Abstract

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 non-glaucomatous 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 IOP. All coding exons of MYOC and OPTN were sequenced. The dataset 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 appear 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 appear to play a major role in the etiology of POAG in this population.

Keywords

myocilin; optineurin; primary open-angle glaucoma; coding variants

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). POAG is responsible for more than half of all of the cases of glaucoma in the world and the blindness of more than 3.3 million people. The number of people who are affected by POAG is steadily continuing to increase, and it is expected to be the cause of blindness for more than 4.4 million people globally by the year 2020.
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 the Southwestern United States found that POAG is highly prevalent in Hispanics.6,7 These studies reported that the prevalence of POAG in Hispanics over age 40 exceeded 4% and over age 80 approached 20%, which is even higher than the reported prevalence in African Americans.2 The clinical phenotype also differed between Hispanics and other U.S. populations. Approximately 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 has elevated IOP.2,6,7 The high frequency of normal IOP on initial screening in the Hispanic populations 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 appear to be associated in patients with POAG and normal intraocular pressure.12,16 Little is known about the genetic etiology of POAG in the Hispanic population. Although MYOC and OPTN have been sequenced in the Brazilian and Spanish populations the role of these genes in POAG in Hispanics of the Southwestern United States has not been reported to date.17-19 Herein we report the contribution of MYOC and OPTN coding variants to POAG risk in a dataset 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). Subjects for this study were recruited from eye clinics located in Sonora, Mexico and Nogales, Arizona, and described themselves as being of 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 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 Additionally, all cases had open angles by gonioscopy. IOP measurements (mmHg) were taken by applanation tonometry. The highest IOP measurements used were those obtained by the ophthalmologists at exam. 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 due to 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 prior to exam.

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) intraocular pressure without treatment less than 22 mmHg; (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 then averaging all cases. Mean IOP for controls was calculated by averaging the IOP of both eyes at ascertainment then averaging all controls.

DNA Analysis

Genomic DNA was extracted from peripheral blood by standard techniques (Gentra, Minneapolis, MN). Primers flanking each exon, (1-3) in MYOC and (1-16) in OPTN, were designed with Primer3 software and are listed in Table 1. All sequencing was performed using conditions that were previously described. All suspected variations were confirmed by bidirectional sequencing. Sequencing was initially performed using cases only. Amplicons containing any non-synonymous coding changes or variants of uncertain pathogenicity in cases were then sequenced in the control dataset.

Statistical Analysis

Sequencing data were examined using Sequencher 4.9 software (Gene Codes Corporation, Ann Arbor, MI) and were analyzed as described previously. 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 Inc., Cary, NC). A p-value of less than 0.05 was considered statistically significant. The 176 POAG case chromosomes sequenced in this study are sufficient to detect over 99.9% of SNPs 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 (SD ±11.2) and 62.7 (SD ±8.3) years, respectively. 52% of POAG cases were female and 66% of POAG controls were female. The mean highest recorded IOPs for controls was
13.8±2.7mmHg, while that of cases was significantly higher at 19.0±6.5mmHg (p<0.0001).
Of the POAG cases for which highest IOP measurements had been obtained for both eyes, a
majority, 59%, had IOPs below 22mmHg.

Sequencing results

**MYOC**—A single novel non-synonymous 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 non-synonymous (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 gene (OPTN): 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) have previously been reported as neutral variants.13,24,25 Our results also indicate that these variants are neutral since 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 at 52 years and a highest recorded IOP of 38mmHg which would be consistent with the phenotype of a myocilin-associated form of POAG where 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 one.26 A study of Brazilian patients by Povoa et al. 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.17 In a similar study on 110 Spanish subjects with POAG, Lopez-Martinez and co-workers 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 study. Although **MYOC** mutations are consistently found in a low percentage of subjects with POAG in populations worldwide, we found no documented pathologic 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 intraocular
pressures on ascertainment. Despite this phenotypic characteristic, we were unable to find evidence that \textit{OPTN} variants play a contributory role in POAG in this population. We found 4 sequence variants in \textit{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 \textit{c.374-194}_374-193\text{insACAC} 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 \textit{OPTN} variants, including M98K, were significantly associated with POAG in this population. Studies of other Hispanic populations also show a low prevalence of \textit{OPTN} variants that cause POAG. Lopez-Martinez et al. failed to find \textit{OPTN} variants that were significantly associated with POAG in an analysis of \textit{OPTN} sequence variants in Spanish POAG patients.\,19 Similarly, a study conducted by Caixeta-Umbelino et al., which examined the role of the multiple \textit{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 \textit{OPTN} in POAG in populations of Hispanic descent.

This study is the first reported survey of \textit{MYOC} and \textit{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.

