Mitochondrial Abnormalities in Patients with Primary Open-Angle Glaucoma

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Purpose. Primary open-angle glaucoma (POAG) is the second most common cause of blindness. It has been linked to mutations in the myocilin (*MYOC*) and optineurin (*OPTN*) genes, although mutations have been found in <5% of patients. The pathologic mechanism(s) of POAG remain unknown but may include retinal ganglion cell apoptosis, which causes progressive damage to axons at the optic nerve head.

Methods. In 27 patients with definite POAG, the *MYOC* and *OPTN* genes were sequenced, the entire mitochondrial (mt)DNA coding region was sequenced, relative mtDNA content was investigated, and mitochondrial respiratory function was assessed.

Results. Only three benign polymorphisms were identified in *MYOC* and *OPTN* in patients with POAG and in control subjects. Conversely, 27 different novel nonsynonymous mtDNA changes were found, only in patients with POAG (not control subjects), 22 of which (found in 14 patients) were potentially pathogenic. Unlike Leber hereditary optic neuropathy, most mtDNA sequence alterations in patients with POAG were transversions—sequence changes that alter the purine/pyrimidine orientation and imply oxidative stress. mtDNA content was relatively increased in 17 patients with POAG compared with age-matched control subjects, also implying a possible response to oxidative stress. Mean mitochondrial respiratory activity was decreased by 21% in patients with glaucoma compared with control subjects ($P < 0.001$).

Conclusions. These results reveal a spectrum of mitochondrial abnormalities in patients with POAG, implicating oxidative stress and implying that mitochondria dysfunction may be a risk factor for POAG. This concept may open up new experimental and therapeutic opportunities. (*Invest Ophthalmol Vis Sci.* 2006;47:2533–2541) DOI:10.1167/iovs.05-1639

Glaucoma is the most common optic neuropathic process affecting humans and the second most common cause of blindness worldwide.[1] Primary open-angle glaucoma (POAG) is the most frequent type of glaucoma, with risk factors that include elevated intraocular pressure (IOP), older age, family history, and black racial ancestry.[2] However, these factors alone do not predict the presence or degree of visual loss.[3] POAG has been linked to mutations in the myocilin[4] (*MYOC*) and optineurin[5] (*OPTN*) genes, although mutations have been found in less than 5% of patients.

The primary pathogenesis of POAG remains unknown but may include chronic retinal ganglion cell apoptosis[6,7] causing progressive damage to axons at the optic nerve head.[8] The subacute retinal ganglion cell apoptosis and axonal injury at the optic nerve head in Leber hereditary optic neuropathy (LHON) are associated with mitochondrial abnormalities.[9,10] Given that all major risk factors for POAG have not yet been identified and that known nuclear genetic abnormalities are associated with only a small fraction of patients, we decided to investigate a possible link between mitochondrial abnormalities and patients with POAG by cataloging mtDNA nucleotide changes, quantifying relative mtDNA content (to evaluate mtDNA duplication or depletion), and assessing mitochondrial respiratory function.

Materials and Methods

Patients and Control Subjects

Patients were eligible for inclusion in this study if they met standard clinical criteria for POAG, including age greater than 40 years, intraocular pressure (IOP) ≥ 21 mm Hg in at least one eye before treatment, open anterior chamber angles bilaterally on gonioscopy, and optic nerve injury characteristic of POAG, including cupping of the optic disc with cup-to-disc ratio ≥0.5 and/or nerve fiber bundle visual field loss on static and/or kinetic perimetry in at least one eye.

Exclusion criteria included historical, neuroimaging, or biochemical evidence of another possible optic neuropathic process affecting either eye, significant visual loss in both eyes not associated with glaucoma, or refusal to participate. Patients were selected from the Glaucoma Clinic at the King Khaled Eye Specialist Hospital after examination by a glaucoma specialist (JM) and obtaining informed consent approved by the institutional review board. This research adhered to the tenets of the Declaration of Helsinki. All patients and control subjects were Middle Eastern Arabs.

Records were reviewed, and full ophthalmic examinations were performed. Patients had either Goldmann manual kinetic perimetry (Haag Streit International, Köniz-Bern, Switzerland) or Humphrey automated white-on-white stimulus static perimetry (Humphrey Field Analyzer II; Carl Zeiss Meditec, Inc., Dublin, CA), or both. Optical coherence tomography was performed (OCT3; Carl Zeiss Meditec, Inc.) on some patients. Fundus photographs were obtained (FF 450 system; Carl Zeiss Meditec, Inc.) on conventional film.

