University Library

MINERVA ACCESS

A gateway to Melbourne's research publications

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:
Shah, RL;Li, Q;Zhao, W;Tedja, MS;Tideman, JWL;Khawaja, AP;Fan, Q;Yazar, S;Williams, KM;Verhoeven, VJM;Xie, J;Wang, YX;Hess, M;Nickels, S;Lackner, KJ;Pärssinen, O;Wedenoja, J;Biino, G;Concas, MP;Uitterlinden, A;Rivadeneira, F;Jaddoe, VWV;Hysi, PG;Sim, X;Tan, N;Tham, YC;Sensaki, S;Hofman, A;Vingerling, JR;Jonas, JB;Mitchell, P;Hammond, CJ;Höhn, R;Baird, PN;Wong, TY;Cheng, CY;Teo, YY;Mackey, DA;Williams, C;Saw, SM;Klaver, CCW;Guggenheim, JA;Bailey-Wilson, JE

Title:
A genome-wide association study of corneal astigmatism: The CREAM consortium

Date:
2018-02-05

Citation:
Shah, R. L., Li, Q., Zhao, W., Tedja, M. S., Tideman, J. W. L., Khawaja, A. P., Fan, Q., Yazar, S., Williams, K. M., Verhoeven, V. J. M., Xie, J., Wang, Y. X., Hess, M., Nickels, S., Lackner, K. J., Pärssinen, O., Wedenoja, J., Biino, G., Concas, M. P. ,... Bailey-Wilson, J. E. (2018). A genome-wide association study of corneal astigmatism: The CREAM consortium. Molecular Vision, 24, pp.127-142

Persistent Link:
https://hdl.handle.net/11343/327023

License:
CC BY-NC-ND

Molecular Vision 2018; 24:127-142 <http://www.molvis.org/molvis/v24/127>
Received 22 September 2017 | Accepted 3 February 2018 | Published 5 February 2018

© 2018 Molecular Vision

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

Rupal L. Shah,[1] Qing Li,[2] Wanting Zhao,[3,4] Milly S. Tedja,[5,6] J. Willem L. Tideman,[5,6] Anthony P. Khawaja,[7,8] Qiao Fan,[3,9] Seyhan Yazar,[10,11] Katie M. Williams,[12] Virginie J.M. Verhoeven,[5,6] Jing Xie,[13,14] Ya Xing Wang,[15] Moritz Hess,[16] Stefan Nickels,[17] Karl J. Lackner,[18] Olavi Pärssinen,[19,20,21] Juho Wedenoja,[22,23] Ginevra Biino,[24] Maria Pina Concas,[25] André Uitterlinden,[5,26] Fernando Rivadeneira,[5,26] Vincent W.V. Jaddoe,[5,27] Pirro G. Hysi,[12] Xueling Sim,[28] Nicholas Tan,[3,29] Yih-Chung Tham,[3] Sonoko Sensaki,[3] Albert Hofman,[5,30] Johannes R. Vingerling,[6] Jost B. Jonas,[15,31] Paul Mitchell,[32,33] Christopher J. Hammond,[12] René Höhn,[17,34] Paul N. Baird,[13,14] Tien-Yin Wong,[3,28,35,36] Chinfsg-Yu Cheng,[3,35,36] Yik Ying Teo,[28,37,38] David A. Mackey,[11] Cathy Williams,[39] Seang-Mei Saw,[3,9,28] Caroline C.W. Klaver,[5,6,40] Jeremy A. Guggenheim,[1] Joan E. Bailey-Wilson,[2] The CREAM Consortium

(The first three and last two authors contributed equally to this work.)

