Genetics

Genetic Variants near *PDGFRA* Are Associated with Corneal Curvature in Australians

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Purpose. Irregularity in the corneal curvature (CC) is highly associated with various eye disorders such as keratoconus and myopia. The sample had limited power to find genomewide significant (5×10^{-8}) hits but good power for replication. Thus, an attempt was made to test whether alleles in the *FRAP1* and *PDGFRA* genes, recently found to be associated with CC in Asian populations, also influence CC in Australians of North European ancestry. Results of initial genomewide association studies (GWAS) for CC in Australians were also reported.

Methods. Two population-based cohorts of 1788 Australian twins and their families, as well as 1013 individuals from a birth cohort from Western Australia, were genotyped using genomewide arrays. Following separate individual analysis and quality control, the results from each cohort underwent meta-analysis.

Results. Meta-analysis revealed significant replication of association between rs2114039 and corneal curvature ($P = 0.0045$). The SNP rs2114039 near *PDGFRA* has been previously implicated in Asians. No SNP at the *FRAP1* locus was found to be associated in our Australian samples. No SNP surpassed the genomewide significance threshold of 5×10^{-8}. The SNP with strongest association was rs2444240 ($P = 3.658 \times 10^{-7}$), which is 31 kb upstream to the *TRIM29* gene.

Conclusions. A significant role of the *PDGFRA* gene in determining corneal curvature in the Australian population was confirmed in this study, also highlighting the putative association of the *TRIM29* locus with CC. (*Invest Ophthalmol Vis Sci.* 2012;53:7131–7136) DOI:10.1167/iovs.12-10489

Refraction of light through the cornea is the preliminary step in vision. This bending of light is highly dependent on the curvature of the cornea. Corneal astigmatism or an irregularity in corneal curvature (CC) leads to blurred uncorrected vision. Keratoconus is a disease characterized by a conical-shaped cornea and irregular astigmatism.[1] Patients with this corneal condition often experience vision distortion, multiple images, and sensitivity to light. In addition to keratoconus, corneal irregularities are also associated with refractive error[2] and Marfan syndrome.[3]

Variation in corneal curvature is dependent on ethnic background,[4,5] geographical as well as environmental conditions,[6] age,[7] and stature.[7] CC is highly heritable,[8] with previous studies revealing heritability estimates ranging between 60% and 95%.[6,8–11] Improved understanding of the genetic architecture of this biometric trait will aid in determining the molecular mechanisms of blinding eye disorders, and contribute to our ocular development and evolutionary biology knowledge.

Genomewide association studies (GWAS) have been successful in revealing the genetic variants behind various complex traits including age-related macular degeneration, type 2 diabetes as examples.[12–15] The only published GWAS for CC is from a Singaporean Asian population, in which the significant associations of single nucleotide polymorphisms (SNPs) in *FRAP1* and *PDGFRA* genes with corneal curvature were reported.[6] As is the case with other quantitative traits, CC is likely to be determined by many genes, with ever larger GWASs likely to lead to the identification of additional associated SNPs.[16] Furthermore, it is unknown whether genes found to be significantly associated with CC in Asian population would be relevant to other racial groups. In

TABLE 1. Demographic Details of Study Participants

	TEST and BATS	Raine Study
Number of subjects	1788	1013
Number of families	857	1013
Mean age, y	22.2	20.0
Range of age	5 to 90	18 to 22
Sex (% female)	1014 (56.7)	497 (49.1)
Mean corneal curvature, mm (SD)	7.63 (0.24)	7.72 (0.24)
Range of corneal curvature, mm	6.77 to 8.41	7.04 to 8.61

Corneal curvature was measured in millimeters (mm); corneal curvatures mentioned here are average of the mean of corneal curvature measured vertically and horizontally for left and right eyes of participants, respectively.

previous studies, ethnic and environmental backgrounds constitute an important determinant of CC.[17-19] Thus, we aimed to test whether *PDGFRA* and *FRAP1* genes found to be associated with CC in a Singaporean Asian population also determine the CC in Australians of Northern European ancestry. We also aimed to report initial GWAS on CC in Australians of Northern European ancestry. We conducted two population-based GWASs on 1788 Australian twins and their families,[20] as well as 1013 unrelated individuals from a population cohort from Western Australia.

