Nuclear gene *OPA1*, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy

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Optic atrophy type 1 (OPA1, MIM 165500) is a dominantly inherited optic neuropathy occurring in 1 in 50,000 individuals[1–3] that features progressive loss in visual acuity leading, in many cases, to legal blindness[4–8]. Phenotypic variations[5] and loss of retinal ganglion cells[9,10], as found in Leber hereditary optic neuropathy (LHON), have suggested possible mitochondrial impairment[11,12]. The OPA1 gene has been localized to 3q28–q29 (refs 13–19). We describe here a nuclear gene, *OPA1*, that maps within the candidate region and encodes a dynamin-related protein localized to mitochondria. We found four different *OPA1* mutations, including frameshift and missense mutations, to segregate with the disease, demonstrating a role for mitochondria in retinal ganglion cell pathophysiology.

We recently identified in *Schizosaccharomyces pombe* a new dynamin-related protein, Msp1, essential for the maintenance of mitochondrial DNA (refs 20,21), as is Mgm1p, its orthologue in *Saccharomyces cerevisiae*[22]. Both proteins have a GTPase and a central dynamin domain conserved among all dynamins[23] and a highly basic amino-terminal domain required for mitochondrial localization[21]. We searched for a human Msp1/Mgm1p homologue in nucleotide databases and found a 5,821-bp cDNA from brain[24] encoding an unknown protein (KIAA0567) whose deduced amino-acid sequence was similar to Msp1 (19% identity) and Mgm1p (17% identity). Sequence and structural similarities between this 960–amino-acid polypeptide and yeast Msp1 and Mgm1 proteins suggest that KIAA0567 is the human orthologue of Msp1. We then retrieved the sequence of the gene, hereafter called *OPA1*, in two overlapping HTG clones from the Whitehead Institute server. *OPA1* spans more than 69 kb and is composed of 29 exons (Fig. 1*a*). We subsequently localized *OPA1* by fluorescence *in situ* hybridization (FISH) on 3q28–29 (Fig. 1*b*).

To test whether OPA1 protein was targeted to mitochondria, we expressed it as a haemagglutinin (HA)-tagged peptide in HeLa cells and compared its expression with that of the mitochondrial network marker Hsp60. Labelling with anti-HA antibodies was coincident to that of Hsp60 in transfected cells (Fig. 2*a*), indicating that OPA1 is a component of the mitochondrial network. Mitochondrial localization of OPA1 was also consistent with the ubiquitous expression of its transcripts in all human tissues examined by northern blot (Fig. 2*b*). Localization on 3q28–29 together with a potential role in mitochondria function made this gene a candidate for OPA1-type dominant optic atrophy (DOA).

