Mitochondrial Abnormalities in Patients with LHON-like Optic Neuropathies

Khaled K. Abu-Amero[1] *and Thomas M. Bosley*[2,3,4]

Purpose. To investigate certain biochemical and molecular characteristics of mitochondria in patients with Leber hereditary optic neuropathy (LHON)-like optic neuropathies.

Methods. Patients who had LHON-like optic neuropathies in both eyes were selected from neuro-ophthalmology clinics. Evaluation included clinical examination, neuroimaging, and assessment of several mitochondrial parameters in the blood, including sequencing the entire mitochondrial (mt)DNA coding region, measuring relative mtDNA content, studying mitochondrial respiratory function in some patients, and sequencing the *OPA1* and *OPA3* genes.

Results. Thirty-five patients (21 men and 14 women; average age at onset 19.0 ± 8.7 years) met inclusion and exclusion criteria for LHON-like optic neuropathies with median visual acuity approximately 20/200. Other hereditary retinopathies and optic neuropathies were unlikely because of inclusion and exclusion criteria, because ERGs were normal, and because no patient had pathogenic sequence changes in the *OPA1* or *OPA3* genes. Compared with control subjects, these patients had more potentially pathogenic nonsynonymous mtDNA changes, greater relative mtDNA content ($P < 0.001$), and less mitochondrial respiratory activity ($P < 0.001$). Only six patients (17%) had primary LHON mutations; however, even the 29 patients without primary LHON mutations had significant evidence of mitochondrial abnormalities. Mitochondrial haplogroup distribution was similar in patients and control subjects.

Conclusions. Primary LHON mutations are less common in patients with LHON-like optic neuropathy selected from a clinical setting than in patients with LHON from multigenerational families. The results suggest that mitochondrial dysfunction plays a role in this type of optic neuropathy whether or not primary LHON mutations are present. This information has implications for diagnostic testing and for future investigations into mechanisms of disease. (*Invest Ophthalmol Vis Sci.* 2006; 47:4211–4220) DOI:10.1167/iovs.06-0295

Leber hereditary optic neuropathy (LHON) is one of the most common hereditary optic neuropathies. It was initially recognized as a maternally inherited optic neuropathy causing roughly symmetric, subacute visual loss in young men[1] and was the first disease to be associated with a mitochondrial (mt)DNA mutation.[2] The mechanism by which mitochondrial abnormalities affect optic nerve viability and the reasons for the age of onset, incomplete penetrance, and male predominance all remain unknown.

The clinical characteristics of LHON overlap in part those of several other optic neuropathy phenotypes, and testing for the presence of primary LHON mutations (*ND1*/G3460A, *ND4*/G11778A, and *ND6*/T14484C) has been helpful in making the diagnosis. Routine mtDNA screening usually covers only primary LHON mtDNA mutations because their prevalence is reported to be 90% or more in families with multigenerational visual loss.[3] However, some patients do not have any mtDNA nucleotide changes,[4] whereas other patients have potentially pathogenic mutations elsewhere in the mitochondrial genome.[5] Primary LHON mutations may be less common when patients with LHON-like optic neuropathies are selected from a clinic setting rather than from multigenerational families.[6]

We investigated prospectively a series of patients presenting to neuro-ophthalmology clinics with bilateral simultaneous or sequential LHON-like optic neuropathies, to assess the frequency and severity of certain mitochondrial abnormalities in the blood. All patients were evaluated clinically and neuroradiologically. We studied the prevalence of primary LHON mutations and other mitochondrial abnormalities by sequencing the entire mtDNA coding region and assessing relative mtDNA content and mitochondrial respiration. Even though other hereditary optic neuropathies (e.g., dominant and recessive optic atrophy) and retinopathies cause chronic rather than acute or subacute visual loss, we also sequenced the *OPA1* and *OPA3* genes and performed electroretinograms (ERGs) to exclude these potential diagnoses.

Methods

Patient Enrollment

Patients were enrolled from the neuro-ophthalmology clinics at the King Khaled Eye Specialist Hospital and the King Faisal Specialist Hospital and Research Centre in Riyadh, Saudi Arabia, after obtaining informed consent approved by the appropriate institutional review board. This research adhered to the tenets of the Declaration of Helsinki.

Patients were eligible for inclusion if they reported acute or subacute visual loss in both eyes simultaneously or sequentially within 1 year, had clinical evidence of relatively symmetric optic neuropathies with central visual loss, and were of age less than 50 years at onset of visual symptoms. Exclusion criteria included exposure to a known optic nerve toxin; pain at onset or recovery of vision more compatible with optic neuritis; historical, neuroimaging, or biochemical evidence of multiple sclerosis or another systemic inflammatory disease; a cause of significant visual loss in either eye independent of optic neuropathy; substantial neurologic disease outside of the optic nerve; evidence on history, examination, or neuroimaging of an alternative medical, surgical, or syndromatic cause of optic neuropathy; or refusal to participate.

Neuroimaging was obtained on a CT or MRI scanner (Magnetom Allegra 3.0 Tesla MRI Scanner or Somatom Sensation 4 CT Scanner; Siemens, Madison, WI). Electroretinograms were performed on an evoked-potential system (Spirit; Nicolet Instrument Corp., Madison, WI), according to the manufacturer's suggested protocol.

From the [1]Mitochondrial Research Laboratory, Department of Genetics and the [2]Neuroscience Department, King Faisal Specialist Hospital and Research Centre, Riyadh, Kingdom of Saudi Arabia; and the [3]Neuro-ophthalmology Division, King Khaled Eye Specialist Hospital, Riyadh, Kingdom of Saudi Arabia.

[4]Present affiliation: Dr. Bosley is currently in the Division of Neurology, Cooper University Hospital, Camden, New Jersey.

Supported by grants from the Prince Salman Center for Disability Research and by the King Abdulaziz City for Science and Technology.

Submitted for publication March 19, 2006; revised June 7, 2006; accepted August 19, 2006.

Disclosure: **K.K. Abu-Amero**, None; **T.M. Bosley**, None

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Khaled K. Abu-Amero, Mitochondrial Research Laboratory, Department of Genetics, KFSH&RC, P.O. Box 3354, MBC-03, Riyadh 11211, Kingdom of Saudia Arabia; kamero@kfshrc.edu.sa.

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 mitochondrial (mt)DNA sequencing consisted of 159 individuals (106 men and 53 women; mean age, 46.3 ± 3.8 years); for relative mtDNA content, 50 individuals (34 men and 16 women; mean age, 24.4 ± 6.3 years); and for mitochondrial functional testing, 62 individuals (39 men and 23 women, mean age, 30.1 ± 7.5 years). Family information was obtained by history. All patients and control subjects were Middle Eastern Arabs.

Sample Collection and DNA Extractions

A single-density gradient (Ficoll-Paque-PLUS; Pharmacia Biotech AB, Uppsala, Sweden) was used for lymphocyte separation and isolation from peripheral blood, as detailed previously.[7] DNA was extracted from whole blood samples of all patients with LHON-like optic neuropathies and control subjects by using a DNA isolation kit (PureGene) from Gentra Systems (Minneapolis, MN).

