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Association of Novel Loci With Keratoconus Susceptibility in a Multitrait Genome-Wide Association Study of the UK Biobank Database and Canadian Longitudinal Study on Aging

Weixiong He, MSc; Xikun Han, PhD; Jue-Sheng Ong, PhD; Alex W. Hewitt, MBBS, PhD; David A. Mackey, MD; Puya Gharahkhani, PhD; Stuart MacGregor, PhD; for the International Glaucoma Genetics Consortium

IMPORTANCE Keratoconus can be a debilitating corneal ectasia in which the cornea thins, bulges, and steepens into a conical shape. Early features of keratoconus include myopia and irregular astigmatism, which affect vision and can be treated with contact lenses, collagen cross-linking, or, in advanced cases, corneal transplant. Recent estimates of the prevalence of keratoconus based on results of Scheimpflug imaging in young adults are as high as 1.2%. However, obtaining very large keratoconus data sets for a genome-wide association study (GWAS) is problematic because few population studies include Scheimpflug imaging and because severe keratoconus is relatively rare.

OBJECTIVE To identify novel keratoconus loci using corneal resistance factor (CRF) and central corneal thickness (CCT).

DESIGN, SETTING, AND PARTICIPANTS This multitrait GWAS used European ancestry CRF data from UK Biobank (UKB) (n = 105 427) and the Canadian Longitudinal Study on Aging (CLSA) (n = 18 307) and European ancestry CCT data from the International Glaucoma Genetics Consortium (IGGC) (n = 17 803). The CRF and CCT variants in published keratoconus data sets (4669 cases and 116 547 controls) were compared. The data set from UKB was compiled March 24, 2020; data were released from the CLSA in July 2020; and IGGC data were available from May 1, 2018.

MAIN OUTCOMES AND MEASURES Association of CRF and CCT variants with keratoconus risk.

RESULTS The GWAS included 4 cohorts: 105 427 UKB European ancestry (56 134 women [53.2%] and 49 293 men [46.7%]; mean [SD] age, 57 [8] years), 5029 UKB South Asian ancestry (2368 women [47.1%] and 2661 men [52.9%]; mean [SD] age, 54 [8] years), 902 UKB East Asian ancestry (622 women [68.9%] and 280 men [31.0%]; mean [SD] age, 53 [8] years), and 18 307 CLSA European ancestry (9260 women [50.6%] and 9047 men [49.4%]; mean [SD] age, 63 [10] years) participants. A total of 369 CRF and 233 CCT loci were identified, including 36 novel CRF loci and 114 novel CCT loci. Twenty-nine CRF loci and 24 CCT loci were associated with keratoconus. Polygenic risk scores (PRS) were constructed using CRF- and CCT-associated variants and published keratoconus variants. The PRS result showed that adding a CRF- or CCT-based PRS to the keratoconus PRS from previously published variants improved the prediction area under the receiver operating characteristic curve (from 0.705 to 0.756 for CRF and from 0.715 to 0.755 for CCT).

CONCLUSIONS AND RELEVANCE These findings support the use of multitrait modeling of corneal parameters in a relatively large data set to identify new keratoconus risk loci and enhance polygenic risk score models.

Supplemental content

Author Affiliations: Author affiliations are listed at the end of this article.

Group Information: A complete list of the members of the International Glaucoma Genetics Consortium appears at the end of this article.

Corresponding Author: Weixiong He, MSc, QIMR Berghofer Medical Research Institute, 300 Herston Rd, Herston, Brisbane, Queensland 4006, Australia (weixiong.he@qimrberghofer.edu.au).

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Keratoconus is the most common corneal ectasia characterized by bilateral, often asymmetric distortion of the cornea with corneal thinning, outward bulging, and conical appearance leading to irregular astigmatism.[1] Onset usually occurs near puberty, with progression of visual impairment in early adult life. Keratoconus is one of the leading causes of corneal transplant,[2] although given a timely diagnosis, many patients are now treated early with collagen cross-linking.[3] The reported prevalence of keratoconus was 1.38 per 1000 in the general population.[1,4] However, the development of Scheimpflug imaging technology and the Belin-Ambrósio enhanced ectasia display score has led to the prevalence being revised, with a Western Australian study of young adults finding a prevalence of 1.2%.[5] Systemic association of keratoconus includes sleep apnea, asthma, and Down syndrome with eye rubbing proposed as a mechanism.[6] However, the causative biological mechanism of keratoconus is still poorly understood.[7]

