Results

Electrophysiological studies

The motor nerve conduction studies revealed moderately slow motor nerve conduction velocities (MCV) with reduced compound muscle action potential (CMAP) amplitude in all examined nerves. The sensory nerve conduction studies showed moderately slow sensory nerve conduction velocities (SCV) with slightly reduced sensory nerve action potential (SNAP) amplitude (Table 1). No temporal dispersions or conduction blocks were observed. These results suggest demyelinating polyneuropathy complicated by axonal sensorimotor polyneuropathy. Because the patient showed hypersensitivity to low-dose VCR (total VCR administered, 3.9 mg), we suspected a pre-existing, inherited neuropathy. Furthermore, electrophysiological studies were performed on her healthy, 51-year-old mother. MCV of the mother was slower in the lower extremities than the upper extremities. CMAP amplitudes were within normal limits. Median nerve distal latency was slightly prolonged. SCV was moderately slow, but this finding was uniform in all examined nerves. SNAP amplitudes were moderately reduced in the upper extremities; SNAP amplitude of the sural nerve was at the lower limit of our normal control data. Temporal dispersions, conduction blocks, and entrapment neuropathies were not observed. These results indicate an electrophysiologically mild demyelinating polyneuropathy (Table 1). These findings suggest that this family may have an inherited demyelinating polyneuropathy.

Resequencing analysis of this family and a control study

The DNA chip resequencing analysis detected a novel c.1057 C>G (p.R353G) missense mutation in the *EGR2* gene. In contrast, the analysis was negative for mutations involving the other 27 CMT or related disease-causing genes. The patient was heterozygous for the c.1057 C>G mutation that substitutes an arginine for glycine at amino acid 353 (p.R353G) in exon 2 of *EGR2* by conceptual translation (Fig. 1a). The mother had the same mutation as the patient (Fig. 1a). We did not observe R353G in 200 control chromosomes or in the 850 chromosomes from 425 patients with inherited neuropathy. In addition, we did not find the R353G mutation in the 1000 Genomes website (http://browser.1000genomes.org), which catalogs human genetic variations using 1,197 samples including 300 East Asian (100 Japanese) samples.

Clinical course of the patient

We changed the chemotherapy regimen after we suspected that the patient had CMT. We chose radiotherapy and rituximab for the treatment of B-cell lymphoma. After 2 months, her symptoms had almost recovered, and she walked normally with only mild numbness in her distal lower limbs.

However, it was difficult to trace the causal agent because she was treated with a combination of chemotherapy agents. According to a previous report [3], there is uncertainty about the neurotoxicity of cyclophosphamide, prednisolone, and rituximab in patients with CMT, while VCR is classified as high risk for such patients. Furthermore, she and her mother's electrophysiological findings were consistent with inherited demyelinating polyneuropathy without the presence of conduction block or temporal dispersion. There were no findings indicated other inherited demyelinating polyneuropathy such as disturbance of lipid metabolism, peroxisomal disorders, hepatic porphyria and amyloidosis besides CMT. The results of her laboratory studies, including liver function tests, renal function tests, serum electrolyte and fasting blood glucose were normal. Her mother was healthy in the past periodic medical checkup, but laboratory

Table 1 Results of the nerve conduction studies

	Nerve	DL (ms)	CMAP amplitude (mV)	MCV (m/s)	SNAP amplitude (μV)	SCV (m/s)
Patient	Median	4.3	1.5	26.9	6.7	45.1
	Ulnar	3.9	2.7	31.8	7.3	45.8
	Tibial	8.2	3.9	23.0	–	–
	Sural	–	–	–	4.2	33.3
Patient's mother	Median	5.0	11.2	44.6	3.9	39.7
	Ulnar	3.3	9.1	50.1	3.1	38.7
	Tibial	4.7	23.6	37.9	–	–
	Sural	–	–	–	5.2	37.6
Control	Median	<4.5	>3.1	>49.6	>7.0	>47.2
	Ulnar	<3.6	>6.0	>50.1	>6.9	>46.9
	Tibial	<5.7	>4.4	>41.7	–	–
	Sural	–	–	–	>5.0	>40.8

DL distal latency, *CMAP* compound muscle action potential, *MCV* motor conduction velocity *SNAP* sensory nerve action potential, *SCV* sensory conduction velocity

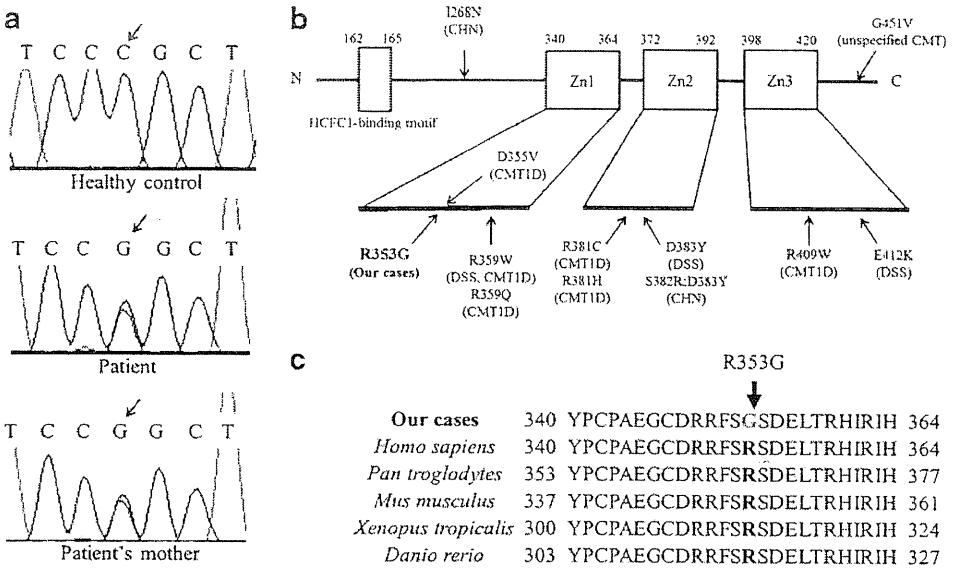

Fig. 1 **a** Chromatograms of the alterations in the *early growth response 2* (*EGR2*) gene that was identified in the patient and her mother, both of whom had the heterozygous transition c.1057 C>G that resulted in R353G. **b** Schematic diagram of the *EGR2* showing previously reported mutations and the R353G alteration. *CHN* congenital hypomyelination neuropathy, *DSS* Dejerine–Sottas disease, *Zn* zinc-finger domains. **c** Comparison of *EGR2* mutations in different species

screening tests were not examined in this report. We strongly suspected VCR-induced neuropathy in CMT with the *EGR2* mutation.

