-related myopathy was suspected in her, and hence, the levothyroxine dose was increased to 50 μg/day; however, her symptoms did not improve. She had a family history of bent spine, i.e., in her elder sister (patient 2, Fig. 1, III-5), mother (Fig. 1, II-3), and maternal aunt (Fig. 1, II-4). Physical examination on arrival revealed a marked atrophy of the paraspinal muscles and abnormal posture (Fig. 2a, b). She also presented with right ptosis, dysarthria, bilateral cataracts, and hearing loss. Her eye movements were normal. But there was moderate weakness of the neck flexion and mild weakness of the proximal limb muscles. Tendon reflexes were symmetrical, and Babinski's sign was absent. She had poor balance with tandem gait without limb ataxia. Sensory systems were intact and Romberg's sign was negative. She scored poorly on the attention and calculation tests that are a part of the Mini-Mental State Examination (score: 25 points).

Laboratory data were as follows: serum CK level was 290 IU/l (normal range 45–163 IU/l), resting blood and cerebrospinal fluid (CSF) lactate levels were normal, thyroid-stimulating hormone levels were slightly low at 0.47 μIU/ml (normal range 0.5–5.0 μIU/ml). Under the administration of 50 μg/day levothyroxine; antithyroglobulin antibody levels were high at 7.0 U/ml (normal range <0.3 U/ml), antithyroid peroxidase antibody levels were high at 46.5 U/ml (normal range <0.3 U/ml), rheumatoid factor levels were high at 152.3 IU/ml (normal value <15.0 IU/ml), antinuclear antibody levels were mildly elevated (titer of 1:80). Autoimmune analyses, including anti-Jo-1, anti-RNP, anti-SS-A, and anti-SS-B, were negative. The oral glucose tolerance test (75 g) was within normal limits, but Holter monitoring revealed high-frequency premature contractions. Pure-tone audiometry indicated sensorineural and high-frequency hearing loss.

Needle electromyographic findings of the biceps brachii and rectus femoris muscles indicated mild myopathic features. Computed tomography (CT) of the thoracic spinal nerve 10 (T10) revealed severe atrophy and fatty degeneration of the paraspinal muscles (Fig. 2c). Brain magnetic

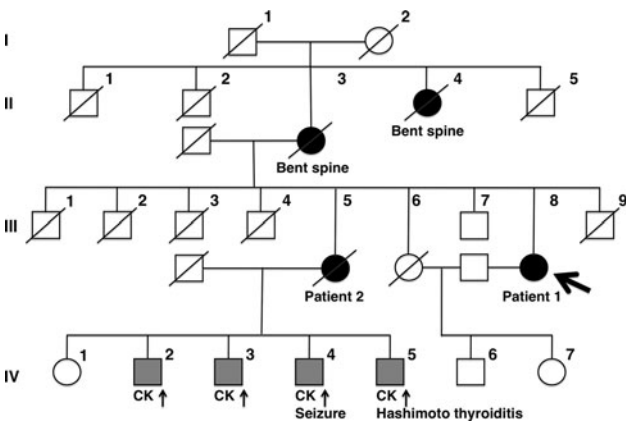


**Fig. 1** Pedigree of the family. The *arrow* indicates the proband. The affected individuals are represented by the *solid black symbols*; *open symbols* represent healthy individuals. *Gray symbols* indicate individuals with elevated CK levels

