A Comprehensive Survey of Mutations in the *OPA1* Gene in Patients with Autosomal Dominant Optic Atrophy

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Purpose. To characterize the spectrum of mutations in the *OPA1* gene in a large international panel of patients with autosomal dominant optic atrophy (adOA), to improve understanding of the range of functional deficits attributable to sequence variants in this gene, and to assess any genotype–phenotype correlations.

Methods. All 28 coding exons of *OPA1*, intron–exon splice sites, 273 bp 5′ to exon 1, and two intronic regions with putative function were screened in 94 apparently unrelated white patients of European origin with adOA by single-strand conformational polymorphism (SSCP)–heteroduplex analysis and direct sequencing. Clinical data were collated, and putative mutations were tested for segregation in the respective families by SSCP analysis or direct sequencing and in 100 control chromosomes. Further characterization of selected splice site mutations was performed by RT-PCR of patient leukocyte RNA. Staining of mitochondria in leukocytes of patients and control subjects was undertaken to assess gross differences in morphology and cellular distribution.

Results. Twenty different mutations were detected, of which 14 were novel disease mutations (missense, nonsense, deletion-frameshift, and splice site alterations) and six were known mutations. Mutations were found in 44 (47%) of the 94 families included in the study. Ten new polymorphisms in the *OPA1* gene were also identified. Mutations occur throughout the gene, with three clusters emerging: in the mitochondrial leader, in the highly conserved guanosine triphosphate (GTP)-binding domain, and in the −COOH terminus. Examination of leukocyte mitochondria from two unrelated patients with splice site mutations in *OPA1* revealed no abnormalities of morphology or cellular distribution when compared with control individuals.

Conclusions. This study describes 14 novel mutations in the *OPA1* gene in patients with adOA, bringing the total number so far reported to 54. It is likely that many cases of adOA are due to mutations outside the coding region of *OPA1* or to large-scale rearrangements. Evaluation of the mutation spectrum indicates more than one pathophysiological mechanism for adOA. Preliminary data suggests that phenotype-genotype correlation is complex, implying a role for other genetic modifying or environmental factors. No evidence was found of pathologic changes in leukocyte mitochondria of patients with adOA. (*Invest Ophthalmol Vis Sci.* 2002;43:1715–1724)

Autosomal dominant optic atrophy (adOA) is the commonest hereditary optic neuropathy with an estimated disease prevalence of between 1 in 10,000[1] and 1 in 50,000.[2] Key clinical features of the disorder are progressive loss of visual acuity, beginning in the first two decades of life, color vision disturbances, paracentral scotomas and bilateral temporal atrophy of the optic nerve.[3,4] Histologic examination of donor eyes suggests that the fundamental pathologic characteristic of adOA is primary degeneration of retinal ganglion cells followed by ascending atrophy of the optic nerve.[5,6] The disease displays great variability in phenotypic expression, both between and within families.[7] The disease was originally reported to show penetrance of greater than 90%,[8,9] but more recent studies suggest that the penetrance may in fact be as low as 43%.[10] Although one adOA pedigree has been reported that maps to chromosome 18q12.2-12.3 (*OPA4*),[11] the predominant genetic locus for this disorder (*OPA1*: Mendelian Inheritance in Man [MIM] 165500) has been mapped to a 1.4-cM interval on chromosome 3q28-q29.[12,13]

The *OPA1* gene has been identified recently from within this interval and has been shown to harbor mutations in patients with adOA.[14,15] The *OPA1* mRNA (GenBank accession AB011139; GenBank is provided in the public domain by the National Center for Biotechnology Information [NCBI], Bethesda, MD, and is available at http://www.ncbi.nlm.nih.gov/genbank/) is 5821 bp long and is split into 29 exons spanning more than 60 kb of genomic DNA. The first 28 exons of the gene encode a dynamin-related large guanosine triphosphatase (GTPase) of 960 amino acids.[14,15] Although the cellular role of OPA1 is unknown, putative functional information has been derived from in silico analysis of the primary protein sequence, which reveals a mitochondrial import motif also observed in dynamin-related large GTPases from other species that are

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most likely orthologues of OPA1.[14,15] Studies in yeast have demonstrated that Dnm1,[16] Mgm1,[17] and Msp1[18] play an important role in the dynamics of mitochondrial morphology and the fusion–fission processes that occur continually throughout the life of a cell. Of the yeast proteins, OPA1 shows greatest similarity (31%–33% over 236 amino acids [aa]) at the primary level to Mgm1 (*Saccharomyces cerevisiae*) and Msp1 (*Schizosaccharomyces pombe*), the precise functions of which are unknown.[18] It is likely that OPA1 is involved in a similar mitochondrial fusion–fission pathway in humans.

Previous studies have reported a variety of mutations in *OPA1* associated with adOA, including missense, nonsense, insertions, deletions, and splicing mutations.[10,14,15,19] These mutations are spread throughout the gene coding sequence, with most of them clustering over the cDNA region corresponding to the GTPase domain (exons 8–16) and the 3′ end of the coding region (exons 27–28), whereas there is a relative paucity of mutations in exons 1 to 7.[10,19]

In this study, we screened, by SSCP or heteroduplex analysis, a large cohort ($n = 94$) of apparently unrelated patients with adOA for mutations in *OPA1*. In addition to the coding exons and associated splice sites, we also screened 273 bp 5′ to the start of *OPA1* exon 1, and 2 intronic regions (in introns 5 and 27) that were predicted by in silico sequence analysis to contain putative exons. We report 14 novel adOA mutations in *OPA1*, including five in hitherto unaffected exons (2, 3, 7, 18) and describe 10 new polymorphic variants. We established the molecular consequence at the RNA level of different splicing mutations in two unrelated patients. Furthermore, we performed mitochondrial staining on isolated leukocytes of selected affected individuals to investigate any gross differences in morphology or cellular distribution of these organelles.

Materials and Methods

Patients, Families, and Samples

Ninety-four white, apparently unrelated patients and families of European origin (UK, $n = 51$; Denmark, $n = 36$; Belgium, $n = 2$; Sweden, $n = 1$; Brazil, $n = 2$; Switzerland, $n = 2$) were recruited in different clinical centers. This study conformed to the tenets of the Declaration of Helsinki, and informed consent was obtained from the subjects after an explanation of the purpose of the study. Diagnosis of adOA was based on the presence of typical clinical features ascertained by clinical ophthalmic tests, including visual acuity, color vision, visual fields, electrophysiology, and optic disc appearance.[4,7] Blood samples were taken and genomic DNA extracted with a kit (Nucleon II; Scotlab Bioscience; Strathclyde, Scotland, UK). Ninety patients were from pedigrees with affected members in at least two subsequent generations, and four were from families with multiple affected siblings only. Thirty-seven of the families had undergone prior marker analysis and had linkage–haplotype data compatible with the disease's mapping to the *OPA1* locus on 3q28.

Mutation Screening

All 28 coding exons of *OPA1* and intron–exon splice sites were screened for mutations by SSCP, heteroduplex analysis, and direct sequencing in one affected individual from each family. Putative mutations were tested for segregation in the respective families by single-strand conformational polymorphism (SSCP)–heteroduplex analysis or direct sequencing and in 100 control chromosomes. PCR products were amplified from patient's DNA using primers located in flanking intron and untranslated region (UTR) sequences.[19] Standard 25-μL PCR reactions were performed for SSCP analysis, heteroduplex analysis, and direct sequencing, using 100 to 200 ng template DNA, 50 mM KCl, 10 mM Tris (pH 8.8), 15 mM $MgCl_2$, 1% Triton X-100, 0.2 mM each dNTP, 0.1 μM each primer, and 0.2 U *Taq* polymerase (Bio-Line, London, UK). Typical cycling conditions were as follows: initial denaturation of 95°C for 5 minutes; 35 cycles of 95°C for 30 seconds, 53°C to 63°C for 30 seconds (temperature optimized for each primer pair), and 72°C for 30 seconds; and a final extension of 72°C for 5 minutes.