Family-based studies suggest that genes are important in determining keratoconus risk,[8] with first-degree relatives of cases having as much as a 67-fold increased risk relative to that of the general population.[9] A genetic contribution to keratoconus is also supported by twin studies.[10,11] Given the evidence for a genetic component to keratoconus, work has begun to identify specific genes.[11] However, obtaining a sufficiently large keratoconus data set for a genome-wide association study (GWAS) is difficult. Previous efforts used only relatively limited sample sizes[12-14]; hence, most genes for keratoconus remain undetected. As an alternative to performing GWAS of keratoconus directly, recent GWASs of quantitative corneal parameters have proven useful for improving our understanding of the biomechanisms underlying keratoconus.[13,15] Central corneal thickness (CCT), a widely measured corneal parameter, is one of the most heritable quantitative traits in humans, with heritability estimates as high as 95%.[16] Because patients with keratoconus typically have thinner corneas, CCT is a directly relevant quantitative phenotype. Corneal resistance factor (CRF), another quantitative corneal parameter, refers to the indication of the overall resistance or elasticity of the cornea. Corneal resistance factor is significantly decreased in eyes with keratoconus.[17] Previous studies[18-20] have suggested CRF is correlated with CCT. As with CCT, genetic factors play an important role in CRF variation.[21] Previous research has found that the prevalence of keratoconus varies by ancestry[22,23] and that CCT and CRF also differ between ancestries.[24-26]

Many CCT and CRF loci have been identified, yet collectively they do not account for the entire heritability of either trait. Identifying additional genes will provide a better understanding of the biology underlying these traits and may provide insights into the pathogenesis of keratoconus. Herein we performed a GWAS for CRF and combined these findings with published CCT GWAS results using a multitrait GWAS approach.[27] We then evaluated the association of our top CRF and CCT variants with keratoconus susceptibility in a recent keratoconus GWAS[28] and identified multiple novel keratoconus variants (**Figure 1**).

Key Points

Question Do large-scale studies of corneal resistance factor (CRF) and central corneal thickness (CCT) increase understanding of keratoconus risk?

Findings In this genome-wide association study of 105 427 participants from UK Biobank, 18 307 from the Canadian Longitudinal Study on Aging, and 17 803 from the European ancestry CCT data from the International Glaucoma Genetics Consortium, 369 CRF and 233 CCT loci (a subset of which affect keratoconus) were identified.

Meaning These findings suggest that polygenic risk score models for keratoconus can be improved using CRF and CCT.

Methods

Study Cohorts

The Canadian Longitudinal Study on Aging (CLSA) and UK Biobank (UKB) database used for this study were approved by local research ethics boards. All participants provided informed written consent. The study methods followed the World Medical Association Declaration of Helsinki ethical standards for medical research[29] and the Strengthening the Reporting of Genetic Association Studies (STREGA) reporting guideline. Additional details are provided in the eMethods in the Supplement.

