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ORIGINAL INVESTIGATION

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Mutation spectrum and splicing variants in the *OPA1* gene

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Abstract Optic atrophy type 1 (OPA1, MIM 165500) is a dominantly inherited optic neuropathy that features low visual acuity leading in many cases to legal blindness. We have recently shown, with others, that mutations in the *OPA1* gene encoding a dynamin-related mitochondrial protein, underlie the dominant form of optic atrophy. Here we report that *OPA1* has eight mRNA isoforms as a result of the alternative splicing of exon 4 and two novel exons named 4b and 5b. In addition, we screened a cohort of 19 unrelated patients with dominant optic atrophy by direct sequencing of the 30 *OPA1* exons (including exons 4b and 5b) and found mutations in 17 (89%) of them of which 8 were novel. A majority of these mutations were truncative (65%) and located in exons 8 to 28, but a number of them were amino acid changes predominantly found in the GTPase domain (exons 8 to 15). We hypothesize that at least two modifications of *OPA1* may lead to dominant optic atrophy, that is alteration in GTPase activity and loss of the last seven C-terminal amino acids that putatively interact with other proteins.

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Introduction

Dominant optic atrophy (DOA), also called Kjer-type optic atrophy after the Danish ophthalmologist who first described it (Kjer 1959), is the most frequent form of hereditary optic neuropathy with a prevalence ranging between 1:12 000 (Kivlin et al. 1983; Kjer et al. 1996) and 1:50 000 (Lyle 1990). It features a decrease in visual acuity, tritanopia (dyschromatopsia characterized by confusion in the blue-yellow hues), sensitivity loss in the central visual fields, and pallor of the optic nerve that is readily seen on fundus examination (Hoyt 1980; Jaeger 1988; Smith 1972; Votruba et al. 1998a). The apparent onset of the disease depends on the degree of visual loss which exhibits great intrafamilial variations ranging from asymptomatic carriers to legally blind patients (Delettre et al. 2000; Votruba et al. 1998b). Histopathology studies have shown diffuse atrophy of the ganglion cell layer that predominates in the central retina and loss of myelin and nerve tissue within the optic nerve (Johnston 1979; Kjer et al. 1983).

There are currently four loci for non-syndromic optic atrophy with two of them, *OPA1* (MIM 165500) and *OPA4* (MIM 605293), being dominant. Linkage analysis has revealed that *OPA1*, mapping to 3q28-q29, is the major locus (Bonneau et al. 1995; Brown et al. 1997; Eiberg et al. 1994; Lunkes et al. 1995; Johnston et al. 1997; Votruba et al. 1997; Votruba et al. 1998c), whereas so far only one family has been found to show the *OPA4* locus at 18q12.2-q12.3 (Kerrison et al. 1999). Recently, a novel dynamin-related protein has been identified in the fission yeast *Schizosaccharomyces pombe* that is essential for the maintenance of mitochondrial DNA and morphology of the mitochondria (Pelloquin et al. 1998; Pelloquin et al. 1999). We have found that the human orthologue of this protein, subsequently named *OPA1*, maps to 3q28-q29 and is mutated in DOA (Delettre et al. 2000). A positional

cloning approach similarly identified this gene as being responsible for OPA1-type DOA (Alexander et al. 2000). Like other members of the dynamin family, *OPA1* has conserved GTPase and central dynamin domains. However, it shares with the S. cerevisiae orthologue Mgm1 a basic N-terminal leader sequence required for its mitochondrial localization (Otsuga et al. 1998; Van der Bliek 1999). Labeling studies have indicated that *OPA1* is a component of the mitochondrial network and that the mitochondrial distribution is abnormal in some *OPA1* mutants (Delettre et al. 2000).

These findings raise a number of questions regarding the pathogenic mechanisms involved in DOA. What is the relationship of OPA1 with Leber's hereditary optic neuropathy, another mitochondrial disease due to mutations of the mitochondrial DNA coding for subunits of the respiratory complex 1 (Howell et al. 1997; Kerrison et al. 1995)? Why are retinal ganglion cells apparently vulnerable to some mitochondrial defects? What is the exact function of *OPA1*? As a prerequisite for further investigations, we screened a panel of 19 unrelated patients with DOA and found eight novel mutations, most of them being truncative ones. In addition, the analysis of leukocyte mRNA revealed eight *OPA1* transcripts resulting from the alternative splicing of exon 4 and two novel exons named 4b and 5b. This suggests that *OPA1* function, like that of other members of the dynamin family, may be highly modulated (Hinshaw 2000).

Materials and methods

Patients and DNA samples

Of 19 unrelated patients seen at various institutions, a majority (16) showed a familial history with autosomal dominant inheritance, and 5 of them were mapped using *OPA1* flanking markers as previously described (Delettre et al. 2000). Three additional patients were isolated cases. Two of them exhibited typical signs of DOA including progressive loss in visual acuity, temporal optic disc atrophy and tritanopia. For the last patient we had no clinical report.