Discussion

This is the first report to describe an *EGR2* mutation that induced VCR hypersensitivity, similar to *PMP22* duplication. The *EGR2* gene located on human chromosome 10q21.1 has two exons that encode a 476 amino acid protein with three zinc finger domains, which is believed to be a transcription factor that regulates myelinogenesis [17, 18]. *EGR2* knockout mice exhibit severe hypomyelination of peripheral nerves due to the blocking of Schwann cell differentiation [19, 20]. Heterozygous mutations in *EGR2* cause myelinopathies, including congenital hypomyelinating neuropathy, Dejerine–Sottas disease, and mild to severe CMT1 [21–26]. Until date, 17 types of *EGR2* mutation have been found (http://www.molgen.ua.ac.be/CMTMutations/Mutations). *EGR2* induces high expression levels of myelin protein components such as *PMP22*, *MPZ*, *DHH*, and *PRX* in Schwann cells [27–30]. Vincristine inhibits axonal transport; thus, an insufficient supply of the myelin protein component necessary for the increased demand created by vincristine may induce a large degree of neurotoxicity. In the present study, we showed a novel R353G mutation in the first zinc finger domain of *EGR2* in a patient with late onset CMT1 who presented with

Table 2 Computational predictions of the pathogenicity on EGR2 mutation within the zinc finger domain

	Mutation	MUPro (SVM score[a])	PolyPhen[b]	PolyPhen2[c]	SIFT[d]
Our patients	R353G	−0.43[e]	2.57[e]	0.90[e]	0.00[e]
Reported mutations	D355V	1.00	2.75[e]	0.97[e]	0.00[e]
	R359W	−0.64[e]	2.79[e]	1.00[e]	0.00[e]
	R359Q	−1.00[e]	1.89[e]	0.92[e]	0.00[e]
	R381C	−0.11[e]	2.79[e]	0.99[e]	0.00[e]
	R381H	−0.24[e]	2.12[e]	0.99[e]	0.00[e]
	S382R	0.35	2.06[e]	0.81[e]	0.00[e]
	D383Y	0.09	2.75[e]	0.99[e]	0.00[e]
	R409W	−0.98[e]	2.69[e]	1.00[e]	0.00[e]
	E412K	−1.00[e]	1.69[e]	0.77[e]	0.00[e]

[a] Support Vector Machine (SVM) scores <0 indicate a decrease in protein stability

[b] PolyPhen scores ≥1.5 indicates a prediction of pathogenic

[c] PolyPhen2 scores of ~1 indicate a prediction of pathogenic

[d] SIFT scores ≤0.05 indicate a prediction of pathogenic

[e] Denotes a pathogenic prediction

a very mild phenotypic expression. Most *EGR2* mutations within the first zinc finger domain cause Dejerine–Sottas disease or severe CMT1 phenotypes (Fig. 1b) [22, 24]. A sequence homology search was performed, which aligned protein sequences from multiple species, using a Constraint-based, Multiple-Alignment tool (COBALT) (http://www.ncbi.nlm.nih.gov/tools/cobalt/). Arginine 353 was conserved among all of the species analyzed (Fig. 1c). It was found that the R353G mutation identified in our patients was located in a remarkably well-conserved sequence of amino acids, suggesting that it may have a potential impact on *EGR2* function. Furthermore, we computationally predicted the effect of the R353G mutation on protein function using the MUpro (http://www.ics.uci.edu/~baldig/mutation.html), PolyPhen (http://genetics.bwh.harvard.edu/pph/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), and SIFT (http://sift.jcvi.org/www/SIFT_seq_submit2.html) algorithms. The algorithms in these programs use evolutionarily conserved species as well as reference sequence alignments, physiochemical differences, and the proximity of various substitutions to predict functional domains and/or structural features. All these programs predicted that the R353G mutation is most likely pathogen-based on the degree of conservation of the affected residues (Table 2). Therefore, the R353G mutation could possibly disrupt various functions. Furthermore, different mutations in the same codon result in divergent CMT phenotypes [26]. The electrophysiological findings were the only abnormal results for the patient's asymptomatic mother with the same *EGR2* mutation. Her neurological findings were normal, including a normal handgrip, the absence of foot deformities, normal and prompt deep tendon reflexes, and normal sensations. It is difficult to diagnose late onset mild CMT based on clinical findings and family history because the disease is heterogeneous. Although we did not perform in vitro functional analysis of the R353G mutation in this study, such further functional studies would illuminate the details of the pathomechanism of the *EGR2* mutation and its relationship with vincristine toxicity in this patient. In order to clarify the pathogenic nature of the *EGR2* mutation and vincristine neurotoxicity, we need to continue the genetic analysis of vincristine-induced neuropathy patients who do not show the CMT phenotype.

VCR-induced neuropathy is a dose-limiting side effect observed in neurologically normal individuals, but it sometimes results in severe neuropathy in patients with CMT. Early recognition of CMT before VCR treatment can prevent severe neurotoxicity. It is very important to use electrophysiological studies to recognize pre-existing CMT before VCR treatment, even if there is no family history or neurological abnormalities. Moreover, the labor and reagent costs of molecular genetic testing have significantly increased along with the increase in the number of genes associated with CMT and related neuropathies that must be screened for mutations. Realistically, it is difficult to perform nerve conduction studies or genetic testing in all patients who receive chemotherapy because of the costs and effort. Because of recent progress in the development of a new generation of genomic sequencing technologies, it will be possible to screen the entire genome/exome sequence for potential risks in all patients before they undergo chemotherapy.

