Association Study in a South Indian Population Supports rs1015213 as a Risk Factor for Primary Angle Closure

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Glaucome is the second leading cause of blindness worldwide. Clinically, it is characterized by progressive retinal ganglion cell damage and distinctive optic neuropathy, \(^\dagger\) with or without elevated intraocular pressure. \(^\dagger\) Primary angle closure glaucoma (PACG), in which the iris abuts most of the trabecular meshwork, is less common than primary open angle glaucoma (POAG). In 2020, it is predicted that POAG worldwide, mostly in Asian countries. \(^\dagger\)–\(^\dagger\) Identifying risk factors for PACG is particularly relevant, as patients with PACG are likely to have progression through primary angle closure suspect (PACS) or primary angle closure (PAC) before reaching PACG. Fellow eyes of patients presenting with acute angle closure are unlikely to suffer progression to glaucoma after laser iridotomy, suggesting that iridotomy may prevent PAC progression in at least some instances.\(^\dagger\)

Genetic factors have been studied extensively for their role in the pathogenesis of PACG. Family history is found to be a consistent risk factor, which suggests a strong genetic predisposition to this disease.\(^9\)–\(^12\) First degree relatives of PACG patients have been estimated to be at 6 to 9 times greater risk of suffering PACG compared to similar relatives of noncases.\(^13\)–\(^14\) All these facts strongly suggest that genetic factors are involved in the pathogenesis of PACG. Indeed, genome wide association studies (GWAS) have identified chromosomal loci and candidate genes statistically associated with PACG.

Recently, Vithana et al. conducted a two-stage GWAS to determine the genetic determinants underlying susceptibility to PACG.\(^15\) The first (discovery) stage included 11,854 PACG cases and 9608 controls. It was followed by a second (replication) stage, comprising 1917 PACG cases and 8943 controls from five different ethnic groups. The study found a genome-wide significant association between PACG and three genetic markers: rs11024102 in \(\text{PLEKHA7}\), rs3753841 in \(\text{COL11A1}\), and rs1015213 between the \(\text{PCMTD1}\) and \(\text{ST18}\) genes, recently have been associated with primary angle closure glaucoma (PACG). We explored the genetic association of these SNPs with subtypes of primary angle closure in a South Indian population.

Methods. The study included three case definitions: primary angle closure/primary angle closure glaucoma (PAC/PACG, \(N = 180\); primary angle closure suspect (PACS, \(N = 171\)), and a combined any-angle closure group. Controls consisted of 411 individuals from South India. Genotyping for all three SNPs was performed using the TaqMan allelic discrimination assay. Genetic association was estimated using a \(\chi^2\) test statistics and logistic regression.

Results. Among the three studied SNPs, significant genetic association was identified for rs1015213 in the PAC/PACG (\(P = 0.002\)) and any-angle closure (\(P = 0.003\)) analyses. However, no significant genetic association was seen when in PACS subjects (\(P = 0.052\)). SNPs rs3753841 and rs11024102 showed no evidence of genetic association with angle-closure phenotypes (\(P > 0.05\)) in South Indian participants.

Conclusions. In our study, rs1015213 (located in the intergenic region between \(\text{PCMTD1}\) and \(\text{ST18}\)) was associated significantly with PAC/PACG, confirming prior reports of an association between this region and angle closure glaucoma. Further work with a larger sample size is necessary to confirm the importance of \(\text{COL11A1}\) and \(\text{PLEKHA7}\) in the pathogenesis of glaucoma.

Keywords: PACG, \(\text{PCMTD1-ST18}\), South India

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Glaucome is the second leading cause of blindness worldwide. Clinically, it is characterized by progressive retinal ganglion cell damage and distinctive optic neuropathy, with or without elevated intraocular pressure. Primary angle closure glaucoma (PACG), in which the iris abuts most of the trabecular meshwork, is less common than primary open angle glaucoma (POAG), though it accounts for nearly 50% of glaucoma-related blindness. \(^3\) In 2020, it is predicted that PACG prevalence will reach 21 million, with roughly 5 million bilaterally blind cases. \(^1\) In fact, it has been estimated that PACG blinds more people than POAG worldwide, mostly in Asian countries. \(^5\)–\(^7\) Identifying risk factors for PACG is particularly relevant, as patients with PACG are likely to have progression through primary angle closure suspect (PACS) or primary angle closure (PAC) before reaching PACG. Fellow eyes of patients presenting with acute angle closure are unlikely to suffer progression to glaucoma after laser iridotomy, suggesting that iridotomy may prevent PAC progression in at least some instances.\(^8\)

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Recently, Vithana et al. conducted a two-stage GWAS to determine the genetic determinants underlying susceptibility to PACG.\(^15\) The first (discovery) stage included 11,854 PACG cases and 9608 controls. It was followed by a second (replication) stage, comprising 1917 PACG cases and 8943 controls from five different ethnic groups. The study found a genome-wide significant association between PACG and three genetic markers: rs11024102 in \(\text{PLEKHA7}\), rs3753841 in \(\text{COL11A1}\), and rs1015213 located between \(\text{PCMTD1}\) and \(\text{ST18}\) on chromosome 8q. These results have implicated specific genetic loci in PACG susceptibility, though further studies are required to confirm these associations.
Furthermore, it is unclear whether these genes simply predispose individuals to iridotrabecular contact (which might be found as an isolated finding in PACS) or whether they are risk factors for later disease stages (i.e., PAC and PACG). The current study was done to replicate the findings of Vithana et al. in a South Indian population. Subanalyses also were conducted to determine the association of these markers in PAC patients with varying disease severity (PACS and PAC/PACG).

