WNT10A exonic variant increases the risk of keratoconus by decreasing corneal thickness

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Abstract

Keratoconus is a degenerative eye condition which results from thinning of the cornea and causes vision distortion. Treatments such as ultraviolet (UV) cross-linking have proved effective for management of keratoconus when performed in early stages of the disease. The central corneal thickness (CCT) is a highly heritable endophenotype of keratoconus, and it is estimated that up to 95% of its phenotypic variance is due to genetics. Genome-wide association efforts of CCT have identified common variants (i.e. minor allele frequency > 5%). However, these studies typically ignore the large set of exonic variants whose minor allele frequency is usually low. In this study we performed a CCT exome-wide association analysis in a sample of 1029 individuals from a population-based study in Western Australia. We identified a genome-wide significant exonic variant rs121908120 ($P = 6.63 \times 10^{-10}$) in $WNT10A$. This gene is 437kb from a gene previously associated with CCT ($USP37$). We showed in a conditional analysis that the $WNT10A$ variant completely accounts for the signal previously seen at $USP37$. We replicated our finding in independent samples from the Brisbane Adolescent Twin Study, Twin Eye Study from Tasmania and the Rotterdam Study. Further, we genotyped rs121908120 in 621 keratoconus cases and compared the frequency to a sample of 1680 unscreened controls from the Queensland twin registry. We found that rs121908120 increases the risk of keratoconus two times (odds ratio 2.03, $P = 5.41 \times 10^{-5}$).
Introduction

Keratoconus is a degenerative eye disease with an incidence of around 1 in 2000 in the general population(1). It is characterized by thinning and weakening of the cornea, and its symptoms range from mild astigmatism and myopia to severe vision distortion. Corneal collagen ultraviolet (UV) cross-linking is a minimally invasive and effective option for management of keratoconus at early stages(2) achieving biomechanical stabilization of the cornea and reducing (or in some cases halting) the disease progression rate. However, it is not uncommon for patients with mild or early stages of keratoconus to be misdiagnosed as cases of astigmatism or myopia and undiagnosed keratoconus can lead to corneal ectasia following laser refractive surgery (LASIK) (3). This makes it particularly important to find biomarkers that can point to keratoconus in its earliest stage.

Previous work has shown that keratoconus risk is affected by both genetic and environmental factors(4, 5). Several strategies have been pursued to identify the genetic risk factors of keratoconus; however, given the low prevalence of the disease, it has been difficult to perform well powered genomic studies(6). In contrast, genome-wide association studies (GWAS) of central corneal thickness (CCT), a highly heritable biometric trait which functions as endophenotype of keratoconus, have successfully identified 27 associated loci(7). Lu et al(7) found that several of these CCT loci were also associated with keratoconus in a case-control analysis.

The identified CCT variants only explain around 8% of the variability of the trait(7). CCT is highly heritable (~90%)(8) and hence there is substantial missing heritability. One possible component of the missing heritability is low frequency variants. The published CCT GWASs to date focused primarily on common variants (i.e. minor allele frequency (MAF) > 5%). This approach ignores a large number of coding exome variants, where the MAF is usually lower. Therefore, to determine the role of low-frequency coding variants in CCT, we evaluated putative functional coding variants from the Illumina Human Exome array. We performed the association using genotype data from 1029 individuals from the Raine cohort(9). We replicated our results in two independent samples from i) the Rotterdam Study(10) and ii) Brisbane Adolescent Twin Study(11, 12). Further, we investigated the significant associations with CCT in a sample of 621 Australian keratoconus cases and 1680 unscreened controls.

Results

We performed an exome-wide association analysis of CCT using data from the Western Australian Pregnancy (Raine) Cohort(9). A sample of 1029 unrelated individuals of European descent and with CCT measures were used to test the association of the 43,435 exonic variants with a MAF>0.25% passing quality control. Sample characteristics are summarized in Table 1. We performed the
association analysis of each variant through linear regression analysis adjusting for sex, age and the first 3 genetic principal components (PCs). The genomic inflation factor ($\lambda$) with respect to the median of $\chi^2$-statistics was 1.006 suggesting no inflation in the test statistics due to population structure(13) (Figure 1a.).

