

Clinical and Genetic Features in a MELAS Child With a 3271T>C Mutation

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A mitochondrial DNA 3271T>C point mutation was reported to be the second most common mutation (following the mutation 3243A>G) in mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) in Japan. This mutation has rarely been reported in other countries. We present an 11-year-old Taiwanese girl with MELAS, who harbored the 3271T>C mutation and had manifested short stature, epilepsy partialis continua, and recurrent basal ganglia infarctions since age 6 years, and rapid intellectual regression, dysarthria, and unsteady gait since age 10 years. The proportion of 3271T>C mutant genomes in various tissues, including urinary sediments, hair follicles, blood leukocytes, and buccal mucosa cells from the patient and her mother, was analyzed by polymerase chain reaction-restriction fragment length polymorphism analysis and quantitative real-time polymerase chain reaction. The proportion of mutant load in the patient's muscles was near 100%. Except for muscle, the highest mutation load was detected in urinary sediments of the patient by both methods. This is the first report involving mutant load analysis with quantitative real-time polymerase chain reaction in the 3271T>C mutation. The results suggest that urinary sediments may be an alternative tissue of choice which can be obtained noninvasively in the diagnosis of mitochondrial DNA 3271T>C mutations. © 2008 by Elsevier Inc. All rights reserved.

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Introduction

Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) is one of the most common clinical entities caused by mitochondrial DNA mutations. A diagnosis of MELAS is based on clinical features, including recurrent stroke-like episodes preceded by headache and vomiting, and magnetic resonance imaging abnormalities of infarct-like lesions that are not confined to the major vascular territories.

At least 30 kinds of gene mutations have been reported in MELAS patients (MITOMAP, at www.Mitomap.org). About 80% of MELAS patients exhibit a heteroplasmic mutation in the dihydrouridine loop of the *tRNA^{Leu(UUR)}* gene at nucleotide position 3243 (3243 mutation). Goto et al. recognized that a T-to-C transition at nucleotide position 3271 (3271 mutation) occurred in the same mitochondrial gene as a secondary common mutation, comprising 7.5% among Japanese MELAS patients [1]. The proportion of heteroplasmy was suggested as one of the underlying factors associated with the clinical diversity of the 3243A>G mutation [2].

We report on a Taiwanese girl who had MELAS syndrome with the 3271 mutation and unusual recurrent lacunar infarctions in the basal ganglia. The heteroplasmic mutation was present in skeletal muscle, blood leukocytes, hair follicles, buccal mucosa cells, and urinary sediments in the patient and her mother, according to polymerase chain reaction-restriction fragment length polymorphism analysis and quantitative real-time polymerase chain reaction analyses.

Case Report

An 11-year-old girl manifested generalized tonic-clonic seizures, easy fatigability, and generalized muscle weakness since age 6 years. She was born healthy to nonconsanguineous parents. Her mother is now 42 years old, and has exhibited migraine headaches for 10 years. There was no family history of neuromuscular diseases. The patient's developmental history was normal.

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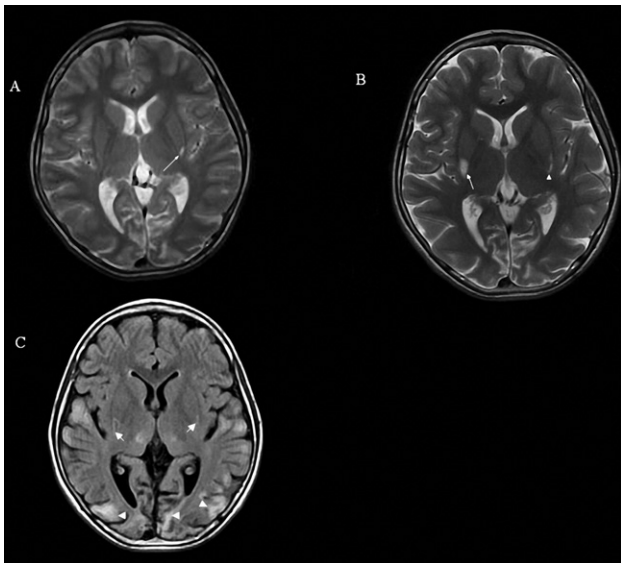


Figure 1. Axial brain magnetic resonance images. (A) A lacunar infarction in the left putamen (arrow) at age 7 years and 8 months. (B) A new infarction (arrow) appears in the right putamen 2 days after complex partial status epilepticus at age 8 years and 4 months. The old lacunar infarction is indicated by arrowhead. (C) Abnormal signal intensity at age 10 years and 4 months (arrowheads). A, T₂-weighted (TR/TE = 4000/101 ms); B, T₂-weighted (TR/TE = 4650/86 ms); and C, T₂-weighted fluid-attenuated inversion recovery (TR/TE = 8802/120 ms) images.

