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Genetic Screening for *OPA1* and *OPA3* Mutations in Patients with Suspected Inherited Optic Neuropathies

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Abstract

Purpose—Autosomal-dominant optic atrophy (DOA) is one of the most common inherited optic neuropathies, and it is genetically heterogeneous, with mutations in both *OPA1* and *OPA3* known to cause disease. About 60% of cases harbor *OPA1* mutations, whereas *OPA3* mutations have only been reported in two pedigrees with DOA and premature cataracts. The aim of this study was to determine the yield of *OPA1* and *OPA3* screening in a cohort of presumed DOA cases referred to a tertiary diagnostic laboratory.

Design—Retrospective case series.

Participants—One hundred and eighty-eight probands with bilateral optic atrophy referred for molecular genetic investigations at a tertiary diagnostic facility: 38 patients with an autosomal-dominant pattern of inheritance and 150 sporadic cases.

Methods—*OPA1* and *OPA3* genetic testing was initially performed using PCR-based sequencing methods. The presence of large-scale *OPA1* and *OPA3* genomic rearrangements was further assessed with a targeted comparative genomic hybridization (CGH) microarray platform. The three primary Leber hereditary optic neuropathy (LHON) mutations, m.3460G>A, m.11778G>A, and m.14484T>C, were also screened in all patients.

Main Outcome Measures—The proportion of patients with *OPA1* and *OPA3* pathogenic mutations. The clinical profile observed in molecularly confirmed DOA cases.

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Results—We found 21 different *OPA1* mutations in 27 of the 188 (14.4%) probands screened. The mutations included six novel pathogenic variants and the first reported *OPA1* initiation codon mutation at c.1A>T. An *OPA1* missense mutation, c.239A>G (p.Y80C), was identified in an 11-year-old African-American girl with optic atrophy and peripheral sensori-motor neuropathy in her lower limbs. The *OPA1* detection rate was significantly higher among individuals with a positive family history of visual failure (50.0%) compared with sporadic cases (5.3%). The primary LHON screen was negative in our patient cohort, and additional molecular investigations did not reveal any large-scale *OPA1* rearrangements or *OPA3* genetic defects. The mean baseline visual acuity for our *OPA1*-positive group was 0.48 logarithm of the minimum angle of resolution (LogMAR) (Mean Snellen equivalent = 20/61, range = 20/20–20/400, 95% confidence interval = 20/52–20/71), and visual deterioration occurred in 54.2% of patients during follow-up.

Conclusions—*OPA*1 mutations are the most common genetic defects identified in patients with suspected DOA, whereas *OPA3* mutations are very rare in isolated optic atrophy cases.

Introduction

Autosomal-dominant optic atrophy (DOA) is one of the most common inherited optic neuropathies seen in the general population, with a minimum prevalence of about 1 in 35,000.[1] DOA classically presents with bilateral, symmetric visual failure from early childhood, with the pathological hallmark being a selective loss of retinal ganglion cells, resulting in optic nerve degeneration.[2, 3] Linkage analysis of large multi-generational pedigrees has revealed genetic heterogeneity with five distinct autosomal loci reported in the literature (*OPA1*, *OPA3*, *OPA4*, *OPA5*, and *OPA7*), but to date, only the causative genes for *OPA1* (3q28-q29, Online Mendelian Inheritance in Man (OMIM) 165500) and *OPA3* (19q13.2-q13.3, OMIM 606580) have been identified.[4] It is now well established that the majority of families with DOA harbor pathogenic *OPA1* mutations, with yield rates of 50–60% in most case series.[1, 5, 6] However, *OPA3* mutations causing a dominantly inherited phenotype have only been described in two French families segregating optic atrophy in association with early-onset cataracts.[7] Interestingly, the Opa1 and Opa3 proteins share the same inner mitochondrial membrane location, and they are jointly involved in the regulation of mitochondrial oxidative phosphorylation, network maintenance, and the sequestration of pro-apoptotic cytochrome *c* molecules within the cristae.[7–9] Disturbed mitochondrial function is therefore central to the underlying disease process in DOA, which is also the defining pathophysiological feature observed in the other common inherited optic nerve disorder, Leber hereditary optic neuropathy (LHON, OMIM 535000).[2, 3] LHON affects between 1 in 31,000 and 1 in 39,000 adults in Northern Europe.[10, 11] It is a primary mitochondrial DNA (mtDNA) disorder, with three point mutations involving complex I subunits of the mitochondrial respiratory chain accounting for about 90% of all cases: m.3460G>A (*MTND1*), m.11778G>A (*MTND4*), and m.14484T>C (*MTND6*).[10, 11] Unfortunately, the complex mechanisms that ultimately preferentially target retinal ganglion cells in both DOA and LHON remain poorly defined.

