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# Association study of multiple gene polymorphisms with the risk of adult-onset primary open-angle glaucoma in a Mexican population

Beatriz Buentello-Volante [a], Celia Elizondo-Olascoaga [b], Antonio Miranda-Duarte [c], Dalia Guadarrama-Vallejo [a], Jesús Cabral-Macias [a], Juan C. Zenteno [a,d,*]

[a] Research Unit-Genetics, Institute of Ophthalmology, "Conde de Valenciana," Mexico City, Mexico
[b] Glaucoma Department, Institute of Ophthalmology, "Conde de Valenciana," Mexico City, Mexico
[c] Department of Genetics, National Rehabilitation Institute, Mexico City, Mexico
[d] Department of Biochemistry, Faculty of Medicine, National Autonomous University of Mexico, Mexico City, Mexico

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ABSTRACT

The aim of this study was to investigate the association of multiple primary open-angle glaucoma (POAG)-risk alleles in a Mexican population for the first time. Genotyping was performed for a total of 26 previously associated alleles located in 11 different genes, including *MYOC*, *CYP1B1*, *OPTN*, *IL1A*, *TNF*, *OPA1*, *EDNRA*, *AGTR2*, *MTHFR*, *GSTM1*, and *GSTT1*. The frequencies of these variants were compared in a group of 218 individuals (118 with POAG and 100 adult controls without the disease). Genomic DNA was extracted from blood leukocytes, and genotyping was performed using PCR followed by direct sequencing. *GSTM1* and *GSTT1* deletion variants were screened by agarose gel analysis. Individual SNP analysis showed that no specific variants conferred an elevated risk for developing POAG. However, the CG genotype for rs5335 polymorphism in *EDNRA* showed a protective effect against the development of POAG, as it provides an estimated odds ratio of 0.5 (95% CI, 0.3–0.9; $p = 0.03$). Moreover, one haplotype consisting of rs1056827 and rs100012 in *CYP1B1* gene was significantly associated with a protective effect against POAG ($p = 0.0045$; OR = 0.3; 95% CI, 0.1–0.7). This is the first case–control investigation of POAG-risk alleles in multiple genes in a Latino population. Although our results support that the analyzed variants are not major risk factors for POAG in this ethnic group, they also point toward a protective effect conferred by *EDNRA* rs5335, as well as by a *CYP1B1* haplotype consisting of rs1056827 and rs100012. Our study emphasizes the importance of genotyping ethnic groups with a complex admixture of ancestral populations for contributing to dissecting the genetics of POAG.



## 1. Introduction

Glaucoma is a group of progressive optic neuropathies in which the axons in the optic nerve are injured, retinal ganglion cell numbers are reduced, and vision is gradually and permanently lost. It comprises a heterogeneous group of disorders with multiple molecular mechanisms underlying its pathogenesis, and it is the second leading cause of irreversible blindness worldwide. Glaucoma is classified according to whether it is congenital or acquired, whether the configuration of the iridocorneal angle is an open or closed angle, and whether a direct cause is recognized (secondary) or not (primary). Primary open-angle glaucoma (POAG) is the most common form in Europe, Africa, South Asia, and Latin America (Quigley, 1996), with reported prevalence rates ranging from 1.1% (Dielemans et al., 1994) to 3.8% (Polansky et al., 2003; Topouzis et al., 2007). POAG may or may not be associated with elevated intraocular pressure (IOP), and it typically has an adult onset (usually >35 years) or juvenile onset (<35 years) (Shields, 2005). Well-recognized risk factors for the development of POAG include elevated IOP, increasing age, African ancestry, and family history of glaucoma (Libby et al., 2005; Manni et al., 2008). Typically, adult-onset POAG is inherited in a multifactorial or complex fashion. It is estimated that family history of glaucoma accounts for a one- to ten-fold risk among the first-degree relatives of an affected individual (Green et al., 2007).

