*Molecular Vision* 2014; 20:691-703 <http://www.molvis.org/molvis/v20/691>
Received 20 January 2014 | Accepted 24 May 2014 | Published 27 May 2014



# First report of *OPA1* screening in Greek patients with autosomal dominant optic atrophy and identification of a previously undescribed *OPA1* mutation

**Smaragda Kamakari,[1] George Koutsodontis,[1] Miltiadis Tsilimbaris,[2] Athanasios Fitsios,[3] Georgia Chrousos[3]**

*[1]Ophthalmic Genetics Unit, OMMA Ophthalmological Institute of Athens, Greece; [2]Department of Ophthalmology, School of Medicine, University of Crete, Iraklion, Greece; [3]Pediatric Ophthalmology Department, MITERA Childrens' Hospital, Athens, Greece*

**Purpose:** To describe the genotype–phenotype correlation in four Greek pedigrees with autosomal dominant optic atrophy (ADOA) and *OPA1* mutations.

**Methods:** Seven patients from four unrelated families (F1, F2, F3, F4) were clinically assessed for visual acuity, color vision, ptosis, afferent pupillary defects, and visual fields and underwent orthoptic assessment, slit-lamp biomicroscopy, and fundus examination to establish their clinical status. Genomic DNA was extracted from peripheral blood samples from all participants. The coding region (exons 1–28), including the intron-exon boundaries of the *OPA1* gene, was screened in the probands of the four families, as well as in seven additional family members (four affected and three unaffected) with PCR and direct DNA sequencing.

**Results:** All patients presented bilateral decrease in best-corrected visual acuity and temporal pallor of the optic disc. The visual fields of the adult patients showed characteristic scotomata. Other signs were present in some patients such as decreased color discrimination and a gray crescent within the neuroretinal rim. After the *OPA1* gene was sequenced, a previously undescribed heterozygous splice-site mutation c.784–1G>T in intron 7 was detected in family F2. In families F1, F3, and F4, a previously reported in-frame deletion c.876_878delTGT/p.(Val294del), the frameshift c.2366delA/p.(Asn789Metfs*11), and splice-site c.1140+5G>C mutations were detected, respectively.

**Conclusions:** This is the first report of molecular characterization of Greek patients with ADOA. Our findings provide additional information regarding the genotype-phenotype correlation and establish the role of the *OPA1* gene in Greek patients with ADOA.

Autosomal dominant optic atrophy (ADOA) is a hereditary optic neuropathy characterized by bilateral and symmetric optic nerve pallor associated with insidious decrease in visual acuity with onset usually in early childhood. ADOA (estimated prevalence between 1/50,000 worldwide [1] and 1/10,000 in Denmark [2-4]) and Leber hereditary optic neuropathy (LHON; prevalence between 1/30,000 and 1/50,000 [5,6]) are the most common forms of inherited optic neuropathies. Even though evidence of dominantly inherited optic neuropathy was presented before 1900, only after the description of 19 families by the ophthalmologist Kjer was ADOA (also called Kjer type optic atrophy) recognized. The diagnostic criteria established in several studies during the past few decades [3,7] include slowly progressive bilateral visual impairment, dyschromatopsia, loss of sensitivity in the central visual field, and temporal optic disc atrophy beginning before the age of 10 years [8]. The precise age of onset is rarely established; most patients are diagnosed when they enter school or only incidentally following the examination of other affected family members [9].

Optic atrophy shows genetic heterogeneity. Thus far, eight genes (*OPA1:* Gene ID: 4976; OMIM: 165500; *OPA2*: Gene ID: 4977; OMIM 311050; *OPA3*: Gene ID: 80207; OMIM 606580; *OPA4*: Gene ID: 58156; OMIM 605293; *OPA5:* Gene ID: 692222; OMIM 610708; *OPA6*: Gene ID: 777778; OMIM 258500; *OPA7/ TMEM126A*: Gene ID: 84233; OMIM 612988; *OPA8: HGNC: Gene ID* 39750) have been implicated. *OPA1* is the most frequently mutated ADOA gene [10,11].

*OPA1* is situated on chromosome 3q28–29, spans approximately 100 kb, and is composed of 30 coding exons. Alternative splicing generates several isoforms. The main isoform is 960 amino acids long, encoded by 28 exons [10]. The OPA1 protein is a ubiquitously expressed mitochondrial protein with similarity to dynamin-related GTPases, located mostly on the mitochondrial inner membrane [12,13]. OPA1 consists of five domains: the mitochondrial target signal (MTS), N-terminal coiled-coil domain, GTPase domain, dynamin central region,

Correspondence to: Smaragda Kamakari; OMMA, Institute of Ophthalmology, Ophthalmic Genetics Unit, 74 Katehaki st, 11525, Athens, Greece; Phone: 00302106755850; FAX: 00302106755851; email: kamakari@hotmail.gr; omma90@otenet.gr

and C-terminal coiled-coil domain. OPA1 seems to have a key role in regulating mitochondrial dynamics and the apoptotic process [14]. To date, more than 250 mutations have been described in the *OPA1* gene [15,16], most of which are localized to the N-terminal leader sequence (exons 1–2), the GTPase domain (exons 8–16), and the C-terminal coiled-coil region (exons 27–28) [17].