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,[8] for all patients and control subjects. Each successfully amplified fragment was directly sequenced with dye termination chemistry (BigDye Terminator ver. 3.1 Cycle Sequencing kit; Applied Biosystems, Inc. [ABI], Foster City, CA), and samples were run on a sequencer (Prism 3100; ABI).

Sequence Analysis of the mtDNA Coding Region

Sequencing results were compared with the corrected Cambridge reference sequence.[5] All fragments were sequenced in both forward and reverse directions at least twice, for confirmation of a detected variant. Patient mtDNA sequences were compared with those from local control subjects, and all sequence variants were compared with the MITOMAP database,[5] the Human Mitochondrial Genome Database (http://www.genpat.uu.se/mtDB/ Uppsala University), GenBank (http://www.ncbi.nlm.nih.gov/GenBank/index.html/ NIH, Bethesda, MD), and Medline listed publications (NIH), all provided in the public domain.

Mitochondrial Haplogroup Analysis

After sequencing the entire mitochondrial DNA and establishing sequence changes for each patient and control, we performed mitochondrial haplogrouping based on the classifications detailed previously.[9,10]

Prediction of Pathogenicity

Pathogenic characteristics of previously undescribed (novel) nonsynonymous mtDNA sequence changes were determined according to a combination of standard criteria[11]; an evaluation of interspecies conservation using the Polyphen database (http://genetics.bwh.harvard.edu/pph/ Brigham and Women's Hospital, Harvard Medical School, Boston, MA), and when necessary, the Mamit-tRNA Web site (http://mamit-trna.u-strasbg.fr/index.html/ Institute of Molecular and Cellular Biology, Strasbourg, France), both sources provided in the public domain; analysis of predicted changes in the hydropathy index, as measured by a commercial software program (Protean program, part of the Lasergene ver 6 software; (DNAStar, Inc. Madison, WI), according to the Kyte-Doolittle method, which predicts the regional hydropathy of proteins from their amino acid sequence (values were assigned for all amino acids and then averaged over a window size of 7); assessment of the possible impact of an amino acid substitution on three-dimensional protein structure using Protean, which also predicts and displays secondary structural characteristics; and assessment of the possible effect of the mtDNA change on protein function using Polyphen[12] and the SIFT (Sorting Intolerant from Tolerant) program (http://blocks.fhcrc.org/sift/SIFT.html/ Fred Hutchinson Cancer Research Center, Seattle, WA), which predicts whether protein substitutions are tolerated.[13]

Therefore, a novel nonsynonymous nucleotide change was considered potentially pathogenic if (1) it was not reported in mitochondrial databases or Medline-listed literature as a confirmed polymorphism; (2) it was not present in local control subjects; (3) it changed a moderately or highly conserved amino acid; (4) it occurred in a region of high interspecies conservation; (5) Protean predicted an alteration of protein structure; (6) it was assessed as possibly or probably pathogenic by PolyPhen; and (7) it was predicted by SIFT to have an effect on protein function. For previously reported nonsynonymous nucleotide changes, consideration was given to pathogenic status determined by others and by mitochondrial databases in addition to these criteria.

Quantification of Heteroplasmy

The heteroplasmy level was determined for each heteroplasmic sequence variant by the primer extension assay described previously.[14] Heteroplasmy level was quantified from fluorescence intensities associated with electrophoretically resolved mutant and wild-type peaks (GeneScan ver. 3.7; 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

Competitive multiplex PCR was performed with two simultaneous primer sets, as described previously.[15] 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 samples were run simultaneously with those of 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 Healthcare, Schenectady, NY). The ratio of *ND1* to β-actin was determined for each patient and control subject by dividing the fluorescence intensity of the *ND1* band by the intensity of the β-actin band.

Measurement of Mitochondrial Functional Testing

Resazurin is a redox-active blue dye that becomes pink and highly fluorescent when reduced. It competes with oxygen for electrons in a standard preparation of circulating lymphocytes, and a change in fluorescence (corrected for background and protein concentration) reflects respiration. Lymphocytes from 19 patients and 62 control subjects were incubated with 6 μM resazurin, without and with mitochondrial inhibition by amiodarone 200 μM, and the fluorescence intensity resulting from resazurin reduction was monitored spectrofluorimetrically over time. Mitochondrial respiratory activity (MRA) was calculated as the difference between uninhibited and inhibited measurements at 240 minutes, taken in triplicate, averaged, and normalized for protein concentration and background activity, as described previously.[7]

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.[16] Similarly, the whole coding region and exon–intron boundaries for the *OPA3* gene were sequenced in all patients by using a protocol described previously.[17]

Statistical Methods

All statistical analyses were performed with commercial software (SPSS for Windows ver 13.0; SPSS Inc, Chicago, IL). Snellen visual acuities were converted to ordinal values, and color vision was quantified on an equal-interval scale as the number of Ishihara color plates identified (of 10) with

each eye. Statistical comparisons included bivariate correlation, independent samples *t*-test, χ^2 test, and receiver operator curve (ROC).

RESULTS

Clinical Description

Table 1 details certain clinical characteristics of 35 patients (21 men and 14 women; mean age at onset 19.0 ± 8.7 years) from 35 different pedigrees who met inclusion and exclusion criteria. They each reported acute or subacute visual loss in both eyes, either simultaneously or sequentially within 1 year, that then remained stable thereafter (except for patient 14, described later). Patients 16 and 19 had type 1 diabetes mellitus, but no patient had pigmentary retinopathy, ptosis, restricted ocular motility, deafness, ataxia, or weakness or reported myotonia, exercise intolerance, palpitations, syncope, cardiac conduction abnormalities, or somatic anomalies. Patients 3 and 13 smoked approximately one pack of cigarettes per day, and patient 7 smoked more than two packs of cigarettes per day. No patient reported a dietary abnormality, drug, or medication associated with visual loss. Fourteen patients reported family members with poor vision, but only patient 7 described several generations with poor vision.

Visual acuity varied from fair to poor, with a median final Snellen visual acuity of approximately 20/200. Visual acuity correlated positively and significantly between the two eyes ($r = 0.74$; $P < 0.001$) and with color vision ($r = 0.62$ OD and 0.58 OS; $P < 0.001$ for either eye), but correlated negatively with age at onset ($r = -0.263$; $P = 0.028$) and did not correlate with sex. All patients had normal erythrocyte sedimentation rates, antinuclear antibodies, and syphilis serology. Three patients (patients 19, 21, and 23) had vitamin B12 levels slightly below the normal range without anemia, megaloblastic changes, other neurologic signs or symptoms, or neuroimaging abnormalities.

Thirty patients had remote visual loss and optic atrophy OU at presentation, whereas five (patients 3, 5, 6, 7, and 35) had swelling of the papillary and peripapillary nerve fiber layer around the time of visual loss that evolved to optic atrophy (Fig. 1).[1,18] Patient 14 experienced substantial recovery of visual acuity OU with doughnut scotomas typical of recovering LHON[19,20] documented in several examinations over 10 months (Fig. 2).

