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Evaluation of Primary Angle-Closure Glaucoma Susceptibility Loci in Patients with Early Stages of Angle-Closure Disease

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Purpose: To investigate whether newly identified genetic loci for primary angle-closure glaucoma (PACG) are associated with early stage angle-closure disease defined as primary angle closure suspect (PACS).

Design: Case-control study.

Participants: A total of 1397 PACS patients and 943 controls of Chinese ethnicity from Singapore and 604 PACS patients and 287 controls of Indian ethnicity.

Methods: The 8 PACG single nucleotide polymorphisms (SNPs; rs11024102 at *PLEKHA7*, rs3753841 at *COL11A1*, rs1015213 located between *PCMTD1* and *ST18* son chromosome 8q, rs3816415 at *EPDR1*, rs1258267 at *CHAT*, rs736893 at *GLIS3*, rs7494379 at *FERMT2*, and rs3739821 mapping in between *DPM2* and *FAM102A*) were genotyped by Taqman assays. The association between SNP genotypes and PACS status was measured using logistic regression. A *P* value of 0.006 was set to account for the testing of 8 genetic loci using a Bonferroni correction. A meta-analysis was conducted to calculate the overall *P* value and accompanying per-allele odds ratios for each SNP analyzed.

Main Outcome Measures: Association of PACG loci with PACS status.

Results: The PACS patients were significantly older in both cohorts (Chinese, $P < 0.001$; Indian, $P = 0.002$), and there were also more women ($P < 0.001$, both Chinese and Indian cohorts). In the Chinese cohort, significant evidence of association was noted at 3 SNPs: rs1015213 [A] in *PCMTD1-ST18* (odds ratio [OR], 2.36; 95% confidence interval [CI], 1.36−4.11; $P = 0.002$), rs3816415 [A] in *EPDR1* (OR, 1.49; 95% CI, 1.19−1.85; $P < 0.001$), and rs3739821 [G] in *DPM2-FAM102A* (OR, 1.40; 95% CI, 1.18−1.65; $P < 0.001$). Only *PCMTD1-ST-18* was replicated modestly in the Indian population ($P = 0.056$). Meta-analysis showed significant evidence of association for *PCMTD1-ST-18* (OR, 1.55; 95% CI, 1.18−2.04; $P = 0.002$) and *DPM2-FAM102A* (OR, 1.27; 95% CI, 1.12−1.45; $P = 0.0002$).

Conclusions: In this study, 2 of 8 PACG-associated loci were associated significantly with PACS status, the earliest stage in the angle-closure glaucoma disease course. The association of these PACG loci with PACS status suggests that these loci may confer susceptibility to a narrow angle configuration. *Ophthalmology 2017;■:1−7* © *2017 Published by Elsevier Inc. on behalf of the American Academy of Ophthalmology*

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Primary angle-closure glaucoma (PACG) is a common subtype of glaucoma, particularly in Asia, affecting 11.7 million people in 2013.[1,2] The earliest stage of the angle-closure disease spectrum is primary angle-closure suspect (PACS) status, which over time can result in the development of glaucomatous optic neuropathy (i.e., PACG). The common feature across the spectrum is the presence of narrow drainage angles, also known as occludable angles, characterized by the apposition of the peripheral iris against the trabecular meshwork on gonioscopy.[3] The apposition results in obstruction of the aqueous outflow, leading to raised intraocular pressure and progressing to irreversible damage to the optic nerve.

Primary angle-closure glaucoma has long been perceived to have a significant genetic basis.[4,5] This was confirmed in a genome-wide association study (GWAS) of PACG conducted in 5 sample collections across Asia and validated in an additional 6 sample collections worldwide.[6] Significant

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associations were identified at 3 genetic loci for PACG: rs11024102 at *PLEKHA7*, rs3753841 at *COL11A1*, and rs1015213 located between *PCMTD1* and *ST18* on chromosome 8q.[6] Recently, an expanded GWAS reported 5 additional new genetic loci: rs3816415 at *EPDR1*, rs1258267 at *CHAT*, rs736893 at *GLIS3*, rs7494379 at *FERMT2*, and rs3739821 mapping between *DPM2* and *FAM102A*.[7] Although all these genes are expressed in tissues of the iridocorneal angle,[6,7] the exact mechanisms by which they cause PACG is not completely understood. Importantly, whether these genetic loci are also associated with the early stages of angle-closure disease is unknown.

In this study, we aimed to investigate whether the newly identified PACG genetic variants are associated with early stages of angle-closure disease, namely PACS status. We hypothesized that if a PACG risk variant were associated significantly with PACS status, then the variant likely would confer genetic predisposition for susceptibility to development of angle closure rather than optic neuropathy.

