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# A genome-wide association study of corneal astigmatism: The CREAM Consortium

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**Purpose:** To identify genes and genetic markers associated with corneal astigmatism.

**Methods:** A meta-analysis of genome-wide association studies (GWASs) of corneal astigmatism undertaken for 14 European ancestry (n=22,250) and 8 Asian ancestry (n=9,120) cohorts was performed by the Consortium for Refractive Error and Myopia. Cases were defined as having >0.75 diopters of corneal astigmatism. Subsequent gene-based and gene-set analyses of the meta-analyzed results of European ancestry cohorts were performed using VEGAS2 and MAGMA software. Additionally, estimates of single nucleotide polymorphism (SNP)-based heritability for corneal and refractive astigmatism and the spherical equivalent were calculated for Europeans using LD score regression.

**Results:** The meta-analysis of all cohorts identified a genome-wide significant locus near the platelet-derived growth factor receptor alpha (*PDGFRA*) gene: top SNP: rs7673984, odds ratio=1.12 (95% CI:1.08–1.16),  $p=5.55 \times 10^{-9}$ . No other genome-wide significant loci were identified in the combined analysis or European/Asian ancestry-specific analyses. Gene-based analysis identified three novel candidate genes for corneal astigmatism in Europeans—claudin-7 (*CLDN7*), acid phosphatase 2, lysosomal (*ACP2*), and TNF alpha-induced protein 8 like 3 (*TNFAIP8L3*).

**Conclusions:** In addition to replicating a previously identified genome-wide significant locus for corneal astigmatism near the *PDGFRA* gene, gene-based analysis identified three novel candidate genes, *CLDN7*, *ACP2*, and *TNFAIP8L3*, that warrant further investigation to understand their role in the pathogenesis of corneal astigmatism. The much lower number of genetic variants and genes demonstrating an association with corneal astigmatism compared to published spherical equivalent GWAS analyses suggest a greater influence of rare genetic variants, non-additive genetic effects, or environmental factors in the development of astigmatism.

Astigmatism is a commonly occurring refractive error that leads to impaired visual acuity if uncorrected and is a risk factor for amblyopia [1-4]. The two major sources of refractive astigmatism in the human eye are the cornea and the crystalline lens. In emmetropic eyes, a low degree of with-the-rule (WTR) corneal astigmatism is typically compensated by a low degree of against-the-rule (ATR) lenticular astigmatism [5]. For individuals with higher levels of refractive astigmatism, corneal astigmatism is usually the major contributor, while lenticular astigmatism is within the normal range [6].

Studies in chicks have recently shown that the eye can compensate for experimentally induced astigmatism through the alteration of corneal curvature [7]. This suggests that the reduction in innate astigmatism seen during infancy in children occurs via active emmetropization [8]. Potential reasons why astigmatism still arises despite the presence of an emmetropization system include (a) astigmatism of too high a degree to be compensated within the juvenile period, (b) astigmatism outside the “operating range” of the emmetropization system, for example, producing a retinal image that is not detected as being caused by astigmatism or that arises at an age beyond that at which emmetropization normally acts, and (c) a failure of the emmetropization response [2].

Several lines of evidence support the role of genetics in the etiology of astigmatism. First, epidemiology studies have shown marked differences in the prevalence of astigmatism across ethnic groups, even after accounting for differences in spherical refractive error. For instance, 78% of native American Tohono O’odham children aged 0–8 have at least 1

diopter (D) of corneal astigmatism, and in Australian children aged 12, at least 1 D of corneal astigmatism was found in 19% of European individuals versus 50% of East Asian individuals [9,10]. Second, corneal and refractive astigmatism have been reported as being moderately/highly heritable (heritability of 0.3–0.6) in twin studies [11,12]. Third, a genetic segregation study in families with high-degree astigmatism found evidence of Mendelian inheritance [13]. Finally, genetic association studies have identified specific genetic variants associated with susceptibility to either refractive and/or corneal astigmatism [14-17]. Despite these latter studies, our understanding of the genetic contribution to astigmatism has lagged behind that of spherical refractive errors, for which dozens of genetic variants have been discovered [18-21].

Previously, the Consortium for Refractive Error and Myopia (CREAM) reported a genome-wide association study (GWAS) of refractive astigmatism that examined approximately two million genetic markers in 45,931 individuals [17]. Only a single marker reached genome-wide significance (rs1401327 in the *NRXN1* gene,  $p=3.92E-8$ ). Reasoning that the paucity of genome-wide significant hits in the previous CREAM study may have been due to phenotypic uncertainty when studying refractive astigmatism that arose from the combination of both corneal and lenticular influence, CREAM has now undertaken a GWAS of corneal astigmatism. Fan et al. [16] performed a GWAS of corneal astigmatism using a discovery sample of 4,254 East Asian individuals and identified a genome-wide significant locus near the *PDGFRA* gene. In view of the success of the Fan et al. [16] study, the current analysis has adopted the same phenotype definition.

TABLE 1. SUBJECT DEMOGRAPHICS OF PARTICIPATING CREAM STUDY GROUPS.

Study	Ancestry	N (cases/controls)	%Female	Age (years)		Corneal astigmatism (D)	
				Mean (SD)	Range	Median (IQR)	Range
ALSPAC	European	2279(985/1294)	53.1	15.5 (0.3)	0.683 (0.469–0.959)	0.000–5.680	
BMES	European	1238(720/518)	41.8	73.3 (7.6)	0.863 (0.565–1.295)	0.155–8.615	
EPIC	European	857(456/401)	58.5	68.7 (7.4)	0.780 (0.527–1.152)	0.075–3.997	
FITSA	European	127(62/65)	100	67.9 (3.1)	0.733 (0.530–1.033)	0.270–2.020	
GenerationR	European	2071(981/1090)	49.9	6.09 (0.4)	0.725 (0.480–0.995)	0.000–3.370	
GHS 1 <sup>1</sup>	European	2398(1003/1395)	48.7	55.9 (10.8)	0.65 (0.400–0.950)	0.050–4.350	
GHS 2 <sup>1</sup>	European	851(383/468)	50.9	55.1 (10.8)	0.65 (0.450–1.000)	0.050–3.800	
RAINE	European	1028(407/621)	50.9	20.0 (0.4)	0.649 (0.445–0.905)	0.280–2.440	
Rotterdam-I	European	5537(2064/3473)	59.3	69.5 (9.2)	0.601 (0.334–1.007)	0.000–9.663	
Rotterdam-II	European	1982(633/1349)	53.8	64.8 (8.0)	0.539 (0.294–0.884)	0.000–6.789	
Rotterdam-III	European	2925(1180/1745)	56.2	57 (6.9)	0.618 (0.356–1.019)	0.000–4.869	
OGP-A <sup>2</sup>	European	92(37/55)	44.6	16.0 (4.5)	0.682 (0.512–0.942)	0.185–3.070	
OGP-B <sup>2</sup>	European	446(181/265)	43.7	50.6 (15.4)	0.650 (0.430–0.970)	0.130–4.240	
TwinsUK	European	419(201/218)	92.7	64 (10.5)	0.729 (0.476–1.105)	0.000–5.432	
BES-610K <sup>3</sup>	Asian	553 (240/313)	65.6	62.1 (8.4)	0.666 (0.407–1.056)	0.000–3.620	
BES-OmniE <sup>3</sup>	Asian	469 (208/261)	60.1	64.7 (9.5)	0.676 (0.429–1.016)	0.000–5.082	
SCES-610K <sup>3</sup>	Asian	1745 (787/958)	48.7	57.6 (9.0)	0.703 (0.476–1.060)	0.109–5.868	
SCES-OmniE <sup>3</sup>	Asian	545 (257/288)	48.6	59.2 (8.8)	0.723 (0.470–1.065)	0.117–5.404	
SCORM	Asian	947 (768/179)	48.6	10.8 (0.8)	1.205 (0.851–1.624)	0.138–3.911	
SIMES	Asian	1778 (750/1028)	51.7	59.5 (10.8)	0.662 (0.432–1.016)	0.078–5.618	
SINDI	Asian	2261 (814/1447)	48.6	56.5 (9.1)	0.614 (0.411–0.912)	0.115–4.727	
STARS	Asian	822 (525/297)	50.0	38.5 (5.3)	1.000 (0.625–1.380)	0.125–3.875	

<sup>1</sup>Association tests were undertaken separately for samples recruited in different waves.<sup>2</sup>Association tests were undertaken separately for different age strata (stratum A, age >3 and <25 years; stratum B, age ≥25 years).<sup>3</sup>Association tests were undertaken separately for samples genotyped on different platforms.