Figure 2 shows the results of the analysis. One single nucleotide polymorphism (SNP) reached the threshold of genome wide significance ($P = 5.0 \times 10^{-8}$): rs121908120 in $WNT10$ on chromosome 2 ($\beta = -23.84 \pm 3.92, P = 6.63 \times 10^{-10}$). $WNT10A$ is expressed in all the ocular tissues reported in the ocular tissue database(14). The SNP rs121908120 causes a missense mutation in $WNT10A$ which results in a change in the amino acid 228 from phenylalanine to Isoleucine. According to SIFT(15) and PolyPhen(16), this missense mutation is deleterious (score 0) and probably damaging (score 0.994), respectively. This variant is 437kb upstream of rs10189064 ($P = 3.11 \times 10^{-4}$) in the $USP37$ gene, which was previously associated with CCT(7). These two variants are in moderate linkage disequilibrium ($R^2 = 0.369$). In order to assess the extent of independent effect of these two SNPs, we performed conditional analysis (i.e. using one as covariate and testing the other and vice versa). The results are summarized in Table 2. Our results show that conditioning rs121908120 by rs10189064 does not reduce the effect ($\beta = -23.75 \pm 5.33$) of the SNP; however the p-value goes down to $9.28 \times 10^{-6}$ probably due to a reduction in sample size, as just 938 individuals had information on the SNP rs10189064. On the other hand, conditioning rs10189064 on the variant in $WNT10A$ removes the effect completely -the effect changes from $\beta = -14.57 \pm 4.02$ ($P = 3.11 \times 10^{-4}$) to $\beta = 0.44 \pm 5.21$ ($P = 0.93$). This suggests that the previously identified associated SNP in $USP37$ is likely due to linkage disequilibrium (LD) confounding with the variant in $WNT10A$.

We replicated these results using data from the Rotterdam Study(10) (Table 2). The total sample size of this replication cohort was $n=4,479$. Although the effects were moderately smaller in these samples, the association signal for rs121908120 was clearly replicated ($\beta = -12.68 \pm 2.75, P = 3.87 \times 10^{-6}$). The results remained similar after conditioning on rs10189064 ($\beta = -10.92 \pm 3.7, P = 3.21 \times 10^{-3}$). In addition, we used available exome data from 147 participants from the Brisbane adolescent twin study (BATS) and Twin Eye Study from Tasmania (TEST)(11, 12). However, this sample only had rs121908120 genotyped. We found that the effect in this sample replicates our finding ($\beta = -28.73 \pm 14.05, P = 0.04$).

Although rs121908120 is the strongest candidate SNP in the region, we used the online tool LocusTrack (17) to look for additional SNPs in high LD with rs121908120, based on the 1000 Genomes phase 3 European ancestry reference set. The only variant (rs146199923) with $r^2=1$ was 100kb downstream of rs121908120, close to the FEV gene (Supplementary Figure 3). Examining, GeneCards(18) and dbSNP, rs146199923 is not a strong candidate SNP as lies in an intergenic region outside conserved transcription factor binding sites and DNaseI hotspots.
We also investigated the effects of exome variants in previous associated loci(7). Figure 1a displays the \textit{p-value} distribution of SNPs within CCT known genes along with the distribution of all SNPs assessed in this study. We did not find any evidence of associated exome variants in these genes.

In addition to per SNP testing, gene-based analysis was done using the optimal unified approach SKAT-O(19). However, the approach did not alter our conclusions, as \textit{WNT10A} \((P = 1.65 \times 10^{-10})\) was the only significant association after correction for multiple testing [Figure 1b]. The top 10 results for the gene-based test are summarized in Table 3. \textit{WNT10A} was not associated in the gene-based analysis if rs121908120 was omitted \((P=0.29)\). Following a similar approach, we performed pathway analysis. However, in order to avoid capturing the same signal as previous experiments we removed all SNPs within the \textit{WNT10A} gene before the analysis. Although no pathways passed the significance threshold, interestingly, the top pathway (GO:2000096, \(P = 2.57 \times 10^{-4}\)) was the one described as the positive regulation of the \textit{Wnt} receptor signaling pathway [Table 4]. Analyses from Lu et al indicated that extracellular matrix and collagen pathways are associated with CCT(7). Analogous to inspecting variants within known associated genes, we examined the distribution of \textit{p}-values in the collagen and extracellular matrix pathways and found an enrichment of small \textit{p}-values for the extracellular matrix \((\lambda=2.14)\) and collagen pathways \((\lambda=2.32)\) [Figure 1c].

Further, we genotyped rs121908120 in 621 keratoconus cases and used data from 1680 individuals from the Queensland twin registry, genotyped on the Illumina HumanCoreExome array, as unscreened controls. The rs121908120 MAF in keratoconus cases was 0.05 while in controls it was 0.024 translating to a 2.03 fold increase in risk (Fisher exact test \textit{p}-value = 5.41x10^{-5}).