She began to have recurrent, episodic headaches from age 6 years. At age 7 years and 8 months, her body length was 113.7 cm (<3rd percentile). Magnetic resonance imaging of the brain revealed a lacunar infarction in the left putamen (Fig 1A). On physical examination at age 8 years and 3 months, her height was 114 cm (<3rd percentile), and she weighed 19 kg (<3rd percentile). Moderately generalized muscle weakness, predominant in the proximal portion, was evident. Deep tendon reflexes were all hypoactive. Her visual field exhibited right upper-quadrant homonymous hemianopia. Her serum creatine kinase level was 845 IU/L (normal range, 26-174 IU/L); her blood lactate level was 4.7 mmol/L (normal range, 0.5-2.2 mmol/L). A glucose-loading test revealed a high lactate baseline level, but no significant elevations were evident at 30 minutes and 60 minutes. Echocardiography and electrocardiography

were normal. An electroencephalogram indicated intermittent theta waves over the occipital areas. The nerve conduction velocity of her extremities was normal. Her bilateral hearing threshold was reduced to 50 dB according to an auditory brainstem response examination. Her performance intelligence quotient was 79, and her verbal intelligence quotient was 55, on the Wechsler Intelligence Scale for Children, 3rd ed. (Chinese version).

Although a visual-field defect was present, no lesion was evident on the bilateral optic radiation and occipital lobes according to neuroimaging. At age 8 years and 4 months, she developed a complex partial status epilepticus that presented with fluctuating confusion, and intermittent clonic seizure in right-side limbs for 1 hour. Postictal weakness was evident, and subsequent magnetic resonance imaging of the brain showed a new lacunar infarction in the right putamen (Fig 1B), but no cortical lesion. A postictal electroencephalogram revealed intermittent generalized theta waves. She had been receiving coenzyme Q₁₀ (5 mg/kg/day) since age 8 years and 4 months (i.e., for 1.5 years). She continued to have exercise intolerance and generalized muscle weakness, although this was not progressive. She experienced another episode of seizure presenting as epilepsy partialis continua, with myoclonic jerks on the right forearm and fingers, which lasted for 1 week at age 9 years and 6 months. Afterwards, she manifested rapid intellectual regression, dysarthria, progressive muscle weakness, and unsteady gait. Her third magnetic resonance imaging of the brain indicated occipital cortical lesions (Fig 1C).

A biopsy from the left biceps brachii muscle at age 8 years and 3 months revealed scattered, ragged-red fibers (18%) and strongly succinate dehydrogenase-reactive blood vessels. All myofibers were cytochrome *c* oxidase-positive. A molecular analysis of mitochondrial DNA of her blood leukocytes revealed a T-to-C transition at nucleotide position 3271 in the mitochondrial *tRNA^{Leu(UUR)}*. We then tested the proportion of mutant genomes in the blood leukocytes, hair follicles, buccal mucosa cells, and urinary sediments of the patient and her parents, using polymerase chain reaction-restriction fragment length polymorphism analysis, as described by Goto et al. [1]. The DNA was extracted by standard protocols. Mitochondrial DNA was amplified and then digested with the restriction enzyme *Af*III. The digested polymerase chain reaction product was electrophoresed, and the proportion of mutant genomes was assessed in a phospho-imager, using Bio-1D software (Wilber Lourmat, Torcy, France). The proportion of mutant load was highest in the urinary sediments of the patient and her mother (87% and 82%, respectively), followed by 81% and 78% in the hair follicles, 77% and 67% in the blood leukocytes, and 70% and 58% in the buccal mucosa cells, respectively. The proportion of mutant loads in the patient's

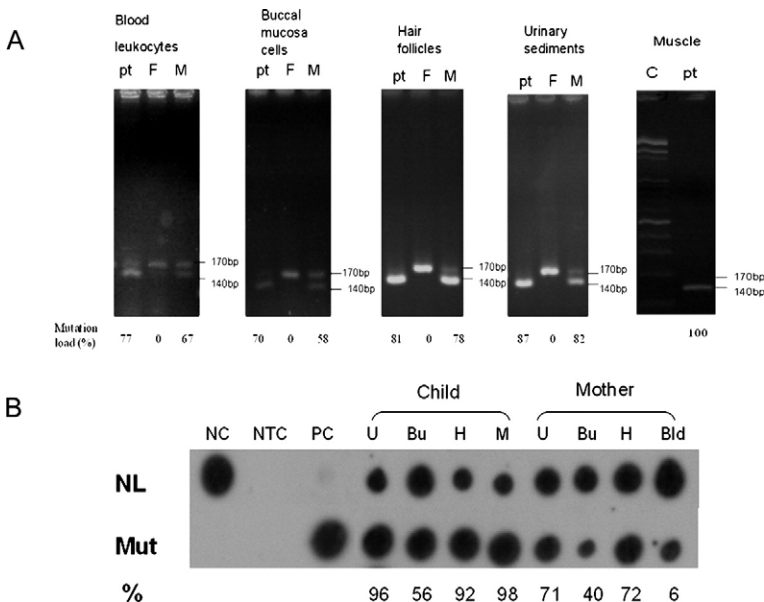


Figure 2. Mutation load of mitochondrial DNA 3271T>C in different tissues of the patient and her parents. (A) Polymerase chain reaction-restriction fragment length polymorphism analysis. F = patient's father; pt = patient; M = patient's mother; 170 bp corresponds to the wild-type band, and 140 bp corresponds to the mutant band formed in 3271 patients with *Af*III digestion. (B) Allele-specific oligonucleotide dot-blot and quantitative real-time amplification refractory mutation systems polymerase chain reaction. NL = normal; Mut = mutant; NC = normal control; NTC = no template control; PC = positive control; U = urinary sediments; Bu = buccal mucosa cells; H = hair follicles; M = muscle; Bld = blood leukocytes; % = percentage of mutant heteroplasmy.