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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

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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

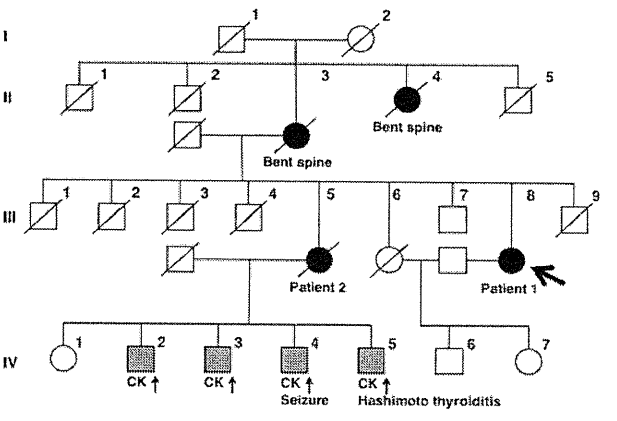

Fig. 1 Pedigree of the family. The *arrow* indicates the proband. The affected individuals are represented by the *solid black symbols*; *open symbols* represent healthy individuals. *Gray symbols* indicate individuals with elevated CK levels

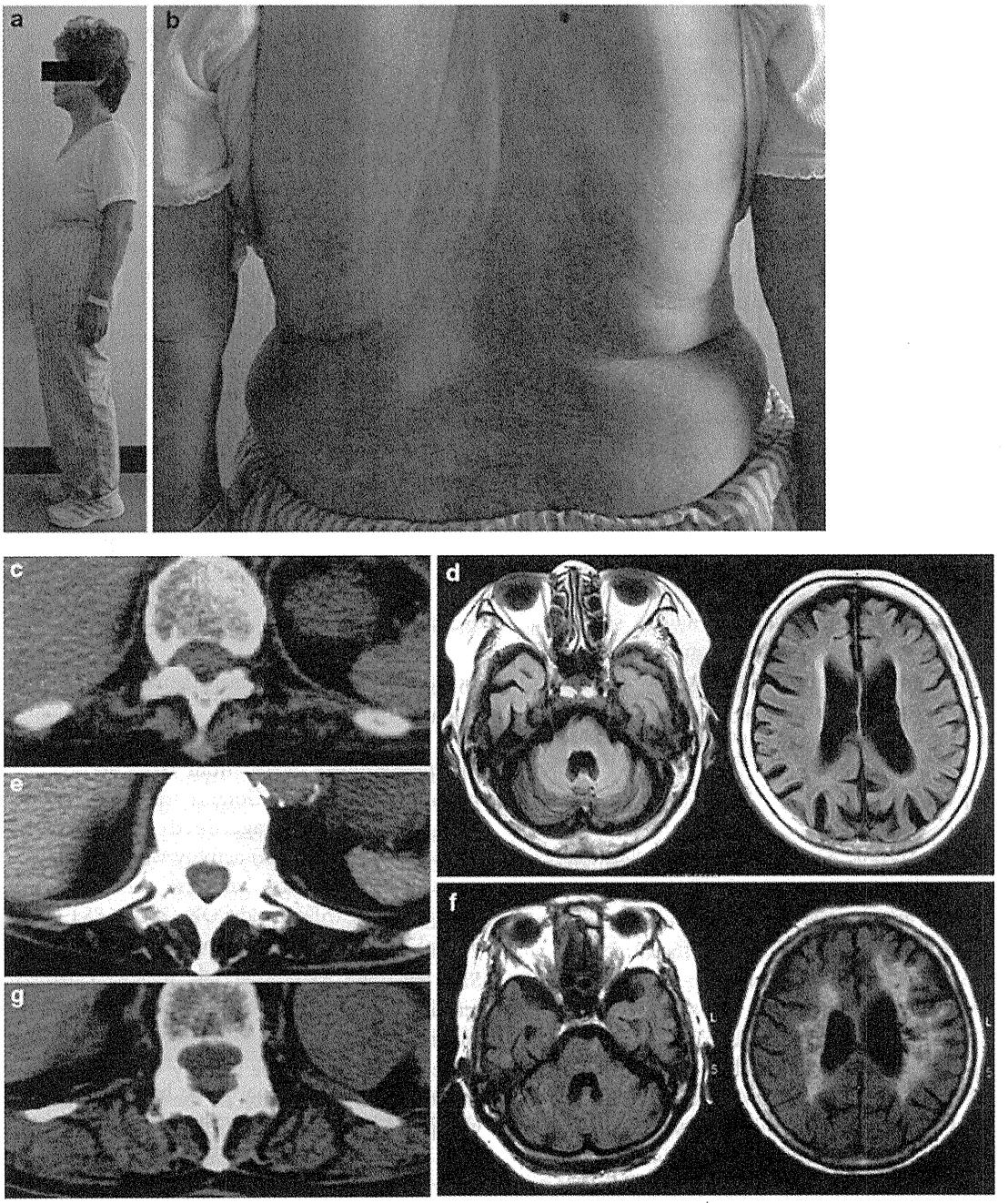

Fig. 2 **a** The full-length figure indicates the posture of patient 1 showing her pushed-out waist. **b** The dorsal view shows the marked atrophy of the paraspinal muscles in patient 1. CT of T10 of **c** patient 1 (age 71), **e** patient 2 (age 82), and **g** a healthy female (age 74) reveals the 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.

