

Variable phenotypes in a family with mitochondrial encephalomyopathy harboring a 3291T > C mutation in mitochondrial DNA

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Abstract We present a Japanese family suffering from mitochondrial encephalomyopathy associated with a T-to-C transition at mitochondrial DNA (mtDNA) nucleotide position 3291. Clinical manifestations of the patients include cerebellar ataxia with myopathy, recurrent headache, and myoclonus and epilepsy. The phenotypic variation among the affected members of a single family and the mutational analysis showing maternal inheritance in a heteroplasmic fashion are consistent with well-recognized phenomena associated with many pathogenic point mutations of mtDNA tRNA genes. The 3291 mutation is a rare mtDNA mutation whose clinical presentation had only been reported in three sporadic cases. This is the first report of a family segregating the 3291 mutation with multigenerational matrilineal recurrence of mitochondrial encephalopathy. Our findings provide conclusive evidence for the pathogenicity of the 3291T > C mutation in mtDNA and its characteristic clinical heterogeneity.

Keywords Mitochondrial encephalomyopathy · Family case · 3291T > C · mtDNA mutation

Introduction

With recent advances in molecular genetic analysis, an increasing number of different nucleotide substitutions in mitochondrial DNA (mtDNA) have been identified in patients with mitochondrial encephalomyopathy. Point mutations in mitochondrial tRNA genes, in particular, are commonly associated with mitochondrial diseases. Currently, more than 90 different mutations within mitochondrial tRNA genes have been characterized as pathogenic (MITOMAP: A Human Mitochondrial Genome Database, <http://www.mitomap.org>). Among the most prevalent of these point mutations are the 3243 and 3271 mutations, which are closely associated with the syndrome of mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS). The 3291 mutation in mtDNA was first identified in a single Japanese patient with a typical MELAS phenotype [1]. Like the 3243 and 3271 mutations, the 3291 mutation in mtDNA is located in the tRNA-Leu(UUR) gene. Thus, this mutation is also thought to be associated with the MELAS phenotype. In contrast to the original patient, the second case associated with the 3291 mutation was reported in an Italian child affected by an apparently isolated, mild myopathy [2]. The third case with the same mutation was an Italian female who suffered from progressive cognitive and behavioral decline, and hearing loss [3]. Here we describe a Japanese family that represents the fourth case—and the first familial case—of mitochondrial encephalomyopathy harboring a 3291T > C mtDNA mutation, and show that the clinical

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manifestations are apparently different from those seen in MELAS.

Case report

Figure 1a shows the pedigree of our patients. A 70-year-old woman (II-4) was admitted to our hospital due to a 6-year history of progressive gait disturbance. At the age of 45 years, she developed bilateral hearing deficits, and at 64 years, she underwent surgery to correct cataracts and glaucoma. Neurological examination of the patient revealed mild external ophthalmoparesis, nystagmus, sensory neural hearing loss, dysarthria, and severe cerebellar ataxia. There was mild proximal muscle weakness of the limbs. Tendon reflexes were reduced generally. Electroencephalogram (EEG) assessment showed diffuse paroxysmal slow activity (3–6 Hz), which was induced by photo-stimulation. No epileptic discharge was found. Needle electromyography showed low amplitude and short motor-unit potential in the proximal extremities. Brain magnetic resonance imaging (MRI) showed atrophy of frontal lobe and cerebellar cortex (Fig. 2). There was no lactate peak on brain magnetic resonance spectroscopy in a voxel placed over a lateral ventricle. Laboratory test results revealed normal lactate levels and slightly increased pyruvic acid levels. Cerebrospinal fluid analysis was normal. The psychometric test showed impaired intelligence, memory, and frontal function.

The proband's mother (I-2) suffered from hearing loss and glaucoma. Since the age of 80, she had slowly become progressively bedridden. She died at the age of 84.