In this study, we screened the *OPA1* gene for mutations in four unrelated Greek families affected with ADOA and identified four different mutations one of which has not been previously described. This is the first report of *OPA1* mutations in Greek patients with ADOA.

## METHODS

*Clinical evaluation:* This study adhered to the tenets of the Declaration of Helsinki and the ARVO statement on human subjects and was approved by the Human Subjects Review Committee at the University Hospital of Heraklion, Crete. Informed written consent was obtained from all study participants. Seven patients from four unrelated Greek families were clinically examined. The inheritance pattern of the four families was autosomal dominant.

All individuals were evaluated for the following clinical ophthalmic parameters: best-corrected visual acuity (BCVA), color vision, orthoptic assessment, eyelid ptosis (positive if the margin of the upper eyelid was more than 2 mm below the superior limbus), presence of relative afferent pupillary defects (RAPD), slit-lamp biomicroscopy of the anterior segment, and fundus examination with particular attention to the optic nerve head characteristics (when possible, fundus photographs were obtained). Assessment of visual fields using automated threshold perimetry were also obtained in the adult patients.

Best-corrected visual acuity was measured using the Snellen chart. The Snellen ratios were then converted to logarithm of the minimum angle of resolution (logMAR) values for the purpose of the study. Color vision was evaluated using Ishihara pseudoisochromatic plates (15 plates). The clinical diagnosis of ADOA was based on a positive family history, a history of gradually bilateral visual impairment beginning at an early age, dyschromatopsia, characteristic abnormalities in the visual fields (cecocentral scotomata), and typical abnormalities of the optic disc (temporal pallor of the optic disc).

*Mutation screening of the OPA1* gene: Total genomic DNA was extracted from whole blood samples on an iPrep purification instrument using the iPrep PureLink gDNA Blood Kit (Invitrogen, Life Technologies, Carlsbad, CA) according to the manufacturer's instructions.

Primer sequences for exons 1–28 and flanking intronic splice sites of the *OPA1* gene were as reported in [17] except for primers of exon 1 which were designed using the Web Primer program. All primer sequences are shown in Table 1. PCR reactions were performed in a 25 µl total reaction volume, containing 50-100 ng genomic DNA, 2.5 µl of 10xPCR buffer (w/o $MgCl_2$), 3 µl of 10 mM dNTPs mix, 0.75 µl of 50 mM $MgCl_2$, 1.75 µl of 10 µM forward primer, 1.75 µl of 10 µM reverse primer and 0.25 µl of 5 U/µl Platinum Taq DNA polymerase (Invitrogen, Life Technologies). Amplification was performed with the following cycling profile: incubation at 94 °C for 5 min followed by 36 cycles of 45 s denaturation at 94 °C, 45 s annealing at 58 °C and 45 s elongation at 72°C. The last cycle was followed by a final extension of 3 min at 72°C. Excess primers and dNTPs were removed using exonuclease I and shrimp alkaline phosphatase, and PCR products were sequenced with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. All sequences were analyzed in forward and reverse directions on an ABI3500 fluorescent sequencer (Applied Biosystems). Nucleotide sequences were compared with the published DNA sequence of *OPA1* gene (GenBank accession number NG_011605.1) and isoform 1 (GenBank accession number NM_015560.1). For the *OPA1* gene, cDNA numbering +1 corresponds to A in the ATG translation initiation codon of *OPA1* isoform 1.

## RESULTS

*Clinical findings:* All patients were Greek and of Greek origin. No patient had a history of neurologic diseases or any other ocular disorders. Seven patients from four families were clinically assessed. The four probands were F1 II:5, F2 II:1, F3 III:24, and F4 III:1. The proband F2 II:1 is the father of patient F2 III:1, patient F3 II:11 is the father of proband F3 III:24, and patient F4 II:1 is the father of proband F4 III:1 (Figure 1A, Figure 2A, Figure 3A, Figure 4A). The age of onset ranged from 5 to 12 years.

Best-corrected visual acuity ranged from 20/25 to 20/400 (0.1–1.3 logMAR units). In ADOA, visual impairment is generally moderate although in some cases it might extend to legal blindness [8]. No patient showed asymmetry in VA, defined as the difference of two lines in Snellen charts, between eyes.

Visual field (automated perimetry) demonstrated cecocentral scotomata in the adult patients (patients F1 II:5, F2 II:1, F3 II:11, F4 II:1). Cecocentral scotomata are the most characteristic feature in people affected with ADOA as shown in Figure 5 (F1 II:5). Color vision testing with the Ishihara

