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OPA1 Gene Mutations in Japanese Patients with Bilateral Optic Atrophy Unassociated with Mitochondrial DNA Mutations at nt 11778, 3460, and 14484

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Purpose: To report mutations in the *OPA1* gene in Japanese patients with bilateral optic atrophy unassociated with mitochondrial DNA mutations at nt 11778, 3460, and 14484.

Methods: Twelve unrelated patients with bilateral optic atrophy and 100 healthy controls were examined. Each exon of the *OPA1* gene was amplified by polymerase chain reaction (PCR). All PCR products were sequenced.

Results: Of the 12 patients, 2 had nonsense mutations of the *OPA1* gene (nt 1039G → T and nt 1096C → T, leading to Glu347Stop and Arg366Stop, respectively). These nonsense mutations were not found in the 100 healthy controls. Two of the patients had silent mutations of *OPA1* gene (nt 1177T → G and nt 1923G → A causing no amino acid change).

Conclusions: The mutations (Glu347Stop and Arg366Stop) of the *OPA1* gene are involved in the pathogenesis of bilateral optic atrophy in Japanese patients. **Jpn J Ophthalmol 2003;47:409–411**

Key Words: Bilateral optic atrophy, Japanese patients, *OPA1* gene mutations.

Introduction

Hereditary optic neuropathies include Leber's hereditary optic neuropathy, autosomal dominant optic atrophy (Kjer type), and autosomal recessive optic atrophy. Leber's hereditary optic neuropathy is commonly associated with mitochondrial DNA mutations at nt 11778, 3460, and 14480. Autosomal dominant optic atrophy is characterized by a progressive loss of visual acuity, scotomas, pallor of the optic disc, and color vision disturbance. Delettre et al[1] and Alexander et al[2] reported on mutations of the *OPA1* gene in patients with autosomal dominant optic atrophy. Toomes et al[3] reported that there was a difference in the causative gene between autosomal dominant optic atrophy and Leber's hereditary optic neuropathy. We examined the *OPA1* genotypes in the 12 unrelated patients with bilateral optic atrophy unassociated with mitochondrial DNA mutations at nt 11778, 3460, and 14484.

Materials and Methods

Twelve unrelated patients with bilateral optic atrophy were examined (Table 1). Of these 12 patients, 3 had positive family history and 9 had negative family history. Bilateral optic atrophy was diagnosed based on the results of evaluating visual acuity, visual field, intraocular pressure, color vision, and visually evoked potentials. Funduscopy, fluorescein fundus angiography, computed

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Table 1. Clinical Features in Patients with Bilateral Optic Atrophy

Patient No.	Age (y)	Sex	Family History	Visual Acuity		Optic Disc	
				R	L	R	L
1	17	M	−	NLP	0.08	Pallor	Pallor
2	35	M	−	0.15	0.2	Temporal pallor	Temporal pallor
3	42	M	−	0.1	0.08	Temporal pallor	Temporal pallor
4	5	F	+	0.3	0.2	Temporal pallor	Temporal pallor
5	54	F	+	0.1	0.15	Temporal pallor	Temporal pallor
6	19	M	+	0.08	0.02	Pallor	Pallor
7	58	M	−	0.04	0.02	Pallor	Pallor
8	10	M	−	0.3	0.2	Temporal pallor	Temporal pallor
9	44	M	−	0.15	0.1	Temporal pallor	Temporal pallor
10	13	M	−	0.5	0.5	Pallor	Pallor
11	17	M	−	0.08	0.08	Temporal pallor	Temporal pallor
12	19	M	−	0.1	HM	Pallor	Pallor

R: right, L: left, M: male, F: female, NLP: no light perception, HM: hand motion.

tomography of the brain and orbit, and electroretinography were also carried out to confirm the diagnosis. One of the authors searched for mutations at nt 11778, 3460, and 14484 in the mitochondrial DNA in the 12 patients, as described previously,[4] and found that these patients had no such mutations. Excluded from the present study were patients with ischemic optic neuropathy, compressive optic neuropathy, or toxic optic neuropathy. One hundred individuals with normal visual acuity, full visual field, normal fundi, and normal color vision in both eyes served as controls. Informed consent was obtained from all individuals or their parents. This study was approved by the Committee on Medical Ethics of Toyama Medical and Pharmaceutical University.

Ten milliliters of peripheral venous blood were collected from each patient and control subject. Genomic DNA was extracted from each sample. Twenty-eight exons of *OPA1* gene were amplified by polymerase chain reaction (PCR), according to the method described by Toomes et al.[3]

A total of 100 ng DNA in 50 μL of a solution containing 10 mM Tris-HCl buffer (pH 8.0), 2.5 mM $MgCl_2$, 50 mM KCl, 0.2 mM dNTPs, and 2.5 U *Taq* polymerase were used for each reaction. After initial denaturation at 95ºC for 5 minutes, 30 cycles of PCR were performed. Each cycle consisted of denaturation at 95ºC for 30 seconds, primer annealing at 55ºC for 30 seconds, and extension at 72ºC for 45 seconds. All PCR products were sequenced with an automated DNA sequencer (ABI 310; Perkin-Elmer Cetus, Foster, CA, USA).

Results

Of the 12 patients with bilateral optic atrophy, 4 had heterozygous substitutions in the *OPA1* gene. Patient 5, a 54-year-old woman, had decreased vision, central scotomas, and pale optic discs in both eyes. Her sister was reported to have bilateral optic atrophy. Patient 5 had a heterozygous substitution of guanine to thymine at nt 1039 (Glu347Stop) in exon10 of the *OPA1* gene (Figure 1A). Patient 9, a 44-year-old man, had decreased vision, central scotoma, and pale optic discs in both eyes. He had a heterozygous substitution of cytosine to thymine at nt 1096 (Arg366Stop) in exon 11 of the *OPA1* gene (Figure 1B). Patient 4 had a heterozygous substitution of adenine to cytosine at nt1177 (no amino acid change) in exon 12 of the *OPA1* gene. Patient 7 had a heterozygous substitution of guanine to adenine at nt 1923 (no amino acid change) in exon 20 of the *OPA1* gene.