It is well known that the risk for developing a multifactorial disease is greatly influenced by the occurrence of allelic polymorphisms in a variety of genes. In recent years, association studies have identified many genetic variants that might contribute to adult-onset POAG. Among these, polymorphisms in genes such as

* Corresponding author. Genetics Department, Institute of Ophthalmology "Conde de Valenciana," Chimalpopoca 14, Col. Obrera, Mexico City, CP 06800, Mexico. Tel.: +52 54421700x3212; fax: +52 54421700x3206.
*E-mail address:* jczenteno@institutodeoftalmologia.org (J.C. Zenteno).

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Myocilin (*MYOC*) (Alward et al., 1998; Mukhopadhyay et al., 2002; Melki et al., 2003; Polansky et al., 2003; Fan et al., 2005; Kumar et al., 2007; Lopez-Martinez et al., 2007; Bhattacharjee et al., 2007; McDonald et al., 2010; Whigham et al., 2011; Rose et al., 2011; Banerjee et al., 2012; Liu et al., 2012), Cytochrome P450 (*CYP1B1*) (Melki et al., 2005; Acharya et al., 2006; Bhattacharjee et al., 2008), Optineurin (*OPTN*) (Leung et al., 2003; Fan et al., 2005; Mukhopadhyay et al., 2005; Sripriya et al., 2006; Caixeta-Umbelino et al., 2009), Interleukin 1, alpha (*IL1A*) (Wang et al., 2006; Mookherjee et al., 2010), Tumor necrosis factor (*TNF*) (Fan et al., 2010; Bozkurt et al., 2012), Optic atrophy 1 (*OPA1*) (Yao et al., 2006; Mabuchi et al., 2007), Endothelin receptor type A (*EDNRA*) (Ishikawa et al., 2005; Kim et al., 2006), Angiotensin II receptor, type 2 (*AGTR2*) (Hashizume et al., 2005), Methylenetetrahydrofolate reductase (*MTHFR*) (Jünemann et al., 2005; Zetterberg et al., 2007; Clement et al., 2009), Glutathione S-transferase Mu1 (*GSTM1*), and Glutathione S-transferase Theta 1 (*GSTT1*) (Juronen et al., 2000; Unal et al., 2007; Abu-Amero et al., 2008; Rocha et al., 2011) have been shown to confer elevated risk for developing POAG. These associations have been successfully replicated in several ethnically different populations, but not in others, suggesting that the effects of these gene variants depend on the genetic background of the subject.

Independent replication of POAG-associated alleles in populations that are not traditionally screened is important for further delineation of the molecular basis of the disease, as well as for the possible identification of ethnic-specific differences in the magnitude with which particular genetic variants modify disease risk. Until now, the studies of association between adult-onset POAG risk and genetic polymorphisms in Latino populations, which are ethnic groups with considerable genetic admixture, has been only focused to single-gene variants (Silva et al., 2009; Caixeta-Umbelino et al., 2009; Kashara et al., 2011; Magalhaes da Silva et al., 2012; Mendoza-Reinoso et al., 2012). The purpose of this study is to present the results of the first association study between POAG and multiple gene polymorphisms in a Mexican population.

## 2. Material and methods

### 2.1. Subjects

Unrelated adult-onset POAG patients and control subjects were recruited at the Institute of Ophthalmology "Conde de Valenciana" in Mexico City, Mexico. The study was approved by the Institutional Review Board of the Institute of Ophthalmology "Conde de Valenciana," Mexico City. Informed consent was signed by all subjects before participation in the study. All the participants were of Mexican Mestizo origin. Mexican Mestizo is defined as a person who was born in Mexico, has a Spanish-derived last name, and has a family of Mexican ancestors going back to the third generation (Gorodezky et al., 2001). Diagnosis of POAG was based on exclusion of congenital glaucoma and secondary causes (pseudoexfoliation syndrome, uveitis, trauma, steroid-induced glaucoma), anterior chamber angle open, grade III or IV gonioscopy, optic nerve and visual field changes compatible with glaucomatous damage, and initial IOP (before treatment) above 21 mmHg. All patients were above 50 years of age and underwent complete ophthalmological evaluations that included medical history, best-corrected visual acuity, slit lamp biomicroscopy, applanation tonometry, gonioscopy, dilated fundoscopy, ophthalmoscopy of the optic disc, computerized visual field (Humphrey Field Analyzer: HFA SITA 24 2 white–white), and central ultrasound pachymetry.