All control subjects were blood donors at the King Faisal Specialist Hospital and Research Centre, 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. Control subjects did not have ophthalmic examinations. The control group for *MYOC* and *OPTN* sequencing consisted of 96 individuals (64 men and 32 women; mean age, 47 ± 5.3 years); for mitochondrial (mt)DNA sequencing, 159 different individuals (106 men and 53 women; mean age, 46.3 ± 3.8 years); for relative mtDNA content, 30 different individuals (18 men and 12 women; mean age, 54 ± 7.2 years); and for mitochondrial functional testing, 64 different individuals (43 men and 21 women; mean age, 45.2 ± 7.5 years).

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TABLE 1. Clinical Characteristics of Patients with POAG

Patient	Age	Sex	IOP OD	IOP OS	c/d OD	c/d OS	VA OD	VA OS	VF OD	VF OS
1	58	M	26	24	.95/.9	.9/.9	LP	20/40	Small, eccentric island of vision	Dense superior arcuate scotoma
2	56	F	24	23	.7/.5	.8/.5	20/60	CF	Superior arcuate scotoma and superonasal step	Temporal island
3	50	M	27	35	.85/.8	.9/.9	20/70	HM	Central island	NA
4	56	M	21	24	.6/.6	.75/.8	20/30	20/400	Normal	Superonasal step
5	81	M	24	22	.95/.9	.8/.8	CF	20/70	Superior arcuate scotoma + superonasal step	Superior arcuate scotoma + superonasal step
6	48	M	28	30	.85/.8	.95/.9	20/40	20/50	Superior and inferior arcuate scotomas	Central island remnant
7	65	F	37	41	.9/.9	.85/.8	NLP	20/60	NA	Inferior arcuate scotoma and inferonasal step
8	63	M	19	41	.4/.4	85/.8	20/30	20/50	Normal	Superonasal step
9	51	F	27	28	.85/.8	.95/.9	20/30	20/400	Superonasal step	Superior arcuate scotoma splitting fixation
10	46	F	26	28	.9/.9	.95/.9	20/100	20/200	Superior and inferior arcuate scotomas and superonasal step	Superior arcuate scotoma and superonasal step
11	46	F	35	27	.7/.6	.4/.4	20/125	CF	Normal (amblyopia)	Small superior arcuate scotoma (CRVO)
12	43	M	40	24	.95/.9	.8/.8	20/50	20/15	Inferior arcuate scotoma	Normal
13	59	F	40	35	1.0/.9	.9/.9	HM	20/30	NA	Superior arcuate scotoma and superonasal step
14	72	M	26	26	.5/.5	.8/.8	CF	20/25	NA (CRVO)	Inferonasal step
15	80	M	28	43	.9/.9	.95/.9	20/70	HM	Central island remnant	NA
16	44	M	26	41	.3/.2	.5/.4	20/50	20/30	Normal	Inferonasal step
17	42	F	17	44	NA	1.0/1.0	20/200	NLP	Central and temporal island remnants	NA
18	68	M	25	29	.75/.7	.9/.85	20/40	20/300	Inferior arcuate scotoma and inferonasal step	Central island remnant
19	56	M	30	45	.5/.4	.85/.8	20/20	20/25	Small superior paracentral scotoma	Dense superior arcuate scotoma and superonasal step
20	60	M	18	27	.95/.8	.8/.8	20/300	20/160	NA	Superonasal step
21	56	M	26	34	.85/.8	.75/.7	20/30	20/50	Superior arcuate scotoma + superonasal step	Superior paracentral scotoma
22	60	F	33		.85/.8	NA	20/40	Pros	Superior and inferior arcuate scotomas and inferonasal step	NA
23	73	M	24	20	NA	.95/.95	LP	20/125	NA	Central island remnant
24	63	M	24	25	.3/.3	.85/.9	20/100	20/125	Inferior paracentral scotoma	Central island remnant
25	55	M	32	40	.85/.8	.95/.9	20/100	20/400	Superonasal step	Superior hemifield scotoma
26	61	F	24	24	.85/.8	.95/.9	20/50	20/30	Superonasal step	Superonasal step
27	51	F	21	26	.4/.4	.7/.6	20/20	20/25	Normal	Superior arcuate scotoma and superonasal step

Age, age at diagnosis in years; IOP, maximum documented intraocular pressure; OD, right eye; OS, left eye; c/d, cup-to-disc ratio in vertical/horizontal dimensions; VA, visual acuity by Snellen plates; Pros, prosthesis; VF, results of Goldmann and/or Humphrey visual field; M, male; F, female; CF, counting fingers; HM, hand motions; LP, light perception; NLP, no light perception; NA, not available; CRVO, central retinal vein occlusion.

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.[11] DNA was extracted from whole-blood samples of all patients with POAG and control subjects by using a DNA isolation kit (Purgene; Gentra Systems, Minneapolis, MN).