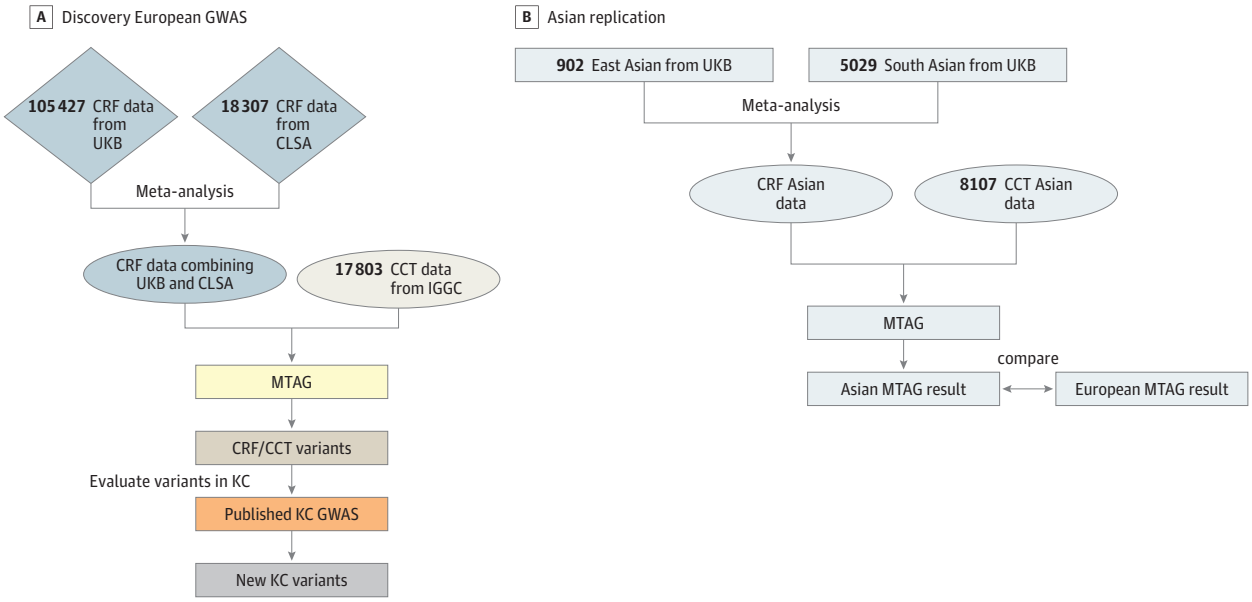
UKB

The UKB is a large-scale UK biomedical database including detailed genetic and phenotypic data from more than 500 000 people aged 40 to 69 years. The CRF measure used here was the mean across both eyes, with rank-based inverse normal transformation applied. Outliers were defined as CRF values larger than 4 SDs from the mean. We also excluded patients with keratoconus (*International Statistical Classification of Diseases and Related Health Problems, Tenth Revision*, diagnostic code H18.6) and their families (n = 145). Our data set (accrued March 24, 2020) included 115 671 people with CRF measurements in at least 1 eye. To evaluate UKB self-reported race and ethnicity (data field 21 000), we used k-means clustering to group the top 20 principal components into 20 clusters and compared them with self-reported race and ethnicity. The UKB individuals with consistent self-reported race and ethnicity and genetic clusters were of European (mostly White British [n = 105 427]), South Asian (mostly Indian, Pakistani, and Bangladeshi [n = 5029]), and East Asian (mostly Chinese [n = 902]) ancestry. The main analysis was conducted using participants of European ancestry, with the Asian groups used for replication.

CLSA

The CLSA is a longitudinal study of 51 338 Canadians aged 45 to 85 years at enrollment. We calculated the mean CRF across both eyes in the baseline and follow-up studies (July 2020 release). Outliers were defined as CRF readings that were more

Figure 1. The Study Design of Applying Quantitative Corneal Parameter Loci to Discover the Novel Keratoconus (KC) Loci



CCT indicates central corneal thickness; CLSA, Canadian Longitudinal Study on Aging; CRF, corneal resistance factor; GWAS, genome-wide association study; IGGC, International Glaucoma Genetics Consortium; MTAG, multitrait analysis of GWAS; and UKB, UK Biobank.

than 4 SDs from the mean. A total of 29 884 CLSA participants had CRF phenotypic data, and 19 669 had genetic data. The GWAS comprised 18 307 people of European ancestry based on their ancestry background and k-means clustering of the top 20 principal components.

International Glaucoma Genetics Consortium

We obtained the summary statistics of the latest CCT GWAS meta-analysis[26] conducted by the International Glaucoma Genetics Consortium (IGGC) (17 803 European and 8107 Asian participants). The individual studies involved, genetic quality control, and phenotype definitions have been described previously.[26]

Statistical Analysis

For the GWAS of the European and Asian CRF cohort from the UKB and CLSA, linear mixed models (BOLT-LMM, version 2.3.2,[30] and GCTA-fastGWA,version 1.93.2 beta[31]) were used, adjusting for age, sex, genotyping array, and the first 10 principal components as covariates, to correct for cryptic relatedness and population stratification. Single-nucleotide variants with imputation quality score of less than 0.3 and minor allele frequency of less than 0.01 were removed. The UKB and CLSA European and Asian GWAS results were meta-analyzed using the inverse variance fixed-effect scheme (METAL software [May 5, 2020]).[32]

We conducted a multitrait analysis of the GWAS of CRF and CCT using MTAG software, version 1.0.8[27] (Figure 1). We used the PLINK (version 1.90b) linkage disequilibrium–clumping procedure to identify statistically independent CRF and CCT variants (P threshold, 5×10^{-8}; r^2 threshold, 0.05; window of 1 megabase [Mb]) from the MTAG output.

We developed a polygenic risk score (PRS) based on the log odds ratio of 36 previously reported genome-wide significant keratoconus top variants.[28] Two additional PRSs based on top CRF and CCT variants were also constructed, weighted by the effect size of the top CRF and CCT variants estimated herein. Before calculating the PRS, we used a screening technique to exclude a small number of variants with an unexpected extreme discordant effect, that is, the corneal thickness–decreasing allele was associated with decreased keratoconus risk. The eliminated variants could have a large influence on keratoconus prediction given their reversed effect.

The PRS was determined using PLINK software, version 1.90b6.8.[33] The area under the receiver operating characteristic curve (AUC) and the correlation between AUCs were calculated using pROC, version 1.17.0.1.[34]

Results

CRF Measurement From UKB and CLSA

In the UKB, participants with at least 1 CRF measurement included in the GWAS consisted of 105 427 European, 902 East Asian, and 5029 South Asian participants (**Table**). In the CLSA, 18 307 European samples with CRF measurement were tested. We conducted GWAS for 4 cohorts: the 105 427 UKB European (56 134 women [53.2%] and 49 293 men [46.7%]; mean [SD] age, 57 [8] years), 5029 UKB South Asian (2368 women [47.1%] and 2661 men [52.9%]; mean [SD] age, 54 [8] years), 902 UKB East Asian (622 women [68.9%] and 280 men [31.0%]; mean [SD] age, 53 [8] years), and 18 307 CLSA European (9260 women [50.6%] and 9047 men [49.4%]; mean [SD] age, 63 [10] years) participants (Table).