We isolated genomic DNA from whole blood (anticoagulated with EDTA) with the informed consent of the patients using a standard salting-out procedure (Miller et al. 1988).

Gene mutation nomenclature used in this article follows the recommendations of den Dunnen and Antonarakis (2001). Gene symbols used in this article follow the recommendations of the HUGO Gene Nomenclature Committee (Povey et al. 2001).

Mutation analysis of the *OPA1* gene in families with DOA

The entire protein coding region and intron/exon boundaries of *OPA1* were amplified by PCR using primer pairs specific for the 28 previously described exons (Delettre et al. 2000). Exons 4b and 5b were amplified using primers 4b S 5′-GTGGTTGGACCAATTTGGTGGT-3′, 4b AS 5′-CCTAAAGGATAATGAGTTTGCC-3′, 5b S 5′-CATCTGTTCCTTTGTTGCACCC-3′ and 5b AS 5′-GGAGTCCATGAACAGATTGAGG-3′. PCR reactions were carried out in a 10-µl volume with 50 ng genomic DNA and the products purified using the Qiaquick purification kit (Qiagen, Valencia, Calif.). Sequencing was done on an ABI 310 capillary sequencer (Applied Biosystems, Foster City, Calif.) using a BigDye terminator kit (Applied Biosystems).

RNA extraction and reverse transcription (RT) PCR

Leukocytes were isolated from 20 ml heparinized blood. Total RNA was then extracted following the APCG single-step method (Chomczynski and Sacchi 1987) and purified on glass beads. Total RNA from two human retinas was extracted with an Rneasy kit (Qiagen, Valencia, Calif.) following the manufacturer's instructions. Total RNA from other tissues were obtained from Stratagene (La Jolla, Calif.). For the reverse transcription, 2 µg total RNA was mixed with 200 U SuperScript II reverse transcriptase (Invitrogen, Groningen, The Netherlands) and 150 ng random oligonucleotides (Promega, Madison, Wis.). As a control, reverse transcription was performed without reverse transcriptase. The *OPA1* coding sequence was then amplified in six overlapping cDNA fragments using the following primers: K1 S 5′-CACCCAGCTTATCTTGCAAGTG-3′, K1 AS 5′-AAAGCGCCCGTAACATACATCG-3′, K2 S 5′-AAACATCTACCTTCCAGCTGCG-3′, K2 AS 5′-TGGATCTACTTCTACTCCTCGG-3′, K3 S 5′-GTCAAATGGACCCTCATGGAAG-3′, K3 AS 5′-CCCAAGCAACCTCTACTGCTTT-3′, K4 S 5′-TGGAAATGATTGCCCAAGCTCG-3′, K4 AS 5′-CAATGCTTTCAGAGCTGTTCCC-3′, K5 S 5′-GGATTGTGCCTGACATTGTG-3′, K5 AS 5′-CACTCAGAGTCACCTTAACTGG-3′, K8 S 5′-CTGTGAGGTCTGCCAGTCTTTA-3′, K8 AS 5′-GCTTGTCACTTTCAGATCCACG-3′.

The conditions used for the PCR reaction were 94ºC for 3 min, 35 cycles of 94ºC for 30 s, 66ºC for 30 s and 72ºC for 1 min, and 72ºC for 10 min. For alternatively spliced transcripts, an upstream primer was used located in exon 3 (K5 S) and a downstream primer covering the boundary between exons 9 and 10 (K5 AS). All PCR products were separated by electrophoresis through 3% NuSieve GTG agarose (FMC Bioproducts, Rockland, Me.). A nested PCR reaction was performed to sequence alternatively spliced transcripts, the first PCR product being subjected to a second amplification using primers K5 S and K5b AS 5′-CTTGCGCTTCTGTTGGGCATA-3′. Four different fragments spanning exons 3 to 5b were obtained in this second PCR reaction. For sequencing, each amplified product was excised from the agarose gel.

For splice site mutations, RT-PCR was performed using primers E10 AS 5′-GGCTAACGGTACAGCCTTCTTT-3′ and 5S 5′-GTCTGCTTGGTGAGCTCATTCT-3′ that amplify a 621-bp fragment containing exons 6–10, and primers K1 AS and K1 S that amplify a 623-bp fragment containing exons 25–28.

Results

Identification of additional exons for *OPA1*

Starting from human RNA, we amplified by RT-PCR the full-length *OPA1* cDNA in several overlapping fragments. Use of primers encompassing exons 3 to 9/10 resulted in eight PCR products in all tissues tested including the human retina, while the absence of products from the direct amplification of total RNA (data not shown) excluded contamination by genomic DNA (Fig. 1). The products included the expected 597-bp fragment and seven additional fragments ranging from 489 bp to 762 bp, indicating the presence of *OPA1* mRNA isoforms.