## METHODS

The research study followed an analysis plan that was agreed upon by members of CREAM before starting work. This plan was designed to standardize methods across participating CREAM groups and to set timelines for the completion of specific tasks. All research groups known to CREAM with relevant genotype and phenotype data were invited to contribute to the study. Ethical approval for the study was obtained locally for each CREAM study group, and participants gave informed consent. The research was carried out in accordance with the tenets of the Declaration of Helsinki.

*Study sample:* The demographics of the participating study groups are shown in Table 1. The participants comprised 22,250 European individuals from 14 studies and 9,120 Asian individuals from 8 studies. There were 5,470 European participants and 947 Asian participants aged <25 years.

*Phenotype definition:* Following Fan et al. [16], cases were defined as participants with corneal astigmatism >0.75 D, and controls were defined as those with corneal astigmatism ≤0.75 D. Corneal astigmatism was averaged between the two eyes, except for participants with data available for only one eye. For the conversion of keratometry readings in millimeters to diopters, we used a conversion factor of 332 divided by the K-reading in mm [22].

*Phenotyping, genotyping, and genetic imputation:* Anterior corneal curvature was measured using keratometry (the keratometer used by each CREAM study group is listed in Appendix 1), and corneal astigmatism was calculated as the difference in curvature between the steepest and flattest meridians. Participants known to have keratoconus, corneal scarring, ocular surgery, or any corneal/ocular condition that would impair keratometry were excluded from the analysis. DNA samples were extracted from blood or saliva and genotyped on a high-density single nucleotide polymorphism (SNP) platform, as previously described [17]. Each CREAM study group imputed non-genotyped markers from an ancestry-matched reference panel from the 1000 Genomes Project [17] using IMPUTE2 [23] or Minimac [24]. Quality-control filtering was performed in accordance with standard GWAS practices [25]. In general, markers with per-study missingness <0.95, minor allele frequency (MAF) <0.05, or a Hardy–Weinberg disequilibrium p value <1×10<sup>-6</sup> were excluded, along with samples with per-study missingness <0.95, extreme heterozygosity, sex mismatch, unaccounted for relatedness, or outlying ancestry [25]. Poorly imputed markers (IMPUTE2 info ≤0.5 or Minimac Rsq ≤0.5) were also excluded.

*Genome-wide association studies and meta-analyses:* Tests of association between corneal astigmatism case/control status and SNP genotype were performed genome-wide by each participating CREAM study group. The analysis was performed using PLINK [26] for marker genotypes coded 0, 1, or 2 or using mach2dat [24] or ProbABEL [27] for marker genotypes coded as imputed dosage on the scale 0–2. Age and sex were included as a continuous and a binary covariate, respectively. The first five major principal components were also included as continuous covariates if there was evidence of population stratification from Q-Q plots or the genomic control inflation factor ( $\lambda_{GC}$ ). For samples of related individuals, the analysis method took account of genetic background by treating this as a random effect in the analysis model. Tests of association were conducted separately for participants of European ancestry and participants of Asian ancestry and for younger (age >3 and <25) and older (age ≥25) participants.

Summary statistics from the participating CREAM study groups were submitted to a central site for meta-analysis. Using the approach implemented in easyQC [28], the summary statistics were evaluated by examining quality control plots and metrics, including effect allele frequency (EAF) plots, p value versus z-score (P-Z) plots, standard error versus sample size (SE-N) plots, effect size (odds ratio) distributions, and genomic control inflation factors. Queries were resolved by discussion with study groups analysts, and, where indicated, imputation or association testing was repeated.

Meta-analyses were performed separately for the four demographic strata—younger/older, European/Asian ancestry individuals. Fixed effects, standard error-weighted meta-analysis [29] was performed initially, followed by a random effects meta-analysis [30] for highly associated markers showing excessive between-study heterogeneity of  $I^2 > 0.5$ , where  $I^2$  is a measure of heterogeneity derived from Cochran's Q statistic [31]. A p value of  $5 \times 10^{-8}$  was adopted for declaring genome-wide significant association in the GWAS meta-analyses [32]. Regional association plots were created using LocusZoom [33]. Conditional analysis was performed on GWAS meta-analysis summary statistics using GCTA-COJO [34].

*Gene-based tests and pathway analysis:* Two gene-based tests, VEGAS2 [35] and MAGMA [36], were used to explore whether specific genes were enriched with strongly associated variants in the GWAS meta-analysis of older European individuals. Attention was restricted to the older European samples because gene-based testing relies on consistent patterns of linkage disequilibrium (LD) across genes, and the sample size was larger for the European meta-analysis compared to that for the Asian cohorts. Markers within 50

kb upstream and downstream of a gene were included in the gene-based tests, with the aim of detecting variants that altered the expression level of genes. The gene-based testing using MAGMA was repeated using an extended flanking region of 200 kb upstream and downstream of genes.

VEGAS2 [35] uses a fast approximation of a permutation-based test to determine whether genes are enriched for highly associated markers and makes use of LD information from an ancestry-matched reference panel to account for association signals shared by markers in LD. The test was implemented to analyze all markers in each gene. MAGMA [36] overcomes the low statistical power inherent when a gene contains many markers, some of which may be in strong LD, by first carrying out a principal components analysis (PCA) for the markers in each gene and then carrying out a per-gene linear regression analysis using the PCA eigenvectors as predictor variables. High statistical power is attained by limiting the regression to the major eigenvalues. Permutation-based p values are calculated to account for the use of a binary outcome as the dependent variable in the linear regression analysis [36].

Gene-set “pathway analysis” was also performed using MAGMA [36]. This was performed using a competitive approach whereby the test statistics for all genes within a gene set were combined to form a joint association statistic. This statistic was compared against that for all other genes not in that set while accounting for the number of SNPs within each gene, gene density, and differential sample size (unequal sample size contributing to each gene) [36]. Gene sets were defined using the Molecular Signatures Database (MSigDB) [37]. Gene definitions and their respective association signals

for genes contributing to gene sets were taken from the MAGMA gene-based analyses with the aim of identifying potential biologic processes that may be influenced by these variants.

*Shared genetic contribution to traits:* LD score regression [38,39] was used to quantify the degree of shared genetic contribution between corneal astigmatism and two related traits, refractive astigmatism and mean spherical equivalent refractive error. GWAS summary statistics for refractive astigmatism and for spherical equivalent refractive error were obtained from previous CREAM studies [17,18]. LD score regression utilizes LD information from an ancestry-matched reference panel and requires large sample sizes; therefore, analyses were limited to European GWAS samples. Specifically, LD score regression was performed using the LDSC program [38,39] for variants present in the HapMap3 CEU reference panel with MAF  $\geq 0.05$ . The prevalence of corneal astigmatism (defined as an amount  $>0.75$  D) in the general population was taken as 42%, which was calculated as the average for the European ancestry population-based studies contributing to this meta-analysis. LD score regression remains valid when two traits are measured in overlapping samples [39], which was the case for these CREAM GWAS samples.

## RESULTS

*Meta-analysis of genome-wide association studies:* Meta-analyses were performed using a fixed effects model for approximately six million genetic variants (approximately 5,500,000 SNPs and 380,000 indels) in each of the four ancestry/age strata (younger European, older European,

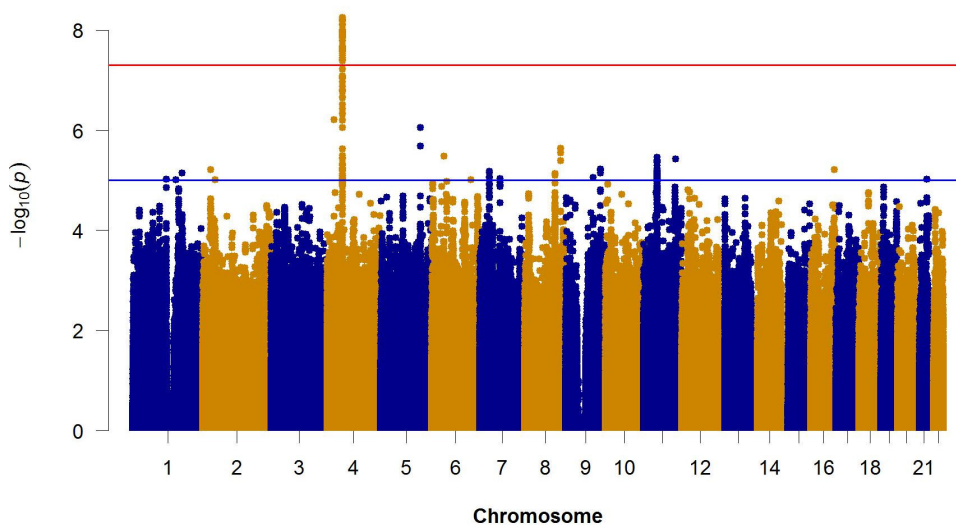


Figure 1. Manhattan plot showing most strongly associated markers in the GWAS fixed-effects meta-analysis for European and Asian participants of all ages combined ( $n=31,375$ ). Red line:  $p=5 \times 10^{-8}$ , blue line:  $p=1 \times 10^{-5}$ .

