

# MELAS syndrome: correlation between clinical features and molecular genetic analysis

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The clinical manifestations and mitochondrial DNA (mtDNA) mutations in a Taiwanese family with a female proband exhibiting mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes syndrome are reported. Clinically, the proband had a stroke-like episode with right hemiparesis, hemianopsia and mental dysfunction as well as short stature, hearing impairments, and elevated lactate levels. Brain magnetic resonance images showed multiple increased signal intensities over the left frontal, parietal and temporal areas. There were no ragged-red fibers, but paracrystalline inclusion bodies were shown in the muscle biopsies under electron microscopic examination. A deficiency of NADH-CoQ reductase was also found in biochemical studies of the muscles. The family survey revealed no abnormal findings except for headache and episodic vomiting in her mother. The molecular analysis of mtDNA disclosed a mutation from A to G at the nucleotide pair 3243 of the mitochondrial transfer RNA<sup>Leu</sup> gene in the blood, hair follicles and/or muscle of the maternal relatives. A characteristic finding of the MELAS family is variation of percentage of mutated mtDNA in various tissues and individuals. However, a higher proportion of mutated mtDNA was noted in the proband than that in the asymptomatic or oligosymptomatic family members. From the data, the variable clinical phenotypes in this MELAS family can be explained at least partly, by the different proportions of mutant mtDNA in the target tissues of the proband and maternal relatives.

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The syndrome of mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS) is characterized by short stature, headache, episodic vomiting, seizure, hemiparesis, hemianopsia, cortical blindness, lactic acidemia and occasional elevated levels of muscle enzymes (1). Patients with MELAS syndrome can be identified based on the distinct clinical features; definite diagnosis, however, usually require the morphologic and biochemical studies of the muscle (2, 3). Recent advances in molecular genetic analysis provide a reliable method for identification of point mutations at the nucleotide pairs of 3243 or 3271 in patients with MELAS syndrome (4–12). Furthermore, the clinical heterogeneity may also be related with the varying proportions of mutant and wild-type mtDNA (11). We recently encountered a patient with clinical features of MELAS syndrome. The molecular analy-

sis was carried out in both symptomatic patient and asymptomatic family relatives to detect the mitochondrial DNA mutations. A comparison was made to search for possible correlation between the clinical manifestations and proportion of the mutant mtDNA in various tissues.

## Case report

This 24-year-old woman was rather healthy until the age of 17 years while she gradually developed bilateral neurosensory type of hearing loss and an impairment of intellectual function. Migrainous headache associated with episodic vomiting was also experienced. At aged 23, she began to suffer from intermittent right hand numbness followed by right hand focal motor seizure. The seizures occurred in the next 3 months with increased frequencies. Three

months later, she was admitted to our hospital due to sudden onset of right limb clumsiness. On evaluation, she was thin and short (body height: 147 cm). The neurological examination showed right hemiparesis with a presence of Babinski sign, mental deficits, neurosensory type of hearing loss and right homonymous hemianopsia. There were neither retinitis pigmentosa, myoclonus, ataxia, heart block nor limitation of eye ball movement. Brain computed tomographic (CT) scan revealed a basal ganglia calcification. However, magnetic resonance images (MRI) exhibited multiple increased signal intensities over the left frontal, parietal and temporal areas in proton and T2-weighted imaging study (Fig. 1). The lactate levels were high with 27.6 mg/dL in serum and 64.1 mg/dL in cerebrospinal fluid. Results of routine hemograms, urinalysis, biochemistries were almost normal in serum except for creatine kinase (1055 U/L) and lactate dehydrogenase (655 U/L). An impression of MELAS was made.

To evaluate the clinical phenotypes of MELAS syndrome and to correlate the clinical manifestations with percentages of the mutant mtDNA, a series of studies were conducted in the proband and other family members.

