Lack of Association Between Primary Angle-Closure Glaucoma Susceptibility Loci and the Ocular Biometric Parameters Anterior Chamber Depth and Axial Length

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PURPOSE. Three susceptibility loci for primary angle-closure glaucoma (PACG) were recently identified: PLEKHA7 rs11024102, COL11A1 rs3753841, and rs1015213 located in the intergenic region between PCMTD1 and ST18. The purpose of this study was to investigate the associations of these loci with the ocular biometric parameters anterior chamber depth (ACD) and axial length (AL).

METHODS. Genotype and ocular biometric data were available for four population-based studies, including three from Singapore (Singapore Chinese Eye Study, Singapore Malay Eye Study, and Singapore Indian Eye Study) and one from China (Beijing Eye Study), exceeding 7000 participants. ACD and AL were measured using the IOLMaster for the Singapore cohorts and optical low-coherence reflectometry (Lenstar 900 Optical Biometer) for the Beijing cohort. Five readings were obtained for each participant and the average was computed. Analysis excluded any eye that was pseudophakic or aphakic.

RESULTS. ACD measurements and genotype data of the three loci were available for 7245, 7243, and 7239 subjects, respectively. We noted nominal evidence of association between single nucleotide polymorphism (SNP) rs1015213 (PCMTD1-ST18) and a shallower ACD when all data were meta-analyzed (β = −0.033, P = 0.021). When multiple testing was considered, the observation was nonsignificant. There was no association between ACD and rs11024102 (PLEKHA7) or rs3753841 (COL11A1). We did not observe significant associations between AL and any of the three SNPs.

CONCLUSIONS. The lack of association between the PACG susceptibility loci with ACD or AL suggests that predilection to PACG may be mediated by factors other than shallow anterior chamber or short eyeball length.

Keywords: genetic, association, glaucoma, quantitative trait, anterior segment OCT

Primary angle-closure glaucoma (PACG) is responsible for substantial visual loss in many Asian countries, such as Singapore,1 China,2,3 Mongolia,4 and India.5,6 PACG has long been suspected to have a substantial hereditable component. This was proven recently in a genome-wide association study (GWAS) of PACG conducted in five sample collections across Asia with validation in a further six sample collections worldwide.7 Genome-wide significant association (P < 5 × 10−8) with PACG was found in three new susceptibility loci (rs5753841 in COL11A1, rs1015213 located between PCMTD1 and ST18, and rs11024102 in PLEKHA7). However, it is not clear how these three loci contribute to the pathogenesis of PACG or whether these genetic variants act via any known anatomical risk factors for PACG.

Previous studies have demonstrated that ocular biometric parameters such as a shallow anterior chamber depth (ACD) and short axial length (AL) are strong risk factors for PACG.8–11 In a community-based study of older Singaporeans, eyes with ACD less than 2.80 mm were 42.5 times more likely to have angle closure than eyes with ACD of at least 3.00 mm.10 Likewise, a shorter AL is also associated with an increased risk for angle closure. Reports have shown that eyes of Chinese patients who had acute angle closure had shorter AL than those...
affected by chronic asymptomatic angle closure, and that both groups had shorter AL than the control group.12,13 The aim of this study was to investigate the association of these three PACG susceptibility loci with the anatomical risk factors ACD and AL. To this end, we sought to test this association in large population-based samples of Asian descent.

METHODS

Descriptions of Study Populations

Ethical approval was obtained from the Singapore Eye Research Institute Institutional Review Board and the Medical Ethics Committee of the Beijing Tongren Hospital for the Singapore and Beijing cohorts, respectively. Written informed consent was obtained from all study participants, and the study adhered to the Declaration of Helsinki.

Singapore Cohorts. Data from the Singapore Malay Eye Study (SiMES), Singapore Indian Eye Study (SINDI), and Singapore Chinese Eye Study (SCES) were analyzed. SiMES, SINDI, and SCES were population-based, cross-sectional studies of ethnic Malay (aged 40–79 years), Indian (aged from 40–80+ years), and Chinese (aged from 40–80+ years) adults residing in the southwestern part of Singapore. Details of the study design, sampling plan, and methods have been previously reported for these studies.14,15 In brief, these three studies were designed to determine the prevalence and impact of major eye diseases in Singaporeans of different ethnicities. An age-stratified (by 10-year age group) random sampling strategy was employed for subject selection from a computer-generated list provided by the Ministry of Home Affairs, Singapore. SiMES was conducted from August 2004 to June 2006 and recruited 3280 participants (78.7% response rate). SINDI was conducted from March 2007 to December 2009 and recruited 3400 participants (75% response rate). SCES was conducted from February 2009 to December 2011 and recruited 3553 participants. The final number of subjects who were genotyped was 3072, 2953, and 1952 for SiMES, SINDI, and SCES, respectively.