SSCP Analysis. PCR products were labeled by incorporation of [^{32}P]-dCTP during amplification.[20] Denatured PCR products mixed with an equal volume of formamide loading buffer containing 40 mM $Ca(OH)_2$ were resolved in nondenaturing 0.5× mutation-detection electrophoresis (MDE) gels (Flowgen, Ltd., Lichfield, UK) in 0.6× Tris-borate EDTA buffer run at 8 to 10 W for 14 to 18 hours. Autoradiographs were exposed at −80°C for 1 to 12 hours.

Heteroduplex Analysis. PCR products were allowed to reanneal and then were analyzed by electrophoresis on MDE gels run at 180 V for 14 to 18 hours on commercial electrophoresis apparatus (600S; Hoeffer Scientific Instruments, San Francisco, CA).[21] Gels were stained in 0.5 μg/mL ethidium bromide and visualized on a UV transilluminator.

Direct Sequencing. PCR products showing conformational variants were sequenced bidirectionally after purification (QIAquick PCR purification kit; Qiagen, Crawley, UK), using fluorescent dideoxynucleotides (BigDye Terminator chemistry; PE Biosystems, Warrington, UK) and separated on an automated sequencer (ABI 377; PE Biosystems).

Mutation Detection in Leukocyte RNA

Leukocytes were isolated from whole blood by density-gradient centrifugation (Ficoll-Paque) according to the manufacturer's instructions (Amersham Pharmacia Biotech, Little Chalfont, UK) and total RNA extracted (Trizol reagent; Invitrogen Life Technologies, Groningen, The Netherlands). Oligo(dT)-primed total leukocyte RNA was reverse transcribed into single-stranded cDNA using Protein A Sepharose beads (Ready-To-Go You-Prime First-Strand Beads; Amersham Pharmacia Biotech) and used to amplify cDNA segments encompassing splice site mutations IVS8+5g→a (cDNA PCR primers exon c6F: 5′-TGTCAGACAAAGAGAAAATTGAC-3′, and exon c10R: 5′-ATCAAACTCCCGAGAACTATC-3′) and IVS12+1g→a (cDNA PCR primers exon c10F: 5′-ACTCTGAGTGAAGGTCCTCAC-3′, and exon c14R: 5′-TCTGCCAGGTCTACTTTGGAC-3′). RT-PCR products were separated on a 2% low-melting-point agarose gel and reamplified after gel excision, by using the same primers with 3 μL melted template. Individual products were then purified using the PCR purification kit (QIAquick; Qiagen) and sequenced bidirectionally from nested primers (for IVS8+5g→a, c6FN: 5′-AACTTCAGGAAGAACTTCTGC-3′, and c10RN: 5′-AGGGCCACATGGTGAGGAC-3′; and for IVS12+1g→a, c10FN: 5′-AGTTCCGGGAGTTTGATCTTAC-3′ and c14RN: 5′-AACGAATATGGTTCTCCTTCC-3′) using fluorescent dideoxynucleotide chemistry (Big Dye) and separation on a DNA sequencer (ABI 377; PE Biosystems).

Identification of Putative Functional Sequences within Introns of *OPA1*

To identify the complete *OPA1* genomic sequence, BLAST analysis of the *OPA1* cDNA sequence (KIAA0567; GenBank AB011139) was performed at the National Center for Biotechnology Information, NCBI (http://www.ncbi.nlm.nih.gov/) against The Human Genome. Genomic sequence NT_005571 was identified to contain the entire *OPA1* cDNA (identity ~100%) split into its 29 exons, with only 1 gap in intron 27, so that the entire gene (corresponding to the cDNA sequence KIAA0567—i.e., from exon 1 to the end of exon 29) spans at least 97 kb of genomic DNA. The intronic sequences and putative 5′ UTR were subjected to NIX analysis at the Human Gene Mapping Project (HGMP) to identify any further expressed sequence tag (EST) matches, putative novel exons, and coding regions.[22]

Mitochondrial Staining of Leukocytes

Leukocytes were isolated from blood of two unrelated patients with splice site mutations in *OPA1* and two control subjects by density-gradient centrifugation (Ficoll-Paque; Amersham Pharmacia Biotech).

TABLE 1. Summary of Mutations in the *OPA1* Gene Causing adOA Identified in This Study

DNA Change*	Exon/Intron	Splice Site Functionality†	Predicted Protein	Unrelated Patients	Country of Origin	Linkage to 3q28‡
Missense						
T1188G	Exon 12		Leu396Arg	2	UK	H
C1508A§	Exon 15		Thr503Lys	3	UK	L
G1712A	Exon 18		Arg571His	1	UK	H
Nonsense						
C112A	Exon 2		Arg38STOP	1	UK	H
A733T	Exon 7		Lys245STOP	1	UK	H
Splice site						
IVS3+1g→c	Intron 3	0.02 > 0.00	?	1	Belgium	H
IVS8+5g→a§	Intron 8	0.98 > 0.34	Minus aa 247-275	1	UK	L
IVS9+3a→t	Intron 9	1.00 > 0.99	?	1	Brazil	H
IVS9del(+1−5)	Intron 9	1.00 > 0.00	?	1	UK	L
IVS12+1g→a	Intron 12	0.99 > 0.02	Minus aa 381-404	3	UK	L, ND, H
IVS27+6t→c/	Intron 27/	0.88 > 0.20	?	1	UK	H
IVS26-10c→g	Intron 26	0.87 > 0.72	?			
Deletion						
112-139del(18bp)	Exon 2		del38RSIYHS	2	Denmark	ND
1016delC‖	Exon 10		Frameshift, 19 novel aa, then STOP	1	UK	H
1296delCAT‖	Exon 13		del432Ile	1	UK	H
1304-1305del(GT)	Exon 13		Frameshift, 8 novel aa, then STOP	1	Denmark	ND
1756-1767del(12bp)	Exon 18		del586RELD	1	Sweden	H
2061delA	Exon 21		Frameshift, 35 novel aa, then STOP	1	UK	H
Deletion of entire exon‖	Exon 20		Frameshift, 13 novel aa, then STOP	1	UK	L
2708del(TTAG)§	Exon 27		Frameshift, 2 novel aa, then STOP	6	UK (5), Brazil (1)	H (5)
2826delT	Exon 28		Frameshift, 22 novel aa, then STOP	14	Denmark	L (5)

* Nucleotide designation beginning 1 at position 56 (translation start) of GenBank entry AB011139.

† Berkeley *Drosophila* genome project splice site predictor (http://www.fruitfly.org/seq_tools/splice.html).

‡ Linkage to 3q28 (L), patients from large families showing linkage to the *OPA1* critical interval with Lod score >3.0; haplotype (H), patients from families too small for linkage analysis, but whose affected members share a haplotype encompassing the *OPA1* critical interval; ND, family untyped (very few individuals).

§ Mutations also reported in independent families (Pesch et al.,[19] Toomes et al.,[10] and Delettre et al.[15]).

‖ Mutations have been reported previously in Alexander et al.[14]

Cells were seeded onto glass coverslips in Dulbecco's modified Eagle's medium, and mitochondria were visualized with mitochondrion-selective dye (MitoTracker CMXRos-H_2; Molecular Probes, Eugene, OR) or immunostained with a monoclonal antibody against subunit I of cytochrome *c* oxidase, as described previously.[23] Coverslips were mounted onto glass slides in fluorescent glycerol-PBS solution (Citifluor; Agar Scientific Ltd., Stansted, UK) supplemented with 1 μg/mL 4,6-diamidino-2-phenylindole (DAPI). Slides were examined with a microscope (Axiophot; Carl Zeiss, Oberkochen, Germany) equipped with filters appropriate for rhodamine, fluorescein, and DAPI. Separate fluorescent images were captured with a cooled, integrated camera (ORCA; Hamamatsu, Hamamatsu City, Japan) with a 1280 × 1024-pixel array and a 12-bit gray-level resolution. The images were recombined, by using image analysis software (Photoshop; Adobe, San Diego, CA).