Although genetic testing for DOA is becoming more widely available, diagnostic services still tend to remain centralized in tertiary academic centers due to patient referral patterns and the expertise required for the interpretation of molecular results. In this study, we describe our experience, at a single tertiary diagnostic facility, with screening for *OPA1* and *OPA3* mutations in patients with suspected inherited optic neuropathies, using standard polymerase chain reaction (PCR) sequencing methods and, more recently, with the application of comparative genomic hybridization (CGH) microarray technology.

Patients and Methods

Optic Atrophy Cohort

Our study cohort consisted of 188 patients with bilateral optic atrophy referred for molecular investigations to the Emory Genetics Laboratory, a tertiary diagnostic facility with College of American Pathologists (CAP), and Clinical Laboratory Improvement Amendments (CLIA) accreditations. All affected individuals had been assessed by experienced neuro-ophthalmologists (VB, NRM, NJN) over a six-year period running from January 1, 2004 to December 31, 2009. A comprehensive examination was performed including: (i) measurement of best-corrected Snellen visual acuity; (ii) assessment of visual fields by static (Humphrey) perimetry, kinetic (Goldmann) perimetry, or both; (iii) color discrimination with Ishihara or Hardy-Rand-Rittler pseudoisochromatic plates; and (iv) dilated fundoscopy. The majority of these patients had been investigated over several years, and extensive investigations, including neuroimaging, had not revealed a compressive, infiltrative or inflammatory etiology. Venous blood samples were collected from all affected individuals, and genomic DNA was extracted from peripheral blood leukocytes using established methods.[1] This study had the relevant institutional approval and complied with the Declaration of Helsinki.

Molecular Genetic Studies

The entire coding regions of the *OPA1* and *OPA3* genes, including flanking exon-intron boundaries, were PCR-amplified with optimised sets of oligonucleotide primers that are available on request. All PCR products were purified and sequenced with BigDye™ terminator cycle chemistries on an ABI3100 Genetic Analyzer (Applied Biosystems). The sequence chromatograms obtained were directly compared with the appropriate Genbank reference sequence using SeqScape™ software v2.1 (Applied Biosystems) and Mutation Surveyor™ (Softgenetics): (i) *OPA1* accession number AB011139 according to mRNA transcript variant 1, NM_011560, and (ii) *OPA3* accession numbers NM_001017989.2 (Transcript 1) and NM_025136.2 (Transcript 2).

All *OPA1* genetic variants identified were confirmed by reverse sequencing and checked against: (i) the eOPA1 Database (http://lbbma.univ-angers.fr/eOPA1/, accessed April 8, 2010); (ii) the Human Genome Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php), accessed April 8, 2010), and (iii) the National Center for Biotechnology Information (NCBI) Database (http://www.ncbi.nlm.nih.gov/gquery/?term=opa1, accessed April 8, 2010). Protein alignment analysis was used to establish the degree of evolutionary conservation at amino acid positions altered by putative novel *OPA1* mutations. The likelihood of a missense substitution being pathogenic was determined with the Polyphen prediction algorithm (http://genetics.bwh.harvard.edu/pph/, accessed April 8, 2010).

To further investigate cases in which PCR-based sequencing had failed to detect a pathogenic *OPA1* variant, we used a high-resolution CGH microarray to detect single and multiple exon deletions and duplications within the *OPA1* and *OPA3* gene regions. As described previously,[12] our assay has been validated on a NimbleGen 385K platform (Roche), using custom-designed intronic oligonucleotide probes to detect copy number variations within the genomic regions of interest and to determine the location of approximate breakpoints.

MtDNA fragments encompassing the primary LHON nucleotide positions: m.3460G>A, m.11778G>A, and m.14484T>C, were generated by PCR amplification of genomic blood DNA under standard cycling conditions. A restriction fragment length polymorphism

(RFLP) protocol was used to analyze the amplified PCR products, with genotyping based on the restriction digest patterns obtained.[13]

Statistical Analysis

The Chi-square test, the independent sample t-test, and one-way analysis of variance (ANOVA) were used for group comparisons with GraphPad™ v.4 statistical software (San Diego, CA). For the purpose of statistical analysis, Snellen ratios were converted to LogMAR (Logarithm of the minimum angle of resolution) decimal values.[14] Patients with visual acuities reduced to counting fingers (CF) were assigned a LogMAR value of 2.0.[15, 16]

