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.

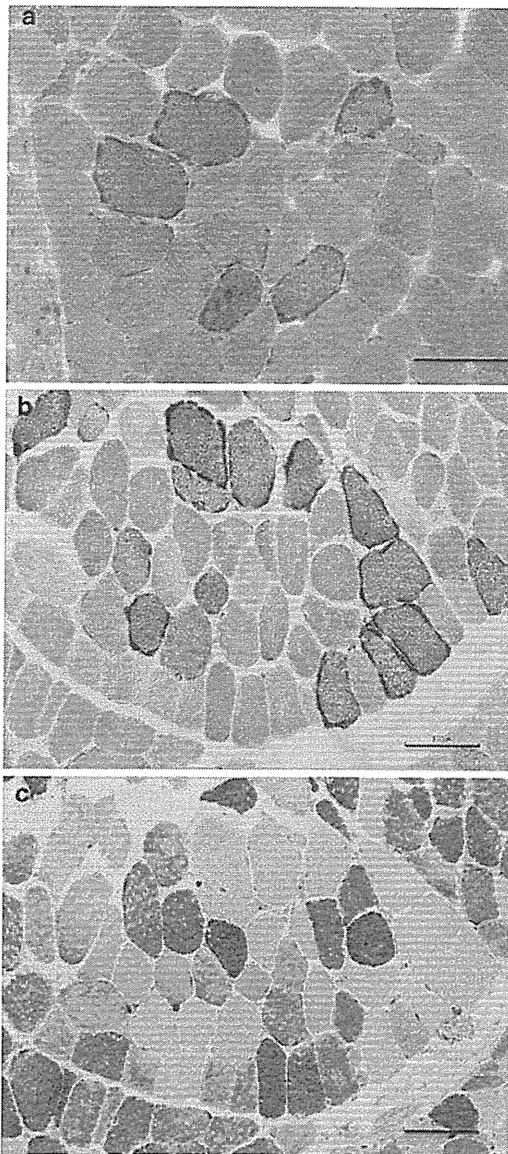

Fig. 3 Histochemical analysis of the right biceps brachii muscle. **a** Gomori trichrome staining reveals typical ragged-red fibers. Histochemical analysis of serial sections of samples stained with **b** SDH or **c** COX shows a number of ragged-blue fibers with COX deficiency. **a**–**c** *Bar* 100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

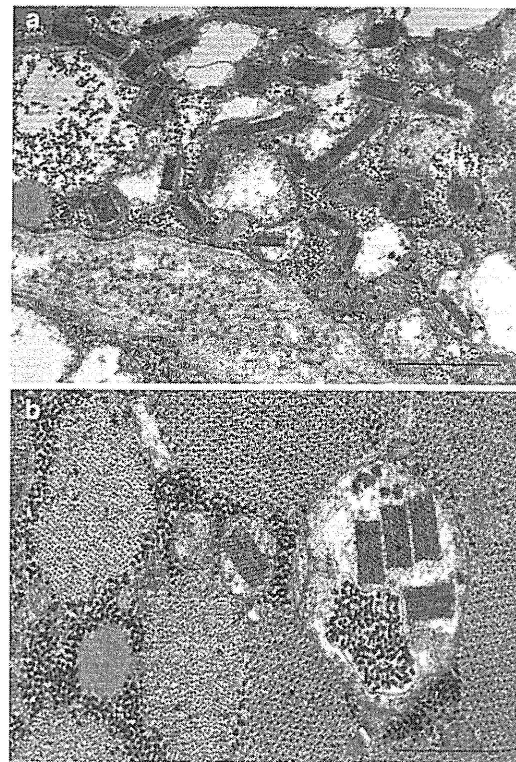

Fig. 4 Electron micrograph of abnormal mitochondria in the right biceps brachii muscle. Abnormal mitochondria with paracrystalline inclusions that are suggestive of mitochondrial myopathy are shown. **a** *bar* 1 μm, **b** *bar* 500 nm