MATERIALS AND METHODS

Ethics Statement

This study was conducted according to the principles expressed in the Declaration of Helsinki. The study was approved by the human research ethics committees of the University of Tasmania, Royal Victorian Eye and Ear Hospital, Queensland Institute of Medical Research, and University of Western Australia. Informed consent was obtained from parents with the child's assent or from adult participants before testing.

Twin Cohorts

In all, 1788 individuals of 857 twin families were recruited from Australia. Recruitment of twins was performed through the Twins Eye Study in Tasmania (TEST) and the Brisbane Adolescent Twin Study (BATS).[20] BATS participants ranged in age from 10 to 40 years and TEST participants ranged in age from 5 to 90 years. Corneal curvature was measured using a commercial automatic refractor/keratometer (Humphrey-598 Automatic Refractor/Keratometer; Carl Zeiss Meditec, Inc., Miami, FL). The difference between curvature values of left and right eyes was not significant (*t*-test, $P = 0.24$).

Saliva or peripheral blood samples from subjects were used to extract DNA, which was genotyped on the Illumina HumanHap 610W Quad arrays (Illumina, Inc., San Diego, CA). The majority of people from the BATS study were genotyped by deCODE Genetics (Reykjavik, Iceland). TEST participants and a small number of BATS individuals were genotyped by the Centre for Inherited Disease Research (CIDR) (Perth, Australia).

Filtering criteria for genotypic data were: minor allele frequency $\geq$ 1%, Hardy-Weinberg Equilibrium Test, $P \geq 10^{-6}$, SNP call rate > 95% or Illumina Beadstudio GenCall score $\geq$ 0.7. Quality control of SNPs gave 559,990 SNPs, which then underwent association testing in the twin cohorts.

Ancestral outliers were corrected by principal component analysis (PCA)[21] using the "smartpca" program (EIGENSOFT 3.0 software; provided in the public domain, nickp@broad.mit.edu). Australian twin data were compared with all populations in HapMap phase 3 and collection of five other GenomEUTWIN populations.[22,23] Only PC1 and PC2 with the highest eigenvalues were considered to identify and filter outliers. PC1 reflects the difference between Africans and others,
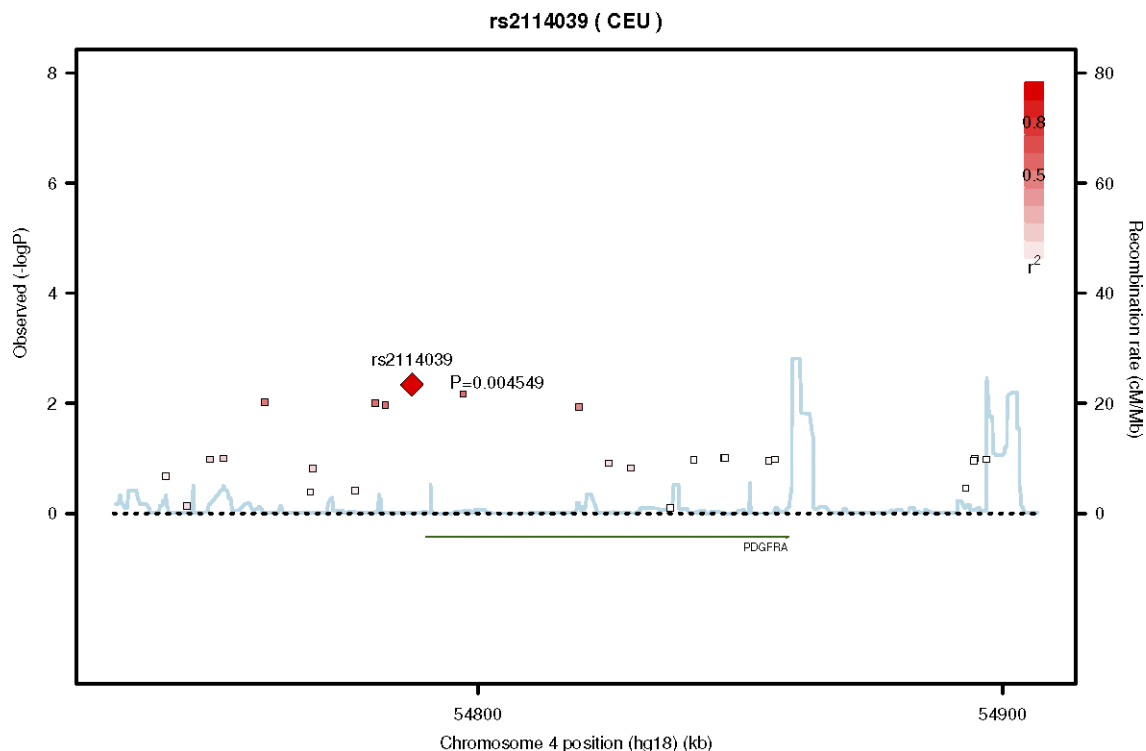