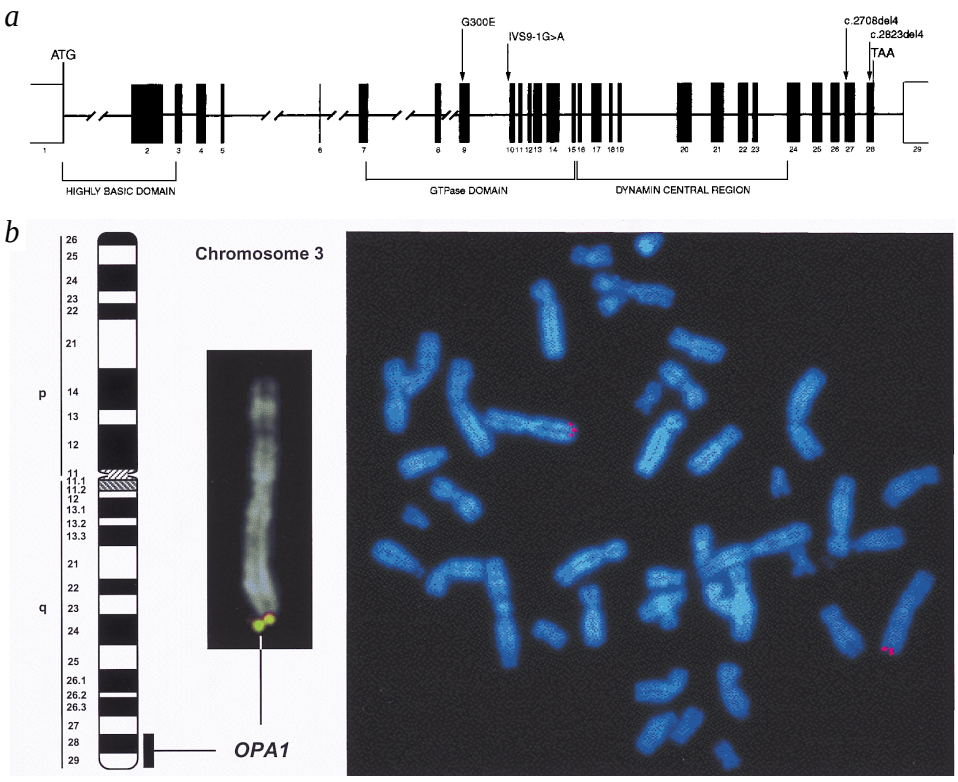

Fig. 1 *OPA1* structure and mapping by FISH. ***a***, Location and orientation of the 29 *OPA1* exons. The portions of the gene that are not shaded in black are untranslated as predicted from the cDNA sequence. Only the 5′ part of the non-coding, 2,878-bp exon 29 is shown. The 5′ boundary of mostly non-coding exon 1 is undetermined. ***b***, FISH with full-length *OPA1* cDNA reveals a double signal on DAPI-stained metaphasic chromosomes (right) and on unstained chromosome 3 (left), indicating its localization to 3q28–29. Diagram depicting human chromosome 3 is shown.

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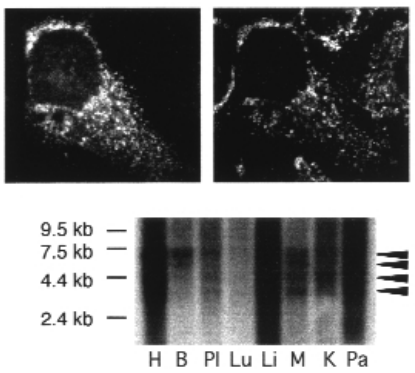

Fig. 2 OPA1 belongs to the mitochondrial network and is expressed ubiquitously. ***a***, HeLa cells were transfected with haemagglutinin (HA)-tagged OPA1 and incubated with anti-HA (left) or anti-HSP60 (right) antibodies. OPA1 reactivity seen in one transfected cell (left) co-localizes with mitochondrial network marker HSP60 (right). Note that surrounding cells visible in the photograph on the right were not transfected. ***b***, Northern blot containing poly(A)$^+$ mRNA from heart (H), brain (B), placenta (Pl), lung (Lu), liver (Li), muscle (M), kidney (K) and pancreas (Pa) was hybridized to a cDNA fragment that spanned the *OPA1* ORF. Four transcripts (arrows) of approximately 4, 5, 6 and 7 kb are visible and exhibit various relative abundances. The sizes of RNA molecular weight standards are shown.

We screened the probands and relatives from six unrelated families with DOA, Kjer's type. We mapped three families (PR1-3) by two-point linkage analysis using two polymorphic markers (centromere-*D3S1601*-*D3S2748*-telomere) that flank the *OPA1* locus. Combined lod scores for the 3 families at each marker ranged from 3.45 to 5.01 for various θ values (Table 1), supporting the contention that the disease gene in these families maps within the *OPA1* locus.