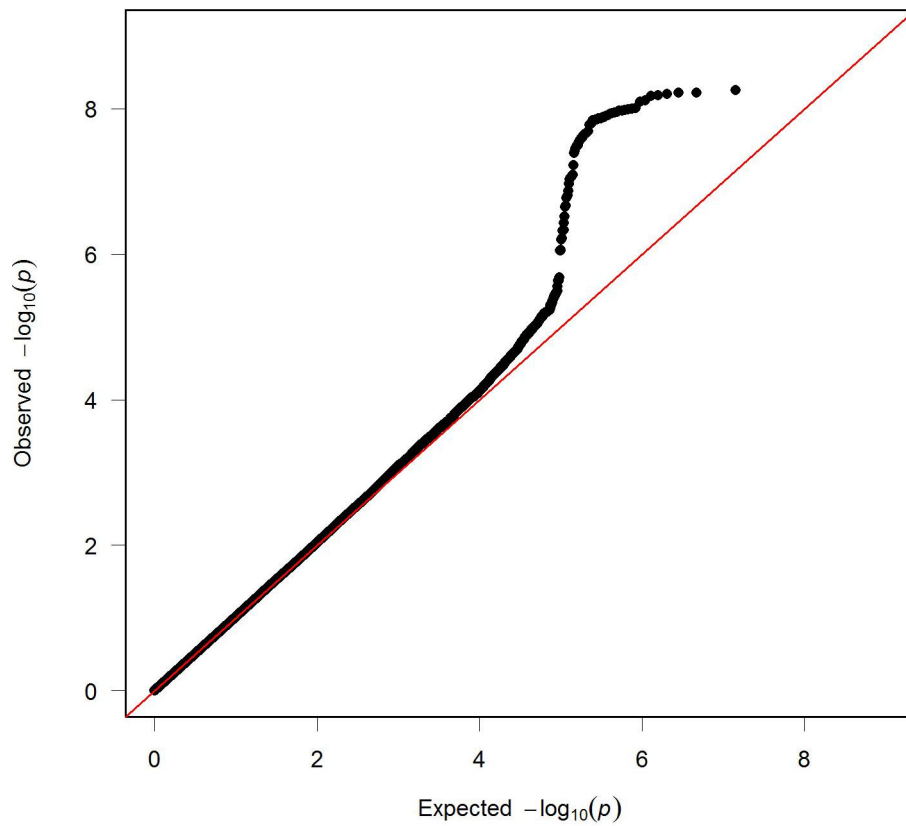


Figure 2. Q-Q plot for the GWAS fixed-effects meta-analysis for European and Asian participants of all ages combined (n=31,375).

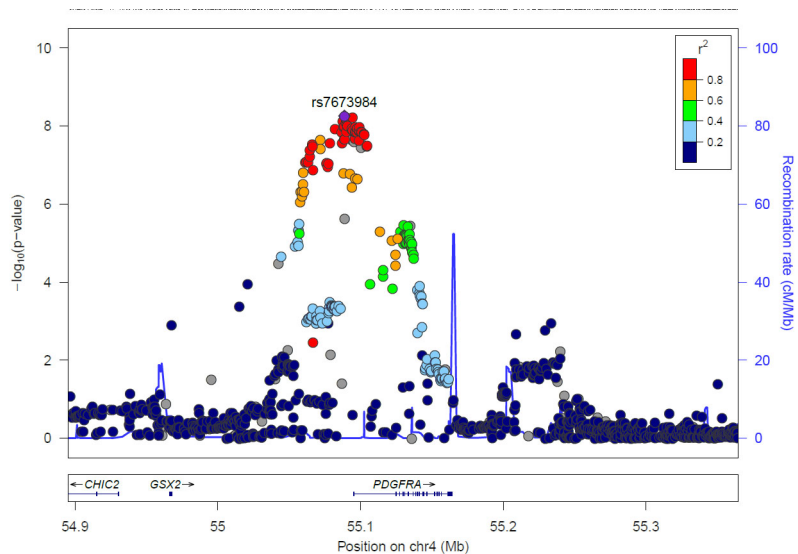


Figure 3. Region plot for the most strongly associated region in the GWAS fixed-effects meta-analysis for European and Asian participants of all ages combined (n=31,375).

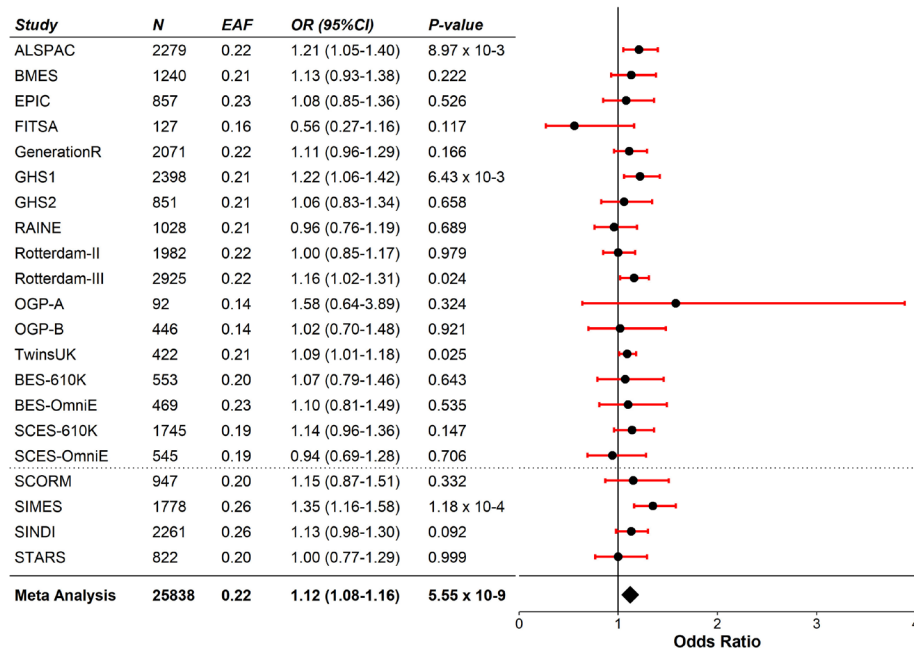


Figure 4. Forest plot and summary table for lead variant rs7673984 across all cohorts. Studies listed above the dotted line are new cohorts not included in the only prior GWAS for corneal astigmatism [16]. EAF=effect allele frequency. (Note that rs7673984 was excluded from the Rotterdam-I cohort analysis during quality control filtering).