The longest product (762 bp) contained a 54-bp insert between exons 4 and 5 and a 111-bp insert between exons 5 and 6, corresponding to parts of introns 4 and 5, respectively. These sequences did not alter the reading frame and possessed the consensus splicing signals conforming to the GT/AG rule (Breathnach and Chambon 1981). They thus represented novel alternatively spliced exons and were named 4b (Genbank AF416919) and 5b (Gen-

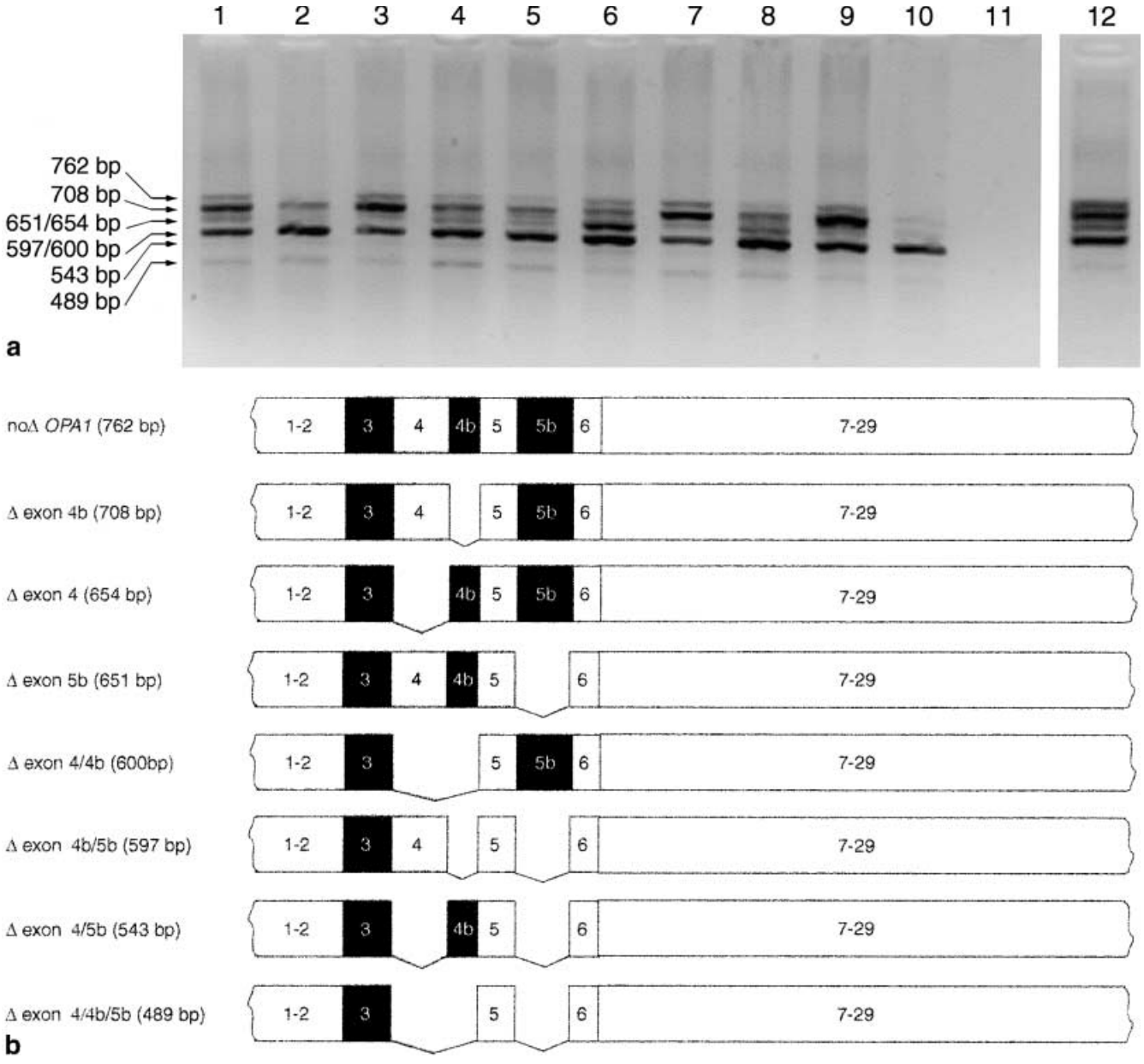

Fig. 1a, b Alternatively spliced exons 4, 4b and 5b. **a** RT-PCR analysis demonstrates multiple *OPA1* transcripts in human tissues. Ethidium bromide-stained agarose gel. Use of K5 S and K5 AS primers resulted in the amplification of six distinct fragments, the size of which is indicated on the left (products 597 bp and 600 bp as well as 651 bp and 654 bp are superimposed). RT-PCR was performed on human thyroid (*lane 1*), retina (*lane 2*), kidney (*lane 3*), lung (*lane 4*), ovary (*lane 5*), skeletal muscle (*lane 6*), liver (*lane 7*), heart (*lane 8*), colon (*lane 9*), fetal brain (*lane 10*), no RNA (*lane 11*) and leukocytes (*lane 12*). **b** Schematic representation of the structure of the eight alternative transcripts found by RT-PCR. The fragment sizes found in **a** and corresponding to each *OPA1* transcript are indicated

bank AF416920). Sequencing of the seven other fragments showed that they resulted from all possible combinations of the presence/absence of exons 4b and 5b as well as the presence/absence of exon 4. As for exons 4b and 5b, the subtraction of exon 4 did not alter the reading frame.