[1]School of Optometry & Vision Sciences, Cardiff University, Cardiff, UK; [2]Computational and Statistical Genomics Branch, National Human Genome Research Institute, National Institutes of Health, Baltimore, MD, USA; [3]Singapore Eye Research Institute, Singapore National Eye Centre, Singapore, Singapore; [4]Centre for Quantitative Medicine, Duke-NUS Medical School, Singapore, Singapore; [5]Department of Epidemiology, Erasmus Medical Center, Rotterdam, The Netherlands; [6]Department of Ophthalmology, Erasmus Medical Center, Rotterdam, The Netherlands; [7]Department of Public Health and Primary Care, Institute of Public Health, University of Cambridge School of Clinical Medicine, Cambridge, UK; [8]NIHR Biomedical Research Centre, Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, London, UK; [9]Centre for Quantitative Medicine, Duke-NUS Medical School, Singapore, Singapore; [10]Institute of Genetics and Molecular Medicine, Medical Research Council Human Genetics Unit, University of Edinburgh, Edinburgh, UK; [11]Centre for Ophthalmology and Visual Science, Lions Eye Institute, University of Western Australia, Perth, Australia; [12]Department of Ophthalmology, King's College London, London, UK; [13]Centre for Eye Research Australia, Royal Victorian Eye and Ear Hospital, Melbourne, Victoria, Australia; [14]Ophthalmology, Department of Surgery, University of Melbourne, Melbourne, Australia; [15]Beijing Institute of Ophthalmology, Beijing Key Laboratory of Ophthalmology and Visual Sciences, Beijing Tongren Eye Center, Beijing Tongren Hospital, Capital Medical University; Beijing, China; [16]Institute for Medical Biostatistics, Epidemiology and Informatics, University Medical Center Mainz, Mainz, Germany; [17]Department of Ophthalmology, University Medical Center Mainz, Mainz, Germany; [18]Institute for Clinical Chemistry and Laboratory Medicine, University Medical Center Mainz, Mainz, Germany; [19]Department of Ophthalmology, Central Hospital of Central Finland, Jyväskylä, Finland; [20]Gerontology Research Center, Faculty of Sport and Health Sciences, University of Jyväskylä, Finland; [21]Department of Health Sciences, University of Jyväskylä, Jyväskylä, Finland; [22]Department of Ophthalmology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland; [23]Department of Public Health, University of Helsinki, Helsinki, Finland; [24]Institute of Molecular Genetics, National Research Council of Italy, Pavia, Italy; [25]Institute for Maternal and Child Health - IRCCS 'Burlo Garofolo', Trieste, Italy; [26]Department of Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands; [27]Department of Pediatrics Erasmus Medical Center, Rotterdam, the Netherlands; [28]Saw Swee Hock School of Public Health, National University of Singapore, Singapore, Singapore; [29]Department of Ophthalmology, National University Hospital, Singapore; [30]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA; [31]Department of Ophthalmology, Medical Faculty Mannheim of the University Heidelberg, Mannheim, Germany; [32]Department of Ophthalmology, Western Sydney Local Health District, Sydney, Australia; [33]Centre for Vision Research, Westmead Institute for Medical Research, University of Sydney, Sydney, Australia; [34]Department of Ophthalmology, Inselspital, University Hospital Bern, University of Bern, Bern, Switzerland; [35]Ophthalmology & Visual Sciences Academic Clinical Program (Eye ACP), Duke-NUS Medical School, Singapore; [36]Department of Ophthalmology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; [37]Department of Statistics and Applied Probability, National University of Singapore, Singapore; [38]Division of Human Genetics, Genome Institute of Singapore, Singapore; [39]School of Social and Community Medicine, University of Bristol, Bristol, UK; [40]Department of Ophthalmology, Radboud University Medical Center, Nijmegen, the Netherlands

Correspondence to: Jeremy A. Guggenheim, School of Optometry & Vision Sciences, Cardiff University, Maindy Road, Cardiff, CF24 4HQ, UK; Phone: +44 (0) 29 2087 4904; email. GuggenheimJ1@cardiff.ac.uk