All patients had brain neuroimaging, including magnetic resonance imaging in 27 and computed tomography in 18. No scan revealed a mass or an alternative cause of optic neuropathy such as evidence of demyelination. Optic nerves and/or optic chiasm appeared relatively small in 11 patients. Twenty-two patients received electroretinograms, all which had waveforms within the institutional normal range for latency and amplitude.

Sequence Analysis of the Mitochondrial DNA Coding Region

Table 2 displays the 41 nonsynonymous (resulting in amino acid change) mtDNA sequence variants detected in patients. Supplementary Table S1, online at http://www.iovs.org/cgi/content/full/47/10/4211/DC1, presents additional detail regarding predicted changes in the hydropathy index, protein structure, and protein function supporting pathogenicity analyses for the nonsynonymous sequence changes listed in Table 2. Table 3 details nonsynonymous nucleotide changes that are known pathogenic (e.g., primary LHON mutations), possibly pathogenic (e.g., secondary LHON mutations, provisional LHON mutations, or somatic mutations), or novel mutations in this patient series. Supplementary Table S2, http://www.iovs.org/cgi/content/full/47/10/4211/DC1, includes nonsynonymous and synonymous mtDNA sequence changes relevant to haplogroup assignment. Supplementary Table S2 excludes the nonsynonymous sequence changes listed in Table 3.

Of the nonsynonymous sequence changes listed in Table 2, 21 (51.3%) were in complex I, 7 (17.1%) in complex III, 5 (12.2%) in complex IV, 6 (14.6%) in complex V, 1 (2.4%) in $tRNA^{glutamine}$, and 1 (2.4%) in 12S rRNA. These mtDNA changes were predominantly transitions rather than transversions, similar to the definite and provisional mtDNA changes previously reported in association with LHON.[5] Five nucleotide changes were heteroplasmic (nt 4040, 7369, 10543, 10591, and 12782), and heteroplasmy levels were near the typical mutation load threshold for pathogenic mtDNA mutations (≥60%)[21,22] for three. Both nt 4040 and 7369 were heteroplasmic in control subjects (range, 18% to 25%), but all other sequences in control subjects were homoplasmic.

Primary LHON mutations nt 3460 and 11778 were found in patients 1 to 6, but nt 14484 was not represented. No primary LHON mutation was found in control subjects. All four secondary LHON mutations (nt 4216, 4917, 13708, and 15257) were present in patients and in control subjects in comparable frequencies. One provisional LHON mutation (nt 10663)[23] was detected in two patients (patients 29 and 35), and two previously reported somatic mutations (nt 6261[24] and 10591[25]) were present in one patient each (patients 28 and 25, respectively). These three mtDNA mutations were not present in control subjects. Sixteen other recognized nonsynonymous mtDNA polymorphisms were detected in both patients and control subjects in comparable frequencies (see Table 2). Nine novel (unreported elsewhere as a mutation or polymorphism to date) homoplasmic nonsynonymous nucleotide changes were identified in patients but not control subjects. Fourteen sequence changes (including nt 11778, 3460, 10663, 6261, 10591, and nine novel mtDNA changes) were considered probably pathogenic.

Age at onset of symptoms, color vision (CV), and frequency of consanguinity did not differ significantly between patients with primary LHON mutations (patients 1–6) and other patients (patients 7–35). However, mean VA of the six patients with primary LHON mutations (slightly <20/400) was significantly worse than that of the 29 patients without primary mutations (slightly better than 20/200; χ^2 $P = 0.009$). All patients with primary LHON mutations were men, whereas women comprised half (14/29) of patients without LHON mutations.

Relative Mitochondrial DNA Content

Table 3 also lists relative mtDNA content for each patient, and Table 4 compares these results with those in control subjects. The mean relative mtDNA content was significantly greater in all patients and in patients without primary LHON mutations than in control subjects ($P < 0.001$ for both comparisons). The optimal relative mtDNA content level to distinguish between control subjects and all patients was 1.76 as calculated by ROC curve (not shown; area under ROC curve 0.77; 95% CI, 0.65–0.89), and 24 patients had relative mtDNA content greater than this value. Relative mtDNA content did not correlate with clinical characteristics (age at onset, sex, VA, or CV) and was not different between patients with pathogenic or potentially pathogenic mtDNA changes and other patients.

Mitochondrial Functional Testing

Table 3 also lists MRA values for each patient, and Table 4 compares these results to control subjects. Mean MRA in all studied patients ($n = 19$) and in patients without primary LHON mutations ($n = 14$) was significantly less than in control

TABLE 1. Clinical Characteristics of Patients

Patients	Age at Onset (y)	Sex	Family History	VA*		Color†		Vision Fields‡	
				OD	OS	OD	OS	OD	OS
1	17	M	No	CF 5 ft.	20/400	0	0	Cecocentral scotoma (22° by I4e)	Cecocentral scotoma (24° by I3e)
2	20	M	No	20/80	CF 5 ft.	0	0	Cecocentral scotoma (14° by I2e)	Cecocentral scotoma (17° by I2e)
3	17	M	Yes	CF 5 ft.	20/400	0	0	Central scotoma (5° by I4e)	Central scotoma (4° by I4e)
4	8	M	No	CF 5 ft.	20/400	0	0	Cecocentral scotoma (20° by I2e)	Cecocentral scotoma (20° by I2e)
5	35	M	No	CF 5 ft.	20/60	0	7	Cecocentral scotoma (13° by I2e)	Cecocentral scotoma (16° by I3e)
6	24	M	Yes	20/80	CF 5 ft.	3	0	Central scotoma (16° by I3e)	Central scotoma (17° by I3e)
7	17	M	Yes	20/50	20/400	6	0	Cecocentral scotoma (21° by I3e)	Cecocentral scotoma (18° by I3e)
8	12	F	No	NLP	HM		0	Unable	Unable
9	18	M	Yes	20/200	20/100	3	3	Cecocentral scotoma (8° by I2e)	Cecocentral scotoma (4° by I2e)
10	17	M	Yes	20/100	20/100	4	2	Diffuse suppression	Diffuse suppression
11	19	F	No	LP	CF 1 ft.		0	Unable	Unable
12	17	M	No	20/40	20/50	9	9	Central scotoma (2° to I2e)	Central scotoma (3° by I2e)
13	14	M	No	20/100	20/50	1	5	Central scotoma (7° by I4e)	Central scotoma (17° by I2e)
14	14	F	No	CF 3 ft.	CF 3 ft.	7	7	Cecocentral scotoma (25° by I2e)	Cecocentral scotoma (24° by I2e)
15	17	M	Yes	20/80	20/50	0	1	Central scotoma (7° by I3e)	Central scotoma (5° by I3e)
16	28	F	No	CF 5 ft.	CF 5 ft.	0	0	Cecocentral scotoma (17° by I3e)	Cecocentral scotoma (15° by I3e)
17	28	F	No	20/200	20/400	0	0	Cecocentral scotoma (15° by I3e)	Cecocentral scotoma (18° by I3e)
18	8	M	No	LP	LP			Unable	Unable
19	13	F	No	20/200	20/200	0	0	Cecocentral scotoma (13° by I14e)	Central suppression and enlarged blind spot
20	16	M	Yes	20/60	20/60	7	7	Central scotoma (5° by I2e)	Small central scotoma
21	15	M	Yes	20/50	20/60	5	5	Small central scotoma	Small central scotoma
22	13	F	No	20/80	20/100	0	0	Cecocentral scotoma (12° by I4e)	Cecocentral scotoma (16° by I4e)
23	11	F	Yes	20/400	20/100	1	1	Central scotoma (15° by I3e)	Central scotoma (14° I2e)
24	41	M	No	20/100	20/80	1	1	Central scotoma (20° by I3e)	Central scotoma (19° I3e)
25	9	F	No	20/100	20/100	0	0	Large central scotoma	Large cecocentral scotoma
26	7	M	No	20/200	20/100	1	1	Small central scotoma	Small central scotoma
27	11	M	No	20/50	20/40	6	5	Small central scotoma	Small central scotoma
28	37	F	Yes	HM	LP	0		Superotemporal island	Unable
29	21	M	Yes	20/200	CF 1 ft.	4	1	Central scotoma (7° by I4e)	Central scotoma (14° by I4e)
30	20	F	No	LP	CF 1 ft.		0	Unable	Unable
31	20	F	No	CF 5 ft.	CF 5 ft.	0	0	Central scotoma (11° by I3e)	Central scotoma (16° by I3e)
32	14	F	No	20/70	20/70	0	0	Small central scotoma	Small central scotoma
33	35	M	Yes	CF 1 ft.	20/100	0	8	Cecocentral scotoma (14° by I14e)	Cecocentral scotoma (15° by I2e)
34	34	F	No	20/400	20/400	1	1	Cecocentral scotoma (19° by I3e)	Cecocentral scotoma (17° by I3e)
35	18	M	Yes	20/200	20/200	0	0	Cecocentral scotoma (14° by I2e)	Cecocentral scotoma (16° by I3e)