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

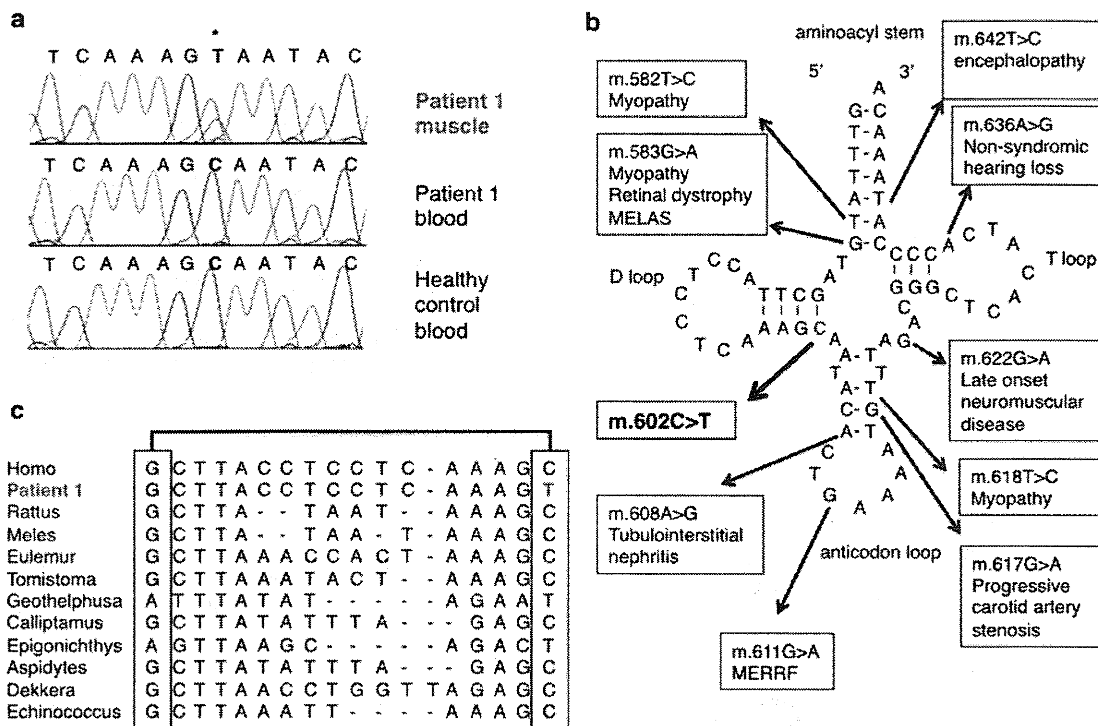

Fig. 5 **a** Sequence chromatogram of the mitochondrial DNA region that encompasses the m.602C>T alteration (*asterisk*) that was obtained from the skeletal muscle of patient 1 (reverse complement). **b** Schematic diagram of the mitochondrial $tRNA^{Phe}$ cloverleaf structure showing previously reported 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,

