Novel Mutations in the *OPA1* Gene and Associated Clinical Features in Japanese Patients with Optic Atrophy

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Purpose: Autosomal dominant optic atrophy (ADOA) is characterized by symmetrical bilateral optic atrophy associated with reduced corrected visual acuity (VA), central or centrocecal scotoma, and color vision disturbances. The disease is genetically heterogeneous, and the *OPA1* gene has been identified as the only causative gene. The aims of this study were to identify and report mutations in the *OPA1* gene in Japanese patients with ADOA and to describe the clinical features associated with the mutations.

Design: Molecular genetic study and observational case reports.

Participants: Nine unrelated Japanese families with optic atrophy and 8 isolated cases of optic atrophy.

Methods: Genomic DNA was extracted from peripheral leukocytes, and all exons containing the open reading frame of the *OPA1* gene and the flanking intron splice sites were sequenced directly. Complete ophthalmologic examinations were performed.

Main Outcome Measures: Direct sequencing of the *OPA1* gene and clinical evaluations including VA, visual field, color vision, and disc appearance.

Results: Ten different heterozygous mutations, including 6 novel mutations, were detected in the *OPA1* gene. The identified mutations included 5 deletions/insertions (c.2061delA, c.2098_2103delCTTAAA, c.2538insT, c.2591insC, and c.2708_2711delTTAG), 4 nonsense mutations (c.112C>T [p.R38X], c.181C>T [p.Q61X], c.946A>T [p.R316X], and c.2713C>T [p.R905X]), and 1 missense mutation (c.1635C>A [p.S545R]). The most common mutation in Caucasians (c.2708_2711delTTAG) was found in 3 unrelated families, suggesting that it is a mutational hot spot. We detected an *OPA1* mutation in 8 of 9 familial cases of optic atrophy and in 4 of 8 cases that were initially considered to be sporadic from the patients' family histories. Examinations of family members of 2 sporadic probands revealed the existence of other family members with the *OPA1* mutations whose phenotype was very mild or within normal limits. This indicates that patients with ADOA sometimes seem to be sporadic because of the extensive variation in the phenotype or, alternatively, a low penetrance of ADOA.

Conclusions: *OPA1* gene mutations are causative in most familial cases of ADOA in Japanese. Sporadic cases of optic atrophy frequently may be caused by *OPA1* mutations in the Japanese population. Molecular genetic examinations are useful in determining the hereditary patterns in some cases of optic atrophy. *Ophthalmology 2006;113:483–488 © 2006 by the American Academy of Ophthalmology.*

Autosomal dominant optic atrophy (ADOA; MIM#165500) is characterized by symmetrical bilateral optic atrophy associated with reduced corrected visual acuity (VA), central or centrocecal scotoma, and blue–yellow color vision disturbances,[1] with an estimated incidence of between 1:50 000[2] and 1:10 000.[3] The visual impairments usually progress very slowly,[4] and the phenotypic severity varies considerably among patients,[5–7] even within the same family. This indicates a low penetrance.[8–11]

Autosomal dominant optic atrophy is genetically heterogeneous, and the *OPA1* gene has been determined to be one of the causative genes.[12,13] The gene is located on chromosome 3q28–q29, containing 31 exons, spanning >90 kilobases, and generating 8 isoforms by alternative

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splicing.[14] It is expressed in various tissues, but most abundantly in the retina and brain.[13] The OPA1 protein is a dynamin-related guanosine triphosphate–ase located in the intermembrane space close to the crista in the mitochondria.[15] The protein is considered to be involved in the fission and fusion processes of mitochondria,[16] which lead to the appropriate distribution of the mitochondria.[7,17] Thus, mutations in the *OPA1* gene are believed to lead to respiratory deficiency.[12] An in vitro study has shown that downregulation of the OPA1 protein using specific small interfering RNA caused fragmentation of the mitochondrial network, resulting in cytochrome *c* release and caspase-dependent apoptosis.[17]