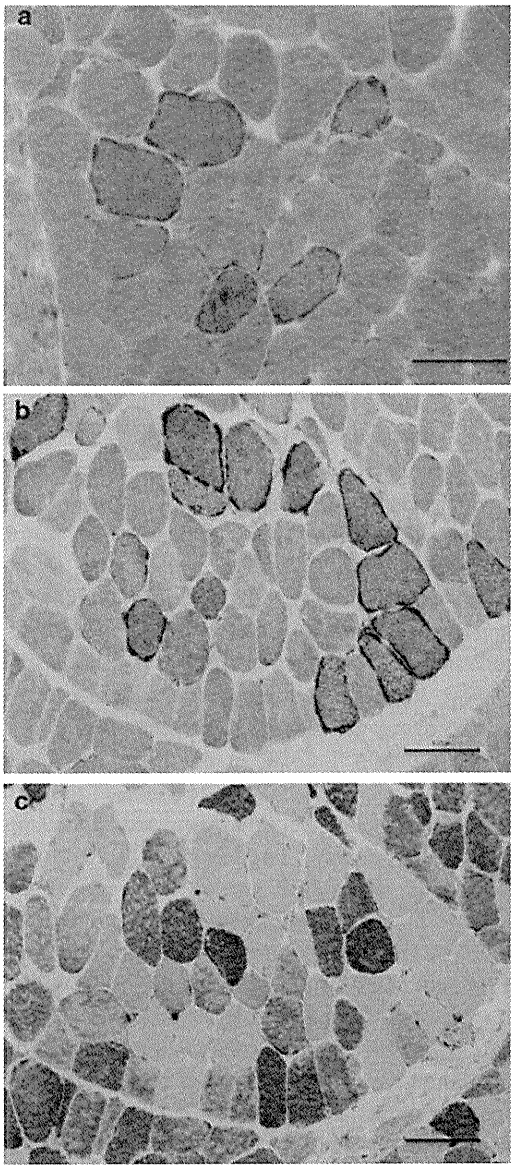

Fig. 3 Histochemical analysis of the right biceps brachii muscle. **a** Gomori trichrome staining reveals typical ragged-red fibers. Histochemical analysis of serial sections of samples stained with **b** SDH or **c** COX shows a number of ragged-blue fibers with COX deficiency. **a**–**c** *Bar* 100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

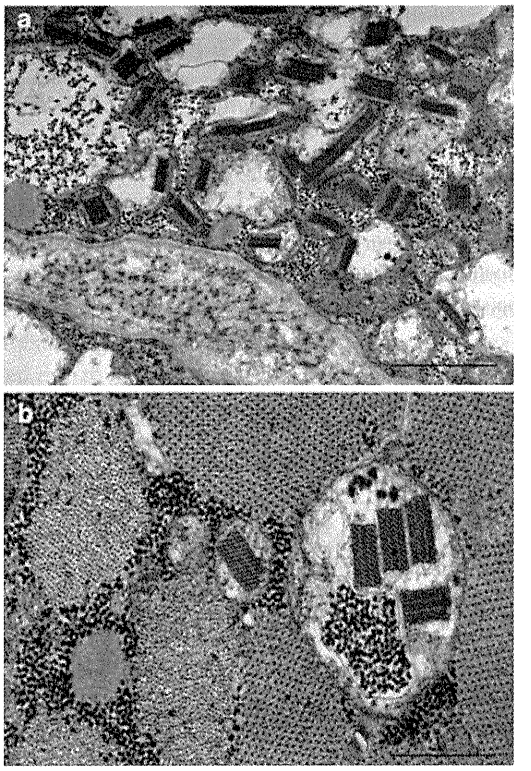

Fig. 4 Electron micrograph of abnormal mitochondria in the right biceps brachii muscle. Abnormal mitochondria with paracrystalline inclusions that are suggestive of mitochondrial myopathy are shown. **a** *bar* 1 μm, **b** *bar* 500 nm

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

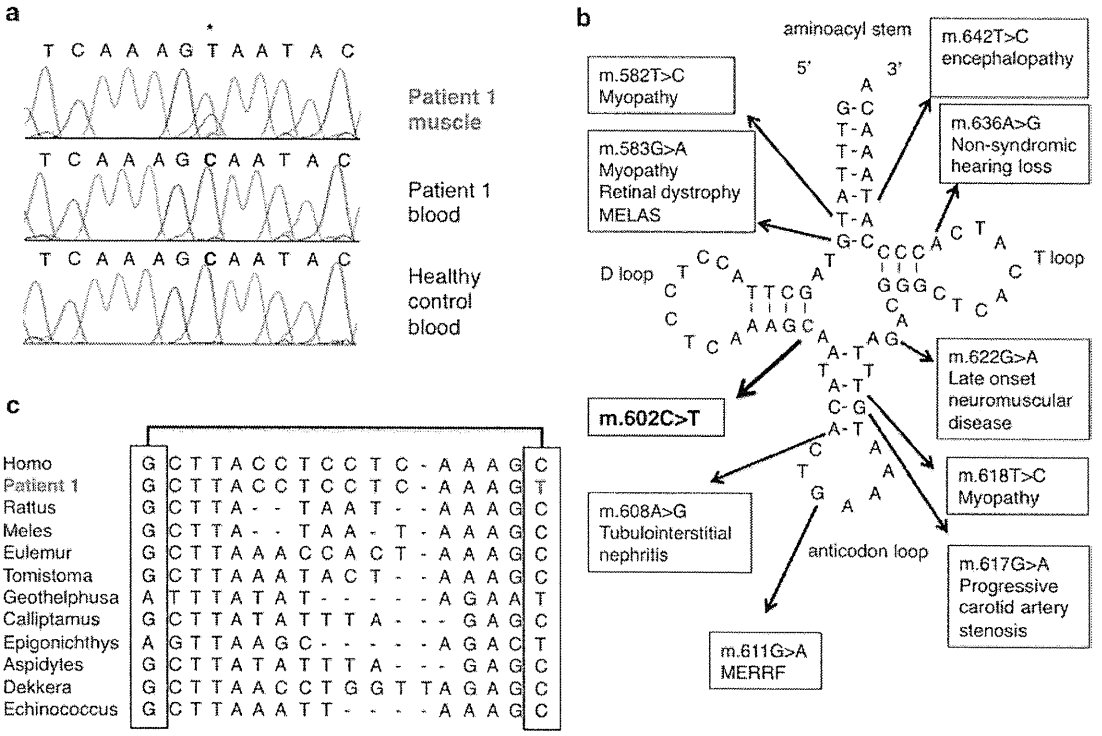

Fig. 5 **a** Sequence chromatogram of the mitochondrial DNA region that encompasses the m.602C>T alteration (*asterisk*) that was obtained from the skeletal muscle of patient 1 (reverse complement). **b** Schematic diagram of the mitochondrial $tRNA^{Phe}$ cloverleaf structure showing previously reported 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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A New Mitochondria-Related Disease Showing Myopathy with Episodic Hyper-creatine Kinase-emia