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**Table 1. PCR primers used in this study.**

| Exon | Primer (5'-3') |
|---|---|
| Exon1 | CACTTCCTGGGTCATTCCTGGA |
| | AGAATTTTCGGGTTGGGTAGGG |
| Exon 2 | CCCTCTCTGATCTTTCTTCCAT |
| | TAATTGGAAAACCAGGAGGA |
| Exon 3 | TATTTGGCATGCAGAGCATC |
| | TCTCTTTCCTCGAGATGACCA |
| Exon 4 | GGGTTGTCATGAGGATTAAACAA |
| | CATGTATTTTTCCTCCATGGTTC |
| Exon 5 | AAAGGCGATTTGATTCTTTGAA |
| | TCTTTCAAGACTACCTACATGAACAA |
| Exon 6 | AAAAATTTAACTTGCTGTACATTCTG |
| | CACCTTCCAAATTTTGCTCTG |
| Exon 7 | TCAAGATTTTGGAAGATTTTAATTTAG |
| | CACACAACGTTAAGCGGTAAAA |
| Exon 8 | CCGTTTTAGTTTTTACGATGAAGA |
| | TTTTTGCTAGTTGGCAAGTTCA |
| Exon 9 | AAAAACTCAGAGCAGCATTACAAA |
| | CCTAAGGAACCTCACTGAGACG |
| Exon 10,11 | CATACGGGCTGTGGGAATTA |
| | CCATAAAACGTCACTGAAATGAA |
| Exon 12,13 | GAATTTTTAGAATACATTTCACCAAAA |
| | TGGATTGCTAAAGAAGAAAACAT |
| Exon 14 | GACACAGGGGTATAATTTGTACTGA |
| | TTCTCGCAACAAAGAATTTGA |
| Exon 15,16 | TTTTGCTTTCTAAATTGTATATTACGC |
| | TGAAAACAGTTCAATTTAAGCTACTC |
| Exon 17 | CATTCGCAGACTTGGTGGTA |
| | TGTCTTAATTTGCTTGCTTCTTT |
| Exon 18 | CCACTTTAACCACTACATCTGGAA |
| | AGCTTATCAGATTTTTCTCTCAACA |
| Exon 19 | TCTGAAAATCATGACAGGGTAAA |
| | CAAGGCAACAATAAATCACTGC |
| Exon 20 | TGATACTTCAGTCAAGCTGTTTTT |

| Exon | Primer (5’-3’) |
|---|---|
| | CAGCTCCTACTCCCTTCAGA |
| Exon 21 | TTTTTCATGTTAACCATTGAAGTATG |
| | GAGGCTGATACCCCAGTATACAA |
| Exon 22 | TTTTTCCATATTTACTAAGCTGTCAA |
| | TCACCACTGTGAACTCAGAACTC |
| Exon 23 | TTCCTTTATTTCAACTGCCTTCA |
| | AATGCCTGAATTAAAATGAACAA |
| Exon 24 | TCAAGCACCAAATTATGAACCA |
| | GCAGATTCCTGCTTCTCAGC |
| Exon 25 | TGTACAACTTCTCAGTGTGGTTGA |
| | GCATATTTTGACAACTGTTGCTT |
| Exon 26 | AAGCTTAGGACATATCTACTGGTTCT |
| | TGGGAAGTATTTTGGCATCC |
| Exon 27 | TCTTTATTCATTTATAAAAACGATGC |
| | AAATGGGAAAGGTGGAAAGG |
| Exon 28 | CCTCCTGATTTGTGATACCTTTG |
| | CAAGCAGGATGTAAATGAAGCA |



*Molecular Vision* 2014; 20:691-703 <http://www.molvis.org/molvis/v20/691>

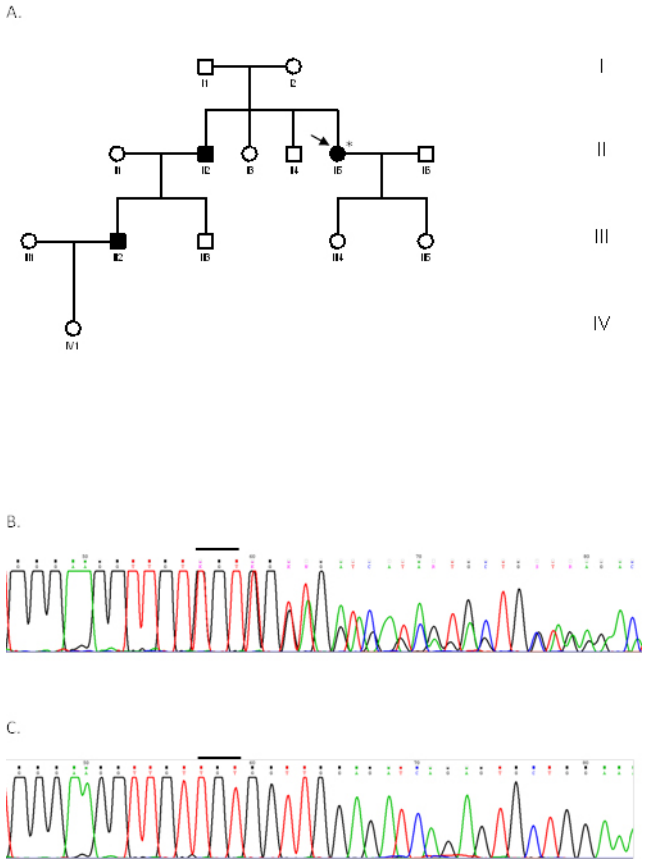


Figure 1. DNA sequence analysis of exon 9 and flanking sequences of the *OPA1* gene in family F1. **A**: Pedigree F1. The arrow indicates the proband. Affected and unaffected individuals are represented with black and open circles, respectively. Males are represented with a quadrant and females with a circle. The asterisk sign indicates individuals clinically and genetically examined. **B**: Mutant sequence in proband F1 II:5. The antisense sequence confirmed the same in-frame mutation. **C**: Normal sequence. The horizontal lines in **B** and **C** indicate deletion of the three nucleotides TGT (c.876_878delTGT) resulting in the in-frame deletion of valine at codon 294 of the OPA1 protein [p.(Valdel294)].

