Chronic Progressive External Ophthalmoplegia with T9957C Mitochondrial DNA Mutation in a Taiwanese Patient

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Abstract-

Purpose: Mitochondrial T9957C mutations have been reported in patients with nonarteritic ischemic optic neuropathy and seizures and in patients with mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes. However, thus far, this mutation has not been reported in patients with chronic progressive external ophthalmoplegia (CPEO).

Case Report: Here we report a female patient with CPEO and agenesis of the corpus callosum. Although no ragged-red fibers were found upon muscle biopsy, sequencing of the entire mitochondrial DNA genome was done. The molecular genetic study revealed a nonsynonymous mitochondrial T9957C mutation. a new genotype of CPEO was identified with varied clinical presentations. Although the effect of the nuclear genome remains unknown, we believe that the nonsynonymous mitochondrial DNA (mtDNA) T9957C mutation may have a role in the clinical manifestations of this patient.

Conclusion: This study extends the phenotype of T9957C mtDNA mutation.

Key Words: chronic progressive external ophthalmoplegia, corpus callosum agenesis, mitochondrial DNA, sequence analysis

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INTRODUCTION

Chronic progressive external ophthalmoplegia (CPEO) is a common phenotype of mitochondrial disease characterized by a limitation of eye movement, ptosis, and muscle weakness. Studies on mitochondrial DNA (mtDNA) have revealed large-scale mitochondrial rearrangement[1,2] and various point mutations in patients with CPEO[3-8]. However, only few mitochondrial studies have been published evaluating mitochondrial complex III mutations[9,10]. Herein we report for the first time, a mitochondrial disease with a CPEO phenotype in a patient harboring the mtDNA T9957C mutation.

CASE REPORT

A 45-year-old Taiwanese woman came to the emergency room complaining of sudden onset of vertigo, nausea, and vomiting; she was admitted to the Neurology ward for impression of peripheral vertigo.

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Her medical history included insidious onset and progressive course of double vision and ptosis since childhood; both her eyes were operated on. In addition, the patient exhibited mental retardation and had performed poorly in elementary school. Family history revealed that the patient's second brother and younger daughter also had the same features of bilateral ptosis as well as mental retardation. Neither ptosis nor ophthalmoplegia was noted in her mother.

Neurological examination revealed decreased visual acuity and external ophthalmoplegia, particularly in upward and downward gazes, but no retinitis pigmentosa. Bilateral ptosis, even after surgical correction, and facial weakness were noted. Neither dysarthria or dysphagia, nor hearing impairment, were present. Mild weakness and generalized hyporeflexia were found in all four limbs.

Laboratory studies revealed normal blood counts, serum electrolytes, and thyroid and liver functions. There was an elevated serum creatine kinase level (379 U/L; reference: 15 to 130 U/L), but fasting blood sugar and lactate were normal. Serum levels of prolactin and growth hormone were normal. Tumor markers, antinuclear antibody, and cryoglobulin were normal, but the parathyroid hormone level was decreased to 0.16 ng/mL (reference: 0.4-1.4 ng/mL). The study of serum acetylcholine receptor antibody was negative.

Nerve conduction velocity, electromyography, repetitive nerve stimulation tests, and electroencephalography were normal. Evoked potential studies, including somatosensory evoked potentials and brainstem auditory evoked potentials, were normal; however, visual evoked potentials revealed a prolongation of P100 latencies in both eyes (right: 143.2 ms, left: 145.6 ms; normal range: <109.6 ms). The electrocardiogram showed a sinus rhythm and transthoracic echocardiography demonstrated a normal left ventricular chamber size and contractility but mild regurgitation in the mitral and tricuspid valves. Brain magnetic resonance imaging (MRI) demonstrated agenesis of the corpus callosum (Figure 1). Magnetic resonance spectroscopy was not available.

Muscle histopathology studies

The muscle specimen taken from the left vastus lateralis was stained with hematoxylin and eosin. The diameter of muscle fibers varied, and the presence of internal nuclei as well as increased connective tissue mass were not noted. Neither ragged-red fibers nor rimmed vacuoles were observed on Gomori-trichrome staining. However, staining with nicotinamide-adenine dinucleotide-tetrazolium reductase and succinate dehydrogenase revealed mitochondrial proliferation in the subsarcolemmal areas. The cytochrome C oxidase (CCO) activity was normal. Further, series ATPase stains