Results

OPA1 Mutations Identified

Using our PCR-based sequencing methods, we identified 21 different *OPA1* mutations in 27 of the 188 probands (14.4%) screened: (i) missense (8/21, 38.1%), (ii) deletion (7/21, 33.3%), (iii) nonsense (4/21, 19.0%), (iv) duplication (1/21, 4.8%), and (v) splice site mutations (1/21, 4.8%) (Table 1, available at http://aaojournal.org). The mutational yield rate was significantly higher among individuals with a positive family history of visual failure (19/38, 50.0%) compared with presumed singleton cases (8/150, 5.3%) (Odds ratio = 17.75, 95% confidence interval (CI) = 6.83–46.13, P < 0.0001). Of the 27 *OPA1*-positive probands, 18 (66.7%) were white Caucasians, and nine (33.3%) were African-Americans.

Six of the 21 *OPA1* mutations found in our patient cohort had not been previously reported. Five of these novel variants were predicted to result in premature termination codons: c93_96dup(AAAA), c.655C>T, c.796_799del(GACA), c.2122del(A), and c.2537T>A. The c.1A>T substitution in exon 1 alters the canonical AUG initiation codon. Large-scale *OPA1* rearrangements were not detected with the targeted CGH microarray analysis in the remaining 161 *OPA1*-negative patients, and no patients harbored one of the three primary LHON mutations.

OPA1 Clinical Profile

Clinical data were available for 32 *OPA1*-positive patients, 17 men and 15 women, with a mean age of 33.1 years (Standard deviation (SD) = 17.9 years, range = 5.0–78.0 years) at the time of their most recent neuro-ophthalmological assessment. The mean age of onset of visual loss was 10.3 years (SD = 8.1 years, range = 1.0–35.0 years), with 90% of all patients becoming symptomatic before the age of 20 years (Figure 1). The mean number of pseudoisochromatic plates correctly identified was 5.4 (SD = 5.5, range = 0–14, 95% CI = 4.0–6.8), with three patients having completely normal color discrimination in both eyes. Visual fields were full in 16 of 64 eyes examined (25.0%). The defects identified in the remaining 48 eyes were classified as: (i) cecocentral (26/48, 54.2%), (ii) central (20/48, 41.6%), and (iii) supero-temporal (2/48, 4.2%). The appearance of the optic nerve head was abnormal in all *OPA1* cases, with diffuse disc pallor observed in 18/64 (28.1%) eyes and a prominent temporal wedge of pallor in the remaining 46/64 (71.9%) eyes. No patients had raised intraocular pressures, marked lenticular opacities, or significant macular or peripheral pigmentary changes.

The clinical profiles of two *OPA1*-positive probands are worthy of further description. A heterozygous missense mutation, c.239A>G (p.Y80C), in exon 2 of the *OPA1* gene was identified in an African-American girl with bilateral poor vision first noted at the age of 4 years when she failed a pre-school screening examination. Because of her family's financial constraints, she did not undergo a neuro-ophthalmological assessment until she was 11 years old, at which time her best-corrected visual acuity was 20/200 in both eyes (OU), with

reduced color perception, and bilateral central scotomas on kinetic perimetry. Her ocular motility and alignment as well as her hearing were normal. She had no gait, speech or cognitive disturbances, but there was clinical evidence of early peripheral sensori-motor neuropathy in her lower limbs. Folate and vitamin B12 levels in her serum were within normal range, and magnetic resonance imaging did not reveal any significant cerebellar or brainstem abnormalities. A retrospective review of clinical records revealed an extensive family history that was significant for several family members being affected with progressive gait ataxia and peripheral neuropathy starting in early adulthood. These neurological deficits affected the proband's mother, three maternal aunts, one maternal cousin, and her deceased maternal grandfather, consistent with an autosomal-dominant spinocerebellar ataxia (SCA) phenotype. Screening for an underlying SCA genetic defect was never performed on any of these affected individuals, and they were not available for further neuro-ophthalmological evaluation.

In the second case, a 43-year-old African-American proband with a paternal family history of poor vision was found to harbor a novel c.1A>T *OPA1* initiation codon mutation. The patient had been referred by his comprehensive ophthalmologist for further investigation of progressive, bilateral decreased vision, in the context of a presumptive diagnosis of normal-tension glaucoma. His best-corrected visual acuity was 20/50 OU, intraocular pressures were 13 mmHg in the right eye, 14 mmHg in the left eye, and kinetic perimetry showed bilateral cecocentral scotomas. Dilated fundoscopy revealed symmetrically cupped optic discs, with cup-to-disc ratios of 0.85 OU, and subtle pallor of the remaining neuroretinal rim. The remainder of his neurological examination was unremarkable.