Table 1. Mutation load of mitochondrial DNA 3271T>C in different tissues of the patient and her parents

A. Polymerase chain reaction-restriction fragment length polymorphism analysis					
Mutation Load (%)	Blood Leukocytes	Buccal Mucosa Cells	Hair Follicles	Urinary Sediments	Skeletal Muscle
Patient	77	70	81	87	100
Mother	67	58	78	82	
Father	0	0	0	0	
B. Allele-specific oligonucleotide dot-blot and quantitative real-time amplification refractory mutation systems polymerase chain reaction					
Mutation Load (%)	Blood Leukocytes	Buccal Mucosa Cells	Hair Follicles	Urinary Sediments	Skeletal Muscle
Patient		56	92	96	98
Mother	6	40	72	71	

skeletal muscle was very close to 100% (Fig 2A, Table 1A). The mutation load analyses of four kinds of tissues from her healthy father were zero (Table 1A). According to allele-specific oligonucleotide dot-blot and real-time amplification refractory mutation systems polymerase chain reaction, as described by Wong and Bole [3], the muscle mutant heteroplasmy was 98%. The proportion of mutant load from the patient and her mother was 96% and 71% in urinary sediments, 92% and 72% in hair follicles, 56% and 40% in buccal mucosa cells, respectively (Fig 2B, Table 1B).

Discussion

To date, only 11 MELAS patients with the 3271 mutation have been reported outside Japan; they were from Europe, North America, and Brazil [4-8]. Our patient is the first non-Japanese patient reported in Asia. The phenotypic expression and the presence of ragged-red fibers and strongly succinate dehydrogenase-reactive blood vessels are similar between the 3271 and 3243 mutations, except for the slightly later onset of this disease [4-9]. As for our patient, the onset of the disease was relatively early, at age 6 years.

In our patient, multiple lacunar infarctions in the basal ganglia comprised the most striking neuroimaging finding. Basal-ganglia involvement in MELAS is commonly seen in terms of chronic infarcts, necrosis, and calcifications, but these lesions are usually accompanied by other cortical lesions. Among those with the 3271 mutation, calcification in the basal ganglia was reported in 26.5% of MELAS patients, but none exhibited a lacunar infarction [9]. In a family with a 3271 mutation, two patients manifested strokes and cortical infarction lesions, without basal ganglia abnormalities on brain computed tomography [6]. Other non-Japanese patients with the 3271 mutation were reported to have no basal ganglia lesions [4-8]. In regard to the 3243 mutation, Koo et al. [10] indicated that only one patient exhibited a solitary basal-ganglion lesion at the initial stage. Accordingly, basal-ganglia infarctions without cortical involvement seem to be very rare in the initial presentation of MELAS patients.

The mutation loads of the 3271T>C mutation in different tissues were investigated by Tay et al. [4] using polymerase chain reaction-restriction fragment length polymorphism analysis, and by Stenqvist et al. [5] with mini-sequencing in 2005. In our study, the heteroplasmy

of the 3271 mutation was found in muscles, urinary sediments, hair follicles, blood leukocytes, and buccal mucosa cells. In terms of the 3243 mutation, Shanske et al. [11] reported that the mean mutant load in urinary sediments was higher than that in blood from 22 patients with the 3243 mutation and 10 maternal relatives. They thought that the higher mutation loads of cells in urinary sediments were attributable to a slower turnover rate than in blood cells. Another type of mitochondrial *tRNA^{Leu(UUR)}* mutation, 3255G>A, in a patient with features of myoclonic epilepsy with ragged-red fibers and Kearns-Sayre syndrome, was also reported to contain the highest mutant load in urinary sediments rather than in leukocytes [12]. At present, the gold standard for mitochondrial DNA mutant load quantification is considered to be quantitative real-time polymerase chain reaction [3]. To our knowledge, this is the first report regarding mutant load analysis with quantitative real-time polymerase chain reaction in 3271 mutation. In our report, we find that the highest proportion of mutant loads is muscle, and the second is urinary sediments in the patient with polymerase chain reaction-restriction fragment length polymorphism analysis and quantitative real-time polymerase chain reaction. The non-invasive genetic testing offers a more attractive alternative to blood sampling in pediatric populations. Thus urinary sediments could be an alternative tissue to detect mutant load in MELAS patients with 3271 mutation.

In summary, recurrent lacunar infarctions in the basal ganglia, without any cortical involvement, can be seen as the initial neuroimaging finding in MELAS patients with 3271 mutation. From our results, the use of urinary sediments as an alternative tissue of choice for the diagnosis of mitochondrial DNA 3271 T>C mutation may be feasible.

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