Sporadic Bilateral Optic Neuropathy in Children: The Role of Mitochondrial Abnormalities

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Purpose. To evaluate a group of patients with isolated, early-onset, bilateral optic neuropathy for genetic and biochemical evidence of mitochondrial diseases.

Methods. This case–control study involved 21 patients, 159 control subjects for mitochondrial (mt)DNA sequencing, and 40 control subjects for relative mtDNA content. Patients were identified who had had decreased vision since childhood due to bilateral optic neuropathy characterized by central visual loss with no other major neurologic or ocular abnormality and no clinical evidence of a mitochondrial syndrome. Clinical examination, electroretinograms, and neuroimaging were performed; the entire mtDNA coding region was sequenced in leukocytes of all patients; relative mtDNA content was assessed; and *OPA1* and *OPA3* nuclear genes associated with dominant and recessive optic atrophy, respectively, were sequenced. Main outcome measures were clinical description, nonsynonymous (NS) mtDNA nucleotide changes, relative mtDNA content, and *OPA1* and *OPA3* nucleotide changes.

Results. Twenty-one unrelated patients (16 male and 5 female; mean age at first examination 13.6 years) had bilateral moderate, relatively symmetric optic neuropathies and normal neurologic examinations other than strabismus in 11 and congenital nystagmus in 9. Four patients had optic nerve hypoplasia. One patient had the nt 11778 primary Leber hereditary optic neuropathy (LHON) mutation, and three others had mtDNA nucleotide changes predicted to be pathologic. The entire group had a small increase (6.7%) in relative mtDNA content of indeterminate statistical significance. No patient had a polymorphism or mutation of *OPA1* or *OPA3*.

Conclusions. A minority of these young patients with sporadic bilateral optic neuropathy had abnormalities of the mitochondrial parameters evaluated. This bilateral optic neuropathy may be due to other genetic, epigenetic, or environmental injury to the optic nerve or to mitochondrial defects not studied. (*Invest Ophthalmol Vis Sci.* 2008;49:5250–5256) DOI:10.1167/iovs.08-2193

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Leber hereditary optic neuropathy (LHON) is a prototypical mitochondrial optic neuropathy and the most common mitochondrial syndrome worldwide.[1] Affected patients report acute or subacute, painless visual loss in one or both eyes leading to bilateral, relatively symmetric optic nerve disease with central visual loss and optic atrophy.[2] To be assigned this diagnosis, patients must have a multigenerational maternal family history; 90% of these individuals have a primary LHON mitochondrial (mt)DNA mutation (e.g., nt 11778, 3460, or 14484).[3] Patients with LHON-like optic neuropathy (LLON) also have subacute visual loss, relatively symmetric optic nerve disease, central visual loss, and optic atrophy in both eyes in the chronic state, but they do not report a multigenerational maternal family history. This group also has mitochondrial abnormalities, although fewer than 20% have primary LHON mutations.[4,5]

The differential diagnosis of optic neuropathy in childhood is extensive[6] and the expense and complexity of a complete diagnostic evaluation often precludes definitive diagnosis. Mitochondrial cytopathies,[7] including LHON,[8] may affect optic nerve function at an age too young for the individual to report loss of vision. These individuals, therefore, describe lifelong bilateral reduced vision rather than subacute visual loss. They are typically young when the disease is diagnosed and usually do not have a multigenerational maternal family history.

Given the strong association of bilateral, symmetric optic neuropathy with mitochondrial disorders, we decided to investigate whether sporadic bilateral optic neuropathies in children are associated with undiagnosed mitochondrial abnormalities.

Methods

Patient Enrollment

Patients were eligible for inclusion in this study if they (1) were 25 years of age or younger; (2) reported lifelong poor vision in both eyes without acute, subacute, or progressive visual changes; (3) had bilateral optic nerve disease without evidence of congenital glaucoma or congenital retinopathy, such as significant optic disc cupping, elevated intraocular pressure (IOP), buphthalmos, retinal pigmentary changes, or arcuate visual field loss; (4) had no other obvious familial, historical, or neuroimaging cause of optic nerve injury; (5) had no other major general medical, ophthalmologic, or neurologic disease; and (6) had no other clinical signs or symptoms of mitochondrial disease.

Exclusion criteria included (1) an abnormal neurologic history or examination (except for strabismus or congenital nystagmus), including birth trauma, developmental delay, mental retardation, or seizures; (2) a cause of significant visual loss in either eye independent of optic neuropathy; (3) evidence on history, examination, or neuroimaging of a medical, surgical, or syndromic cause of optic neuropathy; or (4) refusal to participate. Optic disc size and the potential presence of optic nerve hypoplasia (ONH) were not exclusion criteria. Patients were selected from the Neuro-ophthalmology Clinic at the King Khaled Eye Specialist Hospital, a major national referral site. Institutional review board (IRB)/Ethics Committee approval was obtained. The protocol adhered to the guidelines of the Declaration of Helsinki.