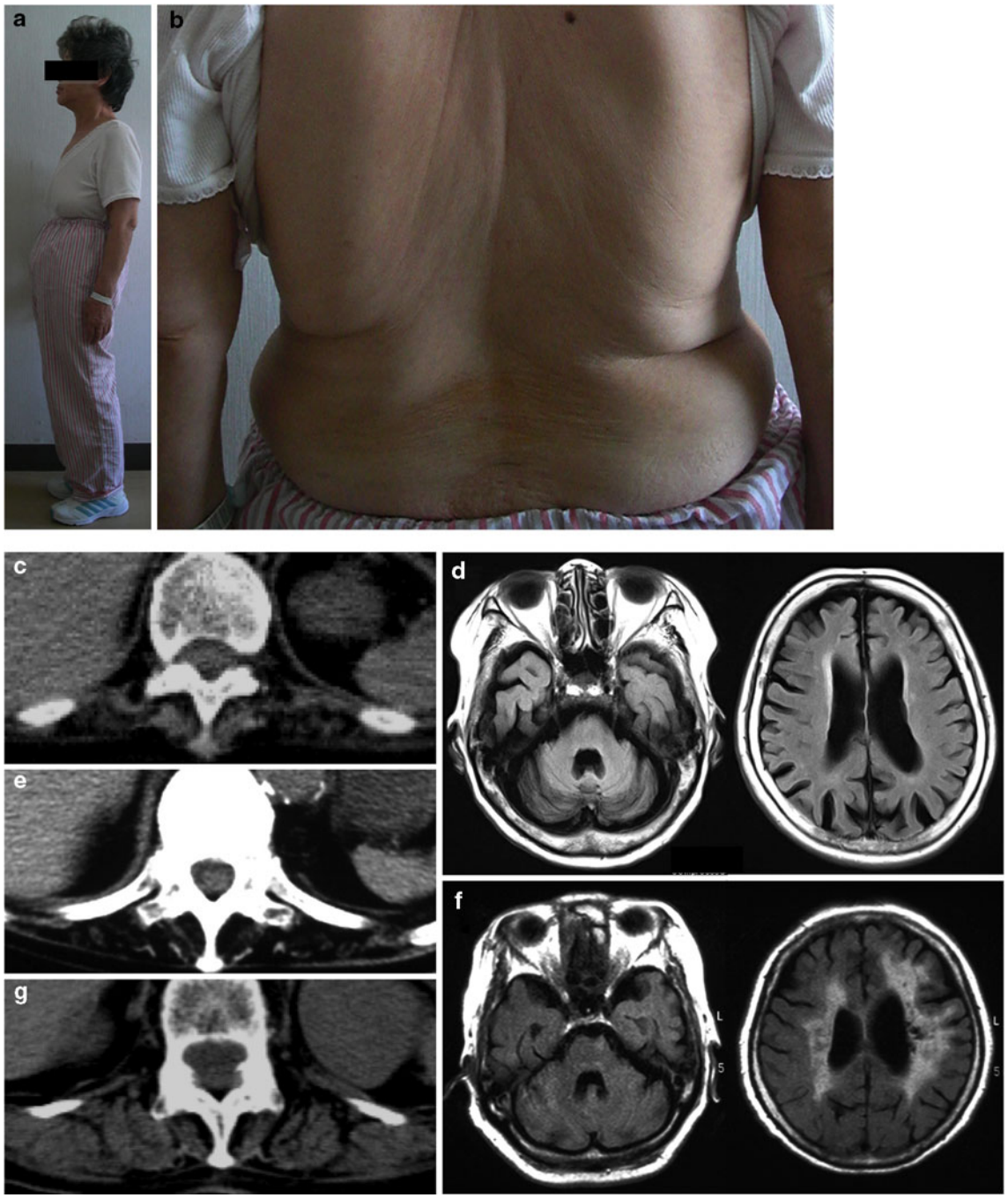


**Fig. 2** **a** The full-length figure indicates the posture of patient 1 showing her pushed-out waist. **b** The dorsal view shows the marked atrophy of the paraspinal muscles in patient 1. CT of T10 of **c** patient 1 (age 71), **e** patient 2 (age 82), and **g** a healthy female (age 74) reveals the profound atrophy of the paraspinal muscles in **c** patient 1 and **e** patient 2, but not in **g** the healthy female. Brain MRI studies revealed several differences between the patients 1 and 2. **d** Axial FLAIR images of patient 1 show moderate cerebellar atrophy and some cerebral cortical atrophy. **f** The same images of patient 2 revealing hyperintense lesions around the white matter

resonance imaging (MRI) with fluid-attenuated inversion recovery imaging showed moderate cerebellar and temporo-parieto-occipital lobe atrophy (Fig. 2d). MR spectroscopy revealed the absence of increased lactate peaks. 123I-IMP single photon emission CT revealed hypoperfusion that was indicative of atrophic brain lesions.

*Patient 2*

The elder sister of patient 1 was an 84-year-old woman with a stooping posture presenting with tremors since the age of 60. In her 70s she started walking with the aid of a walking stick. At 82 years of age, she was hospitalized for

generalized seizures and disturbed consciousness. CT of T10 revealed severe atrophy and fatty degeneration of the paraspinal muscles (Fig. 2e). Brain MRI revealed hyperintense lesions around the white matter (Fig. 2f); elevated serum and CSF lactate levels were also noted at this time. The mitochondrial DNA analysis of the lymphocytes did not indicate MELAS (m.3243A>G) or MERRF (m.8344A>G) mutations. The patient's condition remained undiagnosed and she died at the age of 84. CK levels in all her four sons were found to be elevated and her third son was diagnosed with epilepsy. She and her fourth son had also been previously diagnosed with Hashimoto thyroiditis (Fig. 1).