OD, right eye; OS, left eye; CF, counting fingers; HM, hand motions; LP, light perception; NLP, no light perception.

* Visual acuity by Snellen plates.

† Number of American Optical Company color plates identified correctly out of 10 presented.

‡ Central or cecocentral scotoma on GVF was assessed semi-quantitatively when possible by calculating the average size in degrees of the radius of the scotoma along each primary meridian as drawn with the smallest isopter. "Unable" means that the patient was unable to perform visual field testing.

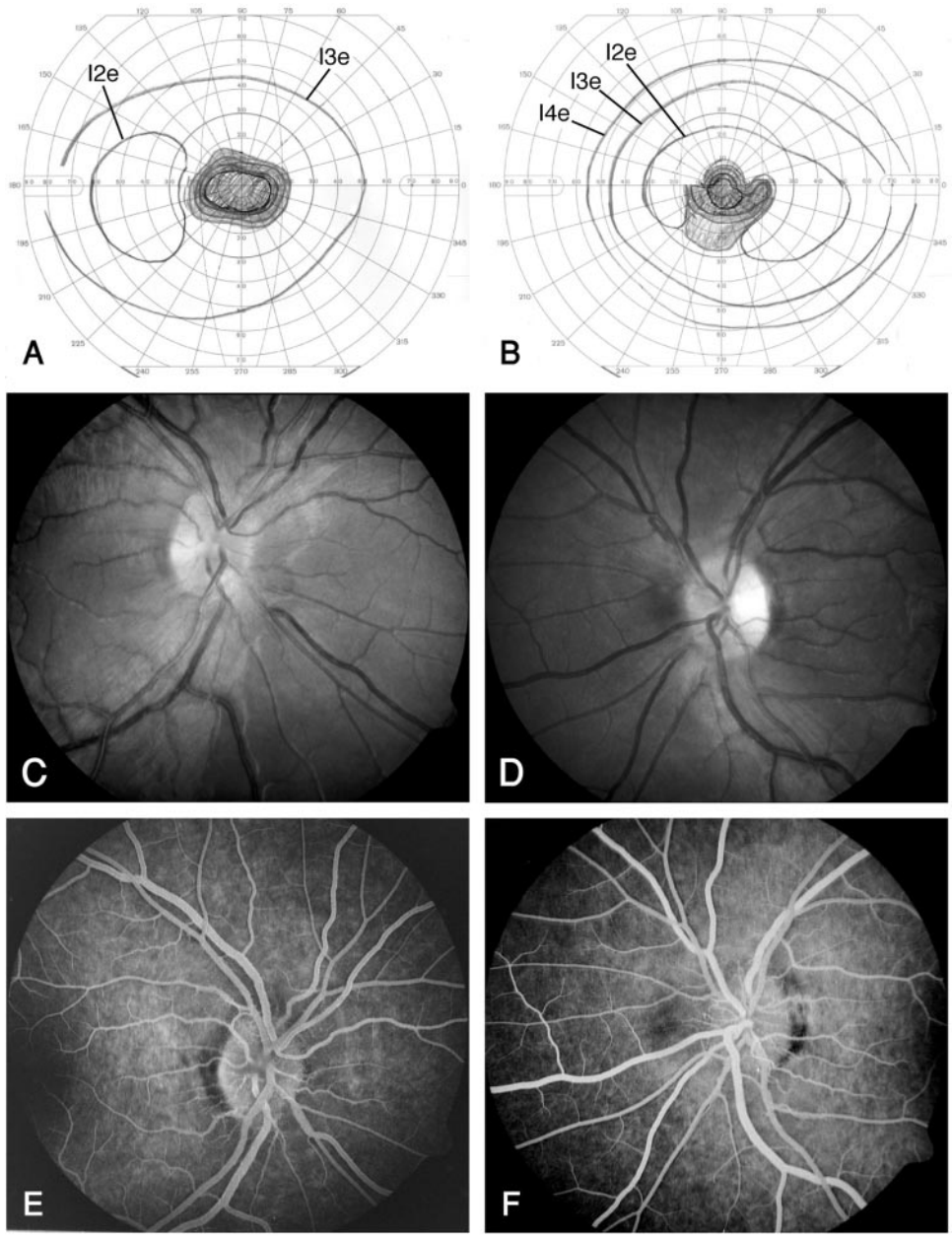

FIGURE 1. Pseudoedema of the optic disc. At presentation, Patient 7 had visual acuities of 20/50 OD and 20/400 OS with cecocentral scotomas both OS (**A**) and OD (**B**). Mild swelling of the papillary and peripapillary nerve fiber layer was documented on fundus photographs OD (**C**) < OS (**D**), and pseudoedema of the optic disc was confirmed by the absence of leakage on intravenous fluorescein angiogram OD (**E**) and OS (**F**). Nerve fiber layer swelling resolved on repeat examination 3 months later.

subjects ($P < 0.001$ for both comparisons). The optimal MRA value to distinguish between control subjects and patients was 21.2 (ROC curve not shown; area under the ROC curve 0.95; 95% CI, 0.88–1.02), and 17 of 19 studied patients had MRA values below this level. MRA levels did not correlate with clinical characteristics (age at onset, sex, VA, or CV) and were not different between patients with pathogenic or potentially pathogenic mtDNA changes and other patients.