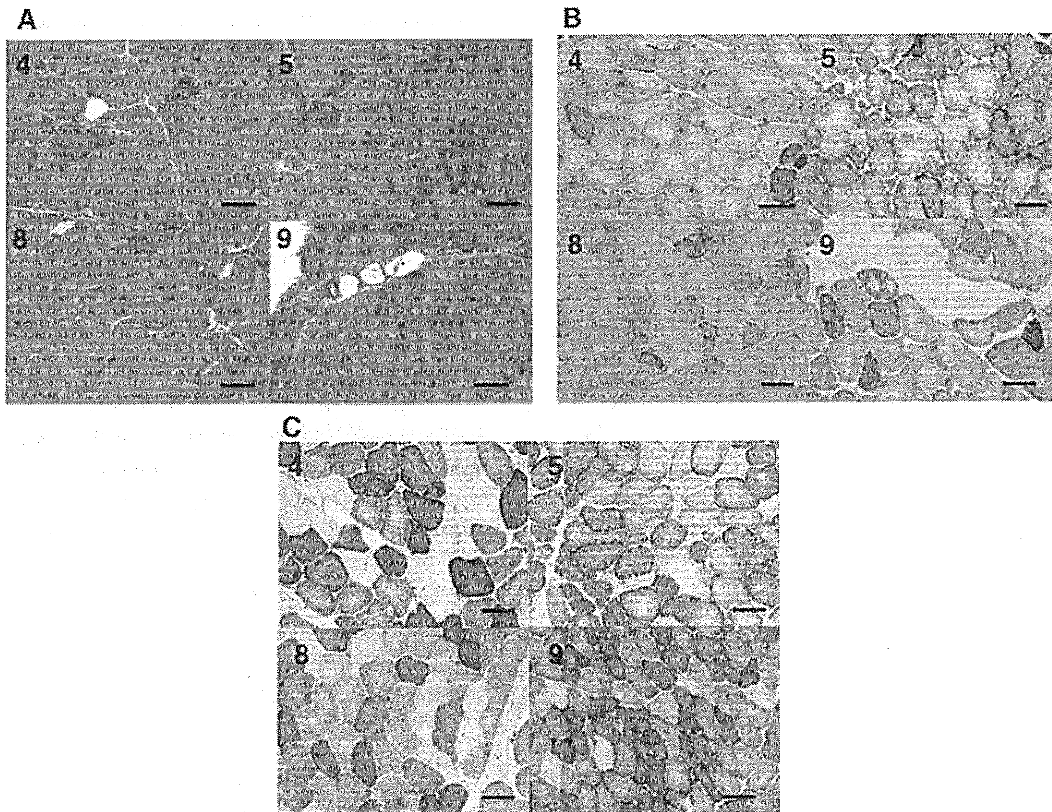

FIGURE 1: Histochemical results following muscle biopsy. Numbers correspond to case index identifiers. (A) Typical ragged-red fibers (1–4%) were detected in all Gomori trichrome-stained samples. (B) Highly expressed fibers were observed (2–8.5%) in succinate dehydrogenase-stained samples. (C) Cytochrome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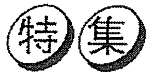

HAM（HTLV-1 associated myelopathy）

HAMの新しい展開*

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Key Words：HTLV-1, task force, epidemiology, high provirus load, treatment

はじめに

HTLV-1-associated myelopathy（HAM）が発見されてすでに四半世紀が経過した[1]．当時，慢性進行性で不治の病と考えられる神経難病の一部が治療可能なウイルス性疾患として新たに分類されたことは，神経内科医にとって大きな驚きであった．次々と新たな知見が積み重ねられ，その臨床像，病理像が明らかとなり，新たな疾患概念が確立していった．HAM発見の経緯については，本特集の納先生の稿，そして，井形先生の近著[2]を参照いただきたい．

HAMに関連した新たな動きとして，2008年（平成20年）6月にHAMが国の「難治性克服研究事業」の対象疾患として正式に指定された．また，昨年（2010年）9月にはHTLV-1感染対策に関する政府特命チームが組織され，本年度からHTLV-1対策が政策課題として本格始動している．すなわち，HAMの診療と研究を取り巻く環境は大きく変貌しようとしている．本稿ではHAMを取り巻く最近の話題について紹介したい．

HTLV-1感染の疫学

HAM患者自身の社会的活動を契機として新たに社会医学的視点での動きが始まり，HTLV-1の健常感染者数，ATL，HAMの両疾患についての全国疫学調査が実施された．

HTLV-1感染者数は20数年前の献血者の抗体陽性数から，全国で120万人と推定されていた．九州・四国，沖縄に多く，地域での対策により今後漸減し，数十年後には新規の感染者はこよなくゼロに近づくとの推計もあり，全国的な感染対策はとられず，また，感染者の全国調査も行われてこなかった．しかし，感染者，患者の実態調査の必要性を訴える声を受けて，研究班が組織され，日本赤十字社の協力を得て2008～2009年の初回献血者の抗体陽性率をもとに感染者数の調査が行われた[3]．その結果，HTLV-1抗体陽性者は全国で108万人であると推計され，20数年前と比較し，予想ほど減少していなかった．さらに地域別にみると，母児間感染などに対する対策を行った九州地区では漸減の傾向が明らかであるのに比べ，積極的な対策が行われていない他の地域，とくに大都市圏ではむしろ増加しており，HTLV-1感染が全国へ拡散している実態が明らかにされた．

HAMについては，1995年に確診例1,103名の患

* New evolution of HAM/TSP research.