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Objective: To elucidate the relationship between mitochondrial DNA (mtDNA) alterations and a mitochondrial disease with a distinct combination of characteristic symptoms, namely episodic hyper-creatine kinase (CK)-emia and mild myopathy.
Methods: We selected 9 patients with mtDNA np8291 alteration from 586 patients suspected to have a mitochondrial disease, and assessed them clinically, pathologically, and genetically. These 9 patients had undiagnosed mitochondrial myopathy with episodic hyper-CK-emia, all showing similar symptoms and progression.
Results: Patients had mild muscle weakness and episodic hyper-CK-emia triggered by infections or drugs. Five of 9 patients were initially diagnosed with other conditions, such as myasthenia gravis, polymyositis, viral myositis, and drug-induced myopathy, because these conditions were acute or subacute, and 9 patients showed the same 16 mtDNA alterations, which have been reported to be nonpathological polymorphisms. Muscle biopsy revealed ragged-red fibers, highly expressed succinate dehydrogenase staining fibers, and cytochrome c oxidase–deficient fibers. Because their mitochondrial sequence data was almost the same, and 9 patients live in widely separated cities in Japan, the alterations may have arisen from a single source.
Interpretation: These findings suggest that mild myopathy with episodic hyper-CK-emia associated with some of the 16 mtDNA alterations or at least with their mitochondria, could be a novel mitochondrial disease. Therefore, we propose that this disease be named as "mitochondrial myopathy with episodic hyper-CK-emia (MIMECK)." These alterations could work concomitantly and probably modify the impact of medications or other environmental factors. We believe these findings provide an insight into a novel aspect of mitochondrial disease pathogenesis.

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Persistently high blood creatine kinase (CK) levels are a hallmark of neuromuscular disease.[1] Serum CK levels show a variable increase in several systemic conditions such as genetic myopathy, viral infections, connective tissue disorders, electrolyte imbalance, and endocrine dysfunction.[2] Idiopathic hyper-CK-emia presents as persistently high serum CK levels with normal neurological, neurophysiological, and neuropathological findings.[3] Persistent asymptomatic hyper-CK-emia progresses to mild or early-stage myopathy in many cases.[4] Furthermore, numerous drugs are reportedly myotoxic. A prospective study on patients from a university hospital revealed 171 cases with high CK levels, the drugs primarily responsible being statins (46.4%), fibrates (14.3%), antiretrovirals (14.3%), and angiotensin-II receptor antagonists (10.7%).[5] Although the mechanisms of drug-induced muscle damage are unclear, an association between mitochondrial function and drug-induced myopathy has been reported.[6–9]

We experienced 9 distinct cases of mitochondrial myopathy in patients with episodic hyper-CK-emia, and diagnosed these as mitochondrial disease. Mitochondrial myopathies usually affect multiple organs and exhibit a broad spectrum of disorders. Numerous mutations and polymorphisms have been reported in the mitochondrial DNA (mtDNA) database (MITOMAP: human mitochondrial genome database; http://www.mitomap.org).[10]

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Over 150 point mutations and innumerable large-scale rearrangements are associated with mitochondrial diseases, which are heterogeneous disorders with a myriad of clinical features.[11] However, neither idiopathic hyper-CK-emia associated with mitochondrial dysfunction nor disease-causing mitochondrial mutations in drug-induced mitochondrial myopathy have been reported. Here we report a novel mitochondrial disease with a distinct combination of characteristic symptoms, namely episodic hyper-CK-emia and mild myopathy. We discuss the relation between mtDNA alterations and this disease.

Patients and Methods

Patients

We studied 586 patients who were referred to our department from South Kyushu (Kagoshima, Miyazaki, Oita, and Okinawa Prefectures), southern Japan, from 1992 to 2009. These patients included those diagnosed with or suspected of having mitochondrial disease—such as mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke (MELAS); myoclonic epilepsy and ragged-red fiber (RRF) disease (MERRF); chronic progressive external ophthalmoplegia (CPEO)—or were patients without a definitive diagnosis. Previously, we reported adult-onset mitochondrial myopathy (4 patients included in this study) with a mtDNA np8291 A-to-G substitution.[12] However, the pathogenesis of this disorder is unclear because np8291 is a noncoding nucleotide located 4 bases before the 5′ end of transfer RNA (tRNA) (Lys). At our institution, an mtDNA np8291 is usually determined by screening patients diagnosed with or suspected of having mitochondrial disease because this alteration is located near np8344, which is the typical MERRF mutation.[13] We focused on this rare alteration and selected only 9 patients (8 families) with mtDNA np8291 alteration from the abovementioned 586 patients; these 9 patients had undiagnosed mitochondrial myopathy with episodic hyper-CK-emia based on clinical findings, all showing similar symptoms and progression. We reassessed these 9 patients clinically, pathologically, and genetically to identify the features of this disease. These 9 patients lived in widely separated cities in the southern part of Japan.

All patients had been referred by their primary physicians or neurologists. Signed, informed consent was obtained for every patient. The Institutional Review Board of Kagoshima University approved this study.

Histopathological Study

All muscle biopsies were obtained from the biceps brachii or quadriceps femoris muscles. The specimens were immediately frozen in isopentane and cooled with liquid nitrogen. Frozen sections (thickness, 8μm) were stained with hematoxylin-eosin, modified Gomori trichrome (mGT), succinate dehydrogenase (SDH), cytochrome *c* oxidase (CCO), periodic acid-Schiff, Sudan black, myosin adenosine triphosphatase (ATPase), and reduced nicotinamide adenine dinucleotide (NADH)-tetrazolium reductase.

mtDNA Analysis

Genomic DNA was extracted from peripheral blood leukocytes and muscles using the Puregene Blood Core Kit C (Qiagen, Tokyo, Japan) or the DNeasy Blood and Tissue kit (Qiagen). MitoChip v2.0 was obtained from Affymetrix (commercially available GeneChip Human Mitochondrial Resequencing array 2.0; Tokyo, Japan). mtDNA from all lymphocyte and skeletal muscle samples were analyzed on separate chips. The entire mtDNA sequence was amplified in 3 overlapping polymerase chain reactions (PCRs) using 50ng genomic DNA in each reaction.[14] Reagents, conditions, and purification were accomplished as described in previous reports.[15] Pooling, DNA fragmentation, labeling, and chip hybridization were performed as per Affymetrix Customseq Resequencing protocol instructions. The chips were washed on the Affymetrix fluidics station using Customseq Resequencing wash protocols. Microarray data for MitoChips v2.0 were analyzed using GeneChip Sequence Analysis Software v4.0 (Affymetrix).[16] We also confirmed key alterations (np8291). In brief, 50ng of the patient's genomic DNA was amplified using a hot-start PCR method and a forward (5′-CATGCCCATCGTCCTAGAA) and reverse primer (5′-TTTGGTGAGGGAGGTAAGTG).[17] PCR products were generated under the following conditions: 15 minutes at 95°C, 42 cycles of amplification (95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 1 minute), and 30 minutes at 72°C.