**TABLE 2. MOST STRONGLY ASSOCIATED MARKER IN EACH REGION IN THE GWAS META-ANALYSIS OF ALL SAMPLES (EUROPEANS AND ASIANS OF ALL AGES COMBINED).**

SNP	Chr	Pos	Effect allele	Other allele	EAF	OR (95%CI)	P value	Nearest gene
rs7673984	4	55,088,761	T	C	0.22	1.12 (1.08–1.16)	5.55×10 <sup>-9</sup>	<i>PDGFRA</i>
rs34751092	4	24,129,037	A	G	0.28	1.09 (1.05–1.13)	6.07×10 <sup>-7</sup>	<i>PPARGCIA</i>
rs630203	5	141,444,269	T	G	0.74	0.92 (0.88–0.95)	8.83×10 <sup>-7</sup>	<i>MRPL11P2</i>
rs75607298	8	128,611,496	A	G	0.72	1.14 (1.08–1.21)	2.28×10 <sup>-6</sup>	<i>CASC11</i>
rs62401199	6	43,813,341	T	C	0.14	1.15 (1.09–1.22)	3.29×10 <sup>-6</sup>	<i>LINC01512</i>
rs753992	11	47,349,846	A	G	0.29	0.91 (0.87–0.95)	3.48×10 <sup>-6</sup>	<i>MADD</i>
rs3214101	11	114,009,408	A	T	0.68	1.08 (1.05–1.12)	3.75×10 <sup>-6</sup>	<i>ZBTB16</i>
rs10985068	9	123,629,724	C	G	0.12	1.13 (1.07–1.19)	5.87×10 <sup>-6</sup>	<i>PHF19</i>
rs62128379	2	26,960,055	T	C	0.85	1.13 (1.07–1.19)	6.00×10 <sup>-6</sup>	<i>KCNK3</i>
rs9939114	16	84,023,972	A	G	0.05	0.54 (0.41–0.70)	6.01×10 <sup>-6</sup>	<i>NECAB2</i>
rs60083876	7	34,228,819	A	T	0.95	1.31 (1.17–1.48)	6.53×10 <sup>-6</sup>	<i>BMPER</i>
rs859362	1	175,495,090	T	C	0.19	1.10 (1.05–1.15)	7.14×10 <sup>-6</sup>	<i>TNR</i>
rs11775037	8	108,317,615	A	G	0.20	1.10 (1.05–1.14)	7.31×10 <sup>-6</sup>	<i>ANGPT1</i>
rs7036824	9	96,149,894	T	C	0.94	0.83 (0.77–0.90)	8.78×10 <sup>-6</sup>	<i>C9orf129</i>
rs142168171	7	71,253,651	I	R	0.09	1.37 (1.19–1.57)	9.14×10 <sup>-6</sup>	<i>CALN1</i>
rs7278671	21	41,047,876	A	G	0.51	0.93 (0.90–0.96)	9.31×10 <sup>-6</sup>	<i>B3GALT5</i>
rs191640722	1	119,264,997	C	G	0.09	0.87 (0.82–0.93)	9.53×10 <sup>-6</sup>	<i>LOC100421281</i>
rs36107906	2	44,162,800	D	R	0.29	1.09 (1.05–1.13)	9.61×10 <sup>-6</sup>	<i>LRPPRC</i>
rs4896367	6	138,807,281	T	C	0.72	1.09 (1.05–1.14)	9.75×10 <sup>-6</sup>	<i>NHSL1</i>
rs35587414	1	153,174,958	T	C	0.15	1.13 (1.07–1.20)	9.79×10 <sup>-6</sup>	<i>LELPI</i>

EAF=effect allele frequency, OR=odds ratio.



TABLE 3. SNP-HERITABILITY ESTIMATED USING LD SCORE REGRESSION (EUROPEANS ONLY).

Trait	No. of Markers	SNP-heritability (SE)	P value
Corneal Astigmatism	1,024,525	0.0555 (0.0381)	0.15
Refractive Astigmatism	1,056,658	0.0136 (0.0218)	0.53
Mean Spherical Equivalent	1,056,658	0.2326 (0.0175)	2.60×10 <sup>-40</sup>

younger Asian, and older Asian). However, none of the markers had a p value below the pre-determined threshold of  $5 \times 10^{-8}$  used to declare genome-wide significance (Appendix 2, Appendix 3, Appendix 4, Appendix 5, Appendix 6, and Appendix 7). Therefore, to increase power, a meta-analysis was performed using data for all four ancestry/age strata, under the assumption that the genetic determinants of corneal astigmatism are consistent across ancestry groups and lifespan. This yielded 49 markers with p values  $< 5 \times 10^{-8}$ , all of which were located in a narrow interval on chromosome 4 close to the *PDGFRA* gene (Figure 1, Figure 2, and Figure 3). This locus has previously been identified in GWAS analyses for corneal astigmatism [16], refractive astigmatism [17], and corneal curvature [15,40,41]. Table 2 lists the most strongly associated marker in each region showing suggestive association, defined as a region with at least one marker with  $p < 1 \times 10^{-5}$ . Both the European and Asian meta-analyses contributed to the association signal at the *PDGFRA* locus; the most strongly associated marker, rs7673984, had an effect size (odds ratio) of OR=1.15 (95% CI:1.07–1.24;  $p=1.76 \times 10^{-4}$ ) in Asians, OR=1.11 (95% CI:1.06–1.16;  $p=5.64 \times 10^{-6}$ ) in Europeans, and OR=1.12 (95% CI:1.08–1.16;  $p=5.55 \times 10^{-9}$ ) in the meta-analysis of Asians and Europeans. The association of rs7673984 in the individual cohorts examined is summarized in Figure 4. Conditional analysis using GCTA-COJO yielded no additional association signals at the *PDGFRA* locus independent of rs7673984.

**Gene-based analyses:** To explore whether specific genes were enriched for markers with low p values in the GWAS meta-analysis, we performed gene-based tests using VEGAS2 [35] and MAGMA [36]. These programs use different approaches to test for such enrichment (see *Methods*). Due to the requirement for an ancestry-matched reference panel, analyses were

conducted using the results of a GWAS meta-analysis of European samples of all ages (however, similar results were obtained when attention was restricted to the meta-analysis of older Europeans). The 10 most strongly associated genes from the VEGAS2 and MAGMA analyses are shown in Appendix 8 and Appendix 9. There was a high degree of overlap between the results of the two programs, with the genes *ACP2*, *CLDN7*, *ELP5*, and *CTDNEP1* showing the strongest association in both analyses (Appendix 8 and Appendix 9). In the MAGMA gene-based test, these four genes and *TNFAIP8L3* achieved  $p < 0.05$  after stringent Bonferroni correction, whereas this was not the case for VEGAS (Appendix 8 and Appendix 9). A further exploratory gene-based analysis that included markers up to 200 kb upstream or downstream of each gene—an approach that has been successful for certain traits [42]—failed to identify any additional genes associated with corneal astigmatism.

**Pathway analysis:** As biologic processes tend to involve multiple genes, a gene-set analysis was performed with MAGMA [36] using the gene-based analysis results for the European samples. Gene-set analyses seek to identify potential biologic mechanisms enriched for genes with markers attaining low p values in the GWAS meta-analysis. However, no gene sets were identified as demonstrating a greater level of association with corneal astigmatism than would be expected by chance (when flanking regions of either  $\pm 50$  kb or  $\pm 200$  kb upstream or downstream of genes were tested).

**SNP heritability and genetic correlation between traits:** LD score regression was used to quantify the heritability explained by commonly occurring genetic variants (“SNP heritability”) and the degree of genetic sharing between corneal astigmatism and two related traits, refractive

TABLE 4. GENETIC CORRELATIONS BETWEEN PAIRS OF REFRACTIVE ERROR TRAITS IN SAMPLES OF EUROPEAN ANCESTRY FROM THE CREAM CONSORTIUM (USING LD SCORE REGRESSION).

Trait Pairs	No. of Markers	Genetic Correlation (SE)	P value
RA and CA	934,512	0.2327 (0.703)	0.7406
MSE and CA	1,024,525	-0.0238 (0.1599)	0.8815
RA and MSE	1,056,658	0.7732 (0.6504)	0.2345

RA=refractive astigmatism, CA=corneal astigmatism, MSE=mean spherical equivalent. P values refer to likelihood of non-zero correlation between traits.

astigmatism and spherical equivalent refractive error (Table 3 and Table 4). The SNP heritability ( $h^2$ ) estimates for corneal and refractive astigmatism (~5% and ~1%, respectively) were lower than for the spherical equivalent (~23%); indeed, the SNP heritability estimates for corneal and refractive astigmatism were not significantly different from zero. The genetic correlation estimates also had high standard errors and therefore yielded very imprecise estimates (Table 4). These hinted at a high genetic correlation between corneal astigmatism and the spherical equivalent; however, in view of the low SNP heritability estimate for the astigmatism traits, these findings imply that much larger sample sizes and/or a more homogeneous population sample is needed to obtain robust findings.