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), $p=5.55\times10^{-9}$. 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)	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[1]	European	2398(1003/1395)	48.7	55.9 (10.8)	0.65 (0.400–0.950)	0.050–4.350
GHS 2[1]	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[2]	European	92(37/55)	44.6	16.0 (4.5)	0.682 (0.512–0.942)	0.185–3.070
OGP-B[2]	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[3]	Asian	553 (240/313)	65.6	62.1 (8.4)	0.666 (0.407–1.056)	0.000–3.620
BES-OmniE[3]	Asian	469 (208/261)	60.1	64.7 (9.5)	0.676 (0.429–1.016)	0.000–5.082
SCES-610K[3]	Asian	1745 (787/958)	48.7	57.6 (9.0)	0.703 (0.476–1.060)	0.109–5.868
SCES-OmniE[3]	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

[1]Association tests were undertaken separately for samples recruited in different waves.[2]Association tests were undertaken separately for different age strata (stratum A, age >3 and <25 years; stratum B, age ≥25 years). [3]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 $<1\times10^{-6}$ 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 (λ_{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 5×10^{-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 ≥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,

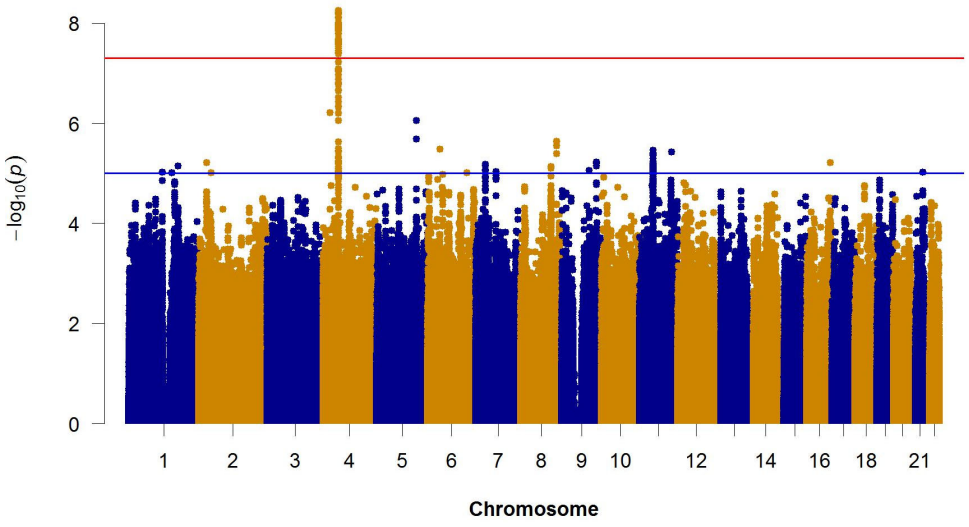


Figure 1. Manhattan plot showing most strongly associated markers in the GWAS fixed-effects meta-analysis for European and Asian participants of all ages combined (n=31,375). Red line: $p=5\times10^{-8}$, blue line: $p=1\times10^{-5}$.

