Nuclear and Mitochondrial Analysis of Patients with Primary Angle-Closure Glaucoma

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Purpose. Certain types of glaucoma are linked to nuclear genetic mutations or to mitochondrial disturbances. In this study, patients with primary angle-closure glaucoma (PACG) were examined for mutations in nuclear genes reported to be associated with glaucoma and for possible mitochondrial abnormalities.

Methods. In patients with PACG, the nuclear genes *MYOC*, *OPTN*, *CYP1B1*, *WDR36*, *OPA1*, and *OPA3* were sequenced, the entire mitochondrial (mt)DNA coding region was sequenced, relative mtDNA content was measured, and mitochondrial respiratory activity (MRA) was assessed.

Results. No novel or previously reported mutations were present in the nuclear genes *MYOC*, *OPTN*, *CYP1B1*, *WDR36*, *OPA1*, and *OPA3* in 29 patients with PACG. Four (13.8%) patients had potentially pathologic mtDNA nucleotide changes not found in control subjects. The patients with PACG did not differ significantly from the control subjects in relative mitochondrial content and had only a small decrease in MRA (2.4%) of indeterminate significance.

Conclusions. These Middle Eastern patients with PACG had no mutations in nuclear genes associated with other types of glaucoma or inherited optic neuropathies. Mitochondrial abnormalities were minimal, and the overall pattern of those abnormalities was distinctly different from that of Leber hereditary optic neuropathy, nonarteritic ischemic optic neuropathy, primary open-angle glaucoma, and optic neuritis. These results are consistent with the hypothesis that anatomic factors may be more important determinants for PACG than the genetic and mitochondrial factors evaluated here. (*Invest Ophthalmol Vis Sci.* 2007;48:5591–5596) DOI:10.1167/iovs.07-0780

The hallmark of primary angle closure glaucoma (PACG) is obstruction of the trabecular meshwork by iris tissue, which prevents exit of aqueous humor, elevates intraocular pressure (IOP), and often damages the optic nerve.[1] Traditionally, PACG has been divided into acute, subacute, and chronic cases according to signs and symptoms at the time of diagnosis.[1] Its prevalence varies greatly depending on the population involved, from a low of 0.1% among Europeans[2,3] to a high of 2.65% among Eskimos older than 40 years,[4] with Asian populations typically having an intermediate prevalence of approximately 0.8%.[5] PACG is less common than primary open-angle glaucoma (POAG) in the Western hemisphere,[6] but its prevalence in some parts of the globe is similar to that of POAG.[7,8]

More than 15 genetic loci and seven genes have been reported in association with POAG,[9] the two most important of which are *MYOC* and *OPTN*.[10,11] Only isolated patients with PACG have been reported to have *MYOC* mutations, including two with combined-mechanism glaucoma.[12,13] A recent study screened 78 Taiwanese patients with acute PACG for occurrence of 67 single-nucleotide polymorphisms (SNPs) on 35 genes associated with various types of glaucoma and found a significant association with one SNP of the *MMP-9* gene, which is important for remodeling of the extracellular matrix.[14] No genetic mutations have been reported in chronic PACG[15] even though anterior chamber depth may be an inherited characteristic[16] and PACG occurs in 1% to 12% of relatives of patients with PACG.[1,17]

A recent study of Middle Eastern patients with POAG found evidence of mitochondrial abnormalities, including potentially pathologic mitochondrial (mt)DNA changes and decreased mitochondrial respiratory function.[18] The purpose of this study was to evaluate patients with PACG in a similar fashion for the presence of mitochondrial abnormalities and for nuclear gene mutations associated with various types of glaucoma (*MYOC*, *OPTN*, *WDR36*, and *CYP1B1*) and certain inherited optic neuropathies (*OPA1* and *OPA3*).[19–21]

Methods

Patients

Patients were eligible for inclusion if they had evidence of glaucomatous optic nerve damage attributable to primary angle closure.[5] Inclusion criteria included (1) clinical documentation of angle closure, defined as the presence of appositional or synechial closure of the anterior chamber angle involving at least 270° by gonioscopy in both eyes; (2) intraocular pressure elevated to a level ≥23 mm Hg measured before or after treatment by Goldmann applanation tonometry; (3) evidence of characteristic glaucomatous optic disc damage with excavation of the disc causing a cup-to-disc ratio (c/d) vertically of at least 0.70 in at least one eye; and (4) characteristic peripheral visual field loss including nerve fiber bundle defects (nasal step, arcuate scotoma, paracentral scotoma) or advanced visual field loss (central and/or temporal island of vision) as tested by a field perimeter (Humphrey Field Analyzer; Carl Zeiss Meditec, GmbH, Oberkochen, Germany), in those patients with vision better than 20/200, or Goldmann manual perimetry, in those with worse vision.