Mitochondrial Haplogroup Analysis

Table 5 shows that haplogroup distributions for patients and for control subjects are comparable. Haplogroup assignment could not be made unequivocally in three patients, possibly because no report to date details the haplogroup spectrum of a broadly representative normal Arabic population. Patients with nt 11778 primary LHON mutation and nt 10663 provisional LHON mutation all had the J1 haplogroup background, as reported previously.[22,23] It was not possible to assign the two patients with nt 3460 an unequivocal mitochondrial haplogroup.

Sequence Analysis of *OPA1* and *OPA3* Genes

There were no polymorphisms, previously recognized mutations, or sequence variants detected in the coding exons, exon–intron boundaries, or the promoter region of the *OPA1* or *OPA3* genes in these patients. These genes were nonpolymorphic in this patient group.

DISCUSSION

We enrolled 35 patients from clinic who experienced acute or subacute, bilateral, persistent optic neuropathies characterized by central visual loss that occurred simultaneously or sequentially within 1 year. Diagnostic criteria were designed to exclude individuals with toxic optic nerve injury, optic nerve compression, systemic disease, optic neuritis, and nonarteritic

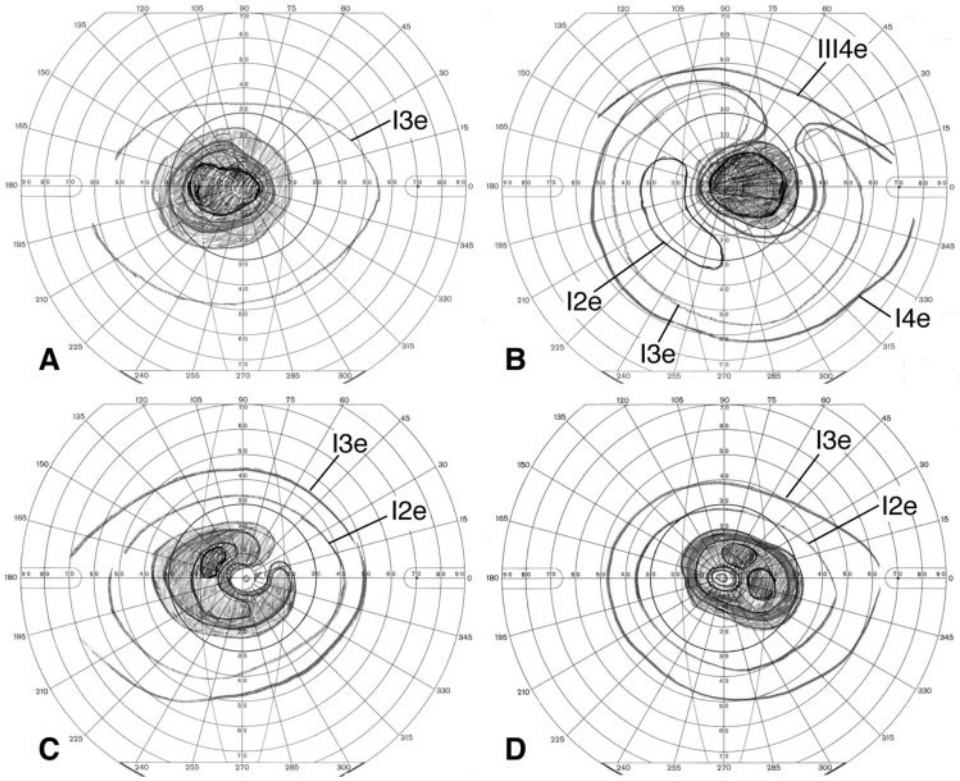

FIGURE 2. Recovery of vision. Montage of Goldmann visual fields of patient 14. Visual fields OS (**A**) and OD (**B**) documented large cecocentral scotomas OU at presentation, when she had a 3-month history of visual loss, VA OD 20/40 and OS counting fingers (CF) at 3 ft., and optic atrophy OU. Within 1 month, her VA was CF at 3 ft. OU, but her visual acuity gradually improved to 20/20 OU by 10 months later, when visual fields OS (**C**) and OD (**D**) showed doughnut scotomas with small central islands of good vision OU.

ischemic optic neuropathy (NAION). Hereditary retinopathies and other hereditary optic neuropathies were unlikely because patients reported subacute rather than chronic visual loss in both eyes, because ERGs were normal, and because sequencing *OPA1* and *OPA3* yielded no mutations. The haplogroup spectrum of patients mirrored that of control subjects. Haplogroup J1 may act as a background risk factor for disease expression in patients with nt 11778 and with the provisional LHON mutation nt 10663, as previously suggested.[22,23]

These patients also had other clinical characteristics reminiscent of LHON,[1] including moderately severe optic nerve disease with central visual field loss beginning around age 19 and with a strong correlation between VA in the two eyes. However, only six patients had primary LHON mutations. These six had additional similarities to patients in previous LHON studies, including preponderance of males, peripapillary telangiectasias and pseudoedema of the optic disc, predominance of the nt 11778 mutation with haplogroup J1 background, and significantly increased relative mtDNA content.[26] Mitochondrial respiration was significantly reduced, in agreement with previous studies of mitochondrial function in LHON patients that documented decreased activity of certain mitochondrial enzymes,[27] impaired complex I respiration,[28] and increased sensitivity to complex I inhibitors.[29] Nevertheless, none of the patients with primary LHON mutations reported a multigenerational history compatible with maternal inheritance.

Even patients without primary LHON mutations had more probably pathogenic mtDNA nucleotide changes, significantly greater relative DNA content, and significantly lower MRA than control subjects. These patients were clinically similar to patients with primary LHON mutations, except that half were women. With or without primary mutations, it seems likely that mitochondrial disturbances contribute to the vulnerability of the optic nerve to this type of injury by mechanisms not yet completely understood and possibly in concert with other factors not yet recognized.[30] The concentration of mitochondria at the optic nerve head implies dependency on some aspect of mitochondrial function,[31] and mitochondria-induced apoptosis may be a final mechanism of optic nerve injury in all patients with LHON-like optic neuropathies.[32,33]

Some of these patients had mtDNA changes predicted to be pathogenic, whereas others had no suspicious mtDNA changes. Patients with no obvious mtDNA abnormalities might have no mitochondrial disease, or they might have mtDNA abnormalities isolated to the optic nerve, conceptually similar to mitochondrial myopathies.[34] However, taken together, these patients had elevated relative mtDNA content and reduced MRA in blood, suggesting systemic oxidative stress comparable to patients with primary LHON mutations.[35] Previous studies have described similar patients with LHON-like optic neuropathies but no identified pathogenic mtDNA mutations after sequencing all[4] or part[6,36] of the mitochondrial coding region. In this setting, unidentified nuclear[37,38] and/or environmental[39,40] factors affecting mitochondrial function may be risk factors for LHON-like optic nerve damage.

