

Evaluation of SNPs on Chromosome 2p with Primary Open Angle Glaucoma in the South Indian Cohort

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PURPOSE. Glaucoma comprises a heterogeneous group of optic neuropathies with a complex genetic basis. It is the second leading cause of irreversible blindness in the world. This study investigates the association of SNPs on chromosome 2p with primary open angle glaucoma (POAG) in a Southern Indian population.

METHODS. Case-control analysis was performed using 220 unrelated POAG cases and 220 age-matched unaffected controls recruited through the Aravind Eye Hospital and its outlying clinics. Five SNPs (rs1533428, rs12994401, rs10202118, rs11125375, and rs11889995) on chromosome 2p were evaluated in these two groups and genotyped using Taq Man SNP genotyping assay. Statistical analysis was performed using the SVS program package by Golden Helix to identify the distributions of allele and genotype frequencies, Fisher exact test *P* values, and odds ratios and to check Hardy-Weinberg equilibrium.

RESULTS. Among the five SNPs screened, SNP rs10202118, showed a *P* = 0.026 for the basic allelic test, *P* = 0.004 for the genotypic test, and *P* = 0.0014 for the recessive model. The second suggestive marker was rs11125375, which also showed *P* = 0.033 for the recessive model. The associated SNPs formed a common disease haplotype. The remaining three SNPs showed insignificant association in this study population.

CONCLUSIONS. This was the first study to demonstrate the association of SNPs on chromosome 2p in patients with POAG in the Indian population. The two tagging SNPs (rs10202118 and rs11125375) on chromosome 2p are the most likely sites underlying the significant association with POAG in this study population. (*Invest Ophthalmol Vis Sci.* 2012;53:1861-1864) DOI:10.1167/iovs.11-8602

Glaucoma is a complex, heterogeneous disease characterized by a progressive degeneration of the retinal ganglion cells and their axons manifesting as optic nerve head cupping and field loss. It is the second most common cause of blindness, affecting over 66 million people worldwide.¹ Most glaucoma

patients are asymptomatic until late in the disease course; therefore, many patients are diagnosed on routine examinations or only after advanced field loss has occurred. The molecular etiology of glaucoma is largely unknown, but there are numerous studies establishing a genetic etiology for this disorder. Mutations in specific genes are associated with the manifestations of open angle glaucoma (OAG), pseudoexfoliation glaucoma, congenital glaucoma, and the anterior segment dysgenesis syndromes. Understanding the molecular basis of glaucoma is important to several aspects of glaucoma diagnosis and management.

The most common form of glaucoma is primary OAG (POAG: MIM 137,760). This is a late-onset and complex disorder that generally is associated with elevated intraocular pressures (IOPs) above 22 mm Hg leading to axonal degeneration and visual field loss.^{2,3} It affects 1% to 4% of all individuals over the age of 40 years, increasing with age and reaching 6% to 7% of individuals over 70 years of age in Caucasian and Indian populations and 10% to 12% in populations of African origin.^{1,4-6} It has been estimated that approximately 12 million Indians are affected, and with a rapidly growing aging population, this figure is predicted to increase to 16 million by 2020.⁷ The IOP increase in this disorder appears to result from an "inefficiency" of the trabecular meshwork leading to decreased aqueous outflow facility. Its multifactorial etiology was first proposed in 1967, and it demonstrates a variable age of onset and severity.⁸ Most studies suggest an autosomal dominant inheritance with incomplete penetrance.⁹ However, the inheritance pattern of this disorder seems to be multifactorial resulting from the interaction of one or more genes and/or environmental stimuli.

To date, there have been over 20 genetic loci and three genes, *MYOC* (myocilin), *OPTN* (optineurin), and *WDR36*, that have been associated with POAG, although mutations in these three genes have been found in less than 10% of all POAG cases.¹⁰⁻¹³ Mutations in *MYOC*, the protein which is secreted into the extracellular matrix of the trabecular meshwork, have been shown to cause a severe form of autosomal dominant juvenile-onset OAG associated with very high IOP.¹⁴ However, while up to 20% of juvenile onset glaucoma might be related to *MYOC* mutations, they have only been found in 3% to 5% of adult-onset POAG patients.¹⁵ Mutations in another gene, optineurin (*OPTN*, *GLC1E*), were initially associated with normal-tension glaucoma, without an elevated IOP.¹⁶ Mutations in *WDR36* (*GLC1G*) have been associated with both types of POAG.¹⁷ Researchers continue to seek to identify the genetic loci that appear to be major risk factors for POAG. One study carried out in an Afro-Caribbean population in Barbados identified strong linkage and association signals on a region of chromosome 2p.¹³ Strong association was demonstrated between POAG and a closely spaced group of markers including rs1533428, rs12994401, rs10202118, rs11125375, and rs11889995. In order to investigate the genetic bases of POAG in India, we tested a possible association between these five SNP markers on chromosome 2p with POAG in South Indian population.

