ORIGINAL ARTICLE

Myocilin and optineurin coding variants in Hispanics of Mexican descent with POAG

Kristin K McDonald¹, Karen Abramson¹, Marco A Beltran², Maria G Ramirez³, Miguel Alvarez³, Alice Ventura⁴, Cecilia Santiago-Turla⁴, Silke Schmidt¹, Michael A Hauser^{1,4} and R Rand Allingham^{1,4}

Coding variants in both myocilin (*MYOC*) and optineurin (*OPTN*) are reported risk factors for primary open-angle glaucoma (POAG) in many populations. This study investigated the contribution of *MYOC* and *OPTN* coding variants in Hispanics of Mexican descent with and without POAG. We conducted a case–control study of unrelated POAG cases and nonglaucomatous controls in a population of Hispanics of Mexican descent. Ascertainment criteria for POAG included the presence of glaucomatous optic neuropathy with associated visual field loss and the absence of secondary causes of glaucoma. Controls had normal optic nerves, visual fields and intraocular pressure. All coding exons of *MYOC* and *OPTN* were sequenced. The data set consisted of 88 POAG cases and 93 controls. A novel nonsynonymous coding variant (R7H) in the first exon of *MYOC* was identified. Other identified variants in *MYOC* and *OPTN* have been previously described and do not seem to contribute to POAG risk. This is the first comprehensive study of *MYOC* and *OPTN* in Hispanics of Mexican descent with POAG. Neither *MYOC* nor *OPTN* sequence variants seem to have a major role in the etiology of POAG in this population.

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INTRODUCTION

Glaucoma is defined as the progressive, irreversible loss of retinal ganglion cells. Primary open-angle glaucoma (POAG) is the most common form of glaucoma. POAG is a complex disorder with known genetic contributions that is often associated with elevated intraocular pressure (IOP).¹ POAG is responsible for more than half of all cases of glaucoma in the world and for blindness in more than 3.3 million people.^{2,3} The number of people who are affected by POAG is steadily continuing to increase, and it is expected to be the cause of blindness in more than 4.4 million people globally by the year 2020.^{4,5}

Individuals of Hispanic descent are at a high risk of developing POAG. Two major population-based studies focusing on ophthalmic disease and vision loss of Hispanics in southwestern United States found that POAG is highly prevalent in Hispanics.^{6,7} These studies reported that the prevalence of POAG in Hispanics over the age of 40 years exceeded 4% and over the age of 80 years approached 20%, which is even higher than the reported prevalence in African Americans.² The clinical phenotype also differed between Hispanics and other US populations. In all, ~80% of Hispanics with POAG were found to have normal IOP on initial screening, as opposed to Caucasian and African American POAG patients, of whom the majority have elevated IOP.^{2,6,7} The high frequency of normal IOP on initial screening in the Hispanic population is shared by the

Japanese population and may represent underlying genetic and environmental differences between populations.⁸

Variants in myocilin (*MYOC*) and optineurin (*OPTN*) have been associated with risk for developing POAG.^{9–12} *MYOC* variants are found in 3–5% of POAG patients and are associated with an early-onset, high-tension form of open-angle glaucoma.^{11,13–15} *OPTN* variants seem to be associated in patients with POAG and normal IOP.^{12,16} Little is known about the genetic etiology of POAG in the Hispanic population. Although *MYOC* and *OPTN* have been sequenced in Brazilian and Spanish populations, the role of these genes in POAG in Hispanics of southwestern United States has not been reported to date.^{17–19} In this paper we report the contribution of *MYOC* and *OPTN* coding variants to POAG risk in a data set of Hispanics of Mexican descent.

MATERIALS AND METHODS

Subject ascertainment

This study adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from all participating individuals after explanation of the nature and risks of the study. The research project was reviewed and approved by the institutional review board of Duke University Medical Center (Durham, NC, USA). Subjects for this study were recruited from eye clinics located in Sonora, Mexico and Nogales, Arizona, and described themselves as being of

¹Center for Human Genetics, Duke University Medical Center, Durham, NC, USA; ²Clinica Oftalmologica, Hermosillo, Sonora, Mexico; ³Oftalmologos Asociados, Nogales, Sonora, Mexico and ⁴Department of Ophthalmology, Duke University Eye Center, Duke University Medical Center, Durham, NC, USA
Correspondence: KK McDonald, Center for Human Genetics, Duke University Medical Center, 905 S. LaSalle Street, Durham, NC, 27710, USA.
E-mail: kkm8@duke.edu

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Hispanic descent, with both parents of Mexican ancestry. These individuals are sometimes termed Mestizos (individuals of mixed European and North American indigenous ancestry). Study subjects include individuals diagnosed with POAG, as well as unaffected spouses and other unaffected individuals.