The proband's first daughter (III-2) is 41 years old and has had palindromic rheumatism since the age of 14 years. She has not experienced convulsion, myoclonic event or stroke-like episodes, but has had recurrent migraine



Fig. 2 The T2 MRI sequences in patient II-4 showed apparent atrophy of the frontal lobe and cerebellar cortex

attacks. Brain MRI showed multiple, small hyper-intense areas in subcortical white matter of the cerebrum on a T2-weighted sequence. Her neurological examination showed no abnormalities.

The proband's second daughter (III-3) is 39 years old and has experienced photo-induced myoclonus since the age 36 years. At that time, atrophy of the cerebellum was noted on MRI. At the age of 38 years, she started taking anticonvulsant medication due to negative myoclonus and photo-induced myoclonus on EEG examination. Her neurological examination showed an absence of tendon reflexes in all limbs and mild truncal ataxia. Muscle strength and mental status were normal.

The proband's first granddaughter (IV-1) is 17 years old. She was delivered with the aid of a vacuum extractor at 37 gestational weeks. At the age of 15 years, loss of

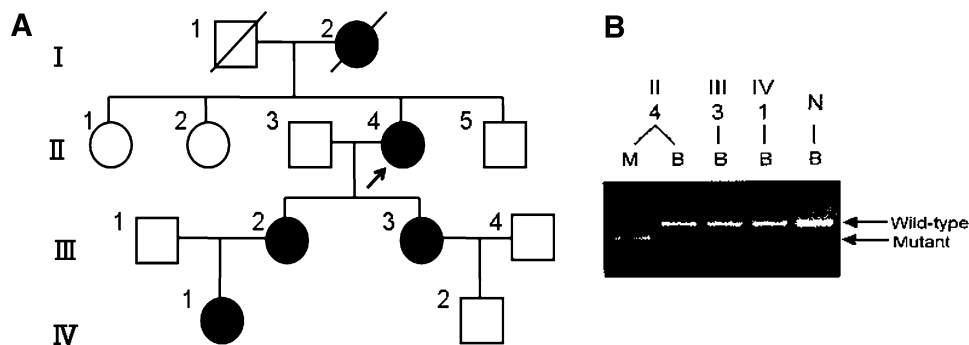


Fig. 1 **a** Pedigree of our patients. Proband is indicated by arrow. Open squares and circles denote healthy relatives; filled squares and circles indicate the affected offspring. **b** PCR–RFLP analysis using a modified set of primers that specifically detects the 3291 mutation. PCR amplification yields a 309-bp product that is cleaved to a 280-bp

product by *Bam*HI digestion in individuals carrying the 3291 mutation, but not in individuals with wild-type mtDNA. PCR products were separated on 4% agarose gel and visualized by ethidium bromide staining. DNA was not obtained from the patients of I-2 and III-2

consciousness lasting a few seconds and myoclonic jerks in her upper extremities and trunk appeared. Two years later, she experienced her first generalized seizure with an abnormal EEG record and started taking antiepileptic drugs. School records indicated below average academic performance. Her neurological examination showed an absence of tendon reflexes in all four limbs and slight cognitive decline. Coordination and muscle strength were normal.

Otherwise, there are no other family members known to have any endocrine or neurological disease.

Materials and methods

A muscle biopsy was performed in the proband patient (Fig. 1a II-4) on the left bicep muscle, and serial frozen sections were stained with hematoxylin and eosin (H&E), modified Gomori trichrome and a range of histochemical stains.

DNA samples were prepared from the muscle biopsy and heparinized blood specimens from the proband and from her symptomatic daughters and grandchild. DNA was extracted from muscle as previously described [1], and from whole blood using standard procedures. PCR-restriction fragment length polymorphism (RFLP) was performed as previously described [1]. Briefly, PCR reactions were performed using a modified primer containing a G-to-C mismatch at 3286 and a G-to-A mismatch at 3289 to generate 309-bp products, which were then digested by *Bam*HI. In the presence of the 3291 mutation, two cleaved fragments of 280- and 29-bp would be expected. The proportions of mutant versus total mtDNA were calculated by densitometric analysis using QuantiScan software (Biosoft, UK).