Hospital records were reviewed, and full neuro-ophthalmic examinations and dilated funduscopic examinations were performed on all

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patients. Color vision (CV) was assessed with Ishihara pseudoisochromatic plates. Patients had either Goldmann manual kinetic perimetry (Haag Streit International, Köniz-Bern, Switzerland); automated, white-on-white stimulus, static perimetry (Humphrey Field Analyzer II; Carl Zeiss Meditec, Inc., Dublin, CA); or both, if they were able to participate. Electroretinograms (ERGs) were performed on an evoked-potential system (Spirit; Nicolet Instrument Corp., Madison, WI), according to the manufacturer's suggested protocol. Brain neuroimaging was obtained on an MRI (Magnetom Allegra 3.0 Tesla) or CT (Somatom Sensation 4; Siemens, Munich, Germany) scanner.

Control Subjects

All control subjects were King Faisal Specialist Hospital and Research Centre blood donors who represented the spectrum of Saudi Arabs and who reported no symptomatic metabolic, genetic, or ocular disorders on an extensive questionnaire regarding family history, past medical problems, and current health. The control group for mtDNA sequencing consisted of 159 individuals (106 males and 53 females, mean age, 46.3 ± 3.8 years) and for relative mtDNA content, 40 different relatively young individuals (16 males and 24 females; mean age, 18.1 ± 2.1 years). Family information was obtained by history. All patients and control subjects were Saudi Arabs.

Sample Collection and DNA Extraction

A single-density gradient (Ficoll-Paque-PLUS; Pharmacia Biotech AB, Uppsala, Sweden) was used for lymphocyte isolation from peripheral blood, as detailed previously.[9] This method ensures a high yield of lymphocytes with little contamination of granulocytes or monocytes. DNA was extracted from whole blood samples of all patients and control subjects with a DNA isolation kit (Puregene; Gentra Systems, Minneapolis, MN).

Mitochondrial DNA Amplification and Sequencing

The entire coding region of the mitochondrial genome was amplified in 24 separate polymerase chain reactions (PCRs) using single set cycling conditions as detailed elsewhere[10] for all patients and control subjects. Primers were designed to avoid amplifying mtDNA-like sequences in the nuclear genome. Each successfully amplified fragment was directly sequenced (BigDye Terminator V3.1 Cycle Sequencing kit; Applied Biosystems, Inc. [ABI], Foster City, CA), and samples were run on the a sequencer (Prism 3100 sequencer; ABI).

Sequence Analysis of the Mitochondrial DNA Coding Region

The full mtDNA genome was sequenced except for the D-loop, and sequencing results were compared to the corrected Cambridge reference sequence.[11] All fragments were sequenced in both forward and reverse directions at least twice for confirmation of any detected variant. All nucleotide variants from both patients and control subjects were compared to the Mitomap database (last updated August 2007),[3] the Human Mitochondrial Genome Database (http://www.genpat.uu.se/mtDB/ provided in the public domain by the Section of Medical Genetics, Department of Genetics and Pathology, Uppsala University, Sweden; last updated November 2007), GenBank (http://www.ncbi.nlm.nih.gov/GenBank/index.html; last updated January 2008), and MedLine listed publications (GenBank and MedLine are provided in the public domain by the National Institutes of Health, Bethesda, MD). Reported homoplasmic synonymous or nonsynonymous (NS) polymorphisms associated with mitochondrial haplogroups[12] were excluded from further consideration.[13]

Prediction of Pathogenicity

Pathologic characteristics of each remaining nucleotide change in patients with PEG and control subjects were assessed according to a combination of standard criteria[14]; an evaluation of interspecies conservation using the PolyPhen database (http://genetics.bwh.harvard.edu/pph/ provided in the public domain by the Division of Genetics, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, and the Bork Group, EMBL, Heidelberg, Germany), and the Mamit-tRNA Web site (http://mamit-trna.u-strasbg.fr/index.html, provided in the public domain by the Institute of Molecular and Cell Biology, Strasbourg, France), when a sequence variant is detected in the tRNA region; assessment of the possible impact of an amino acid substitution on three-dimensional protein structure (Protean program, part of Lasergene ver. 6 software; DNAStar, Inc., Madison, WI), which predicts and displays secondary structural characteristics; and assessment of the possible effect of the mtDNA change on protein function using PolyPhen.[15] Therefore, an NS sequence change was considered potentially pathologic if it met all of the following criteria, When applicable: (1) It was not a haplogroup-determining polymorphism; (2) it was not reported in mitochondrial databases or available literature as an established polymorphism; (3) it was not found in at least 100 control subjects of matching ethnicity; (4) it changed a moderately or highly conserved amino acid; (5) Protean predicted an alteration of protein structure; and (6) it was assessed as possibly or probably pathologic by PolyPhen. For previously reported NS nucleotide changes, consideration was given to pathologic status determined by others and by mitochondrial databases in addition to these criteria.