#### Material and methods

Six persons in 2 generations of the family were evaluated. The clinical investigations included age, sex, short stature, headache, episodic vomiting, hemiparesis, hemianopsia, stroke-like episode, ophthalmoplegia, optic atrophy, retinitis pigmentosa, myoclonus, ataxia, hearing impairment, seizure and mental abnormality. Muscle biopsied specimens obtained from the right vastus lateralis muscle of I1, I2,

II2 and II3 were stained with hematoxylin-eosin (HE), modified Gomori-trichrome (GT), succinate dehydrogenase and nicotinamide adenine dinucleotide-tetrazolium reductase (NADH-TR) for light microscopic examination and some of the tissues were prepared for electronmicroscopic studies. The activities of the respiratory chain enzymes were assayed on mitochondria isolated from the muscle biopsied specimen of the proband (13).

The mitochondrial DNAs (mtDNAs) were isolated from blood cells, hair follicles and/or muscle biopsies of the proband and the family members according to the method described previously (14). For restriction analysis of the 3243-mutation, a 1159 base pair (bp) of mtDNA fragment ranging from nt 2678 to nt 3836 was amplified by polymerase chain reaction (PCR) according to the protocol developed in this laboratory (15, 16). The PCR cycle consisted of 1 min denaturation of DNA at 94°C, 1 min annealing of DNA and primers at 56°C and 1 min of primer extension at 72°C. Amplification was usually done for 30 cycles and the DNA fragment was digested with restriction enzyme *Apa* I which recognized the sequence GGGCCC resulted from the 3243-mutation and gave rise to two additional fragments (591 bp and 568 bp). The *Apa* I-digested PCR products were subjected to electrophoresis on a 1.5% agarose gel at 100 V for 2 h. After electrophoresis, the gel was soaked in doubly-distilled water containing 0.5 µg/ml ethidium bromide for 20 min and was subsequently destained in doubly-distilled water with slow agitation. The gel was then photographed under trans-illumination with short-wavelength UV radiation. The intensity of fluorescence is proportional to the total amount of DNA in each band in the agarose gel. Thus, after photo-