pseudoisochromatic plates ranged from severely compromised (1/15 correct plates) to quite normal (14/15 correct plates). A reliable color test from the youngest patient, F2 III:1, was not possible. Color vision examination in previous studies has shown that mixed color defect accounts for more than 80% of the color deficits documented [8]. Afferent pupillary defects were uncommon in the group with only one patient exhibiting a trace of afferent pupillary defect in both eyes (F2 II:1).

The orthoptic assessment documented normal ocular motility in all patients, and there was no upper eyelid ptosis. We paid considerable attention to these features because additional associated clinical manifestations (chronic progressive external ophthalmoplegia, ataxia, sensorineural deafness, sensory-motor neuropathy, and myopathy [18,19]), the syndromic forms of ADOA or ADOA plus [16], account for 20% of all DOA cases with *OPA1* mutation [20] and could be missed, particularly in patients with a mild to moderate degree of ptosis or ophthalmoplegia [19]. External ophthalmoplegia and neurologic signs occur from the third decade of life, while visual failure occurs in the first decade [20]. Therefore, some of our probands could manifest handicaps later in life that were not present in the current examination. In addition, almost one third of the family members manifested either pure DOA or DOA plus phenotypes although they carry the same *OPA1* mutation [20].

The fundus examination revealed moderate to marked pallor of the temporal quadrant of each optic disc in all patients. An example of this feature is shown in patient F3 III:24 (Figure 6). The degree of temporal pallor was symmetric in every individual. Ophthalmologic fundus examination in patients with ADOA typically discloses bilateral and symmetric temporal of diffuse pallor of the optic disc [21].

One optic nerve examined, in patient F3 III:24, had a gray crescent situated temporally (gray pigment within the

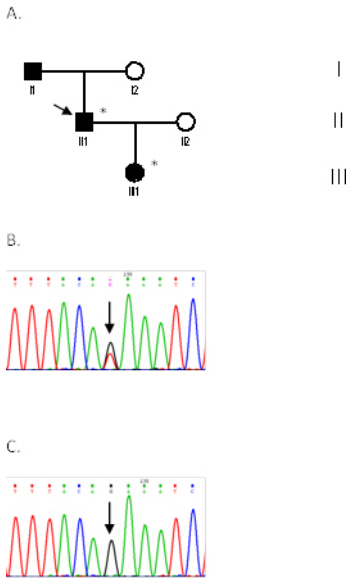


Figure 2. DNA sequence analysis of exon 8 and flanking sequences of the *OPA1* gene in family F2. **A**: Pedigree F2. The arrow indicates the proband. Affected and unaffected individuals are represented with black and open circles, respectively. Males are represented with a quadrant and females with a circle. The asterisk sign indicates individuals both clinically and genetically examined. **B**: Mutant sequence in proband F2 II:1. The antisense sequence confirmed the same splicing site mutation. **C**: Normal sequence. The arrows in **B** and **C** indicate the position of the G to T substitution at the 3′ acceptor splice site of intron 7 (c.784–1G>T).

