MUTATION IN BRIEF

Fourteen Novel OPA1 Mutations in Autosomal Dominant Optic Atrophy Including Two De Novo Mutations in Sporadic Optic Atrophy

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The *OPA1* gene, encoding a dynamin-related GTPase that plays a role in mitochondrial biogenesis, is implicated in most cases of autosomal dominant optic atrophy (ADOA). Sixty-nine pathogenic *OPA1* mutations have been reported so far. Most of these are truncating mutations located in the GTPase domain coding region (exons 8-16) and at the 3¢-end (exons 27-28). We screened 44 patients with typical ADOA using PCR-sequencing. We also tested 20 sporadic cases of bilateral optic atrophy compatible with ADOA. Of the 18 *OPA1* mutations found, 14 have never been previously reported. The novel mutations include one nonsense mutation, 3 missense mutations, 6 deletions, one insertion and 3 exon-skipping mutations. Two of these are *de novo* mutations, which were found in 2 patients with sporadic optic atrophy. The recurrent c.2708_2711delTTAG mutation was found in 2 patients with a severe congenital presentation of the disease. These results suggest that screening for *OPA1* gene mutations may be useful for patients with optic atrophy who have no affected relatives, or when the presentation of the disease is atypical as in the case of early onset optic atrophy.

KEY WORDS: autosomal dominant optic atrophy; ADOA; Mitochondria; *OPA1*; Optic atrophy

INTRODUCTION

The optic nerve is formed by about a million axons that run from the lateral geniculum to the retina.

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Degeneration of the ganglion cells of the retina, leading to optic atrophy, may arise from a number of causes, such as genetic disease, vascular impairment, trauma, glaucoma, inflammation, brain tumour, toxic neuropathy, or vitamin deficiency. The most frequent form of hereditary optic atrophy, or autosomal dominant optic atrophy (ADOA, MIM# 165500), was first described by Kjer in 1959. The frequency of the disease, estimated to have a worldwide prevalence of 1:50,000 [Lyle, 1990], was found to be as high as 1:12,000 in Denmark [Eiberg et al., 1994; Kjer et al., 1996]. The disease, which generally appears in childhood, is characterised by a progressive decrease in visual acuity, dyschromatopsia that often predominates in the blue-yellow hues (tritanopia), loss of sensitivity in the central visual field (central, paracentral or coecocentral scotomas), and optic nerve pallor. A gene located at 3q28-q29 [Eiberg et al., 1994], identified in most cases of ADOA, has been named *OPA1* (optic atrophy 1) [Alexander et al., 2000; Delettre et al., 2000]. This gene was first believed to contain 29 exons, 28 of which encoded a mitochondrial dynamin-related GTPase, composed of 960 amino acids. It was later demonstrated that *OPA1* possesses 2 additional, alternatively spliced exons, named 4b and 5b (encoding 18 and 37 amino acid sequences respectively), making a total of 30 coding exons [Delettre et al., 2001]. It has been recently confirmed that *OPA1* is involved in the biogenesis and maintenance of mitochondria in mice [Misaka et al., 2002], as well as in humans [Olichon et al., 2002]. Sixty-nine *OPA1* gene mutations, mainly family-specific, have been described so far, and the results of these studies support the hypothesis that haploinsufficiency with null mutations and the functional loss of one allele may represent a major mechanism of ADOA [Pesch et al., 2001; Thiselton et al., 2001; Toomes et al., 2001; Delettre et al., 2002; Marchbank et al., 2002]. We report here 14 new *OPA1* mutations found in 44 patients with a family history of optic atrophy. Two of these are de novo mutations, which were found in 2 patients affected with sporadic optic atrophy.

PATIENTS AND METHODS

Patients

Sixty-four unrelated patients of Caucasian origin were recruited from different Medical Centres. The diagnosis of optic atrophy was based on ophthalmologic tests of visual acuity, color vision, and visual fields, together with an investigation of optic disc anomaly. All patients presented progressive loss of visual acuity, centrocecal scotoma and bilateral nerve pallor. Dyschromatopsia was also found in patients with conserved partial visual acuity. Forty-four of the patients had a family history of dominant optic atrophy. The other 20 patients presented the minimal criteria for ADOA disease in the absence of affected relatives. 23 LHON primary and secondary mutations were previously excluded in all 20 sporadic cases.