Quantification of Heteroplasmy

Heteroplasmy level was determined for each heteroplasmic sequence variant by the primer extension assay described previously.[16] Heteroplasmy level was quantified from fluorescence intensities associated with electrophoretically resolved mutant and wild-type peaks (Genescan 3.7 software program; ABI). Percentage heteroplasmy was calculated using the following equation: [fluorescent band intensity for the mutant/(fluorescent band intensity for the wild-type + fluorescent band intensity for the mutant)] × 100.

Determination of Relative Mitochondrial DNA Content

Relative mitochondrial DNA content may be adjusted upward in certain tissues in the setting of compromised mitochondrial function.[17] Competitive multiplex PCR was performed with two simultaneous primer sets as described previously,[18] a technique that has been applied successfully to a variety of tissues,[19,20] including blood of patients with LHON[21] and several other optic neuropathies.[4,22,23] One pair was designed to amplify a 450 bp fragment of the ND1 mitochondrial gene and the other pair to amplify a 315-bp fragment of the β-actin nuclear gene, which served as an internal control. Control subjects were run simultaneously with patients. PCR products were separated on 1% agarose gel at 100 V for 1 hour, and the intensity of the two bands was quantified by the use of gel imager (Typhoon 9410; GE-Biosciences, Schenectady, NY). The ratio of ND1 to β-actin was determined for each patient and control by dividing the fluorescence intensity of the ND1 band by the intensity of the β-actin band.

Sequence Analysis of *OPA1* and *OPA3* Genes

The 31 coding exons, exon–intron boundaries, and promoter regions of the OPA1 gene were amplified by PCR from genomic DNA for all patients and subjected to direct sequencing as described previously.[24] The whole *OPA3* gene was sequenced in all patients by using the protocol described previously.[25]

Statistical Methods

All statistical analyses were performed with commercial software (SPSS for Windows, ver. 15.0; SPSS Inc, Chicago, IL). Snellen visual acuities were converted to ordinal values, and CV was quantified on an equal interval scale as the number of a possible 10 Ishihara color plates identified with each eye. Statistical comparisons included bivariate

TABLE 1. Clinical Characteristics and Neuroimaging Results

Patient	Sex	Age	Family History	CT	MRI	Neuroimaging Results
1	M	25	No	Yes	Yes	Small ONs and chiasm
2	M	17	No	Yes	Yes	Normal ONs and chiasm
3	F	14	No	Yes	No	Poor views of ONs
4	F	23	No	Yes	No	Normal
5	M	16	Yes	Yes	Yes	Tortuous left ON
6	M	9	No	No	Yes	Normal
7	F	6	No	No	Yes	Slightly small ONs and chiasm
8	F	14	Yes	No	Yes	Mild ON and chiasmal hypoplasia
9	M	25	No	No	Yes	Normal
10	M	11	No	No	Yes	Small ONs
11	M	6	No	Yes	Yes	Normal
12	M	12	No	Yes	No	Small ONs
13	M	18	Yes	Yes	No	Poor views but grossly normal
14	M	16	No	Yes	No	Normal
15	M	14	No	Yes	Yes	Small ONs and chiasm
16	M	20	No	Yes	Yes	Very small ON and chiasm
17	M	9	No	No	Yes	Normal
18	F	12	Yes	Yes	Yes	Normal ONs but small chiasm
19	M	10	No	Yes	Yes	Normal ONs and chiasm
20	M	3	Yes	No	No	Not done (affected brother with normal MRI)
21	M	5	No	Yes	No	Normal

Family history, family history of poor vision; CT, computed tomography performed; MRI, magnetic resonace imaging performed; ON, optic nerve.

correlation, independent-samples *t*-test, and the Fisher exact analysis. Bonferroni correction was applied where appropriate.

RESULTS

Clinical Characteristics

We identified 21 unrelated patients (16 male and 5 female; mean age at first examination, 13.6 ± 6.3 years) with early onset optic neuropathy who met inclusion and exclusion criteria. No patient had any other significant general medical problem, such as type 1 diabetes mellitus, pigmentary retinopathy, ptosis, cataract, restricted ocular motility, deafness, ataxia, diffuse weakness, or somatic anomalies; or reported myotonia, exercise intolerance, palpitations, syncope, or cardiac conduction abnormalities. All patients had normal erythrocyte sedimentation rates, antinuclear antibodies, and syphilis serology. ERG was normal in all 21 patients.

Table 1 includes clinical characteristics and neuroimaging results of these individuals. Ten patients (48%) had consanguineous parents, but this prevalence of consanguinity is not unusual in the region, and consanguinity did not correlate with other clinical parameters. Five patients described a family history of poor vision, but this typically consisted of an isolated individual who wore glasses. None had an obvious multigenerational or maternal inheritance pattern. Family members were not examined or evaluated genetically. All patients had brain neuroimaging, including computed tomography (CT) in 14 and magnetic resonance imaging (MRI) in 14. No scan revealed a mass, disseminated demyelination, or a developmental anomaly of the brain that might provide an alternative explanation for poor vision. In general, the pregeniculate afferent visual system appeared somewhat small but otherwise intact.