To date, 93 different *OPA1* mutations in 191 families with ADOA have been reported.[12,13,18–26] We have searched for mutations in the gene in 17 unrelated Japanese probands with optic atrophy and identified 10 heterozygous mutations, including 6 novel mutations in 11 probands. An *OPA1* mutation was detected in 8 of 9 probands with known family histories, indicating that most Japanese familial cases with optic atrophy are associated with *OPA1* mutations. A mutation in the gene was identified in 4 of 8 probands who had clinical characteristics of ADOA but were initially considered to be sporadic from their family histories. Additional examinations of the family members of 2 probands revealed others with the *OPA1* mutation. These family members had very mild or subclinical phenotypes. This indicates that the inheritance pattern of patients with ADOA can appear to be sporadic because of the low penetrance of the mutation, and that sporadic-appearing cases with optic atrophy should be analyzed genetically to rule out *OPA1* mutations.

Patients and Methods

Patients

Seventeen unrelated Japanese probands with optic atrophy and some of their family members (families 42, 92, 169, 247, 397, 407, and 543) were studied. Nine probands had family histories (families 92, 169, 241, 407, 519, 526, 534, 543, and 667), with 8 showing an apparent autosomal dominant inheritance pattern (families 92, 169, 241, 407, 519, 534, 543, and 667). Eight probands were considered initially to be sporadic from their family histories (families 42, 52, 247, 250, 397, 485, 507, and 598). They were observed in the Department of Ophthalmology of Nagoya University or Hamamatsu University, Japan. Patients with optic atrophy who had inconsistent clinical characteristics of ADOA, such as those with acute onset, unilateral involvement, retinal atrophy, or systemic symptoms, were excluded.

Peripheral blood was obtained with informed consent, and genomic DNA was extracted using the QIAamp Blood Maxi Kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. The protocol of the study adhered to the provisions of the Declaration of Helsinki and was approved by the local ethics committee.

Mutation Analysis

All coding exons with flanking intronic sequences of the *OPA1* gene were amplified by polymerase chain reaction (PCR) (Ampli-*Taq* Gold polymerase, Applied Biosystems, Foster City, CA) with primers as described.[18] The PCR products were purified using the High Pure PCR Purification Kit (Boehringer Mannheim, GmbH, Mannheim, Germany) and then directly sequenced with a DNA sequencing kit (Dye Terminator Cycle Sequencing Ready Reaction Kit, Applied Biosystems) and an automated DNA sequencer (model 3100, Applied Biosystems). Identified DNA sequence variants were confirmed by sequencing both DNA strands from at least 2 different PCR products. The identified mutations and coding polymorphisms were assayed in 100 control chromosomes (25 male and 25 female healthy Japanese individuals) by direct sequencing. The mutated nucleotides were numbered according to the *OPA1* cDNA sequence[27] (GenBank accession no. AB011139) and the *OPA1* genomic sequence (GenBank accession no. NT_005612).

Partial alignment of the amino acid sequences of 5 eukaryotic *OPA1* homologs selected using an online analysis tool (available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=homologene) was constructed and analyzed. The website was accessed on October 1, 2004. The proteins (GenBank accession numbers) used were *Homo sapiens* OPA1 (NP_570849.1), *Mus musculus* Opa1 (NP_598513.1), *Drosophila melanogaster* CG8479 (NP_610941.1), *Anopheles gambiae* 1270645 (XP_309360.1), and *Caenorhabditis elegans* eat-3 (NP_495986.2).

Clinical Evaluations

The ophthalmologic examination included best-corrected VA, ophthalmoscopy, fundus photography, Goldmann kinetic perimetry, and color vision testing with Farnsworth panel D-15 plates.

Results

Mutation Analysis

A summary of the mutations and polymorphisms in the *OPA1* gene identified in this study is listed in Table 1 (available at http://aaojournal.org). Direct sequencing of the *OPA1* gene of 17 unrelated probands with optic atrophy revealed 10 different heterozygous mutations in the 12 probands. The sequence variants are listed in Table 1. An *OPA1* mutation was detected in 8 of 9 probands who had family histories, and 7 of the 8 had an autosomal dominant inheritance pattern. An *OPA1* mutation was identified in 4 of 8 probands who initially were considered to be sporadic by their family histories.