FIGURE 1. Association of variants at the *PDGFRA* locus. The top SNP rs2114039 has a *P* value 4.549×10^{-3}. The *red shading* shows the degree of linkage disequilibrium between rs2444240 and neighboring SNPs. The *light blue line* displays the rate of recombination with scale on *right-hand axis*.
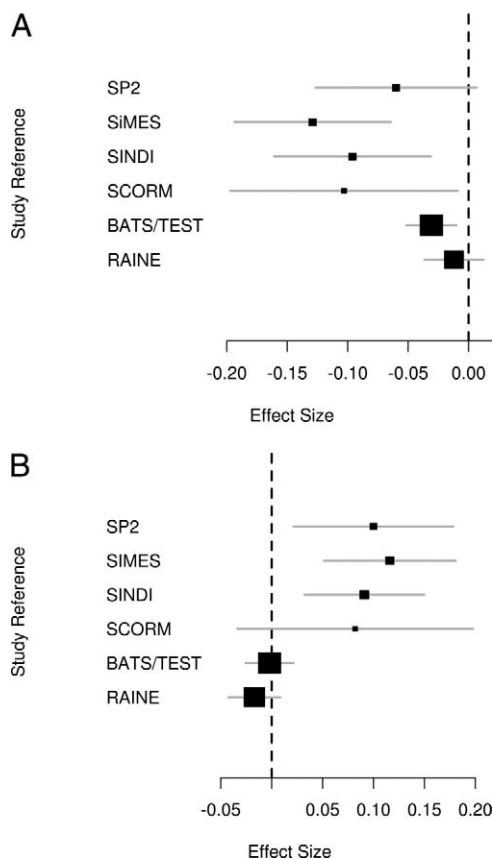
FIGURE 2. Forrest plot, showing effect size distribution in different studies for (**A**) *PDGFRA* SNP rs2114039 and (**B**) *FRAP1* SNP rs6540964. The *top four studies* are reported by corneal curvature studies in an Asian population. The *last two studies* demonstrate the effect size distribution for SNPs found in our analysis of corneal curvature GWAS on Australians with Northern European Ancestry.

whereas PC2 reflects the difference between East Asian populations with others. As expected, the Australian population clustered with Europeans with some individuals also showing Asian and African ancestry. To remove Australian population outliers like Australians with African or Asian ancestry, the mean and SD of collective European populations, that is, Hapmap CEU (Utah residents with Northern and Western European ancestry from the CEPH collection), TSI (Toscans in Italy), and GenomEUTWIN (European Twin Population), was calculated for reference PC1 and PC2 scores. Any individual that fell away from the mean by >6 times their SD on PC1 and PC2 were removed, with the remainder considered for further analysis.

Using the "MACH v1.0.16b" and "mimimac" packages, imputation of Australian Twin genotyped data was carried out using the 1000 Genomes haplotypes available for 283 European ancestry individuals in the August 2010 release of 1000 Genomes project.[24,25] Approximately 482,000 common genotyped markers were used in imputation, which gave 11,914,767 imputed SNPs. Following quality control ($R^2 > 0.3$ for each SNP), 8,016,011 SNPs underwent association analysis. The MERLIN program (Center for Statistical Genetics, Ann Arbor, MI) was used to perform association testing, taking into account relatedness of the twins and their families.[26] We used age and sex as covariates.

Raine Eye Health Study

The 21-year review of the Western Australian Pregnancy Cohort (Raine) Study investigated ophthalmic health and established the Raine Eye Health Study (REHS). The Raine Study is one of the largest ongoing prospective cohort studies of pregnancy, childhood, adoles-

cence, and young adulthood. In 1989, 2900 pregnant women were recruited at 16- to 18-weeks' gestation into a randomized clinical trial at King Edward Memorial Hospital, Perth, Western Australia for investigating effects of intensive ultrasound and Doppler studies in pregnancy outcomes. The cohort has been evaluated in detail during childhood (1, 2, 3, 5, 8, and 10 years) and adolescence (14 and 17 years). Raine participants underwent a comprehensive ocular examination for the first time at the 21-year follow-up. The ocular examination included measurements of visual acuity, cycloplegic auto refraction, as well as several ocular biometric variables and multiple ophthalmic photographs (anterior and posterior segments). A total of 1344 subjects were examined in the 24-month period from March 2010 to February 2012.