The patients were cognitively normal without a history of developmental delay, seizures, or cerebral palsy and without a focal abnormality on neurologic examination outside of the visual system. No patient had a developmental abnormality of the anterior or posterior globe. Table 2 details neuro-ophthalmic examinations. Visual acuity (VA) ranged from 20/30 to CF at 3′ with a mean of 20/200. The VAs in an individual's two eyes correlated strongly ($r = 0.931$; $P < 0.0001$). Mean CV was less than 3 of 10 pseudoisochromatic plates, and color vision correlated strongly with VA ($r = 0.51$; $P = 0.003$). Most patients had flat, pale optic disks typical of optic atrophy (OA) that were roughly symmetric in appearance in the two eyes (Fig. 1A). Some fundi had an appearance reminiscent of dominant OA (Fig. 1B), but four (patients 3, 4, 11, and 15) had small optic disks (Fig. 1C) more typical of ONH. Optic disc diameter did not correlate with VA, color vision, or ocular motility. Dilated funduscopic examination revealed no obvious pigmentary retinopathy, macular disruption, or other retinal abnormality that might explain poor vision. No patient had congenital cataracts, elevated IOP in either eye, or an optic disc appearance or visual field loss more typical of glaucoma. No patient who could perform confrontation or formal VFs had a major arcuate or altitudinal VF defect. Some patients with moderately reduced VA in both eyes had no central scotoma documented on Goldmann VF. Goldman VF and tangent screen testing sometimes fail to detect central VF loss in metabolic optic neuropathies,[26–28] and this medically unsophisticated population, some of whom had congenital nystagmus, also had relatively poor fixation. All patients had full ocular motility, although nine had some degree of strabismus (four with esodeviation and five with exodeviation), one had dissociated vertical deviation in both eyes, and nine had congenital nystagmus of varying amplitude. The presence of congenital nystagmus was modestly correlated with color vision ($r = 0.445$; $P = 0.011$), but this result is best considered informative rather than definitive given the number of statistical tests performed. Congenital nystagmus was not correlated with VA, optic disc diameter, or strabismus.

Detection of Mitochondrial Abnormalities

Table 3 lists the 11 NS mtDNA sequence variants detected in these patients that had not been reported as haplogroup-specific polymorphisms,[13] nine of which were transitions and two transversions. Five of these were novel, whereas no novel mtDNA sequence change was present in control subjects.

TABLE 2. Neuro-ophthalmic Examination

Patient	VA		Color		Fundi	VF	Ocular Alignment	Nystagmus
	OD	OS	OD	OS				
1	20/80	20/60	1	1	Diffuse temporal pallor OU with large optic cups	Central scotoma OD, ? OS	Orthophoric	None
2	20/50	20/40	9	9	Mild temporal pallor OU	Small central scotomas OU	Orthophoric with DVD OU	Small amplitude horizontal pendular
3	20/200	20/200	5	5	Small discs without pallor	Full to Goldmann OU	Modest ET	Small amplitude horizontal pendular with latent
4	20/400	CF 5′	0	1	Small, pale discs OU	Full to Goldmann OU	Orthophoric	None
5	20/200	20/100	3	3	Diffuse optic atrophy OD>OS	Large blind spots OU	Orthophoric	None
6	20/30	20/50	4	1	Diffuse optic atrophy OU	Full to confrontation OU	Mild EP	None
7	CF 3′	CF 3′			Moderate diffuse optic atrophy OU	Unable OU	Orthophoric	None
8	20/80	20/80	1	1	Diffuse optic atrophy OU	Full to GVF OU	Orthophoric	None
9	20/80	20/80	3	3	Moderate temporal pallor OU	Tiny central scotoma OS	Orthophoric	Minimal amplitude horizontal pendular
10	CF 5′	CF 5′	0	0	Diffuse optic atrophy OU	Central scotomas OU	Orthophoric	None
11	20/200	20/100			Small discs with diffuse optic atrophy OU	Unable OU	Modest XT	Moderate amplitude horizontal pendular
12	CF 5′	CF 5′	0	0	Diffuse optic atrophy OU	Unable OU	Modest ET	None
13	20/100	20/100	6	6	Mild temporal pallor OU	Full to Goldmann OU	Orthophoric	Small amplitude horizontal pendular
14	20/100	20/200	5	4	Wedge-shaped temporal pallor OU with large optic cups	Full to Goldmann OU	Mild EP	None
15	20/400	CF 3′	0	0	Severe ONH OS with increased pallor inferiorty	Large cecocentral scotomas OU	Modest XT	None
16	20/200	20/400	0	0	Mild temporal pallor OU	Central scotomas OU	Orthophoric	Modest amplitude horizontal pendular
17	20/200	20/200	6	9	Diffuse optic atrophy OU	Full to confrontation	Orthophoric	None
18	20/200	20/200			Diffuse optic atrophy OU	Cecocentral scotomas OU	Modest XT	Small amplitude horizontal pendular
19	20/200	20/200			Wedge-shaped temporal pallor with large cups	Full to confrontation OU	Orthophoric	Modest amplitude horizontal pendular
20	Poor	Poor	0	0	Diffuse optic atrophy OU	Unable OU	Modest XT	None
21	20/100	20/100			Mild temporal pallor	Unable OU	Modest XT	Modest amplitude, slow horizontal pendular

Color, color vision, number of Ishihara plates identified of a possible 10; CF, count fingers VA at distance indicated; Poor, unable to fixate and follow; ET, esotropia; EP, esophoria; XT, exotropia; DVD, dissociated vertical deviation.