Sequence Analysis of *MYOC* and *OPTN*

The coding exons, exon–intron boundaries, and promoter regions in the *MYOC* and *OPTN* genes were amplified by PCR from genomic DNA for all patients and control subjects and subjected to direct sequencing, as described previously.[12]

DNA Amplification and Sequencing

The entire coding region of the mitochondrial genome was amplified in 24 separate polymerase chain reactions (PCRs), by using single-set cycling conditions, as detailed elsewhere[13] for all patients and control subjects. Using a sequencing kit (BigDye Terminator ver. 3.1 Cycle Sequencing kit; Applied Biosystems [ABI], Foster City, CA), each successfully amplified fragment was directly sequenced (Prism 3100; ABI). All fragments were sequenced in both forward and reverse directions at least twice for confirmation of any detected variant.

Sequence Analysis of the Mitochondrial DNA Coding Region

Patient mtDNA sequences were compared with those from local control subjects, and all sequence variants were compared with the MitoMap database,[14] the Human Mitochondrial Genome Database (http://www.genpat.uu.se/mtDB/ provided in the public domain by the Department of Genetics and Pathology, Uppsala University, Uppsala, Sweden), GenBank (http://www.ncbi.nlm.nih.gov/GenBank/index.html/ provided in the public domain by the National Center for Bio-

TABLE 2. MYOC and OPTN Variants in Patients with POAG and Control Subjects

Gene	Location	Sequence Change	Codon Change	Patient(s)	Patients with POAG (%) (n = 27)	Control Subjects (%) (n = 96)	P
MYOC	Exon 3	2259 G→T	G324V	1, 2, 12, 14, 15, 20, 24	25.9	29.2	0.74
OPTN	Exon 4	412 G→A	T34T	27	3.7	6.3	0.61
OPTN	Exon 5	469 G→C	Q53H	24	3.7	10.4	0.28

The entire *MYOC* and *OPTN* genes were sequenced as detailed in Methods, and the only detected sequence variants are shown.

P, prevalence in POAG group versus control group.

technology Information, Bethesda, MD), and Medline-listed publications (National Center for Biotechnology Information).

Prediction of Pathogenicity

Pathogenic characteristics of reported nonsynonymous mtDNA changes (those that change an amino acid in the resultant protein) were accepted from mitochondrial databases and Medline-listed literature. Pathogenic characteristics of a previously undescribed (novel) nucleotide change were determined according to a combination of standard criteria[15]; an evaluation of interspecies conservation using the Polyphen database (http://genetics.bwh.harvard.edu/pph/ provided in the public domain by Harvard University, Cambridge, MA), and when necessary, the Mamit-tRNA Web site (http://mamit-trna.u-strasbg.fr/index.html/ provided in the public domain by the University of Strasbourg, Strasbourg, France); an analysis of predicted changes in the Hydropathy Index, as measured by the 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 = 7); an 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 an assessment of the possible effect of the mtDNA change on protein function using PolyPhen[16] and the SIFT (Sorting Intolerant From Tolerant) program (http://blocks.fhcrc.org/sift/SIFT.html/ provided in the public domain by the Fred Hutchinson Cancer Research Center, Seattle, WA), which predicts whether protein substitutions are tolerated.[17]

Therefore, a novel 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.

Quantification of Heteroplasmy

The heteroplasmy level was determined for each heteroplasmic sequence variant by primer extension assay, as described previously.[18] The heteroplasmy level was quantified from fluorescence intensities associated with electrophoretically resolved mutant and wild-type peaks by using a computer software program (GeneScan ver. 3.7; ABI). The percentage of heteroplasmy was calculated with the equation: [fluorescent band intensity for the mutant/(fluorescent band intensity for the wild-type + fluorescent band intensity for the mutant)] × 100.

Determination of Relative mtDNA Content

Competitive multiplex PCR was performed with two simultaneous primer sets as described previously.[19] 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. 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 a gel imager (Typhoon 9410; GE Healthcare, Piscataway, NJ). 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.

Mitochondrial Functional Testing

The resazurin assay for mitochondria respiratory function has been described elsewhere.[11] 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 the change in fluorescence reflects respiration. Lymphocytes from each patient and control were incubated with 6 μM resazurin without and with mitochondrial inhibition by 200 μM amiodarone, 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.[11]

RESULTS

Clinical Information

Table 1 details the clinical characteristics of 27 patients with POAG (mean age at onset, 57.9 ± 10.7 years; 17 men and 10 women) from 27 different families who met inclusion and exclusion criteria. They had been observed for an average of more than 5 years in a glaucoma clinic caring for patients with relatively advanced disease. Ten patients reported a family history of poor vision, but family members were not examined or evaluated genetically. No patient had type 1 diabetes mellitus, ptosis, restricted ocular motility, deafness, ataxia, weakness, myotonia, exercise intolerance, palpitations, or syncope.