Ocular biometric parameters including CC were measured (IOLMaster v.5; Carl Zeiss Meditec AG, Jena, Germany). Three measurements of CC within 0.3D within each meridian with careful alignment and focus were recorded. Complete data were available from 1013 participants for analysis. The average age of the participants was 20.0 (range: 18–22) years. DNA samples from previous assessments and consents for GWAS studies were available for the participants.

Individual genotype data for 1494 participants were extracted from the genomewide array (Illumina 660 Quad Array). Briefly, the genotyping was performed on the Illumina BeadArray Reader at the Centre for Applied Genomics (Toronto, Ontario, Canada) using 250 nanograms of DNA. Any pair of individuals who were related with a π > 0.1875 (in between second- and third-degree relatives; e.g., between half-sibs and cousins) was investigated, and the individual with the higher proportion of missing data was excluded from the "clean" data set (68 individuals excluded). Individuals who had low genotyping success (i.e., missing data) were excluded from the "clean" data set; a threshold of absent data >3% was used for exclusion (16 individuals excluded). Additionally, if they had high levels of heterozygosity then they were also excluded (heterozygosity < 0.30 excluded 3 individuals) because this may indicate sample contamination. In terms of genotyping success rates, we also excluded SNPs that did not satisfy a Hardy–Weinberg equilibrium P value > 5.7×10^{-7} (919 markers), a call rate > 95% (97,718 markers), and a minor allele frequency > 0.01 (1%) (119,246 markers, including CNVs). To account for population stratification, the first five principal components were calculated using a subset of 42,888 SNPS that were not in LD with each other. PCA was conducted using the EIGENSTRAT program (provided in the public domain, aprice@hsph.harvard.edu).[21]

The MACH v1.0.16 (http://www.sph.umich.edu/csg/yli/mach/index.html) software was used for GWAS imputation on the 22 autosomes. Once the data were cleaned, a two-step process was carried out using the CEU samples from HapMap phase2 build 36 release 22 (http://hapmap.ncbi.nlm.nih.gov/index.html.en) as a reference panel.

With PLINK[27] as an interface with R (provided in the public domain by the R Foundation for Statistical Computing, Vienna, Austria, available at http://www.r-project.org/), a linear regression model was used to examine the association between SNPs and keratometry adjusted for age, sex, and the first two principal components, which account for population stratification in this cohort.

Meta-Analysis Method

Meta-analysis increases the power to identify associations by increasing the sample size. The β-coefficients strategy of the program METAL was used to conduct meta-analysis.[28]