**ACKNOWLEDGMENTS**

This work would have been impossible without the generous participation of POAG patients and their families. The study was supported by NIH grants R01EY013315 (M.A.H.), R01 EY019126 (M.A.H.), and R01 EY015543-04 (R.R.A.).

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Table 1

| Exon | PCR Forward primer sequence | PCR Reverse primer sequence | PCR product size (bp) |
|------|-----------------------------|-----------------------------|----------------------|
| 1a   | ATCTTGCTGGCACCGTGAAG       | TCTCTGGTTTGGGTTTCC          | 614                  |
| 1b   | GACAGTCTAGCTAGGAAGG        | GAAGTGAT CGCTGCTTT          | 663                  |
| 2    | AGCAAGACAGGAGTTTACCCG     | AGGGCTTGTAGGGAAAGG          | 555                  |
| 3a   | TGCGATAACTGAGGCCGTAGA      | GCCTACCTCGTGCTGTAAT         | 663                  |
| 3b   | GCCCGAAGACTCTGAACAT        | GCGCCCTAGACCTCAATCC          | 679                  |
| 3c   | GCCCGGAACTTTGAAACAT        | CAGGCCAACGCTCTAGTT          | 719                  |
| 4    | CGGACAGGAGGGTGTTGTA       | CAGGACCCGCGCGAGGCT          | 554                  |
| 2    | AAGCAGAGTGGGAGTTTACTCA    | TCCCATGCAAATCTTCAA          | 500                  |
| 3    | CCCATTCCTCTCTCTCTTT       | GCCGCAGCTGAGGCTGTA          | 633                  |
| 4    | TAAGTATAGCAATCGCCA         | AGTGCAAAAGGATGGCATTT        | 340                  |
| 5    | CACCATGACTAAGTCCAATTCT    | GGAGTTGAGACAGTAAGAT         | 340                  |
| 6    | ATGCTGCCCACCTCTTTAT       | CAACTCTTGGCTGTGTTGTA        | 340                  |
| 7    | CATCTGATGTTGGAAGCT        | TATCTGGAAAGATCTGTA          | 340                  |
| 8    | ATACGTGAAAGGCCATTTGTC     | GTGGTGCAACATCTGGAA          | 300                  |
| 9    | GATCTTGTATTCCTAATTTGA     | TTGAATCAGTTGCTGACT          | 282                  |
| 10   | TTGATTCCACCACCGCTTTCT     | GCTCACACATTAACTGGAAC        | 400                  |
| 11   | TGCACTTACAAAACCTACAG      | TAGGACTTCTACAGTAAGT         | 400                  |
| 12   | TTGATGCAGAAGAATGCTAG      | GATTTAGTGAGGATTCAG          | 340                  |
| 13   | ATGCTGCCCAGGCTCTCCTC     | CACATCTGCTTCCAAATGCG        | 420                  |
| 14   | GGATACAGCAGACTACCTCCTC   | TCAGGAAGCTTTGAGGACAG        | 300                  |
| 15   | GCTCATTTGTTGTCATGTTC      | GGAATCATTGTAGAGAATG         | 240                  |
| 16   | TGGCCATCTCCTTTCTTCAGT     | AAAAGCCAAACTCTGGAGG         | 269                  |

*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 | SNP ID | Allele | Minor Allele Frequency |
|------|----------|----------------|--------------|--------|--------|------------------------|
| MYOC | Exon 1   | G > A          | R7H          | Unlisted | A      | 0.01 0.00            |
| MYOC | Exon 1   | G > A          | R76K         | rs2234926 | A      | 0.11 0.09           |
| MYOC | Exon 1   | C > T          | G122G        | Unlisted | T      | 0.01 N/A            |
| MYOC | Exon 3   | G > T          | T285T        | Unlisted | T      | 0.01 N/A            |
| MYOC | Exon 3   | T > C          | Y347Y        | rs61730974 | C      | 0.02 N/A            |
| MYOC | Exon 3   | A > G          | K398R        | rs56314834 | G      | 0.01 0.00           |
| OPTN | Intron 2  | 4bp (ACAC)     | c.374-194_374-193insACAC | rs67406260 | ACAC  | 0.30 0.31           |
| OPTN | Exon 4   | G > A          | T34T         | rs2234968 | A      | 0.27 0.26           |
| OPTN | Exon 5   | T > A          | M98K         | rs11258194 | A      | 0.03 0.03           |
| OPTN | Intron 6  | T > C          | c.553-5T>C   | rs2244380 | C      | 0.10 0.13           |

N/A= not applicable. Control individuals were not genotyped across amplicons in which only previously reported and consistently neutral polymorphisms were found.

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

#88 POAG cases were sequenced across all myocilin (MYOC) and optineurin (OPTN) exons

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