## DISCUSSION

This GWAS for corneal astigmatism in a combined sample of Europeans and Asians identified a single genome-wide significant locus in the promoter region of the *PDGFRA* gene, replicating the previous discovery of this corneal astigmatism locus by Fan et al. [16] in a predominantly Asian sample. Therefore, despite a fourfold increase in sample size ( $n=31,370$  versus  $n=7,719$ ) compared to the only previous GWAS meta-analysis for corneal astigmatism [16], the standard, single-marker GWAS analysis performed here did not identify any new loci. GWAS analyses for spherical equivalent and other morphological traits in equivalently sized samples have identified dozens of independent risk loci [18,19]. This paucity of GWAS loci for corneal astigmatism mirrors that observed in a previous large-scale GWAS for refractive astigmatism [17]. Our LD score regression-based SNP heritability estimates for corneal astigmatism ( $h^2 \sim 5\%$ ) and refractive astigmatism ( $h^2 \sim 1\%$ )—the first ever estimates for these traits—were also much lower than those for the spherical equivalent ( $h^2 \sim 23\%$ ), suggesting that common, additively acting SNPs make a relatively minor contribution to the development of astigmatism. In the study by Fan et al. [16] that originally identified the association between SNPs close to the *PDGFRA* gene and corneal astigmatism, the authors speculated that the underlying causal mechanism was common to populations of diverse ancestry and not specifically to those of Asian origin. This was based on the knowledge that their GWAS included individuals of Indian ancestry, who are more closely genetically related to Europeans than East Asians [16]. Our findings support this theory.

The association between *PDGFRA* SNPs and corneal astigmatism has been replicated in a previous study of Europeans ( $n=1968$ ) but not in another smaller study ( $n=1013$ ) [41,43]. Variants at this locus were not associated with

refractive astigmatism in GWAS meta-analyses of  $n=45,287$  participants [17] yet were associated with corneal curvature in an Asian sample [15] and with both corneal curvature and axial length (but not refractive error) in a European sample [41]. This complex series of findings suggests a role for *PDGFRA* in the regulation of eye size and corneal astigmatism; however, the underlying mechanism of action remains uncertain.

In contrast to the single-marker analyses, gene-based analysis did provide new insight into the genetic basis of corneal astigmatism, implicating the genes *ACP2*, *CLDN7*, *CTDNEP1*, *ELP5*, and *TNFAIP8L3*. Three of these five genes—*CLDN7*, *CTDNEP1*, and *ELP5*—are tightly clustered on chromosome 11, with their respective (gene-based) association signals sharing many variants in common. Therefore, a parsimonious interpretation is that only one of the genes has a causal association with astigmatism and that the other two genes are false-positive associations detected due to the signal from the causal gene. Of the three genes, *CLDN7*, which encodes the claudin-7 membrane protein [44], appears to be the most biologically plausible candidate. Claudins are responsible for tight junction formation and function [45], with claudin-7 being the subtype present in human corneal epithelium and endothelium [46]. Currently, how claudin-7 may contribute to the development of corneal astigmatism is unclear. The acid phosphatase 2, lysosomal gene (*ACP2*) is located on chromosome 17 and codes for the beta subunit of the degradative enzyme, lysosomal acid phosphatase (LAP). Interestingly, LAP activity is enhanced in keratoconic corneas [47,48]. The *TNFAIP8L3* gene located on chromosome 15 codes for TNF alpha-induced protein 8 like 3, which is preferentially expressed in secretory epithelial cells [49]. *TNFAIP8L3* is implicated as a negative regulator of inflammation (and carcinogenesis) through its role in TNF $\alpha$  and phospholipid signaling. Based on this evidence, the *CLDN7*, *ACP2*, and *TNFAIP8L3* genes are promising susceptibility genes for corneal astigmatism. It is important to note that while the statistical support for the above three genes was much stronger in the MAGMA analysis than in the VEGAS2 analysis, the two software programs similarly ranked the most strongly associated genes. This commonality between the MAGMA and VEGAS2 results provides greater confidence that the findings are robust than would be the case for findings identified using either software program alone, as the statistical models and hypothesis tests used by the two programs differ, especially regarding the adjustment for variants in LD.

The strengths of this investigation are that data from multiple population samples were combined and

meta-analyzed and that gene-based and pathway-based follow-up analyses were undertaken to leverage new biologic insights into the genetics of astigmatism. The weaknesses were that although the samples included both European and Asian ancestry individuals, trans-ethnic meta-analysis [50] was not performed due to the small size of the Asian sample compared to the European sample and that the age spectrum of the participants was very broad. The latter point is an important consideration because astigmatism does not remain constant during life, with changes in both magnitude and orientation occurring with age [1]. For example, in childhood, astigmatism tends to be WTR, whereas in older adults this orientation typically changes to ATR. Our study design sought to overcome some of this variation by considering only the magnitude of corneal astigmatism (i.e., no consideration of astigmatism axis) and by using a case-control classification scheme, with the aim of reducing the impact of the subtle changes in astigmatism that commonly occur with age.

In conclusion, this GWAS meta-analysis for corneal astigmatism replicated the discovery of a genome-wide significant locus near the *PDGFRA* gene [16] and provided strong evidence that this locus is important in both Asians and Europeans (Figure 4). Three novel candidate genes, *CLDN7*, *ACP2*, and *TNFAIP8L3*, were identified using gene-based analyses that leveraged data from across genomic regions rather than from examining one genetic marker at a time. These novel genes warrant further investigation to understand their role in the pathogenesis of corneal astigmatism. Finally, exploiting the recently introduced LD score regression technique, we estimated the SNP heritability of corneal astigmatism (and refractive astigmatism) to be much lower than that for spherical equivalent refractive error (Table 3) [51]. This implies that astigmatism must be under greater influence of rare genetic variants or environmental risk factors than spherical equivalent or that the common genetic variants that contribute to astigmatism have non-additive effects.

#### **APPENDIX 1. INSTRUMENT FOR MEASURING CORNEAL CURVATURE.**

To access the data, click or select the words “[Appendix 1](#)”

#### **APPENDIX 2. MANHATTAN PLOTS FOR THE SEPARATE ANCESTRY/AGE STRATA FIXED EFFECTS META-ANALYSES.**

Manhattan plots for the separate ancestry/age strata fixed effects meta-analyses. Y-axes show negative  $\log_{10}$  p-values and X-axes show genomic position. Red line corresponds to  $P = 5 \times 10^{-8}$ , blue line corresponds to  $P = 1 \times 10^{-5}$ . Panel A, European ancestry, aged >25 years; B, European ancestry,

aged <25 years; C, Asian ancestry, aged >25 years; D, Asian ancestry, aged <25 years. To access the data, click or select the words “[Appendix 2](#)”

#### **APPENDIX 3. QUANTILE-QUANTILE PLOTS FOR THE SEPARATE ANCESTRY/AGE STRATA FIXED EFFECTS META-ANALYSES.**

Y-axes show observed negative  $\log_{10}$  p-values and X-axes show expected negative  $\log_{10}$  p-values according to the null hypothesis of no genetic association. Red line is the line of unity ( $y = x$ ). Panel A, European ancestry, aged >25 years; B, European ancestry, aged <25 years; C, Asian ancestry, aged >25 years; D, Asian ancestry, aged <25 years. To access the data, click or select the words “[Appendix 3](#)”

#### **APPENDIX 4. MOST STRONGLY ASSOCIATED MARKER IN EACH REGION IN THE GWAS META-ANALYSIS OF ALL EUROPEANS AGED >25 YEARS.**

To access the data, click or select the words “[Appendix 4](#)”

#### **APPENDIX 5. MOST STRONGLY ASSOCIATED MARKER IN EACH REGION IN THE GWAS META-ANALYSIS OF ALL EUROPEANS AGED <25 YEARS.**

To access the data, click or select the words “[Appendix 5](#)”

#### **APPENDIX 6. MOST STRONGLY ASSOCIATED MARKER IN EACH REGION IN THE GWAS META-ANALYSIS OF ALL ASIANS AGED >25 YEARS.**

To access the data, click or select the words “[Appendix 6](#)”

#### **APPENDIX 7. MOST STRONGLY ASSOCIATED MARKER IN EACH REGION IN THE GWAS META-ANALYSIS OF ALL ASIANS AGED <25 YEARS.**

To access the data, click or select the words “[Appendix 7](#)”

#### **APPENDIX 8. TOP 10 GENES FROM VEGAS2 GENE-BASED ASSOCIATION TEST WITH $\pm 50$ KB BUFFERS FOR ALL EUROPEANS.**

Start and stop positions listed include  $\pm 50$ kb buffers. nSNPs: number of variants included in gene region. Test Statistic: gene-based  $\chi^2$  test statistic to nSNPs degrees of freedom. P-value: obtained from Test Statistic and adjusting for LD between variants. FDR: false discovery rate (likelihood of gene association being a false positive result). Top SNP: variant within gene locus with strongest association signal from previous SNP-based association test. Genes shown in bold were also identified with MAGMA (Appendix 9). To access the data, click or select the words “[Appendix 8](#)”