Patient 1 was examined using pathological, biochemical, and genetic analyses. The Institutional Review Board of Kagoshima University approved this study. Patient 1 gave the written and informed consent for her participation in this study.

### *Histochemical and immunohistochemical studies*

Frozen biopsies of the biceps brachii muscle specimens were obtained from patient 1. The specimens were sliced into 8 μm sections and placed on aminosilane-coated slides. Histochemical and immunohistochemical procedures were performed as previously described [13].

### *Biochemical studies*

Enzyme activity levels, blue native polyacrylamide gel electrophoresis (BN-PAGE), and other biochemical measurements of the frozen muscle specimens from patient 1 were performed as previously described [6, 33, 36].

### *Mitochondrial DNA analysis*

In case of patient 1, the total DNA was extracted from the peripheral blood leukocytes and the frozen muscle specimens using the DNeasy Blood & Tissue kit (Qiagen). MitoChip v2.0 (The GeneChip® Human Mitochondrial Resequencing Array 2.0), which provides a standard assay for the complete sequence analysis of human mitochondrial DNA, was obtained from Affymetrix. The patient's entire mitochondrial DNA was sequenced using MitoChip v2.0 as previously described [37]. Analysis of the microarray data obtained with MitoChip v2.0 was performed using GeneChip Sequence Analysis Software v4.0 (Affymetrix) [24].

In order to reveal the mutations that were confirmed by MitoChip v2.0, a 465-base pair PCR product that spanned all of the mutation sites was screened by DNA sequencing. In brief, 50 ng of the patient's genomic DNA was amplified using the hot-start PCR method and a forward (5′-CACCATTCTCCGTGAAATCA-3′) and reverse primer (5′-AGGCTAAGCGTTTTGAGCTG-3′) [5, 29]. Each PCR product was generated under the following conditions: 15 min at 95°C, 42 cycles of amplification (95°C for 30 s, 61°C for 30 s, and 72°C for 1 min), and 30 min at 72°C. Using a presequencing kit (USB, Cleveland, OH, USA), the patient's PCR products with abnormal elution profiles were purified, and the appropriate PCR products from relatives and control chromosomes were obtained and sequenced by dye-terminator chemistry using an ABI Prism 377 sequencer (Applied Biosystems, Foster City, CA, USA). The resulting sequences were then aligned and any mutations were evaluated using the Sequencher sequence alignment program (Gene Codes, Ann Arbor, MI, USA).

The polymorphic and pathogenic natures of the confirmed mutations were checked against two databases: the MITOMAP (http://www.mitomap.org/) and GiiB-JST mtSNP database (http://mtsnp.tmig.or.jp/mtsnp/index.shtml).

## Results

### Histological and immunohistochemical characterizations

The muscle fibers ranged from 10 to 80 μm in diameter. Sixty-nine of the 609 Gomori trichrome stained muscle fibers (11.3%) were ragged-red fibers (Fig. 3a). Cytochrome *c* oxidase (COX) activity was deficient in many of the ragged-blue fibers that were stained with succinate dehydrogenase (SDH) and COX (233 of 881 muscle fibers, 26.4%) (Fig. 3b, c), and no blood vessels showing strong SDH reactivity were observed. In NADH dehydrogenase-reactive sections, focal decreases and increases in oxidative enzyme activities were observed. Adenosine monophosphate (AMP) deaminase activity was normal. The random checkerboard distribution of the histochemical fiber types was preserved as shown in the ATPase-reactive sections. Acid phosphatase activity was slightly high in some fibers. Muscle fiber glycogen contents appeared normal and the lipid contents were slightly high in some fibers. Electron microscopy showed abnormal proliferation of mitochondria with paracrystalline inclusions (Fig. 4).

### Biochemical studies

All respiratory chain enzyme activities, which are expressed as a percentage of the normal control values relative to the citrate synthase activity, were greater than 20% (Table 1). BN-PAGE revealed no abnormalities in either the respiratory chain complexes or their molecular assembly structures.