For each proband, we examined the entire coding sequence by direct sequencing of PCR-amplified exons. We identified 4 different mutations in the heterozygous state that were not found in 100 control chromosomes (Fig. 3*b*). Mutations segregated with the disease phenotype, except in family MT3, in which one individual was asymptomatic. In exon 9, we found one missense mutation in family PR2, c.899G→A, that changes glycine to glutamic acid at codon 300. Another mutation in the last nucleotide of intron 9 (IVS9-1G→A) that abolishes the acceptor splice site was found in family PR4. This mutation results in either skipping of exon 10 with no frameshift in the following exon 11 or a frameshift with a premature stop at first codon of exon 10 (329) if the newly ectopic acceptor splice site 1-bp downstream of the original is functional. In exon 27, we found in 3 families (MT1, MT2 and MT3) a 4-bp deletion, c.2708delTTAG, that causes 2 amino-acid substitutions (Val903Gly, Arg904Asp) and a premature stop at codon 905. This mutation was present in asymptomatic carrier II-2 from family MT3, but was fully penetrant in families MT1 and MT2. This was expected, as minimally affected patients

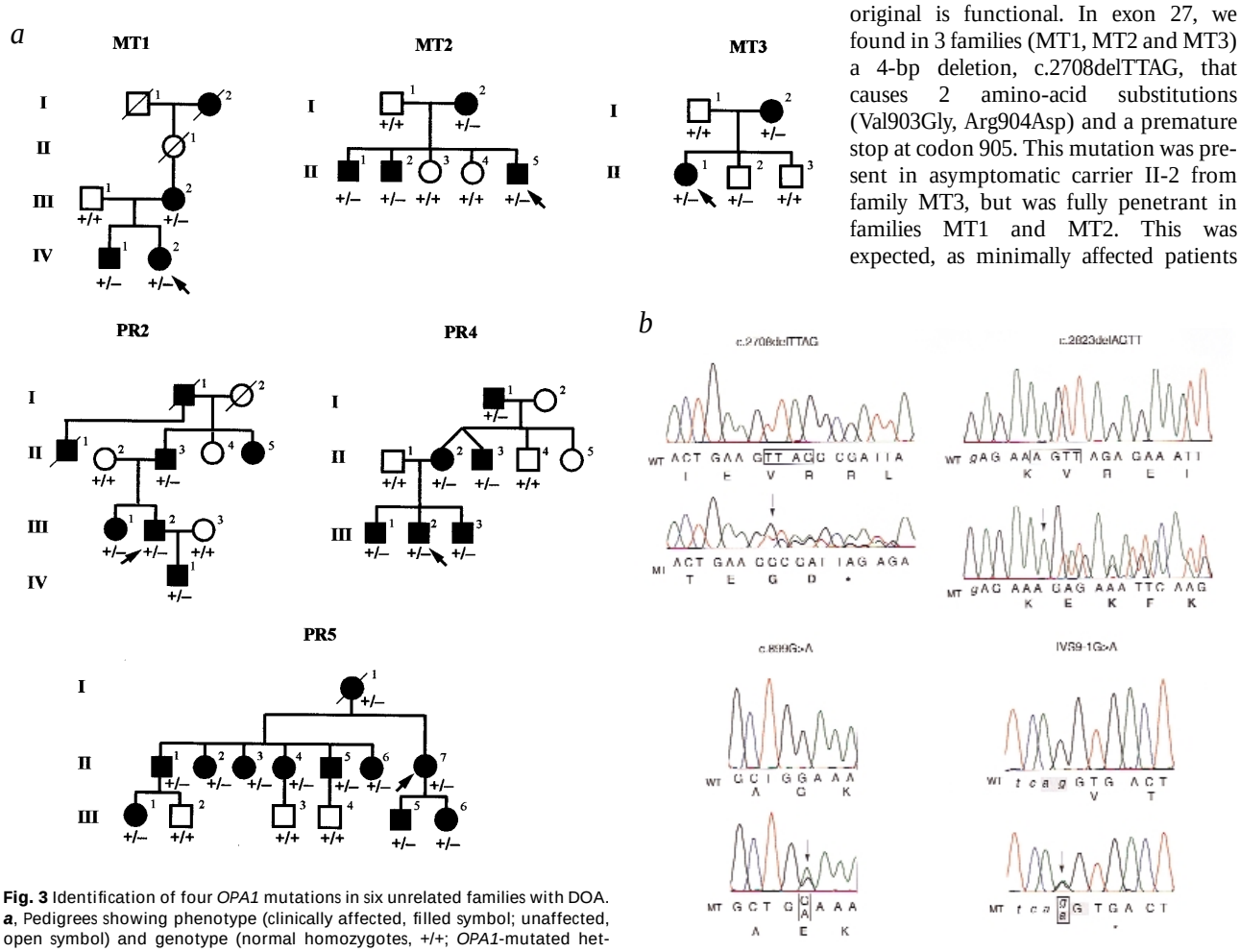

Fig. 3 Identification of four *OPA1* mutations in six unrelated families with DOA. ***a***, Pedigrees showing phenotype (clinically affected, filled symbol; unaffected, open symbol) and genotype (normal homozygotes, +/+; *OPA1*-mutated heterozygotes, +/–) of living family members. Arrows indicate probands. ***b***, Electropherograms of *OPA1* cDNA sequencing showing wild-type (WT, top) and mutant (MT, bottom) sequences containing the four mutations (arrowhead) found in the six DOA families. Boxed nucleotides (top) indicate deleted nucleotides found in mutated alleles containing