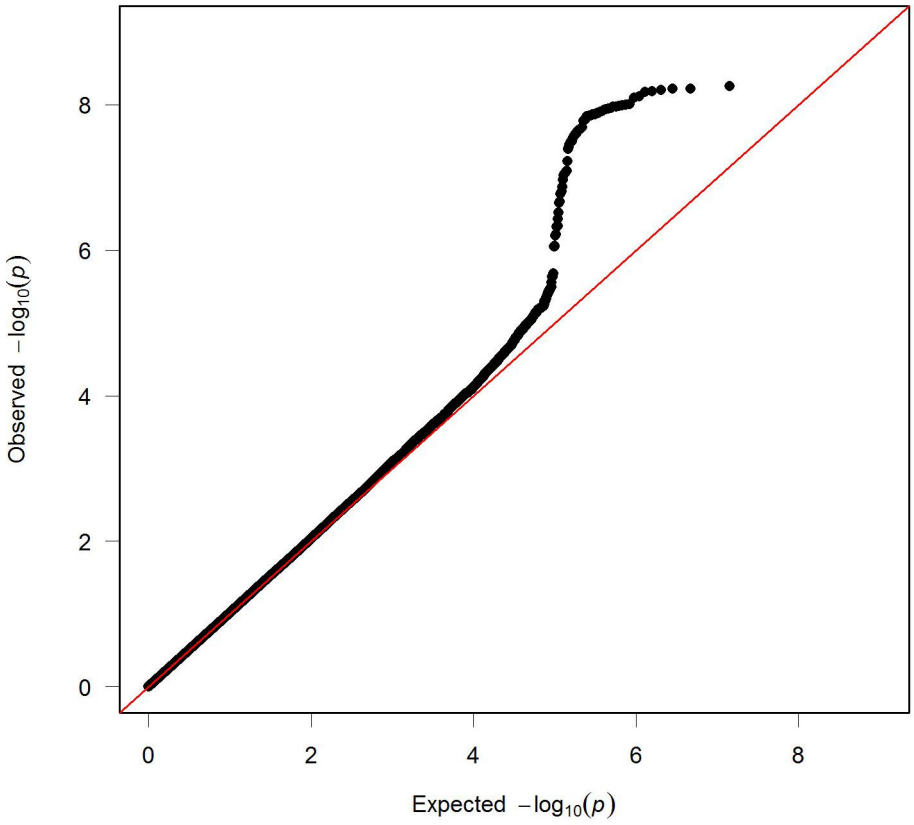


Figure 2. Q-Q plot for the GWAS fixed-effects meta-analysis for European and Asian participants of all ages combined (n=31,375).

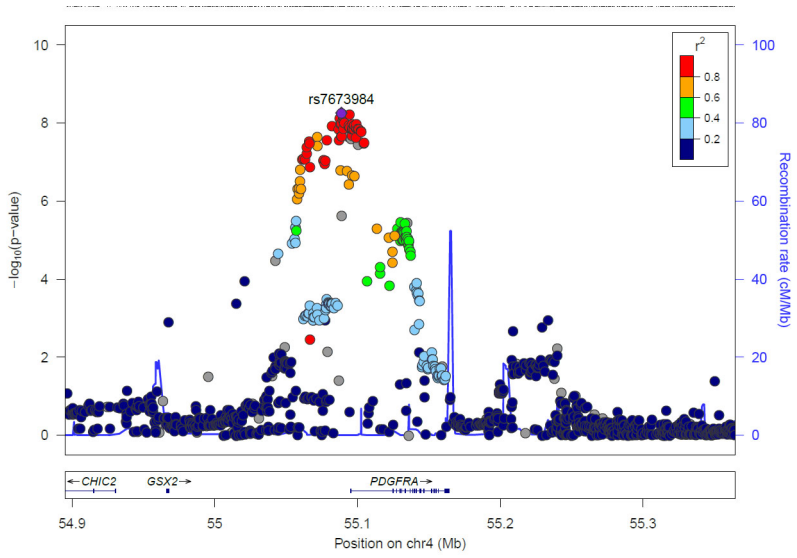


Figure 3. Region plot for the most strongly associated region in the GWAS fixed-effects meta-analysis for European and Asian participants of all ages combined (n=31,375).