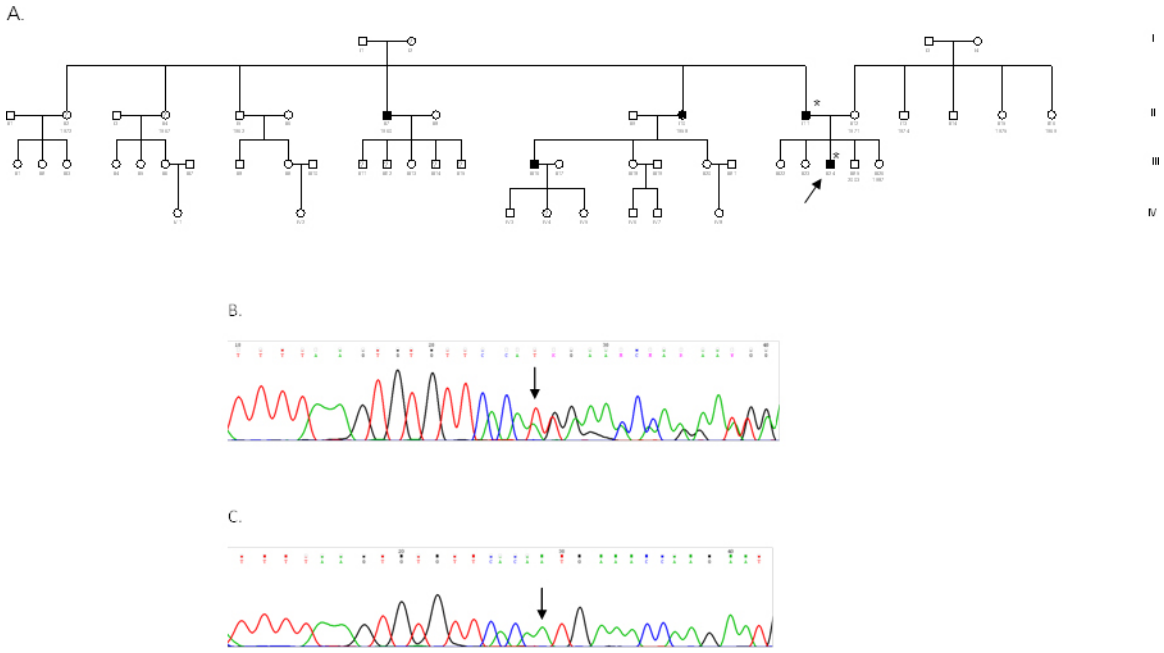


Figure 3. DNA sequence analysis of exon 24 and flanking sequences of the *OPA1* gene in family F3. **A**: Pedigree F3. The arrow indicates the proband. Affected and unaffected individuals are represented with black and open circles, respectively. Males are represented with a quadrant and females with a circle. The asterisk sign indicates individuals both clinically and genetically examined. Questions marks indicate probably affected family members. **B**: Mutant sequence in proband F3 III:24. The antisense sequence confirmed the same in-frame mutation. **C**: Normal sequence. The arrows in **B** and **C** indicate the deletion of nucleotide A (c.2366delA) resulting in the frameshift p.(Asn789Metfs*11) mutation of the OPA1 protein.

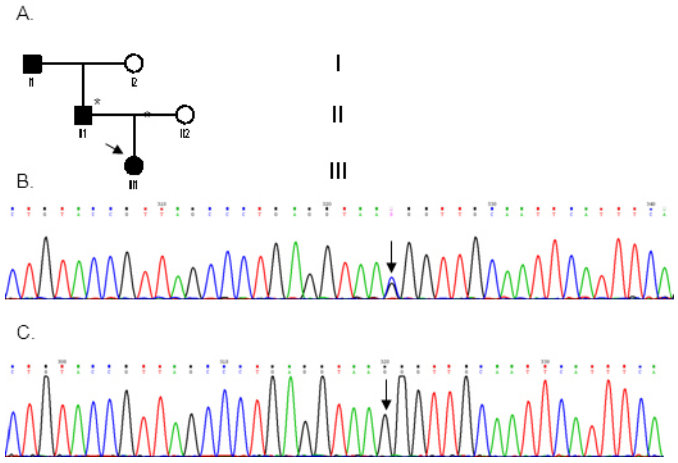


Figure 4. DNA sequence analysis of exon 11 and flanking sequences of the *OPA1* gene in family F4. **A**: Pedigree F4. The arrow indicates the proband. Affected and unaffected individuals are represented with black and open circles, respectively. Males are represented with a quadrant and females with a circle. The asterisk sign indicates individuals both clinically and genetically examined. **B**: Mutant sequence in proband F4 III:1. **C**: Normal sequence. The arrows in **B** and **C** indicate the position of the G to C substitution at the fifth nucleotide of the 5′ donor site of intron 11.

substance of neuroretinal rim tissue; Figure 7). This finding has been reported in previous studies by others [22,23]. None of the participants presented anterior segment abnormalities. No electrophysiological investigation was performed since electrophysiology does not contribute to the diagnosis of ADOA [9].

*Mutation analysis:* We identified one unreported and three previously reported *OPA1* gene mutations in the probands of four unrelated families. Specifically, proband F1 II:5