Genetic Power Calculation

GWAS power (type 1 error cutoff = 5×10^{-8}) and Replication Power for two SNP test (cutoff = 0.05/2) was calculated using the Genetic Power Calculator program (http://pngu.mgh.harvard.edu/~purcell/gpc/).[29]
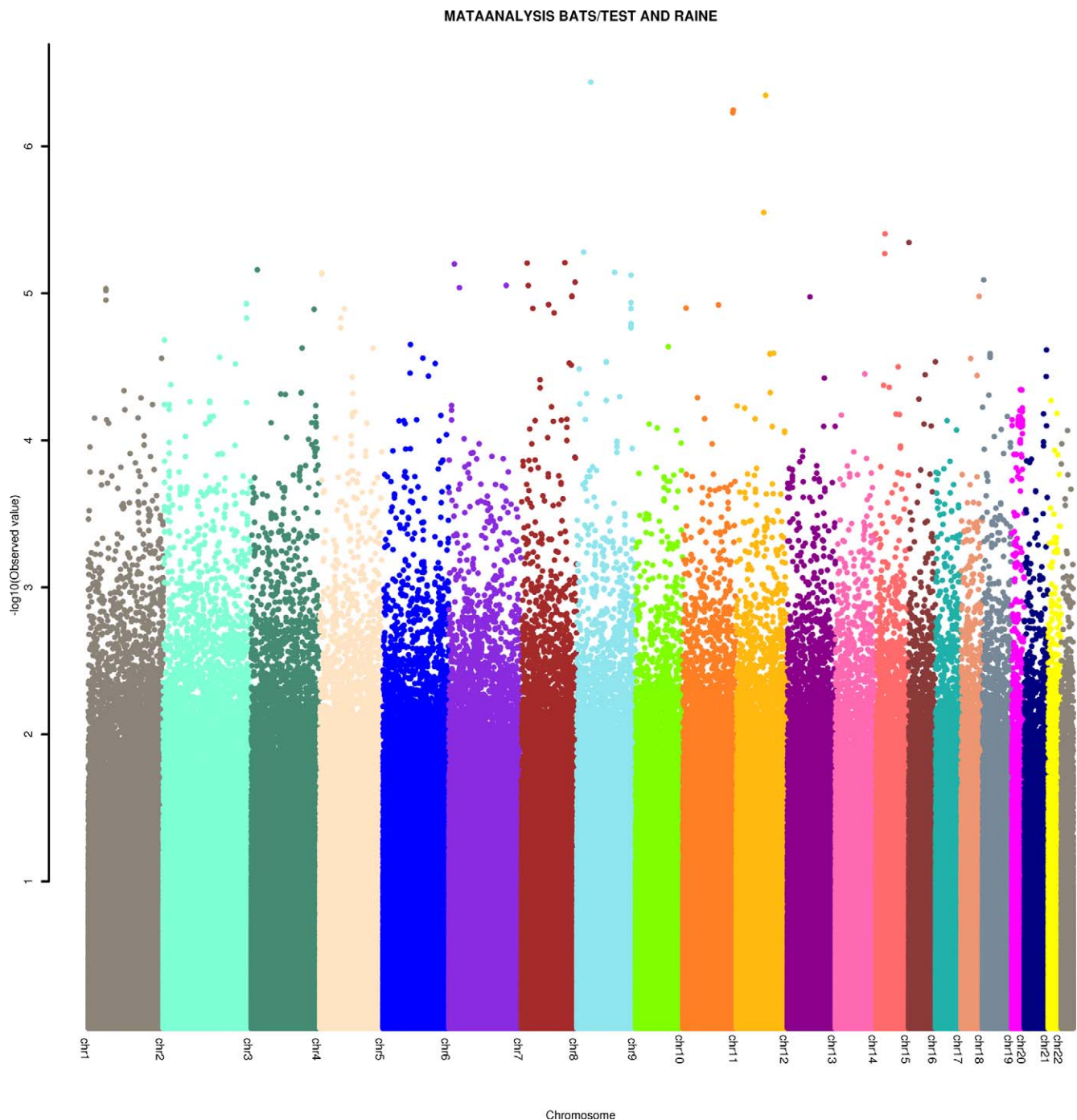
MATAANALYSIS BATS/TEST AND RAINE

FIGURE 3. Manhattan plot, which shows SNP *P* value distribution of meta-analysis results with respect to the chromosome.

RESULTS

After quality control, the population sizes of the BATS/TEST and Raine studies were 1788 and 1013, respectively. Summary statistics for both the populations under study are shown in Table 1.

With consideration of combined sample size, the GWAS power to detect the variant that could explain at least 1% variation of CC is very low (power = 30%), whereas replication power for significant replication cutoff 0.05/2 is >99%. Thus, our primary emphasis was on replication of *FRAP1* and *PDGFRA* genes, reported genomewide association with CC in Asians, in an Australian population of Northern European ancestry. We tested whether the most significant SNP in each gene was

associated in our European ancestry samples. We found that SNP rs2114039 in *PDGFRA* was associated with corneal curvature (*P* = 0.0045). Figure 1 shows the recombination profile between the SNPs and *PDGFRA* locus. The effect size of the trait-increasing allele was 0.02275 mm per copy of the T allele. The effect sizes across different studies are shown in Figure 2A. However, SNP rs6540964 in gene *FRAP1* was not associated with curvature (*P* = 0.2984); effect sizes are shown in Figure 2B.

Although our study does not have enough power to detect a genomewide-associated variant, we report the initial findings. The Independent association test on BATS/TEST and Raine data yielded a best SNP rs4552334 ($P = 2.5 \times 10^{-6}$) and rs11930632 ($P = 2.47 \times 10^{-6}$), respectively. We followed this analysis by

meta-analysis of outcomes from the 1,704,858 SNPs common in both studies.

Results from the meta-analysis across the genome are displayed in Figure 3. The 25 most significant SNPs from the meta-analysis are shown in Supplemental Table S1 (see Supplementary Material and Supplementary Table S1, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-10489/-/DCSupplemental).