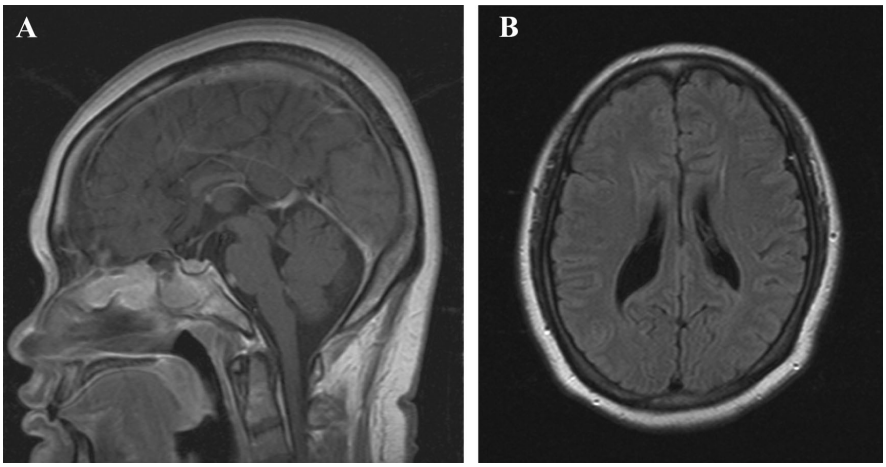

Figure 1. Brain magnetic resonance imaging (MRI) showing agenesis of the corpus callosum in sagittal T1-weighted image (A) and axial fluid attenuated inversion recovery (FLAIR) image (B).

showed a mosaic pattern of fiber types.

Molecular genetic analysis

The blood sample was obtained from the patient and was prepared for mitochondrial DNA analysis after informed consents were obtained. Full-length mtDNA analysis was performed as described by Tanaka. et al.[11]

Amplification of DNA

A two-step polymerase chain reaction (PCR) process was used in our study. In the first step, six PCR fragments were obtained from amplification of the entire mitochondrial genome. In the second step, 60 overlapping segments were amplified.

1. First-step PCR

By using symmetric PCR, the entire mitochondrial genome was amplified as six fragments, A-F, each approximately 3.0 kb in size. The oligonucleotide primers, synthesized and purified by gel filtration, were obtained from Mission Biotech Co., Ltd. (Taiwan). PCR amplification was carried out in a final reaction volume of 40 μL containing 200 ng human genomic DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM concentration of each dNTP, 0.5 or 1.0 μM concentration of each primer, and 1 unit of Pfu DNA polymerase (Fermentas, Life Sciences). PCR conditions were as follows: Initial denaturation at 94˚C for 5 min, followed by 40 cycles of denaturation at 94˚C for 15 s, annealing at 52-62˚C for 15 s, and extension at 72˚C for 3 min with a final extension of 10 min at 72˚C. The amplified fragments were visualized using electrophoresis on a 1% agarose gel stained with ethidium bromide.

2. Second-step PCR

The first PCR DNA templates for the sequence analysis of the entire mitochondrial genome were amplified as 60 overlapping segments (1-60), each approximately 600-1000 base pairs (bp), by a symmetric PCR method. The FL primer was a 38-mer oligonucleotide, consisting of an 18-base sequence of a universal forward sequencing primer (-21M13, 5'-TGTAAACGCG-GCCAGT-3') connected by its 5' side to the 3' side of a 20-base L-strand-specific sequence. PCR amplifications were carried out in a final reaction volume of 20 μL, containing 200 ng of the first PCR product, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM concentration of each dNTP, 1.0 μM concentration of each primer, and 0.5 units of Pfu DNA polymerase (Fermentas, Life Sciences). The PCR conditions used were the same as that of the first PCR except the annealing temperature used was 60˚C. Second-step PCR fragments were analyzed by electrophoresis on a 1.5% agarose gel stained with ethidium bromide. Sequence reactions were performed by use of the second PCR template, -21M13 forward primer, and a BigDye Terminator Cycle Sequence Ready Reaction Kit version 1.0, 3.0, or 3.1 (Applied Biosystems, Foster City, CA, USA). PCR conditions were as follows: Initial denaturation at 96˚C for 5 min, followed by 25 cycles of denaturation at 96˚C for 10 s, annealing at 50˚C for 5 s, and extension at 62˚C for 4 min. After the sequence reaction, excess dye terminators were removed by gel filtration on a MultiScreen-PCR HV Plate (Millipore). The purified DNA samples were dried and suspended in either the template suppression reagent (TSR) or formamide (Applied Biosystems). The dissolved DNA samples were heated at 95˚C for 2 min for denaturation and then immediately cooled on ice. Sequences were analyzed with an automated DNA sequencer, Prism 310 or 377 (Applied Biosystems), and analysis was performed by use of Sequencing Analysis Program (version 4.1; Applied Biosystems). Complete sequences were aligned, assembled, and compared with the program DNAstar (DNAstar, Inc., Madison, WI, USA).