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MATERIALS AND METHODS

Study Subjects

This study was approved by the Institutional Review Board of Aravind Eye Hospital and adhered to the tenets of the Declaration of Helsinki on human trials. The nature of the study was discussed, and informed consent was obtained from all the study participants before participation in the study. Patients with POAG who were of Indian ethnic origin were recruited from the Glaucoma services of the Aravind Eye Hospital, Madurai, India. A total of 440 clinically well-characterized POAG cases and age-matched controls were recruited to this study. The controls were recruited from the general ophthalmology clinic of the Aravind Eye Hospital and had no history of glaucoma and no ocular or systemic disease. The inclusion criteria included POAG based on optic disc changes typical of glaucoma, matching visual field defects by Humphrey's autoperimetry, and open iridocorneal angles on gonioscopy, irrespective of the level of intraocular pressure. The mean IOPs of POAG patients were OD 22.91 ± 10.45 mm Hg (SD) and OS 23.27 ± 10.43 mm Hg (SD), and the cup to disc ratios were OD 0.79 ± 0.12 (SD) and OS 0.89 ± 0.94 (SD). The IOP of each control was <19 mm Hg.

Clinical diagnosis involved a detailed workup for medical and family history of glaucoma and ocular diseases. Ophthalmic evaluation included best-corrected Snellen visual acuity, measurement of IOPs by Goldmann applanation tonometry, anterior chamber angle evaluation by Goldman two-mirror gonioscope, and optic disc and retinal nerve fiber examination by 90-diopter indirect lens. A Humphrey auto perimeter was used to evaluate the patient's visual fields.

Genotyping

DNA was extracted from peripheral blood leukocytes by modified protocol of Miller et al.¹⁸ Five SNPs, rs1533428, rs12994401, rs10202118, rs11125375, and rs11889995, on chromosome 2p were selected for this study based on the results seen in Barbados Family Study of Glaucoma study.¹³ Each SNP was genotyped using a TaqMan-based SNP genotyping assay according to the manufacturer's instructions. In brief, each 5- μ L reaction mixture containing TaqMan universal PCR Master mix, no AmpErase UNG, 20 \times SNP genotyping assay, MilliQ

water, and 1 μ L of DNA (40 ng/ μ L) was prepared in a 384-well plate and then amplified and analyzed in an ABI (ABI, Foster City, CA) 7900 HT Fast real-time PCR sequence detection system. Markers rs1533428, rs12994401, rs10202118, rs11125375, and rs11889995 were independently genotyped and verified by two independent readers.

Statistical Analysis

Fisher's exact test was carried out to test for association of genotypes and alleles over different models. Odds ratios and 95% confidence intervals were also calculated to estimate risk effects for heterozygous and homozygous marker alleles. Hardy-Weinberg equilibrium (HWE) of each SNP in control and in affected individuals was also examined using a Fisher's exact test, all as implemented in the Golden Helix SVS software suite 7 (Golden Helix, Bozeman, MT). Results were corrected for multiple testing with Bonferroni or false discovery rate control (FDR) corrections using the same program. Linkage disequilibrium, haplotype blocks, tagging SNPs, and HWE were examined using Haploview v3.32, and association was calculated using the SVS implementation. *P* values <0.05 after correction for multiple testing using a Bonferroni correction were considered to be statistically significant. The exact correction is somewhat difficult to estimate, given the interdependence of the various tests, the lack of polymorphism in rs11889995, and the a priori odds that this locus is associated with POAG. For the initial series of tests we chose to correct for 20 tests (three genotypes and allelic test for each of the five markers examined), requiring *P* < 0.0025 . This correction was increased to 24 tests for the specific models (adding the genotypic and dominant models for two markers), requiring *P* < 0.0021 for significance. This correction was judged to be conservative in view of the dependence of genotypes on allele frequencies.