Results and discussion

H&E-stained muscle biopsies showed marked variation in fiber size, ranging from 15 to 115 μ m in diameter. Some fibers had internal nuclei. Scattered, ragged red fibers were observed with modified Gomori trichrome stain, and a succinate dehydrogenase (SDH) reaction revealed many ragged red fibers (RRF) (Fig. 3a). Several strongly SDH-reactive blood vessels (SSVs) were highlighted (Fig. 3b). Staining for cytochrome *c* oxidase (COX) activity demonstrated the presence of scattered COX-deficient fibers, and showed that SSVs were COX-negative (Fig. 3c). The muscle biopsy also showed RRF/COX-positive fiber, as evident from Fig. 3. The proportions of RRF and of COX defective muscle fibers found in our patient were 4.2 and 5.6%, respectively. The above findings are suggestive of mitochondrial disease.

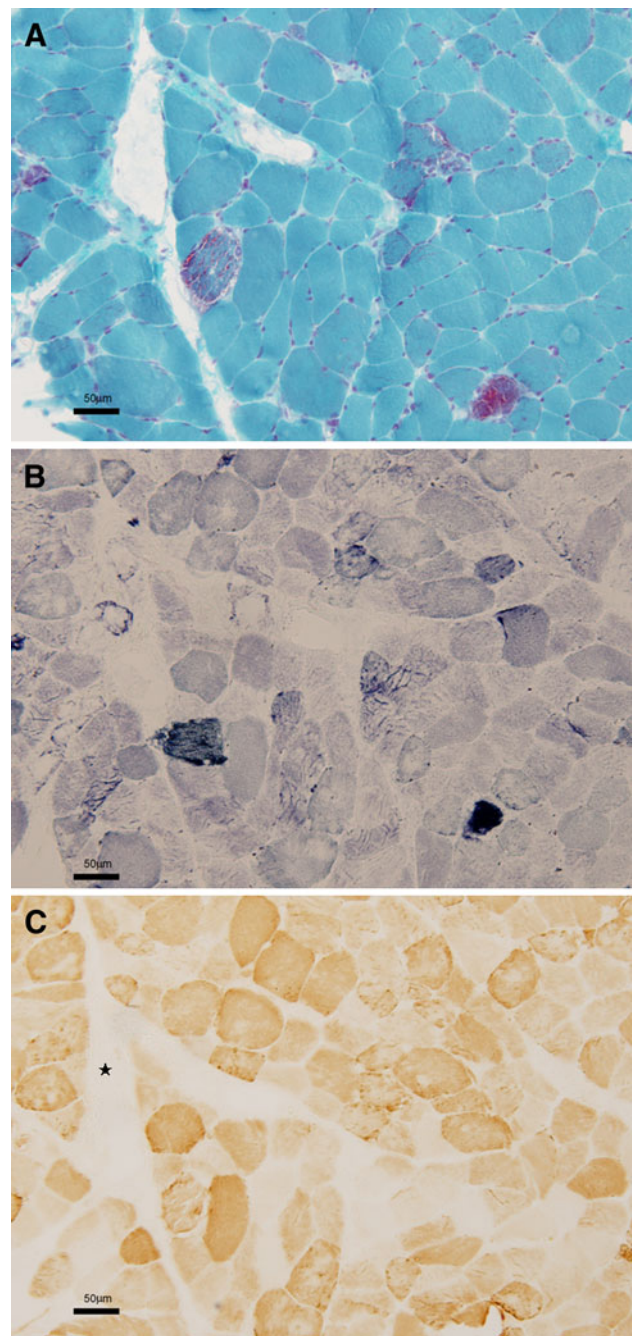


Fig. 3 Light microscopy of muscle biopsy from patient II-4. **a** Modified Gomori trichrome staining shows several ragged red fibers. **b** Succinate dehydrogenase reaction (SDH) shows numerous hyperintense fibers. Strongly SDH-reactive blood vessel (SSV) is highlighted. **c** Cytochrome *c* oxidase (COX) reaction shows several negative fibers. Asterisk indicates that SSV is COX-negative. Scale bars represent 50 μ m (color figure online)