c.2708del4 and c.2823del4. Wild-type (normal setting) and changed (bold face) deduced amino acids are shown. ***c***, Amino acid alignments of G1 and G2 motifs in the GTPase domain of representative dynamin family members. Only a short segment of the GTPase domain is shown. A conserved glycine at position 300 that belongs to the G1 motif and is mutated in DOA is indicated (*).

Table 1 • Pairwise lod scores between *OPA1* and two polymorphic DNA markers on chromosome 3q

Penetrance = 75%		Recombination fraction (θ)		
Locus	Pedigree	0	0.05	0.10
D3S1601	PR2	0.60	0.54	0.47
	PR4	1.41	1.28	1.15
	PR5	3.00	2.72	2.43
	Total	5.01	4.54	4.05
D3S2748	PR2	0.60	0.54	0.47
	PR4	NI	NI	NI
	PR5	3.58	3.29	2.98
	Total	4.18	3.83	3.45

and asymptomatic carriers (with no detectable optic atrophy in fundus) have been described in DOA (refs 5,19). Moreover, mutation c.2708delTTAG was absent from an additional 200 control chromosomes. In family PR5, we found a second 4-bp deletion in exon 28 (c.2823delAGTT) that causes a delayed stop, downstream of the original one, resulting in the replacement of the last 19 amino acids of the protein with 24 novel amino acids at the carboxy terminus (NH_2-EKFKKNLMLSLKLFIRRNKLKSYS-COOH). We also identified three other sequence changes that are probably polymorphisms. These were the missense changes Asn158Ser (c.473A→G) and Glu907Gly (c.2720A→G), which do not segregate with the disease and the silent variation Ala703Ala (c.2109C→T).

The presence of one mutation, c.2708delTTAG, found in three of six apparently unrelated families suggests that some families may have common ancestors. Founder effect in the British Isles has been reported from haplotype studies[8,19]. The affected ascendants from the three families with c.2708delTTAG all originated from northern French provinces and Belgium.