RESULTS

A total of 440 samples including 220 POAG cases and 220 age-matched controls were recruited for the genetic analysis. The mean IOP of POAG was OD 22.91 ± 10.45 mm Hg (SD) and OS 23.27 ± 10.43 mm Hg (SD), cup to disc ratio was OD 0.79 ± 0.12 (SD) and OS 0.89 ± 0.94 (SD), and age was 56.80 ± 11.74 years. The IOP in control group was <19 mm Hg, cup to disc

TABLE 1. Distribution of Genotype Frequencies of Four SNPs on Chromosome 2p in POAG Cases and Controls

SNP	Genotype	POAG Cases <i>n</i> (%)	Controls <i>n</i> (%)	<i>P</i> Values*	Odds Ratio (95% CI)
rs12994401	CC	120 (54.55)	120 (54.55)	1.00	1.01 (0.69-1.47)
	CT	83 (37.73)	82 (37.27)	0.92	1.02 (0.69-1.51)
	TT	16 (7.27)	18 (8.18)	0.85	0.88 (0.44-1.78)
Alleles	C	323 (73.41)	322 (73.19)	0.87	1.02 (0.76-1.38)
	C	323			
	T	115 (26.14)	118 (26.82)		0.97 (0.72-1.31)
rs1533428	AA	114 (51.82)	114 (51.82)	1.00	1.00 (0.68-1.45)
	AG	75 (34.09)	86 (39.09)	0.32	0.80 (0.54-1.19)
	GG	31 (14.09)	20 (9.09)	0.135	1.64 (0.90-2.97)
Alleles	A	303 (68.86)	314 (71.36)	0.46	0.88 (0.66-1.18)
	G	137 (31.14)	126 (28.64)		1.13 (0.84-1.50)
rs10202118	CC	33 (15)	12 (5.45)	0.0014*	3.06 (1.53-6.09)
	CT	79 (35.90)	91 (41.36)	0.28	0.79 (0.54-1.17)
	TT	108 (49.09)	117 (53.18)	0.44	0.84 (0.583-1.23)
Alleles	T	295 (67.05)	325 (73.86)	0.026	0.71 (0.53-0.96)
	C	145 (32.95)	115 (26.14)		1.39 (1.04-1.85)
rs11125375	AA	92 (41.82)	98 (44.54)	0.56	0.88 (0.60-1.29)
	AG	85 (38.64)	95 (43.18)	0.33	0.82 (0.56-1.20)
	GG	42 (19.09)	25 (11.36)	0.033	1.83 (1.07-3.12)
Alleles	A	269 (61.14)	291 (66.14)	0.10	0.79 (0.60-1.04)
	G	169 (38.41)	145 (32.95)		1.26 (0.96-1.66)

Fisher's exact test was used to compare the genotype and allele frequencies between cases and controls.

* *P* < 0.0025 is significant, corresponding to a *P* < 0.05 after Bonferroni correction for 20 tests (three genotypes and allelic for each of the five SNPs tested).

TABLE 2. Association Results of rs10202118 and rs11125375 from POAG Cases and Controls by Fisher's Exact Test

SNPs	rs10202118		rs11125375	
	Case (<i>n</i> = 220)	Control (<i>n</i> = 220)	Case (<i>n</i> = 219)	Control (<i>n</i> = 218)
Risk allele frequency	33% (C)	26% (C)	38% (G)	33% (G)
Genotypic <i>P</i> values	0.004		0.08	
Allelic <i>P</i> values	0.026		0.10	
Recessive <i>P</i> values	0.0014*		0.033	
Dominant <i>P</i> values	0.44		0.56	
OR _{HOM}	3.06 (1.53, 6.1)		1.83 (1.07, 3.13)	
OR _{HET}	1.17 (0.81, 1.71)		1.13 (0.77, 1.65)	

Fisher's exact test was used to compare the genotype and allelic *P* values, dominant, recessive model *P* values and odds ratio of genotypes between cases and controls. OR_{HOM}, homozygous odds ratio; OR_{HET}, heterozygous odds ratio.

* *P* < 0.0021 was considered to be significant, corresponding to a *P* < 0.05 after Bonferroni correction for 24 tests (adding testing for two additional models for each marker to those shown in Table 1).

ratio range was 0.3 to 0.5 and the mean age of the control group was 64.08 ± 4.52 years (SD).