The prevalence of primary LHON mutations in this series of patients with LHON-like optic neuropathies is substantially less than the 90% figure frequently mentioned for patients with LHON[1] that derives largely from studies of multigenerational families.[3] In fact, these results may be typical of a clinical setting. In another series of 32 individuals with LHON-like optic neuropathies enrolled from a clinic, rather than from multigenerational families, there were primary LHON mutations in only 5 (16%) patients.[6] In addition, a large commercial laboratory identified primary LHON mutations in only 11.4% of submitted samples (343/3000 samples had nt 11778, 3460, or 14484; Athena Diagnostics, Inc., Worcester, MA, personal communication, February 2006). These data and the information presented herein suggest that fewer than one in five patients with LHON-like optic neuropathies selected from doctors' offices have primary LHON mutations and that most do not describe a recognizable family history.

TABLE 2. Analysis of Nonsynonymous Sequence Changes

Nucleotide Substitution	Amino Acid Substitution	Location	Prevalence of Nucleotide Change: Patients (%)	Prevalence of Nucleotide Change: Controls (%)	Heteroplasmy* (%)	Novel	Interspecies Conservation	Pathogenicity Prediction†
1393 G→A	—	12S rRNA	2.9	16.3	N/A	No	Low	Nonpathologic
3460 G→A	A52T	Functional domain of ND1	5.7	0	N/A	No	High	Pathologic
4040 C→G	T245Stop	Transmembrane domain of ND1	2.9	6.3	18–20	Yes	Moderate	Nonpathologic
4216 T→C	Y304H	Transmembrane domain of ND1	51.4	46.5	N/A	No	Low	Nonpathologic
4381 A→G	—	Position 53 of tRNA glutamine just before the TψC loop	2.9	0	N/A	Yes	Moderate	Pathologic
4917 A→G	N150D	Functional domain of ND2	5.7	2.5	N/A	No	High	Nonpathologic
5319 A→T	T284S	Functional domain of ND2	2.9	0	N/A	No	Moderate	Nonpathologic
6261 G→A	A120T	Transmembrane domain of COI	2.9	0	N/A	No	Moderate	Pathologic
7369 C→G	S489C	Outside the functional domain of COI	5.7	6.3	25	Yes	Low	Nonpathologic
7623 C→T	T13I	Functional domain of COII	2.9	0	N/A	Yes	High	Pathologic
8656 A→T	T44S	Membrane proton channel CF(0) of ATPase6	2.9	0	N/A	Yes	Low	Nonpathologic
8701 A→G	T59A	Outside the functional domain of ATPase6	31.4	31.4	N/A	No	Low	Nonpathologic
8836 A→G	M104V	Transmembrane domain of the membrane proton channel CF(0) of ATPase6	2.9	0	N/A	No	High	Pathologic
8860 A→G	T112A	Transmembrane domain fragment 3 of the ATPase6 gene	91.4	83.6	N/A	No	Low	Nonpathologic
9053 G→A	S176N	Outside the transmembrane domain of the ATPase6 gene	25.7	31.4	N/A	No	Low	Nonpathologic
9055 G→A	A177T	Membrane proton channel CF(0) of ATPase6	2.9	0	N/A	No	Low	Nonpathologic
9660 A→C	M152L	Functional domain of COIII	2.9	0	N/A	Yes	High	Pathologic
9667 A→G	N154S	Functional domain of COIII	2.9	1.9	N/A	No	Low	Nonpathologic
10398 A→G	T114A	Outside the transmembrane domain of ND3 gene	77.1	66.7	N/A	No	Low	Nonpathologic
10543 A→G	H25R	Catalytic domain of NADH dehydrogenase (ND4L)	5.7	0	70	Yes	High	Pathologic
10591 T→G	F41C	Catalytic transmembrane domain of ND4L	2.9	0	75	No	High	Pathologic
10663 T→C	V65A	Transmembrane domain of ND4L	5.7	0	N/A	No	High	Pathologic
11778 G→A	R340H	Functional domain of ND4	11.4	0	N/A	No	High	Pathologic
11874 C→A	T372N	Transmembrane functional domain of ND4	2.9	0	N/A	Yes	High	Pathologic
12346 C→T	H4Y	Outside the transmembrane domain of ND5 gene	25.7	32.7	N/A	No	Low	Nonpathologic
12403 C→T	L23F	Outside the transmembrane domain of ND5 gene	31.4	35.2	N/A	No	Low	Nonpathologic
12782 T→G	I149S	Transmembrane domain of ND5	2.9	0	65	Yes	High	Pathologic
12950 A→G	N205S	Outside the transmembrane domain of ND5 gene	31.4	41.5	N/A	No	Low	Nonpathologic
13379 A→C	H348P	Conserved domain of ND5	2.9	0	N/A	Yes	Moderate	Pathologic
13681 A→G	T449A	Outside the functional domain of ND5	5.7	0	N/A	No	Low	Nonpathologic
13708 G→A	A458T	C-terminus of ND5	45.7	46.5	N/A	No	Moderate	Nonpathologic
13934 C→T	T533M	Outside the transmembrane domain of ND5 gene	28.6	28.9	N/A	No	Low	Nonpathologic
14110 T→C	F592L	In the Transmembrane domain of ND5 gene	31.4	16.3	N/A	No	Low	Nonpathologic
14582 A→G	V31A	Transmembrane domain of ND6	2.9	2.5	N/A	No	Low	Nonpathologic
14766 TVC	I7T	N-terminal domain of cytochrome B (CYTB)	88.6	74.8	N/A	No	Low	Nonpathologic
14798 TVC	F18L	N-terminal domain of cytochrome B (CYTB)	31.4	1.2	N/A	No	High	Nonpathologic
14862 C→T	A39V	N-terminal domain of CYTB	40	32.7	N/A	No	Low	Nonpathologic
15257 G→A	D171N	N-terminal domain of CYTB	5.7	7.5	N/A	No	High	Nonpathologic
15326 A→G	T194A	N-terminal domain of CYTB	91.4	72.9	N/A	No	Low	Nonpathologic
15452 C→A	L236I	Transmembrane domain of CYTB	51.4	61	N/A	No	Low	Nonpathologic
15674 T→C	S310P	C-terminal domain of CYTB	2.9	0	N/A	Yes	High	Pathologic

* Mixture of mutant and normal mtDNA. N/A, not applicable to this sequence variant because it was found in a homoplasmic status.

† See Supplementary Table S1 http://www.iovs.org/cgi/content/full/47/10/4211/DC1 for more information supporting pathogenicity prediction.