The mutations reported here are likely to impair OPA1 function. Glycine at codon 300, located in the G1 motif of the GTPase domain, is conserved among all members of the dynamin super family including dynamins, dynamin-related proteins from Dnm1/Drp1 and Mgm1/Msp1 families, and several prokaryotic proteins such as Yjda, an *Escherichia coli* protein of unknown function thought to be distantly related to dynamins[23] (Fig. 3*c*). In dynamins, GTP promotes the disassembly of molecular structures called spirals that might be important in vesicle formation. The substitution of glycine 300 with glutamic acid or the skip of exon 10 will therefore probably compromise the GTPase activity in a similar function of OPA1. Frameshift mutation c.2708del4 encodes a truncated protein lacking the 56 C-terminal amino acids, accounting for 5.82% loss of the original protein. Although non-truncating, frameshift mutation c.2823del4 might have similar consequences because of the new 24–amino-acid C terminus. To examine the pathophysiologic effect of these C-terminal changes, we labelled monocytes of patients with mutation c.2708del4 (MT1 family) with anti-HSP60 antibodies (Fig. 4). Although the mitochondrial network of the age-matched control was finely homogenous throughout the cell, that of patient III-2 was heterogenous, with clumps in discrete areas of the cytoplasm (Fig. 4). Mitochondrial network of patient IV-1, who was less severely affected than his mother (III-2), also showed a heterogenous appearance, although milder. This indicates that the distribution of mitochondria in these patients is abnormal, a phenomenon that was also described in transfection experiments of another dynamin-related protein, human DRP1 (ref. 25). Therefore, alteration in *OPA1* may cause impairment in integrity and/or function of the mitochondria as observed in yeast, in which loss of function affects the maintenance of mitochondrial DNA and leads to respiratory deficiency[20].

It is plausible that *OPA1* defects in DOA share some pathogenic characteristics with LHON, in which there are decreased activities of mitochondrial respiratory complexes I, III and IV, and loss of retinal ganglion cells[11,12]. Future experiments based on comparison of wild-type and mutated *OPA1* will show whether this is the case in human retinal cells. In addition, it will be important, for therapeutic prospects, to identify the putative factors that may interfere with the pathogenic mechanism in a protective manner in asymptomatic carriers. Finally, the finding that *OPA1*-type DOA, as LHON, is caused by impairment of a mitochondrial protein is relevant to other forms of non-syndromic optic atrophy. This is the case for the dominantly inherited *OPA4*, linked to 18q12.2–q12.3, in which the currently unknown gene might encode another mitochondrial protein[26–28].

Methods

Patients and families. We followed six families in outpatient clinics. All had familial histories of DOA. Most patients exhibited typical signs of DOA (also called Kjer disease[1,2]), including progressive loss of visual acuity since childhood, temporal optic disc atrophy (as shown in Fig. 5), tritanopia and centrocoecal scotoma. There were intrafamilial variations in the severity of the disease with visual acuity ranging from 1/10 to 9/10, and optic disc