Findings of molecular genetic study

Our molecular genetic study showed that the full-length mtDNA sequence revealed six nonsynonymous mutations at the T5442C, A8701G, T9957C, A10398G, A14766C, and A15326G positions (Table). The mtDNA T9957C mutation resulted in a phenylanalanine to leucine amino acid change at the CO III polypeptide's C-terminal. The other five mutation sites were considered to be polymorphisms.

Table. *Nonsynonymous mutation in sequence analysis of mitochondrial DNA (mtDNA) and comparison with our patient to the revised Cambridge Reference Sequence for human mitochondrial DNA (rCRS)*

	Mutation position	Base	rCRS patient	Location	Amino acid changes	Synonymous	Interpretation
1	T5442C	T	C	ND2	Phe → Leu	Nonsynonymous	polymorphism
2	A8701G	A	G	ATPase 6	Thr → Ala	Nonsynonymous	polymorphism
3	T9957C	T	C	COIII	Phe → Leu	Nonsynonymous	ROS ↑
4	A10398G	A	G	ND3	Thr → Ala	Nonsynonymous	polymorphism
5	T14766C	T	C	Cyt b	Ile → Thr	Nonsynonymous	polymorphism
6	A15326G	A	G	Cyt b	Thr → Ala	Nonsynonymous	polymorphism

ROS: reactive oxygen species.

DISCUSSION

Our patient presented with bilateral ptosis, external ophthalmoplegia, mild limb muscle weakness, and mental retardation. Although she had poor visual acuity and prolonged visual evoked potential, there was neither optic atrophy nor retinitis pigmentosa. In addition, endocrine screening revealed normal results except for a decreased parathyroid hormone level. Parathyroid hormone had been found to have an effect on the metabolism of skeletal muscle[12].

Brain MRI studies usually show white matter lesions and cortical atrophy in CPEO patients[13,14]. Occasionally, agenesis of the corpus callosum without white matter change, similar to our patient, has also been reported[15,16]. Recently, mitochondrial encephalomyopathy was reported to associate with diabetes mellitus and cataract and corpus callosum atrophy; however, the investigation for the mtDNA 3243 point mutation was negative[17]. Miller et al.[18] also reported a patient with corpus callosum agensis, dysmorphism, and fetal neonatal lactic acidosis with decreased complex I and IV activity in muscle. The abnormal gene product may be attributed to a nonsense mutation in a ribosomal protein-the MRPS16 gene.

In addition, mitochondrial diseases with corpus callosum agenesis have also been found in mitochondrial neurogastrointestinal encephalomyopathy, a rare autosomal recessive disorder due to thymidine phosphorylase gene mutations[19,20]. Most patients with mitochondrial myopathy show ragged-red fibers and the presence of a mosaic of CCO deficient fibers[21]; however, ragged-red fibers or CCO-deficient fibers have not been detected in some patients with CPEO using muscle biopsy[22]. After large-scale mtDNA rearrangements screening, mtDNA deletion (nucleotides 7990-15434) were found[22]. Therefore, if mitochondrial disease is highly suspected clinically, but histopathology and histochemistry are normal, larger scale mtDNA screening or whole sequence analysis is warranted.

The genetic defects in mitochondrial CPEO are usually associated with large-scale deletion of mtDNA[1,2,23]. Total sequence analysis of mtDNA for the patient in the present study revealed a T9957C mutation, which may result in a F251L amino acid substitution from phenylalanine to leucine or may induce intrinsic uncoupling of CCO[24]. The family history of our patient strongly suggests a maternal inheritance pattern of bilateral ptosis since early adulthood. To our knowledge, there have been only three case reports dealing with T9957C mtDNA mutation: One patient had mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke syndrome (MELAS)[25], the second had nonarteritic ischemic optic neuropathy (NAION)[10], and the third had hypertrophic cardiomyopathy[26]. In the hybrids harboring a Complex III mtDNA mutation (T9957C) associated with MELAS, no major decrease of either CCO activity or total respiration, nor any change of mitochondrial membrane potential or enhanced reactive oxygen species, were noted[27]. Recently, another report has appeared dealing with this mutation as a polymorphism in Jewish populations[28]. Therefore, the real effect of T9957C mtDNA remains uncertain.

In conclusion, our patient presented with CPEO and agenesis of the corpus callosum possibly due to an

mtDNA mutation of T9957C. Although nuclear DNA defects may have also influenced the clinical features we observed, we still believe that mtDNA T9957C mutation played a crucial role in the clinical manifestations of this patient. Further investigation of the nuclear DNA is warranted.

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