Table. Characteristics of CRF From Individuals in the UKB and CLSA[a]

Variable	UKB			CLSA European (n = 18 307)
	European (n = 105 427)	East Asian (n = 902)	South Asian (n = 5029)	
Sex, No. (%)				
Women	56 134 (53.2)	622 (68.9)	2368 (47.1)	9260 (50.6)
Men	49 293 (46.7)	280 (31.0)	2661 (52.9)	9047 (49.4)
Age at recruitment, mean (SD), y	57 (8)	53 (8)	54 (8)	63 (10)
CRF measurement (right), mean, mm Hg				
First visit	10.75 (1.97)	10.48 (2.09)	10.39 (2.00)	9.83 (1.92)
Second visit	10.67 (2.17)	10.36 (2.31)	10.38 (2.13)	10.11 (1.95)
CRF measurement (left), mean, mm Hg				
First visit	10.67 (2.04)	10.46 (2.14)	10.27 (2.00)	9.59 (1.97)
Second visit	10.94 (2.33)	10.61 (2.90)	10.71 (2.58)	9.91 (2.00)

Abbreviations: CLSA, Canadian Longitudinal Study on Aging; CRF, corneal resistance factor; UKB, UK Biobank.

[a] Participants include individuals with at least 1 CRF measurement and no keratoconus.

We then compared the effect size of independent clumped variants of UKB European participants across different cohorts (eFigure 1 in the Supplement). We removed rare variants (minor allele frequency, <0.01). The effect size of leading variants was highly concordant between UKB European and CLSA European cohorts (Pearson *R*, 0.93 [95% CI, 0.91 to approximately 0.94]) (eFigure 1C in the Supplement). We also observed correlated effect sizes between UKB European and South Asian cohorts (Pearson *R*, 0.59 [95% CI, 0.51 to approximately 0.66]) (eFigure 1A-B in the Supplement).

MTAG Results

Next, we used MTAG to combine the CRF and CCT GWASs in European participants (eFigures 2 and 3 in the Supplement). We observed a strong genetic correlation (0.69 [SE, 0.049]) between CCT and CRF input data sets. Using MTAG, we identified 369 genome-wide significant independent variants for CRF and 233 for CCT (r^2 threshold, 0.05; $P < 5 \times 10^8$) (eTables 1 and 2 in the Supplement). By excluding previously reported loci,[21,35,36] 36 CRF variants and 114 CCT variants were not associated at genome-wide significance ($P = 5 \times 10^{-8}$) with the input data; the MTAG approach exploited the high genetic correlation between the input traits and suggested that these subthreshold signals were likely real (eTables 3 and 4 in the Supplement).

Cross-Ancestry Effects of Top Variants

To assess the cross-ethnic effects of CRF and CCT hits, we compared the effect sizes between European and Asian participants. We meta-analyzed the CRF summary statistics of UKB East and South Asian cohorts (n = 5931). The genetic correlation between CRF summary statistics from the UKB Asian groups and CCT summary statistics from the IGGC Asian cohort (n = 8107) was 0.40 (SE, 0.43). We then performed MTAG analysis on both input data sets as the replication for the results from the European cohorts. Although the power was low in the Asian cohorts, we still observed good concordance in effect size across ancestries (Pearson *R* for CRF, 0.70 [95% CI, 0.53-0.82]; Pearson *R* for CCT, 0.76 [95% CI, 0.58-0.87]) (**Figure 2**). Cross-ancestry effects were also seen in a genetic correlation analysis (eTable 7 in the Supplement).