Inclusion criteria for the control group were: age above 55 years, IOP below 21 mmHg, open anterior chamber angle, and optic nerve without abnormalities suggestive of glaucoma. They were recruited from the outpatient care unit of the same institution.

### 2.2. Sample collection and DNA analysis

A total of 26 polymorphic variants located in 11 genes (Table 1) were genotyped in both the POAG and control groups. All these variants were selected from previous studies in which they showed statistical association with POAG or at least a higher frequency in the POAG group (see references in Table 1). Genomic DNA was extracted from 200 μl of whole blood using a QuickGene-810 semiautomated DNA extraction system (Autogen, Holliston, MA, USA). Polymorphic variants were screened by polymerase chain reaction (PCR) followed by direct DNA sequencing using a BigDye Terminator DNA sequencing kit on a 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Oligonucleotide sequences and PCR conditions are shown in supplementary Table 1. *GSTM1* and *GSTT1* deletion variants were screened only by PCR and agarose gel analysis.

### 2.3. Statistical analysis

Comparisons of continuous variables were tested by Student's *t*-test, and corrected chi-squared statistics were applied for categorical variables. Uni- and multivariate, non-conditional logistic regressions were conducted to determine risk magnitude, comparing each allele, and with genotype as the main effect employed as a binary variable. Odds ratios (ORs) and 95% confidence intervals (95% CI) were reported. An alpha level of 0.05 was used for significance. STATA ver.10.0 statistical software package was utilized for calculations. Allele frequencies, Hardy–Weinberg equilibrium (HWE) and haplotype association analyses were assessed with Haplo View 4.0 software (Daly Lab, Broad Institute, Cambridge, MA). For the haplotype construction, all the alleles were tested, except those showing HWE deviation. Only haplotypes demonstrating statistical significant differences were reported.

## 3. Results

A total of 218 individuals, 118 POAG patients and 100 control subjects, were genotyped. The mean age at recruitment was 67.9 years in cases and 71 years in the controls. The gender distribution between cases and controls was not significantly different ($p = 0.11$). Other demographic data in both groups are shown in Table 2.

Most SNPs followed Hardy–Weinberg equilibrium in the control group. Our results were separated into two groups of SNPs: those that were not polymorphic in this particular population (i.e., only one genotype was observed in the cases and controls) and included seven variants (Supplementary Table 2). The second group included nineteen polymorphisms with allelic differences among the cases and controls (Supplementary Table 3). Most SNPs in this group followed HWE in the control group (marked with asterisks in Supplementary Table 3). Genotypic frequencies analysis in this group of 19 variants showed that three homozygote genotypes, AA for rs2234926 in *MYOC*, GG for rs1800440 in *CYP1B1*, and TT for rs1800587 in *IL1A*, were shown to have a tendency to be associated with POAG (Table 3). Although ORs conferred by these particular variants ranged from 1.6 to 3.2, no statistical significance was reached ($p = 0.09–0.9$). Similar results were obtained when the analysis was performed adjusting for variables as gender, age and diabetes mellitus (Table 3, last two columns). The most consistent finding was genotype CG for EDNRA SNP rs5335, which demonstrated a significant association with POAG ($p = 0.03$; OR = 0.5; 95% CI, 0.3–0.9) (Table 3). Thus, this particular SNP genotype confers protection for POAG development. Interestingly, a significant association between genotypes of rs1801708 in EDNRA and POAG risk was observed; however, as this variant was not in HWE in our