All eight transcripts were expressed in the 11 tissues tested although with some variation in their levels of expression (Fig. 1). In retina, fetal brain, heart, skeletal muscle, lung and ovary a combination of the transcripts 3/4/5/6 and 3/5/5b/6, 597 and 600 bp, respectively, showed the highest levels of expression, while in kidney, liver and colon the highest levels were observed for transcript 3/4/5/5b/6 (708 bp). Except in skeletal muscle, the combination of transcripts 3/4b/5/5b/6 and 3/4/4b/5/6 (654 bp and 651 bp) was slightly expressed. In all tissues, transcripts 3/5/6 and 3/4b/5/6 were weakly expressed.

Exons 4b and 5b encoded novel 18- and 37-amino acid sequences, respectively, with no significant homology to other proteins in the NCBI database. Secondary structure predictions for *OPA1* by the Coil program (Lupas et al. 1991) suggested two possible coiled-coil regions. Domains encoded by exons 6-7 and exons 27-28 were predicted to have a 100% and 95% probability of coiled-coil

formation, respectively. Analysis of the amino acid-deduced sequences of exons 4b and 5b revealed that exon 5b amino acids had a 90% probability of forming a coiled-coil structure. The addition of exon 5b could therefore modify the interaction of the dynamin coded by exon 6-7, just N-terminal to the GTPase domain.

Screening of DOA patients in *OPA1*

We screened 19 unrelated patients and found 17 mutations giving a detection rate of 89%. Of these, 15 were different (Table 1), of which 8 were novel and 7 had been previously described (Alexander et al. 2000; Delettre et al. 2000; Pesch et al. 2001; Toomes et al. 2001). They were distributed in the GTPase domain, the dynamin central region and the coiled-coil C-terminal domain. The mutations segregated with the disease in nine families for which additional members (45) were available and none was detected in a panel of 100 chromosomes from normal individuals.

Splicing mutations

One mutation affecting the 3′ splice site and four affecting the 5′ splice site were identified. The 3′ splicing mutation, c.985–1G>A, is recurrent (Delettre et al. 2000). In the family described in this study there was an asymptomatic carrier. We found by RT-PCR analysis that the mutation resulted in the skipping of exon 10 (not shown). Of mutations affecting 5′ splice sites, one was a change in the first nucleotide of intron 12 (c.1212+1G>T) and was found in two families; RNA was not available for transcript analysis. Another was a change in the fifth nucleotide of intron 8 (c.870+5G>A). Sequencing of the mutated transcript obtained by RT-PCR indicated skipping of exon 8 (not shown). The two other 5′ splicing mutations were substitutions of the last nucleotide of the upstream exon: one was in exon 9 (c.984G>A, family MT5) with no change in the encoded amino acid (Lys328Lys) and the other was in exon 26 (c.2707G>C, family PR6) leading to a conserved amino acid substitution (Val903Leu). Therefore, these two latter mutations are unlikely to have a pathogenic effect through the amino acid sequence. However, since guanine at position –1 belongs to the donor splice site consensus sequence (found in 77% of human genes), inefficient splicing with occurrence of an abnormal mRNA could be expected. Indeed, for both mutations we found two transcripts (Fig. 2). The longer one was not mutated while the shorter one lacked exon 9 for c.984G>A or exon 26 for c.2707G>C. Density measurement of the cDNA fragments indicated an equal proportion of normal and mutated cDNA for c.984G>A (ratio normal allele/mutated allele 1.07) while there was twice as much normal cDNA as mutated cDNA for c.2707G>C (ratio 2.0).

All five mutations would be expected to change the OPA1 protein. The lack of exons 8, 9 and 10 led to the deletion of 29, 38 and 27 amino acids from the GTPase domain, respectively, with no frameshift. Skipping exon 26 caused a frameshift resulting in the addition of three novel amino acids (NH_2-LCD-COOH) after codon 871 followed by a premature stop at codon 875 with the loss of the last 89 C-terminal amino acids (9.3% of the OPA1 protein). Finally, c.1212+1G>T was presumed to cause either retention of intron 12 with the addition of 18 novel amino acids (NH_2-VSIVKTCILFYCYCVKHL-COOH) followed by a stop or splicing at c.1212+5 with the addition of nine novel amino acids (NH_2-VNCDIRHGS-COOH) followed by a stop. In both cases, the last 556 C-terminal amino acids (57.9% of the OPA1 protein) were lost.