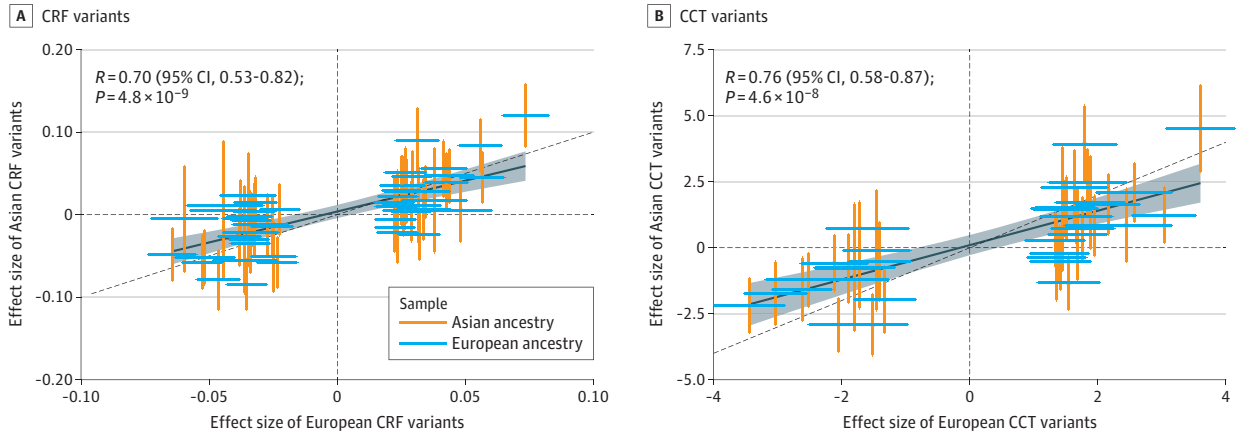
CRF and CCT Variants in Keratoconus

We then looked up the variants of European MTAG results for CRF and CCT in the recently published keratoconus GWAS.[28] The genetic correlation (based on MTAG output) between keratoconus and CRF was −0.52 (SE, 0.04) and between keratoconus and CCT was −0.36 (SE, 0.06), based on the linkage disequilibrium score regression method. We observed a negative correlation when comparing the effect size of independent CRF and CCT variants and keratoconus GWAS (**Figure 3**). Of the top CRF and CCT loci, 354 CRF variants and 221 CCT variants were available in the keratoconus GWAS. Twenty-nine CRF variants near or within the gene included *FNDC3B* (OMIM 611909), *THBS4* (OMIM 600715), *CRYBA2* (OMIM 600836), *INVS* (OMIM 243305), *MAMDC2* (OMIM 612879), and *SEPT9* (OMIM 604061), which reached the study-wide significance for keratoconus after Bonferroni correction but not genome-wide significance (ie, variants where $P < 5 \times 10^{-8}$ to $P < .05/354 = 1.41 \times 10^{-4}$ genome-wide significant loci were already reported in the previous keratoconus GWAS) (eTable 5 in the Supplement). These variants are likely to represent novel keratoconus variants. Using the same method, we identified 24 novel keratoconus variants from CCT variants ($P < 5 \times 10^{-8}$ to $P < .05/221 = 2.26 \times 10^{-4}$) near or within genes such as *FNDC3B*, *CCDC80* (OMIM 608298), *SKI* (OMIM 164780), *CD34* (OMIM 142230), *ZBTB38* (OMIM 612218), *SMC5* (OMIM 609386), and *RP11-493L12.3* (OMIM 600138) (eTable 6 in the Supplement).

Influence of Top Loci on Mendelian Disease Genes

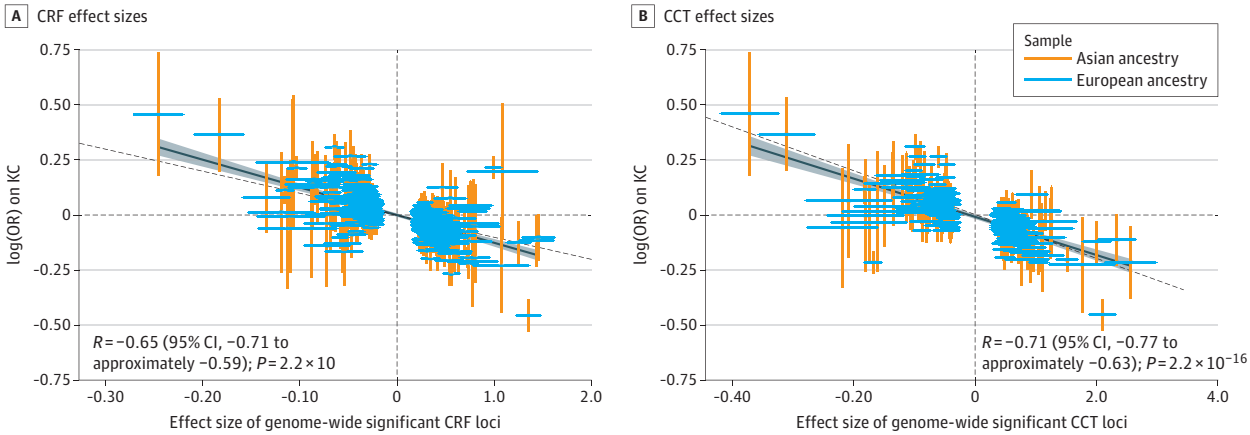
We examined whether the CRF- and CCT-associated loci are nearby (<1 Mb) rare mendelian disease genes. Of the CRF and CCT loci, we discovered that 213 CRF and 134 CCT loci are near genes associated with the rare cornea or connective tissue diseases. We also identified several CRF and CCT loci harbored within 1 Mb of mendelian genes associated with rare corneal or connective tissue diseases. These included *FECD7* (OMIM 613271), *TCF4* (OMIM 602272), *SLC4A11* (OMIM 610206), *UBIAD1* (OMIM 611632), associated with corneal dystrophy; *MPV17* (OMIM 137960), associated with Charcot-Marie-Tooth disease; *ZNF513* (OMIM 613598) and *PCARE* (OMIM 613425), involved in retinitis pigmentosa; *CRYBA2*, a cataract gene; *PLOD1* (OMIM 153454) (779 kilobase [kb] downstream to