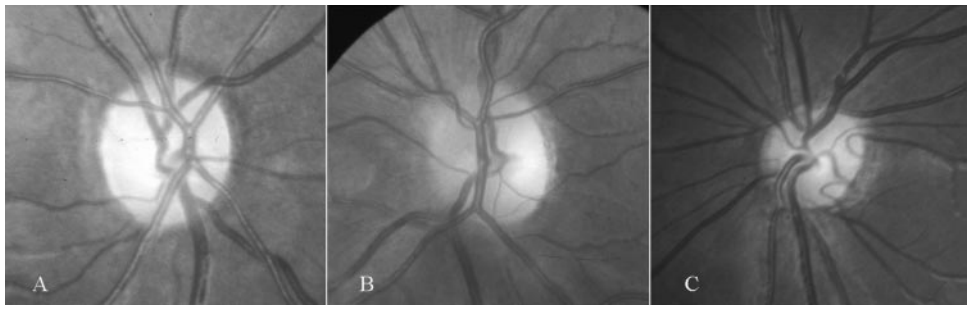

FIGURE 1. (**A**) Right optic disc of patient 6 with diffuse pallor and nerve fiber layer loss. (**B**) Left optic disc of patient 14 with wedge-shaped temporal pallor reminiscent of dominant optic atrophy with predominantly temporal nerve fiber layer loss. (**C**) Left optic disc of patient 3 with optic nerve hypoplasia and striking temporal and nasal nerve fiber layer loss.

TABLE 3. Analysis of Nonsynonymous Sequence Changes

Nucleotide Substitution	AA Substitution	Location	Base Substitution Type	Controls (%)	Heteroplasmy (%)	Novel	Interspecies Conservation	Protean Prediction	Polyphen Prediction	Summary
3236 A>G	—	In the acceptor stem of tRNA leucine	Transition	0	N/A	Yes	High	N/A	N/A	Pathologic
4640 C>A	57 Ile>Met	Outside the TM domain of *ND2* gene	Transversion	0	N/A	No	Low	No	Benign	Nonpathologic
4960 C>T	164 Ala>Val	In the TM domain of *ND2* gene	Transition	1.9	N/A	No	Low	No	Benign	Nonpathologic
5098 T>G	210 Ile>Ser	In the TM domain of *ND2* gene	Transversion	0	30	Yes	Low	No	Benign	Nonpathologic
7520 G>A	—	In the acceptor stem of tRNA Aspartic acid	Transition	0	N/A	Yes	High	—	—	Pathologic
8405 A>G	14 Thr>Ala	In the TM domain of *ATPase8* gene	Transition	0	N/A	Yes	Low	No	Benign	Nonpathologic
8460 A>G	32 Asn>Ser	In the TM domain of ATPase8 gene	Transition	0	30	No	Low	No	Benign	Nonpathologic
9544 G>A	113 Gly>Glu	Outside TM domain of COIII	Transition	0	N/A	No	High	Yes	Probably damaging	Pathologic
10611A>G	48 Thr>Ala	In the TM domain of ND4L gene	Transition	0	45	Yes	Low	No	Benign	Nonpathologic
11696G>A	313 Val>Ile	In the TM domain of ND4 gene	Transition	0	N/A	No	Low	No	Benign	Nonpathologic
11778G>A	340 Arg>His	Functional domain of ND4 gene	Transition	0	N/A	No	High	Yes	Probably damaging	Pathologic

Base substitution type: Transversion, a mutation in which a purine/pyrimidine replaces a pyrimidine/purine base pair or vice versa (G:C > T:A or C:G, or A:T > T:A or C:G); Transition, a mutation in which a purine/pyrimidine base pair is replaced with a base pair in the same purine/pyrimidine relationship (A:T > G:C or C:G > T:A). Controls (%), percentage of controls with this nucleotide substitution. Previous reports of sequence variants were found in the MITOMAP database, the Human Mitochondrial Genome Database (http://www.genpat.uu.se/mtDB), GenBank (http://www.ncbi.nlm.nih.gov/Genbank/index.html), and MedLine listed publications. Interspecies conservation was assessed with PolyPhen (http://genetics.bwh.harvard.edu/pph/), which determines interspecies conservation for an altered amino acid by performing alignment with all available amino acid sequences for other species, and the Mamit-tRNA website (http://mamit-trna.u-strasb.f) when the sequence variant was in a tRNA region. Protean predicts and displays secondary structural characteristics. Yes, nucleotide change will alter protein secondary structure; No, change will not alter secondary structure. PolyPhen prediction of pathogenicity was assessed utilizing the PolyPhen (Polymorphism Phenotyping) database (http://genetics.bwh.harvard.edu/pph/). Probably damaging, a high confidence of affecting protein function or structure; possibly damaging, a likelihood of affecting protein function or structure; benign, changes that most likely lack phenotypic effect; unknown, PolyPhen could not make a prediction due to lack of data. None of these nucleotide changes was heteroplasmic. Summary, see Prediction of Pathogenicity in Methods. TM, transmembrane; N/A, not applicable, because the database is not designed to predict this type of sequence change. Reported haplogroup specific NS sequence changes were excluded from this table and further analyses.