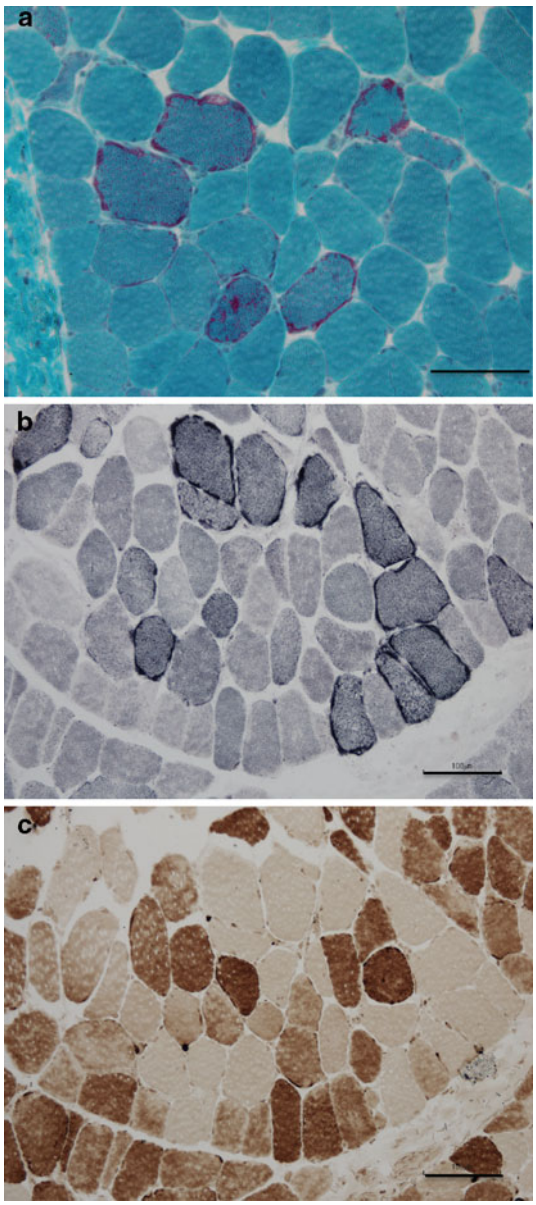


**Fig. 3** Histochemical analysis of the right biceps brachii muscle. **a** Gomori trichrome staining reveals typical ragged-red fibers. Histochemical analysis of serial sections of samples stained with **b** SDH or **c** COX shows a number of ragged-blue fibers with COX deficiency. **a**–**c** *Bar* 100 μm

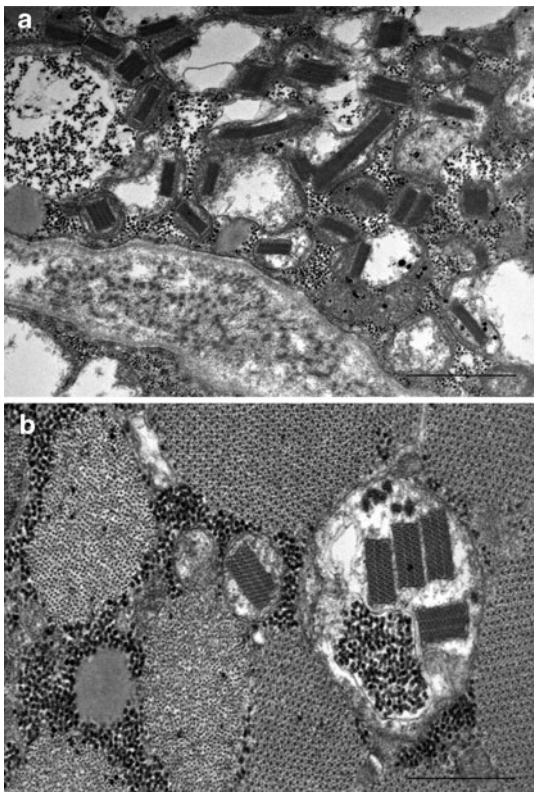


**Fig. 4** Electron micrograph of abnormal mitochondria in the right biceps brachii muscle. Abnormal mitochondria with paracrystalline inclusions that are suggestive of mitochondrial myopathy are shown. **a** *bar* 1 μm, **b** *bar* 500 nm