Table 1 *OPA1* mutations detected in this study

Nucleotide change	Amino acid change/effect on mRNA	Location	Protein domain	No. of families	Reference
c.794delTTGA	Frameshift Ile265	Exon 8	GTPase domain	1	Toomes et al. (2001)
c.869G>A	Arg290Gln	Exon 8	GTPase domain	1	Alexander et al. (2000); Toomes et al. (2001)
c.870+5G>A	Splice defect with skipping of exon 8	Intron 8	GTPase domain	1	Toomes et al. (2001)
c.984G>A	Splice defect with skipping of exon 9	Exon 9	GTPase domain	1	Novel
c.985–1G>A	Splice defect with skipping of exon 10	Intron 9	GTPase domain	1	Delettre et al. (2000)
c.1096C>T	Arg366STOP	Exon 11	GTPase domain	1	Alexander et al. (2000)
c.1212+1G>T	Splice defect with putative frameshift	Intron 12	GTPase domain	2	Novel
c.2029delC	Frameshift Leu677	Exon 21	Dynamin central region	1	Novel
c.2131C>T	Arg711STOP	Exon 21	Dynamin central region	1	Novel
c.2354A>G	Gln785Arg	Exon 23	Dynamin central region	1	Pesch et al. (2001)
c.2681insT	Frameshift Leu894	Exon 26	–	1	Novel
c.2707G>C	Splice defect with skipping of exon 26	Exon 26	–	1	Novel
c.2708delTTAG	Frameshift Val903	Exon 27	Coiled-coil region	2	Delettre et al. (2000); Pesch et al. (2001); Toomes et al. (2001)
c.2816T>C	Leu939Pro	Exon 27	Coiled-coil region	1	Novel
c.2855insT	Frameshift Phe952	Exon 28	Coiled-coil region	1	Novel

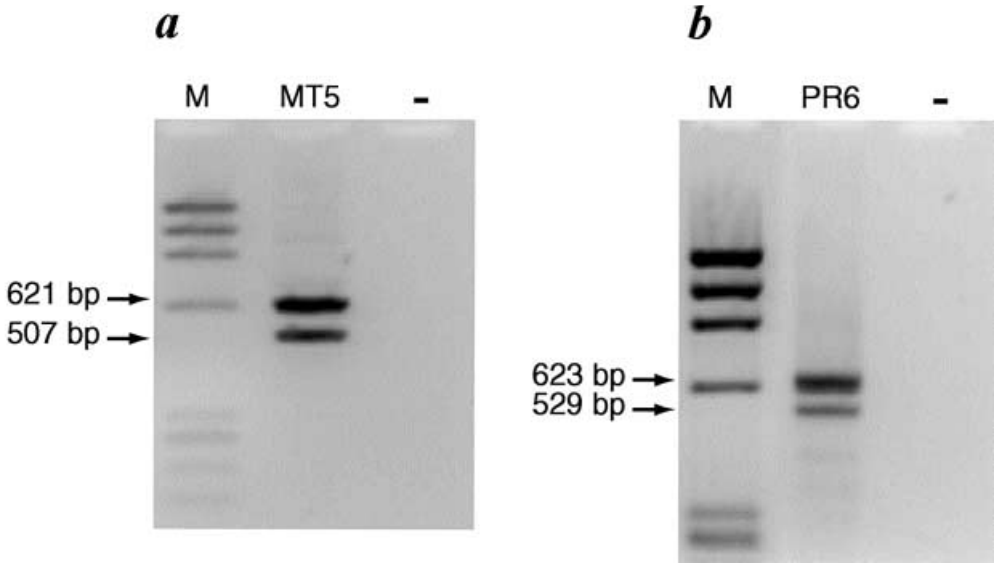

Fig. 2a, b Effects of the mutation of the last nucleotide of exon 9 and exon 26 on the splicing of *OPA1* mRNA. Ethidium bromide-agarose gel showing the RT-PCR products from leukocyte mRNA of patients MT5 (**a**) and PR6 (**b**). As a control PCR was performed directly on RNA (–). The longest fragments (621 bp in MT5 and 623 bp in PR6) were derived from the normal transcripts while the shortest fragments were from the mutant transcript (507 bp in MT5, 529 bp in PR6) indicating skipping of exon 9 (MT5) or 26 (PR6). (*M* HaeIII ϕX174 DNA marker)