Methods

Study Populations and Case Definition

Ethics approval was obtained from the Singapore Eye Research Institute Institutional Review Board and the study adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all study participants. The newly identified PACG genetic variants were tested in both a Chinese and a South Indian PACS cohort, with the latter cohort used for validation of the primary finding from the Chinese cohort. All the study participants from Singapore were of Chinese ethnicity. The PACS participants were recruited from the glaucoma clinics, and controls (without angle closure) were recruited from within a population-based sample, the Singapore Chinese Eye Study (SCES),[8] based on robust clinical criteria. Primary angle-closure suspect status was defined as the pigmented posterior trabecular meshwork not being visible on nonindentation gonioscopy for at least 180° in the primary position and intraocular pressure of less than 21 mmHg without peripheral anterior synechiae or glaucomatous optic neuropathy.[9]

The SCES is a population-based, cross-sectional study of Chinese adults residing in the southwestern part of Singapore. An age-stratified (by 10-year age group) random sampling strategy was used for participant selection from an initial computer-generated list of ethnic Chinese names of adults 40 to 80 years of age or older provided by the Ministry of Home Affairs of Singapore.[8] Of the 3353 participants who were recruited from January 2009 through December 2011, the final number genotyped was 1952. A control was defined as having an intraocular pressure of less than 21 mmHg with open angles (on gonioscopy) in all quadrants, healthy optic nerves, normal visual fields, and no previous intraocular surgery.

The replication cohort comprised 604 PACS participants and 287 controls of South Indian ancestry recruited from Aravind Eye Hospital, Tamil Nadu, India. The same diagnostic criteria for PACS participants and controls were used as defined above for the Singaporean Chinese population.

Genotyping

Genotyping of the PACG-associated single nucleotide polymorphisms (SNPs) in PACS participants' samples (Chinese and Indian) and in Indian controls was carried out using Taqman assays. We genotyped these 8 SNPs in PACS participants: rs11024102 (*PLEKHA7*), rs1015213 (*PCMTD1-ST18*), rs3753841 (*COL11A1*), rs79394014 (*CHAT*), rs736893 (*GLIS3*), rs3816415 (*EPDR1*), rs3739821 (*DPM2-FAM102A*), and rs7494379 (*FERMT2*). We selected the 8 SNPs and genes associated with PACG through GWA studies because they were the most robustly replicated loci.

Methods of genotyping and data quality control for SCES have been described previously.[10,11] In brief, participants were genotyped using the Illumina Human610-Quad BeadChip (Illumina, Inc., San Diego, CA) with the following quality control criteria: samples were excluded if they had a per-sample call rate of less than 95% or showed evidence of admixture, cryptic relatedness, high heterogeneity, or gender discrepancy. The final number of participants passing quality checks was 1949. Additionally, details of genotyping and case definition for the PACG patients of Singaporean Chinese descent who were included in the GWAS to identify the PACG susceptibility loci have been described previously.[6,7]

Statistical Analysis

Statistical analysis was performed using a commercially available statistical software package (SPSS for Windows version 20.0; IBM-SPSS, Chicago, IL). Individual SNP genotypes were coded according to the number of copies of the variant allele present: 0 for the wild-type genotype, 1 for heterozygous carriers of the minor allele, and 2 for individuals homozygous for the effect allele. Association testing was carried out using a 1-degree-of-freedom score-based test using logistic regression additionally adjusted for age and gender. This model assumed a trend-per-copy effect of the variant allele. A P value of less than 0.006 was considered statistically significant after a Bonferroni correction factor of 8 was applied to correct for the number of loci tested. A meta-analysis was conducted using inverse-variance weights for each sample collection that calculated an overall Z statistic, its corresponding P value, and accompanying per-allele odds ratios (ORs) for each SNP analyzed.

We also performed a subanalysis within the Chinese PACS cohort by including only older PACS participants, the cutoff age based on the mean age of these participants (i.e., 68 years). This was to minimize the likely effect of age in our PACS cohort. In addition, we also evaluated the combined effects of the 8 PACG loci in the PACS cohort; an additive model was used to construct both unweighted and weighted genetic risk scores. The unweighted genetic risk score was calculated by summation of the risk alleles across the 8 variants. The weighted genetic risk score was calculated by multiplying the number of risk alleles at each locus (0, 1, 2) for the corresponding effect size per allele, as reported by Khor et al.[7]

Results

Of a total of 1412 Chinese PACS participants who were enrolled, genotyping data were available for 1397. Of the 1949 SCES samples with genotyping data, 943 were specially selected open-angle controls. The Chinese PACS participants were significantly older (mean age, 68.1±8.2 years vs. 56.4±8.9 years; $P < 0.001$), and there were proportionately more women within the PACS group than among controls (77.3% vs. 49.0%; $P < 0.001$). Likewise, the Indian PACS participants were significantly older (53.8±9.6 years vs. 51.7±8.1 years; $P = 0.002$) and included more women than the control group (79.8% vs. 51.6%; $P < 0.001$).

In the Chinese PACS cohort, significant evidence of association was noted for PACS status at 3 of the 8 SNPs investigated, namely, rs1015213 [A] in *PCMTD1-ST18* (OR, 2.36; 95% confidence