RESULTS

Novel *OPA1* Mutations

Altogether, 20 different mutations were identified that segregate with disease in families with adOA and/or are not present in 100 control chromosomes (Table 1). We have previously described three of the mutations unique to individual families: 1016delC, 1296delCAT, 1848del(exon 20).[14] The 17 newly identified mutations comprise three missense, two nonsense, six splice site, and six microdeletion mutations (two in-frame, four frameshift) spread throughout the coding portion of the gene (Table 1). Three of these mutations have been reported by other groups: the missense C1508A in exon 15 was recently identified in one family[10] and segregates with disease in three of our families from the United Kingdom, the splice site change IVS8+5g→a has also been previously identified in one British family,[10] and the recurrent mutation 2708del(TTAG), found in six of our families, has been reported in 10 different families in other studies.[10,15,19]

In total, 14 of the mutations are reported herein for the first time, five of which are present in novel locations within the gene: two in exon 2 and one each in the donor splice site of exons 3, 7, and 18 (Table 1). An in-frame deletion in exon 2, 112-139del(18bp), detected in a Danish patient, removes six amino acids (RSIYHS), including the first predicted cleavage site of the mitochondrial leader sequence,[14] and may therefore affect the correct targeting of the OPA1 protein to the mitochondria. The two novel missense mutations (Table 1; Leu396Arg, Arg571His) both alter conserved amino acid residues: Arg571 is conserved between orthologues in other species,[14] whereas Leu396 lies close to a GTP-binding motif in the dynamin-related domain, is highly conserved across orthologues, and is also related to human GTPases, such as dymple and dyn2.[14] One common frameshift mutation 2826delT in exon 28, which creates a novel OPA1 −COOH terminus, was identified in 14 Danish patients (Table 1).

Testing for reduced splice site functionality in the six putative splice site mutations predicted a reduced splicing efficiency to varying degrees (Table 1). Those sequence alterations affecting the highly conserved consensus G at position +1 of the donor splice site IVS3+1g→c, IVS9del(+1-5), IVS12+1g→a are all predicted to completely abolish the respective splice site (Table 1). RT-PCR of total leukocyte RNA from two affected individuals from one large family segregating the IVS12+1g→a mutation revealed complete skipping of

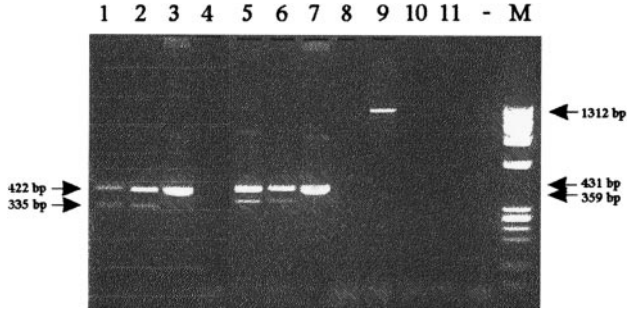

FIGURE 1. RT-PCR amplification products from total lymphocyte RNA of patients with adOA from two families harboring splice site mutations in the *OPA1* gene, G1 (IVS8+5g→a) and M1 (IVS12+1g→a). *Lanes 1* to *4*: products obtained using PCR primers exon c6F and c10R; *lanes 5* to *9*: products obtained using PCR primers exon c10F and c14R. PCR templates are as follows: *lane 1*: Total lymphocyte cDNA from affected individual in family G1; *lane 2*: total lymphocyte cDNA from asymptomatic but mutation-carrying individual in family G1; *lane 3*: *OPA1* gene cDNA (KIAA0567); *lane 4*: human control genomic DNA (expected product size of 11.2 kb too large to amplify with *Taq* polymerase); *lane 5*: Total lymphocyte cDNA from affected individual in family M1; *lane 6*: total lymphocyte cDNA from second affected individual in family M1; *lane 7*: *OPA1* gene cDNA (KIAA0567); *lane 8*: human control genomic DNA (no product); *lane 9*: human control genomic DNA (expected amplification product of 1312 bp); *lane 10*: no DNA control (primers c6F/c10R); *lane 11*: no DNA control (primers c10F/c14R); *lane M*: size marker ϕX174/*Hae*III. *Arrows*: (*left*) sizes of RT-PCR products from IVS8+5g→a–carrying cDNA (*lanes 1* and *2*); (*right*) sizes for IVS12+1g→a cDNA and control genomic DNA (*lanes 5*, *6*, *9*).

exon 12 in mature transcripts (Fig. 1). The IVS8+5g→a mutation segregating in one family large enough to show prior linkage to the *OPA1* locus is predicted to reduce the strength of the corresponding donor splice site, but not completely eradicate it (Table 1). RT-PCR of total leukocyte RNA from two disease haplotype-bearing individuals from this family, both of whom harbor the mutation indicated skipping of exon 8 in both cases (Fig 1), as has been reported for this mutation in an independent study.[10] This is especially interesting, because one of these two individuals had obvious clinical signs and symptoms of adOA, whereas the other had reduced penetrance for the disease. Unfortunately, fresh blood could not be obtained for RNA analysis from the patients with putative splicing mutation IVS9+3a→t or the double alteration IVS27+6t→c/IVS26-10c→g. Although these sequence alterations affect less-conserved nucleotides, the changes segregate with adOA in the small families in which they were detected (five affected members and three affected members respectively), are predicted to affect splicing efficiency (Table 1), and were not found in more than 50 control individuals.

OPA1 Mutation Profile

This study brings the total number of adOA mutations so far reported in *OPA1* to 54 and highlights the high rate of new mutations in this gene. Our findings enhance the adOA mutation profile of *OPA1* and pinpoint novel residues and domains critical to its function (Fig. 2). We provide further evidence for a strong clustering of missense mutations in or close to the GTP-binding domain (~exons 8–16) of OPA1, emphasizing that OPA1 GTPase activity is particularly critical for retinal ganglion cell development and function. The sole missense mutation 2354A→G so far reported outside this region in exon 23,[19] together with the four in-frame deletions so far identified in exon 2—112-139del(18bp), this study; exon 13, 1296delCAT[14]; exon 17, 1651delTGC[19]; and exon 18, 1756-1767del(12bp), this study—are all predicted to result in a nontruncated OPA1 polypeptide, but may lead to an altered conformation of the protein.

Our study revealed two novel adOA mutations within the predicted mitochondrial leader sequence (~exons 1–2) of the *OPA1* gene[14,15]: a nonsense mutation Arg38STOP and an in-frame deletion of six amino acids of the first of three predicted cleavage sites for mitochondrial import peptidases.[14] Examination of the putative salmon, *Drosophila melanogaster*, and *Caenorhabditis elegans* OPA1 orthologous protein sequences (protein accession numbers BAA32279, AAF58275, and CAA87771, respectively) reveals only one of these sites, corresponding approximately to the third in humans from amino acid residues 100 to 110. In the murine orthologue (AB044138), however, the first and third sites are retained (data not shown). Future work may elucidate whether the first cleavage site is indeed functional only in mammals.