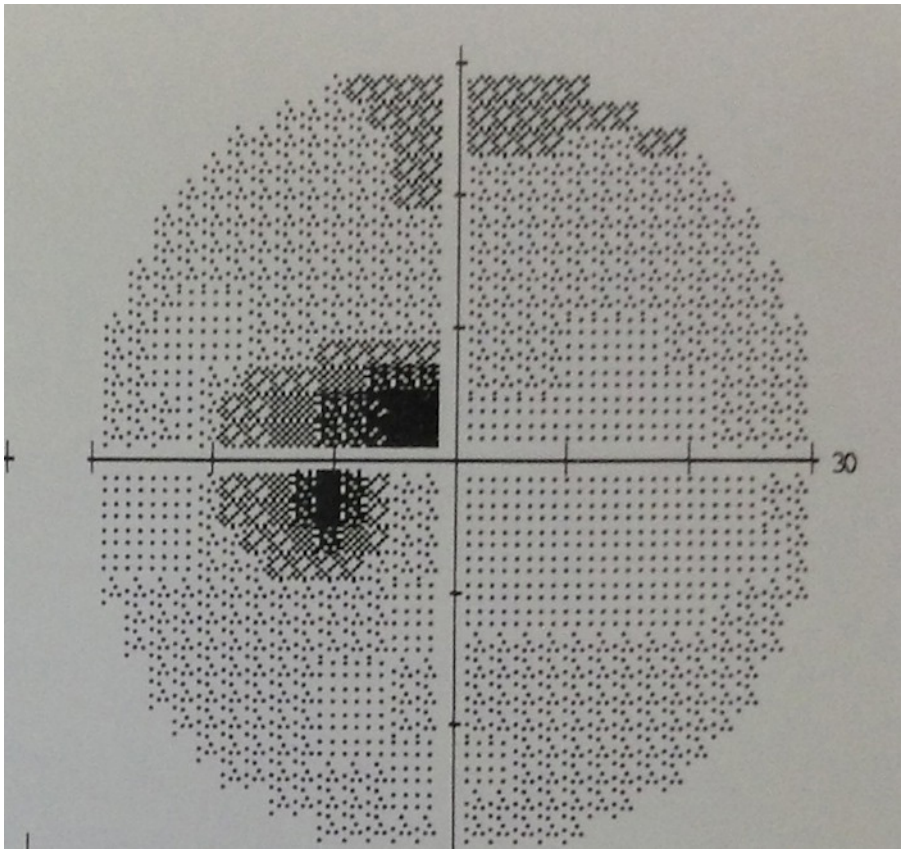


Figure 5. Left Humphrey 30–2 visual field showing a cecocentral scotoma of the proband F1 II:5.

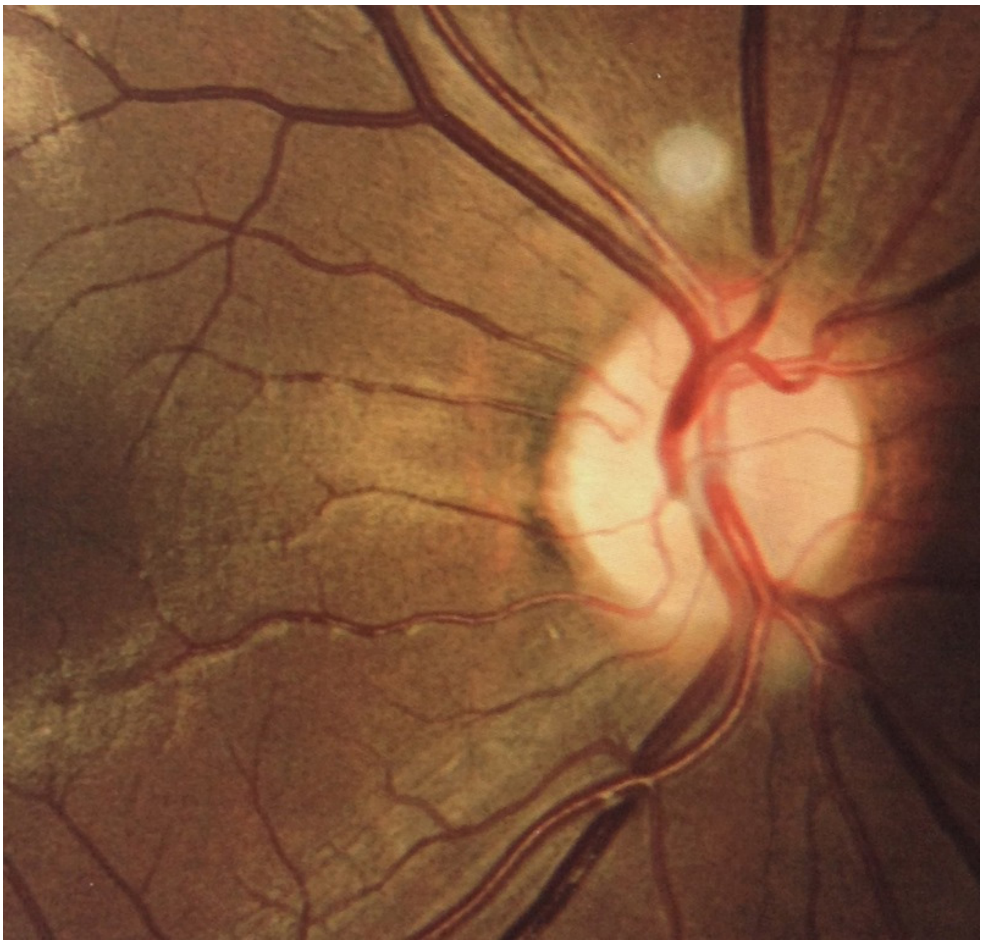

Figure 6. Fundus photograph of the right eye shows optic nerve temporal pallor in the patient F3 III:24 with dominant optic atrophy.

(family F1) had a heterozygous 3-bp in-frame deletion, c.876_878delTGT, in exon 9 that is predicted to result in a premature termination codon (p.(Val294fsx667); Figure 1B). This mutation has been previously reported once [20]. The proband has two children who appeared unaffected. Only one was genetically tested and found to be negative for the mutation.

Proband F2 II:1 (family F2) had a heterozygous splicing site mutation, c.784–1G>T, in intron 7 (Figure 2B). This mutation has not been previously described. He has one affected child (F2 III:1) who was found positive for the same mutation. This mutation was not detected in 100 unrelated healthy control individuals.