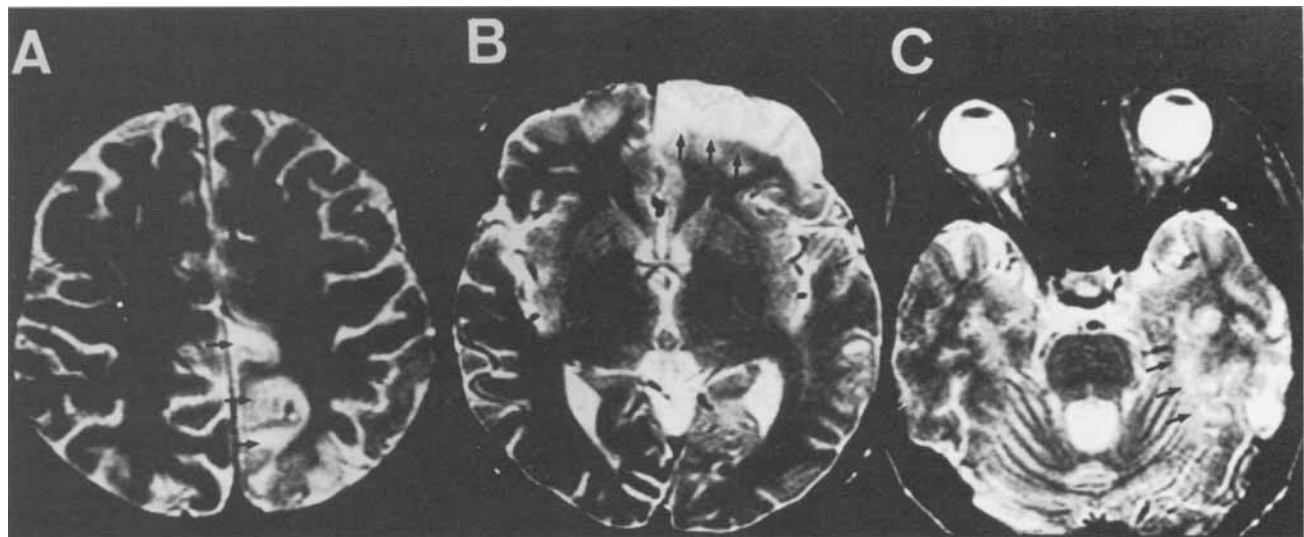


Fig. 1. T2-weighted axial MR image (2000/80 msec) shows high signal intensity (arrows) in the (A) left parietal, (B) left frontal and (C) left temporal areas.

Table 1. Clinical data and mitochondrial DNA mutations in a MELAS family

Subjects	I1	I2	II2	II3	II4	II5
Age (Y)/Sex	52/M	48/M	24/F	20/F	19/M	17/M
Short stature	-	-	+	-	-	-
Headache	-	+	+	-	-	-
Episodic vomiting	-	+	+	-	-	-
Hemiparesis	-	-	+	-	-	-
Hemianopsia	-	-	+	-	-	-
Stroke-like episode	-	-	+	-	-	-
Ophthalmoplegia	-	-	-	-	-	-
Optic atrophy	-	-	-	-	-	-
Retinitis pigmentosa	-	-	-	-	-	-
Myoclonus	-	-	-	-	-	-
Ataxia	-	-	-	-	-	-
Hearing impairment	-	-	+	-	-	-
Seizures	-	-	+	-	-	-
Mental deficits	-	-	+	-	-	-
Ragged-red fibers	-	-	-	-	NA	NA
Paracrystalline inclusion bodies	-	-	+	-	NA	NA
Mitochondrial DNA mutation at 3243*						
Blood (%)	-(0)	+(11)	+(32)	+(19)	+(21)	+(27)
Hair follicles (%)	-(0)	+(8)	NA	+(32)	+(14)	+(27)
Muscle (%)	-(0)	+(51)	+(67)	+(43)	NA	NA

Y: years, M: male, F: female, NA: not available, \*: numbers in parentheses indicate the percentages of mutant mtDNA in various tissues.

graphy, the negative film was scanned with a densitometer (Molecular Dynamics, Sunnyvale, CA), and

the relative quantity of the mutant DNA (*Apa* I-digested fragments) and wild-type DNA (uncut fragment) can be estimated by the ratio of their intensities on the negative film.

For restriction analysis of the 3271-mutation, a 223 bp fragment was amplified from the mtDNA of each subject by PCR technique using the following pair of primers: 5'-<sub>3079</sub>GGAGTAATCCAGGTCGGT<sub>3096</sub>-3' and 3'-<sub>3272</sub>AATtcCAGTCTCCAAGTTAAGGAGAAGAAT<sub>3301</sub>-5'. The small letters in the second primer indicate a G to T mismatch at nt 3275 and a T to C mismatch at nt 3276. The mismatches were specially designed to create a *Bfr* I (*Aff* II) recognition site after amplification of the DNA segment encompassing the putative 3271 point mutation in the mtDNA of the MELAS patients. The PCR product of the 3271-mutant mtDNA could be cleaved by *Bfr* I into a 197 bp and a 26 bp fragments, and the digested DNA mixture was further analysed as described above.

**Results**

Table 1 shows the clinical manifestations of six subjects in the family. The proband had typical features of MELAS syndrome including headache, episodic vomiting, hemiparesis, hemianopsia, stroke-like episode, hearing impairment, seizures and mental