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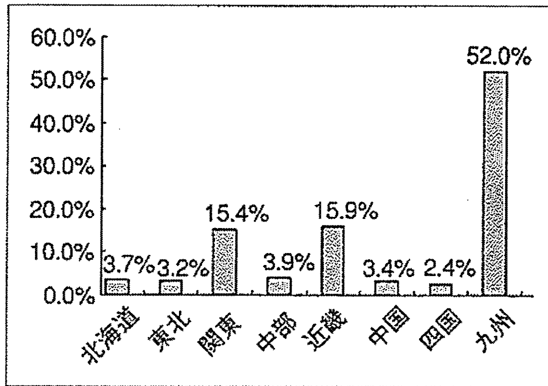

図1　地方別のHAM患者数の比率

者が報告されている．今回15年ぶりにHAM全国疫学調査が実施された[4]．2007年，2008年の2年間に通院，入院したことのあるHAM患者を対象に，全国の神経内科診療施設にアンケート調査を施行した．回収率33.5%の時点でHAM患者は790人が登録された．内部コントロールとして同時に調査した筋萎縮性側索硬化症(ALS)患者は1,002人が登録され，これまでの調査でALSの有病率は人口10万人あたり約4人と想定され，それとの比較により，HAMの有病率は人口10万人あたり3人，全国でおよそ3,600人のHAM患者がいるものと推定された．患者の分布は九州・沖縄地方で52.0%を占めていたが，関東15.4%，近畿地方15.9%と大都市圏でも多くのHAM患者が集計され(図1)，前回調査と比較して大都市圏での比率の増加が明らかであった．

最近の傾向をみるためにHAM患者を1994年以前の診断例と1995年以後の診断例に分け比較した．診断年におけるHAM患者の年齢比較では，1994年以前に診断された65歳以上の高齢者は1.3%でしかなかったが，1995年以降診断された患者では65歳以上の高齢者が26%を占めていた．近年，高齢になって診断される例が増加していることが示された．また，地域別には1995年以降の診断が近畿で54例(+3%)，関東76例(+12.9%)が新たに診断され，大都市圏での診断が増えており(表1)，HTLV-1感染者の全国への拡散傾向を裏づける結果となっている．発症数を年別にみると，1995年以降，実数として30人前後が新規にHAMを発症しており，2004年を除いて大きな変動はみられない(図2)．背景にあるHTLV-1キャリア数がほとんど変化していないことを

表1　HAM発症者の地方別比率の変動

地方	1994年以前 発症患者数(%)	1995年以後 発症患者数(%)	変動率(%)
北海道	9(2.8%)	10(2.2%)	−0.6%
東北	9(2.8%)	14(3.0%)	+0.2%
関東	44(13.8%)	78(16.8%)	+3.0%
中部	4(1.3%)	24(5.2%)	+3.9%
近畿	46(14.5%)	71(15.3%)	+0.8%
中国	9(2.8%)	14(3.0%)	+0.2%
四国	9(2.8%)	10(2.2%)	−0.6%
九州	176(55.5%)	233(50.2%)	−5.3%
合計	317	464	

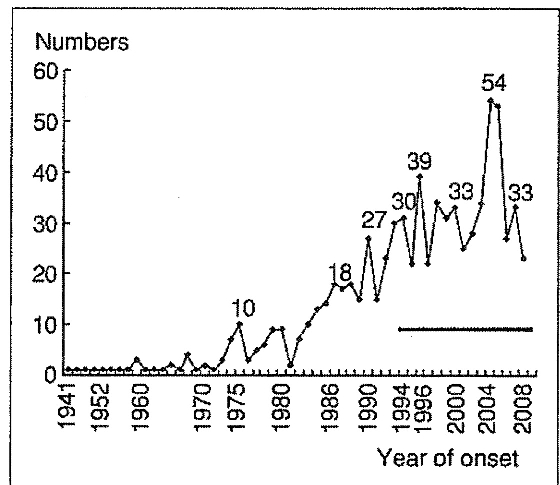

図2　HAM患者発症数の年次変化

背景に，毎年一定の割合でHAMも新規に発症し続けていることを示している．

HAM研究の流れ

HAMの疾患概念確立の過程で重要な役割を果たしたものの一つは神経免疫学の主要な研究対象である多発性硬化症(multiple sclerosis：MS)とHTLV-1との関連を示唆する論文がNature誌上に発表されたことで[5]，HTLV-1感染と神経疾患との関連を探っていた鹿児島大学のスタッフには，カリブ海に浮かぶ島々に鹿児島で注目している患者と同様の患者がMSと診断されて存在していることを示唆する報告として大いに注目することとなった．実際，HAMの発見以前にはMSと診断されていた患者も少なからずいたことは，その後のHAMの研究に大きく寄与した．これまでに進められてきたMSの研究テーマはそのままHAMの研究に応用され，特異な自己増殖反応の存在[6]，サイトカインのTh_1優位性[7]，HAM発症

