A Novel Mutation of the *OPA1* Gene in a Japanese Family with Optic Atrophy Type 1

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Purpose: To report a novel mutation of the *OPA1* gene in a Japanese family with optic atrophy type 1 (OPA1) and to describe the clinical features of this family.

Methods: Standard ocular examinations were performed on the proband and his two affected sons. The DNA sequence of all exons and splice sites of the *OPA1* gene was determined to detect mutations.

Results: The proband and his sons had a heterozygous mutation of the *OPA1* gene in the third nucleotide of intron 12 (IVS12+3A→T). Clinically, each patient had reduced visual acuity (onset within the first 6 years of life) and optic nerve pallor. The proband showed bilateral central scotomas and generalized dyschroatopsia. This is the first report of *OPA1* gene mutation in Japanese patients with familial optic atrophy.

Conclusions: A mutation of the *OPA1* gene was detected in a Japanese family with OPA1, which follows the same pattern as reported in Western countries. It is suggested that mutations of the *OPA1* gene contribute to the development of optic nerve atrophy regardless of ethnic groups. Screening for the *OPA1* gene mutation will be useful for diagnosis of OPA1 in Japanese patients. **Jpn J Ophthalmol 2002;46:336–340** © 2002 Japanese Ophthalmological Society

Key Words: DNA sequencing, *OPA1* gene, splice site mutation, type 1 optic atrophy.

Introduction

Optic atrophy type 1 (OPA1, MIM165500) is a dominant hereditary optic neuropathy resulting in progressive loss of visual acuity, color vision deficits, a centrocecal scotoma, and optic nerve pallor with an insidious onset within the first decade of life.[1–3] OPA1 is the most prevalent inherited optic neuropathy with an incidence of approximately 1:10,000 live births.[4] Histopathological studies showed that OPA1 is primarily a degeneration of the ganglion cell layer in the retina ascending to optic atrophy.[5] OPA1 was first mapped to 3q28-qter by Eiberg et al in 1994.[6] Subsequently, OPA1 linkage to the locus was confirmed, and the candidate region was narrowed by several researchers to the 1.4-cM interval on 3q28–q29.[7–12] In 2000, two groups independently identified mutations of the *OPA1* gene, which is located within the region, in families with OPA1.[13,14] The *OPA1* gene consists of 29 exons encoding a 960 amino acid protein (Figure 1) initially isolated from a brain cDNA library.[15] The OPA1 protein is the human orthologue of yeast Mgm1p, which is a member of the dynamin family and essential for mitochondrial distribution.[14,16] The OPA1 protein was shown to be a component of the mitochondrial network and ubiquitously expressed in various human tissues examined by Northern blot.[13,14]

To date, various kinds of mutations, such as missense mutations, nonsense mutations, in-frame deletions, frameshift mutations and splice site mutations, have been identified to be responsible for OPA1[13,14,17,18] (Figure 1). In this study, we examined
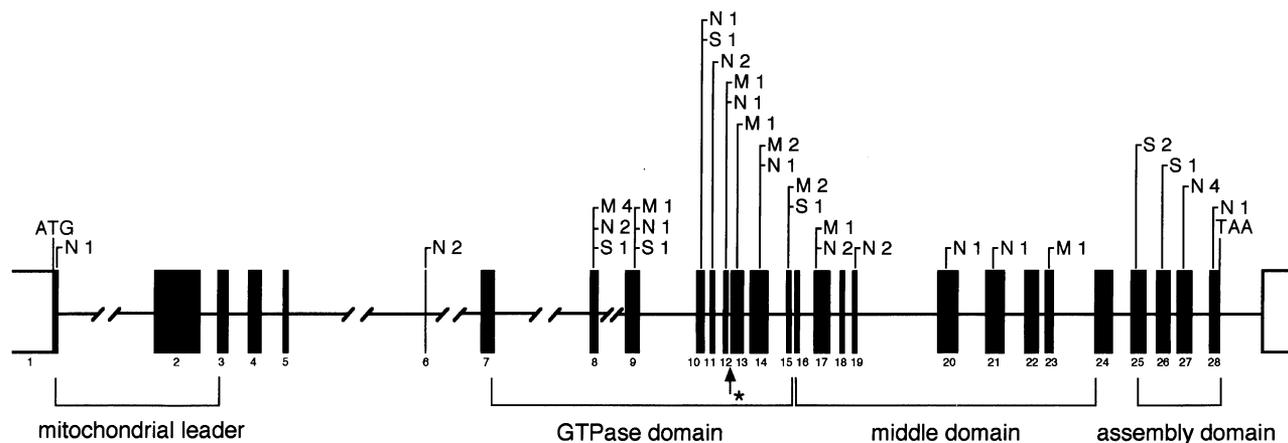
Figure 1. Schematic presentation of the *OPA1* gene structure. Coding exons are shown in solid boxes. The reported mutations are shown above. M: missense mutations and in-frame deletions, N: nonsense and frameshift mutations, S: splice site mutations, Numbers: numbers of mutations identified in each exon and its boundaries. The arrow with an asterisk indicates the position of IVS12+3A→T.