### Mitochondrial DNA analysis

Using MitoChip v2.0, 37 missense variants were detected in the mitochondrial DNA of the peripheral blood lymphocytes. All of these variants show polymorphisms and are listed in the MITOMAP and GiiB-JST mtSNP databases. Two additional missense variants were detected in the mitochondrial DNA of the muscle homogenate; the variants were m.602C>T in the $tRNA^{Phe}$ gene and m.16111C>G in the D-loop. The variant m.16111C>G is listed as a polymorphism, but the variant m.602C>T is not reported in either database. The m.602C>T variant was also confirmed by direct sequencing. The sequence chromatogram showed a heteroplasmic m.602C>T transition in the muscle homogenate mitochondrial $tRNA^{Phe}$ gene (Fig. 5a). The proportion of mutant mitochondrial DNA in the muscle was 64.7 ± 1.2% (mean ± SD; the operation was performed thrice). Mutant mitochondrial DNA was not detected in the blood lymphocytes when measured using real-time amplification refractory mutation system quantitative PCR analysis (RT-ARMS qPCR), as previously described [2, 10]. Healthy Japanese controls ($n = 100$) did not show these mutations in their blood lymphocytes, at least not within the limits of Sanger's method for DNA sequencing.

## Discussion

A novel mitochondrial $tRNA^{Phe}$ gene mutation was identified in a patient with late-onset predominant axial myopathy and cerebellar ataxia (patient 1). She presented with a maternal history of bent spine, and her elder sister presented with elevated lactate levels, severe paraspinal muscle atrophy, and epilepsy. Furthermore, the sister's four

**Table 1** Enzymatic activities for mitochondrial respiratory complexes in patient 1

| | CI activity (CI/CS) | CII activity (CII/CS) | CIII activity (CIII/CS) | CIV activity (CIV/CS) | CS activity |
|---|---|---|---|---|---|
| Patient 1 | 0.1938 (0.7027) | 0.2723 (0.9874) | 1.2737 (4.6192) | 0.0579 (0.21) | 0.2757 |
| Control | 0.3194 (1.6183) | 0.2751 (1.3444) | 1.3132 (6.5512) | 0.0826 (0.3840) | 0.2151 |
| Patient 1/control ratio | 60.7% (43.4%) | 98.9% (73.4%) | 97.0% (70.5%) | 70.1% (54.7%) | |

Enzymatic activities for individual mitochondrial respiratory complexes are given in nmol/min protein, and represent percentage of normal control ($n = 10$) mean relative to a reference enzyme of citrate synthase (CS)

The activities are relatively low in complex I and complex IV compared with other complexes