Exclusion criteria were (1) secondary angle closure glaucoma; (2) the presence of pseudoexfoliation syndrome even if coexistent with angle closure; (3) another cause of optic nerve injury affecting either eye; (4) significant visual loss in both eyes not associated with glaucoma; (5) inability to visualize the optic fundus for optic disc assessment; or (6) refusal to participate. Patients were Middle Eastern Arabs selected from the Glaucoma Clinic at King Khaled Eye Specialist

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Hospital (KKESH) after examination by a glaucoma specialist (JM) and informed consent approved by the KKESH Institutional Review Board. The research adhered to the tenets of the Declaration of Helsinki. Family members were not evaluated clinically or genetically.

Records were reviewed, and full ophthalmic examinations were performed. Every patient received laser iridotomy and antiglaucoma medications, and filtering surgery was performed when IOP remained elevated. Patients had either Goldmann manual kinetic perimetry (Haag Streit International, Köniz-Bern, Switzerland) or automated, white-on-white stimulus, static perimetry (Humphrey Field Analyzer II; Carl Zeiss Meditec, GmbH), or both. Optical coherence tomography (OCT) of the optic nerve including average nerve fiber thickness assessment and optic disc topography was performed (OCT3 System, Humphrey Systems (Carl Zeiss Meditec, GmbH) on some patients. Optic disc photographs were obtained by digital photography (FF 450 system; Carl Zeiss Meditec, GmbH).

Control Subjects

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. No ophthalmic examination was performed on these individuals. The control group for mtDNA sequencing consisted of 159 individuals (106 men and 53 women; mean age, 46.3 ± 3.8 years). For relative mtDNA content, 28 individuals (19 men and 9 women; mean age, 59.2 ± 3.3 years) and for mitochondrial respiration testing, 55 individuals (39 men and 16 women; mean age, 54.8 ± 4.6) were examined. Family information was obtained by history. All patients and control subjects were Middle Eastern Arabs.

Sample Collection and DNA Extraction

Single-density gradient (Ficoll-Paque-PLUS; Pharmacia Biotech AB, Uppsala, Sweden) was used for lymphocyte separation from peripheral blood as detailed previously.[22] DNA was extracted from whole blood samples of all patients with PACG and control subjects (Puregene DNA isolation kit; Gentra Systems, Minneapolis, MN).

Sequence Analysis of Nuclear Genes

The coding exons, exon–intron boundaries, and promoter regions in the *MYOC*, *OPTN*, *CYP1B1*, and *WDR36* genes were amplified by PCR from genomic DNA of all patients and control subjects and subjected to direct sequencing, as described previously.[23] 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] Similarly, the whole coding region and exon–intron boundaries for the *OPA3* gene were sequenced in all patients by a protocol described previously.[21]

mtDNA Amplification and Sequencing

The entire coding region of the mitochondrial genome of all patients and control subjects was amplified in 24 separate polymerase chain reactions (PCRs) in single-set cycling conditions, as detailed elsewhere.[25] Primers were designed to avoid amplifying mtDNA-like sequences in the nuclear genome. Each successfully amplified fragment was directly sequenced with a kit (BigDye Terminator, ver. 3.1 cycle sequencing kit; Applied Biosystems, Inc., Foster City, CA), and samples were run on a sequencer (Prism 3100; Applied Biosystems).