\textbf{Discussion}

Our study identified a missense mutation (rs121908120) in \textit{WNT10A} associated with CCT and keratoconus. Previous GWAS of CCT identified rs10189064 in the \textit{USP37} gene(7), which is in moderate LD with this newly found variant rs121908120. However, the SNP in \textit{WNT10A} has a bigger effect on CCT, and completely accounts for the signal previously seen at \textit{USP37} in the analyzed samples.

Aside from the \textit{USP37}/\textit{WNT10A} region, we did not detect association of exonic variants in or near CCT associated loci from a previous study focusing on common variation(7). This indicates that the tagged SNPs are not in LD with the previously identified common variants in those genes, or the sample size is too small to detect any association.

\textit{WNT10A} belongs to the WNT gene family. This family consists of structurally related genes encoding secreted signaling molecules that have been implicated in oncogenesis and in several developmental processes, including regulation of cell fate and patterning during embryogenesis(20, 21). Studies have
shown that corneal endothelial cell fate is maintained by the hedgehog and WNT pathways(22, 23). The corneal endothelium is responsible for maintaining the transport of fluids and solutes to the corneal stroma (which accounts for up to 90% of the total corneal thickness). A reduced endothelial cell density can have an impact on this fluid regulation leading to stromal swelling and scarring due to excess fluid(24, 25), which has also been described as a complication of keratoconus(26).

A handful of studies have described structural changes in the corneal epithelium in keratoconic eyes (27-29). The corneal epithelium is an extremely thin layer composed of epithelial tissue covering the front of the cornea. Cornea epithelial cells renew continuously from limbal stem cells (LSCs) in order to maintain transparency for light transmission. A deficiency in LSCs can lead the cornea into a non-transparent or keratinized skin like epithelium(30). Molecular analysis of the Wnt signaling pathway in limbal stem cells have shown that WNT2, WNT6, WNT11, WNT16B are over-expressed in the limbal region, while the expression of WNT3, WNT7A, WNT7B and WNT10A is upregulated in the central cornea (mature corneal epithelium)(20). Based on this, we examined the p-value distribution of WNT genes and SNPs within them. We observed that in aggregate these genes tend to have small p-values, although non-significant (Figure 1a, Figure 1b and Table 5). The latter suggests that might be a matter of power or fine mapping of causal variants to see significant associations within these genes.

The strong association of WNT10A found in keratoconus adds evidence to its possible role in cornea stability. In addition, studies indicate that mutations in WNT10A are also a risk factor for ectodermal dysplasia including odonto-onycho-dermal dysplasia(31, 32). This syndrome is associated with abnormalities of skin as well as epidermally derived structures including, hair, teeth, nails, tongue, and sweat glands. Hair including eyelashes is typically thin and sparse. Ocular features include chronic tearing, photophobia, and keratitis(33).

Other diseases that show corneal thinning as clinical feature include connective tissue disorders and osteogenesis imperfecta(34, 35). Wnt signalling is essential for maintaining bone density and the homeostasis in connective tissue(36-38). Our finding adds evidence on the link of these disorders to corneal thinning.

Pathway analyses performed by Lu et al(7) associated collagen and extracellular matrix pathways to CCT. Collagen fibrils are a major component of the cornea’s extracellular matrix (39) and are the building blocks of the corneal stroma and Bowman’s layer(40, 41).Our study was underpowered to detect significant pathway associations. However, we observed smaller p-value in these pathways than the expected from a uniform distribution.

In conclusion, our findings indicate that WNT10A plays a role in the corneal thickness homeostasis and that the mutation rs121908120 is a risk factor for keratoconus. Also, this finding adds evidence to the association of WNT10A to odonto-onycho-dermal dysplasia and the link of connective tissue
disorders with corneal thinning. Furthermore, suggestive results on the association of WNT genes, and the fact that they are expressed in the different ocular tissues, indicate that may be a matter of extending the sample size or a finer mapping of variants to detect their association.

Materials & Methods

Our study consisted of two phases: in the first phase, we performed exome-wide association with CCT using the Raine study sample as discovery and the Rotterdam Study, the Brisbane adolescent twin study (BATS) and Twin Eye Study of Tasmania (TEST) for replication. In the second phase, we investigated the associated variant in Australian keratoconus patients and unscreened controls from the Queensland Twin Registry (QTwin).

Raine
Sample

Recruitment of the Western Australian Pregnancy (Raine) cohort has previously been described elsewhere in detail(42). In brief, between 1989 and 1991 2,900 pregnant women were recruited prior to 18-weeks gestation into a randomized controlled trial to evaluate the effects of repeated ultrasound in pregnancy. Children have been comprehensively phenotyped from birth to 21 years of age (average ages of one, two, three, six, eight, ten, fourteen, seventeen and twenty-one) by trained members in the Raine research team. Most of the children are of Caucasian ethnicity. Data collection included questionnaires completed by the child’s primary carer and by the adolescent from age 14, physical assessments by trained assessors at all follow up years, DNA collection from year 14 follow-up. The study was conducted with appropriate institutional ethics approval, and written informed consent was obtained from all mothers.