POAG cases were unrelated and met the following inclusion criteria: (1) glaucomatous optic nerve damage in both eyes and (2) glaucomatous visual field defects in at least one eye. Glaucomatous optic nerve damage was defined as being present when two or more of the following criteria were identified: (a) vertical or horizontal cup/disc ratio ≥ 0.7 ; (b) superior or inferior neuroretinal rim cup/disc ratio ≤ 0.1 ; (c) focal notching of the superior or inferior neuroretinal rim; (d) asymmetry of the cup/disc ratio ≥ 0.2 without asymmetric refraction; and/or (e) optic disc hemorrhage. IOP was recorded but was not used as an inclusion criterion. Visual fields were performed using standard automated perimetry.⁴ In addition, all cases had open angles by gonioscopy. IOP measurements (mm Hg) were taken by applanation tonometry. The highest IOP measurements used were those obtained by ophthalmologists at the time of examination. The patients were in many cases indigent and were seen on one occasion by the study ophthalmologists; no past medical history was obtained for these individuals because of the potential for variability in the manner in which IOP measurements were taken at other locations. However, for those individuals who were regular patients of the study ophthalmologists, the highest IOP measurement was the highest IOP seen by the coauthors over time. IOP measurements were not taken for patients who had ocular surgery before their examination.

Exclusion criteria included other identifiable forms of glaucoma due to or associated with exfoliation syndrome, pigmentary dispersion syndrome or traumatic glaucoma. The controls used for this study were well matched to cases by ethnicity, and met the following criteria: (1) IOP without treatment < 22 mm Hg; (2) no evidence of glaucomatous optic nerve disease; and (3) normal automated visual field testing (Humphrey SITA standard or equivalent). All examiners are board-certified ophthalmologists. All clinical data, including visual field tests and optic nerve photos, were reviewed by a board-certified glaucoma fellowship-trained ophthalmologist (RRA). Blood samples for DNA extraction were obtained from a total of 88 POAG cases and 93 POAG controls

and were processed and stored at the DNA repository at the Center for Human Genetics, Duke University Medical Center. Mean IOP for cases was calculated by averaging the highest recorded IOP of both eyes where available, thereafter averaging all cases. Mean IOP for controls was calculated by averaging the IOP of both eyes at ascertainment, thereafter averaging all controls.

DNA analysis

Genomic DNA was extracted from peripheral blood by standard techniques (Gentra, Minneapolis, MN, USA). Primers flanking each exon, 1–3 in *MYOC* and 1–16 in *OPTN*, were designed with Primer3 Plus software (Humana Press, Totowa, NJ, USA) and are listed in Table 1.²⁰ All sequencing procedures were performed using conditions that were previously described.²¹ All suspected variations were confirmed by bidirectional sequencing. Sequencing was initially performed using cases only. Amplicons containing any nonsynonymous coding changes or variants of uncertain pathogenicity in cases were then sequenced in the control data set.

Statistical analysis

Sequencing data were examined using Sequencher 4.9 software (Gene Codes Corporation, Ann Arbor, MI, USA) and were analyzed as described previously.²² Briefly, allele frequencies for each coding and intronic variant in POAG cases and controls were compared using a two-sided Fisher's exact test using SAS/Genetics version 9.1 (SAS Institute, Cary, NC, USA). A *P*-value of < 0.05 was considered statistically significant. The 176 POAG case chromosomes sequenced in this study are sufficient to detect over 99.9% of single-nucleotide polymorphisms with a minimum allele frequency of 5%.²³

RESULTS

The mean age of onset for 88 POAG cases and age of exam for 93 controls were 62.4 (s.d. ± 11.2) and 62.7 (s.d. ± 8.3) years, respectively. In all, 52% of POAG cases and 66% of POAG controls were female. The mean highest recorded IOP for controls was 13.8 ± 2.7 mm Hg, whereas that of cases was significantly higher at 19.0 ± 6.5 mm Hg