Deletion/insertion mutations

In exon 8 we found a 4-bp deletion, c.794delTTGA, that caused changes in the 41 amino acids from codons 265 to 305 and a premature stop at codon 306. This mutation led to the loss of 68.2% of the C-terminal part of the protein, including most of the GTPase domain. We found another deletion in exon 21, c.2029delC, resulting in a frameshift after codon 676 replacing eight amino acids (NH_2-YKKNFPAL-COOH) before a stop at codon 685 with the loss of 29.6% of the C-terminal part of the protein. Two novel insertions were identified. One in exon 26, c.2681insT, that caused the substitution of eight amino acids from codons 894 to 901 (NH_2-FKATTYKY-COOH) before a stop at codon 902 with the loss of 7.0% of the C-terminal part of the protein, and the other in exon 28, c.2853insT, causing substitution of one amino acid (Ile953His) followed by a stop at codon 954 with the loss of the last seven C-terminal amino acids. We also found two families with the c.2708delTTAG mutation that we had previously described (Delettre et al. 2000).

Nonsense mutations

One novel nonsense mutation was detected in exon 21, Arg711Stop, resulting from a C>T transition at position c.2131. This mutation led to the loss of 26.0% of the C-terminal part of the protein. In one patient we also found the previously described c.1096C>T (Arg366Stop) mutation (Alexander et al. 2000).

a **Gln785Arg**

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Human OPA1          TENAIENMVGPDWKKRWLYWKNRTQEQCVHNETKNELEKMLKCNEEHPAYLAS
Rat RN protein      TENAIENMIGPDWKKRWLYWKNRTQEQCVHNETKNELEKMLKVNDEHPAYLAS
Salmon T00394       TDSVINDMVGPDWKQRWMSWKNRSPEQHTRNETRNELERLLKLHEDHTAYLAN
Drosophila CG8479   TEETLAQMFGPGQMRRITHWQYLTQDQCKRRSVKNELDKILKNDTKHLPTLTH
Yeast Msp1          VENCVKPYKY-DVEVNDDEWK—SSRGQA-----EKLLQRELGLCQSALEKIKN
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b **Leu939Pro**

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Human OPA1          KNVKEVLEDFAEDGEKKIKLLTGKRVQLAEDLKKVREIQEKLDAFIEALHQEK
Rat RN protein      KNVKEVLEDFAEDGEKKVKLLTGKRVQLAEDLKKVREIQEKLDAFIEALHQEK
Salmon T00394       KNVKEVLEDFGEDNERKVHLITGRRVQLAEDLKKVREIQEKLEAFIEALHKEK
Drosophila CG8479   KEIKAVLDEFSDDEEKKGYLLTGKRVLLAEELIKVRQIQEKLEEFINSLNQEK
Yeast Msp1          RELDQRLIHSLSSEQLNAFVNENPLLKSQLQLQHKRQCLELALQKINSLVILE
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Fig. 3a, b *OPA1* missense mutations involved conserved amino acids. Protein alignment depicting the amino acid sequences in part of the dynamin central region (**a**) and of the coiled-coil region (**b**) of human OPA1 and orthologs including the rat RN protein (AAB51724), cherry salmon GTP-binding protein (T00394), CG8479 *Drosophila melanogaster* (AAF58275) and yeast *S. pombe* Msp1 (NP011542). Glu785Arg (**a**) was from family PR2; Leu939Pro (**b**) was from family ST1

Table 2 Polymorphisms in the *OPA1* gene

Location	Nucleotide change	Alteration in polypeptide	Frequency
Exon 3	c.420G>T	Val140Val	G 98%, T 2%
Exon 4	c.473A>G	Asn158Ser	A 39%, G 61%
Intron 4	c.556+178G>T	Intronic	–
Intron 4b	c.557–19T>C	Intronic	–
Intron 8	c.870+4T>C	Intronic	–
Intron 8	c.870+32T>C	Intronic	–
Exon 11	c.1096C>A	Arg366Arg	C 97%, A 3%
Intron 14	c.1443+23G>A	Intronic	–
Intron 14	c.1444–75T>C	Intronic	–
Intron 18	c.1770+16T>G	Intronic	–
Intron 18	c.1770+51T>G	Intronic	–
Exon 21	c.2109C>T	Ala703Ala	C 43%, T 57%
Intron 21	c.2166+115G>T	Intronic	–
Intron 21	c.2167–83T>G	Intronic	–
Intron 23	c.2355+16T>A	Intronic	–
Intron 23	c.2355–158delG	Intronic	–
Intron 26	c.2707+25T>A	Intronic	–
Exon 27	c.2715A>G	Arg905Arg	A 98.5%, T 1.5%

Missense mutations

Three missense mutations were identified. One of them, Arg290Gln in exon 8, has been described previously (Alexander et al. 2000). The two others, Gln785Arg in exon 23 and Leu939Pro in exon 27, were novel. The substituted amino acids were conserved in the OPA1 proteins from human and yeast species (Fig. 3), suggesting that these changes may impair the function of the protein.

Polymorphisms

We identified 18 polymorphisms, most of them in the intronic sequences (Table 2).