Molecular analyses

Blood samples were taken from patients and family members, after obtaining informed consent. Genomic DNA was extracted from blood samples using the High Pure PCR Template Preparation Kit (Roche Diagnosis, Mannheim, Germany). Thirty primer couples (available on request) were used to amplify the 30 coding exons, including exon-intron junctions. PCR reactions were carried out under standard conditions with 100 ng of genomic DNA in a 50 µl volume: 1.5 mM MgCl2, 75 mM Tris-HCl (pH 9 at 25°C), 20 mM (NH4)2SO4, 0.01% Tween 20, 50 pmol of each primer, 200 µM of each dNTP and 2 units of Hot GoldStar (Eurogentec, Seraing, Belgium). Each of the 30 cycles consisted of a denaturation step of 30 s at 94°C, a hybridisation step of 30 s at 58°C, and an extension step of 1 min at 72°C.

The purified PCR products were sequenced using a Ceq2000 DNA sequencer (CEQ DTCS-Quick Start Kit, Beckman Coulter, Fullerton, CA, USA). Multiple sequence alignments were performed using CLUSTALW software. In order to analyse the splicing alteration of the c.983A>G mutation, total RNA was extracted from blood samples (Trizol, Invitrogen Life Technologies, Groningen, The Netherlands). cDNA was obtained using poly-T as a primer and SuperScript RNA polymerase (Invitrogen, Cergy Pontoise, France). PCR was performed with primers D08 5'ACGCAAGATCATCTGCCACGG and R011 5'AACGGTACAGCCTTCTTTCAC that amplified a 282-bp fragment overlapping exons 8 to 11. PCR products were analysed on a 1% agarose gel and sequenced to confirm the c.983A>G mutation.

RESULTS AND DISCUSSION

New and recurrent mutations in *OPA1*

The analysis of the entire coding region of the *OPA1* gene by direct sequencing of PCR-amplified exons in 64 unrelated patients revealed mutations in 27/44 patients with ADOA (61%) and in 2/20 patients with sporadic optic atrophy (10%). All these patients had the same clinical presentation. We found 18 different heterozygous mutations (2 nonsense, 3 missense, 5 splice-site mutations, 7 deletions and 1 insertion). Fourteen of these mutations have not been previously reported (Table 1). No such mutations were detected in a control study of 200 chromosomes. In all familial cases, the mutations cosegregated with the disease phenotype. The absence of *OPA1* gene mutations in 39% of the 44 ADOA patients strongly suggests the possible implication of other *OPA1* gene mutations (such as large-scale rearrangements, promoter mutations) or the involvement of other genes. Unfortunately, the size of analyzed families was not large enough to perform linkage analysis with *OPA1* and *OPA4* microsatellites.

Table 1. *OPA1* Mutations Iidentified in the Present Study

DNA CHANGE	EXON	RNA AND PREDICTED PROTEIN	NUMBER OF PATIENTS
NONSENSE			
c.1096T>C	11	R366X	1
c.1861T>C*	20	Q621X	1
MISSENSE			
c.815T>C*	8	L272P	1
c.1409A>G*	14	N470G	1
c.1721T>C*	18	L574P	1
SPLICE SITE			
c.870+5G>A	8	In-frame skipping of exon 8a, loss of 29 aa	2
c.983A>G*	9	In-frame skipping of exon 9b, loss of 38 aa	1
c.984G>A	9	In-frame skipping of exon 9a, loss of 38 aa	1
c.1140+1G>A*	11	Possible in-frame skipping of exon 11, loss of 25 aa	1
c.2818+1G>T*	27	Possible in-frame skipping of exon 27, loss of 37 aa	1
DELETION			
c.635_638del4*	6	D211fsX236	1
c.1251_1252del 3*	13	T417fsX429	2
c.1590-22_1593del26*	17	K529fsX534	1
c.1879_1880del2*	20	E626fsX633	1
c.2098_2103del6*	21	LK700-701del	1
c.2366delA*	24	N789fsX799	2
c.2708_2711del2	27	V903fsX905	9
INSERTION			
c.1569_1570insT*	16	F523fsX561	1

*Novel mutations.
a: shown by RT-PCR in previous studies; b: shown by RT-PCR in this study
The +1 of the cDNA nucleotide numbering is at the “A” of the ATG translation start (GenBank entry ABO11139).