No conserved protein motifs or domains were evident on bioinformatic analysis of the primary sequence of the –COOH-terminal region of OPA1, which corresponds to a third mutation cluster (exons 27 and 28) and has unknown function (Fig 2). Bioinformatic analysis of the protein primary structure has revealed two coiled–coil domains in OPA1: one spanning residues 226 to 268 and one from 940 to 969.[24] Coiled–coil domains are thought to be intrinsically involved in protein interactions and therefore highlight domains of OPA1 for future investigation by yeast-2-hybrid/pull-down assay for binding partners. The second coiled–coil is completely removed by the common 2708del(TTAG) mutation in exon 27 (data not shown), but is only marginally reduced in probability score by the 2826delT mutation in exon 28.[25]

Prevalence of Mutations in *OPA1* in the Current Study

Overall, *OPA1* mutations were detected in 44 of 94 patients with adOA, for a detection frequency of 47%. It is possible that some of the remaining cases represent nonallelic genetic het-

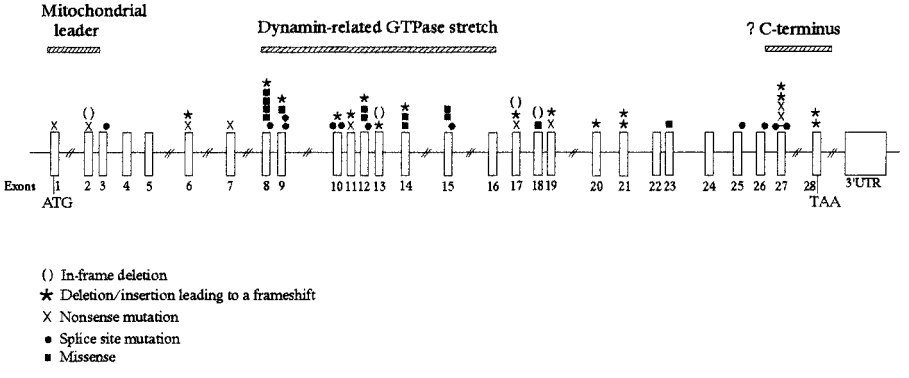

FIGURE 2. Summarization of the type and genetic location of all the different *OPA1* mutations found to date that cause adOA. This highlights a clustering of mutations over the dynamin-related GTPase domain, carboxyl terminus, and mitochondrial leader peptide of OPA1.

erogeneity, autosomal recessive, or X-linked forms of optic atrophy.[25] Of the patients with mutations, 16% (7/44) were from individual families linked to chromosome 3q28-qter with lod scores greater than 3, and 57% (25/44) were from small families with lod scores lower than 3 or a haplotype compatible with linkage to chromosome 3q28-qter. The remaining 27% (12/44) of the mutations were from pedigrees for which no marker data were available, but which showed a strong family history of optic atrophy compatible with autosomal dominant inheritance. No mutations were discovered in families with affected siblings in only one generation. When considering the total number of families in each of these categories, it is apparent that mutations are found in 70% (26/37) of pedigrees showing linkage–haplotype data consistent with disease segregation with the *OPA1* locus and in only 34% (18/53) with a family history in the absence of marker data. This finding is important, because it suggests that it may be worth screening a patient for a mutation in *OPA1* only if haplotype–linkage data suggest dominant inheritance of optic atrophy or if there is a clinical picture strongly suggestive of adOA in the absence of a clear-cut dominant history.

In addition, we identified one family of 15 affected members that shows no evidence of linkage to the chromosome 3q28-qter *OPA1* locus (D3S3669: $Z_{max} = 0.19$, $\theta_{max} = 0.33$; D3S3642: $Z_{max} = 0.16$, $\theta_{max} = 0.35$; D3S3590: $Z_{max} = 0.97$, $\theta_{max} = 0.19$; D3S3562: $Z_{max} = 0.48$, $\theta_{max} = 0.50$; where Z is Lod Score and θ is recombination fraction) and in which the chromosome 18 *OPA4* locus has also been excluded (data not shown). Including only the known affected individuals from this family in the linkage analysis excludes the chromosome 3q28 locus (D3S3669: $Z_{max} = 0.27$, $\theta_{max} = 0.14$; D3S3590: $Z_{max} = 0.25$, $\theta_{max} = 0.23$). It is possible that this family represents a new as yet unmapped genetic locus for adOA. The clinical phenotype in this family is very similar to the chromosome 3–associated phenotype[7] and also overlaps the phenotype of the single family mapping to the OPA4 locus on chromosome 18.[11]

Mutation Screening of Additional ORFs in *OPA1*

Because mutations were not identified in 11/37 (30%) index patients from families mapping to 3q28 through linkage–haplotype analysis, we investigated the possible existence of additional as yet undetected exonic sequences or open reading frames (ORFs) lying within the known *OPA1* introns and 5′ UTR, which are not present in the original full-length mRNA sequence AB011139 (derived from brain) but may contribute to alternative splice isoforms or tissue-specific transcripts. NIX analysis of these *OPA1* extraexonic regions predicted several sequence segments containing putative ORFs, three of which were examined further, in introns 5 and 27 and extending 5′ from exon 1.

NIX analysis revealed 99% identity to EST BG470279 overlapping and extending 5′ to exon 1 of *OPA1* (as defined by the start of mRNA sequence AB011139). This EST matched the *OPA1* mRNA over the mitochondrial leader (96%) and extended 5′ for a further 195 bp. This region contained an ORF in-frame with the OPA1 protein sequence (BAA29453), potentially adding a further 63 amino acids to the 5′ of OPA1 or, alternatively, containing regulatory sequences. However, we discovered no SSCP band shifts on screening 273 bp 5′ to *OPA1* exon 1 in adOA index patients.

A 111-bp stretch of DNA in intron 5 (IVS5+7613-+7724) gave an excellent exon prediction by three different programs (GRAIL, GENSCAN, and Genefinder[22]), with predicted splice sites matching the Kozak consensus[26] and a predicted ORF of 37 amino acids. Although this polypeptide sequence showed no significant match by BLAST analysis to any known protein sequence, the predicted exon (putative exon 5a) identified two human ESTs (99% identity; AA334558, embryo; AW996319, breast) which both contained contiguous sequence 3′ and 5′ to the region of identity matching exons 5 and 6 of the *OPA1* gene (AB011139). The putative exon also identified four mouse ESTs by sequence similarity search (BB254844, 89%, cerebellum; BB395916, 90%, cerebellum; BB546811, 93%, eye; BE303971, 96%, ductal carcinoma), which show 92% to 98% identity to mouse mRNA for large GTP-binding protein AB044138, the presumed murine orthologue of human *OPA1* (88% identity over the entire length at the nucleotide level, 96% at the amino acid level). This may be indicative of conserved, hence functionally important, sequences.

NIX analysis of intron 27 predicted a marginal exon (FEX, HEXON, FGENE[22]) spanning a 365-bp stretch of DNA (IVS27+1041-+1406; putative exon 27a). Putative acceptor and donor splice sites were present (scores 0.97 and 0.89 respectively; http://www.fruitfly.org/seq_tools/splice.html). This predicted exon translates into an ORF of 121 amino acids, showing no significant similarity to known polypeptides. However, this nucleotide stretch shows 100% identity to an EST (accession T96104) and to UniGene cDNA cluster Hs.331315 (complementary strand). There is also a polyadenylation signal 205 bp downstream of this predicted exon, which could represent the 3′ end of an alternative *OPA1* transcript.

RT-PCR of fetal brain cDNA (Stratagene, La Jolla, CA), adult retina cDNA library lysates (gift from Jeremy Nathans, Johns Hopkins Medical School, Baltimore, MD), and normal leukocyte cDNA (prepared as above from control blood) using cDNA primers designed to amplify from exon 5a to exon 7, and exon 26 to exon 27a, produced no amplification product. This suggests that these putative exons are not present in contiguity with exon 7 (for 5a) or exon 26 (for 27a) in the library aliquots studied. Furthermore, SSCP analysis of patients with adOA, in whom no *OPA1* mutation had been identified, revealed no sequence alterations in putative exons 5a or 27a.