Twelve patients had a history of glaucoma surgery, and nine had hand motions or worse visual acuity (VA) in one eye. VA did not correlate with maximum IOP, age, or sex within this group of patients. Results of optical coherence tomography testing and fundus photographs agreed well with clinical assessment of optic disc cupping (data not shown).

Sequence Analysis of *MYOC* and *OPTN*

Table 2 shows the results of sequencing MYOC and OPTN in patients with POAG and control subjects. In the MYOC gene, only one sequence variant (the novel change 2259 G→T in Exon 3, which resulted in codon change G324V) was detected in seven patients with POAG (25.9%) and 28 control subjects (29.2%; P = 0.74), implying that it is likely a non–disease-causing polymorphism in this ethnic population.

TABLE 3. Analysis of Nonsynonymous mtDNA Sequence Changes

Nucleotide Substitution	Amino Acid Substitution	Location	Base Substitution Type	% of Controls (n = 159)	Heteroplasmy Level (%)	Interspecies Conservation	Hydropathy Index Change		Protean Prediction	PolyPhen Prediction	SIFT Prediction	Summary
							From	To				
3574 C→A	90 Pro→Thr	Functional domain of ND1 gene	Transversion	0	N/A	High	0.64	0.54	Yes	Possibly damaging	Yes	Pathologic
3715 G→C	137 Ala→Pro	Functional domain of ND1 gene	Transversion	0	N/A	High	−0.73	−0.36	Yes	Probably damaging	Yes	Pathologic
3851 C→G	182 Ala→Gly	Transmembrane domain of ND1 gene	Transversion	0	N/A	High	−0.89	−0.64	No	Benign	No	Nonpathologic
3903 C→A	199 Asp→Glu	Functional domain of ND1 gene	Transversion	0	N/A	High	0.64	0.64	Yes	Probably damaging	Yes	Pathologic
3995 A→G	230 Asn→Ser	Transmembrane domain of ND1 gene	Transition	1.9	N/A	Low	−0.62	−0.92	No	Benign	Yes	Nonpathologic
4936 C→A	156 Thr→Asn	Transmembrane domain of ND2 gene	Transversion	0	65	Moderate	−2.36	−2.04	Yes	Possibly damaging	Yes	Pathologic
5194 C→T	242 Pro→Leu	Transmembrane domain of ND2 gene	Transition	0	N/A	Moderate	−0.64	−1.24	No	Benign	No	Nonpathologic
6186 C→A	95 Pro→Thr	Functional domain of COI gene	Transversion	0	N/A	High	0.91	0.81	Yes	Probably damaging	Yes	Pathologic
6219 C→A	106 Pro→Thr	Transmembrane domain of COI gene	Transversion	0	N/A	High	−1.46	−1.56	Yes	Possibly damaging	Yes	Pathologic
6391 A→T	163 Asn→lle	Transmembrane domain of COI gene	Transversion	0	N/A	High	−1.34	−2.23	Yes	Possibly damaging	Yes	Pathologic
6459 T→C	186 Trp→Arg	Transmembrane domain of COI gene	Transition	0	N/A	High	−2.22	−1.82	Yes	Possibly damaging	Yes	Pathologic
6877 C→A	325 Ala→Asp	Transmembrane domain of COI gene	Transversion	0	N/A	High	−0.69	−0.1	Yes	Possibly damaging	Yes	Pathologic
7827 T→C	81 Leu→Pro	Transmembrane domain of COII gene	Transition	0	N/A	High	−1.06	−0.17	Yes	Possibly damaging	Yes	Pathologic
7834 C→A	83 lle→Met	Transmembrane domain of COII gene	Transversion	3.8	N/A	Low	−0.57	−0.28	Yes	Benign	No	Nonpathologic
8516 T→A	51 Trp→Ter	Functional catalytic domain of ATPase 8	Transversion	0	70	High	2.34	—	—	—	—	Pathologic
8660 C→A	45 Thr→Asn	Outside the functional domain of COIII gene	Transversion	0	10	Moderate	0.19	0.5	Yes	Benign	No	Nonpathologic
8996 C→G	157 Ala→Gly	Transmembrane domain of ATPase 6	Transversion	0	N/A	High	−1.17	−0.92	Yes	Benign	No	Nonpathologic
9030 C→A	168 His→Gln	Catalytic domain of ATPase 6 gene	Transversion	0	N/A	High	−0.92	−0.89	Yes	Probably damaging	Yes	Pathologic
9081 C→A	185 Asn→Lys	Catalytic domain of ATPase 6 gene	Transversion	1.9	N/A	Low	−0.23	−0.19	No	Benign	No	Nonpathologic
9244 C→A	13 Pro→His	Functional domain of COIII gene	Transversion	0	N/A	High	0.83	1.01	Yes	Probably damaging	Yes	Pathologic
9277 C→A	24 Ala→Asp	Transmembrane domain of COIII gene	Transversion	0	N/A	High	−1.67	−1.08	Yes	Probably damaging	Yes	Pathologic
10053 A→C	—	Located in the Aco-stem of tRNA glycine	Transversion	0	70	High	—	—	—	Probably damaging	—	Pathologic
10135 A→G	26 Gln→Arg	Functional domain of ND3 gene	Transition	0	65	High	0.09	0.2	Yes	Probably damaging	Yes	Pathologic
10791 T→A	11 Leu→Ter	Transmembrane domain of ND4	Transversion	0	70	High	−1.47	—	—	Probably damaging	—	Pathologic