TABLE 3. Mitochondrial Abnormalities by Patient

Patient	mtDNA Sequence Changes*	Mitochondrial Haplogroup†	Relative mtDNA Content‡	MRA§
1	4216, 11778, 13708, 14862	J1	4.70	17.25
2	4216, 11778, 13708	J1	2.70	13.33
3	4216, 4917, 11778, 13708	J1	3.10	14.30
4	4216, 11778, 13708	J1	2.40	NA
5	3460	Other	2.61	15.27
6	3460	Other	2.61	19.33
7	None	M1a	3.35	NA
8	None	M1a	2.03	14.73
9	None	M1a	1.48	NA
10	None	M1a	1.76	NA
11	None	M1a	2.70	13.37
12	4216, 13708	J1	3.20	NA
13	None	Other	2.50	NA
14	4216, 13708, 15257	J2	2.78	18.27
15	4216, 13708	J1	1.74	NA
16	4216, 13708	J1	1.40	NA
17	4216, 13708	J1	2.50	NA
18	4216, 13708	J1	1.75	18.00
19	7369	L3e	0.94	NA
20	4381, 15674	M1	1.76	22.43
21	13379	M1	1.68	18.27
22	4216, 13708	J1	3.15	NA
23	4216, 12782, 13708, 15257	J2	1.72	NA
24	10543	Hv	1.67	22.7
25	4216, 10591, 13708	J1	1.53	NA
26	4216, 4917, 10543	T2	1.90	14.43
27	8836	M1a	1.27	NA
28	4216, 4917, 6261	T2	1.48	18.45
29	4040, 4216, 10663, 13708	J1	2.65	NA
30	11874	L3e	2.27	19.57
31	8656	M1a	2.62	18.77
32	None	M1a	3.20	NA
33	9660	M1a	2.56	20.01
34	4216, 7623, 13708	J1	1.95	13.56
35	4216, 10663, 13708	J1	3.01	20.13

* Listed are nonsynonymous mtDNA nucleotide changes that are known to be pathogenic (e.g., primary LHON mutations), are possibly pathogenic (e.g., secondary LHON mutation, provisional LHON mutations, or somatic mutations), or are novel in this patient series. Nonsynonymous polymorphisms and nonsynonymous mtDNA changes important to haplogroup designation with no pathogenic significance are listed in Supplementary Table S2 http://www.iovs.org/cgi/content/full/47/10/4211/DC1. Underscored nucleotide changes are known or suspected to be pathogenic (see Table 2 and Supplementary Table S1 http://www.iovs.org/cgi/content/full/47/10/4211/DC1).

† Haplogrouping was based on previously established haplogroup specific polymorphisms.[9] "Other" signifies that mitochondrial haplogroup could not be assigned unambiguously to one of the major known haplogroups based on the sequence changes detected. Patients 20 and 21 could not be grouped beyond M1.

‡ The optimal values to distinguish between controls and patients were >1.76 for relative mtDNA content.

§ The optimal values to distinguish between controls and patients were <21.2 for MRA.

The diagnosis of LHON depends on the presence of acute or subacute, relatively symmetric, generally persistent optic neuropathies characterized by central visual loss that occur simultaneously or sequentially within 1 year without another likely diagnostic possibility. Leber described a maternal inheritance pattern; however, some patients with primary LHON mutations deny a family history. Primary LHON mutations have been considered a hallmark feature of LHON,[2] but some patients[4]

TABLE 4. Comparison of Control Subjects with Patients

	Control Subjects	All Patients	*P**	Patients without Primary LHON Mutations	*P*†
Mean relative mtDNA content ± SD (95% CI)	1.62 ± 0.28 (1.52–1.72)	2.30 ± 0.76 (2.04–2.57)	<0.001	2.16 ± 0.66 (1.90–2.41)	<0.001
Mean MRA ± SD (95% CI)	22.53 ± 0.95 (22.26–22.80)	17.48 ± 2.97 (16.05–18.91)	<0.001	18.05 ± 3.02 (16.31–19.79)	<0.001

* Comparison of all 35 patients to controls by independent samples *t*-test. (See Methods for number of controls studied.)

† Comparison of the 29 patients without primary mutations to controls.

TABLE 5. Frequency of Mitochondrial Haplogroups for Patients and Control Subjects

Haplogroup	Patients (%) (n = 35)	Controls (%) (n = 159)	P*
J1	14 (40)	53 (33.3)	0.452
J2	2 (5.7)	13 (8.2)	0.621
M1a	9 (25.7)	63 (39.6)	0.123
M1	2 (5.7)	14 (8.8)	0.547
L3e	2 (5.7)	11 (6.92)	0.796
T2	2 (5.7)	12 (7.5)	0.704
Hv	1 (2.9)	11 (6.9)	0.732
Other†	3 (8.6)	4 (2.5)	0.908

* Comparison of frequency of haplogroups in patients to that in controls by Chi-Square test.

† A mitochondrial haplogroup could not be assigned unambiguously based on the single nucleotide polymorphisms detected.

and families[41,42] with LHON-like optic neuropathies do not have primary mutations. Pseudoedema of the optic disc[18] and a doughnut scotoma during recovery[20] are strongly linked to LHON, but were present in our study in patients without a primary LHON mutation or a maternal inheritance pattern. The clinical characteristics of LHON and its association with primary LHON mutations are well recognized. This report identifies similar clinical characteristics in patients with LHON-like optic neuropathies who do not have a maternal family history and associates these characteristics with a broader spectrum of mitochondrial abnormalities.

We report a fairly small group of patients from a restricted ethnic population, and this type of evaluation should be repeated in other centers. If these results are confirmed, the demographic importance of LHON-like optic neuropathies has been substantially underestimated. Extrapolating from data regarding primary LHON mutations, the prevalence of visual loss from mitochondria-associated LHON-like optic neuropathies might approach 20 per 100,000 individuals,[43] and this diagnosis may be responsible for as much as 10% of blindness in individuals under age 65.[44] Given this possibility, sequencing the entire mitochondrial genome and measuring relative mitochondrial content may be warranted when patients with LHON-like optic neuropathies do not have primary LHON mutations. Resazurin testing of mitochondrial respiratory activity is also a simple, reproducible procedure that can add an in vivo functional assessment to the evaluation of patients with LHON-like optic neuropathies and other mitochondrial syndromes.[7]

Acknowledgments

The authors thank Gamal Mohamed, PhD, and Barry Milcarek, PhD, for statistical assistance and Vincente M. Cabrera, PhD, for assistance with haplogrouping.