Baseline and Long-Term Visual Acuity

The mean LogMAR visual acuity for our *OPA1*-positive cohort was 0.48 (Mean Snellen equivalent = 20/61, range = 20/20–20/400, 95% CI = 20/52–20/71). There was no statistically significant difference in mean LogMAR visual acuity between Caucasian and African-American patients (P = 0.1756) or between the various *OPA1* mutational subtypes (P = 0.1443). Longitudinal data were available for 24 patients. The mean follow-up duration for these patients was 8.5 years (SD = 9.0 years, range = 0.5–35.8 years), during which time best-corrected visual acuity worsened in 13 individuals (54.2%). The rate of visual loss ranged from 0.010 to 0.400 LogMAR/years, with a mean rate of 0.070 LogMAR/years (SD = 0.098 LogMAR/years). At their last follow-up visits, mean visual acuity for these 24 patients was 0.66 LogMAR (Mean Snellen equivalent = 20/91, range = 20/20-CF, 95% CI = 20/68–20/124). This was significantly worse compared with a mean visual acuity of 0.45 LogMAR at their baseline visits (Mean Snellen equivalent = 20/56, range = 20/20–20/200, 95% CI = 20/48–20/66, P = 0.0049) (Figure 2).

OPA3 Genetic Testing

OPA3 sequencing was performed for 123 *OPA1*-negative patients and revealed two previously reported, common single nucleotide polymorphisms: c.1–38g>a in the 5′ untranslated region,[17] and c.231T>C (p.A77A) in exon 2.[8] A subgroup of optic atrophy patients (N = 52) with a higher clinical index of suspicion for DOA was investigated further using our CGH assay, and no large-scale deletions or duplications involving *OPA3* exonic regions were identified.

Discussion

OPA1 comprises 30 coding exons spread over 100 Kb of genomic DNA. It is a highly polymorphic gene with more than 200 pathogenic variants known to cause disease.[18, 19] In this study, we further extend the mutational spectrum of *OPA1* and report six novel

mutations in families with non-syndromal optic atrophy. We also describe the first *OPA1* initiation codon mutation, c.1A>T, which is a rare molecular occurrence but has been reported in other inherited ocular disorders.[20–23] Interestingly, the affected proband had been labelled as having normal-tension glaucoma prior to his neuro-ophthalmological assessment – a common misdiagnosis among DOA patients, likely due to the frequently excavated appearance of the optic nerve cup.[24, 25] Abrogation of the highly conserved AUG code is expected to have a deleterious effect on mRNA translation, either requiring an alternative downstream start sequence or resulting in a null allele. The next AUG sequence is located 238 base pairs downstream from the original *OPA1* initiation codon, and if a viable mRNA transcript is translated, a severely truncated protein product would result (p.W2fsX13). It is more likely that this mRNA transcript would be unstable and would therefore be eliminated by nonsense-mediated decay mechanisms.[26] Both scenarios implicate haploinsufficiency as the underlying disease mechanism for this c.1A>T mutation.

Our overall *OPA1* detection rate was 14.4%, but this was significantly higher (50.0%) when only probands with a positive family history were considered, a figure comparable with previously published case series.[1, 5, 6] The remaining families are likely to harbor other as yet unidentified nuclear genes, DOA being a genetically heterogeneous disorder with multiple implicated autosomal loci.[4] The relatively low yield obtained for presumed singleton cases (5.3%) is not surprising, as this group is less homogeneous and more likely to have included non-genetic causes of optic atrophy.

Although the distinct clinical presentations of DOA and LHON usually allow for their easy differentiation, there is a degree of phenotypic overlap between these two most common inherited optic neuropathies. In rare cases, DOA patients have been described with an acute and even reversible disease course,[27, 28] and a proportion of LHON patients can present with slowly progressive visual loss.[29, 30] However, we excluded a primary LHON mutation as the causative molecular defect among the *OPA1*-negative patients in this study.

A major limitation of standard sequencing methods is the inability to detect genomic rearrangements, with large-scale deletions and duplications being major causes of human disease. In two reports, the frequency of *OPA1*-negative probands with these mutational subtypes varied between 10–20%,[1, 31] but these were highly selected familial subgroups with affected individuals spanning two or more generations. The multiplex ligation probe amplification (MLPA) kit used in these studies targeted 20 of the 30 *OPA1* exons, whereas our CGH platform is a more sensitive assay covering the entire coding region. Even so, we did not identify any *OPA1* deletions or duplications with this method, with the caveat that our cohort included a relatively high proportion of sporadic optic atrophy cases. Our findings therefore suggest that targeted screening of the *OPA1* gene is likely to represent a more cost-effective strategy. A search for large-scale rearrangements should be reserved primarily for patients with characteristic DOA features and a clear autosomal-dominant pattern of inheritance, after direct PCR-based sequencing has failed to identify any pathogenic *OPA1* variants.