に関与するHLA(human leukocyte antigen)遺伝子[8]など，次々とHAMに特徴的な免疫動態が明らかにされていった．治療の面でもステロイド剤に反応して症状の改善が認められたことを背景に，血漿交換療法[9]，インターフェロン-α[10]，γ-グロブリン大量療法[11]，リンパ球除去術[12]，ペントキシフィリン[13]などが試みられ，ある程度の治療効果が認められている．これらの治療法の多くはMSをはじめとする免疫性神経疾患の治療法としてその免疫動態をコントロールすることを目指して行われていた治療法である．

一方，近年の分子生物学を背景としたウイルス学の進歩に呼応して，HAMの研究は免疫動態の解析からHTLV-1の動態そのものにその対象を移してきた．これまでに，ウイルス遺伝子のサブタイプによりHAM発症のリスクが異なること[14]，HAMでは末梢血単核球中のプロウイルス量が健常キャリアに比較し約10倍多く，HAMの最大の発症リスクであること[15]，PBMC中のプロウイルス量の変動は疾患活動性と連動していること[16]，髄液中，さらに脊髄組織中のプロウイルス量が炎症の活動性と連動していること[17][18]が明らかとなった．さらには，脊髄炎症病巣でウイルスは浸潤Ｔ細胞に感染しており[19]，ウイルスmRNAの発現[20]が証明されている．いずれの結果もウイルスの生体内での動態はHAMの発症，病勢と深く関連していることを示している．それに呼応して，免疫学の領域でもウイルスを標的とした免疫反応の特異性について精力的に解析が行われた．髄液中の抗HTLV-1抗体，とくにIgM抗体価の上昇[21]，髄腔内での抗体産生亢進[22]はHAM患者髄腔内でHTLV-1に対する液性免疫が持続的に生じていることを示している．細胞性免疫については，HTLV-1の調節遺伝子産物であるTaxを認識する細胞傷害性Ｔ細胞がHAM患者末梢血から高率に分離できることが明らかにされた[23]．実際，このTax11～19を認識する細胞傷害性Ｔ細胞がHAM患者のPBMCs中で活性化しており，プロウイルス量と相関し，さらにプロウイルスの変動に連動して動いていることも明らかとなった[24]．これらの結果は，HAM患者ではHTLV-1に対する強い免疫応答が生じていることを示している．

これらの研究はHAMがHTLV-1感染症であるという事実を明らかにし，ウイルス量を減らすことがHAM治療の目標となることを示している．

HAM研究の新たな展開

すでに臨床試験に入っている具体的な治療法として，細胞内でのHTLV-1遺伝子発現を標的にプロスルチアミンやペントサンの治療効果が報告されており，本特集で中村先生が紹介されている．一方，ウイルス学の領域ではHTLV-1の細胞間感染拡大がウイルスシナプスの形成という特殊な機序を介して行われていることが明らかとなり[25][26]，ウイルス感染受容体の解析やウイルスシナプス形成の分子機構が精力的に進められている．HAMは前述のように，感染者生体内でウイルス増生を背景に発症しており，生体内でこのウイルスシナプスを介した感染拡大が生じていることがうかがわれる．ウイルス感染拡大阻止はHAMの治療法として理想的で，その治療標的分子を探索する目的で，近年，一気に普及したマイクロアレー法，レクチンアレー法などによる細胞膜蛋白表面の遺伝子発現，糖鎖分子の網羅的解析が進められている．また，プロテオーム解析を含めたこれらのポストゲノムの網羅的解析により，病態の理解，疾患活動性，重症度，予後予測などに有用なバイオマーカーの探索が進行中である．さらに，HTLV-1感染者のごく一部にのみHAMは発症し，疾患感受性にかかわる複数の因子があることは明らかで，そのいくつかはすでに報告されている[27]～[29]．次世代シーケンサーを用いた網羅的ゲノム解析も開始されることになった．

一方で，今回の疫学調査から明らかとなったHAM患者の全国的な拡散傾向から，HAM診療の経験に乏しい医療施設でも的確な診療が可能となる診断・治療マニュアルの作成や臨床研究体制の整備など，より日常診療の現場に沿った研究と対策が必要とされている．