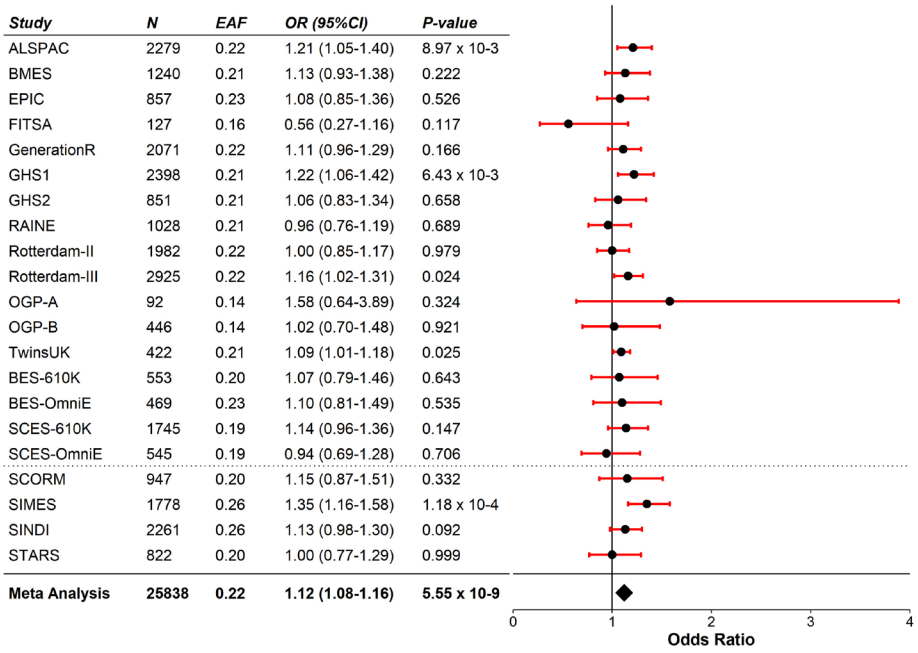


Figure 4. Forest plot and summary table for lead variant rs7673984 across all cohorts. Studies listed above the dotted line are new cohorts not included in the only prior GWAS for corneal astigmatism [16]. EAF=effect allele frequency. (Note that rs7673984 was excluded from the Rotterdam-I cohort analysis 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^{-9}	*PDGFRA*
rs34751092	4	24,129,037	A	G	0.28	1.09 (1.05–1.13)	6.07×10^{-7}	*PPARGC1A*
rs630203	5	141,444,269	T	G	0.74	0.92 (0.88–0.95)	8.83×10^{-7}	*MRPL11P2*
rs75607298	8	128,611,496	A	G	0.72	1.14 (1.08–1.21)	2.28×10^{-6}	*CASC11*
rs62401199	6	43,813,341	T	C	0.14	1.15 (1.09–1.22)	3.29×10^{-6}	*LINC01512*
rs753992	11	47,349,846	A	G	0.29	0.91 (0.87–0.95)	3.48×10^{-6}	*MADD*
rs3214101	11	114,009,408	A	T	0.68	1.08 (1.05–1.12)	3.75×10^{-6}	*ZBTB16*
rs10985068	9	123,629,724	C	G	0.12	1.13 (1.07–1.19)	5.87×10^{-6}	*PHF19*
rs62128379	2	26,960,055	T	C	0.85	1.13 (1.07–1.19)	6.00×10^{-6}	*KCNK3*
rs9939114	16	84,023,972	A	G	0.05	0.54 (0.41–0.70)	6.01×10^{-6}	*NECAB2*
rs60083876	7	34,228,819	A	T	0.95	1.31 (1.17–1.48)	6.53×10^{-6}	*BMPER*
rs859362	1	175,495,090	T	C	0.19	1.10 (1.05–1.15)	7.14×10^{-6}	*TNR*
rs11775037	8	108,317,615	A	G	0.20	1.10 (1.05–1.14)	7.31×10^{-6}	*ANGPT1*
rs7036824	9	96,149,894	T	C	0.94	0.83 (0.77–0.90)	8.78×10^{-6}	*C9orf129*
rs142168171	7	71,253,651	I	R	0.09	1.37 (1.19–1.57)	9.14×10^{-6}	*CALN1*
rs7278671	21	41,047,876	A	G	0.51	0.93 (0.90–0.96)	9.31×10^{-6}	*B3GALT5*
rs191640722	1	119,264,997	C	G	0.09	0.87 (0.82–0.93)	9.53×10^{-6}	*LOC100421281*
rs36107906	2	44,162,800	D	R	0.29	1.09 (1.05–1.13)	9.61×10^{-6}	*LRPPRC*
rs4896367	6	138,807,281	T	C	0.72	1.09 (1.05–1.14)	9.75×10^{-6}	*NHSL1*
rs35587414	1	153,174,958	T	C	0.15	1.13 (1.07–1.20)	9.79×10^{-6}	*LELP1*