Beijing Cohort. The Beijing Eye Study (BES) was a population-based, cross-sectional study of Chinese adults (aged 40+ years) in urban communities in the Haidian district in the north of central Beijing and in rural communities in the village area of Yufa of the Daxing district, south of Beijing.16 In the year 2001 when the first survey was carried out, 4439 participants were recruited (83.4% response rate). The BES was repeated in 2006 and 2011. In 2011, when ACD and AL were measured, the study included all participants from the previous two surveys and added subjects who fulfilled the eligibility criteria of age of 50+ years, who lived in the study region, and who had not participated in the previous surveys. A total of 3468 participants (78.8% response rate) were recruited in the 2011.17 The final number of subjects who were genotyped was 988. Measurement of ACD and AL was performed using optical low-coherence reflectometry (Lenstar 900 Optical Biometer; Haag-Streit, Koeniz, Switzerland). The examination was performed by experienced clinical technicians. Five measurements were performed, and the mean value was taken for further statistical analysis.

Measurements of Ocular Parameters

Singapore Cohorts. ACD and AL were measured by noncontact partial-coherence laser interferometry (IOLMaster, Carl Zeiss Meditec, Dublin, CA). Five readings were obtained, and the mean value was used for analysis. The biometric measurements were excluded from any eye that was pseudophakic or aphakic. As there was good correlation between biometric data for the two eyes, analysis was performed using only data for the right eyes.

Beijing Cohort. ACD and AL were measured by optical low-coherence reflectometry (Lenstar 900 Optical Biometer; Haag-Streit) for the right eyes of study participants in the survey in 2011. Five measurements were performed, and the mean value was used for analysis.17 Good correlation between IOLMaster and Lenstar measurements for ACD/AL has been demonstrated, and no statistical differences in ACD/AL measurements were observed in a previous study.18 Therefore, the use of different measurement devices is unlikely to affect the result of analysis.

Genotyping and Data QC

Methods of genotyping and data quality control (QC) for SiMES, SINDI, SCES, and BES have been described previously.19–22 In brief, participants of all four studies were genotyped using the Illumina Human610-Quad BeadChip (Illumina, Inc., San Diego, CA) with the following QC criteria: Samples were excluded if they had a per sample call rate <95% or showed evidence of admixture, cryptic relatedness, high heterogeneity, or sex discrepancy. Final number of subjects passing quality checks was 2542, 2538, 1949, and 927 for SiMES, SINDI, SCES, and BES, respectively. Genotype at the three loci of interest (rs3753841, rs1015213, and rs11024102) was analyzed using PLINK (version 1.07).23

Statistical Analysis

Linear regression was performed for primary association testing 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, and 2 for homozygous variants. A trend test incorporated within a linear regression model was used for primary association testing between genotypes and ACD/AL as quantitative traits, adjusting for age, sex, and population admixture (reflected by principal components).

Meta-analysis was performed with PLINK (version 1.07) using the fixed effects model on primary analysis and was verified with the random effects model when significant heterogeneity was observed. For the meta-analysis, a combined point estimate of the overall effect size ($\beta$ coefficient) and its corresponding $P$ value were obtained. The Cochran’s $Q$ and accompanying $I^2$ statistic were used to assess intercohort heterogeneity. For the final analysis, a Bonferroni correction factor of 3 was applied to correct for number of loci studied, resulting in a $P$ value threshold of 0.0017 to be considered statistically significant experiment-wide.

RESULTS

Table 1 summarizes the descriptive statistics of all cohorts after genotyping and sample QC.

A total of 7245, 7243, and 7239 subjects who had complete genotype and ACD (including covariates) measurement data were tested for association between the three PACG susceptibility loci (rs35753841, rs1015213, and rs11024102) and ACD, respectively (Table 2). We found nominal evidence of association between SNP rs1015213 (PCMTD1-ST18) and a shallower ACD when all data were meta-analyzed ($\beta = -0.035$, $P = 0.021$). There was no significant heterogeneity of the effect size across all sample collections ($P_{\text{heterogeneity}} = 0.87$, $I^2 = 0$). The observation was
Table 1. Characteristics of the Study Population

<table>
<thead>
<tr>
<th></th>
<th>SIMES</th>
<th>SINDI</th>
<th>SCES</th>
<th>BES</th>
</tr>
</thead>
<tbody>
<tr>
<td>n*</td>
<td>2542</td>
<td>2538</td>
<td>1949</td>
<td>927</td>
</tr>
<tr>
<td>Age†</td>
<td>59.09 (11.04), 40–80</td>
<td>58.04 (10.01), 43–80</td>
<td>58.99 (9.98), 44–111</td>
<td>53.11 (9.35), 40–81</td>
</tr>
<tr>
<td>Sex‡</td>
<td>0.51</td>
<td>0.49</td>
<td>0.49</td>
<td>0.65</td>
</tr>
<tr>
<td>ACD†</td>
<td>5.10 (0.38), 1.78–4.25</td>
<td>3.22 (0.46), 1.74–5.39</td>
<td>2.95 (0.37), 1.99–5.09</td>
<td>2.41 (0.32), 1.29–3.61</td>
</tr>
<tr>
<td>AL†</td>
<td>23.57 (1.05), 20.61–30.53</td>
<td>23.38 (1.11), 19.14–32.76</td>
<td>23.61 (1.37), 20.39–33.27</td>
<td>23.03 (1.11), 19.90–30.36</td>
</tr>
</tbody>
</table>

* Number of subjects with genotype data after quality check.
† Mean (standard deviation), range.
‡ % female.