Sequence Analysis of the mtDNA Coding Region

The full mtDNA genome was sequenced except for the D-loop, and sequencing results were compared with the corrected Cambridge reference sequence.[26] All fragments were sequenced in both forward and reverse directions at least twice, for confirmation of any detected variant. All nucleotide variants from patients and control subjects were compared with the MITOMAP database (last updated August 2007),[27] the Human Mitochondrial Genome Database (http://www.genpat.uu.se/mtdb; last updated March 2007; provided in the public domain by the Institute for Genetics and Pathology, Uppsala University, Uppsala, Sweden), GenBank (http://www.ncbi.nlm.nih.gov/GenBank/index.html; last updated July 2007), and the MedLine listed publications. Reported homoplasmic synonymous or nonsynonymous (NS) polymorphisms associated with mitochondrial haplogroups[28] were excluded from further consideration.[29]

Prediction of Pathogenicity

Pathologic characteristics of each remaining nucleotide change in both patients with PACG and control subjects were estimated according to a combination of standard criteria,[30] an evaluation of interspecies conservation according to the PolyPhen database (http://genetics.bwh.harvard.edu/pph/ Brigham and Women's Hospital, Harvard Medical School, Boston, MA), and the Mamit-tRNA Web site (http://mamit-trna.u-strasbg.fr/index.html/ Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France) when necessary; assessment of the possible impact of an amino acid substitution on three-dimensional protein structure on computer (Protean program, part of the Lasergene V.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.[31] Therefore, a nonsynonymous nucleotide change was considered potentially pathologic if it met standard criteria[30] and all the following criteria (when adequate information was available for databases to make predictions): (1) It changed a moderately or highly conserved amino acid, (2) Protean predicted an alteration of protein structure, and (3) it was assessed as possibly or probably pathologic by PolyPhen.

Quantification of Heteroplasmy

Heteroplasmy level was determined for each heteroplasmic sequence variant by the primer extension assay described previously.[32] The level was quantified from fluorescence intensities associated with electrophoretically resolved mutant and wild-type peaks (Genescan, ver. 3.7 software; ABI). The percentage of heteroplasmy was calculated with the following equation: [fluorescent band intensity for the mutant/(fluorescent band intensity for the wild-type + fluorescent band intensity for the mutant)] × 100. This assay reliably detects mutant alleles present at ratios as low as 1% and 3%. The variability of the assay is typically ≤5%.[32]

Determination of Relative mtDNA Content

Competitive multiplex PCR was performed with two simultaneous primer sets, as described previously,[33] a technique that has been applied successfully to a variety of tissues,[34,35] including the blood of patients with LHON (Leber hereditary optic neuropathy)[8] and other optic neuropathies.[18,36,37] 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 a 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 Respiration

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 change in fluorescence (corrected for background and protein concentration) reflects respiration. Lymphocytes from patients and control subjects were incubated with 6 μM resazurin, without and with mitochondrial inhibition by amiodarone 200 μM, and the fluorescence intensity

TABLE 1. Clinical Characteristics of Patients

Patient	Age/ Sex	Family History	VA		IOP		C/D Ratio		VF	
			OD	OS	OD	OS	OD	OS	OD	OS
1	58/F	−	20/160	20/160	24	40	0.5/0.5	.85/.8	Superior arcuate scotoma with nasal step	Superior arcuate scotoma with nasal step
2	46/F	+	20/50	20/50	33	27	.85/.8	.3/.3	Central island	Generalized depression
3	75/M	−	20/40	3/200	24	54	.75/.7	.95/.9	Inferior arcuate scotoma with nasal step	Temporal island
4	56/F	−	NLP	20/30	39	22	.98/.9	.5/.5	Unable	Normal
5	62/F	−	HM	20/40	33	27	.95/.8	.65/.6	Unable	Small nasal step
6	65/F	−	20/60	20/60	32	38	.7/.6	.7/.6	Superior arcuate scotoma with nasal step	Central island
7	63/F	+	HM	20/30	18	17	.85/.8	.5/.5	Unable	Normal
8	61/M	+	20/25	20/30	17	18	.8/.7	.85/.8	Central island	Central island
9	62/M	−	20/40	20/50	23	22	.85/.8	.9/.85	Central island	Central island
10	50/F	−	20/300	20/30	44	16	.85/.8	.3/.3	Superior arcuate scotoma with nasal step; inferior arcuate scotoma	Normal
11	64/F	+	20/20	20/20	30	28	.9/.85	.95/.9	Superior arcuate scotoma with nasal step, inferior paracentral scotoma	Central and temporal island
12	59/M	−	HM	20/30	40	26	.98/.95	.95/.9	Unable	Central island remnant
13	56/F	−	20/30	20/30	52	36	.85/.8	.4/.4	Inferior arcuate scotoma with nasal step; inferior arcuate scotoma	Normal
14	76/M	−	20/25	LP	40	55	.7/.6	.98/.95	Generalized depression	Unable
15	62/M	−	20/20	20/40	20	30	.5/.5	.95/.9	Normal	Central island
16	58/F	+	HM	20/20	48	29	.98/.9	.85/.8	Unable	Superior arcuate scotoma with nasal step
17	66/F	−	NLP	20/60	50	26	NA	.8/.6	Unable	Nasal step
18	68/F	+	4/200	20/40	34	37	.7/.6	.3/.3	Superior arcuate scotoma with nasal step	Nasal step
19	71/M	−	20/40	NLP	47	81	.95/.9	NA	Central island	Unable
20	59/F	+	20/60	20/30	24	40	.3/.3	.7/.6	Generalized depression	Superior arcuate scotoma with nasal step
21	69/F	−	20/50	20/50	23	53	.7/.6	.95/.9	Generalized depression	Central island
22	54/M	+	20/60	20/40	25	20	.75/.7	.2/.2	Central island	Normal
23	67/F	−	20/50	NLP	48	37	.85/.8	NA	Central island remnant	Unable
24	88/F	−	HM	20/50	34	26	.95/.9	.75/.7	Central and temporal islands	Superior nasal step
25	66/F	−	20/200	20/200	32	36	.95/.8	.8/.75	Inferior arcuate scotoma with nasal step	Superior arcuate scotoma with nasal step
26	57/M	−	20/30	20/30	27	20	.7/.5	.5/.5	Small nasal step	Normal
27	53/M	+	20/30	20/30	42	41	.9/.8	.95/.9	Central island	Central island
29	61/M	−	20/30	HM	20	26	.85/.8	.98/.9	Central island	Unable
29	59/M	+	20/30	20/70	25	30	.75/.6	.85/.8	Normal	Central island