Using a presequencing kit (USB, Cleveland, OH), we purified patients' PCR products and sequenced them with dye-terminator chemistry using an ABI377 automated sequencer (Applied Biosystems, Tokyo, Japan). We aligned the resulting sequences and evaluated mutations and alterations using the Sequencher sequence alignment program (Gene Codes, Ann Arbor, MI).

Results

Clinical Features

We present the case histories of only 3 among the 9 patients in detail, because all 9 patients had similar clinical features (Table 1).

CASE 1. This 71-year-old woman had a significant family history. Her sister had previously reported similar symptoms but was not included in this study. Our patient noticed slight muscle weakness at the age of 40 years, and by her late 60s she often felt lethargic. At the age of 70 years, general weakness, dysphagia, and dysarthria appeared several weeks after a bout of common cold. She was initially diagnosed with myasthenia gravis, but the symptoms were resolved almost completely without medication upon admission. Her serum CK level increased transiently up to 360IU/liter (normal range, 45–163IU/liter). She exhibited mild proximal dominant muscle weakness, and hypothyroidism was detected after admission.

CASE 2. This 57-year-old woman had reported muscle weakness and an inability to run fast while still in school. By the age of 40 years, she was experiencing limb

TABLE 1: Clinical Characteristics of Mitochondrial Myopathy Patients with Episodic Hyper-CK-emia

Case	Age/Sex	Onset (yr)	CK (Usual) (IU/liter)	CK (Episodic) (IU/liter)	Subacute Onset	Dysphagia	Myalgia	Muscle Weakness	RRF (%)	SDH (%)	CCO (%)	Trigger	Initial Diagnosis
1	71/F	69	150	360	+	+	−	Mild	1.5	3	2	Common cold	MG
2	57/F	41	100	617	+	−	+	Mild	1	2.5	2	Common cold	Viral myositis
3	64/M	62	181	593	+	+	+	Mild	1.5	2	2.5	Lamivudine	Drug-induced myopathy
4	59/F	54	180	209	−	−	−	Mild	4	6	8	−	
5	71/M	65	270	11708	+	+	+	Moderate	2.5	8.5	7	−	PM
6	50/F	47	98	985	+	+	+	Mild	2	3	2.5	−	PM
7	70/F	50	67	527	−	−	+	Mild	4	7.5	3	−	
8	38/M	35	328	1478	+	−	+	Moderate	4	6	5.5	−	
9	42/F	39	200	1089	−	−	−	Mild	2	5	5	−	

Serum CK levels during the course of the disease are indicated in 2 columns: (1) usual condition and (2) maximum episodic value (normal range 45–163IU/liter). Trigger indicates the event-precipitating symptoms. CCO = cytochrome *c* oxidase-deficient fibers; CK = creatine kinase; F = female; M = male; MG = myasthenia gravis; PM = polymyositis; RRF = ragged-red fibers; SDH = highly expressed succinate dehydrogenase staining fibers.

myalgia with every bout of common cold. She exhibited proximal dominant muscle weakness and elevated serum CK levels (691U/liter) upon admission. Thereafter, she gradually developed mild proximal dominant muscle weakness, but her serum CK level normalized. Although easily fatigued, she could manage day-to-day activities without support. Her 29-year-old daughter (data not shown) showed no evidence of muscle weakness; however, she complained of tiredness and exhibited an elevated serum CK level (more than 1,000U/liter).

CASE 3. This 64-year-old man was a chronic hepatitis B patient. By the age of 62 years, he had gradually developed dysarthria and dysphagia following lamivudine treatment for hepatitis B. However, he did not complain of limb weakness. Laboratory examination revealed normal blood lactate and pyruvate levels (9.8mg/dl and 0.8mg/dl, respectively), elevated lactate and normal pyruvate levels in the cerebrospinal fluid (21.4mg/dl and 1.0mg/dl, respectively), and an elevated serum CK level of 593U/liter. We initially suspected drug-induced myopathy. After discontinuing lamivudine, several symptoms improved slightly but dysphagia persisted.

We present a summary of patient characteristics and clinical findings in Table 1. The patient age ranged from 38 to 71 years, with the age of onset ranging from 30 to 60 years. All 9 patients had mild or moderate muscle weakness. Four of the 9 patients had a relevant clinical family history, and Case 7 was the mother of Case 8. Mild muscle weakness was observed in 7 patients. Varying serum CK levels were observed, and 5 of the 9 patients were initially diagnosed in other hospitals with other conditions, such as myasthenia gravis, polymyositis, viral myositis, and drug-induced myopathy. The mode of onset in 6 patients was acute or subacute. Seven patients experienced dysphagia or myalgia. Elevation in serum CK levels and myalgia resolved after lamivudine was discontinued.

Histopathological Study

Muscle biopsies from all patients indicated myopathic changes. Histopathological studies revealed a moderate variation in muscle fiber size but no necrotic fibers. Several RRFs (1–4%) were detected in all mGT-stained samples. Highly expressed fibers (2.0–8.5%) were observed in SDH-stained samples, but strongly SDH-reactive blood vessels were not detected in any sample. CCO-deficient fibers (2%–8%) were detected in all samples (Fig).

mtDNA Analysis

Sequencing of the entire mtDNA of 9 patients revealed the same 16 alterations: np200, np257, np1442, np4612, np5127, np6332, np7389, 9bp deletion between np8281 and 8289, np8291, np10403, np11151, np11969,