Current Single-Nucleotide Polymorphism Catalog of *OPA1*

During the mutation screening, 16 single-nucleotide polymorphism SNPs were found within the *OPA1* gene, 10 of which are reported herein for the first time (Table 2). Two of these sequence changes, C500T and IVS7del(+49-51), were identified in single adOA families and were not found in 100 control chromosomes. That C500T, although resulting in the nonconservative amino acid change Pro167Leu, clearly did not segregate with disease in the family in which it was detected on repeated analysis makes it likely to be a rare polymorphism. This amino acid substitution is a known polymorphic variant in other genes.[27,28] The IVS7del(+49-51) alteration was found in a patient who also harbored the 2708del(TTAG) frameshift mutation (Table 1). This work extends the current SNP catalog for *OPA1* to 27, of which 9 are cSNPs.[10,15,19]

Morphology and Cellular Distribution of Mitochondria in Leukocytes of Patients and Control Subjects

To investigate whether *OPA1* mutations had an effect on morphology and cellular distribution of mitochondria, we stained mitochondria of leukocytes from two unrelated patients (harboring mutation IVS12+1g→a and IVS8+5g→a) and normal individuals with mitochondrion-selective dye (MitoTracker CMXRos-H_2; Molecular Probes). The dye showed that mitochondria were present as discrete entities in the cytosol around the nucleus of these circulating cells (Fig. 3). There was no notable difference in mitochondrial morphology and distribution between patients and control subjects. Immunostaining

TABLE 2. Polymorphic Variants Found in the *OPA1* Gene in This Study

DNA Change	Consequence	Location	Unrelated OA Patients (*n*)	Control Allele Frequency (%)	Comment	Restriction Enzyme Assay
Exonic						
A473G*	Asn158Ser	Exon 4	>5	A/53 G/47		+*Cje*I site
C500T	Pro167Leu	Exon 4	1	C/100 T/0	No familial segregation	+*Bfa*I site
C580T*	Ala192Val	Exon 5	2	C/98 T/2		+*Mwo*I site
A1609C*	Ala536Ala	Exon 17	5	A/94 C/6		+*Hae*III site
C2109T*	Ala703Ala	Exon 21	>5	ND		−*Hae*III site
Intronic						
IVS7del(+49−51)	gttgtgt→gt-gtg	Intron 7	1	0		+*Afl*III site
IVS8+32c→t*	caatta→caacta	Intron 8	>5	ND		+*Bfa*I site
IVS10+18a→g	tctaag→tctgag	Intron 10	4	ND		+*Mnl*I site
IVS13+32a→g	actgat→actggt	Intron 13	2	A/99 G/1	Creates splice site 0.91 putative splice enhancer†	+*Bsr*I site
IVS18+16t→g	actcgt→acgcgt	Intron 18	>5	T/96 G/2		+*Mlu*I site
IVS18+51t→g	gtttgc→gtgtgc	Intron 18	>5	T/50 G/50		−*Ttb*III11 site
IVS25-142t→c	tatatc→tacatc	Intron 25	>5	ND		No change
IVS25-112a→g	tgagta→tgggta	Intron 25	>5	ND		−*Sca*I site
IVS26+26t→a*	ttattt→taattt	Intron 26	>5	ND		+*Sse*91 site
IVS27+23t→c	tctacc→tccacc	Intron 27	1	ND	No splice site created	No change
IVS27+108g→a	cacggt→cacagt	Intron 27	2	G/97 A/3		−*Bsr*I site

ND, not determined.
* Previously reported in Pesch et al.,[19] Toomes et al.,[10] and Delettre et al.[15]
† Correct donor site 1.00.

with a monoclonal antibody against the mitochondrial DNA-encoded subunit I of cytochrome *c* oxidase produced an identical staining pattern (data not shown).

Clinical Features of Pedigrees with adOA *OPA1* Mutations

Clinical data for 11 families in the United Kingdom with *OPA1* mutations in whom affected and unaffected members were examined are summarized in Table 3. There was widespread intra- and interfamily variability in the phenotype. Seven of 11 families had at least one individual with clinical onset of disease before the age of 6 years. Conversely, the clinical diagnosis was made in individuals in four families as late as their third (families A1, K1, T1; Table 3) or fifth (family G1) decade. Visual acuity and disc appearances showed no notable association with type or intragenic location of mutations. Visual acuity ranged from 6/9 to hand-movement perception, and disc appearances ranged from subtle atrophy to total pallor. Comparing the clinical data for different families segregating the same mutation—for example, B1 and L1 (C1508A, predicting the Thr503Lys substitution) and B3 and C7 (2708del(TTAG), predicting a frameshift deletion at codon 902)—it does appear that these families share more similarities in clinical symptom severity than families with other mutations. This is especially true for the C1508A mutation where several affected members were examined from each family (Table 3). This may in part be due to the shared genetic context of the disease allele in the families with the C1508A mutation, who display a common disease haplotype at the *OPA1* locus (data not shown). This suggests that these families may have common ancestors, consistent with their broadly similar clinical profile (Table 3), and also that genetic background may be a key factor influencing the clinical variability in expression of individual mutations. Patients with mutations in the latter sections of the gene—for example, exon 27: 2708del(TTAG) or splice IVS27+6t→c/IVS26-10c→g— do not have a milder phenotype than patients with mutations predicted to severely truncate the protein (Table 3).

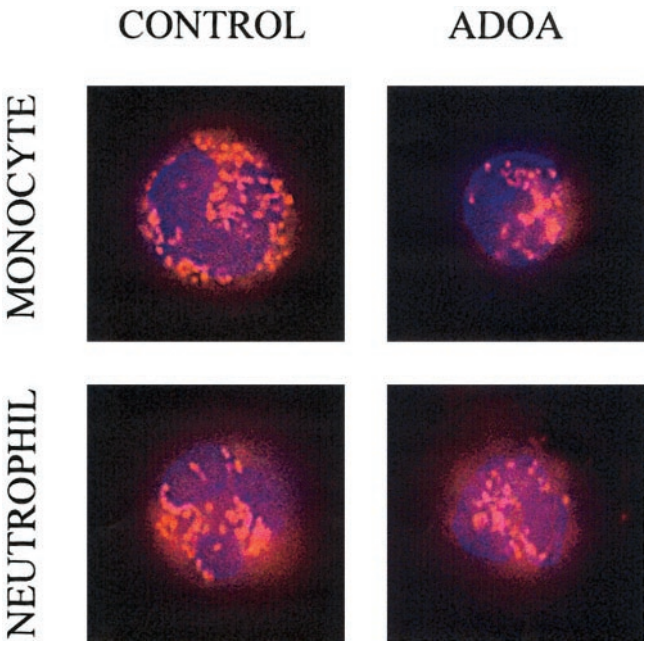

FIGURE 3. Mitochondrial distribution in leukocytes. Monocytes and neutrophils of a normal individual and a patient were stained with a mitochondrion-selective dye (*red*). Cells were counterstained with DAPI to reveal the nucleus (*blue*). No difference in mitochondrial distribution was observed between patients and control subjects.