## APPENDIX 9. TOP 10 GENES FROM MAGMA GENE-BASED ASSOCIATION TEST WITH $\pm 50$ KB BUFFERS FOR ALL EUROPEANS.

Start and stop positions listed include  $\pm 50$ kb buffers. nSNPs: number of variants included in gene region. Z Statistic: gene-based test statistic. P-value: obtained from *Z Statistic* under the assumption of a normally distributed model. FDR: false discovery rate (likelihood of gene association being a false positive result). Genes shown in bold were also identified with VEGAS2 (Appendix 8). To access the data, click or select the words “Appendix 9”

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**ALSPAC.** The Avon Longitudinal Study of Parents and Children (ALSPAC) team and authors are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses. The UK Medical Research Council and Wellcome Trust (Grant ref: 102215/2/13/2) and the University of Bristol provide core support for ALSPAC. GWAS data was generated by Sample Logistics and Genotyping Facilities at Wellcome Sanger Institute and LabCorp (Laboratory Corporation of America) using support from 23andMe. This publication is the work of the authors and JAG and CW will serve as guarantors for the contents of this paper. This research was specifically funded by NIHR Senior Research Fellowship SRF-2015–08–005 (CW) and a Wellcome Trust ISSF Populations Pilot Award (grant 508353/509506). Ethical approval for the ALSPAC study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees. Please note that the ALSPAC study website contains details of all the data that is available through a fully searchable data dictionary: <http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/>. ALSPAC children with available genotype data and corneal curvature phenotype information formed the GWAS sample (Table 1). A description of the ALSPAC study cohort is available [52]. **BES.** The Beijing Eye Study (BES) was supported by National Natural Science Foundation of China (grant # 81770890). This publication is the work of the authors and YXW and JBJ will serve as guarantors for the contents of this paper. The study was approved by the Medical Ethics Committee of the Beijing Tongren Hospital. A description of the BES study cohort is available [53]. **BMES.** The Blue Mountains Eye Study (BMES) acknowledge funding from the National Health and Medical

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## REFERENCES

1. Read SA, Collins MJ, Carney LG. A review of astigmatism and its possible genesis. *Clin Exp Optom* 2007; 90:5-19. .
2. Kee CS. Astigmatism and its role in emmetropization. *Exp Eye Res* 2013; 114:89-95. .
3. Mozayan E, Lee JK. Update on astigmatism management. *Curr Opin Ophthalmol* 2014; 25:286-90. .
4. Somer D, Budak K, Demirci S, Duman S. Against-the-rule (ATR) astigmatism as a predicting factor for the outcome of amblyopia treatment. *Am J Ophthalmol* 2002; 133:741-5. .
5. Dunne MC, Elawad ME, Barnes DA. A study of the axis of orientation of residual astigmatism. *Acta Ophthalmol (Copenh)* 1994; 72:483-9. .
6. Keller PR, Collins MJ, Carney LG, Davis BA, vanSaarloos PP. The relation between corneal and total astigmatism. *Optom Vis Sci* 1996; 73:86-91. .
7. Chu CH, Kee CS. Effects of optically imposed astigmatism on early eye growth in chicks. *PLoS One* 2015; 10:e0117729-.
8. Gwiazda J, Scheiman M, Mohindra I, Held R. Astigmatism in children: changes in axis and amount from birth to six years. *Invest Ophthalmol Vis Sci* 1984; 25:88-92. .
9. Harvey EM, Dobson V, Miller JM, Schwiegerling J, Clifford-Donaldson CE, Green TK, Messer DH. Prevalence of corneal astigmatism in Tohono O'odham Native American children 6 months to 8 years of age. *Invest Ophthalmol Vis Sci* 2011; 52:4350-5. .
10. Huynh SC, Kifley A, Rose KA, Morgan IG, Mitchell P. Astigmatism in 12-year-old Australian children: comparisons with a 6-year-old population. *Invest Ophthalmol Vis Sci* 2007; 48:73-82. .
11. Sanfilippo PG, Hewitt AW, Hammond CJ, Mackey DA. The heritability of ocular traits. *Surv Ophthalmol* 2010; 55:561-83. .
12. Parssinen O, Kauppinen M, Kaprio J, Koskenvuo M, Rantanen T. Heritability of refractive astigmatism: a population-based twin study among 63- to 75-year-old female twins. *Invest Ophthalmol Vis Sci* 2013; 54:6063-7. .
13. Clementi M, Angi M, Forabosco P, Di Gianantonio E, Tenconi R. Inheritance of astigmatism: evidence for a major autosomal dominant locus. *Am J Hum Genet* 1998; 63:825-30. .
14. Lopes MC, Hysi PG, Verhoeven VJ, Macgregor S, Hewitt AW, Montgomery GW, Cumberland P, Vingerling JR, Young TL, van Duijn CM, Oostra B, Uitterlinden AG, Rahi JS, Mackey DA, Klaver CC, Andrew T, Hammond CJ. Identification of a candidate gene for astigmatism. *Invest Ophthalmol Vis Sci* 2013; 54:1260-7. .
15. Han S, Chen P, Fan Q, Khor CC, Sim X, Tay WT, Ong RT, Suo C, Goh LK, Lavanya R, Zheng Y, Wu R, Seielstad M, Vithana E, Liu J, Chia KS, Lee JJ, Tai ES, Wong TY, Aung T, Teo YY, Saw SM. Association of variants in FRAP1 and PDGFRA with corneal curvature in Asian populations from Singapore. *Hum Mol Genet* 2011; 20:3693-8. .