Figure 2. Effect Size of Corneal Resistance Factor (CRF) and Central Corneal Thickness (CCT) Variants



Effect sizes for CRF and CCT top loci in European and Asian ancestry data sets are shown. The error bars represent 95% CIs, which are large owing to the sample size of the Asian group. Dashed lines represent the y=x line, solid lines represent the linear regression line of effect sizes around ancestries, and shaded areas represent 95% CIs around the linear regression line.

Figure 3. Comparison of Effect Size Between Corneal Resistance Factor (CRF) and Central Corneal Thickness (CCT) Top Loci and Keratoconus (KC) Loci



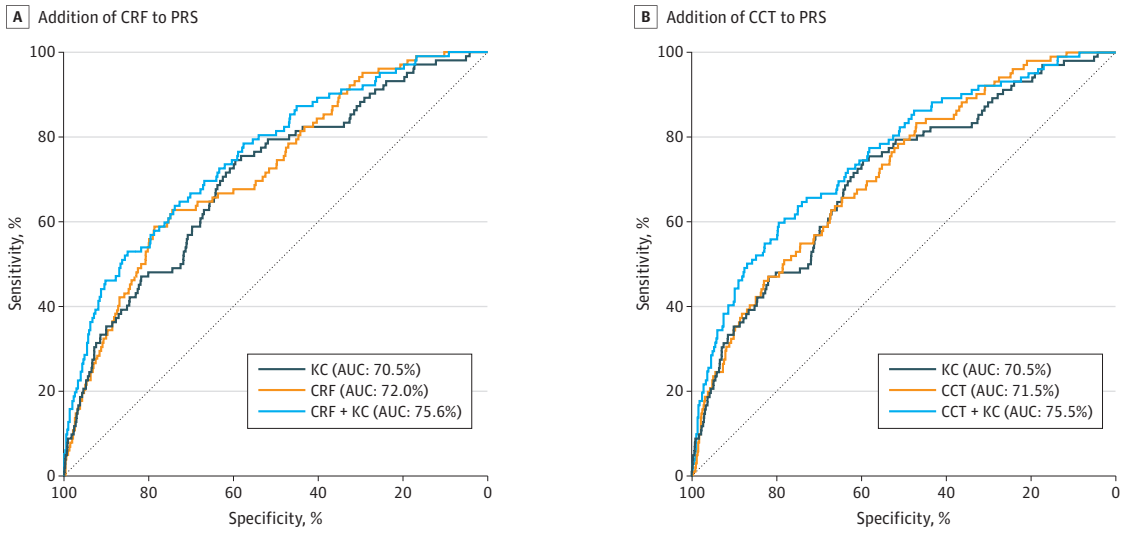
We normalized the β value of CCT to make it on the same scale as CRF and KC. The error bars represent 95% CIs for variant effect sizes. Dashed lines represent the y=x line, solid lines represent the linear regression line of effect sizes between CRF and CCT top loci and KC loci, and shaded areas represent 95% CIs around the linear regression line. OR indicates odds ratio.

rs59862925, 394 kb downstream to rs79174949), involved in Ehlers-Danlos syndrome; *EFEMP1* (OMIM 601548) (54 kb downstream to rs143247366), involved in Doyne honeycomb degeneration of retina; *DMP1* (OMIM 600980), the hypophosphatemic rickets gene; and *ANTXR1* (OMIM 606410), related to GAPO (growth retardation, *a*lopecia, *p*seudoanodontia, and progressive *o*ptic atrophy) syndrome. Some of the connections are consistent with the previous result (*COL5A1* [OMIM 120215], *ZNF469* [OMIM 612078], *COL8A2* [OMIM 120252], *AGBL1* [OMIM 615496], *SMAD3* [OMIM 603109], *DCN* [OMIM 125255], *KERA* [OMIM 603288], and *TGFB2* [OMIM 190220]).[26]