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^{-40}

younger Asian, and older Asian). However, none of the markers had a p value below the pre-determined threshold of 5×10^{-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 $<5\times10^{-8}$, 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 $p<1\times10^{-5}$. 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; $p=1.76\times10^{-4}$) in Asians, OR=1.11 (95% CI:1.06–1.16; $p=5.64\times10^{-6}$) in Europeans, and OR=1.12 (95% CI:1.08–1.16; $p=5.55\times10^{-9}$) 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 *PDGRFA* 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 ±50 kb or ±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 ~5%) and refractive astigmatism (h^2 ~1%)—the first ever estimates for these traits—were also much lower than those for the spherical equivalent (h^2 ~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 *PDGRFA* 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 TNFα 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 ±50KB BUFFERS FOR ALL EUROPEANS.

Start and stop positions listed include ±50kb 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 ±50KB BUFFERS FOR ALL EUROPEANS.

Start and stop positions listed include ±50kb 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"

ACKNOWLEDGMENTS AND STUDY INFORMATION

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 Corportation 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 Research Council of Australia (NHMRC) Senior Research Fellowship 1138585 (PNB). The Centre for Eye Research Australia (CERA) receives Operational Infrastructure Support from the Victorian Government. Details of the BMES cohort have been published previously [54]. **EPIC.** The European Prospective Investigation of Cancer (EPIC)-Norfolk infrastructure and core functions are supported by grants from the Medical Research Council (G1000143) and Cancer Research UK (C864/A14136). The clinic for the third health examination was funded by Research into Aging (262). Mr Khawaja was a Wellcome Trust Clinical Research Fellow at the time of analysis. The EPIC-Norfolk Eye Study was performed following the principles of the Declaration of Helsinki and the Research Governance Framework for Health and Social Care. The study was approved by the Norfolk Local Research Ethics Committee (05/Q0101/191) and East Norfolk & Waveney NHS Research Governance Committee (2005EC07L). All participants gave written, informed consent. A description of the EPIC study cohort is available [55]. **FITSA.** Finnish Twin Study on Aging (FITSA) is a study of genetic and environmental effects on the disablement process in older female twins. The study cohort of 13,888 adult twin pairs started in 1975. Altogether 103 MZ and 114 DZ twin pairs (424 individuals, all women of European ancestry) aged 63–76 years living in Finland took part in multiple laboratory examinations in 2000 and 2003, and responded in questionnaires in 2011. Before the examinations, the subjects provided a written informed consent according to the Declaration of Helsinki. The study protocol was approved by the ethics committee of the Central Hospital District of Central Finland. FITSA was supported by ENGAGE (FP7-HEALTH-F4–2007, 201,413); European Union through the GENOMEUTWIN project (QLG2-CT-2002–01254); the Academy of Finland Center of Excellence in Complex Disease Genetics (213506, 129680); the Academy of Finland Aging Programme; and the Finnish Ministry of Culture and Education and University of Jyväskylä, Silmäsäätiö Foundation and Evald & Hilda Nissi Foundation. For FITSA the contributions of Emmi Tikkanen, Samuli Ripatti, Markku Kauppinen, Taina Rantanen and Jaakko Kaprio are acknowledged. A description of the FITSA cohort has been published [56]. **Generation R.** The Generation R study is conducted by the Erasmus Medical Centre in close collaboration with the School of Law and Faculty of Social Sciences of the Erasmus University Rotterdam, the Municipal Health Service Rotterdam, the Rotterdam Homecare Foundation, and the Stichting Trombosedienst & Artsenlaboratorium Rijnmond (Star-MDC), Rotterdam. We gratefully acknowledge the contribution of the children and parents, as well as the participating general practitioners, hospitals, midwives,