16. Fan Q, Zhou X, Khor CC, Cheng CY, Goh LK, Sim X, Tay WT, Li YJ, Ong RT, Suo C, Cornes B, Ikram MK, Chia KS, Seielstad M, Liu J, Vithana E, Young TL, Tai ES, Wong TY, Aung T, Teo YY, Saw SM. Genome-wide meta-analysis of five Asian cohorts identifies PDGFRA as a susceptibility locus for corneal astigmatism. *PLoS Genet* 2011; 7:e1002402-.
17. Li Q, Wojciechowski R, Simpson CL, Hysi PG, Verhoeven VJ, Ikram MK, Hohn R, Vitart V, Hewitt AW, Oexle K, Makela KM, MacGregor S, Pirastu M, Fan Q, Cheng CY, St Pourcain B, McMahon G, Kemp JP, Northstone K, Rahi JS, Cumberland PM, Martin NG, Sanfilippo PG, Lu Y, Wang YX, Hayward C, Polasek O, Campbell H, Bencic G, Wright AF, Wedenoja J, Zeller T, Schillert A, Mirshahi A, Lackner K, Yip SP, Yap MK, Ried JS, Gieger C, Murgia F, Wilson JF, Fleck B, Yazar S, Vingerling JR, Hofman A, Uitterlinden A, Rivadeneira F, Amin N, Karssen L, Oostra BA, Zhou X, Teo YY, Tai ES, Vithana E, Barathi V, Zheng Y, Siantar RG, Neelam K, Shin Y, Lam J, Yonova-Doing E, Venturini C, Hosseini SM, Wong HS, Lehtimäki T, Kahonen M, Raitakari O, Timpson NJ, Evans DM, Khor CC, Aung T, Young TL, Mitchell P, Klein B, van Duijn CM, Meitinger T, Jonas JB, Baird PN, Mackey DA, Wong TY, Saw SM, Parssinen O, Stambolian D, Hammond CJ, Klaver CC, Williams C, Paterson AD, Bailey-Wilson JE, Guggenheim JA. Cream Consortium. Genome-wide association study for refractive astigmatism reveals genetic co-determination with spherical equivalent refractive error: the CREAM consortium. *Hum Genet* 2015; 134:131-46. .
18. Verhoeven VJ, Hysi PG, Wojciechowski R, Fan Q, Guggenheim JA, Hohn R, MacGregor S, Hewitt AW, Nag A, Cheng CY, Yonova-Doing E, Zhou X, Ikram MK, Buitendijk GH, McMahon G, Kemp JP, Pourcain BS, Simpson CL, Makela KM, Lehtimäki T, Kahonen M, Paterson AD, Hosseini SM, Wong HS, Xu L, Jonas JB, Parssinen O, Wedenoja J, Yip SP, Ho DW, Pang CP, Chen LJ, Burdon KP, Craig JE, Klein BE, Klein R, Haller T, Metspalu A, Khor CC, Tai ES, Aung T, Vithana E, Tay WT, Barathi VA. Consortium for Refractive Error and Myopia, Chen P, Li R, Liao J, Zheng Y, Ong RT, Doring A, Diabetes Control Complications Trial/Epidemiology of Diabetes Interventions Complications Research Group, Evans DM, Timpson NJ, Verkerk AJ, Meitinger T, Raitakari O, Hawthorne F, Spector TD, Karssen LC, Pirastu M, Murgia F, Ang W, Wellcome Trust Case Control Consortium, Mishra A, Montgomery GW, Pennell CE, Cumberland PM, Cotlarciuc I, Mitchell P, Wang JJ, Schache M, Janmahasatian S, Igo RP, Jr., Lass JH, Chew E, Iyengar SK, Fuchs' Genetics Multi-Center Study Group, Gorgels TG, Rudan I, Hayward C, Wright AF, Polasek O, Vataavuk Z, Wilson JF, Fleck B, Zeller T, Mirshahi A, Muller C, Uitterlinden AG, Rivadeneira F, Vingerling JR, Hofman A, Oostra BA, Amin N, Bergen AA, Teo YY, Rahi JS, Vitart V, Williams C, Baird PN, Wong TY, Oexle K, Pfeiffer N, Mackey DA, Young TL, van Duijn CM, Saw SM, Bailey-Wilson JE, Stambolian D, Klaver CC, Hammond CJ. Genome-wide meta-analyses of multiethnicity cohorts identify multiple new susceptibility loci for refractive error and myopia. *Nat Genet* 2013; 45:314-8. .
19. Kiefer AK, Tung JY, Do CB, Hinds DA, Mountain JL, Francke U, Eriksson N. Genome-wide analysis points to roles for extracellular matrix remodeling, the visual cycle, and neuronal development in myopia. *PLoS Genet* 2013; 9:e1003299-.
20. Tkatchenko AV, Tkatchenko TV, Guggenheim JA, Verhoeven VJ, Hysi PG, Wojciechowski R, Singh PK, Kumar A, Thinakaran G. Consortium for Refractive Error and Myopia, Williams C. APLP2 Regulates Refractive Error and Myopia Development in Mice and Humans. *PLoS Genet* 2015; 11:e1005432-.
21. Pickrell JK, Berisa T, Liu JZ, Segurel L, Tung JY, Hinds DA. Detection and interpretation of shared genetic influences on 42 human traits. *Nat Genet* 2016; 48:709-17. .
22. Bennett AG, Rabbetts RB. *Clinical Visual Optics*. Second Edition ed. London: Butterworths; 1989.
23. Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet* 2009; 5:e1000529-.
24. Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR. MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. *Genet Epidemiol* 2010; 34:816-34. .
25. Fan Q, Verhoeven VJM, Wojciechowski R, Barathi VA, Hysi PG, Guggenheim JA, Hohn R, Vitart V, Khawaja AP, Yamashiro K, Hosseini SM, Lehtimäki T, Lu Y, Haller T, Xie J, Delcourt C, Pirastu M, Wedenoja J, Gharahkhani P, Venturini C, Miyake M, Hewitt AW, Guo X, Mazur J, Huffman JE, Williams KM, Polasek O, Campbell H, Rudan I, Vataavuk Z, Wilson JF, Joshi PK, McMahon G, St Pourcain B, Evans DM, Simpson CL, Schwantes-An T-H, Igo RP, Mirshahi A, Cougnard-Gregoire A, Bellenguez C, Blettner M, Raitakari O, Kahonen M, Seppala I, Zeller T, Meitinger T. Consortium for Refractive E, Myopia, Ried JS, Gieger C, Portas L, van Leeuwen EM, Amin N, Uitterlinden AG, Rivadeneira F, Hofman A, Vingerling JR, Wang YX, Wang X, Tai-Hui Boh E, Ikram MK, Sabanayagam C, Gupta P, Tan V, Zhou L, Ho CEH, Lim We, Beuerman RW, Siantar R, Tai ES, Vithana E, Mihailov E, Khor C-C, Hayward C, Luben RN, Foster PJ, Klein BEK, Klein R, Wong H-S, Mitchell P, Metspalu A, Aung T, Young TL, He M, Parssinen O, van Duijn CM, Jin Wang J, Williams C, Jonas JB, Teo Y-Y, Mackey DA, Oexle K, Yoshimura N, Paterson AD, Pfeiffer N, Wong T-Y, Baird PN, Stambolian D, Wilson JEB, Cheng C-Y, Hammond CJ, Klaver CCW, Saw S-M, Rahi JS, Korobelnik J-F, Kemp JP, Timpson NJ, Smith GD, Craig JE, Burdon KP, Fogarty RD, Iyengar SK, Chew E, Janmahasatian S, Martin NG, MacGregor S, Xu L, Schache M, Nangia V, Panda-Jonas S, Wright AF, Fondran JR, Lass JH, Feng S, Zhao JH, Khaw K-T, Wareham NJ, Rantanen T, Kaprio J, Pang CP, Chen LJ, Tam PO, Jhanji V, Young AL, Doring A, Raffel LJ, Cotch M-F, Li X, Yip SP, Yap MKH, Biino G, Vaccargiu S, Fossarello M, Fleck B, Yazar S, Tideman JW, Tedja M, Deangelis MM, Morrison M, Farrer L, Zhou X, Chen W, Mizuki N, Meguro A, Makela KM. Meta-analysis of gene-environment-wide association scans accounting for

- education level identifies additional loci for refractive error. *Nat Commun* 2016; 7:11008-.
26. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007; 81:559-75. .
  27. Aulchenko YS, Struchalin MV, van Duijn CM. ProbABEL package for genome-wide association analysis of imputed data. *BMC Bioinformatics* 2010; 11:134-.
  28. Winkler TW, Day FR, Croteau-Chonka DC, Wood AR, Locke AE, Mägi R, Ferreira T, Fall T, Graff M, Justice AE, Luan Ja, Gustafsson S, Randall JC, Vedantam S, Workalemahu T, Kilpeläinen TO, Scherag A, Esko T, Kutalik Z, Heid IM, Loos RJJ, The Genetic Investigation of Anthropometric Traits C. Quality control and conduct of genome-wide association meta-analyses. *Nat Protoc* 2014; 9:1192-212. .
  29. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics* 2010; 26:2190-1. .
  30. Magi R, Morris AP. GWAMA: software for genome-wide association meta-analysis. *BMC Bioinformatics* 2010; 11:288-.
  31. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003; 327:557-60. .
  32. Sham PC, Purcell SM. Statistical power and significance testing in large-scale genetic studies. *Nat Rev Genet* 2014; 15:335-46. .
  33. Pruim RJ, Welch RP, Sanna S, Teslovich TM, Chines PS, Gliedt TP, Boehnke M, Abecasis GR, Willer CJ. LocusZoom: regional visualization of genome-wide association scan results. *Bioinformatics* 2010; 26:2336-7. .
  34. Yang J, Ferreira T, Morris AP, Medland SE, Madden PAF, Heath AC, Martin NG, Montgomery GW, Weedon MN, Loos RJ, Frayling TM, McCarthy MI, Hirschhorn JN, Goddard ME, Visscher PM, Genetic Invest AT. Meta-A DIGR. Conditional and joint multiple-SNP analysis of GWAS summary statistics identifies additional variants influencing complex traits. *Nat Genet* 2012; 44:369-U170. .
  35. Mishra A, Macgregor S. VEGAS2: Software for More Flexible Gene-Based Testing. *Twin Res Hum Genet* 2015; 18:86-91. .
  36. de Leeuw CA, Mooij JM, Heskes T, Posthuma D. MAGMA: generalized gene-set analysis of GWAS data. *PLOS Comput Biol* 2015; 11:e1004219-.
  37. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci USA* 2005; 102:15545-50. .
  38. Bulik-Sullivan BK, Loh P-R, Finucane HK, Ripke S, Yang J. Schizophrenia Working Group of the Psychiatric Genomics C, Patterson N, Daly MJ, Price AL, Neale BM. LD Score regression distinguishes confounding from polygenicity in genome-wide association studies. *Nat Genet* 2015; 47:291-5. .
  39. Bulik-Sullivan B, Finucane HK, Anttila V, Gusev A, Day FR, Loh P-R. ReproGen C, Psychiatric Genomics C, Genetic Consortium for Anorexia Nervosa of the Wellcome Trust Case Control C, Duncan L, Perry JRB, Patterson N, Robinson EB, Daly MJ, Price AL, Neale BM. An atlas of genetic correlations across human diseases and traits. *Nat Genet* 2015; 47:1236-41. .
  40. Mishra A, Yazar S, Hewitt AW, Mountain JA, Ang W, Pennell CE, Martin NG, Montgomery GW, Hammond CJ, Young TL, Macgregor S, Mackey DA. Genetic variants near PDGFRA are associated with corneal curvature in Australians. *Invest Ophthalmol Vis Sci* 2012; 53:7131-6. .
  41. Guggenheim JA, McMahon G, Kemp JP, Akhtar S, St Pourcain B, Northstone K, Ring SM, Evans DM, Smith GD, Timpson NJ, Williams C. A genome-wide association study for corneal curvature identifies the platelet-derived growth factor receptor alpha gene as a quantitative trait locus for eye size in white Europeans. *Mol Vis* 2013; 19:243-53. .
  42. Brodie A, Azaria JR, Ofran Y. How far from the SNP may the causative genes be? *Nucleic Acids Res* 2016; 44:6046-54. .
  43. Yazar S, Mishra A, Ang W, Kearns LS, Mountain JA, Pennell C, Montgomery GW, Young TL, Hammond CJ, Macgregor S, Mackey DA, Hewitt AW. Interrogation of the platelet-derived growth factor receptor alpha locus and corneal astigmatism in Australians of Northern European ancestry: results of a genome-wide association study. *Mol Vis* 2013; 19:1238-46. .
  44. Hewitt KJ, Agarwal R, Morin PJ. The claudin gene family: expression in normal and neoplastic tissues. *BMC Cancer* 2006; 6:186-.
  45. Tsukita S, Furuse M. Pores in the wall: claudins constitute tight junction strands containing aqueous pores. *J Cell Biol* 2000; 149:13-6. .
  46. Inagaki E, Hatou S, Yoshida S, Miyashita H, Tsubota K, Shimura S. Expression and distribution of claudin subtypes in human corneal endothelium. *Invest Ophthalmol Vis Sci* 2013; 54:7258-65. .
  47. Sawaguchi S, Yue BY, Sugar J, Gilboy JE. Lysosomal enzyme abnormalities in keratoconus. *Arch Ophthalmol* 1989; 107:1507-10. .
  48. Maruyama Y, Wang X, Li Y, Sugar J, Yue BY. Involvement of Sp1 elements in the promoter activity of genes affected in keratoconus. *Invest Ophthalmol Vis Sci* 2001; 42:1980-5. .
  49. Cui J, Hao C, Zhang W, Shao J, Zhang N, Zhang G, Liu S. Identical expression profiling of human and murine TIPE3 protein reveals links to its functions. *J Histochem Cytochem* 2015; 63:206-16. .
  50. Wang X, Chua HX, Chen P, Ong RT, Sim X, Zhang W, Takeuchi F, Liu X, Khor CC, Tay WT, Cheng CY, Suo C, Liu J, Aung T, Chia KS, Kooner JS, Chambers JC, Wong TY, Tai ES, Kato N, Teo YY. Comparing methods for performing trans-ethnic meta-analysis of genome-wide association studies. *Hum Mol Genet* 2013; 22:2303-11. .