all protein-coding exons and their splice-sites by DNA sequencing analysis in the proband of an OPA1 family and found a novel mutation at a splice site. This mutation co-segregated with the disease in this family. This is the first report of a mutation of the *OPA1* gene in a Japanese family with OPA1.

Materials and Methods

Informed consent was obtained from all the subjects who participated in this study. The proband, his sons, his wife (Figure 2) and 110 Japanese controls ranging from 17 to 92 years of age (mean = 53.0 years) were analyzed. All individuals examined had been under observation in the Department of Ophthalmology, Teikyo University School of Medicine, Tokyo. Ophthalmological examinations included testing for best-corrected visual acuity, refraction, Goldmann visual fields, assessment of color vision with Ishihara pseudoisochromatic plates, and visual evoked potential (VEP). The proband and his sons were diagnosed as having optic nerve atrophy based on the presence of decreased visual acuity and optic nerve pallor observed by ophthalmoscopy. The 110 unrelated controls included 97 normal individuals and 13 primary open-angle glaucoma patients.

Genomic DNA was extracted from leucocytes of the peripheral blood samples from all participants using the QIAamp DNA blood kit (QIAGEN, Tokyo) according to the manufacturer's protocol. Exons 1 through 28, which encode the OPA1 protein, of the *OPA1* gene were amplified by polymerase chain reaction (PCR) with TaKaRa *Taq* (Takara,

Tokyo) and primers in a 50-μL reaction mixture containing 100 ng of genomic DNA. PCR conditions were as follows: 10 minutes at 95°C for denaturing; 30 cycles of 95°C for 45 seconds, annealing temperature for 45 seconds, 72°C for 1 minute; and a final extension step at 72°C for 7 minutes. The annealing temperatures are listed in Table 1. PCR products were purified with a QIAquick PCR purification kit (QIAGEN). Nucleotide sequences were determined by direct sequencing of both strands of the PCR product from the proband with a DNA sequencing kit and an ABI3700 DNA sequencer (Big Dye Cycle Sequencing Ready Reaction, PE Applied Biosystems, Foster City, CA, USA) using the same primers as used in PCR. To examine the presence of the detected sequence variant, the nucleotide sequences of the sense strands of exon 12, exon 13 and intron 12 from other family members and controls were determined.

Results

DNA Sequence

The proband and his sons had a heterozygous A→T mutation (Figure 2) at the third nucleotide of intron 12. The IVS12+3A→T mutation was not detected in either the proband's wife or the control individuals.

Clinical Characteristics

The proband was a 21-year-old man when he first visited the Department of Ophthalmology, Teikyo University School of Medicine, in 1976. His decreased
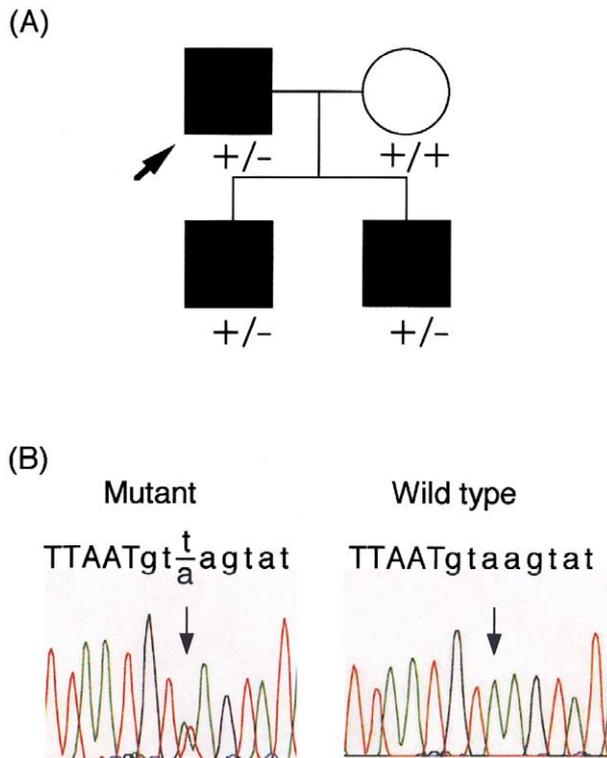
(A)