and pharmacies in Rotterdam. The Generation R study is made possible by financial support from the Erasmus Medical Centre, Rotterdam; the Netherlands Organisation for Scientific Research (NWO); the Netherlands Organisation for Health Research and Development (ZonMw); the Dutch Ministry of Education, Culture and Science; the Dutch Ministry of Health, Welfare, and Sports; the European Commission (DG XII); and UitZicht (Grant 2013–24). The study protocol was approved by the Medical Ethical Committee of the Erasmus Medical Centre, Rotterdam (MEC 217.595/2002/20), and written informed consent was obtained from all participants. Research was conducted according to the declaration of Helsinki. A description of the Generation R study has been published [57]. **Gutenberg Health Study (GHS 1 and GHS 2).** The Gutenberg Health Study is a population-based, prospective, observational cohort study in mid-western Germany that includes consecutive follow-ups every five years. The primary study aim is to evaluate and improve cardiovascular risk stratification and the general health status of the population. The baseline examination included a total of 15,010 participants aged 35 to 74 years and took place from 2007 to 2012. The participants were randomly drawn and equally stratified for sex, residence (urban or rural) and for each decade of age. Exclusion criteria were the following: insufficient knowledge of German and physical or mental inability to participate in the examinations in the study center. The study protocol and study documents were approved by the local ethics committee of the Medical Chamber of Rhineland-Palatinate, Germany (reference no. 837.020.07; original vote: 22.3.2007, latest update: 20.10.2015). According to the tenets of the Declaration of Helsinki, written informed consent was obtained from all participants before their entry into the study. The Gutenberg Health Study is funded through the government of Rhineland-Palatinate ("Stiftung Rheinland-Pfalz für Innovation," contract AZ 961–386261/733), the research programs "Wissen schafft Zukunft" and "Center for Translational Vascular Biology (CTVB)" of the Johannes Gutenberg-University of Mainz, the National Genome Network "NGFNplus" by the Federal Ministry of Education and Research, Germany (A301GS0833) and its contracts with Boehringer Ingelheim and PHILIPS Medical Systems. We thank all study participants for their willingness to provide data for this research project and we are indebted to all coworkers for their enthusiastic commitment. A description of the ophthalmic arm of the GHS has been published [58]. **OGP.** The Ogliastra Genetic Park (OGP) study authors would like to express their gratitude to all the study participants for their contributions, to the municipal administrations for their economic and logistic support and, to the whole OGP team, which includes interviewers, computer and laboratory technicians, research scientists, physicians and nurses. This research was supported by grant from the Italian Ministry of Education, University and Research (MIUR) no: 5571/DSPAR/2002. The research protocol of the study was approved by the institutional review board of the Italian Ministry of Education, University and Research. It adheres to the tenets of the declaration of Helsinki, furthermore written informed consent was obtained from all participants. A description of the OGP study cohort has been published [59]. **RAINE** (Western Australian Pregnancy Cohort). We are grateful to all the study participants. We also thank the Raine Study and Lions Eye Institute (LEI) research staff for cohort coordination and data collection. The core management of the Raine Study is funded by The University of Western Australia (UWA), The Telethon Institute for Child Health Research, Raine Medical Research Foundation, UWA Faculty of Medicine, Dentistry and Health Sciences, Women's and Infant's Research Foundation and Curtin University. Genotyping was funded by Australian National Health and Medical Research Council (NHMRC) project grant 1021105. Support for the REHS was provided by LEI, the Australian Foundation for the Prevention of Blindness and ORIA. SY is supported by NHMRC CJ Martin Early Career Fellowship (#1111437). A description of the RAINE Eye Health Study study cohort is available [60]. **Rotterdam Study (RS1, RS2, RS3).** The Rotterdam Study is a prospective population-based cohort study in the elderly living in Ommoord, a suburb of Rotterdam, the Netherlands. In brief, the Rotterdam Study consists of 3 independent cohorts: RS1, RS2, and RS3. For the current analysis, 5,328 residents aged 55 years and older were included from RS1, 2,009 participants aged 55 and older from RS2, and 1,970 aged 45 and older from RS 3. 99% of subjects were of European ancestry. Participants underwent multiple physical examinations with regular intervals from 1991 to present, including a non-dilated automated measurement of refractive error using a Topcon RM-A2000 autorefractor. All measurements in RS-1–3 were conducted after the Medical Ethics Committee of the Erasmus University had approved the study protocols and all participants had given a written informed consent in accordance with the Declaration of Helsinki. The Rotterdam Study was supported by the Dutch governmental Innovational Research Incentives Scheme Grant (VICI 91815655); Horizon2020 ERC Consolidator Grant (648268); Erasmus Medical Center and Erasmus University, Rotterdam, The Netherlands; Netherlands Organization for Health Research and Development (ZonMw); UitZicht; the Research Institute for Diseases in the Elderly; the Ministry of Education, Culture and Science; the Ministry for Health, Welfare and Sports; the European Commission (DG XII); the Municipality of Rotterdam; the