TABLE 3. (*continued*). Analysis of Nonsynonymous mtDNA Sequence Changes

Nucleotide Substitution	Amino Acid Substitution	Location	Base Substitution Type	% of Controls (n = 159)	Heteroplasmy Level (%)	Interspecies Conservation	Hydropathy Index Change		Protean Prediction	PolyPhen Prediction	SIFT Prediction	Summary
							From	To				
10920 C→G	54 Pro→Arg	Functional domain of ND4 gene	Transversion	0	N/A	Moderate	−0.29	0.03	Yes	Possibly damaging	Yes	Pathologic
11768 A→C	337 Thr→Pro	Functional domain of ND4 gene	Transversion	0	N/A	Moderate	1.94	2.04	Yes	Probably damaging	Yes	Pathologic
11867 C→A	370 Pro→Thr	Transmembrane domain of ND4 gene	Transversion	0	N/A	High	−0.33	−0.43	Yes	Probably damaging	Yes	Pathologic
12084 C→T	442 Ser→Phe	Transmembrane domain of ND4 gene	Transition	1.9	N/A	Moderate	−1.67	−2.07	Yes	Benign	No	Nonpathologic
12359 C→A	8 Thr→Asn	Transmembrane domain of ND5 gene	Transversion	0	N/A	High	−0.31	0	Yes	Possibly damaging	Yes	Pathologic
13448 C→A	371 Thr→Asn	Transmembrane domain of ND5 gene	Transversion	4.4	N/A	Low	−0.76	−0.44	No	Benign	Yes	Nonpathologic
13886 T→C	517 Leu→Pro	Functional domain of ND5	Transition	3.1	N/A	Low	−0.3	0.3	Yes	Benign	Yes	Nonpathologic
14580 A→C	32 Leu→Val	Transmembrane domain of ND6 gene	Transversion	1.2	50	High	−2.06	−2.1	No	Benign	Yes	Nonpathologic
15674 T→C	310 Ser→Pro	C-terminal domain of CYTB gene	Transition	0	N/A	High	0.61	0.7	Yes	Possibly damaging	Yes	Pathologic
15848 A→G	368 Thr→Ala	Transmembrane domain of CYTB gene	Transition	0	N/A	Low	−2.21	−2.49	Yes	Benign	No	Nonpathologic

See the Methods section for a description of the assessment of interspecies conservation, hydropathy index, Protean prediction, Polyphen prediction, SIFT prediction, and a summary pathogenicity prediction. For "Protean Prediction" and "SIFT Prediction," "Yes" indicates a prediction of probably pathogenicity while "No" indicates a prediction that the nucleotide change is probably not pathogenic.

In the *OPTN* gene, sequence variants 412 G→A in exon 4 and 469 G→C in exon 5 were each detected in one patient. The 412 G→A variant did not change an amino acid (T34T) and was a previously reported polymorphism.[20] The novel 469 G→C sequence variant resulted in a codon change (Q53H) but was also found with a similar prevalence in control subjects, implying that it also was probably a non–disease-causing polymorphism in this population.

Sequence Analysis of the Mitochondrial Coding Region

Table 3 details all nonsynonymous mtDNA changes detected in patients with POAG that were not previously reported as polymorphisms in mitochondrial databases and Medline-listed literature. These 34 mtDNA sequence changes spanned the mitochondrial coding region; 17 (50%) were in complex I, 2 (5.9%) in complex III, 9 (26.5%) in complex IV, 5 (14.7%) in complex V, and 1 (2.9%) in $tRNA^{glycine}$. Seven nonsynonymous mtDNA changes were detected in both patients with POAG and control subjects and were predicted to be benign. Twenty-seven of these changes were detected in patients with POAG but not in control subjects, and 22 altered moderately or highly conserved amino acids and were predicted to be damaging to the corresponding protein structure and/or function. The presence of these mtDNA sequence changes did not correlate with clinical characteristics (age, sex, IOP, VA, or degree of optic disc cupping).