Table 1 PCR primers for sequencing genomic DNA

	Exon	PCR forward primer sequence	PCR reverse primer sequence	PCR product size (bp)
<i>MYOC</i>	1a	ATCTTGCTGGCAGCGTGAA	TCTCTGGTTGGGTTTCC	614
	1b	GACAGCTCAGCTCAGGAAGG	GAAGGTGATCGCTGTGCTTT	663
	2	AGCAAAGACAGGGTTTCACC	AGGGCTTTGTAGGGAAAGG	555
	3a	TGCGATAACTGAGGCGTAGA	GCCTCATCGGTGCTGTAAAT	663
	3b	GTCCAGAAGTGTCTAAGA	CGCCCTCAGACTACAATTCC	679
	3c	GCCTGGGACAACCTGAACAT	CAGGCACAAGCCTCTCAGTT	719
<i>OPTN</i>	1	CGGACAGCGAGGGTGGGTA	CAGGACCCGCCGAGGCTT	554
	2	AAGCAGAGTGGGGATTACTCA	TTCCCATGCAAATCTTCAAA	500
	3	CCCCATTTCCCAAATCCTTA	GAGGCAGCTGAGAGGTTGAT	633
	4	TAAGTATTAGCAATCGCCAA	AGTGCAAAGGGATGGCATTT	340
	5	CATCAGATCAAGTCCACTTT	GGAGTCTAGACACGTAAGAT	340
	6	ATGGTGCCAGCCTTAGTTT	CAATCCTTGGCTTGTTGA	340
	7	CATCTGAATGTTTGAAGCT	TATTCTGGAAGATCCTGGT	340
	8	ATACTGAACAGGCGATTGTC	GTGGTTGCACAATCCTGGAA	300
	9	GATCCTTTATCCCAATTGTA	TTGAATTCAGTTGCTGGACT	282
	10	TTGATTACACGACGCTCTT	GCTCACACATTAAGTGAAC	400
	11	TGCATTATAAACCTACAG	TAGGACTCCTTCAGATAAGT	400
	12	TTGAGAGTAAGAAATGCTAG	GATTTAGTGAAGGATTCATG	340
	13	ATGTTGCCAGGCTTGCTCTC	CACCATTCCTTCCAATGCG	420
	14	GGATACAGCACTACCTCCTC	TCAGGAACGCTTTGGACAG	300
	15	GCTCAGTGTGTCTGTTTC	GGAATCCATTGTAGAGAATTG	240
	16	TCGCCATCTGTTCTTCAAGT	AAAAGCACAACTCTTGGAGG	269

Abbreviations: *MYOC*, myocilin; *OPTN*, optineurin.

Table 2 MYOC and OPTN sequence variants found in POAG patients and control Hispanics of Mexican Descent

Gene	Location	Sequence change	Codon change ^a	SNP ID	Allele	Minor allele frequency	
						POAG ^b	Control ^c
MYOC	Exon 1	G>A	R7H	Unlisted	A	0.01	0.00
	Exon 1	G>A	R76K	rs2234926	A	0.11	0.09
	Exon 1	C>T	G122G	Unlisted	T	0.01	NA
	Exon 3	G>T	T285T	Unlisted	T	0.01	NA
	Exon 3	T>C	Y347Y	rs61730974	C	0.02	NA
	Exon 3	A>G	K398R	rs56314834	G	0.01	0.00
OPTN	Intron 2	4 bp (ACAC)	c.374-194_374-193insACAC	rs67406260	ACAC	0.30	0.31
	Exon 4	G>A	T34T	rs2234968	A	0.27	0.26
	Exon 5	T>A	M98K	rs11258194	A	0.03	0.03
	Intron 6	T>C	c.553-5T>C	rs2244380	C	0.10	0.13

Abbreviations: NA, not applicable; POAG, primary open-angle glaucoma; SNP, single-nucleotide polymorphism.

Control individuals were not genotyped across amplicons in which only previously reported and consistently neutral polymorphisms were found.

^aThe sequence files used to number residues were NM_000261.1 for MYOC and NM_001008211.1 for OPTN.

^b88 POAG cases were sequenced across all myocilin (MYOC) and optineurin (OPTN) exons.