Genotyping of the five selected SNPs in the chromosome 2p16 region showed that rs11889995 was not polymorphic in any of the samples examined. Among the four remaining SNPs, only rs10202118 showed suggestive allelic association, with an odds ratio for the C allele of 1.39 (95% CI: 1.04–1.85), although the results did not withstand correction for multiple testing (allelic *P* < 0.026). However, the genotypic *P* values did show statistically significant association with POAG (Table 1), with the CC genotype for rs10202118 giving a *P* < 0.0014 and the GG genotype for rs11125375 showing suggestive association with *P* < 0.033. After correcting for 20 total tests, only association of the CC genotype of rs10202118 remained significant. The odds ratios reflected this association with the CC rs10202118 genotype giving an OR = 3.06 (95% CI: 1.53–6.09), and the GG genotype for rs11125375 giving an OR = 1.83 (95% CI: 1.07–3.12). While markers rs11125375 and rs1533428 were slightly out of HWE (*P* < 0.027 and *P* < 0.007, respectively), rs10202118 and rs12994401 were in HWE.

Association tests of specific inheritance models gave similar results (Table 2), with a SNP genotypic *P* = 0.004 for rs10202118 and *P* = 0.08 for rs11125375. Association under a recessive model confirmed the homozygous risk allele results, while no significant association was seen under a dominant model. All markers were in HWE in controls. Markers rs10202118 and rs12994401 were in HWE overall, while markers rs11125375 and rs1533428 showed mild deviation from HWE with *P* = 0.027 and *P* = 0.008, respectively. Markers rs1533428, rs10202118, and rs11125375 showed deviation from HWE in cases with *P* = 0.002, *P* = 0.005, and *P* = 0.007 respectively. All markers were in HWE in controls. None of the remaining SNPs showed significant association with POAG.

A linkage disequilibrium plot calculated with the samples from this study showed markers rs10202118 and rs11125375 had significant linkage disequilibrium, while rs1533428 was strongly associated with rs11125375 but not rs10202118 (Fig. 1). Marker rs12994401 did not show association with any of the other markers studied in this population. The haplotype association values for the CA haplotype of rs10202118 and rs11125375 yielded a *P* = 0.042 and OR = 1.79 (95% CI: 1.01–3.17), and no other haplotype of alleles at these markers showed significant association, although the TA haplotype of rs10202118 and rs11125375 showed results suggestive of a protective effect, with *P* = 0.68 and OR = 0.77 (95% CI: 0.59–1.02). However, the combined recessive model for the CC genotype at rs10202118 and the GG genotype at rs11125375 gave a *P* = 0.0014 with a corresponding OR = 3.06 (95% CI:

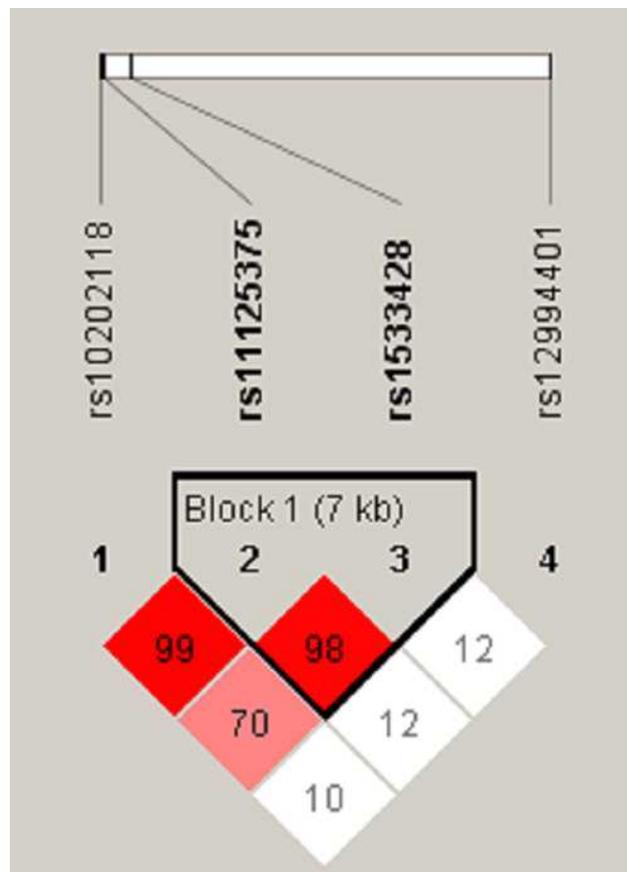


FIGURE 1. Haplotype linkage disequilibrium plot. The linkage disequilibrium (*D'*) as calculated for the combined POAG and control groups using the Haploview program. Markers rs10202118 and rs11125375 showed very significant association, while rs11125375, but not rs10202118, was in linkage disequilibrium with rs1533428. rs12994401 did not show association with any of the other markers studied in this population.