PCR–RFLP analysis using a modified set of primers that specifically detects the 3291T \rightarrow C mutation in mtDNA showed maternal inheritance of the mutation in a heteroplasmic manner (Fig. 1b). These findings demonstrate that the 3291 mutation is pathogenic in this family.

Previously, we identified a novel point mutation of the tRNA-Leu(UUR) gene in a single patient affected by the typical MELAS phenotype [1]. The second case involving the 3291 mutation was reported by Uziel in an Italian child with isolated, mild myopathy [2]. The third case with the same mutation was revealed by Salsano in an Italian female who presented with cognitive and behavioral disturbances, and hearing loss [3]. Here we describe the third case with an apparent family history. The symptoms of the proband include severe cerebellar ataxia, myopathy, mild ophthalmoparesis, hearing loss, and asymptomatic EEG abnormality. COX-negative SSV in biopsied muscle of the patient (Fig. 3) is compatible with the histological findings of myoclonic epilepsy and ragged red fiber (MERRF) rather than MELAS [4]. Furthermore, clinical manifestations in the patient's second daughter and the patient's first grandchild are consistent with the MERRF phenotype. In contrast, the patient's first daughter had recurrent migraine-like attacks. These findings indicate that the same 3291 mutation in mtDNA can be associated with a wide spectrum of phenotypes, including MELAS and MERRF. Interestingly, the symptoms within the family become apparent at successively earlier ages as the condition is passed to subsequent generations. Thus, not only the disease manifestations, but the age of onset is also variable.

MtDNA is transmitted only from females to their offspring, but a single female can bear offspring who harbor different levels of mutant mtDNA. During oogenesis, the mutant and normal mtDNA segregating units are dispersed through repeated cell divisions, resulting in the protoocytes segregating their mtDNA genotypes toward predominantly normal or mutant mtDNAs [5]. Some of the phenotypic variability, especially that seen between patients with the same mtDNA mutation, is undoubtedly attributable to differences in the level of heteroplasmy within an individual [6]. Moreover, the distribution of the mutant mtDNA in different tissues varies widely among individuals [7].

Analysis of mtDNA from peripheral leukocytes of our patients estimated the proportions of mutant versus total mtDNA as followed: II-4 11%, III-3 16% and IV-1 27%. The amount of detection of the point mutation in blood samples was age dependent (Fig. 1b). A similar tendency was also evident for two previously reported patients with neuromuscular disorders associated with the 3291 mutation. Analysis using blood samples is not as sensitive, particularly in older subjects. The absence of the mutation

in the blood samples suggests that there is a preferential selection process for normal (wild type) mtDNA over time. This may be related to the rate of cell division and energy requirements of each tissue. By contrast, muscle is composed of highly differentiated, post-mitotic syncytial cells, in which no selection against cells containing defective mitochondria can occur. The proband patient was demonstrated to have the same mutation at high abundance (74%) in the biopsied muscle (Fig. 1b).

Mitochondrial DNA is highly susceptible to mutation and polymorphisms are common in the general population [8]. The question arises, therefore, whether a mutation detected in the mitochondrial genome of a patient with a suspected mitochondrial disorder is a rare polymorphism or a pathogenic mutation. Only recurrent mutations occurring in different populations and segregating with the disease phenotype in multigenerational pedigrees may be undoubtedly classified as pathogenic mutations. Our findings provide conclusive characterization of the 3291 T > C as a rare pathogenic mutation of mtDNA.

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