51. Guggenheim JA, St Pourcain B, McMahon G, Timpson NJ, Evans DM, Williams C. Assumption-free estimation of the genetic contribution to refractive error across childhood. *Mol Vis* 2015; 21:621-32. .
52. Boyd A, Golding J, Macleod J, Lawlor DA, Fraser A, Henderson J, Molloy L, Ness A, Ring S, Davey Smith G. Cohort Profile: the ‘children of the 90s’—the index offspring of the Avon Longitudinal Study of Parents and Children. *Int J Epidemiol* 2013; 42:111-27. .
53. Wang YX, Zhang JS, You QS, Xu L, Jonas JB. Ocular diseases and 10-year mortality: the Beijing Eye Study 2001/2011. *Acta Ophthalmol* 2014; 92:e424-8. .
54. Schache M, Richardson AJ, Mitchell P, Wang JJ, Rochtchina E, Viswanathan AC, Wong TY, Saw SM, Topouzis F, Xie J, Sim X, Holliday EG, Attia J, Scott RJ, Baird PN. Genetic association of refractive error and axial length with 15q14 but not 15q25 in the Blue Mountains Eye Study cohort. *Ophthalmology* 2013; 120:292-7. .
55. Khawaja AP, Chan MP, Hayat S, Broadway DC, Luben R, Garway-Heath DF, Sherwin JC, Yip JL, Dalzell N, Wareham NJ, Khaw KT, Foster PJ. The EPIC-Norfolk Eye Study: rationale, methods and a cross-sectional analysis of visual impairment in a population-based cohort. *BMJ Open* 2013; 3:e002684-.
56. Kaprio J, Koskenvuo M. Genetic and Environmental Factors in Complex Diseases: The Older Finnish Twin Cohort. *Twin Res* 2012; 5:358-65. .
57. Jaddoe VW, Mackenbach JP, Moll HA, Steegers EA, Tiemeier H, Verhulst FC, Witteman JC, Hofman A. The Generation R Study: Design and cohort profile. *Eur J Epidemiol* 2006; 21:475-84. .
58. Hohn R, Kottler U, Peto T, Blettner M, Munzel T, Blankenberg S, Lackner KJ, Beutel M, Wild PS, Pfeiffer N. The ophthalmic branch of the Gutenberg Health Study: study design, cohort profile and self-reported diseases. *PLoS One* 2015; 10:e0120476-.
59. Biino G, Balduino CL, Casula L, Cavallo P, Vaccargiu S, Parracciani D, Serra D, Portas L, Murgia F, Pirastu M. Analysis of 12,517 inhabitants of a Sardinian geographic isolate reveals that predispositions to thrombocytopenia and thrombocytosis are inherited traits. *Haematologica* 2010; 96:96-101. .
60. Yazar S, Forward H, McKnight CM, Tan A, Soloshenko A, Oates SK, Ang W, Sherwin JC, Wood D, Mountain JA, Pennell CE, Hewitt AW, Mackey DA. Raine eye health study: design, methodology and baseline prevalence of ophthalmic disease in a birth-cohort study of young adults. *Ophthalmic Genet* 2013; 34:199-208. .
61. Hofman A, Brusselle GG, Darwish Murad S, van Duijn CM, Franco OH, Goedegebure A, Ikram MA, Klaver CC, Nijsten TE, Peeters RP, Stricker BH, Tiemeier HW, Uitterlinden AG, Vernooij MW. The Rotterdam Study: 2016 objectives and design update. *Eur J Epidemiol* 2015; 30:661-708. .
62. Lavanya R, Jeganathan VS, Zheng Y, Raju P, Cheung N, Tai ES, Wang JJ, Lamoureux E, Mitchell P, Young TL, Cajucom-Uy H, Foster PJ, Aung T, Saw SM, Wong TY. Methodology of the Singapore Indian Chinese Cohort (SICC) eye study: quantifying ethnic variations in the epidemiology of eye diseases in Asians. *Ophthalmic Epidemiol* 2009; 16:325-36. .
63. Foong AW, Saw SM, Loo JL, Shen S, Loon SC, Rosman M, Aung T, Tan DT, Tai ES, Wong TY. Rationale and methodology for a population-based study of eye diseases in Malay people: The Singapore Malay eye study (SiMES). *Ophthalmic Epidemiol* 2007; 14:25-35. .
64. Pan CW, Wong TY, Lavanya R, Wu RY, Zheng YF, Lin XY, Mitchell P, Aung T, Saw SM. Prevalence and risk factors for refractive errors in Indians: the Singapore Indian Eye Study (SINDI). *Invest Ophthalmol Vis Sci* 2011; 52:3166-73. .
65. Saw SM, Shankar A, Tan SB, Taylor H, Tan DT, Stone RA, Wong TY. A cohort study of incident myopia in Singaporean children. *Invest Ophthalmol Vis Sci* 2006; 47:1839-44. .
66. Dirani M, Chan YH, Gazzard G, Hornbeak DM, Leo SW, Selvaraj P, Zhou B, Young TL, Mitchell P, Varma R, Wong TY, Saw SM. Prevalence of refractive error in Singaporean Chinese children: the strabismus, amblyopia, and refractive error in young Singaporean Children (STARS) study. *Invest Ophthalmol Vis Sci* 2010; 51:1348-55. .
67. Moayyeri A, Hammond CJ, Valdes AM, Spector TD. Cohort Profile: TwinsUK and healthy ageing twin study. *Int J Epidemiol* 2013; 42:76-85. .

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