Of the 10 identified mutations, 5 (c.2061delA, c.2098_2103delCTTAAA, c.2538insT, c.2591insC, and c.2708_2711delTTAG) were deletions or insertions, and 4 were frameshifts resulting in premature terminations. Four mutations (c.112C>T [p.R38X], c.181C>T [p.Q61X], c.946A>T [p.R316X], and c.2713C>T [p.R905X]) generated early stop codons, and the other was a missense mutation (c.1635C>A [p.S545R]).

Six of the mutations (p.Q61X, p.R316X, p.S545R, c.2061delA, c.2538insT, and c.2591insC) were novel. Five of these (p.Q61X, p.R316X, c.2061delA, c.2538insT, and c.2591insC) were considered to be pathogenic null mutations because the mRNA containing the predicted premature termination would most likely be subject to nonsense-mediated decay without being translated into a protein. The novel missense mutation p.S545R was interpreted as likely to be pathogenic because the amino acid was changed from neutral to basic in codon 545 by the mutation. It was also likely to be pathogenic because the serine at residue 545 is highly conserved among all 5 close eukaryotic homologs ranging from *H. sapiens* to *C. elegans* (Fig 1).

None of the mutations were present in 100 control alleles. We confirmed that some of the mutations were segregated with the

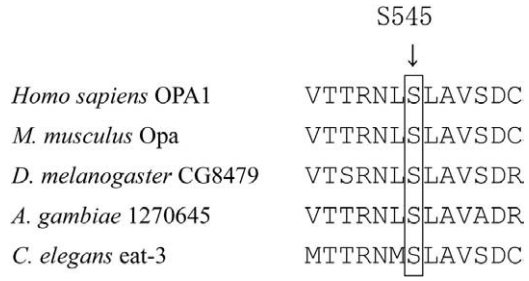

Figure 1. Partial amino acid sequence alignment surrounding the site of the p.S545R mutation in 5 eukaryotic OPA1 homologues: *Homo sapiens* OPA1 (NP_570849.1), *Mus musculus* Opa1 (NP_598513.1), *Drosophila melanogaster* CG8479 (NP_610941.1), *Anopheles gambiae* 1270645 (XP_309360.1), and *Caenorhabditis elegans* eat-3 (NP_495986.2). The arrow indicates the position of the residue altered by the novel missense mutation p.S545R.

disease in affected and unaffected members of families 42 (c.2708_2711delTTAG), 92 (p.S545R), 169 (c.2538insT), 247 (p.Q61X), 397 (c.2098_2103delCTTAAA), 407 (c.2708_2711-delTTAG), and 543 (c.2708_2711delTTAG).

In the screening, we detected 4 coding and 2 intronic polymorphic variants. All of them have already been reported.[14,18,19,22,24] Three isocoding changes (c.321G>A [p.S107S], c.1923G>A [p.A641A], and c.2109C>T [p.A703A]) were found in patients with other pathogenic recessive mutations as well as in normal control subjects. A missense change in exon 4, c.473A>G [p.N158S], was probably not a pathogenic variant because it was found in patients with other pathogenic recessive mutations in *OPA1* as well as in normal control individuals at a high frequency. The asparagine at residue 158 was conserved poorly among the 5 eukaryotic homologues (data not shown). The allelic frequencies of the 4 coding polymorphisms in patients with optic atrophy and in 50 normal individuals are listed in Table 1 (available at http://aaojournal.org).

Two intron changes (IVS8+32T>C and IVS26+25A>T) were identified in patients with other pathogenic recessive mutations in *OPA1* as described above. These 2 changes were predicted not to affect the splicing by an online splice site prediction software (available at http://www.fruitfly.org/seq_tools/splice[28]) that was accessed on October 1, 2004, and they were interpreted as nonpathogenic.

Clinical Findings

The clinical characteristics of the patients with *OPA1* mutations are summarized in Table 2. The phenotypic severity varied considerably—for example, VAs of the 21 patients with the *OPA1* mutations ranged from normal (1.5) to severely reduced (hand movements). Symmetrical temporal pallor of the optic discs was observed in the patients; however, the more severe cases showed diffuse optic disc atrophy, whereas some very mild cases did not show any apparent signs of optic atrophy. Visual field (VF) tests using Goldmann kinetic perimetry showed central scotoma, centrocecal scotoma, paracentral scotoma, blind spot enlargement, and normal VFs in different patients. The results of color vision tests using panel D-15 plates showed a tendency to blue–yellow defects in the patients; however, they varied from normal to failure without any axis, and the number of patients who showed a typical blue–yellow defect was not high.