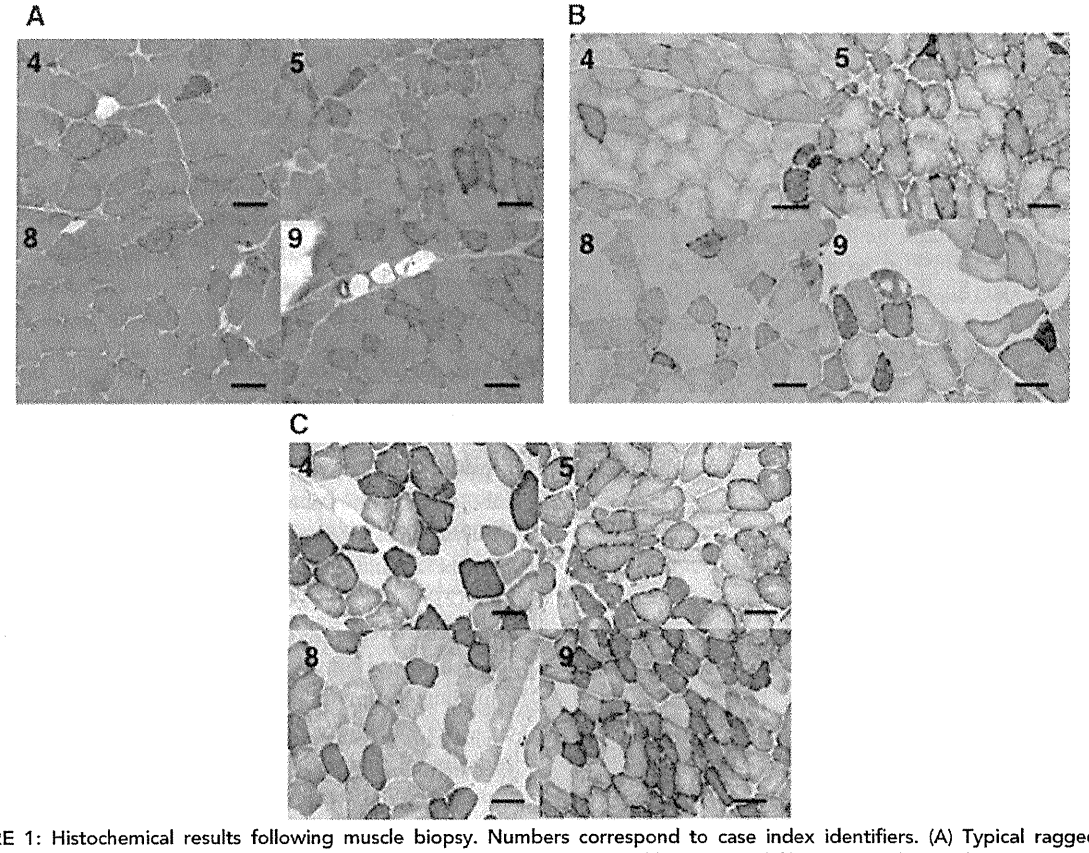

FIGURE 1: Histochemical results following muscle biopsy. Numbers correspond to case index identifiers. (A) Typical ragged-red fibers (1–4%) were detected in all Gomori trichrome-stained samples. (B) Highly expressed fibers were observed (2–8.5%) in succinate dehydrogenase-stained samples. (C) Cytochrome c oxidase-deficient fibers (2–8%) were detected in all samples. Bar = 100μm.

np13105, np16325, np16390, and np16523 (Table 2). All patients had the same 16 polymorphisms. In addition, Patient 4 had 3 additional mtDNA alterations (np3834, np4718, and np7375). These 16 mtDNA alterations have previously been reported as nonpathological polymorphisms. Six substitutions caused coding polymorphisms; other substitutions were observed in the 12S ribosomal RNA, a hypervariable site, and the displacement loop (D-loop). The mtDNA transition at np8291 has been reported and was considered to be a rare polymorphism. The frequency of mtDNA transition at np8291 was detected in only 2 of 600 controls (0.3%), including healthy subjects and patients with other neuromuscular disorders. Two positive patients had diabetes mellitus or myotonic dystrophy.[12] We could not detect any mtDNA alteration as a disease-associated mutation. The sequencing results of lymphocyte and skeletal muscle mtDNA were identical. All mtDNA variants in all patients were homoplasmic mtDNA alterations.

Discussion

We describe patients with novel mitochondrial myopathy characterized by episodic muscle weakness and elevated serum CK levels triggered by infections, drugs, or stressful situations. Furthermore, we demonstrate an association between mtDNA alterations, thus providing a novel aspect of mitochondrial disease pathogenesis.

Five of the 9 patients were initially diagnosed with other diseases, such as myasthenia gravis, polymyositis, viral myositis, or drug-induced myopathy. Disease onset was acute or subacute, and the patients experienced dysphagia or myalgia when on medication or during a bout of common cold. Case 3, an index case of this study, was admitted to the hospital following gradual development of dysarthria and dysphagia after lamivudine treatment for chronic hepatitis B. Initially, we suspected drug-induced myopathy because several symptoms, apart from dysphagia, were slightly improved after lamivudine was discontinued.

Mitochondrial dysfunction is a well-known side effect of nucleoside analogs, the best-known example being zidovudine, which is used mainly to manage human immunodeficiency virus infections.[18] In zidovudine-induced myopathy, molecular analysis of muscle biopsy shows depletion of mtDNA caused by drug-induced inhibition of mtDNA polymerase γ.[19] Following the muscle biopsy report of Case 3 that revealed RRFs, highly expressed

TABLE 2: Total mtDNA Sequencing Identified 16 Alterations Previously Reported as Polymorphisms, 10 Alterations in the MITOMAP Database, and 9 in the GiiB-JST mtSNP Database

Gene Product	Nucleotide Number	Base Change	Amino Acid Change	MITOMAP Database	GiiB-JST mtSNP Database
Hypervariable segment 2	200	A to G		Reported polymorphism	
Hypervariable segment 2	257	A to G		Reported polymorphism	Reported polymorphism
12S ribosomal RNA	1442	G to A			Reported polymorphism
NADH dehydrogenase 2	4612	T to C	M to T		Reported polymorphism
NADH dehydrogenase 2	5127	A to G	N to D		Reported polymorphism
Cytochrome *c* oxidase 1	6332	A to G	Synonymous		
Cytochrome *c* oxidase 1	7389	C to T	Y to H	Reported polymorphism	
Noncoding nucleotides 7	8272	9bp deletion		Reported polymorphism	
Noncoding nucleotides 7	8291	A to G		Reported polymorphism	Reported polymorphism
NADH dehydrogenase 3	10403	A to G	Synonymous	Reported polymorphism	Reported polymorphism
NADH dehydrogenase 4	11151	C to T	A to V	Reported polymorphism	
NADH dehydrogenase 4	11969	G to A	A to T	Reported polymorphism	
NADH dehydrogenase 5	13105	A to G	I to V	Reported polymorphism	Reported polymorphism
D-loop	16325	T to G			Reported polymorphism
D-loop	16390	G to A		Reported polymorphism	
D-loop	16523	A to G			Reported polymorphism

D-loop = displacement loop; GiiB-JST mtSNP = human mitochondrial genome single nucleotide polymorphism database (http://mtsnp.tmig.or.jp/mtsnp/index.shtml); MITOMAP = human mitochondrial genome database (http://www.mitomap.org); mtDNA = mitochondrial DNA; NADH = reduced nicotinamide adenine dinucleotide.