Use of a PRS for Keratoconus

We constructed a PRS using the β values of the lead CRF loci as weights. We then repeated this for the lead CCT loci. We tested the predictive ability of the PRS in 102 cases with keratoconus and 142 493 controls from the UKB European samples (*International Statistical Classification of Diseases and Related Health Problems, Tenth Revision*, diagnostic code H18.6). The CRF- and CCT-associated variants typically demonstrated a strong negative correlation with keratoconus risk, but there were a few clear outliers, including the well-known association at *ZNF469* at which the CCT-increasing allele actually increases keratoconus risk.[37] We hence inspected plots of the CRF and CCT variant effect sizes against the keratoconus variant effect sizes and removed 7 CRF and 7 CCT variants with strongly discordant direction of effects (95% CI bounds outside expected quadrants) (eFigure 4 in the Supplement), resulting in 362 CRF and 226 CCT variants being included in the PRS. The AUC of the PRS for the CRF variant was 0.720 and the AUC of the PRS for the CCT variant was 0.715. To assess whether CRF and CCT variants would help improve the prediction of

Figure 4. Comparison of the Area Under the Receiver Operating Characteristic Curve (AUC) of Different Keratoconus (KC) Polygenic Risk Score (PRS) Models



Our model was tested in 102 patients with KC and 142 493 controls from the UK Biobank database. The AUCs were significantly increased when adding CRF and CCT PRS to the keratoconus PRS. CCT indicates central corneal thickness; CRF, corneal resistance factor.

keratoconus, we tested the predictive ability of 36 lead variants from the study by Hardcastle et al[28] in the UKB keratoconus case-control set. The AUC of PRS for 36 keratoconus variants was 0.705, but the AUC improved significantly ($P = 9.4 \times 10^{-4}$) when adding the CRF-based PRS to the model (AUC = 0.756 for the PRS CRF variants plus the PRS 36 keratoconus variants). We also observed significant ($P = 5.9 \times 10^{-4}$) AUC improvement of PRS (AUC = 0.755) from the PRS CCT variants plus the PRS 36 keratoconus variants (**Figure 4**).

Discussion

In this study, we integrated the CRF measures from the UKB and CLSA cohorts with CCT from the IGGC cohort using a multitrait approach. We discovered 369 genomic areas linked with CRF variations and 233 genomic areas linked with CCT variations, including 36 new CRF loci and 114 new CCT loci. We also identified a total of 29 CRF and 24 CCT variants that are likely to be the keratoconus variants.

The genetic correlation between CRF and CCT is moderately high (0.69 [SE, 0.049]). We hence found pleiotropy in many genes associated with both traits. For example, *ST7L* has been reported to be associated with CCT[26] and glaucoma,[26,38] and the findings of the present study confirm its association with CRF. *ST7L* is clustered in a tail-to-tail manner with the *WNT2B* gene in a genomic region known to be deleted and rearranged in many kinds of cancers.[39] *HERC2* was also associated with both CRF and CCT loci in our study. *HERC2* is known to affect several eye functions.[40-42] Shah et al[43] suggested that *HERC2* is associated with refractive astigmatism and myopia; myopia is a risk factor for keratoconus.[44] Moreover, rs1129038[45] in *HERC2* affects both blue eyes and CRF and CCT in our study. *CHRNB1* encodes acetylcholine receptor subunit beta[46] and has been associated with the slow-channel syndrome[46] and Parkinson disease.[47,48] We also found several genes near the novel CRF and CCT variants that are associated with rare corneal or connective tissue disorders. Our finding confirms the previously reported CCT genes that are associated with collagen and extracellular matrix.[26] We identified CRF-associated variants in mendelian disorder genes such as *DCN* (OMIM 125255) and *KERA* (OMIM 603288). We found a total of 54 genes nearby 1 or multiple CRF variants and 55 genes nearby 1 or multiple CCT variants that relate to rare eye and connective tissue disorders, which can be regarded as the candidate gene in quantitative corneal parameter studies. Variants near *CD34* (rs2745950 and correlated variants) were also associated with both CRF and CCT and with keratoconus.[28] *CD34* encodes CD34 protein, an important adhesion molecule; this gene is expressed in human corneal keratocytes and may play a role in anchoring the keratocytes in their microniche between the collagen lamellae.[49]

Previous studies[35,50,51] have demonstrated the statistically significant correlation among CRF and CCT and keratoconus. Our results demonstrate that both CRF and CCT can be leveraged as reliable endophenotypes of keratoconus, consistent with previous studies.[21,35]

Of the novel keratoconus loci, most of the CRF-associated genes overlapped with CCT-associated genes, but 5 loci (*THBS4*, *MXRA7*, *SEC24D*, *PTRH2*, and *GLIS3)* were only associated with CRF (eTables 5 and 6 in the Supplement). Four of these (*THBS4*, *PTRH2*, *SEC24D*, and *GLIS3*) are involved in the extracellular matrix pathways[52-55] previously implicated in both CCT regulation and keratoconus susceptibility.[26,28,35,37] The function of *MXRA7* remains unknown, but *MXRA6* in this family was related to myofibroblast differentiation and extracellular matrix formation[56] (eResults in the Supplement).