We detected an *OPA1* mutation in 4 of 8 probands who initially were considered to be sporadic cases from their family histories (family 42, III-2; family 52, III-6; family 247, III-3; and family 397, III-1). However, additional genetic and clinical examinations of other family members of 2 of the patients revealed that there were other family members with the same mutations whose phenotype was very mild or within normal limits, with relatively good VAs and without any subjective symptoms. For example, the 20-year-old proband (III-1) of family 397 with the c.2098_2103delCTTAAA mutation had corrected VAs of 0.2 (right eye) and 0.1 (left eye) associated with bilateral temporal

Table 2. Clinical Characteristics in Patients with *OPA1* Mutations

Family	Case	Gender	Age (yrs)	Visual Acuity (Right/Left)	Visual Field (Right/Left)	Color Vision (Right/Left)	Disc Appearance	Mutation
42	III-2	F	27	0.4/0.4	CS/CS	N/T	SP	c.2708_2711delTTAG
52	III-6	F	51	HM/HM	ND/ND	ND/ND	DA	c.2061delA
92	III-2	F	49	0.06/0.06	CS/CS	I/I	DA	p.S545R
92	IV-5	F	22	0.06/0.07	CS/CS	ME/T	TP	p.S545R
92	IV-4	M	24	0.04/0.05	CS/CS	T/ND	DA	p.S545R
169	III-1	M	50	0.3/0.3	N/N	T–I/I	TP–DA	c.2538insT
169	III-3	M	46	0.3/0.3	N/N	T–I/T	TP–DA	c.2538insT
247	II-2	M	83	0.3/0.3	BSE/BSE	T–I/T–I	N	p.Q61X
247	III-1	M	55	0.7/0.7	N/N	N/N	TP	p.Q61X
247	III-3	F	51	0.01/0.01	CC/CC	I/I	DA	p.Q61X
247	IV-1	F	25	1.0/1.0	N/N	N/N	N	p.Q61X
397	II-4	F	45	1.5/1.5	N/N	N/N	N–SP	c.2098_2103delCTTAAA
397	III-1	M	20	0.2/0.1	CCS/CCS	T/T	TP	c.2098_2103delCTTAAA
407	II-8	F	46	0.1/0.09	CCS/CCS	ME–T/ND	TP–DA	c.2708_2711delTTAG
407	III-3	F	7	0.15/0.15	CS/CS	T–I/ND	TP	c.2708_2711delTTAG
519	III-3	M	35	0.5/0.2	N/N	N/T	TP	p.R905X
526	III-1	M	22	0.15/0.15	N/N	ME/N	TP	c.2591insC
534	III-5	M	71	0.1/0.15	BSE/BSE	ND/ND	TP	p.R316X
543	II-3	M	65	0.05/0.07	CCS/CCS	ND/ND	TP–DA	c.2708_2711delTTAG
543	III-2	F	33	0.3/0.3	PCS/PCS	ND/ND	TP	c.2708_2711delTTAG
667	III-2	F	53	0.06/0.1	CS, BSE/CCS	T/I	TP	p.R38X

BSE = bind spot enlargement; CC = concentric constriction; CCS = centrocecal scotoma; CS = central scotoma; DA = diffuse atrophy; F = female; HM = hand movements; I = irregular pattern; M = male; ME = minor error; N = normal; ND = not determined; PCS = paracentral scotoma; SP = subtle pallor; T = tritanopia; TP = temporal pallor.