社会医学としてのHAM

HTLV-1研究は血液悪性腫瘍として血液学の領域で，ヒトの初めての腫瘍ウイルスとしてウイルス学の領域で，そして，新たな神経疾患とし

て神経内科学の領域で，それぞれ独立して研究が進められていたが，一方で，世界的には研究者が領域の垣根を越えて交流する場として，早期より国際HTLV会議が開催され，基礎科学から臨床・疫学までが一会場に集まり，研究成果を共有していた．国内でも交流する場の必要性が認識され，領域の枠を超えた交流の具体的な形として4年前にHTLV-1研究会が組織され，HTLV-1感染症としての共通の認識が育まれていくことになった．今回のHAMに関する新たな展開の端緒となったのは，HAM患者自身が起こしたHAM, ATLの窮状を訴える社会活動で，患者会が組織され，NPO法人に発展し，マスコミを通じてその活動は報道された．診療にあたっている医師，研究者は共通の認識として対策の必要性を理解し活動をサポートするとともに，患者会と厚労省の関連部局を交えた「専門家会議」を組織し議論を重ねた．幸い厚労省の部局の枠を超えた理解を得ることとなり，政府による「HTLV-1特命チーム」発足へとつながった．この一連の経緯は種々の難病対策について，一つの行動モデルを提示していると思われる．社会から置き去りにされがちな難病患者を理解し，サポートし続けることの必要性をあらためて認識するとともに，今回のHTLV-1対策の成果としてHAMが難病ではなくなる日がくることを祈念したい．

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ORIGINAL ARTICLE

Histopathological differences between human T-lymphotropic virus type 1-positive and human T-lymphotropic virus type 1-negative polymyositis

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Keywords
cytochrome c oxidase; human T-lymphotropic virus type 1; mitochondrial abnormality; polymyositis

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Abstract

Objectives: Epidemiological studies show that human T-lymphotropic virus type 1 (HTLV-1) is closely associated with polymyositis (PM). However, the pathogenic roles of HTLV-1 in PM remain unknown. The present study aims to assess skeletal muscle morphology in the presence of HTLV-1 infection to compare the histopathological findings of HTLV-1-positive and HTLV-1-negative PM.

Methods: Among the 68 patients with inflammatory myopathy diagnosed through muscle biopsy over the previous 10 years at Kagoshima University Hospital, we retrospectively selected 21 patients with PM not associated with any other disease; we evaluated HTLV-1 positivity through serology, confirmed it by nested polymerase chain reaction using DNA extracted from muscles, and then assessed the tissue viral load. Meticulous histopathological examination was carried out using routine histochemical and immunohistochemical staining, and specimens from selected cases were examined by electron microscopy.

Results: The clinical and histopathological findings of muscle biopsy specimens of HTLV-1-positive (n = 11) and HTLV-1-negative PM cases (n = 10) were compared. Compared with HTLV-1-negative patients, HTLV-1-positive patients showed protracted clinical courses, prominent endomysial infiltrates, infrequent necrotic fibers and prominent regenerative activities. Furthermore, they showed frequent cytochrome c oxidase deficiency and ultrastructural abnormalities in mitochondria.

Conclusions: These differences are significant, but not specific to HTLV-1-positive PM. Therefore, HTLV-1 might induce the clinical and histopathological modifications of PM observed in the present study. (Clin. Exp. Neuroimmunol. doi: 10.1111/j.1759-1961.2011.00017.x, January 2011)

Introduction

Human T-lymphotropic virus type 1 (HTLV-1), the first human retrovirus to be identified, is a causative agent of adult T-cell leukemia (ATL). HTLV-1 has been reported to be associated with a particular type of chronic progressive myelopathy: HTLV-1-associated myelopathy/tropical spastic paresis (HAM/TSP).[1] HTLV-1 infection has also been linked to other inflammatory diseases, such as uveitis, arthritis, bronchoalveolitis, Sjögren's syndrome and myositis.[2] Epidemiological studies show a high incidence of seropositivity for HTLV-1 among polymyositis (PM) patients. However, whether HTLV-1 is a direct causative agent or it plays a role in the pathogenesis of PM remains unknown.[2,3] In addition, HTLV-1 infection is closely related to inclusion body myositis (IBM).[4–6]

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PM and IBM are two types of inflammatory myopathies. IBM can be distinguished from PM by the presence of rimmed vacuoles, cytoplasmic inclusions and amyloid deposits in addition to inflammatory changes. However, because of the large similarity between PM and early IBM, modified criteria for diagnosis were introduced by Dalakas and Hohlfeld in 2003, in which careful follow up and repeated biopsies are required to avoid misdiagnosing early IBM as PM.[7,8]

To determine the clinical and histopathological differences between HTLV-1-positive and HTLV-1-negative PM, we retrospectively evaluated patients with PM not associated with any other disease and carried out a comparative study.