Table 2. Association Results Between Three PACG Susceptibility Loci and ACD and AL Parameters

<table>
<thead>
<tr>
<th>Chr</th>
<th>SNP</th>
<th>BP</th>
<th>A1</th>
<th>Cohort</th>
<th>n</th>
<th>EAF</th>
<th>β</th>
<th>SE</th>
<th>P</th>
<th>Phet</th>
<th>I²</th>
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<tbody>
<tr>
<td>1</td>
<td>rs3753841</td>
<td>103152506</td>
<td>G</td>
<td>SCES</td>
<td>1690</td>
<td>0.32</td>
<td>0.006</td>
<td>0.014</td>
<td>0.67</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SINDI</td>
<td>2209</td>
<td>0.2889</td>
<td>0.019</td>
<td>0.011</td>
<td>0.094</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BES</td>
<td>826</td>
<td>0.2983</td>
<td>0.014</td>
<td>0.016</td>
<td>0.392</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>All</td>
<td>7245</td>
<td>0.0093</td>
<td>0.16</td>
<td>0.76</td>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>8</td>
<td>rs1015213</td>
<td>53050094</td>
<td>A</td>
<td>SCES</td>
<td>1690</td>
<td>0.011</td>
<td>−0.041</td>
<td>0.064</td>
<td>0.52</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>SINDI</td>
<td>2207</td>
<td>0.04055</td>
<td>−0.021</td>
<td>0.025</td>
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<td>BES</td>
<td>826</td>
<td>0.02427</td>
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<td>0.046</td>
<td>0.139</td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>All</td>
<td>7243</td>
<td>0.0153</td>
<td>0.021</td>
<td>0.87</td>
<td>0</td>
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</tr>
<tr>
<td>11</td>
<td>rs11024102</td>
<td>16965181</td>
<td>G</td>
<td>SCES</td>
<td>1690</td>
<td>0.37</td>
<td>−0.0007</td>
<td>0.013</td>
<td>0.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SINDI</td>
<td>2205</td>
<td>0.37</td>
<td>−0.014</td>
<td>0.011</td>
<td>0.19</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>BES</td>
<td>826</td>
<td>0.4326</td>
<td>−0.023</td>
<td>0.015</td>
<td>0.119</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>All</td>
<td>7239</td>
<td>−0.009</td>
<td>0.148</td>
<td>0.65</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Chr, chromosome number; SNP, SNP ID; BP, base-pair position; A1, effect allele; n, number of subjects included in the analysis; EAF, effect allele frequency; β, per-allele change in ACD/AL; SE, standard error for ascertainment of β; P, P value for association; Phet, P value for heterogeneity between cohorts; I², I² index for heterogeneity between cohorts.

Statistically nonsignificant when multiple testing was considered. No significant association was found between the other two loci (rs3753841 and rs11024102) and ACD in the meta-analysis (Table 2). The regional association analysis for the three loci and ACD is illustrated in Supplementary Figures S1, S2, and S3.

In the association testing between the three PACG susceptibility loci (rs3753841, rs1015213, and rs11024102) and AL, a total of 6902, 6900, and 6896 subjects who had complete genotype and AL (including covariates) measurement data were included, respectively (Table 3). We found that locus rs11024102 (PLEKHA7) was significantly associated with longer AL in the meta-analysis of all collections using the fixed effects model (β = 0.051, P = 0.009). However, significant heterogeneity of effect sizes was observed (Phet = 0.01, I² = 69.19%). Therefore meta-analysis using the random effects model was conducted; the results did not show significant association between rs11024102 (PLEKHA7) and AL (Prandomeffects = 0.38). No significant association was found between the other two loci (rs3753841 and rs1015213) and AL in individual cohorts or in the meta-
anterior chamber width 25; smaller anterior chamber area and addition to ACD and AL, there are several novel imaging-based susceptibility genes, dynamic (as opposed to static) mechanisms. One of the PACG-associated variants confers risk via these dynamic physiological factors such as changes in iris volume26; greater iris thickness, area, and curvature27; and larger lens vault.28 Although not correlated with ACD or AL, these PACG-associated loci may be associated with these other anatomical parameters. Recent evidence also suggests that dynamic physiological factors such as changes in iris volume with dilation29,30 and choroidal expansion/effusion31 may have a role in angle-closure pathogenesis. We speculate that at least one of the PACG-associated variants confers risk via these dynamic (as opposed to static) mechanisms. One of the susceptibility genes, PLEKHA7, encodes a plekstrin homology domain containing protein, proposed to regulate apical junctional complexes (AJCs).32,33 As AJCs control epithelial and endothelial paracellular permeability, PLEKHA7 may be involved in the pathophysiology of angle closure related to aberrant fluid dynamics. With the identification of newer ocular biometric and dynamic risk factors for angle closure and progression in angle-closure disease should now be performed.

Acknowledgments

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