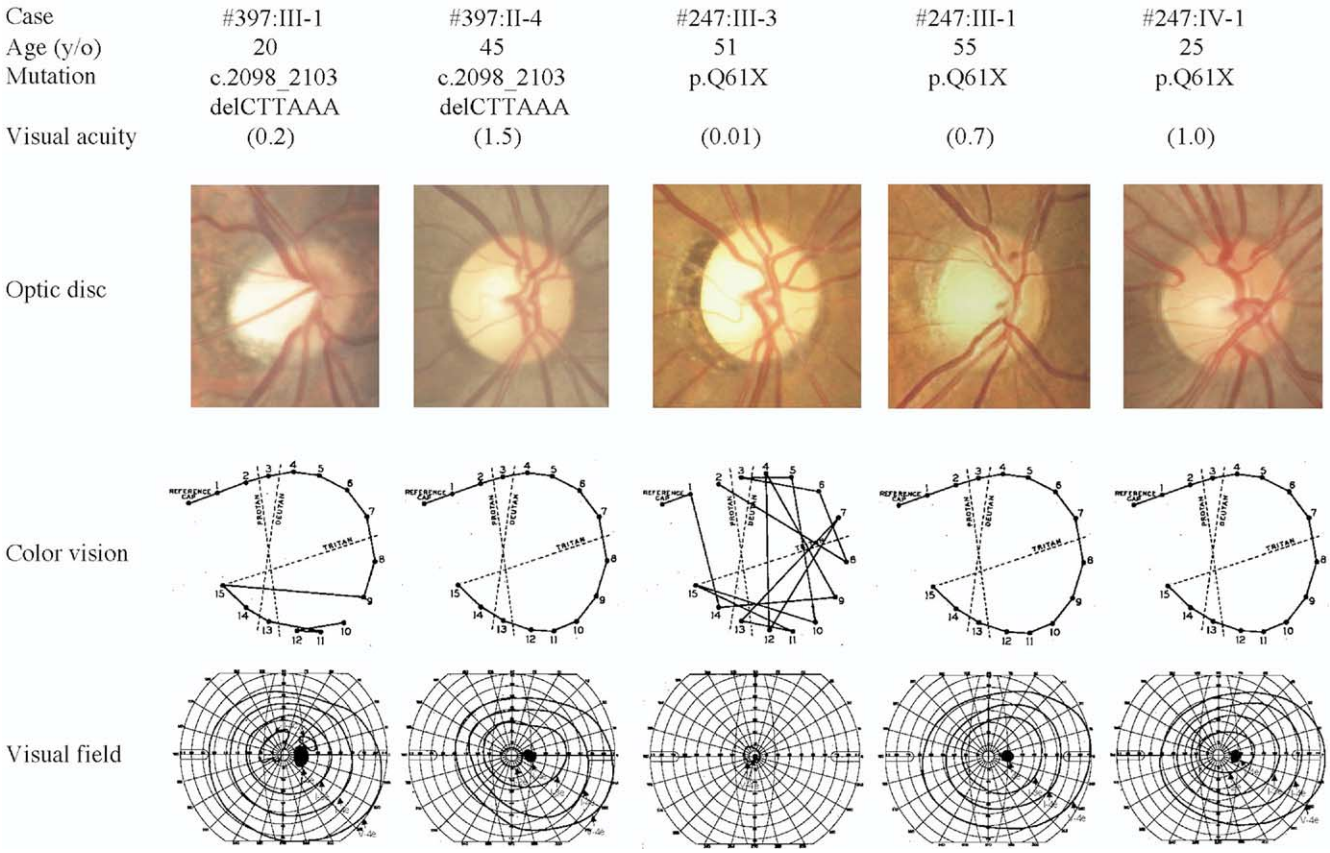

Figure 2. Disc appearance, Goldmann kinetic visual field, and color vision tested with Farnsworth panel D-15 plates in the right eyes of patients with *OPA1* mutations in 2 families (247 and 397). Clinical severity varies considerably among patients with the same mutation within the same family. y/o = years old.

pallor of the optic discs (Fig 2). The optic discs of his 45-year-old mother (II-4) with the same mutation had normal coloration to very slight color changes, and her corrected VAs were 1.5 in both eyes, with normal VFs and normal color vision (Fig 2).