^c93 controls were sequenced across all MYOC and OPTN exons except for those which only contained known polymorphisms in the cases.

($P < 0.0001$). Of the POAG cases for which highest IOP measurements had been obtained for both eyes, a majority, 59%, had IOP below 22 mm Hg.

Sequencing results

MYOC. A single novel nonsynonymous coding variant, R7H, was identified in MYOC. This variant was identified in one allele of a single affected individual with an age at onset of 52 years. Five additional coding variants were identified: three synonymous (G122G, T285T and Y347Y) and two nonsynonymous (R76K and K398R; Table 2). None of these variants had allele frequencies that differed significantly between cases and controls ($P > 0.05$).

OPTN. Four variants were identified in the optineurin (OPTN) gene: two intronic (c.374-194_374-193insACAC and c.553-5T>C), one synonymous (T34T) and one nonsynonymous (M98K). None of these variants were significantly associated with POAG in this population ($P > 0.05$).

DISCUSSION

Two previous studies examining eye disease in Hispanics in the southwestern United States found that POAG is highly prevalent in Hispanics. Disease-associated MYOC variants have been reported in 3–5% of POAG cases from many different populations,^{11,13–15} including the Brazilian and Spanish populations.^{17,19} This study was undertaken to determine the role of MYOC and OPTN variants in POAG in the Hispanic population of Mexican descent. Sequence analysis of 88 POAG patients revealed six MYOC and four OPTN sequence variants. Of the six MYOC variants, five (G122G, T285T, Y347Y, R76K and K398R) were previously reported as neutral variants.^{13,24,25} Our results also indicate that these variants are neutral as they are not found at significantly different frequencies in cases and controls. It is unclear whether the novel MYOC variant, R7H in exon 1, is a disease-associated variant because it was found as a heterozygous change in only one individual. The individual with the R7H variant had an age of onset of POAG of 52 years and a highest recorded IOP of 38 mm Hg, which would be consistent with the phenotype of a myocilin-associated form of POAG in which IOP is almost always significantly elevated. However, the majority of disease-associated

variants in myocilin are found in exon 3, although disease-associated variants have been reported in exon 1.²⁶ A study of Brazilian patients by Pova *et al.*¹⁷ found the MYOC variant Cys433Arg in 3.1% of POAG patients and in 5.2% of POAG patients with a family history of the disease. In a similar study on 110 Spanish subjects with POAG, Lopez-Martinez *et al.* found that 2.7% had causative MYOC variants, including Gln368Stop, Ala445Val and Tyr479His.¹⁹ We did not find these variants from either study in our research. Although MYOC mutations are consistently found in a low percentage of subjects with POAG in populations worldwide, we found no documented pathological mutations in this Hispanic population of Mexican descent.

Variants in OPTN were originally identified in families with normal tension glaucoma.¹² Two population-based studies found that 80% of POAG cases had normal IOPs on ascertainment.^{6,7} Despite this phenotypic characteristic, we were unable to find evidence that OPTN variants have a contributory role in POAG in this population. We found four sequence variants in OPTN. Two variants, T34T and c.553-5T>C, have previously been reported to be neutral polymorphisms.^{12,18,21,27,28} The M98K variant was initially reported to be associated with POAG risk, but the results of subsequent studies are conflicting.^{1,12,16,19,21,29–33} c.374-194_374-193insACAC has not been reported in the context of previous glaucoma studies, but is listed in dbSNP (<http://www.ncbi.nlm.nih.gov/projects/SNP/>). None of these OPTN variants, including M98K, were significantly associated with POAG in this population. Studies on other Hispanic populations also show a low prevalence of OPTN variants that cause POAG. Lopez-Martinez *et al.* failed to find OPTN variants that were significantly associated with POAG in an analysis of OPTN sequence variants in Spanish POAG patients.¹⁹ Similarly, a study conducted by Caixeta-Umbelino *et al.*, which examined the role of multiple OPTN variants in the development of POAG in a Brazilian population, did not find any association between the evaluated variants and POAG risk.¹⁸ Our findings also support the findings of these studies regarding the role of OPTN in POAG in populations of Hispanic descent.

This study is the first reported survey of MYOC and OPTN sequence variants in a Hispanic population of Mexican descent. Our results suggest that the contribution of these genes to the high prevalence of POAG in this population is small. These results should be confirmed with a larger sample size of this population.

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