More than 70% of the mtDNA sequence changes in Table 3 (25/34) were transversions. Significantly more patients with POAG had transversions (17/27) than transitions (8/27; χ^2 $P < 0.003$). The presence of transversions, however, did not correlate with clinical characteristics.

Seven nonsynonymous mtDNA sequence changes were present in a heteroplasmic status (see Table 3). The heteroplasmy levels were 55% for nucleotide (nt) 4936, 68% for nt 8516, 21% for nt 8660, 70% for nt 10053, 70% for nt 10135, 65% for nt 10791, and 25% for nt 14580.

Table 4 lists the 52 synonymous mtDNA sequence changes detected in the patients with POAG, all of which were transitions and all of which were present in control subjects.

TABLE 4. Synonymous mtDNA Sequence Changes in Patients with POAG

Nt	Gene	Nucleotide Change	Patient(s)	Novel
3396	ND1	T→C	21	No
3594	ND1	C→T	7, 3	No
3768	ND1	A→G	26	No
3834	ND1	G→A	26	No
3847	ND1	T→C	17, 10, 24	No
3918	ND1	G→A	1, 20	Yes
3948	ND1	A→G	15	Yes
4104	ND1	A→G	3	No
4146	ND1	C→T	23	Yes
4203	ND1	A→G	9, 13	No
4257	ND1	A→G	8	No
5105	ND2	T→C	18	Yes
5981	COI	T→C	24	Yes
6182	COI	G→A	1	Yes
6671	COI	T→C	6	No
6680	COI	T→C	6	No
7256	COI	C→T	17, 11	Yes
7424	COI	A→G	3	No
8251	COII	G→A	14, 3, 9	No
8754	ATPase 6	C→T	3	Yes
9266	COIII	G→A	18	Yes
9380	COIII	G→A	23	No
9494	COIII	A→G	26	No
9587	COIII	A→G	19	Yes
9708	COIII	T→C	22	No
9899	COIII	T→C	3	No
9950	COIII	T→C	17, 21	No
10589	ND4L	G→A	11	No
10640	ND4L	T→C	9, 13	No
10915	ND4	T→C	9, 13	No
11002	ND4	A→G	25	No
11251	ND4	A→G	12, 25	No
11719	ND4	G→A	1, 26	No
11881	ND4	C→T	3, 7	No
11893	ND4	A→G	4	No
12372	ND5	G→A	16	No
12570	ND5	A→G	8, 25	Yes
13650	ND5	C→T	7	No
13722	ND5	A→G	7	No
14094	ND5	T→C	26	No
14179	ND6	A→G	4	Yes
14182	ND6	T→C	20	No
14470	ND6	T→C	15	No
14635	ND6	T→C	7	Yes
14905	CYTB	G→A	3, 7	No
14911	CYTB	C→T	11	No
14929	CYTB	C→G	26	Yes
14971	CYTB	T→C	3, 7 16	Yes
15043	CYTB	G→A	6	No
15217	CYTB	G→A	3, 7, 8	No
15670	CYTB	T→C	22	No
15862	CYTB	T→C	4	Yes

Novelty was established as described in the Methods. All synonymous mtDNA sequence changes were also found in controls. All sequences were homoplasmic. The base substitution type of all sequences was transition.

Relative Mitochondrial DNA Content

Relative mtDNA content was not significantly different between patients with POAG (mean, 0.81 ± 0.25 [SD]; 95% CI 0.70–0.91) and simultaneous control subjects (mean 0.73 ± 0.12; 95% CI 0.67–0.79; $P < 0.25$). However, the variation of relative mtDNA content was greater in patients with POAG (SD = 0.25) than in the control subjects (SD = 0.12). The optimum value of the ratio to distinguish between the two groups was 0.76 as calculated by receiver operator characteristic curve (ROC curve not shown; area under the ROC curve 0.685, 95% CI 0.525–0.844), and 17 patients with POAG had relative mtDNA content greater than this level. Relative mtDNA content did not correlate with clinical characteristics or with the presence of either mtDNA transitions or transversions.

Mitochondrial Functional Testing

Figure 1 displays the distribution of MRA levels for control subjects and patients with POAG in box plots. MRA in control subjects (mean 22.48 ± 0.88 [SD]; 95% CI 22.26–22.70; $P < 0.001$) was significantly greater than patients with POAG (mean 17.8 ± 2.78; 95% CI 16.69–18.89; $P < .002$). The optimum MRA value to distinguish between the two groups was 21.39 (area under the ROC curve 0.948, 95% CI 0.891–1.004), and 24 patients with POAG had MRA levels less than this. MRA did not correlate with clinical characteristics and did not predict the presence of either mtDNA transitions or transversions.

Table 5 summarizes pertinent mitochondrial data by patient, including nonsynonymous mtDNA sequence changes, relative mtDNA content, and MRA values (in ascending order).