Netherlands Genomics Initiative/NWO; Center for Medical Systems Biology of NGI; Lijf en Leven; Henkes Stichting; Landelijke Stichting voor Blinden en Slechtzienden; Oogfonds; MaculaFonds. We acknowledge Ada Hooghart, Corina Brussee, Riet Bernaerts-Biskop, Patricia van Hilten, Pascal Arp, Jeanette Vergeer, Marijn Verkerk; Sander Bervoets for their valuable contributions. A description of the Rotterdam study has been published [61]. **SCES, SIMES and SINDI.** The Singapore Chinese Eye Study (SCES), Singapore Malay Eye Study (SiMES) and Singapore Indian Eye Study (SINDI) were supported by the National Medical Research Council (NMRC), Singapore (grants 0796/2003, 1176/2008, 1149/2008, STaR/0003/2008, 1249/2010, CG/SERI/2010, CIRG/1371/2013, and CIRG/1417/2015), and Biomedical Research Council, Singapore (08/1/35/19/550 and 09/1/35/19/616). Ching-Yu Cheng is supported by an award from NMRC (CSA/033/2012). Descriptions of the SCES, SIMES and SINDI cohorts have been published [62-64]. **SCORM.** The Singapore Cohort Study of the Risk Factors for Myopia (SCORM) was supported by the Biomedical Research Council (BMRC) 06/1/21/19/466. A description of the SCORM cohort has been published [65]. **STARS.** The Singaporean Chinese in the Strabismus, Amblyopia, and Refractive Error Study (STARS) was supported by National Medical Research Council (NMRC), Singapore (grants 1176/2008). A description of the STARS cohort has been published [66]. **TwinsUK.** The TwinsUK adult twin registry based at St. Thomas' Hospital in London is a volunteer cohort of over 10,000 twins from the general population. Twins largely volunteered unaware of the eye studies, gave fully informed consent under a protocol reviewed by the St. Thomas' Hospital Local Research Ethics Committee. TwinsUK is funded by the Wellcome Trust and the European Community's Seventh Framework Programme (FP7/2007–2013). The study also receives support from the National Institute for Health Research Clinical Research Facility at Guy's and St. Thomas' National Health Service Foundation Trust and National Institute for Health Research Biomedical Research Centre at Guy's and St. Thomas' National Health Service Foundation Trust and King's College London. Keratometry was obtained using the VX-120 ocular diagnostic device (Visionix®, Luneau Technology Group). A description of the TwinsUK study cohort is available [67]. **CREAM Meta-analyses.** This work was supported in part by the Intramural Research Program of the National Human Genome Research Institute, National Institutes of Health.

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, Lehtimaki 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, Lehtimaki 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, Vatavuk 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 multiancestry 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, Lehtimaki 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, Vatavuk 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 JWL, 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 RJF, 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, Shimmura 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, Balduini 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. .

Articles are provided courtesy of Emory University and the Zhongshan Ophthalmic Center, Sun Yat-sen University, P.R. China. The print version of this article was created on 5 February 2018. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.