Mutations in Families with Reduced Penetrance

Penetrance is not clearly defined in the literature and is used in different ways by different investigators. A fully penetrant individual shows symptoms and signs of the disease and carries a disease-associated mutation. We have defined nonpenetrance in an individual patient as the detection of a disease-associated mutation in an individual who shows no symptoms or clinical signs of the disease on examination. Partial penetrance is taken as meaning an individual who is asymptomatic but has a func-

TABLE 3. Clinical Features in 11 British Families with OA and Mutations in the *OPA1* Gene

Pedigree	Lod score or Haplotype Suggestive of Linkage to Chromosome 3	Mutation and Nucleotide Change	Exon/Intron	Predicted Protein	Total of Family Members Examined (*n*)	Symptomatic Affected Patients Examined (*n*)	Nonpenetrant Carriers Identified (*n*)	Age Range at Exam (y)	Clinically Affected Patients					
									Age Range at Onset of Symptomatic Clinical Disease (y)	Mean Age of Onset of Symptomatic Clinical Disease (y)	Best VA Range	Disc†	Color Vision	Visual Field Defect
A1	4.52	Splice site IVS12+1g→a	Intron 12	Skip exon 12	20	10	None	2–68	2–29	8.6	6/12–4/60	S to T	MD	—
B1*	3.61	Missense C1508A	Exon 15	Thr503Lys	17	7	None	23–68	2–8	5.3	6/24–HM	S to T	SD	CC to S
L1*	7.32	Missense C1508A	Exon 15	Thr503Lys	29	9	1	12–60	1–8	4.9	6/24–1/60	T to A	MD	CC to S
B3	Singleton	Deletion 2708del(TTAG)	Exon 27	Val902fs	1	1	None	31	7	7	6/36	T	T	CC
C7	Haplotype	Deletion 2708del(TTAG)	Exon 27	Val902fs	3	2	None	35–51	7–9	8	6/18–6/36	T to A	T	CC
C5	Haplotype	Nonsense C112A	Exon 2	Arg38STOP	6	4	None	12–45	2–15	7.5	6/18–6/60	T to A	SD	CC to S
F3	Haplotype	Splice site IVS27+6t→c/ IVS26−10c→g	Intron 27/ Intron 26	Unknown	5	2	None	42–54	5	5	6/18–4/60	T	MD	—
G1	2.51	Splice site IVS8+5g→a	Intron 8	Skip exon 8	17	7	None	41–76	3–41	16.83	6/9–6/18	N to A	MD	CC to S
K1	3.61	Deletion, entire exon	Exon 20	Glu617fs	15	6	2	36–61	7–25	10	6/18–6/60	S to A	N to MD	CC to S
P1	2.20	Splice site IVS9del(+1−5)	Intron 9	Unknown	16	2	1	4–44	4–9	7	6/9–6/24	T to A	MD	CC
T1	Haplotype	Deletion 2061delA	Exon 21	Glu687fs	5	2	None	36–76	12–20	16	6/36–6/60	A	A	CC

HM, Hand movements.

* Haplotype suggests may be branch of same family.

† Disc appearance: N, normal; S, subtle pallor; T, temporal pallor; A-total atrophy. Color vision: T, tritanopia; MD, mixed dyschromatopsia; SD, severe dyschromatopsia; A, achromatopsia. Visual fields: CC, centrocecal; ST, subtotal. Program 30-2 (Humphrey Instruments, San Leandro, CA).

tional deficit evident in clinical examination, albeit very mild. The person may be subclinically affected, requiring more sophisticated tests, such as perimetry, to make the diagnosis of affected status.[29] It is clearly difficult to draw the boundaries between reduced (or partial) penetrance and expressivity, and, indeed, variable expressivity may be a reflection of reduced penetrance.

Based on these definitions, an estimate of penetrance for the two families in whom it was possible to examine all affected and unaffected individuals showed highly differing values ranging from 100% (pedigree A1, splice site mutation intron 12: IVS12+1g→a) to 67% (pedigree K1, deletion of entire exon 20). From the 11 pedigrees in Table 3, we detected four cases of nonpenetrance in three pedigrees (K1, L1, and P1) after clinical examination of symptomatic and asymptomatic family members.

We found six further individuals in five adOA-affected pedigrees (not tabulated in Table 3) who were asymptomatic and who carried the disease-associated mutation, but for whom clinical data are insufficient to make the distinction between nonpenetrance and partial penetrance on these criteria: one patient: exon 18, G1712A; one patient: exon 18, 1756-1767del; three patients from two families: exon 27, 2708del(TTAG); and one patient: exon 28 (2826delT). The mutation exon 27 2708del(TTAG) has been associated with reduced penetrance.[10] However, the presence of reduced penetrance in family P1 with mutation IVS9del(+1-5) suggests that a predisposition to reduced penetrance is not solely due to mutations located 3′ to the GTPase domain of *OPA1*.

DISCUSSION

Current Spectrum of *OPA1* Gene Mutations: A Key Role for the Carboxyl Terminus?

An increasing range of mutations in the *OPA1* gene are being identified in families with adOA.[10,19] In this study we identified 14 novel *OPA1* mutations in an independent cohort of 94 patients or pedigrees with adOA, 37 of which showed prior linkage–haplotype evidence consistent with a map location including the *OPA1* interval. We also addressed the possibility that clinical symptoms of the disease may be the result of altered mitochondrial integrity or subcellular localization. We also examined our clinical data for any evidence of genotype–phenotype correlation and made attempts to address the observed low detection rate of *OPA1* mutations in patients with adOA.

Of the 54 *OPA1* mutations reported to cause adOA to date, the majority (26, or almost 50%) cause premature truncation of the OPA1 protein or create novel –COOH termini. This number may in fact be larger, owing to the introduction of frameshifts by some of the 11 splice site mutations, which has not yet been demonstrated because patients' RNA was unavailable (Table 1).[10,19] Recent studies have shown that some missense and nonsense mutations, previously presumed to exert their pathogenic effects solely on either the resultant amino acid change or protein truncation, may instead lead to aberrant splicing by disruption of exon-splicing enhancers (ESEs) or suppressors (ESSs).[30] Whether this means that most cases of adOA caused by mutations in *OPA1* effectively result from null mutations that may halve the amount of normal OPA1 protein in the cell (haploinsufficiency) and this is sufficient to cause disease awaits the results of future experiments. Many mutations do not truncate the protein, which may either be rapidly degraded or interfere in a dominant–negative manner with the OPA1 polypeptide arising from the normal gene copy. Therefore, there is possible evidence for more than one disease mechanism underlying adOA.

The cluster of mutations resulting in loss or abnormality of the −COOH terminus of OPA1 highlights the physiological importance of this protein domain. The occurrence of a founder mutation—exon 28, (2826delT)—in the −COOH terminus may account for the high frequency of adOA in Denmark.[24] That adOA mutations in the 3′ coding exons 27 and 28 which disrupt the ORF can produce a phenotype of clinical severity comparable to those in regions of known functional significance highlights the integral contribution of the OPA1 –COOH terminus to the correct cellular function of this protein. The –COOH terminus of the dynamin family of GTPases is not well conserved, apparently imparting unique functional attributes to each specific member, and it may be that this region of OPA1 is intrinsically essential to its specific role in the mitochondria.[31] This may explain how mutations affecting only the very –COOH end of OPA1 protein could give rise to disease.

Low Detection Rate of *OPA1* Gene Mutations in adOA Pedigrees

In total, SSCP and heteroduplex analysis of all coding exons of *OPA1* identified mutations in 44 of 94 adOA patients, an overall detection rate of 47%. This is comparable with the results of other recent studies with rates ranging from 32.1% to 57%.[10,19] This low detection rate may be ascribed to a combination of factors: the screening methods used are not 100% effective (in our case, SSCP– heteroduplex analysis); large-scale mutations (deletions, insertions) are undetectable by these same methods, which may account for a significant number of cases; or mutations residing in unscreened portions of the gene (e.g., introns, 5′ UTR–promoter regions, 3′ UTR). However, the mutation detection methods we used are known to be at least 80% effective,[32] which alone would not account for the 53% of mutations left undetected. If there are *OPA1* mutations present in the remaining patients, it is likely that they reside in noncoding portions of the gene involved in regulatory processes or nondetection may be due to gross DNA rearrangements that require other methods (e.g., Southern blot analysis, RT-PCR) for their detection.