In family 247, the 51-year-old proband (III-3) with the p.Q61X mutation had severe diffuse total optic disc atrophy associated with poor corrected VAs of 0.01 in both eyes and nonaxial color visual defects. Her VFs were severely contracted to $<10°$ of the center by Goldmann kinetic perimetry using the V-4 target in each eye (Fig 2). She and her relatives stated that her vision had been severely impaired from childhood. However, her 55-year-old brother (III-1) with the same mutation had no subjective symptoms, with corrected VAs of 0.7 in both eyes, normal VFs, and normal color vision. His fundi showed mild temporal pallor of optic discs (Fig 2). The proband's 83-year-old father (II-2) with the same mutation had reduced corrected VAs of 0.3 in both eyes associated with blue–yellow color visual defects. However, he had dense cataracts in both eyes, which most likely accounted for his visual reduction. The proband's 25-year-old daughter (IV-1) had the same mutation; however, her optic discs were normal in appearance, and her corrected VAs were 1.0 in both eyes. She also had normal VFs and color vision (Fig 2). These results indicated that the true hereditary pattern of the 2 families was autosomal dominant, but the probands had been considered to be sporadic because the clinical features of other affected members were too mild. Unfortunately, we could not examine any other family members of probands 42 III-2 and 52 III-6, who stated that there was no other family member with eye disease or visual disturbance, and that their deceased parents had good VAs without any complaints.

Discussion

Autosomal dominant optic atrophy is genetically heterogeneous,[29] and another uncloned causative gene, *OPA4,* located on human chromosome 18q12.2–12.3, has also been reported to cause ADOA.[30] However, it is generally considered that most patients with the typical clinical features of ADOA are associated with *OPA1* gene mutations, because previous linkage analysis showed that most families with ADOA were linked to the *OPA1* locus,[29,31] and because Delettre et al detected *OPA1* gene mutations in 17 of 19 unrelated cases presenting with a reliable diagnosis of ADOA.[14] Our results showed that *OPA1* gene mutations were detected in 8 of 9 familial cases of optic atrophy, with 7 of the 8 having autosomal dominant inheritance, and would also indicate that defects in the *OPA1* gene are the major cause of ADOA.

The 93 mutations that have been reported in the *OPA1* gene include 38 deletion/insertion mutations, with 33 of them causing frame shifts resulting in premature stop codons. There were 13 nonsense, 21 splice site, and 21 missense mutations.[12,13,18–26] Thus, 67 (72%) of the reported mutations were truncative mutations or splice site mutations, and this was also true in our patients, in whom most of the mutations (7/9) were truncative. From these results, the mechanism of haplo-insufficiency, defined as the

inability of one copy of the wild-type allele to produce sufficient levels of protein to confer the normal phenotype, was suggested to be involved in the pathogenesis of ADOA.[13,32] A decreased level of the OPA1 protein may cause an abnormality of the mitochondria, resulting in insufficient energy support. The retinal ganglion cells are most likely affected because the *OPA1* gene is predominantly expressed in the ganglion cell layer of the retina[18] (Invest Ophthalmol Vis Sci 42[suppl]:654, 2001) and because the papillomacular retinal nerve fibers require high levels of energy supply for the axoplasmic transport.[13]

However, the exact pathological mechanism for the optic disc atrophy is probably more complicated. For example, the proband of family 247 with the p.Q61X mutation, which is truncative, showed very severe clinical features, whereas her brother, father, and daughter with the same mutation had a mild phenotype. This may indicate that some environmental or genetic factors other than the *OPA1* gene mutation have an influence on the ultimate phenotype in the proband.

All 3 affected members of family 92 with the novel missense mutation p.S545R were affected severely. Although we have not confirmed it by biochemical analysis, this would indicate that residue 545 is important for the function of the protein, such as an involvement in the self-assembly of the OPA1 protein[33] or interaction with other proteins. Alternatively, the expected amino acid substitution from neutral to basic might cause critical structural changes of the molecule.