SDH staining fibers, and CCO-deficient fibers, this case was diagnosed with mitochondrial myopathy.

Muscle biopsy from the other patients revealed several RRFs, highly expressed SDH staining fibers, and CCO-deficient fibers. Histochemical parameters showed relatively mild alterations, and the low frequency of CCO-deficient fibers and RRFs might have been influenced by age-related changes. However, we could not explain the histochemical findings in Cases 8 and 9 as age-related changes because these were younger patients; hence, we surmise that their histochemical findings could be associated with their clinical features and the pathogenetic property of mtDNA alterations. Accordingly, we diagnosed all 9 cases as mitochondrial disease of similar genetic background and clinical findings.

Six patients in this study had experienced severe myalgia at some point in time; this is characteristic of recurrent myoglobinuria associated with mtDNA mutation.[20–22] In contrast, elevated serum CK levels were relatively low in these patients and recurrence rates were also

low; no patient had a history of voiding dark brown urine or acute renal failure. Furthermore, serum CK levels had normalized without medication at follow-up examinations. We believe that mild muscle weakness and the minor, episodic elevation in CK levels observed in our patients could be caused by mitochondrial dysfunction, as indicated by histochemical findings.

Patients in this study originated from 8 different families, but they had the same 16 mtDNA polymorphisms and a similar phenotype. In addition, all patients originated from the southern part of Japan. These results suggest that this disease is of mitochondrial origin, caused by mtDNA alterations, and transmitted by maternal inheritance, leading to the possibility that a common source exists or had existed in southern Japan. At the same time, these mitochondrial diseases were less likely to be associated with nuclear DNA. We evaluated all mtDNA alterations listed in MITOMAP and GiiB-JST (human mitochondrial genome single nucleotide polymorphism database; http://mtsnp.tmig.or.jp/mtsnp/index.shtml), the largest publicly available compendium of mtDNA polymorphisms. We found the following 16 alterations: np200, np257, np1442, np4612, np5127, np6332, np7389, 9bp deletion between np8281 and 8289, np8291, np10403, np11151, np11969, np13105, np16325, np16390, and np16523. However, each alteration previously reported in MITOMAP and GiiB-JST had been described as a nonpathological alteration.

The 16 polymorphisms are probably because of a rare haplotype that is probably derived from the B4f1 haplogroup of the East Asian mtDNA haplogroups that share 14 of the 16 polymorphisms (np200, np257, np1442, np4612, np5127, np6332, np7289, 9bp deletion between np8281 and 8289, np8291, np11969, np13105, np16325, np16390, and np16523).[23]

In addition, oxidative phosphorylation complex activity was studied in a previous study that included 4 of the 9 patients from this study; the activity of complex IV relative to that of citrate synthetase was reduced to about 50% in normal controls in this previous study.[12] Mitochondrial disease is usually caused by a pathological mtDNA rearrangement, with mtDNA mutations being classified as depletion, deletion/duplication, and point mutations. Nevertheless, a previous study reported that retrospective screening of 2,000 patients suspected of mtDNA disorders for common point mutations and large deletions identified mutations in only 6% of the patient population.[24] Mitochondrial myopathies with isolated skeletal muscle involvement and mtDNA mutation are relatively rare. However, many patients could live normally with pure myopathy but still harbor unknown genetic defects in the mtDNA. A previous study reported exercise intolerance due to mutations in the cytochrome *b* gene of mtDNA;[25] the clinical manifestations included progressive exercise intolerance, proximal limb weakness, and in some cases, myoglobinuria.

In several reports, double disease-associated mutations were detected in the same patients with Leber's hereditary optic neuropathy (LHON);[26–28] these mutations may have some influence on the symptoms of LHON. Another study reported that some polymorphisms adjacent to the 3243A>G mutation had different effects on the clinical phenotype, muscle pathology, and respiratory chain enzyme activity.[29] Yet another pathogenesis has been suggested; antiretroviral therapy causes peripheral neuropathy, a pathogenesis in which nucleoside reverse transcriptase inhibitor (NRTI)-associated mitochondrial dysfunction, inflammation, and nutritional factors have been implicated. Owing to its well-documented potential for inducing mitochondrial dysfunction and oxidative stress, NRTI therapy could be considered as a significant environmental challenge, which, when superimposed on genetic susceptibility, leads to a toxicity phenotype. The environmentally determined genetic expression (EDGE) concept provides a framework for considering the combinations of genetic and environmental exposure that define the thresholds for expression of specific phenotypes in an individual. This concept holds that genetic variations in expressed proteins have different effects in different environmental contexts, and that disease or toxicity phenotype is determined by the functional magnitude of the genetic change and the severity of the environmental exposure.[30]

In summary, the findings of distinct clinical features, mitochondrial pathologic changes and the same mitochondrial genetic background in all patients suggest that this disease could be a novel mitochondrial disease. Although we did not identify the key pathogenic mutations, this disease should be associated with some of the 16 mtDNA alterations or at least with their mitochondria. Therefore, we propose that this disease be named as "mitochondrial myopathy with episodic hyper-CK-emia (MIMECK)." We believe that this study provides an insight into a novel aspect of mitochondrial disease pathogenesis.

Furthermore, pharmacogenetic studies on drug-induced and associated mtDNA alterations could contribute to research leading to the discovery and design of novel drugs that would eliminate the negative side effects associated with current therapies. Further genetic and clinical studies, especially involving persons of another race and from other geographic areas, will clarify the pathogenesis of this disease.

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Potential Conflict of Interest

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