Discussion

The focus of this study was to analyze the mutation spectrum of *OPA1*. By screening the cDNAs from human leukocytes, we identified two novel exons (4b and 5b) which, together with exon 4, were alternatively spliced and defined eight mRNA isoforms. We described eight novel mutations in *OPA1* that were predominantly truncative.

We demonstrated that *OPA1*, like other dynamin and dynamin-related protein transcripts, exhibits splicing variations. At least 36 variants have been described in mammalian tissues in the large dynamin family (Cao et al. 1998; McNiven et al. 2000). Members of this family such as dynamin-2 (Dyn2), dynamin-like protein-1 (DLP1) and yeast vacuolar protein-sorting defect (Vps1) have variable N-terminal regions flanking the GTPase domain or the pleckstrin homology and the GTPase-effector domains (McNiven et al. 2000). Dynamins such as Dyn1 and Dyn3 also have splicing variants in the C-terminal domain. *OPA1* has splicing variants in the region N-terminal to the GTPase domain but lacks variants in the central dynamin and C-terminal domains. Its splicing region corresponds to a part of the protein with unknown function located between the mitochondrial leader sequence and the GTPase domain. Distinct cytoplasmic distribution and developmental expression have been described in some splicing variants of Dyn1, Dyn2 and Dyn3 (Cao et al. 1998). Whether this is also the case for *OPA1* will have to be determined.

The biochemical properties of these splicing variants remain unknown. In the OPA1 protein, the largest part of exon 5b showed a high probability of coiled-coil formation, as did exon 6. Given that coiled-coil motifs are involved in self-assembly of dynamins (Okamoto et al. 1999) and assuming that regions spanning exons 5b to 6 in *OPA1* show this structure, the addition of exon 5b may strengthen the protein-protein interactions or modulate the specificity of these interactions as, for example, in agrin in which a 4-amino acid insertion creates a specific heparin-binding site (Gesemann et al. 1996).

Variations in tissue expression of *OPA1* mRNA splicing isoforms were seen. There was a large predominance of PCR products mixing 3/4/5/6 and 3/5/5b/6 variants in retina, fetal brain and heart, and to a lesser extent in skeletal muscle, lung and ovary. This expression pattern was in contrast to that of liver, kidney and colon where the longest transcripts 3/4/5/5b/6 were predominantly expressed. Results from sequencing indicated that in the retina, the 3/4/5/6 variant was more abundant than the 3/5/5b/6 variant. Northern blots also show multiple transcripts (Alexander et al. 2000; Delettre et al. 2000) which likely result from alternative polyadenylation sites. It is interesting to note that the highest levels of expression in Northern blots are found in retina, brain, testis, heart and muscle, a distribution which roughly corresponds to that of the 3/4/5/6 and 3/5/5b/6 variants. These tissues are those that are usually involved in mitochondrial dysfunction.

In this study, we screened by direct sequencing the entire protein-coding region including exons 4b and 5b in 19 unrelated DOA patients. The diagnosis of DOA was considered as certain in 18 of them. We found mutations in 17 (89%) of these patients, indicating that *OPA1* is the major gene of the dominant form of non-syndromic optic atrophy. The lower detection rate (32.1% and 57%) found by Pesch et al. (2001) and Toomes et al. (2001), respectively, reflects the use of SSCP/heteroduplex analysis instead of direct sequencing and suggests that patients with other types of optic atrophy were included. In our study, the two patients with no detected mutation (an isolated case and a nuclear family with autosomal dominant inheritance) may belong to families mapping to *OPA4*, the second locus for DOA on chromosome 18q12 (Kerrison et al. 1999), or may have had a change in regulatory regions (promoter, untranslated regions and intronic sequences) or a large deletion that would not have been detected by our PCR-based approach. This latter hypothesis is particularly relevant to *OPA1* for which haplotype insufficiency could be a disease mechanism (see below).