Three sequence changes were present in a heteroplasmic state with heteroplasmy levels less than 50%, and all these were considered nonpathologic.[29] The nt 11778 primary LHON mutation was present in one patient, and three other NS mtDNA nucleotide changes were considered potentially pathologic because they changed moderately or highly conserved amino acids and were predicted to alter the corresponding protein structure and function (see the Methods section). Two of the potentially pathologic changes were among the five novel mtDNA nucleotide alterations.

Table 4 details by patient all NS mtDNA nucleotide changes in Table 3. Eleven patients had no mtDNA sequence change other than previously described polymorphisms (see the Methods section; patients 1–11) and six patients had only NS mtDNA sequence changes predicted to be benign (patients 12–17). The remaining four patients (patients 18–21) each had a single nucleotide change that was predicted to be pathologic. Patient 18 had the nt 11778 primary LHON mutation, even though she reported lifelong poor vision with no acute episode of visual loss in either eye. She had one brother with poor vision but denied a multigenerational maternal family history. The presence of mtDNA nucleotide changes predicted to be pathologic did not correlate with VA, CV, optic disc size, or the presence of strabismus or congenital nystagmus.

Table 4 also details relative mtDNA content for each patient. Mean relative mtDNA content was slightly greater in patients (1.87 ± 0.23; 95% confidence interval [CI], 1.76–1.97) than in control subjects (1.74 ± 0.20; 95% CI, 1.70–1.80; $P = 0.046$). This 6.7% difference is statistically nonsignificant after Bonferroni correction and is best interpreted as indeterminate, given the post hoc indication of a minimum sample size approximately three times that studied to attain 80% power of avoiding false-negative interpretation. Nevertheless, patients 12, 13, and 18 had relative mtDNA content more than 2 SD above the control mean. Relative mtDNA content was not different between patients with no mtDNA nucleotide changes, patients with mtDNA nucleotide changes predicted to be nonpathologic, and patients with mtDNA nucleotide changes predicted to be pathologic. Relative mtDNA content level did not correlate with VA, CV, optic disc size, or the presence of strabismus or congenital nystagmus. The four patients with ONH (3, 4, 11, and 15) had no mtDNA changes predicted to be pathogenic and relative mtDNA content levels not far above the normal 95% CI.

Table 4. Mitochondrial DNA Nucleotide Changes and Relative mtDNA Content by Patient

Patient	Nucleotide Changes	Relative mtDNA Content
1	None	2.1
2	None	1.89
3	None	1.84
4	None	1.78
5	None	1.76
6	None	1.68
7	None	1.76
8	None	1.69
9	None	1.71
10	None	1.75
11	None	1.78
12	11696	2.2
13	8460	2.28
14	5098, 8405	1.95
15	4640	1.85
16	10611	1.68
17	4960	1.74
18	11778	2.52
19	9544	1.58
20	7520	1.81
21	3236	1.83

Patients are organized according to characteristics of nucleotide substitutions from Table 3 for each patient. Patients 1–11 had no noteworthy mtDNA changes; patients 12–17 had NS mtDNA changes not thought to be pathologic; patients 18– 21 had NS mtDNA changes thought likely to be pathologic. Relative mtDNA Content, ratio of ND1 to β-actin.

Sequence Analysis of *OPA1* and *OPA3* Genes

No polymorphisms or mutations were found in either the *OPA1* or the *OPA3* gene in any patient, and control subjects had only established polymorphisms reported previously.[22]

Discussion

We report 21 young, unrelated individuals with decreased vision in both eyes since early childhood due to bilateral, symmetric optic nerve disease. Anterior and posterior globes were normal other than optic disc appearance, and mean VA was approximately 20/200 with a range from 20/30 to counting fingers. The group included four patients with ONH, but optic disc diameter did not correlate with afferent or efferent visual function. Neurologic examination was otherwise normal except for the presence of strabismus in slightly more than half and congenital nystagmus in slightly less than half. Neuroimaging was unremarkable except for small optic nerves and chiasm in some.

These patients have an unclassified form of optic neuropathy. The diagnosis of LHON or LLON was inappropriate on both clinical and genetic grounds. They did not have developmental delay or obvious neurologic disease outside of the optic nerve on examination or neuroimaging. They did not have progressive visual loss or a mutation in *OPA1* or *OPA3* that would imply the diagnosis of dominant or recessive OA. They also did not have a mitochondrial cytopathy,[7] or an obvious syndromic or metabolic optic neuropathy.[30] Rather, this study describes a group of patients who incurred sporadic, moderate, symmetrical optic neuropathy in utero or in the first several years of life. It seems likely that strabismus and congenital nystagmus were secondary to poor vision.