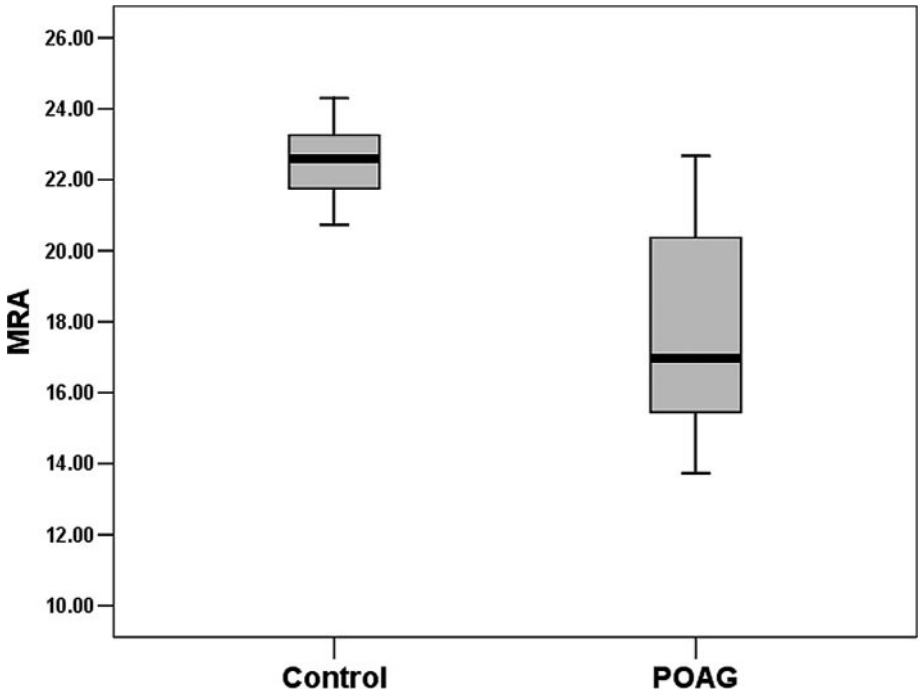

FIGURE 1. MRA in control subjects and patients with POAG. Box plot shows median, interquartile range, and outliers of MRA for 64 control subjects and 27 patients with POAG.

DISCUSSION

We evaluated 27 patients who met age, funduscopic, visual field, and anterior segment criteria that define POAG in contrast to other causes of optic nerve disease and other types of glaucoma. All these patients had documentation of elevated IOP during follow-up. Their nerve fiber bundle visual field loss would be atypical for hereditary optic neuropathies, and these patients were unusually old for LHON and other hereditary optic neuropathies. They were in an appropriate age range for nonarteritic anterior ischemic optic neuropathy (NAION),[13] but a swollen optic disc was never observed, and characteristic glaucomatous optic nerve cupping was diagnosed by a glaucoma specialist (JM). No patient reported a maternal family history of visual loss.

No patient with POAG had a nucleotide change in *MYOC* or *OPTN* that was likely to be pathologic. All three nucleotide changes in these nuclear genes were present in control subjects and were likely to be polymorphisms in this population. The prevalence of *MYOC* and *OPTN* mutations in Arabic patients with POAG has not been evaluated before, but these results are not surprising, given the reported low prevalence of *MYOC* and *OPTN* mutations in open-angle glaucoma in other populations.[21]

No patient with POAG had a primary LHON mutation. The presence of primary LHON mutations has been investigated previously in normal-tension glaucoma[12] but not in POAG. In addition, no patient had a secondary, intermediate, or provisional LHON mutation. Of course, LHON, which involves the subacute injury of the majority of optic nerve fibers,[9] often in a young adult male with a maternal family history of visual loss, is a very different optic neuropathy from POAG, which causes the gradual loss of optic nerve fiber bundles over a period of years in an older individual.

However, several other mitochondrial abnormalities were detected in patients with POAG. More than 60% (17/27) of patients with POAG had nonsynonymous mtDNA changes not considered LHON mutations and not found in control subjects, most of which were predicted to cause pathologic changes. Most (17/27) had relatively increased mtDNA content, which may suggest a compensatory response to oxidative stress.[22] In