Proband F3 III:24 (family F3) had a heterozygous, one nucleotide deletion, c.2366delA in exon 24 (Figure 3B). This frameshift mutation results in the substitution of ten amino acids starting with methionine at codon 789 and in a premature stop at codon 799 of the OPA1 protein. This mutation has been previously reported [24]. We screened four relatives of the proband: his affected father, who was found positive for the mutation, and his three unaffected siblings (F3 III:22, F3 III:25, F3 III:26), one of whom was revealed to be positive for the mutation (F3 III:26). The proband's younger brother (3 years old) was under the expected age of onset (F3 III:25).

Proband F4 III:1 (family F4) had a heterozygous, splice-site mutation, c.1140+5G>C, in intron 11 (Figure 4B). This mutation has been previously reported [25,26]. We also screened her affected father (F4 II:1), who had the same mutation. Clinical and genetic findings are summarized in Table 2.

## DISCUSSION

Greek patients with suspected ADOA have never been screened for *OPA1* mutations. This is the first report of clinical and genetic characterization of Greek patients with identification of *OPA1* mutations.

In this study, we included four unrelated Greek families with clinically diagnosed optic atrophy and screened the entire coding and flanking sequences of the *OPA1* gene in the four probands. We identified four different mutations as

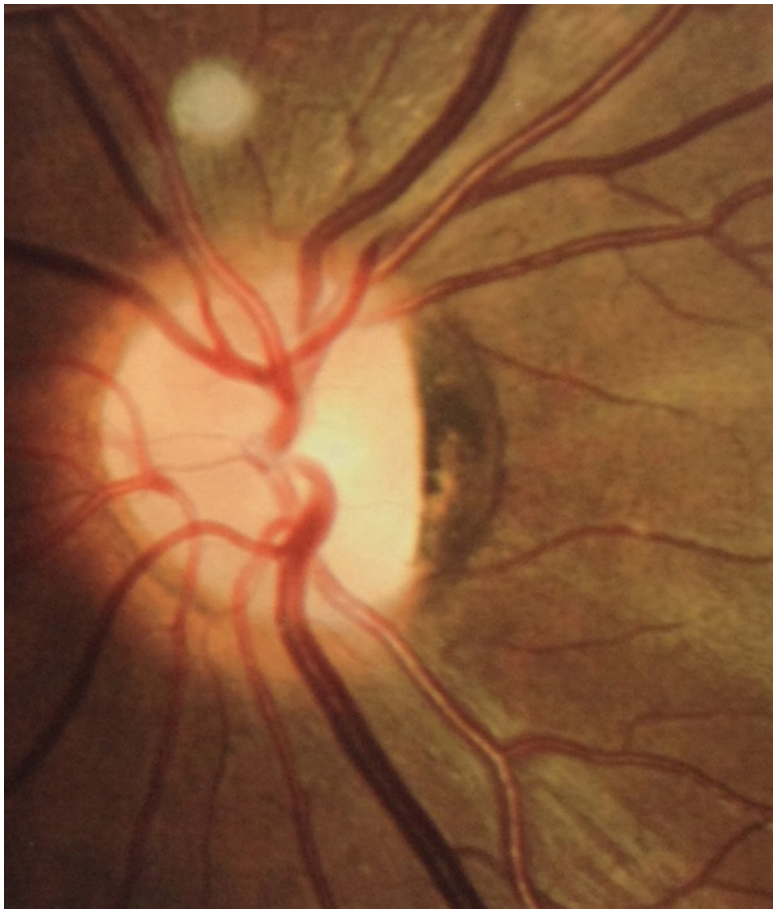

Figure 7. Fundus photograph of the left optic disc showing gray crescent situated temporally in patient F3 III:24.

follows: a previously undescribed heterozygous splice-site mutation, c.784–1G>T, in intron 7, a previously reported heterozygous in-frame deletion mutation, c.876_878delTGT/p.(Val294del), in exon 9 leading to a one amino acid deletion, a previously reported frameshift mutation, c.2366delA/p.(Asn789Metfs*11), in exon 24 and a previously reported splice-site mutation, c.1140+5G>C, in intron 11 of the *OPA1* gene. Our genetic analysis included seven additional members of the four families (four affected and three unaffected).

More than 250 *OPA1* mutations have been reported to date. At the protein level, 40% of mutations lead to premature translation termination, 27% are missense mutations, 27% are splicing mutations, and 6% are either deletions or duplications [25]. In the present study, the four mutations detected include an in-frame deletion mutation, a frameshift mutation, and two splice-site mutations.