1.53–6.1), similar to those for the CC rs10202118 genotype alone. Examination of the genotypes showed that this reflected the exclusive co-occurrence of the homozygous CC genotype at rs10202118 with the homozygous GG genotype at rs11125375 in this population.

DISCUSSION

Here we have confirmed association of alleles at markers on chromosome 2P with POAG in a South Indian population. Previously, Jiao et al.¹³ performed linkage analyses in 146 multiplex families and identified a locus with a major impact on the susceptibility to POAG in the Afro-Caribbean population in Barbados, West Indies. As part of that study, a case-control association study demonstrated strong association of alleles at rs12994401 and rs1533428 on chromosomes 2p with POAG, with significant association also seen with alleles at rs10202118 and suggestive association with rs11125375. Genetic associations are biologically meaningful if they are replicated in different ethnic populations. Association at the chromosome 2p locus was confirmed in an African-American population by Liu et al.¹⁹ who also saw suggestive results in a Ghanaian population. Association was also seen in a Chinese population by Chen et al. (personal communication, 2010, see Chen et al., ASHG 2010, abstract 979).²⁰ However, there was no significant association of alleles at rs12994401 and rs1533428 with POAG in two studies in Japanese and Korean populations.^{21,22} The

authors note that these two SNPs are common variants in the Japanese and Korean population, as well as in the Afro-Caribbean population, and interpret their findings as suggesting that these two SNPs might be markers of a neighboring susceptibility gene responsible for POAG in the Afro-Caribbean population rather than directly responsible for the increased risk.

This study was designed to recapitulate this analysis in a South Indian population including 220 POAG cases and 220 control subjects. Although no association was seen with alleles at rs12994401 or rs1533428, there was significant association with alleles at rs10202118 and suggestive association with rs11125375, consistent with the suggestion that these sequence variations might not be causative, but rather be in linkage disequilibrium with as yet unknown sequence changes that affect susceptibility to POAG.

While rs10202118 only showed a $P = 0.026$ for the basic allelic test, it showed a $P = 0.004$ for the genotypic test and $P = 0.0014$ for the recessive model, both of which withstand Bonferroni correction for multiple testing. Marker rs11125375, which also showed a $P = 0.033$ for the recessive model, did not withstand Bonferroni correction for multiple testing. However, it is suggestive in light of the previous association (in Barbados) and the association of the closely positioned rs10202118 in this population.

The haplotype analysis, which showed strong linkage disequilibrium between rs10202118 and rs11125375 as well as between rs1533428 and rs11125375 but not among other markers (Fig. 1) was distinctly different from that seen in the Barbadian population, in which there was linkage disequilibrium across the region.¹³ The Yoruban population also showed smaller more loosely associated marker blocks as estimated by HapMap data. This is also somewhat different from the Caucasian population, in which all the markers are fairly strongly associated into a single haplotype block. The first three markers also formed a tight haplotype block showing moderate disequilibrium with an adjacent block including the remaining markers in the Chinese population as well. Thus, analysis of the association in the South Indian population may narrow the associated region more closely than those in other studied populations could.

This is the first report to show the association of SNPs rs10202118 and rs11125375 with POAG in an Indian population and confirms the findings of Jiao et al.,¹³ Liu et al.,¹⁹ and Chen et al.²⁰ The results suggest that these two SNP markers either might influence risk for POAG, or more likely, might be in linkage disequilibrium with markers that do. As POAG is a multifactorial disease, the specific genes responsible for its pathogenic mechanism remain to be identified. It awaits confirmation by larger studies providing additional statistical power. In addition, while this study provides insight into the loci contributing to POAG, further studies are required to elucidate whether this locus contributes to optic neuropathy in POAG in other ethnic populations.

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