Even though we excluded patients with systemic signs of overt mitochondrial dysfunction, we found that patients 18 to 21 had pathologic or potentially pathologic mtDNA nucleotide changes and patients 11, 12, and 18 had substantially elevated relative mtDNA content. In fact, one female with lifelong poor vision (patient 18) had the nt 11778 primary LHON mutation with bilateral moderate optic nerve injury. These observations indicate that mitochondrial function may be abnormal in a portion of patients with this clinical presentation.

The pathologic role of mitochondrial abnormalities in spontaneous optic neuropathies has become increasingly evident over the past two decades.[1] The patients described in this report have a comparable severity of symmetric optic nerve injury as patients with LHON and LLON but report lifelong rather than subacute visual loss. However, the mitochondrial changes documented were less frequent and severe than mitochondrial abnormalities found in a similar evaluation of patients with LLON[4] or in other spontaneous optic neuropathies such as nonarteritic ischemic optic neuropathy,[10,31] primary open-angle glaucoma,[22] and optic neuritis.[32] These observations, if confirmed, provide additional perspective regarding the range of influence of mitochondrial abnormalities in the pathogenesis of spontaneous optic neuropathies and suggest a useful clinical guideline for predicting the likelihood of mitochondrial disease based on the timing of optic nerve injury.

The current nosology of congenital, nonhereditary, optic neuropathies distinguishes small optic nerves (termed ONH) from pale optic nerves (termed OA).[6] This classification assumes that ONH generally arises from a prenatal perturbation of the developing visual system and receives support from the frequent association of ONH with other CNS developmental malformations.[33] By contrast, OA is considered a sign of either postnatal or late intrauterine injury,[6] where only a fraction of patients have small optic disks. Only four patients in this group had small optic discs, but our entire patient group had a similar distribution of visual acuity and a similar incidence of strabismus and nystagmus as reported in patients with ONH.[34] The four patients with ONH did not differ from the other 17 with regard to visual function or identified mitochondrial abnormalities. No patient with ONH had a mtDNA nucleotide change predicted to be pathologic or strikingly elevated relative mtDNA content. Our patient numbers are small, but these results may imply that patients with ONH are relatively unlikely to have a mitochondrial mechanism to their optic nerve disease. In reality, ONH is often accompanied by some degree of atrophy, and the clinical significance of the distinction between ONH and congenital OA is still ambiguous.[6]

This study evaluated only two mitochondrial parameters (sequencing the mitochondrial genome and measuring relative mtDNA content) in a relatively small number of patients from one ethnic group, and results reported herein may not be pertinent to patients from other ethnic groups. In addition, we may have failed to detect mitochondrial abnormalities of nuclear origin or environmental derangements affecting mitochondrial function during development. Therefore, our findings require confirmation by studying these and other mitochondrial parameters in other ethnic groups before they can be more generally applied in predicting likelihood of mitochondrial disease. Nevertheless, these observations provide insight into the limited influence of mitochondrial abnormalities in the pathogenesis of sporadic, childhood-onset, bilateral optic neuropathy.