The c.2708_2711delTTAG mutation is the most common mutation in the *OPA1* gene and has been identified in 27 Caucasian families.[12,14,18,19,22,25] Initially, a founder effect was considered for the mutation because all 3 families with the mutation originated from northern French provinces and Belgium.[12] However, later haplotype analysis of 5 English and Australian families with the same mutation suggested that it was a mutation hot spot.[19] Although we did not perform a haplotype analysis, our results—the same c.2708_2711delTTAG mutation was identified in 3 unrelated Japanese families, far from Europe and in a different race—would strongly support the idea that the mutation arose independently in multiple families.[19]

It is notable that we detected an *OPA1* mutation in 4 of 8 probands with optic atrophy who were considered to be sporadic cases from their family histories. After additional family examinations in 2 families (247 and 397), we concluded that the true hereditary patterns of the families were autosomal dominant. The probands with mutations initially had been considered to be sporadic because the clinical features of other family members with the mutations were too mild to be recognized or within normal range. Regarding the other 2 cases (family 42, III-2, and family 52, III-6), we could not determine whether de novo mutations accounted for the optic atrophy[25] or the clinical phenotype of other family members was very mild. In any case, our results indicate that some ADOA patients seem to be isolated due to the low penetrance of the disease, and that sporadic-appearing cases of optic atrophy may be caused by *OPA1* mutations. Thus, it is important to perform a detailed family examination to determine the hereditary patterns in cases of optic atrophy, even when they were considered to be sporadic from their family histories. Nonetheless, only molecular genetic examination can permit the exact determination of the hereditary patterns in these cases.

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Table 1. Mutations and Polymorphisms in the *OPA1* Gene Identified in This Study

Nucleotide Variants*	Exon	Predicted Change	Frequency among the Analyzed Probands	Frequency among Control Individuals	Reference
Mutations					
c.112C>T	2	p.R38X	1/17 heterozygous (3%)	0/50 (0%)	Thiselton et al[†]
c.181C>T	2	p.Q61X	1/17 heterozygous (3%)	0/50 (0%)	Novel
c.946A>T	9	p.R316X	1/17 heterozygous (3%)	0/50 (0%)	Novel
c.1635C>A	17	p.S545R	1/17 heterozygous (3%)	0/50 (0%)	Novel
c.2061delA	20	p.E687fsX722	1/17 heterozygous (3%)	0/50 (0%)	Novel
c.2098_2103delCTTAAA	21	p.L700_K701del	1/17 heterozygous (3%)	0/50 (0%)	Baris et al[‡]
c.2538insT	25	p.L846fsX854	1/17 heterozygous (3%)	0/50 (0%)	Novel
c.2591insC	25	p.Q864fsX871	1/17 heterozygous (3%)	0/50 (0%)	Novel
c.2708_2711delTTAG	27	p.V903fsX905	3/17 heterozygous (9%)	0/50 (0%)	Delettre et al,[§] Pesch et al,[‖] Toomes et al,[¶] Delettre et al,[#] Thiselton et al,[†] Baris et al[‡]
c.2713C>T	27	p.R905X	1/17 heterozygous (3%)	0/50 (0%)	Toomes et al[¶]
Polymorphisms					
c.321G>A	2	p.S107S	2/17 heterozygous (6%)	3/50 heterozygous (3%)	Pesch et al[‖]
c.473A>G	4	p.N158S	2/17 heterozygous (6%) 13/17 homozygous (76%)	19/50 heterozygous (38%) 22/50 homozygous (44%)	Pesch et al[‖] Delettre et al,[#] Thiselton et al[†]
c.1923G>A	20	p.A641A	1/17 heterozygous (3%)	1/50 heterozygous (1%)	Yamada et al**
c.2109C>T	21	p.A703A	5/17 heterozygous (15%) 10/17 homozygous (59%)	20/50 heterozygous (20%) 26/50 homozygous (52%)	Pesch et al[‖] Toomes et al,[¶] Delettre et al,[#] Thiselton et al[†]
IVS8+32T>C			8/17 heterozygous (24%)	ND	Toomes et al,[¶] Delettre et al,[#] Thiselton et al[†]
IVS26+25T>A			7/17 heterozygous (21%) 10/17 homozygous (59%)	ND	Toomes et al[¶] Delettre et al[#]

ND = not determined.
*Nucleotides numbered from the ATG initiator codon based on a cDNA reference (GenBank accession no. AB011139).
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