Age, age in years; family history, −/+, family history of glaucoma recorded or reported; −, no family history recorded or reported; IOP, maximum documented intraocular pressure; C/D, cup-to-disk ratio in vertical/horizontal dimensions; VA, visual acuity by Snellen plates; VF, results of Goldmann and/or Humphrey visual field perimetry; HM, hand motions; LP, light perception; NLP, no light perception; NA, not available.

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.[22] Mitochondrial metabolic activity has been assessed using resazurin in synaptosomes from spinal cord–injured animals[38] and neonatal rat cerebellum[39] and in isolated yeast mitochondria.[40] The current technique has been validated in systemic mitochondrial disorders[22] including LHON-like optic neuropathies.[36]

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. Statistical comparisons included bivariate correlation, independent-samples *t*-test, and the Fisher exact analysis.

RESULTS

Clinical Information

Table 1 details the clinical characteristics of 29 unrelated patients with PACG (17 women and 12 men; mean age, 62.45 ± 8.40 years) who met inclusion and exclusion criteria. Patients were enrolled between August 2005 and December 2006 from a glaucoma clinic caring for patients with relatively advanced disease. They had been observed for an average of more than 5 years. Family history was positive for glaucoma in 10 pa-

TABLE 2. Nonsynonymous mtDNA Nucleotide Changes

Nucleotide Substitution	Amino Acid Substitution	Base Substitution Type	Location	Heteroplasmy (%)	Interspecies Conservation	Pathogenicity Prediction
6880 C>G	T326ter	Transversion	TM domain of COI	20%	Moderate	Nonpathologic
8660 C>A	T45N	Transversion	Outside the functional domain of ATPase6	N/A	Low	Nonpathologic
8806 C>A	P94T	Transversion	Outside the functional domain of ATPase6	N/A	High	Pathologic
9795 T>A	F197I	Transversion	Outside the functional domain of COIII	N/A	High	Pathologic
10239 A>C	T61P	Transversion	TM domain of ND3	N/A	High	Pathologic
13826 G>T	G497V	Transversion	TM domain of ND5	N/A	High	Pathologic
14051 C>A	S572Y	Transversion	Outside the TM domain of ND5	N/A	Low	Nonpathologic
14113 T>C	F593L	Transition	TM domain of ND5	N/A	Low	Nonpathologic
14171 A>G	I168T	Transition	TM domain of ND6	N/A	Low	Nonpathologic
14966 A>C	N74H	Transversion	Outside the functional domain of CYTB	N/A	High	Nonpathologic
15048 G>C	G101A	Transversion	Outside the functional domain of CYTB	N/A	Moderate	Nonpathologic