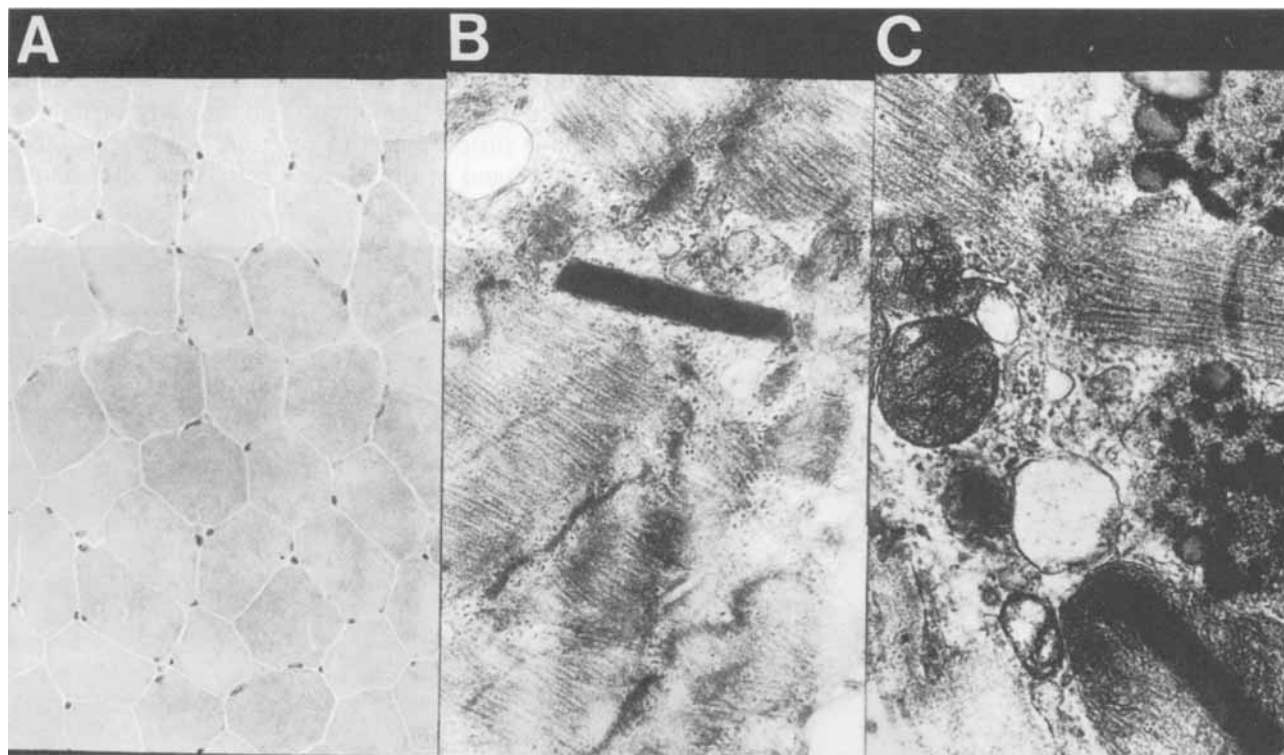


Fig. 2. Muscle biopsy from the right vastus lateralis. (A) Modified Gomori-trichrome stain showed a normal appearance of muscle fibers (x 300 before reduction). (B) Electron micrography showed an inclusion body (x 12000 before reduction). (C) Electron micrography showed a paracrystalline inclusion body with enlarged mitochondria (x 20000 before reduction).

deficits. However, no definite clinical symptom was found in her family members except for intermittent headache and vomiting in her mother. There were no ragged-red fibers in muscle biopsies in the proband and her family members. The SDH and NADH-TR activity stains were also normal. However electron microscopic examination of the muscle biopsies revealed some paracrystalline inclusion bodies with increased lipid droplets and abnormal mitochondria in the proband (Fig. 2). The respiratory chain enzymes activities in the muscle from the proband showed low level of Complex I + III (120.4; controls  $226.0 \pm 119.6$ ) and normal levels in Complex IV (222.8; controls  $270.7 \pm 133.0$ ) and Complex II + III (143.6; controls  $150 \pm 43.5$ ).