(B)

Mutant   Wild type

TTAATgt$\frac{t}{a}$agtat   TTAATgtaagtat

Figure 2. (**A**) Pedigree of the optic atrophy type 1 (OPA1) family. Affected individuals are shown in filled symbols. Genotypes are shown below the individual symbols where + and − represent the absence and the presence of the mutation, respectively. An arrow indicates the proband. (**B**) Electropherograms of OPA1 sequencing. The mutant position is indicated by arrows. The intron sequence is shown in small letters.

Figure 3. (**A**) Left optic disc of the proband showing complete pallor. (**B**) Goldmann visual field of left eye. Fundus appearance and visual field were similar in both eyes.

visual acuity had been noticed upon his enrollment in elementary school. In our initial examinations, his visual acuity was 0.1(0.2X+1.25 D), OD and 0.1(0.15X-0.25 D cyl+1.0 D Ax20°), OS. Eye positions and eye movements were normal. Intraocular pressure was 17 mm Hg in both eyes. He had bilateral atrophy of optic discs, central scotoma, and generalized dyschromatopsia detected by fundoscopic examination, Goldmann visual field measurements and Ishihara pseudoisochromatic plates, respectively. In 2001, his visual acuity had slightly decreased to 0.06 (n.c.), OU while visual fields and optic disc appearance were stable. The visual field and fundus photograph of his left eye only are shown in Figure 3.

The proband's elder son was examined at our hospital when he was 6 years old in 1998. His visual acuity was 0.06(0.15X+1.5 D cyl+2.0 Ax90°), OD and 0.07(0.15X+1.0 D cyl+1.0 Ax95°), OS. His eye positions were parallel with correction whereas there was 30 prism diopter esotropia without correction. Eye
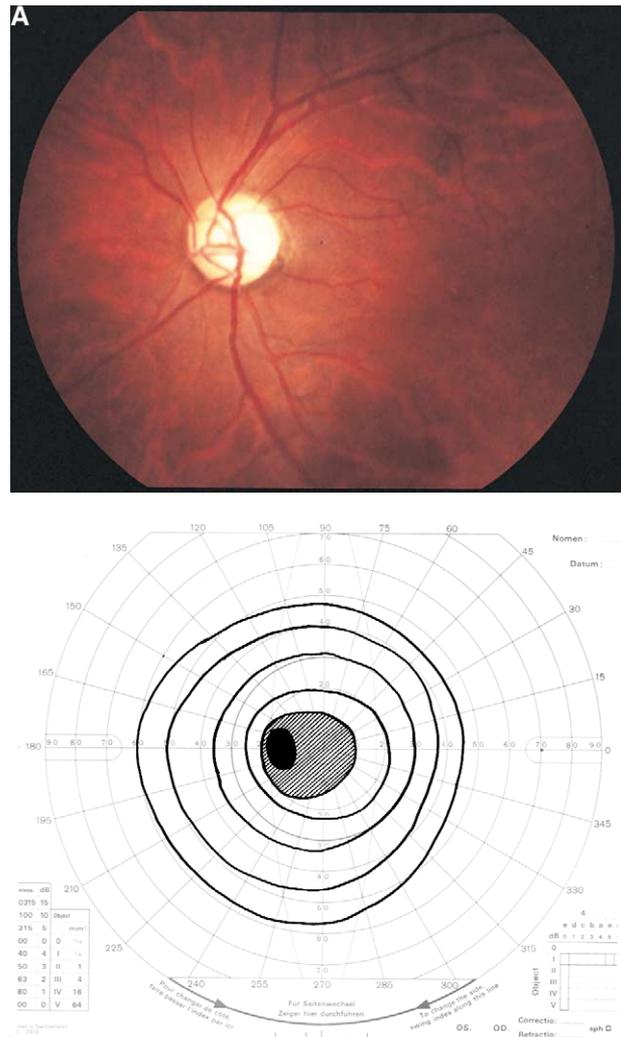
movements were normal. Temporal pallor of the optic discs and no VEP response were observed in both eyes.

The proband's younger son was examined at our hospital in 1999 when he was 3 years old. His visual acuity was 0.08(0.1X+1.5 D), OD and 0.08(0.1X+0.75 D cyl+0.75 D Ax95°), OS. His eye positions were parallel and eye movements were normal. As was the case with his brother, temporal pallor of the optic discs and no VEP response were observed.