METHODS

All subjects were recruited from the Pondicherry and Madurai Glaucoma Clinics of the Aravind Eye Hospital. Both clinics are located in the South Indian state of Tamil Nadu. All subjects read and signed informed consent except for illiterate subjects, who had the information leaflet read out to them and provided a thumb impression. The study complied with the tenets of the Declaration of Helsinki, and ethics approval was received from the Institutional Review Board of the Aravind Eye Hospital, Madurai, Tamil Nadu, India.

Study Subjects

All subjects, including cases and controls, underwent a comprehensive ophthalmic examination, including slit-lamp examination, gonioscopy, visual acuity, and Goldmann application tonometry. Fundus examination was performed to assess for optic nerve head and retinal nerve fiber layer findings consistent with glaucoma.

The case group included primary angle closure suspects (PACS, N = 171), or either primary angle closure or primary angle closure glaucoma (PAC/PACG, N = 180). These stages of angle closure were defined as described by the ISGEO classification system, though PAC and PACG were collapsed into a single category given that reliable visual fields were not available in all subjects. All cases had at least 2 quadrants of iridotrabecular contact (ITC), which prevented visualization of any pigmented trabecular meshwork. Subjects were characterized as having primary angle closure (PAC/PACG) if any of the following characteristics were present: peripheral anterior synchiae (PAS), elevated IOP >21 mm Hg, cup-to-disc ratio of 0.7 or greater, notching of the optic nerve, or other findings suggesting glaucomatous optic neuropathy. Angle closure patients without any of the aforementioned characteristics were classified as PACS. An “any-angle closure” group also was defined, and consisted of PACS and PAC/PACG cases.

The control group comprised of 411 ethnically matched subjects (PACS, N = 171), or PAC/PACG cases (43 males and 128 females) with a mean age 51.5 ± 7.8 years, and 171 PACS cases (43 males and 128 females) with a mean age 51.5 ± 7.2 years. The control group contained 411 subjects (165 males and 246 females) with a mean age of 56.9 ± 7.9 years.

Genotyping

Genomic DNA was extracted using peripheral blood by a modification of the method described by Miller et al. or from saliva using the OrageneDNA/Saliva extraction kit (DNA Genotek, Inc., Ottawa, Ontario, Canada). Extracted DNA was stored at −20°C until use. Genotyping of each of the three single nucleotide polymorphisms (SNPs; rs11024102, rs3753841, and rs1015213) was performed using a real-time based allelic discrimination TaqMan SNP assay (Applied Biosystems, Foster City, CA). Real-time PCR was performed using 2.5 μL of TaqMan Universal PCR master mix, 0.125 μL of 40X primer probe, and 0.375 μL MilliQ water and 2 μL of DNA sample (20 ng/μL). PCR cycling conditions consisted of 50°C for 2 minutes, 95°C for 10 minutes, and 40 cycles of amplification (95°C denaturation for 15 seconds, 60°C annealing/extension for 1 minute). Reactions were performed in 384-well MicroAmp Optical reaction plates using the ABI 7900 HT Fast Real-time thermo-cycler. The post assay analysis was performed using the associated SDS (Sequence Detection Systems) software version 2.3. Genotypes on the allelic discrimination plot with a quality value ≥96% and a Ct value between 21 and 30 were included in the results.

Power Calculations and Statistical Analysis

Power calculations were performed with the statistical package QUANTO, Version 1.2.4 using the following assumptions: an additive genetic model, per-allele odds ratios (OR) equal to those of the combined analyses from Vithana et al. risk allele frequencies (RAF) equal to the Gujarati Indian in Houston (GII) HapMap population (these were within 0.01 of CEU HapMap frequencies for all three SNPs), baseline risk of affection ranging from 1% to 5%, 1-sided P value thresholds of 0.05 (nominal significance [NomS]) and 0.0167 (experiment-wise significance [EWS]), and either 180 or 351 cases and 411 controls. The estimated power was nearly identical for baseline risks between 1% and 5% for all SNPs. Hence, we reported the mean power across all baseline risk values.