Mitochondrial DNA analysis

Whole blood was obtained from all the family members except for II1, hair follicles from 5 family members (II1, II2, II3, II4 and II5) and muscle biopsies from 4 family members (II1, II2, II2 and II3). The PCR products digested with *Apa* I were cleaved into 3 fragments including the wild-type undigested fragment (1159 bp) and two newly generated fragments (591 bp and 568 bp) in various tissues of the family members except for the blood cells, hair follicles and muscle of II1 (Fig. 3). The data indicated an A to G transition at nucleotide position 3243 in her maternal lineage relatives. The percentage of mutant mtDNA was lower in the blood cells and hair follicles

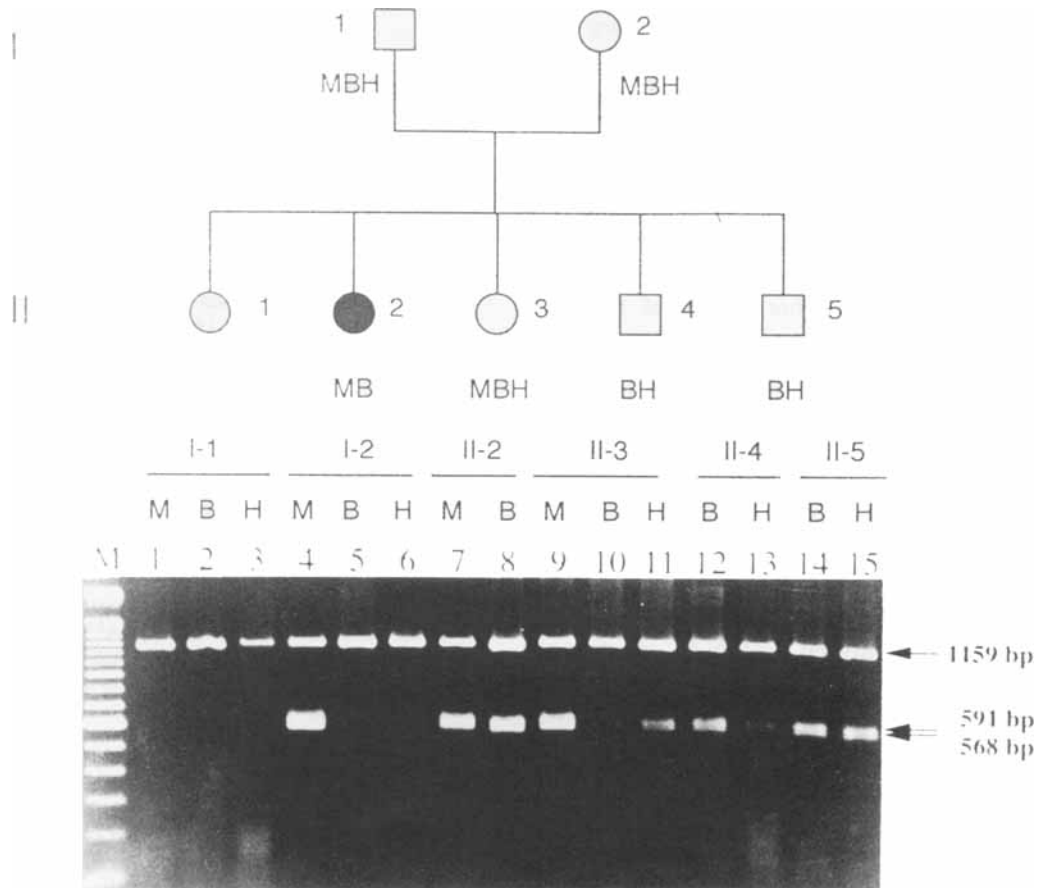


Fig. 3. Restriction analysis of the mtDNAs from various tissues of a Taiwanese MELAS family members. A 1159 bp PCR product was obtained from the specified tissues of the family members indicated by using a pair of primers H2678-2696 and L3836-3817. After digestion with *Apa* I, the PCR product can be cleaved into two fragments (591 bp and 568 bp) in maternal lineage relatives. Lanes 1-3 indicate the mtDNAs from the muscle, blood cells and hair follicles of her father (II1); Lanes 4-6 indicate the mtDNAs from the muscle, blood cells and hair follicles of her mother (II2); Lanes 7 and 8 indicate the mtDNAs from the muscle, and blood cells of the proband (II2); Lanes 9-11 indicate the mtDNAs from the muscle, blood cells and hair follicles of her sister (II3); Lanes 12 and 13 indicate the mtDNAs from the blood cells, and hair follicles of her younger brother (II4) and Lanes 14 and 15 indicate the mtDNAs from the blood cells and hair follicles of her youngest brother (II5). Lane M represents the 100 bp DNA ladder as marker. (Abbreviations: M = muscle, B = blood, H = hair)

than that in the muscles. Furthermore, the percentage of mutant mtDNA in blood and muscle were higher in the proband than that in the family members (Table 1).

In the restriction analysis of the 3271 mutation, the 223 bp fragments from various tissues of the family members were not cleaved by *Bfr* I into two new fragments of 197 bp and 26 bp. The data indicated no point mutation at the 3271st nucleotide pair.

### Discussion

In the report, we describe a Taiwanese family in which a female proband had a full-brown MELAS syndrome and her maternal relatives were either oligosymptomatic or asymptomatic. The molecular genetic analysis demonstrated the presence of an A to G transition at the nucleotide pair 3243 of mtDNA in her maternal lineage family members. The mtDNA mutation was heteroplasmic and the proportions of wild-type versus mutant mtDNAs varied among different individuals and various tissues including the