The one amino acid deletion p.(Val294del) was detected in family F1. Several lines of evidence support the pathogenicity of this mutation. Specifically, valine at residue 294 is highly conserved among all five close eukaryotic homologs, including mouse, salmon, Xenopus, *Drosophila melanogaster*, and *Caenorhabditis elegans*. This mutation is located in the highly conserved GTPase domain and has been previously reported once in two related patients [20]. The authors report that this mutation has not been detected in a panel of 150–300 age-matched controls. Furthermore, a similar mutation involving deletion of Val294 as well as its preceding residue Val293 [p.(Val293_Val294del; c.877_882delGTGGTT)] has been detected in a patient with DOA and not found in a panel of 1,000 control chromosomes [26]. The absence of the genetic change involving deletion of Valine 294 in a large number of control chromosomes strongly supports that this is a disease-causing mutation. The two previously reported related patients (age 59 and 75) who carried the same mutation as our patient (age 59) presented childhood onset optic atrophy as well as extraocular features including neuropathy, hereditary spastic paraplegia, and migraine (but not ptosis or ophthalmoplegia) whereas two additional affected members of the family (children of the 59-year-old patient) presented isolated optic atrophy. We did not observe any indication of neurologic signs or symptoms or other extrabulbar ophthalmological manifestations (eyelid

Table 2. Clinical and genetic characterization of probands and their relatives with ADOA.

| Family pedigree | Gender | Age at examination | Age of onset | Visual acuity (Right/left) | Optic nerve head | Color vision (OD=Right Eye, OS=Left Eye) | Mutation | Exon | Type of mutation |
|---|---|---|---|---|---|---|---|---|---|
| F1II5 | F | 59 | 7 | 1.0/1.3 | temporal pallor | 0/15 OD<br>0/15 OS | c.876_878delTGT, p.(Val294del) | 9 | In-frame deletion |
| F2II1 | M | 45 | 12 | 0.8/0.8 | temporal pallor | 14/15 OD<br>14/15 OS | c.784–1G>T | Intron 7 | Splice site Novel |
| F2III1 | F | 5 | 5 | 0.6/0.6 | temporal pallor | - | | | |
| F3II11 | M | 50 | 10 | 0.1/0.2 | temporal pallor | 1/15 OD<br>1/15 OS | c.2366delA,p.(Asn789Metfs*11) | 24 | Frameshift |
| F3III24 | M | 15 | 10 | 0.2/0.3 | temporal pallor | 1/15 OD<br>1/15 OS | | | |
| F4II1 | M | 43 | 10 | 0.4/0.3 | temporal pallor | 14/15 OD<br>14/15 OS | c.1140+5G>C | Intron 11 | Splice site |
| F4III1 | F | 6 | 6 | 0.4/0.2 | temporal pallor | 14/15 OD<br>14/15 OS | | | |

ptosis or ophthalmoplegia) in the past medical history of our patient carrying the same *OPA1* mutation. Her unaffected daughter tested negative for this mutation, which further supports that Val294del is the disease-causing mutation. Finally, nonsense mutations affecting neighboring amino acids Arg290 and Glu297, located within the same GTPase domain, have also been reported [27-29].

Among the reported mutations of the *OPA1* gene, more than 20% are splice-site mutations, which are presumed to result in the in-frame skipping of exons or premature termination of *OPA1* translation. The previously undescribed splice-site mutation c.784–1G>T detected in family F2 affects the critical guanine at the 3′ acceptor splice site of intron 7. Schimpf et al. [25] reported a similar mutation c.784–1G>A and showed that this caused in-frame skipping of exon 8 (K262-R290del, Human Genome Mutation Database, CS080724). Of note, cDNA analysis of the c.784–1G>T mutation, presented in this study, similarly causes in-frame skipping of exon 8 (data not shown).

A second splice-site mutation, c.1140+5G>C was detected in family F4. This mutation has been previously reported [25,26] and through cDNA analysis was shown to lead to the complete skipping of exon 11 thus causing the deletion of the corresponding amino acids Leu356_Glu380 of the OPA1 protein [25].

The last mutation c.2366delA/p.(Asn789Metfs*11) has been previously detected in only two patients [24] and is a frameshift mutation in exon 24 resulting in the substitution of ten amino acids, and in a premature stop at codon 799, leading to the loss of the 162 C-terminal amino acids of the OPA1 protein. This mutation was tested in five members of family F3, including the affected father and his four children, one affected son 15 years old, one son under the age of onset, and two apparently unaffected daughters in their early 20s. Both affected members carried the mutation. One of the presumed unaffected daughters (21 years old), never clinically evaluated previously, surprisingly tested positive for the mutation. Her positive genetic result requires further investigation; however, she has not agreed to be clinically examined. The son, under the age of onset of the illness, tested negative for the mutation, thus excluding the risk of him being affected and relieving the parents of anxiety and frequent unnecessary ophthalmological examinations. This once more proves the value of genetic testing for detecting presymptomatic carriers or excluding the risk of being affected.

Although more than 250 *OPA1* gene mutations have been reported to date, mutations in Greek patients have not been reported. We detected four *OPA1* mutations, one of which was previously unreported. The current study is the first report of mutations causing isolated ADOA in Greek families enabling genetic counseling of the probands and their families.

## ACKNOWLEDGMENTS

We are grateful to Dr. Christophoros Giatzakis for critical reading and comments of the manuscript. The project was financially supported by the EU FP6, Integrated Project ‘EVI-GENORET’ (LSHG-CT-2005-512036; S.K)

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Articles are provided courtesy of Emory University and the Zhongshan Ophthalmic Center, Sun Yat-sen University, P.R. China. The print version of this article was created on 27 May 2014. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.

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