References

1. Newman NJ. Hereditary optic neuropathies: from the mitochondria to the optic nerve. *Am J Ophthalmol*. 2005;140:517–523.
2. Wallace D, Singh G, Lott M, et al. Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. *Science*. 1988;242:1427–1430.
3. Mackey DA, Oostra RJ, Rosenberg T, et al. Primary pathogenic mtDNA mutations in multigeneration pedigrees with Leber hereditary optic neuropathy. *Am J Hum Genet*. 1996;59:481–485.
4. Fauser S, Luberichs J, Besch D, Leo-Kottler B. Sequence analysis of the complete mitochondrial genome in patients with Leber's hereditary optic neuropathy lacking the three most common pathogenic DNA mutations. *Biochem Biophys Res Commun*. 2002;295:342–347.
5. Brandon MC, Lott M, Nguyen KC, et al. MITOMAP: A human mitochondrial genome database—2004 update. Available at: http://www.mitomap.org. *Nucleic Acids Res*. 2005;33:D611–D613.
6. Dogulu CF, Kansu T, Seyrantepe V, et al. Mitochondrial DNA analysis in the Turkish Leber's hereditary optic neuropathy population. *Eye* 2001;15:183–188.
7. Abu-Amero KK, Bosley TM. Detection of mitochondrial respiratory dysfunction in circulating lymphocytes using resazurin. *Arch Pathol Lab Med*. 2005;129:1295–1298.
8. Rieder MJ, Taylor SL, Tobe VO, Nickerson DA. Automating the identification of DNA variations using quality-based fluorescence re-sequencing: analysis of the human mitochondrial genome. *Nucleic Acids Res*. 1998;26:967–973.
9. Herrnstadt C, Elson JL, Fahy E, et al. Reduced-median-network analysis of complete mitochondrial DNA coding-region sequences for the major African, Asian, and European haplogroups. *Am J Hum Genet* 2002;70:1152–1171.
10. Kivisild T, Reidla M, Metspalu E, et al. Ethiopian mitochondrial DNA heritage: tracking gene flow across and around the gate of tears. *Am J Hum Genet*. 2004;75:752–770.
11. Chinnery PF, Howell N, Andrews RM, Turnbull DM. Mitochondrial DNA analysis: polymorphisms and pathogenicity. *J Med Genet*. 1999;36:505–510.
12. Sunyaev S, Ramensky V, Koch I, et al. Prediction of deleterious human alleles. *Hum Mol Genet*. 2001;10:591–597.
13. Ng PC, Henikoff S. Accounting for human polymorphisms predicted to affect protein function. *Genome Res*. 2002;12:436–446.
14. 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.
15. Kao SH, Chao HT, Liu HW, et al. Sperm mitochondrial DNA depletion in men with asthenospermia. *Fertil Steril*. 2004;82:66–73.
16. 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.
17. Anikster Y, Kleta R, Shaag A, et al. 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;69:1218–24.
18. Smith JL, Hoyt WF, Susac JO. Ocular fundus in acute Leber optic neuropathy. *Arch Ophthalmol*. 1973;90:349–354.
19. Acaroglu G, Kansu T, Dogulu CF. Visual recovery patterns in children with Leber's hereditary optic neuropathy. *Int Ophthalmol*. 2001;24:349–355.
20. Stone EM, Newman NJ, Miller NR, et al. Visual recovery in patients with Leber's hereditary optic neuropathy and the 11778 mutation. *J Clin Neuroophthalmol*. 1992;12:10–14.
21. Smith KH, Johns DR, Heher KL, Miller NR. Heteroplasmy in Leber's hereditary optic neuropathy. *Arch Ophthalmol*. 1993;111:1486–1490.
22. Black GC, Morten K, Laborde A, Poulton J. Leber's hereditary optic neuropathy: heteroplasmy is likely to be significant in the expression of LHON in families with the 3460 ND1 mutation. *Br J Ophthalmol*. 1996;80:915–917.
23. Brown MD, Starikovskaya E, Derbeneva O, et al. The role of mtDNA background in disease expression: a new primary LHON mutation associated with Western Eurasian haplogroup J. *Hum Genet*. 2002;110:130–138.
24. Petros JA, Baumann AK, Ruiz-Pesini E, et al. mtDNA mutations increase tumorigenicity in prostate cancer. *Proc Natl Acad Sci USA*. 2005;102:719–724.
25. Taylor RW, Barron MJ, Borthwick GM, et al. Mitochondrial DNA mutations in human colonic crypt stem cells. *J Clin Invest*. 2003;112:1351–1360.
26. Yen MY, Chen CS, Wang AG, Wei YH. Increase of mitochondrial DNA in blood cells of patients with Leber's hereditary optic neu-

ropathy with 11778 mutation. *Br J Ophthalmol*. 2002;86:1027–1030.

27. Cock HR, Cooper JM, Schapira AH. Functional consequences of the 3460-bp mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. *J Neurol Sci*. 1999;165:10–17.
28. Brown MD, Trounce IA, Jun AS, et al. Functional analysis of lymphoblast and cybrid mitochondria containing the 3460, 11778, or 14484 Leber's hereditary optic neuropathy mitochondrial DNA mutation. *J Biol Chem*. 2000;275:39831–39836.
29. Carelli V, Ghelli A, Bucchi L, et al. Biochemical features of mtDNA 14484 (ND6/M64V) point mutation associated with Leber's hereditary optic neuropathy. *Ann Neurol*. 1999;45:320–328.
30. Howell N. LHON and other optic nerve atrophies: the mitochondrial connection. *Dev Ophthalmol*. 2003;37:94–108.
31. Barron MJ, Griffiths P, Turnbull DM, et al. The distributions of mitochondria and sodium channels reflect the specific energy requirements and conduction properties of the human optic nerve head. *Br J Ophthalmol*. 2004;88:286–290.
32. Polster BM, Fiskum G. Mitochondrial mechanisms of neural cell apoptosis. *J Neurochem*. 2004;90:1281–1289.
33. Zanna C, Ghelli A, Porcelli AM, et al. Apoptotic cell death of cybrid cells bearing Leber's hereditary optic neuropathy mutations is caspase independent. *Ann NY Acad Sci*. 2003;1010:213–217.
34. Lightowlers RN, Chinnery PF, Turnbull DM, Howell N. Mammalian mitochondrial genetics: heredity, heteroplasmy and disease. *Trends Genet*. 1997;13:450–455.
35. 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.
36. Horvath J, Horvath R, Karcagi V, et al. Sequence analysis of Hungarian LHON patients not carrying the common primary mutations. *J Inherit Metab Dis*. 2002;25:323–324.
37. Zeviani M, Spinazzola A, Carelli V. Nuclear genes in mitochondrial disorders. *Curr Opin Genet Dev*. 2003;13:262–270.
38. Carelli V, Giordano C, d'Amati G. Pathogenic expression of homoplasmic mtDNA mutations needs a complex nuclear-mitochondrial interaction. *Trends Genet*. 2003;19:257–262.
39. Sadun A. Acquired mitochondrial impairment as a cause of optic nerve disease. *Trans Am Ophthalmol Soc*. 1998;96:881–923.
40. Carelli V, Ross-Cisneros FN, Sadun AA. Mitochondrial dysfunction as a cause of optic neuropathies. *Prog Retin Eye Res*. 2004;23:53–89.
41. Nikoskelainen EK, Huoponen K, Juvonen V, et al. Ophthalmologic findings in Leber hereditary optic neuropathy, with special reference to mtDNA mutations. *Ophthalmology*. 1996;103:504–514.
42. Lamminen T, Huoponen K, Sistonen P, et al. mtDNA haplotype analysis in Finnish families with leber hereditary optic neuroretinopathy. *Eur J Hum Genet*. 1997;5:271–279.
43. Man PY, Griffiths PG, Brown DT, et al. The epidemiology of Leber hereditary optic neuropathy in the North East of England. *Am J Hum Genet*. 2003;72:333–339.
44. Mackey DA, Buttery RG. Leber hereditary optic neuropathy in Australia. *Aust N Z J Ophthalmol*. 1992;20:177–184.