Consistent with the natural history of DOA,[2–4] the vast majority of patients with *OPA1* mutations in this study became symptomatic in the first two decades of life, with visual deterioration occurring in about half of all cases during long-term follow-up. However, the rate of disease progression varied markedly in our cohort, and genetic counselling of *OPA1* carriers regarding their visual prognosis remains difficult. This is further compounded by the recent observation from a large multi-center survey that up to 20% of *OPA1* carriers develop significant neuromuscular complications, in addition to their bilateral optic nerve dysfunction.[32] These so-called DOA+ variants have been linked to the accumulation of secondary mtDNA abnormalities in skeletal muscle, with clinical presentations including

bilateral sensorineural hearing loss, ataxia, peripheral neuropathy, myopathy, progressive external ophthalmoplegia, or combinations of these features.[32–34] In our study, we identified a c.239A>G (p.Y80C) *OPA1* mutation in an 11-year-old African American girl with both visual failure and a peripheral sensori-motor neuropathy. No muscle biopsies were available for further investigations but the observed pattern of multi-system organ dysfunction is highly suggestive of an underlying mitochondrial biochemical defect, and optic neuropathy is a well-recognised feature of the autosomal-dominant spinocerebellar ataxias.[2, 35, 36] Unfortunately, other affected individuals from this three-generational pedigree were not available for further evaluation. Thus, segregation of this pathogenic *OPA1* variant with both optic atrophy and spinocerebellar degeneration requires additional confirmation. To date, the c.239A>G (p.Y80C) mutation has been identified only in DOA patients with pure optic nerve involvement (eOPA1 database,[18] Dr. Patrizia Amati-Bonneau, unpublished data).

Optic atrophy secondary to an *OPA3* mutation can manifest in two specific clinical settings, most commonly as an autosomal-recessive, relentlessly progressive neurodegenerative disease with 3-methylglutaconic aciduria (Costeff syndrome),[8, 17, 37] or, very rarely, as a milder autosomal-dominant disorder associated with the development of premature lenticular opacities.[7] Heterozygous *OPA3* point mutations causing DOA and cataracts have been identified in two unrelated French families in which functional studies of affected individuals showed increased susceptibility to apoptosis in cultured fibroblasts.[7] To our knowledge, ours is the first study to investigate systematically the frequency of *OPA3* abnormalities, including both large-scale rearrangements and point mutations, in DOA. Our findings indicate that *OPA3*-related disease in patients with isolated optic atrophy is very rare. However, despite the application of targeted CGH microarray technology, our screening protocols for *OPA1* and *OPA3* cannot entirely exclude the possibility that discrete mutations in the promoter or intronic regions have been missed, causing functional effects on gene expression and mRNA splicing.

Genetic testing can play a determining role in the investigation of patients with suspected inherited optic neuropathies, and the identification of an underlying molecular defect has important ramifications for patients who are often young and of reproductive age. Because the provision of a molecular diagnostic service is resource intensive, for patients with a clinical diagnosis of DOA, it seems reasonable to limit the initial screen to PCR-based sequencing methods that have lower running costs and that will detect the majority of pathogenic variants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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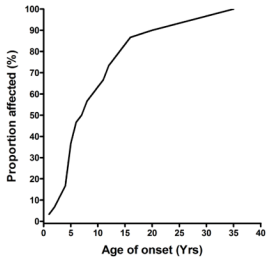

Figure 1.
Age of onset of visual failure among patients with confirmed *OPA1* mutations.

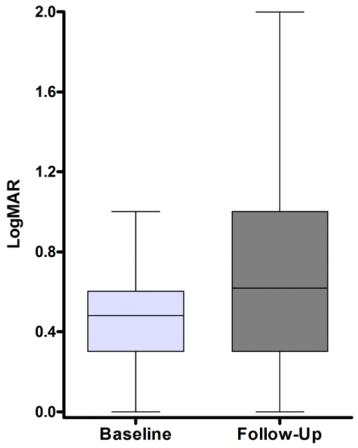

Figure 2.

Comparison of LogMAR visual acuity for *OPA1*-positive patients between their baseline and latest ophthalmological assessments (N = 24, P = 0.0049). The whiskers represent the minimum and maximum LogMAR values, the ends of the boxes are the upper and lower quartiles, the vertical length of the boxes indicate the interquartile range, and the line within the boxes represent the median LogMAR values for each group. LogMAR: Logarithm of the minimum angle of resolution.