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). Heteroplasmy (%), percentage of mutant DNA present; N/A, not applicable because the nucleotide change was always found in the homoplasmic state. Interspecies Conservation, assessed using PolyPhen, 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 Web site when necessary. Pathogenicity prediction, a sequence variant was considered potentially pathogenic if it satisfied all the following conditions: changed a moderately or highly conserved amino acid; Protean predicted an alteration of protein structure; and it was assessed as possibly or probably pathogenic by PolyPhen. Previous reports of these sequence variants were not found in the MITOMAP database, the Human Mitochondrial Genome Database, GenBank, or MedLine-listed publications. None of these nonsynonymous sequence changes were found in ethnicity-matched control subjects (n = 159). Each was present in one patient (1/29; 3.4%).

tients, although family members were not examined and the type of glaucoma was not ascertained.

Five patients had acute angle-closure episodes and went on to develop the chronic form after customary treatment with laser iridotomy and glaucoma medications. Twenty-one had painless, chronic angle-closure glaucoma, and three had symptoms suggestive of intermittent angle closure with haloes, intermittent blurry vision, ocular pain, or headache. Eleven patients had a visual acuity (VA) of hand motions or worse in at least one eye. Mean maximum documented IOP was 38.8 mm Hg. All 29 patients had undergone laser iridotomy to treat pupillary block, and 13 had a filtering procedure, either alone or combined with cataract extraction. Results of OCT and fundus photography agreed well with clinical assessment of optic disc appearance (data not shown).

Sequence Analysis of *MYOC*, *OPTN*, *WDR36*, *CYP1B1*, *OPA1*, and *OPA3*

No novel or previously reported sequence mutation was found in *MYOC*, *OPTN*, *WDR36*, *CYP1B1*, *OPA1*, or *OPA3* in patients with PACG or control subjects. Patients had no previously reported or novel polymorphisms in any of these nuclear genes, and control subjects had only polymorphisms reported previously.[18]

Sequence Analysis of the Mitochondrial Coding Region

The prevalence of synonymous and NS mtDNA nucleotide changes in patients with PACG was not different from that in control subjects (Fisher exact analysis, $P = 0.24$ for synonymous nucleotide changes and $P = 0.55$ for NS changes). Table 2 details the 11 NS mtDNA changes found in patients with PACG after excluding all synonymous mtDNA changes, established NS polymorphisms, and NS mtDNA sequence changes relevant primarily to haplogroup designation. None of these mtDNA sequence changes had been reported, and none was found in ethnicity-matched control subjects. Nine were transversions and two were transitions. One was heteroplasmic (nt 6880 with 20% heteroplasmy) and was considered nonpathologic. Four NS mtDNA sequence changes were considered probably pathologic according to the criteria described in the Methods section.

Table 3 details mtDNA sequence changes by patient. Nineteen (patients 1–19) had none of the NS mtDNA sequence changes listed in Table 2, whereas six (patients 20–26) had NS sequence changes predicted to be nonpathologic. The four potentially pathologic mtDNA sequence changes were present in four patients (26–29). Patient 26 had two NS mtDNA sequence changes from Table 2: one predicted to be pathologic (nt 8806) and one predicted to be nonpathologic (nt 8660). All four patients with potentially pathologic mtDNA changes were male, but they did not differ from other patients in clinical characteristics such as age, family history, IOP, and VA.

Relative mtDNA Content and Mitochondrial Functional Testing

Table 3 also details relative mtDNA content and MRA by patient. Mean relative mtDNA content in patients with PACG (1.13 ± 0.20; 95% CI 1.06–1.21) was not significantly different from that in control subjects (1.16 ± 0.16; 95% CI 1.09–1.22; $P = 0.67$). MRA in patients with PACG (20.63 ± 1.14; 95% CI 20.20–21.07) was 2.4% lower than in control subjects (21.14 ± 1.03; 95% CI 20.86–21.41; $P = 0.044$), which probably is not clinically relevant. This difference is statistically nonsignificant after Bonferroni correction but is best interpreted as indeterminate, given post hoc indication of a minimal sample size approximately three times that studied to attain 80% power of avoiding false-negative interpretation.