Missense and nonsense mutations

A new nonsense mutation, the Q621X mutation, is expected to lead to the loss of the 340 C-terminal amino acids. Three novel missense mutations, i.e. L272P, L574P and N470G, involve residues that are strongly conserved in the *OPA1* orthologs of mouse, salmon, *Drosophila melanogaster* and *Caenorhabditis elegans.*

Splice-site mutations

We detected three new splice-site mutations. Mutation c.983A>G, at the antepenultimate nucleotide position of exon 9, modifies the consensus sequence of the 5' donor splice site of intron 9, resulting in an in-frame skipping of exon 9 (Figure 1). This result was confirmed by sequencing of the RT-PCR product. The 2 other novel splice-site mutations, both affecting the 5' donor site of the introns c.1140+1G>A from exon 11, and c.2818+1G>T from exon 27, may be expected to produce in-frame skipping of exons 11 and 27 respectively.

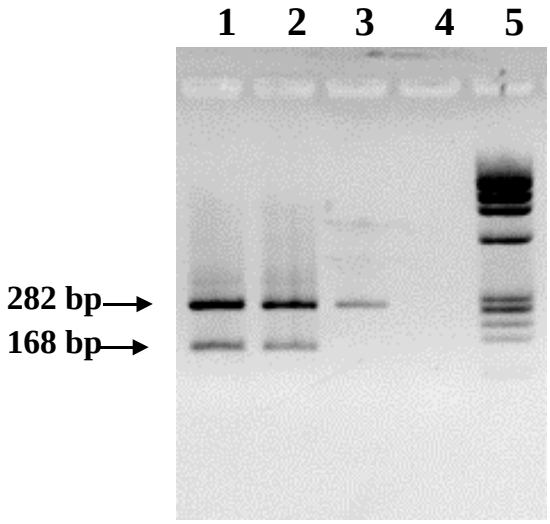

Figure 1: RT-PCR products of the splice mutant A983G from affected daughter (lane 1), affected mother (lane 2), normal control (lane 3), and DNA-free control (lane 4). Lane 5: Molecular weight marker PhiX174/Hae III.

In-frame deletions and insertions

All the deletions found in this study were new mutations, except for the recurrent c.2708_2711delTTAG mutation, which was found in 9 unrelated patients. A frameshift deletion in exon 6 (c.635_638delAAGA) leads to the substitution of 13 amino acids after codon 211, and to the appearance of a stop codon at position 236, resulting in the loss of the 725 C-terminal amino acids of the protein. The second deletion in exon 13 (c.1251_1252delTA) leads to the substitution of 12 amino acids after codon 417, and to the appearance of a stop codon at position 429, resulting in the loss of the 531 C-terminal amino acids of the protein. The third deletion, c.1590-22_1593del26, removes the first 4 nucleotides of exon 17 and the last 22 nucleotides of intron 16, including its acceptor splice site. The fourth deletion, c.1879_1880delAG in exon 20, is a frameshift resulting in the substitution of six amino acids and a premature stop at position 633. This results in the loss of 328 amino acids in the C-terminal position. The fifth deletion, c.2098_2103delCTTAAA in exon 21, results in the loss of two conserved amino acids, L700_K701del. The sixth novel deletion, c.2366delA, which is a frameshift in exon 24, results in the substitution of 10 amino acids, and in a premature stop at codon 799, leading to the loss of the 162 C-terminal amino acids.

Finally, we describe a new 1-bp insertion (1569_1570insT) in exon 16. To our knowledge, this is the first mutation to be reported in exon 16. This frameshift mutation leads to the substitution of 37 amino acids after codon 523, and to the introduction of a premature stop at position 561, causing the loss of the 400 C-terminal amino acids.

Taking into account the mutations recently reported [Fingert et al., 2002; Shimizu et al., 2002], and the large deletion encompassing the *OPA1* locus [Marchbank et al., 2002], the 14 new mutations reported in this study bring

the total number of *OPA1* mutations to 83. All these mutations were detected in 139 familial or isolated cases of optic atrophy. Among the mutations, 38 (46%) are C-terminal truncating mutations, 20 (24%) are amino acid substitutions and 24 (29%) are in-frame deletions (although only 6 of these were verified by RT-PCR, the other 18 may be considered as putative in-frame deletions). Many of these mutations are private, except for the c.2708_2711delTTAG deletion found in 27 unrelated familial or isolated cases. This deletion, which accounts for about 32% of the mutations reported so far in *OPA1*, could be a mutational hot spot. Interestingly, no founder effect has been identified [Toomes et al., 2001]. The mutations are not evenly distributed over the *OPA1* gene. In fact, almost 50% of the mutations are localized in only 4 of the 28 exons, i.e. exons 8, 9, 12 and 27. This finding may be expected to facilitate rapid, primary screening for optic atrophy since the direct sequencing of exons 8, 9, 12 and 27 should enable the detection of about half of the mutations in the *OPA1* gene.