As expected, meta-analysis did not reveal any genomewide-associated SNPs: the most associated genotyped SNP rs2444240 had a P value of 3.658×10^{-7} at 120.040 Mb (build 37) on chromosome 11. This SNP had a slightly stronger signal in the Raine study, as shown in Table 2. The nearest gene to these SNPs is *TRIM29* on the chromosome 11q23.3 region (NCBI build 37), which spans only 26 kb. The recombination profile between the SNPs and *TRIM29* locus is shown in Supplemental Figure S1 (see Supplementary Material and Supplemental Fig. S1, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-10489/-/DCSupplemental).

DISCUSSION

We have conducted a genomewide association study on corneal curvature in an Australian population of Northern European ancestry. Our sample showed that SNPs near *PDGFRA*, which were very recently shown to play a role in CC in Asians, also play a role in samples of Northern European Ancestry. The allele frequency at rs2114039 is similar ($\sim$0.3) across the range of European and Asian ancestry samples in the HapMap3 data. The variance explained by rs2114039 in our European ancestry sample for BATS/TEST and Raine study was 0.6% and 0.1%, respectively, which is somewhat lower than that seen in the Asian studies (1.8%, 11.1%, 4.9%, and 7.5% for SP2, SIMES, SINDI, and SCORM study, respectively). However, the estimates of variance explained in the Asian studies are probably an overestimate of the true effect size due to this SNP being selected as one of the most significant results from a genomewide scan.[30] An estimate of the effect size in an independent Asian population would allow us to determine if the effect size at this SNP truly differs in the two populations.

Although our study did not identify any genomewide significant loci, meta-analyzing across two Australian studies led to a more significant top SNP, with rs2444240 in *TRIM29* achieving a P value of 3.658×10^{-7}. Larger sample sizes are required to unambiguously identify novel loci. With reference to past literature, we found involvement of some of the genes near or within the top 25 SNPs with some related traits.[31–34] A gene expression profile of human keratoconus suggests significant expression of the *TRIM29* gene.[31] This differential expression of the *TRIM29* gene could be responsible for the conical shape of the cornea. Apart from this, a linkage study on Ashkenazi Jewish families also supports a possible role of *TRIM29* in variation of CC by their report of linkage between 11q23 loci and myopia.[32] In addition, a gene expression study on focal loss of retinal ganglion cells suggests significant downregulation of the *BCL11B* gene.[34] Studies on myopia in chicks already established that the flattened cornea is an outcome of ganglion cells' destruction.[33] Further study needs to be done to investigate the possible role of *TRIM29* and *BCL11B* genes in determining CC.

In conclusion, our study of CC showed that the SNP in *PDGFRA*, recently implicated in this trait in Asians, also underlies trait variation in Australians of Northern European ancestry. The other gene reported to be associated with CC in Asian populations, *FRAP1*, did not show a significant effect in our samples. Although our study was underpowered to detect novel loci, we found some evidence that SNPs near *TRIM29* may play a role in determining CC. Our findings of SNPs at

TABLE 2. Top Four SNPs and Their Association Results

Marker	Chr	Coordinate, build 36	Nearest Gene	Alleles*	BATS/TEST Effect	SE	BATS/TEST P	RAINE Effect	SE	RAINE P	Weighted Effect	SE	Meta Analysis P
rs2444240	11	119545652	*TRIM29*	T/G	0.030631	0.009544	1.28×10^{-3}	0.044733	0.011125	5.71×10^{-5}	−0.0364	0.0071	3.66×10^{-7}
rs494965	11	119558860	*TRIM29*	T/C	−0.0309	0.009392	1.12×10^{-3}	−0.04317	0.010944	9.35×10^{-5}	0.0365	0.0073	4.50×10^{-7}
rs470606	11	119533412	*TRIM29*	T/G	0.029068	0.009541	2.2×10^{-3}	0.045626	0.011163	4.41×10^{-5}	−0.0359	0.0071	5.66×10^{-7}
rs470373	11	119531481	*TRIM29*	T/C	0.029068	0.009541	2.2×10^{-3}	−0.04492	0.010922	4.61×10^{-5}	0.0362	0.0073	5.90×10^{-7}

* The first letter in the Alleles column is the effect allele for specified SNP (e.g., for SNP rs2444240, the T is effect allele not G).

TRIM29 and other regions should be replicated in further studies, with meta-analyses likely to prove important in further dissecting this important endophenotype.

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