Discussion

The first locus for autosomal dominant optic atrophy (ADOA) was mapped to 3q28–q296. In most ADOA families reported so far, the disease had

been mapped to the OPA1 locus on chromosome 3.[6–12,19] However, heterogeneity within the group of ADOA has been suggested by linkage studies.[19,20] Kerrison et al identified a second locus for ADOA on 18q12.2–q12.3.[21] After the responsible gene for OPA1 was identified, the prevalence of *OPA1* gene mutations in ADOA patients was reported to be 32.1% and 57%, respectively, by two groups.[17,18] Because the mutations were reported only from Europe, Australia and Cuba,[13,14,17,18] the prevalence of *OPA1* gene mutations in ADOA patients in Japan is unknown. Although we could not confirm that the disease is transmitted through three generations, we diagnosed the proband and his sons as having ADOA based on the inheritance trait and the clinical findings indicating optic atrophy since their childhood. No mutations shared by all 3 patients were detected in coding regions and splice sites of the *OPA1* gene except the IVS12+3A→T substitution. Although there is a possibility that this substitution is a very rare polymorphism, lack of the substitution in 220 control chromosomes and its co-segregation with the disease strongly suggests that this is a disease-causing mutation.

The specificity and accuracy of splicing ultimately reside in the nucleotide sequence of the primary transcript. If a splice aberration occurs, cryptic splice sites within genes can be activated.[22,23] The analysis of 90 donor sites revealed that the consensus sequence for the donor site is 5′-$^A/_C$AGgt$^a/_g$agt-3′ and that 80% of the sixth nucleotide (measured relative to the consensus sequence above), where the IVS12+3A→T substitution occurred, is A or G while the probability of T is 6%.[24] The wild-type sequence for the exon12-intron12 junction of *OPA1* gene is consistent with the consensus sequence except for the third nucleotide. Breathnach et al noted that if the second and third nucleotides of a particular donor sequence are not AG, the sixth nucleotide almost always is purine.[24] Moreover, similar A to T substitutions at the same position of the donor site have been reported to be disease-causing mutations for cystic fibrosis[25] and X-linked retinitis pigmentosa.[26] Thus, the substitution of IVS12+3A→T appears to cause splicing aberration resulting in either skipping of exon 12 or insertion of the intron sequence.

The dynamin family members are functionally heterogeneous large GTPases sharing their common structure characterized by a GTPase domain followed by a middle domain and an assembly domain[16] (Figure 1). In addition to the common structure, the OPA1 protein, like its homologue, has a putative mitochondrial leader at the N-termini[14,16] (Figure 1). The dynamin family proteins have three consensus GTP-binding motifs in their GTPase domain to catalyze the GTP hydrolysis.[13,16] It is demonstrated that the assembly domain of dynamin binds to itself; the GTPase domain and middle domain self-assemble into spirals.[27,28] Because dynamin self-assembles by interaction of the three conserved domains, it is also likely that the OPA1 protein forms multimers via these domains. More than a half of the *OPA1* gene mutations identified so far cluster in the GTPase domain[13,14,17,18] (Figure 1). In this study, the mutation in the Japanese family with OPA1 was also detected in the GTPase domain. The mutations in the GTPase domain may abolish GTPase activity and thereby lead to optic nerve atrophy. Alternatively, the mutations in the GTPase domain may cause the disease by affecting the self-assembly of the OPA1 protein, assuming that the OPA1 protein forms multimers like dynamin.

Leber's hereditary optic neuropathy (LHON) is also known as major optic neuropathy caused by mitochondrial DNA mutations which are cytoplasmically transmitted and reflect the maternal inheritance of the disease.[29–31] LHON is clinically distinct from OPA1. For example, the onset of LHON is more acute and usually later (mean = 20–24 years[32]) than that of OPA1. However, once optic discs fall into atrophy, it is hard to distinguish between OPA1 and LHON, especially when the family history is unclear. Gene analysis may be useful for diagnosis in such cases. Pesch et al reported that neither the type nor the site of mutations correlated with severity of symptoms.[18] The existence of asymptomatic carriers of the *OPA1* gene mutations[14,17] indicates that the phenotypes can vary widely between patients sharing the same mutation. Most of the mutations identified are unique to a family.[13,14,17,18] The mutation identified in this study was also different from the previously reported mutations. To apply gene analysis of *OPA1* gene to clinical diagnosis, further investigation should be focused on the prevalence of mutation and founder effect in Japan.

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