Discussion

In the present study, Patient 5 had a positive family history for optic atrophy, but her hereditary pattern could not be identified. The *OPA1* gene in her sister could not be analyzed, because informed consent could not be obtained. Patient 9 had a negative family history for optic atrophy. We found a nonsense mutation (Glu347Stop) in Patient 5, another nonsense mutation (Arg366Stop) in Patient 9, and two polymorphisms (Arg393Arg in Patient 4, and Ala641Ala in Patient 7). To our knowledge, the *OPA1* gene mutation, Glu347Stop, has rarely been reported. The mutation (Arg366Stop) in patients with autosomal dominant optic atrophy, as demonstrated in Patient 9 in the present study, had been previously reported by Alexander et al[2] and Toomes et al.[3] Although a cosegregation study was not performed, it is likely that the two nonsense mutations (Glu347Stop and Arg366Stop) shown in the present study may be involved in the

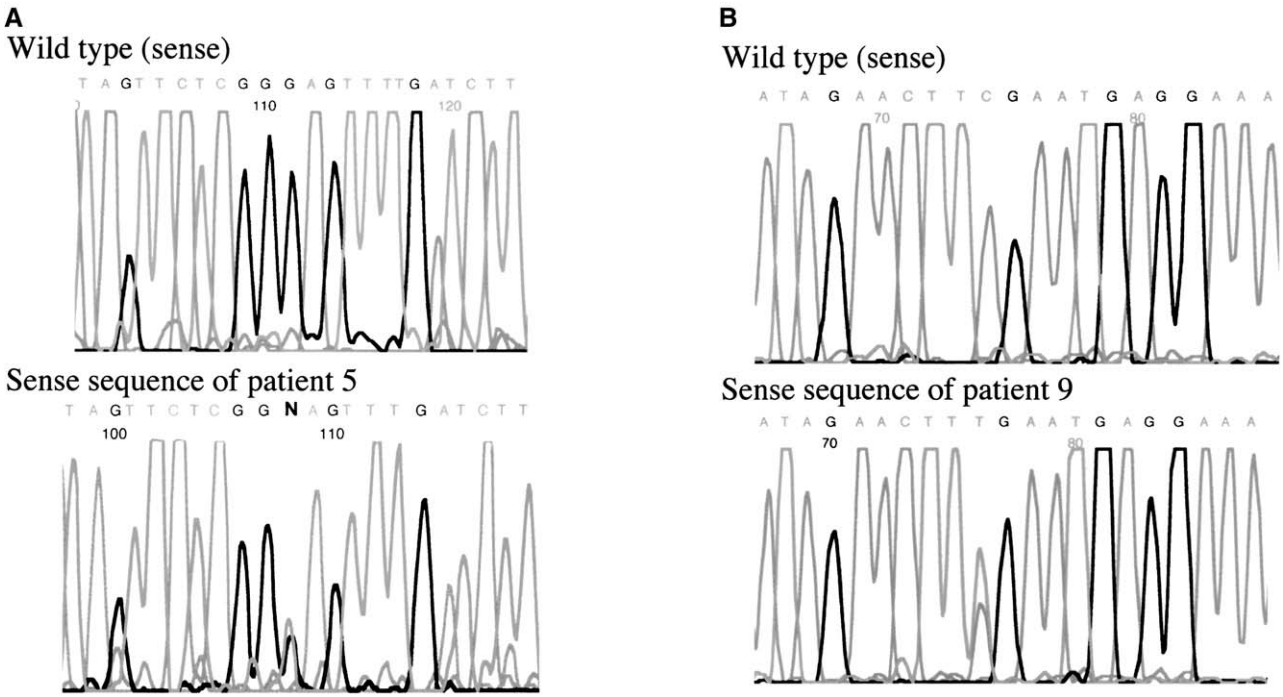

Figure 1. (**A**) Sense sequences around codon 347 in exon 10 of the *OPA1* gene in healthy control (Upper) and Patient 5 (Lower). Substitution of guanine to thymine at nt 1039 in Patient 5 results in change of amino acid at codon 347 from glutamine to stop codon. (**B**) Sense sequences around codon 366 in exon 11 of the *OPA1* gene in normal control (Upper) and Patient 9 (Lower). Substitution of cytosine to thymine at nt 1096 in Patient 9 results in change of amino acid at codon 366 from arginine to stop codon.

pathogenesis of optic atrophy. These mutations were not found in the 100 healthy controls.

The *OPA1* gene, isolated from a brain cDNA library, is located on chromosome 3q and composed of 29 exons encoding a 960 amino acid polypeptide.[5] The OPA1 polypeptide contains a typical sequence leader at the N-terminus and GTPase domain.[6] It is possible that the OPA1 polypeptide is synthesized within the endoplasmic reticulum and transported into the mitochondria, where it catalyzes the GTP hydrolysis.[6] Although the exact function of the OPA1 polypeptide is not known, the OPA1 in the mouse seems to control mitochondrial morphology.[7] It is likely that OPA1 dysfunction may cause mitochondrial impairment, leading to optic atrophies.

It is unlikely that the polymorphisms in Patients 4 and 7 in the present study may be associated with optic atrophies, because both polymorphisms (nt 1177 adenine to cytosine and the nt 1923 guanine to adenine) produced no amino acid change.

The screening of the *OPA1* gene may be helpful for diagnosis in Japanese patients with bilateral optic atrophy.

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