TABLE 3. Nonsynonymous mtDNA Sequence Changes, Relative mtDNA Content, and Mitochondrial Respiration Activity by Patient

Patient	NS mtDNA Sequence Changes	Relative mtDNA Content	MRA
1	None	1.50	21.5
2	None	0.83	21.6
3	None	0.82	19.8
4	None	0.80	20.2
5	None	1.20	17.8
6	None	1.20	21.4
7	None	0.88	19.8
8	None	1.20	21.5
9	None	1.20	19.6
10	None	1.20	18.6
11	None	1.24	21.4
12	None	1.48	19.8
13	None	1.10	18.6
14	None	0.80	20.8
15	None	1.20	21.8
16	None	1.20	20.8
17	None	1.24	21.4
18	None	1.28	21.6
19	None	1.20	21.4
20	14171	1.30	20.8
21	14051	1.20	20.5
22	14113	1.15	19.8
23	6880	1.24	17.8
24	14966	0.90	20.4
25	15048	1.46	20.8
26	8660, 8806*	1.12	21.8
27	9795*	0.92	20.8
28	13826*	1.20	21.4
29	10239*	0.85	21.5

NS, nonsynonymous. See Results for group analysis of relative mtDNA content and MRA. Patient order was determined by the character of the nucleotide change from none (patients 1–19) to benign (patients 20–25) to potentially pathologic (patients 26–29).

* Potentially pathogenic nonsynonymous mtDNA sequence changes.

DISCUSSION

We evaluated 29 patients with PACG diagnosed by closure of the anterior chamber angle, history of elevated IOP, and funduscopic and visual field changes compatible with glaucomatous optic nerve damage. Because these patients were seen at a tertiary eye referral center, they had relatively advanced disease, with 14 being legally blind in one eye, two thirds with severe central or peripheral visual field loss, and almost half requiring a glaucoma surgical procedure. Compared with a group of patients with POAG evaluated previously in a similar fashion,[18] these patients with PACG had slightly worse VA and slightly more surgical interventions. These patients did not meet criteria for POAG because of anterior chamber configuration, and they also did not have the clinical characteristics of other types of glaucoma or other spontaneous optic neuropathies, such as LHON or dominant optic atrophy.[41]

No patients had a novel or previously described mutations in *MYOC*, *OPTN*, *WDR36*, *CYP1B1*, *OPA1*, and *OPA3*. These results extend previous negative studies of *MYOC* in Chinese patients with chronic PACG[15] and of *MYOC*, *OPTN*, *CYP1B1*, and *OPA1* in Chinese patients with acute PACG.[14] *MYOC*, *OPTN*, *WDR36*, and *CYP1B1* have been reported in only a small fraction of patients with POAG, and it remains possible that these genes are abnormal with a small prevalence in Arabic patients with PACG or in patients with PACG who are of other ethnicities. Also, other nuclear genes may be important in PACG. For example, a particular SNP of *MMP-9* has been associated with acute PACG,[14] and a genetic localization was reported almost a decade ago in an uncommon variant of angle closure glaucoma characterized by nanophthalmos,[42] a condition marked by a very short globe, severe hyperopia, and high lens/globe volume ratio.

We found evidence of only mild mitochondrial abnormalities in these patients with PACG. Ten patients had a total of 11 novel mtDNA nucleotide changes, and four had mtDNA sequence changes that were predicted to be pathologic. Relative mtDNA content was unchanged from control subjects, and patients with PACG as a group had a small and possibly insignificant decrease in MRA. These results contrast with the substantial mitochondrial abnormalities documented in a group of patients with POAG,[18] in which the frequency of NS mtDNA nucleotide changes that were novel and/or potentially pathologic was double that of the PACG group, and the defect in MRA was almost 10-fold greater. The absence of substantial mitochondrial abnormalities in PACG suggests that not all types of glaucoma are associated with mitochondrial changes.

Optic nerve injury in PACG has been attributed primarily to elevated IOP caused by anatomic changes in the anterior[1] and posterior[43] globe, in contrast with the molecular and biochemical abnormalities suspected in POAG.[44] Perhaps for this reason, the role of nuclear genetic and mitochondrial abnormalities in PACG has received limited attention, even though PACG may be present in 3.9 million people around the world by 2010.[45] If confirmed in other studies involving different ethnic groups, the results reported herein imply that these particular nuclear genetic and mitochondrial factors may indeed be less important than anatomic and dynamic factors that result in closure of the anterior chamber angle to determine who is at risk for visual loss in PACG. We report a relatively small number of Middle Eastern Arab patients, and these genetic and mitochondrial studies should be confirmed and extended, because ethnicity may be important in the clinical characteristics of PACG.[46,47]

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