Polygenic risk scores have been successfully applied to estimate risk and response to therapies of several eye diseases; for example, a PRS for glaucoma was used for disease risk

stratification,[38] and an age-related macular thickness study combined a PRS with 1-year outcome for estimating the response to aflibercept therapy.[57] A keratoconus PRS may help identify persons at high risk who might benefit from corneal imaging, allowing for earlier diagnosis and treatment (eg, corneal collagen cross-linking).[58] We evaluated the prediction power of MTAG-derived PRS models based on our CRF and CCT lead variants and on 36 published keratoconus variants.[28] For CCT- and CRF-based PRS, we removed a few extreme outlier variants such as the well-known *ZNF469* variant[37] rs9938149, which decreases CCT but also reduces keratoconus risk and conflicts with the strong negative correlation between CRF and CCT and keratoconus[1,37] (eResults and eFigure 5 in the Supplement). To avoid rs9938149-like CRF and CCT variants with similarly discordant effects on keratoconus from adversely affecting the PRS, we removed 7 variants from CRF and CCT data. Adding the CRF PRS to the keratoconus PRS enhanced the prediction of keratoconus, whereas the CCT PRS achieved similar prediction accuracy with fewer variants. The improved PRS performance for keratoconus can potentially benefit clinical screening and treatment.

Limitations

The first concern about our result was the incomplete replication in the Asian population, likely attributable to low power. To gain power for GWAS, we meta-analyzed the South Asian and East Asian groups from UKB. Previous work suggested the CRF distributions in Indian and Chinese populations were similar,[59] but herein we observed differences in the mean CRF measurement between UKB East and South Asian groups. We noticed the concordance of the effect size of some CRF/CCT variants between European and Asian populations; however, as the result of the limited sample size, most of these variants have large standard errors of the β value in the Asian replication cohort. In the future, larger studies are warranted to accurately characterize the cross-ethnic effect sizes for other variants with a lower magnitude of association. Second, because we did not have access to the keratoconus GWAS with the UKB samples removed to formally test the estimate, there was an overlap between the UKB data set we used to test the PRS and the previously published keratoconus meta-analysis used to identify the 7 discordant variants; this could conceivably bias the identification of the 7 variants, although given that the UKB data set comprises only 2% of the full published keratoconus GWAS sample set, the bias is expected to be minimal.

Conclusions

In this GWAS, we identified variants and genes associated with CRF and CCT, enhancing our understanding of the biological processes underpinning CRF, CCT, and keratoconus. Our findings suggest an association between our PRS model based on CCT and CRF and improved accuracy for identifying keratoconus. These findings underscore the power of multitrait GWAS in identifying new disease-related variants using quantitative traits.

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Author Affiliations: QIMR Berghofer Medical Research Institute, Brisbane, Australia (He, Han, Ong, Gharahkhani, MacGregor); School of Medicine, University of Queensland, Brisbane, Australia (He, Han); Centre for Eye Research Australia, University of Melbourne, Royal Victorian Eye and Ear Hospital, East Melbourne, Australia (Hewitt); School of Medicine, Menzies Institute for Medical Research, University of Tasmania, Hobart, Australia (Hewitt); Lions Eye Institute, Centre for Ophthalmology and Visual Science, University of Western Australia, Perth, Australia (Mackey).

Author Contributions: Mr He had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Concept and design: He, Mackey, Gharahkhani, MacGregor.
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Drafting of the manuscript: He, MacGregor.
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Members of the International Glaucoma Genetics Consortium: Tin Aung, PhD, Kathryn P. Burdon, PhD, Ching-Yu Cheng, PhD, Jamie E. Craig, DPhil, Angela J. Cree, BSc, Puya Gharahkhani, PhD, Christopher J. Hammond, MD, Alex W. Hewitt, PhD, René Höhn, MD, Pirro Hysi, PhD, Adriana I. Iglesias Gonzalez, PhD, Jost Jonas, PhD, Anthony Khawaja, PhD, Chiea-Cheun Khor, PhD, Caroline C.W. Klaver, PhD, Francesca Pasutto, PhD, Stuart MacGregor, PhD, David A. Mackey, PhD, Paul Mitchell, PhD, Aniket Mishra, PhD, Calvin Pang, PhD, Louis R. Pasquale, MD, Francesca Pasutto, PhD, Henriette Springelkamp, PhD, Gudmar Thorleifsson, PhD, Unnur Thorsteinsdottir, PhD, Cornelia M. van Duijn, PhD, Ananth Viswanathan, PhD, Veronique Vitart, PhD, Janey L. Wiggs, PhD, Robert Wojciechowski, MD, Tien Wong, PhD, Terri L. Young, PhD, and Tanja Zeller, PhD.

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