The *OPA1* gene has been shown to express alternative transcripts in a variety of tissues.[14,15] To address the possibility that additional exons and ORFs exist in the introns or 5′ or 3′ UTRs of the original brain-derived transcript (GenBank entry AB011139), we extracted these sequences through computational analysis from The Human Genome at NCBI and subjected them to NIX analysis in the HGMP.[22] Introns 5 and 27 contained exons predicted by more than one program, with ORFs and EST matches. Although we did not find any adOA mutations in these putative exons and could not link them to the known *OPA1* transcript sequence in human retina, fetal brain cDNA libraries or leukocyte cDNA, further studies may reveal functional significance for these *OPA1*-intragenic sequences and others like them. Even if such intronic sequences do not normally contribute to the *OPA1* transcript profile, pseudoexon activation, caused by mutations in intronic nucleotides, is becoming more widely recognized as a genetic disease mechanism.[33] Screening of 273 nucleotides, matching EST BG470279 and extending the ORF 5′ to the putative start of *OPA1* exon 1, revealed no further mutations in our panel of patients.

Mitochondrial Integrity and Subcellular Localization in Lymphocytes of Patients and Control Subjects

The pathophysiology and clinical symptoms observed in adOA show overlap with those occurring in Leber hereditary optic neuropathy,[34] caused by mutations in mtDNA-encoded genes for subunits of complex I of the respiratory chain.[35] Therefore, it is tempting to speculate that mutations in *OPA1* leading to disturbances of mitochondrial integrity may compromise the energy supply to the highly energy-demanding optic nerve tissue, so that it degenerates and visual impairment ensues. Delettre et al.[15] reported that the mitochondrial network is disorganized in leukocytes from patients with *OPA1.* Their micrograph of control leukocytes immunostained with anti-HSP60 showed general cytoplasmic staining with no evidence of a mitochondrial network, whereas the antibody seemed to reveal clumps of mitochondria in patients' cells. Our results are very different. Instead of a general cytoplasmic staining of control leukocytes, we clearly observed a discrete mitochondrial network in the cytosol with the mitochondrion-selective dye (Fig. 3) and with an antibody against cytochrome *c* oxidase subunit I (data not shown). Patients' cells showed a mitochondrial network that was indistinguishable from that of normal individuals and, moreover, similar to the results reported by Delettre et al.[15] Blood monocytes and granulocytes have extra-large nuclei, folded and lobed, respectively, that leave little room for the cytoplasm in these cells. We thus believe that the distribution of mitochondria observed in patients by both groups, actually represents the typical pattern expected for normal blood monocytes. Therefore, we conclude that these *OPA1* mutations have no observable effect on the mitochondrial distribution in leukocytes. The contradictory results of Delettre et al.[15] could in part be explained by the fact that different patients and mutations were studied but are more likely due to differences in methodology. The absence of any mitochondrial network in their control cells suggests that the cellular structures were not properly fixed before antibody incubation. If adOA caused by *OPA1* mutations is a mitochondriopathy, our findings failed to provide evidence that the disease is readily observable in patients' leukocytes. Either the mitochondrial abnormalities are limited to the cells of the retinal ganglion cell layer or optic nerve or are more subtle than our methods can detect. Systematic characterization of mitochondria staining patterns across a more comprehensive array of patient *OPA1* mutations and control individuals is warranted to clarify this issue. Nevertheless, we conclude that diagnosis of adOA based on mitochondrial morphology or distribution in blood leukocytes is currently inadvisable.

OPA1 Gene Founder Effects and Mutation Hot Spots in adOA

One frameshift mutation 2826delT in exon 28 has been identified in 14 Danish patients (Table 1). Statistical analysis of patient haplotypes versus control subjects strongly indicates this mutation to be a founder allele in the Danish population, which may explain the higher prevalence of adOA in Denmark.[24]

Of 38 British families previously analyzed by haplotype and linkage disequilibrium analysis, 36 had haplotypes compatible with a founder effect.[36] We have found mutations in 15 of these families. The gene *OPA1* lies closest to marker D3S2305, contrary to the predicted likely position by linkage disequilibrium, which placed the gene at approximately 400 kb either side of D3S1523. The inaccuracy of the founder prediction may be in part due to a change in marker order used in the founder prediction versus the marker order now accepted through physical mapping.[24] Marker D3S2305, then placed between D3S3642 and D3S3590, using the best available genetic data then available (GeneMap 1997 [http://www.ncbi.nlm.nih.gov/genemap97]), is now known to lie between D3S3590 and D3S3562. This change in marker order over a critical part of the haplotype has the effect of breaking up the core founder haplotype so that only 15 families appear to share a core haplotype across the *OPA1* disease interval.

Examination of our revised marker allele data for microsatellite markers spanning the D3S3669-D3S3562 interval reveals that three families with a mutation in exon 15 (C1508A, two of which are B1 and L1 in Table 3) share a common haplotype for markers D3S3642, D3S3590, and D3S3562, encompassing a ~640kb genomic interval (data not shown). Of the 12 remaining families in whom we have found mutations and who were analyzed in the assessment of the founder effect,[36] only three others show a core haplotype from markers D3S1523 to D3S2305 that would be consistent with a founder effect. However, these three families have three different splice site mutations: IVS8+5g→a (family G1), IVS12+1g→a (family A1), and IVS27+6t→c/-10c→g (family F3), illustrating that a common haplotype does not always guarantee common ancestry.

The 2708del(TTAG) frameshift mutation has been found in white patients of various nationalities (Australian, France, Belgian, German, British, Brazilian; Table 1),[10,15,19] which may indicate that it represents a mutation hot spot. Two novel recurrent mutations detected in this screen are located in exon 12: missense T1188G in two British families and splice site IVS12+1g→a in three British families (Table 1). We found no evidence of shared haplotypes for *OPA1*-linked markers in these families, implying novel mutation hot spots in this gene.

Intra- and Interfamily Variability in Expression of adOA

Preliminary data suggest that phenotype–genotype correlations in patients with adOA with mutations in the *OPA1* gene appear to be weak and indicate that other factors, such as genetic background, modifier loci, and/or environmental influences play an important role in phenotypic expression. There was a fairly frequent occurrence of asymptomatic carriers in our family cohort with mutations, notably in two families with the 2708del(TTAG) mutation, as has been reported previously.[10] Furthermore, we found cases of nonpenetrance in families with three other mutations, with a variable penetrance estimate in appropriate large families from 100% for the IVS12+1g→t mutation, resulting in exon 12 skipping, to 67% for the del(exon20) frameshift mutation. Thus it appears that adOA may be a monogenic disorder due primarily to the disruption of a single gene, but has a complex phenotypic expression owing to other genetic, epigenetic or environmental factors impacting greatly on the clinical outcome.[37]

There are now 27 polymorphisms described within exons and introns of the *OPA1* gene, including 10 that are novel in this study. Sequence variants, such as these, although not individually associated with adOA, may contribute to subtle variations in structure and function, or may influence gene expression and the phenotypic expression of individual mutations. Variable penetrance in genetic disease has been shown in some instances to be affected by polymorphisms within the wild-type allele at the disease locus.[38] Although we do not yet know the mechanism of reduced penetrance in adOA caused by mutations in the *OPA1* gene, a catalog of benign sequence variants will undoubtedly add to our future understanding of how this gene and its encoded protein operate in health and disease.