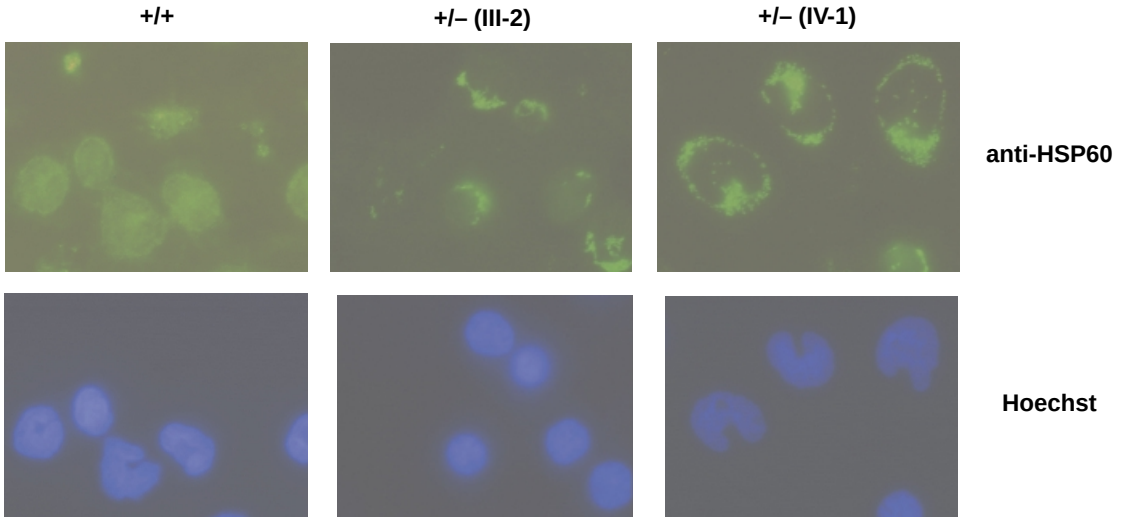

Fig. 4 Mitochondrial network is disorganized in *OPA1* mutants. Monocytes from a normal individual (+/+) and from c.2804del4 members from family MT1 (+/–) are shown, including those of patient III-2 (severely affected) and patient IV-1 (minimal disease), were incubated with anti-HSP60, a marker of the mitochondrial network, and Hoechst. Clumps of mitochondria are present in both patients.

Fig. 5 Fundus photograph of right eye of 29-year-old patient II-5 from family MT2 showing the macular area and the optic disc with retinal vessels converging to it. Atrophic temporal side of the optic disc (proximal to centre of photograph) is white and pale, whereas the nasal side remains yellow-orange.

atrophy that varied from full disc atrophy to normal disc appearance. In family MT3, patient I-2 had asymmetrical involvement and patient III-2 was asymptomatic, whereas patient II-1 was severely affected with visual acuity score at 2.5/10.

Amplification and sequencing of PCR products. We isolated genomic DNA from whole blood (anti coagulated with EDTA) with the informed consent of the patients using a standard salting out procedure[29]. We amplified DNA with *Taq* DNA polymerase (Roche) using intronic primer pairs representing each exon (primer sequences are available on request). Purified PCR products (15–40 ng) were cycle-sequenced using BigDye terminator system (Perkin Elmer), and analysed on an ABI Prism 310 capillary sequencer (Perkin Elmer). We performed screening of control chromosomes by SSCA of PCR products on a Hydrolink MDE gel (FMC Bioproducts) and revealed by silver staining.

In situ hybridization. We labelled plasmid DNA from clone AB011139 by nick-translation using DIG-11-dUTP (Life Technologies) and hybridized to human metaphase chromosomes as described.

Northern-blot analysis. We hybridized a blot (Clontech) containing 2 μg of poly(A)$^+$ mRNA from 8 different tissues with the ^{32}P-labelled OPA1 cDNA (coding sequence only) as a probe. Final wash was at 65 °C for 20 min in 0.1×SSC, 0.1% SDS.

OPA1 *in vitro* expression. We transfected HeLa cells with the pCDNA3-OPA1-HA-6HIS plasmid that directs expression of OPA1 fused to a haemagglutin-histidine tag. After 36 h of incubation, cells were fixed in 3.7% formaldehyde in PBS for 20 min at 4 °C, washed in PBS, permeabilized by 100% methanol at –20 °C for 10 min followed by 0.25% Triton X-100 in PBS for 5 min and finally washed in PBS. We then incubated cells with primary anti-HA (1/700, Roche), anti-Hsp60 (1/50, LK2, Sigma) and secondary anti-rabbit Alexa 594 (1/500) and anti-mouse Alexa 488 (1/500) antibodies (Molecular Probes).

Monocytes mitochondrial network characterization. We isolated white blood cells by Dextran sedimentation and centrifugation using Ficoll-Hypaque as described[30]. The interface band containing monocytes was then transferred in 24-well tissue culture plates and incubated for 1 h at 37 °C. Non-adherent cells were washed off and adherent monocytes were then fixed and processed for anti-Hsp60 immunolabelling as described above. Nuclear counterstaining of the cells was obtained by a 10-min incubation in Hoechst (1 μg/ml) in PBS.

GenBank accession numbers. *Homo sapiens* dynamin-1 cDNA, L07807; *H. sapiens DRP1* cDNA, AB006965; *H. sapiens* HTG clones for *OPA1*, AC023594 and AC025847; *H. sapiens OPA1* cDNA, AB011139; *S. pombe* Msp1 cDNA, Y07891; *S. cerevisiae MGM1* cDNA, Z75119; *E. coli* Yjda cDNA, J05620.

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