*CI* complex I, *CII* complex II, *CIII* complex III, *CIV* complex IV

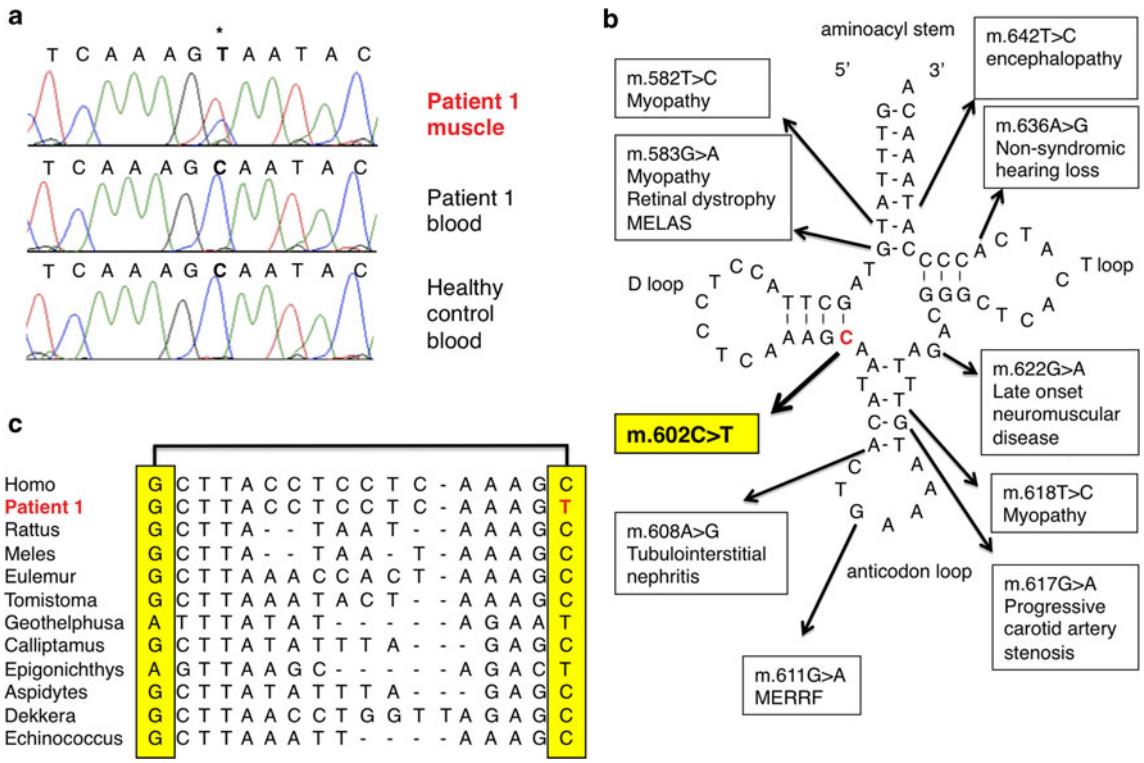


**Fig. 5** **a** Sequence chromatogram of the mitochondrial DNA region that encompasses the m.602C>T alteration (*asterisk*) that was obtained from the skeletal muscle of patient 1 (reverse complement). **b** Schematic diagram of the mitochondrial $tRNA^{Phe}$ cloverleaf structure showing previously reported mutations and the m.602C>T alteration in the D-stem. **c** Comparison of mitochondrial $tRNA^{Phe}$ from different species. Base pairs, including the 602 nucleotides, are shown in *boxes*

sons presented with elevated CK levels, among which one had epilepsy. Patient 1 also presented with other symptoms associated with mitochondrial disease, including mild blepharoptosis, cataracts, hearing loss, and arrhythmia. Morphological examination revealed many ragged-red fibers and a partial deficiency in COX activity. One of the major diagnostic criteria for respiratory chain disorders in adults is less than 20% activity in any of the tissue complexes, but the data of the present study did not fulfill this condition [4]. However, the activities of complexes I (43.4%) and IV (54.7%) were lower than those of the other complexes. The decreased activities of complexes I and IV are probably due to the deficiency in COX activity that was measured in the muscle fibers. These clinical, morphological, and biochemical manifestations indicate that the patient most likely had a mitochondrial disease.

The marked atrophy of the paraspinal muscles was the most interesting feature found in patients 1 and 2. Axial myopathy has been defined as muscle weakness that is limited to the spinal and neck muscles [21]. Therefore, the symptoms of patient 1 are incompatible with pure axial myopathy because of the muscle weakness and mitochondrial abnormalities that were observed in the biceps brachii muscle. The most characteristic feature of axial myopathy is the remarkable atrophy of the paraspinal muscles rather than the atrophy of the muscles of the limbs, which is

different from the clinical symptoms of conventional mitochondrial myopathy. Thus, based on the available evidence, we believe that patients 1 and 2 can be diagnosed with mitochondrial predominant axial myopathy.

Axial myopathy may occur secondary to various diseases. However, only five cases of mitochondrial axial myopathy associated with the prominent involvement of the extensor muscles of the spine have been previously reported (Table 2) [8, 11, 28, 30, 32]. All these cases presented with abnormal trunk flexion that developed during walking and disappeared when the patient was in a supine position. In the cases described here, only patient 2 presented with camptocormia. These common symptoms, including late-onset, mildly elevated serum CK levels, ragged-red fibers, and the partial deficiency in COX activities, were observed in patient 1 and also in the above mentioned cases. However, biochemical analysis was performed in only one case that showed deficiencies in complexes I and III [32]. No case has been previously reported that describes a family history of similar symptoms. In addition, no genetic cause of any mitochondrial axial myopathy has been previously reported.