Phenotypic variability

All reports of the *OPA1* mutations in ADOA have emphasized the great intra- and inter-familial phenotypic variability, ranging from asymptomatic carriers to severely impaired individuals [Delettre et al., 2001; Pesch et al., 2001; Toomes et al., 2001; Thiselton et al., 2002]. In our study, we found an interesting case of early onset optic atrophy at birth. The patient, a 10 year-old boy, suffered from optic atrophy with nystagmus, bilateral visual acuity of 2/10, and papillary atrophy. His father presented the same severe and early presentation of the disease, whereas his grandfather had developed optic atrophy only at the age of 40. Since all these patients were found to carry the recurrent 2708-2711del mutation, there was no clear indication of any relationship between phenotype and genotype. This suggests the involvement of other genetic or epigenetic factors in the pathogenesis of ADOA. To our knowledge, this is the first report of an *OPA1* gene mutation in very early onset and severe presentation of optic atrophy.

***De novo* mutations**

In our study, we tested 20 patients identified as having optic atrophy without any family history of eye disorder. In these sporadic cases, all the main primary an secondary mutations of Leber's hereditary optic neuropathy (LHON) in mitochondrial DNA were excluded before *OPA1* sequencing. Interestingly, de novo mutations of *OPA1* were identified in two of the patients.

The first patient was a 20-year old woman who had had an infantile onset of the disease, at age 6, with a decrease of visual acuity followed by progressive aggravation of the condition. At the time of examination, her visual acuity was 6/10 with the right eye, and 5/10 with the left eye. She had typically bilateral, temporal papillary pallor, and tritanopia. Delayed latencies in patterned visual evoked potentials revealed defective conduction of the optic nerves. She was found to carry the c.635_638delAAGA mutation in exon 6. Her parents and two sisters were unaffected and none of them were found to carry the mutation. The second patient, a 30 year-old woman, had been 20 when optic atrophy was diagnosed. Clinical examination, analysis of visual evoked potentials and MRI confirmed the diagnosis of isolated bilateral optic neuropathy. She was found to carry the c.1721T>C mutation in exon 18, whereas neither of her parents carried this mutation. In both cases, false paternity was excluded by testing 4 informative microsatellite markers on chromosomes 4, 7, 15 and 19.

To our knowledge, this is the first report of de novo mutations in the *OPA1* gene in patients affected with sporadic optic atrophy.

Sporadic cases in autosomal dominant diseases can be caused by de novo mutations, which may be explained by mutational hot spots or advanced paternal age. In the 2 cases in our study, the de novo mutations were not located in hot spots, and one father was aged 29 and the other 38 when the children were born. Alternatively, the disease may simply appear to be sporadic because of incomplete penetrance of the trait. In 3 large families, carrying the c.2708_2712del and the L396R mutations, the penetrance has been estimated at 43%, 62% and 77% [Delettre et al., 2001; Toomes et al., 2001]. This incomplete penetrance suggests that, in small families, ADOA may easily appear to be sporadic. Several studies undertaken before the discovery of *OPA1* have evaluated possible causes of idiopathic bilateral optic atrophy [Morissey et al., 1995; Chan et al., 1996]. After eliminating multiple sclerosis, LHON mutations, and other classical causes of optic atrophy, such as toxic neuropathy, 60 to 90 % of the cases of the disease remain unexplained. Our results suggest that mutations of the *OPA1* gene may be

found in about 10% of the cases of sporadic optic atrophy. Thus, the systematic search for *OPA1* mutations, as currently carried out for LHON mutations, should prove useful in sporadic cases of optic atrophy.

CONCLUSION

We have reported 14 novel *OPA1* mutations occurring in different clinical phenotypes. In particular, our results suggest that *OPA1* may be involved in severe ADOA occurring at birth, as well as in sporadic cases of the disease. Up to now, the search for *OPA1* mutations has been mainly performed in patients with known dominant transmission of the disease or in patients with a phenotype strongly suggestive of ADOA. The investigation of *OPA1* mutations in sporadic cases and in patients with severe early onset of the disease, in addition to the search for large-scale rearrangements of the gene, should lead to a better understanding of the mutational and phenotypic spectrum of optic atrophy.

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