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References

1. Kjer B, Eiberg H, Kjer P, Rosenberg T. Dominant optic atrophy mapped to chromosome 3q. II: clinical and epidemiological aspects. *Acta Ophthalmol Scand.* 1996;74:3–7.
2. Lyle WM. *Genetic Risks*. Waterloo, Ontario, Canada: University of Waterloo Press; 1990.
3. Hoyt CS. Autosomal dominant optic atrophy: a spectrum of disability. *Ophthalmology* 1980;87:245–251.
4. Votruba M, Moore AT, Bhattacharya SS. Clinical features, molecular genetics and pathophysiology of dominant optic atrophy. *J Med Genet.* 1998;35:793–800.
5. Johnston PB, Gaster RN, Smith VC, Tripathi R. A clinicopathologic study of autosomal dominant optic atrophy. *Am J Ophthalmol.* 1979;88:868–875.
6. Kjer P, Jensen OA, Klinken L. Histopathology of eye, optic nerve and brain in a case of dominant optic atrophy. *Acta Ophthalmol* (*Copenh*). 1983;61:300–312.
7. Votruba M, Fitzke FW, Holder GE, Carter A, Bhattacharya SS, Moore A. Clinical features in affected individuals from 21 pedigrees with dominant optic atrophy. *Arch Ophthalmol* 1998;116:351–358.
8. Kivlin JD, Lovrien EW, Bishop T, Maumenee IH. Linkage analysis in dominant optic atrophy. *Am J Hum Genet.* 1983;35:1190–1195.
9. Kjer P. Infantile optic atrophy with dominant mode of inheritance: a clinical and genetic study of 19 Danish families. *Acta Ophthalmol Scand.* 1959;37(suppl 54):1–146.
10. Toomes C, Marchbank NJ, Mackey DA, et al. Spectrum, frequency and penetrance of *OPA1* mutations in dominant optic atrophy. *Hum Mol Genet*. 2001;10:1369–1378.
11. Kerrison JB, Arnould VJ, Ferra Zsallum JM, et al. Genetic heterogeneity of dominant optic atrophy, Kjer type: identification of a second locus on chromosome 18q12.2–12.3. *Arch Ophthalmol.* 1999;117:805–810.
12. Eiberg H, Kjer B, Kjer P, Rosenberg T. Dominant optic atrophy (OPA1) mapped to chromosome 3q region. I: linkage analysis. *Hum Mol Genet.* 1994;3:977–980.
13. Jonasdottir A, Eiberg H, Kjer B, Kjer P Rosenberg T. Refinement of the dominant optic atrophy locus to a 1.4cM interval on chromosome 3q28-3q29, within a 3-Mb YAC contig. *Hum Genet.* 1997;99:115–120.
14. Alexander C, Votruba M, Pesch UEA, et al. *OPA1*, encoding a dynamin-related GTPase, is mutated in dominant optic atrophy linked to chromosome 3q28. *Nat Genet.* 2000;26:211–215.
15. Delettre C, Lenaers G, Griffoin J, et al. Nuclear gene *OPA1*, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy. *Nat Genet.* 2000;26:207–210.
16. Bleazard W, McCaffery JM, King EJ, et al. The dynamin-related GTPase DnmI regulates mitochondrial fission in yeast. *Nat Cell Biol.* 1999;1:298–304.
17. Pelloquin L, Belenguer P, Menon Y, Ducommun B. Identification of a fission yeast dynamin-related protein involved in mitochondrial DNA maintenance. *Biochem Biophys Res Commun.* 1998;251:720–726.
18. Van der Bliek AM. A mitochondrial division apparatus takes shape. *J Cell Biol.* 2000;151:F1–F4.
19. Pesch UEA, Leo-Kottler B, Mayer S, et al. *OPA1* mutations in patients with autosomal dominant optic atrophy and evidence for semi-dominant inheritance. *Hum Mol Genet*. 2001;10:1359–1368.
20. Orita M, Iwahara H, Kanazawa H, Hayashi K, Sekiya T. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. *Proc Natl Acad Sci USA*. 1989;86:2766–2770.
21. Keen J, Lester D, Inglehearn CF, Curtis A, Bhattacharya SS. Rapid determination of single base mismatches as heteroduplexes on Hydrolink gels. *Trends Genet.* 1991;7:5.
22. Williams GW, Woollard PM, Hingamp P. NIX: A nucleotide identification system at the Human Genome Mapping Project (HGMP)-Resource Center, Cambridge, UK; 1998; available at http://www.hgmp.mrc.ac.uk/NIX/. (Accessed September 2001.)
23. Taanman J-W, Bodnar AG, Cooper JM, et al. Molecular mechanisms in mitochondrial DNA depletion syndrome. *Hum Mol Genet.* 1997;6:935–942.
24. Thiselton DL, Alexander C, Morris A, et al. A frameshift mutation in exon 28 of the *OPA1* gene explains the high prevalence of dominant optic atrophy in the Danish population: evidence for a founder effect. *Hum Genet.* 2001;109:498–502.

25. Assink JJ, Tijmes NT, ten Brink JB, et al. A gene for X-linked optic atrophy is closely linked to the Xp11.4-Xp11.2 region of the X chromosome. *Am J Hum Genet.* 1997;61:934–939.
26. Mount SM. Genomic sequence, splicing, and gene annotation. *Am J Hum Genet.* 2000;67:788–792.
27. Forsberg L, de Faire U, Marklund SL, Andersson PM, Stegmayr B, Morgenstern R. Phenotype determination of a common Pro-Leu polymorphism in human glutathione peroxidase 1. *Blood Cells Mol Dis.* 2000;26:423–426.
28. Masellis M, Basile VS, Meltzer HY, et al. Lack of association between the T→C 267 serotonin 5-HT6 receptor gene (HTR6) polymorphism and prediction of response to clozapine in schizophrenia. *Schizophr Res.* 2001;47:49–58.
29. Del Porto G, Vingolo EM, Steindl K, et al. Clinical heterogeneity of dominant optic atrophy: the contribution of visual function investigations to diagnosis. *Graefes Arch Clin Exp Ophthalmol.* 1994; 232:717–727.
30. Maquat LE. The power of point mutations. *Nat Genet.* 2001;27: 5–6.
31. McNiven MA, Cao H, Pitts KR, Yoon Y. The dynamin family of mechanoenzymes: pinching in new places. *Trends Biochem Sci.* 2000;25:115–120.
32. Jones AC, Austin J, Hansen N, et al. Optimal temperature selection for mutation detection by denaturing HPLC and comparison to single-stranded conformation polymorphism and heteroduplex analysis. *Clin Chem.* 1999;45:1133–1140.
33. Metherell LA, Akker SA, Munroe PB, et al. Pseudoexon activation as a novel mechanism for disease resulting in atypical growth-hormone insensitivity. *Am J Hum Genet.* 2001;69:641–646.
34. Riordan-Eva P, Sanders MD, Govan GG, Sweeney MG, Da Costa J, Harding AE. The clinical features of Leber's hereditary optic neuropathy defined by the presence of a pathogenic mitochondrial mutation. *Brain* 1995;118:319–337.
35. Wallace DC, Singh G, Lott MT, et al. Mitochondrial DNA mutations associated with Leber's hereditary optic neuropathy. *Science* 1988; 242:1427–1430.
36. Votruba M, Moore AT, Bhattacharya SS. Demonstration of a founder effect and fine mapping of dominant optic atrophy locus on 3q28-qter by linkage disequilibrium method: a study of 38 British Isles pedigrees. *Hum Genet.* 1998;102:79–86.
37. Dipple KM, McCabe ER. Phenotypes of patients with "simple" Mendelian disorders are complex traits: thresholds, modifiers, and systems dynamics. *Am J Hum Genet.* 2000;66:1729–1735.
38. Vithana EN, Abu-Safieh L, Allen MJ, et al. A human homolog of yeast pre-mRNA splicing gene, *PRP31*, underlies autosomal dominant retinitis pigmentosa on chromosome 19q13.4 (*RP11*). *Mol Cell.* 2001;8:375–381.