This study is unable to conclusively prove or disprove the pathogenicity of the m.602C>T mutation. However, three reasons that support the pathogenicity of this mutation are apparent. First, the heteroplasmic m.602C>T point mutation disrupts a conserved Watson–Crick cytosine–guanine (C–G) base pairing within the D-stem of the mitochondrial $tRNA^{Phe}$ gene, which would most likely affect the stability of the secondary structure of mitochondrial tRNA (Fig. 5b). Almost 94% of mitochondrial tRNA pathogenic mutations occur in this stem structure, and the disruption of Watson–Crick C–G base pairing is a significantly more common feature of pathogenic mutations than neutral variants [23]. Second, after performing a sequence homology search using CLUSTALW (http://clustalw.ddbj.nig.ac.jp/top-j.html), it was determined that this base pairing is largely conserved in other species as C–G or adenine–thymine base pairings (Fig. 5c). Third, the mutation is heteroplasmic and present in the affected skeletal muscles but not in the peripheral blood lymphocytes. Almost all pathogenic mitochondrial tRNA mutations in clinically affected tissues have a high proportion of heteroplasmy compared with unaffected tissues [23].

However, the decreased activities of complexes I and IV that were observed during the biochemical examination cannot be completely explained by the disruption in mitochondrial protein synthesis that could have been caused by the mitochondrial tRNA mutation. In addition, data obtained from the single muscle fiber analyses were limited due to the small sample size, and therefore, are not sufficient to prove the pathogenicity of the m.602C>T mutation.

Any additional evidence of the pathogenicity of the cybrid cells was not obtained. Therefore, 10 points (out of a maximum score of 20 points) was applied to the scoring criteria of the mitochondrial tRNA mutations listed in MITOMAP, which indicated that the m.602C>T mutation is possibly pathogenic [23].

The mechanism of late-onset axial myopathy induced by mitochondrial dysfunction is unclear. Nine pathogenic mutations in the mitochondrial $tRNA^{Phe}$ gene have been previously described in various diseases (Fig. 5b), including a late-onset neuromuscular disease but not axial myopathy [7, 9, 12, 14, 17, 18, 22, 25, 34, 35]. A probable etiological mechanism for the presentation of such a myopathy in the elderly is the accumulation of mitochondrial tRNA pathogenic mutations that affect aging tissues [9]. If it is possible to get any information on the pathological status of the primarily affected muscles, this would perhaps be as informative as the differential involvement of the biceps and paraspinal muscles. Unfortunately, these data could not be obtained due to the remarkable fatty degeneration of the paraspinal muscles.

The patients described in this report are characterized by the combination of axial myopathy and CNS involvement. One report about a parkinsonian patient with mitochondrial axial myopathy suggested that mitochondrial dysfunction

**Table 2** Clinical characteristics of patients with paraspinal muscle atrophy from mitochondrial myopathy

| Age/sex [Ref.] | Onset age | Family history | CK (IU/l) | RRF | COX deficiency | mtDNA mutation | Neurological deficit |
|---|---|---|---|---|---|---|---|
| 73/F [patient 1] | 63 | + | 290 | + | + | 602C>T | Cerebellar ataxia |
| 84/F [patient 2] | 60 | + | 474 | NE | NE | NE | Encephalopathy |
| 65/M [32] | 59 | − | 245 | + | + | NR | − |
| 65/M [30] | 62 | NR | NR | + | + | NR | Parkinsonism |
| 78/M [11] | 78 | NR | 501 | + | + | NR | − |
| 64/M [28] | NR | NR | Elevated | + | + | NR | − |
| 55/M [8] | NR | NR | Normal | + | + | NR | − |

*M* male, *F* female, *CK* creatine kinase, *RRF* ragged-red fiber, *NR* not reported, *NE* not evaluated, *COX* cytochrome *c* oxidase, *mtDNA* mitochondrial DNA, *Ref* reference

may lead to both axial myopathy and parkinsonism [30]. In the patients described here, CNS involvement was similar to that observed in myoclonus epilepsy with ragged-red fiber (MERRF) due to the accompanying cerebellar atrophy and epilepsy. In fact, MERRF has been previously reported to be associated with pathogenic mutations of the mitochondrial $tRNA^{Phe}$ gene [22].

Finally, mitochondrial dysfunction might be implicated in the development of Hashimoto thyroiditis in patients 1 and 2 and in the fourth son of patient 2; the relationship between mitochondrial diseases and Hashimoto thyroiditis has been previously described [3, 20, 26, 27].

In summary, this is the first report about familial mitochondrial disease with late-onset predominant axial myopathy and encephalopathy, which were confirmed by clinical and histological findings. This case expands the phenotypic spectrum of mitochondrial diseases. Future studies on the novel mitochondrial $tRNA^{Phe}$ 602C>T mutation may contribute to the understanding of late-onset predominant axial myopathy and encephalopathy.

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