References

1. Newman NJ. Hereditary optic neuropathies: from the mitochondria to the optic nerve. *Am J Ophthalmol.* 2005;140:517–523.
2. Newman NJ. From genotype to phenotype in Leber hereditary optic neuropathy: still more questions than answers. *J Neuroophthalmol.* 2002;22:257–261.
3. Brandon MC, Lott M, Nguyen KC, et al. MITOMAP: A human mitochondrial genome database: 2004 update (database issue). URL: http://www.mitomap.org. *Nucleic Acids Res.* 2005;33:D611–D613.
4. Abu-Amero KK, Bosley TM. Mitochondrial abnormalities in patients with LHON-like optic neuropathies. *Invest Ophthalmol Vis Sci.* 2006;47:4211–4220.
5. Dogulu CF, Kansu T, Seyrantepe V, Ozguc M, Topaloglu H, Johns DR. Mitochondrial DNA analysis in the Turkish Leber's hereditary optic neuropathy population. *Eye* (*Lond*). 2001;15:183–188.
6. Hoyt CS, Good WV. Do we really understand the difference between optic nerve hypoplasia and atrophy? *Eye* (*Lond*). 1992;6:201–204.
7. Taban M, Cohen BH, David Rothner A, Traboulsi EI. Association of optic nerve hypoplasia with mitochondrial cytopathies. *J Child Neurol.* 2006;21:956–960.
8. Barboni P, Savini G, Valentino ML, et al. Leber's hereditary optic neuropathy with childhood onset. *Invest Ophthalmol Vis Sci.* 2006;47:5303–5309.
9. Abu-Amero KK, Bosley TM. Detection of mitochondrial respiratory dysfunction in circulating lymphocytes using resazurin. *Arch Pathol Lab Med.* 2005;129:1295–1298.
10. Bosley TM, Abu-Amero KK, Ozand PT. Mitochondrial DNA nucleotide changes in non-arteritic ischemic optic neuropathy. *Neurology.* 2004;63:1305–1308.
11. Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. *Nat Genet.* 1999;23:147.
12. Abu-Amero KK, Gonzalez AM, Larruga JM, Bosley TM, Cabrera VM. Eurasian and African mitochondrial DNA influences in the Saudi Arabian population. *BMC Evolutionary Biol.* 2007;7:32.
13. Bandelt HJ, Salas A, Bravi CM. What is a 'novel' mtDNA mutation: and does 'novelty' really matter? *J Hum Genet.* 2006;51:1073–1082.
14. Chinnery PF, Howell N, Andrews RM, Turnbull DM. Mitochondrial DNA analysis: polymorphisms and pathogenicity. *J Med Genet.* 1999;36:505–510.
15. Sunyaev S, Ramensky V, Koch I, Lathe W 3rd, Kondrashov AS, Bork P. Prediction of deleterious human alleles. *Hum Mol Genet.* 2001;10:591–597.
16. Fahy E, Nazarbaghi R, Zomorrodi M, et al. Multiplex fluorescence-based primer extension method for quantitative mutation analysis of mitochondrial DNA and its diagnostic application for Alzheimer's disease. *Nucleic Acids Res.* 1997;25:3102–3109.
17. Lee HC, Wei YH. Mitochondrial biogenesis and mitochondrial DNA maintenance of mammalian cells under oxidative stress. *Int J Biochem Cell Biol.* 2005;37:822–834.
18. Kao SH, Chao HT, Liu HW, Liao TL, Wei YH. Sperm mitochondrial DNA depletion in men with asthenospermia. *Fertil Steril.* 2004;82:66–73.
19. Wu CW, Yin PH, Hung WY, et al. Mitochondrial DNA mutations and mitochondrial DNA depletion in gastric cancer. *Genes Chromosomes Cancer.* 2005;44:19–28.
20. Taanman JW, Bodnar AG, Cooper JM, et al. Molecular mechanisms in mitochondrial DNA depletion syndrome. *Hum Mol Genet.* 1997;6:935–942.
21. Yen MY, Chen CS, Wang AG, Wei YH. Increase of mitochondrial DNA in blood cells of patients with Leber's hereditary optic neuropathy with 11778 mutation. *Br J Ophthalmol.* 2002;86:1027–1030.
22. Abu-Amero KK, Morales J, Bosley TM. Mitochondrial abnormalities in patients with primary open-angle glaucoma. *Invest Ophthalmol Vis Sci.* 2006;47:2533–2541.
23. Abu-Amero KK, Bosley TM. Increased relative mitochondrial DNA content in leucocytes of patients with NAION. *Br J Ophthalmol.* 2006;90:823–825.
24. Thiselton DL, Alexander C, Taanman JW, et al. A comprehensive survey of mutations in the OPA1 gene in patients with autosomal dominant optic atrophy. *Invest Ophthalmol Vis Sci.* 2002;43:1715–1724.
25. Anikster Y, Kleta R, Shaag A, Gahl WA, Elpeleg O. Type III 3-methylglutaconic aciduria (optic atrophy plus syndrome, or Costeff optic atrophy syndrome): identification of the OPA3 gene and its founder mutation in Iraqi Jews. *Am J Hum Genet.* 2001;69:1218–1224.
26. Kline LB, Glaser JS. Dominant optic atrophy: the clinical profile. *Arch Ophthalmol.* 1979;97:1680–1686.
27. Hoyt CS. Autosomal dominant optic atrophy: a spectrum of disability. *Ophthalmology.* 1980;87:245–251.
28. Brodsky MC, Baker RS, Hamed LM. *Pediatric Neuro-ophthalmology*. New York: Springer-Verlag; 1996.
29. Taylor RW, Taylor GA, Morris CM, Edwardson JM, Turnbull DM. Diagnosis of mitochondrial disease: assessment of mitochondrial DNA heteroplasmy in blood. *Biochem Biophys Res Commun.* 1998;251:883–887.
30. Sadun AA. Metabolic optic neuropathies. *Semin Ophthalmol.* 2002;17:29–32.
31. Abu-Amero KK, Bosley TM. Mitochondrial DNA abnormalities in NAION. *Br J Ophthalmol.* 2007;91:1561.
32. Bosley TM, Constantinescu CS, Tench CR, Abu-Amero KK. Mitochondrial changes in leukocytes of patients with optic neuritis. *Mol Vis.* 2007;13:1516–1528.
33. Brodsky MC. Congenital optic disk anomalies. *Surv Ophthalmol.* 1994;39:89–112.
34. Garcia ML, Ty EB, Taban M, David Rothner A, Rogers D, Traboulsi EI. Systemic and ocular findings in 100 patients with optic nerve hypoplasia. *J Child Neurol.* 2006;21:949–956.