blood cells, hair follicles and muscles. The percentages of mutant mtDNAs in hair follicles were nearly equal to that in the blood. Determination of the proportion of mutated mtDNA in hair follicles can possibly be used to predict that in blood. Furthermore, the muscle had a relatively higher proportion of mutant mtDNA than that of the blood and hair follicles. The constant finding of higher levels of mutant mtDNA in muscle has been reported previously (10, 11, 18) and may be due to a) selective increase of mitochondria with deficient respiratory-chain function, b) a growth disadvantage or self selection by the bone marrow and hair follicles. The proband also had a higher proportion of mutated mtDNA in both muscle and blood cells than her oligosymptomatic and asymptomatic family members. In this MELAS family, the clinical presentations were almost normal which can be possibly explained by the lower percentage of mutated mtDNAs. Ciafaloni et al. (11) also reported a significant higher proportion of mutant mtDNA in the muscle of MELAS patients than their maternal relatives and they suggested a threshold hypothesis. The proportion of mutated mtDNA needed to cause disease in muscle is usually between 50% and 96% in MELAS (10, 11). In some families, however, the variation of clinical manifestations can not be completely explained by variable proportion and distribution of mutated mtDNAs (18). Several mechanisms have been proposed to explain these observations: a) coexistence of more than one mutation in the mtDNA, b) nuclear genes can also modulate the effect of mtDNA mutation.

The proband had short stature, headache, epi-

sodic vomiting, hemiparesis, hemianopsia, stroke-like episodes, seizure, mental deficits and hearing impairments, but no ragged-red fibers were shown in muscle biopsy. However, the paracrystalline inclusion bodies were found in electronmicroscopic study. The lack of ragged-red fibers in the proband is an unusual feature but had been seen in previous reports (2). Magnetic resonance images of the proband revealed an increased signal intensity in the frontal, parietal and temporal areas. These findings suggest that MRI is better than CT scan in demonstration of brain lesions in patients with MELAS syndrome. Moreover, these MRI changes were hard to be explained by the vascular territories, particularly in the large arteries. There were some pathological studies showing changes in small arteries and capillaries and strong SDH activities in vessel walls and many proliferated mitochondria in the endothelia and smooth muscle cells (19, 20). The involvement of small arterioles and capillaries may be considered in the explanation of MRI changes.

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