Using 351 cases, the power to detect a per-allele OR of 1.5 with a population risk allele frequency of 0.09 for rs1015213 (PCMTD1-ST18) was 0.79 for nominal significance and 0.62 for experiment-wise significance. For rs3753841 (COL11A1, RAF = 0.55, OR = 1.20), the estimated power was 0.53 for NomS and 0.34 for EWS. The statistical power to detect an association signal at rs11024102 (PLEKHA7, RAF = 0.28, OR = 1.22) was 0.55 at NomS and 0.27 at EWS. When using 180 cases from the PACG/PAC group only and 411 controls, the power to detect an association for rs1015213 was 0.64 at NomS and 0.45 at EWS. For rs3753841 and rs11024102, the power for NomS was approximately 0.40, and between 0.23 and 0.25 for EWS.

All statistical analyses were performed using STATA software version 11.0 (STATA Corporation, College Station, TX). Categorical variables were expressed as frequencies (percentages). Continuous variables were expressed as mean ± SD and compared across groups using Student’s t-test. For each SNP, genotype and allele frequency were calculated. Genotype frequencies of all the three SNPs were consistent with Hardy-Weinberg’s expectations among controls (P > 0.05). A χ² test was used to evaluate genotypic association. Logistic regression was used to calculate ORs with 95% confidence intervals (CI) for allelic association tests. For logistic regression association analyses, a one-sided P value less than 0.05 was considered NomS and a 1-sided Bonferroni-adjusted P value of 0.05/3 (0.0167) was used for EWS.

RESULTS

The study included a total of 180 PAC/PACG cases (76 males and 104 females) with a mean age 55.6 ± 7.8 years, and 171 PACS cases (43 males and 128 females) with a mean age 51.5 ± 7.2 years. The control group contained 411 subjects (165 males and 246 females) with a mean age of 56.9 ± 7.9 years.
The any-angle closure group consisted of all 351 cases (119 men and 232 women) from the PAC/PACG and PACS groups. All subjects were of South Indian origin. Study sample characteristics are shown in Table 1.

The genotype and allele frequencies for each of the SNPs rs1015213, rs3753841, and rs11024102 were calculated in controls and a case group consisting of PAC/PACG cases only, PACS cases only, or any-angle closure group. Among the three studied SNPs, significant genetic association was identified for rs1015213 when cases were defined as subjects with PACS and PAC/PACG subjects (allelic \(P = 0.213\)) and controls and PAC/PACG subjects (allelic \(P = 0.127\)), or control and cases in the any-angle closure group (allelic \(P = 0.116\), Table 3). The frequency of the C allele of rs3753841 was higher in cases (47% PACS, 48% PAC/PACG, and 47.5% in the any-angle closure group) than in controls (44.3%). SNP rs11024102 also did not show statistical evidence of genetic association in analyses consisting of controls and PAC/PACG subjects (allelic \(P = 0.079\), Table 4). The frequency of the C allele was higher in cases (34.5% PACS, 35.1% PAC/PACG, 34.8% any-angle closure group) than controls (31.4%).

### DISCUSSION

Three loci defined by SNP markers rs11024102 in PLEKHA7, rs3753841 in COL11A1, and rs1015213 located in an
The other two SNPs (rs3753841 in \textit{COL11A1} and rs11024102 in \textit{PLEKHA7}) were not found to be associated in our study with any of the case groups. SNP rs3753841 lies within the coding region of \textit{COL11A1} causing a nonsynonymous (c.3968C > T) change that leads to substitution of proline to leucine at 1325 amino acid position (NM_001854.5). The rs11024102 locus contains the \textit{PLEKHA7} gene, which encodes a pleckstrin-homology-domain-containing protein 7 involved in the stability and maintenance of adherence junction’s (AJ) between the cells.\textsuperscript{21} 22

There was no statistical evidence for an association of rs3753841 (\textit{COL11A1}) and rs11024102 (\textit{PLEKHA7}) with angle closure in our study. However, these SNPs had comparatively small effect sizes in the original GWAS (per-allele OR = 1.34 per T allele),\textsuperscript{6} and our power to detect association at the SNPs was limited even at nominal significance levels (using 180 cases, the power was 0.4 at \textit{P} = 0.05 and 0.24 at \textit{P} = 0.0167). The original GWAS study included 3771 PACG cases and 18,551 controls, whereas our study contains 180 PAC/PACG cases and 411 controls. Interpopulation differences in LD patterns also may have contributed to the discrepancies between the studies. Gene-locus-based replication study designs can be more powerful than single SNP analyses when the tested SNP is in LD with the causal variant, or when there is genetic heterogeneity. Hence, a more thorough search for potentially causal variants should be undertaken with a more highly powered study and more harmonized diagnostic criteria to confirm these loci in the South Indian population.

Overall, our study in a South Indian population replicates findings of a large GWAS implicating rs1015213 (\textit{PCMTD1}-\textit{ST18}) in the pathogenesis of PAC. On the other hand, we found no statistical evidence of association for SNPs rs3753841 (\textit{COL11A1}) and rs11024102 (\textit{PLEKHA7}) with angle closure phenotypes. The nonsignificant associations of \textit{PLEKHA7} and \textit{COL11A1} may have been be influenced by a relative lack of statistical power, which could be overcome by increasing the sample size in future studies. Therefore, large studies groups will be required to confirm these associations in different ethnic groups and correctly interpret negative findings.

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