TABLE 5. Mitochondrial Abnormalities in Patients with POAG

Patient	Nonsynonymous mtDNA Change(s)	Relative mtDNA Content	MRA
1	3851	0.85	13.73
2	None	0.88	14.13
3	6219	1.20	14.80
4	14580	1.19	14.97
5	3574, 3903, 11867	1.11	15.20
6	None	0.95	15.27
7	None	0.88	15.40
8	4936	0.96	15.50
9	None	0.98	15.53
10	11768	0.51	15.60
11	6459, 6877, 7834	0.69	16.00
12	3995, 13448	0.83	16.30
13	9277, 10791, 13886	0.57	16.33
14	None	0.99	16.97
15	6459, 12084, 12359	0.76	17.73
16	None	1.13	18.43
17	None	0.83	18.50
18	8660	0.67	18.50
19	7827, 8516, 10135	0.61	19.53
20	6186, 6219, 8996, 9081	1.13	20.10
21	5194	0.69	20.27
22	10920, 15848	0.62	20.47
23	None	0.76	20.53
24	15674	0.77	21.00
25	6391, 9244, 10053	0.85	21.63
26	9030	0.95	22.37
27	3715	0.66	22.67

Nonsynonymous mtDNA changes lists by patient the nucleotide changes referred to in Table 3. See the Methods and Results sections for more information regarding relative mitochondrial content and MRA.

addition, almost 90% (24/27) of patients with POAG had reduced MRA, a measure of mitochondrial respiration.[11] The coincidence of changes in the mitochondrial genome and in mitochondrial respiration raises the argument that the optic neuropathy of POAG is broadly associated with mitochondrial abnormalities. In fact, mitochondrial disturbances were much more frequent than *MYOC* or *OPTN* mutations in this Arabic population.

The list of optic neuropathies currently associated with mitochondrial abnormalities includes LHON, certain patients with optic neuritis and multiple sclerosis, Wolfram's syndrome, dominant optic atrophy (DOA),[9] and NAION.[13] The maternal inheritance pattern of LHON led to initial mtDNA investigations, but even primary LHON mutations are incompletely penetrant with erratic reporting of family history.[23] The penetrance of mtDNA changes reported herein may be somewhat less than primary LHON mutations with later presentation of symptoms and more interaction with other risk factors such as nuclear mitochondrial changes, anatomy of the anterior globe, IOP, race, and age, which is itself related in part to mitochondrial changes.[24] These factors may obscure maternal inheritance in our patients and other POAG populations.[25]

The mechanisms by which mitochondrial abnormalities may place the optic nerve at risk remain uncertain.[9] The high concentration of mitochondria at the optic nerve head implies dependency on some aspect of mitochondrial function,[26] and mitochondrial disease in LHON, for example, has been linked to abnormalities in complex I activity.[23] In contrast, some patients with LHON do not have primary LHON mutations[20,27] or, at times, any mtDNA change at all.[28] Patients with Wolfram's syndrome[29] or NAION[13] have widely distributed mtDNA changes, and patients with dominant optic atrophy have mutations of *OPA1* affecting a dynamin-related mitochondrial wall protein rather than a component of the electron chain.[30] One hypothesis suggests that progressive optic nerve damage in POAG is the result of optic nerve fiber apoptosis.[6] Mitochondria-induced apoptosis, which may be a mechanism of injury in experimental glaucoma[31] and other optic neuropathies,[32] may also be a pathologic factor in POAG.

mtDNA transition changes comprise almost 70% (23/33) of the primary, secondary, intermediate, and provisional LHON mutations reported currently in MitoMap.[14] Similarly, 75% (12/16) of nonsynonymous mtDNA changes found in a group of 19 NAION patients were transitions.[13] Unlike patients with LHON and NAION, patients with POAG had a high frequency of mtDNA transversion changes (25/34; 73.5%). The guanine base has the lowest oxidation potential of the four DNA nucleobases, making G:C→T:A or G:C→C:G transversion mutations frequent in the setting of oxidative stress.[33] The presence of mtDNA transversions in POAG may be evidence that alternative mitochondrial damage and repair mechanisms are involved in the generation of mtDNA mutations in patients with POAG as part of a response to oxidative stress early in development. Transversions, in turn, may contribute to the unique optic nerve injury in POAG.

Mitochondrial pathology was variable in this POAG group, but mitochondrial abnormalities of one type or another were present in every individual. It is intriguing that potentially pathogenic mtDNA sequence changes (particularly transversions), increased relative mtDNA content, and reduced MRA levels all imply oxidative stress. Oxidative stress has also been reported to induce human trabecular meshwork degenerative changes that favor increased intraocular pressure.[34] Therefore, oxidative stress early in development and/or throughout life could precipitate both metabolic and anatomic sequelae that increase the risk of optic nerve damage in POAG.

This report describes a relatively small number of patients with POAG from a restricted ethnic population. However, if these results are confirmed in other populations, knowledge that mtDNA mutations and/or mitochondrial dysfunction are present in POAG may lead to a better understanding of glaucoma pathophysiology.[35] Clever approaches are now available for studying mitochondrial disease in the eye, and a novel in vitro treatment has already been devised for the metabolic defect of at least one mtDNA mutation.[36] These tools might be applicable to investigating and treating POAG.

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