Methods

Patients

Of all patients who underwent muscle biopsy during the past 10 years at Kagoshima University Hospital, South Kyushu, Japan, 68 patients were reported to be diagnosed with inflammatory myopathy; of these patients, 21 were carefully selected for the present study, as shown in Fig. 1.

The 21 selected patients were re-evaluated using the modified criteria introduced by Dalakas and Hohlfeld,[7] which list the following essential requirements for diagnosing PM: (i)elevated serum creatine kinase (CK); (ii) electromyography (EMG) abnormalities; and (iii) muscle biopsy abnormalities. Muscle biopsy abnormalities are crucial and include degeneration, regeneration, necrosis and foci of lymphocytic inflammatory cells with primary inflammation [i.e. $CD8^+$ cells surrounding healthy intact non-necrotic muscle fibers expressing major histocompatibility complex (MHC)-1 without rimmed vacuoles].

Informed consent was obtained from all patients. The institutional review board for regulating clinical research approved the present study.

Muscle biopsy

All muscle biopsies were obtained by open surgical procedures from the biceps brachii or quadriceps femoris. Muscle specimens for histopathological examination were snap-frozen by isopentane chilled in liquid nitrogen; 7-μm-thick sections were routinely stained with hematoxylin and eosin (HE), modified Gomori trichrome, periodic acid-Schiff (PAS), Sudan black, myosin ATPase, NADH-tetrazolium reductase, cytochrome c oxidase (CCO), adenosine monophosphate (AMP) deaminase, acid phosphatase and succinate dehydrogenase (SDH).[9] Select sections were

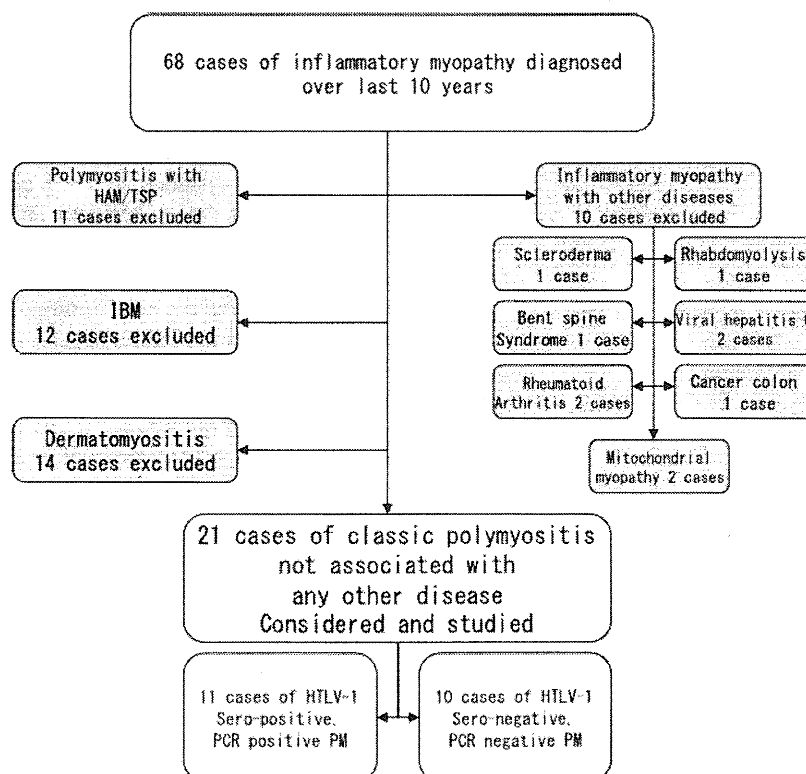

Figure 1 Classification of final diagnoses after the initial diagnosis. The subjects reported with diagnoses other than polymyositis (PM) or PM associated with any other disease were excluded from further analysis. HAM/TSP, human T-lymphotropic virus type 1-; HTLV-1, human T-lymphotropic virus type 1-associated myelopathy/tropical spastic paresis; IBM, inclusion body myositis; PCR, polymerase chain reaction.

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