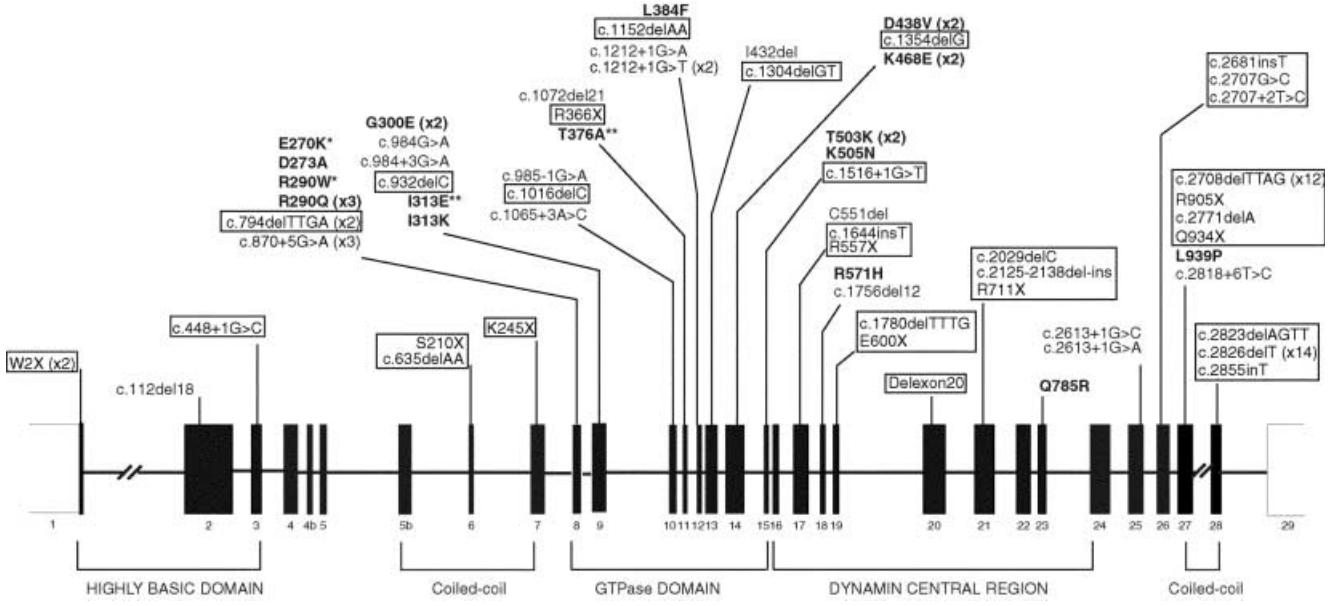

Fig. 4 *OPA1* mutations. Structure of human *OPA1* (black boxes and numbers depicting exons) and positions of mutations identified in DOA patients. Missense mutations are in bold, and truncative mutations that cause loss of a C-terminal part of the protein are boxed. In-frame deletions either by deletion of nucleotides or by exon skipping resulting from splicing mutations are in plain text. The numbers in parentheses indicate the frequency. Exons encoding the different domains of the protein are bracketed. *Mutations segregating in one family with a compound heterozygosity; **mutations segregating in the same family

To date 62 *OPA1* mutations have been identified (Fig. 4) in 201 patients with DOA (Alexander et al. 2000; Delettre et al. 2000; Pesch et al. 2001; Thiselton et al. 2001; Toomes et al. 2001). We report here 15 mutations (8 novel and 7 previously described), 9 (60%) of them being truncative (leading to a stop codon) and 3 (20%) being in-frame deletions. Among the mutations described so far, half are truncative and 24% are in frame-deletions, suggesting that haplotype insufficiency may be a disease mechanism in *OPA1* DOA. A few mutations were present in the first seven exons of the gene compared with downstream regions. With regard to this question, it is interesting to note that mutations in the spliced exons 4, 4b and 5b may not generate a phenotype, since other isoforms can compensate for the mutated one. RT-PCR analysis of splicing mutations in families MT5 (c.984G>A) and PR6 (c.2707G>C), showed that the mutated allele for the truncative mutation c.2707G>C was downregulated compared with the normal allele while for the in-frame deletion c.984G>A, there was an equal proportion of mutated and normal alleles, suggesting a regulation of the truncated transcript, either at the splicing level or by faster degradation.

Missense mutations account for 26% (20% in this study) and are predominantly located in the GTPase domain which contains 81% of them. These mutations change highly conserved amino acids, presumably critical for the GTPase activity of *OPA1*. In Dyn1 protein, it has been shown that three domains (GTPase, central and coiled-coil C-terminal regions) are involved in intra- and intermolecular interactions (Okamoto et al. 1999; Smirnova et al. 1999). The GTPase cycle controls these interactions and drives the release and subsequent binding to adjacent molecules. Therefore, impairment of GTPase activity would not only lead to modification of GTPase function, but also to alteration of specific interactions of *OPA1* with itself and other proteins.

Of particular interest is the fact that the most downstream mutation found (c.2855insT) caused the loss of the last seven C-terminal amino acids with a full disease phenotype. It is possible that a number of truncative mutations located at the last three exons of the gene, and representing 35% of all described families, only act by the loss of the last seven amino acids. In vitro studies with recombinant proteins lacking either one of these seven amino acids or a portion the C-terminus while keeping the last seven amino acids are underway to address this question.

Overall, these results (modification of GTPase activity and possible alterations in protein-protein interactions) suggest that there may be various pathogenic mechanisms in play in *OPA1* DOA. Putative dimerization (in C-terminus) and trimerization (N-terminal to the GTPase domain) domains also indicate that a dominant negative effect in some cases is possible, although haplotype insufficiency is more probable in many cases. Future in vitro and in vivo studies will try to clarify these questions.

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