Phenotypes

At age 21, participants were invited for an eye study. CCT was obtained from the Pupil Center Pachymetry readout obtained by anterior segment tomography of each dilated eye taken with an Oculus Pentacam (Optikgerate GmbH, Wetzlar, Germany)(9).

Exome array

A total of 1825 participants were genotyped using the Illumina HumanExome-12v1_A array includes 247,870 markers. Approximately 90% of the markers are coding variants selected from >12,000 exome and genome sequences representing multiple ethnicities and complex traits. The remaining 10% comprises variants that have been associated with complex traits in previous Genome Wide Association Studies (GWAS), ancestry-informative markers, markers for identity-by-descent estimation, random synonymous SNPs and HLA tags(43). Genotype calling was carried out in two
steps. First, we called genotypes using Illumina GenomeStudio GenTrain clustering algorithm, together with the Illumina HumanExome-12v1_A product files. Quality control in the initial genotypes was done by excluding samples with a calling rate below 95%, and variants with a GeneTrain score <0.15, calling rate <0.95 or heterogeneity excess <-0.3 or >0.2. We performed principal component (PC) analysis, and excluded samples that were above 6s.d from the centroid of the 1000 Genomes(44) European population (GBR+CEU+FIN) PC1 and PC2. In the second step we used zCall(45) with the default parameters to improve calling of rare variants on the remaining samples. We excluded variants with calling rate < 99%, or which deviate from Hardy-Weinberg equilibrium (HWE) P < 10^{-6}, resulting in 235,619 variants and 1563 individuals passing quality control (QC).

**Statistical analysis:**

Among the genotyped individuals passing QC, 1029 unrelated individuals (proportion of identity by descent <0.2) counted with CCT phenotype data. To ensure that the variants tested had at least 5 copies of the minor allele, we restricted the analyses to just those variants with a MAF > 0.25% (i.e. 43,435 SNPs). Single SNP based analysis was carried out using linear regression in plink(46, 47) and adjusting by sex, age and the first 3 PCs. The genotype cluster plot for the top associated variant rs121908120 is displayed in Supplementary Figure 1.

Gene and pathway based association analyses were performed using SKAT-O(19), which performs association test of SNP sets and optimally combines the burden test and the nonburden sequence kernel association test (SKAT). Gene-based SNP sets were created using the SNP-gene annotation file from the Illumina Human-Exome bead-chip. Pathways were based on the Gene Ontology database(48). Pathway-based SNP sets were composed by the SNPs within the genes involved in each particular pathway.

We performed conditional analysis of rs10189064 and rs121908120 using Plink(46). Given that the Illumina HumanExome-12v1_A does not contain rs10189064 among the tagged SNPs, we extracted the rs10189064 genotype from 938 individuals that were also genotyped in the Human660W-Quad bead chip for previous experiments. Genotyping and quality control details for the Human660W-Quad bead chip in the Raine sample are described elsewhere(7). In brief 1593 individuals were genotyped in 2009 using the Human660W-Quad bead chip, as part of quality control (QC), the data were filtered by single nucleotide polymorphism (SNP) call rate <0.95, a Hardy-Weinberg equilibrium (HWE) p-value < 10^{-6} and a minor allele frequency (MAF) > 1%. To exclude population outliers, a principal component analysis (PCA) was carried out using SNPs with genotyping rate >0.98.
The Rotterdam Study

Samples

The Rotterdam Study is a population-based study held in Rotterdam, the Netherlands (10). It consists of three cohorts. The original cohort, RS-I, started in 1990 and includes 7,983 subjects aged 55 years and older. The second cohort, RS-II, was added in 2000 and includes 3,011 subjects aged 55 years and older. The last cohort, RS-III, includes 3,932 subjects of 45 years of age and older and started in 2006. The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the “Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study)”. All participants provided written informed consent to participate in the study and to obtain information from their treating physicians.

Phenotype

CCT was measured using ultrasound pachymetry (Allergan Humphrey 850, Carl Zeiss Meditec, Dublin, CA, USA; subset of RS-I), and using a non-contact biometer (Lenstar LS900, Haag-Streit, Köniz, Switzerland; subset of RS-I, RS-II, and RS-III).

Genotyping and association

Genotyping of SNPs was performed using the Illumina Infinium II HumanHap550 array (RS-I), the Illumina Infinium HumanHap 550-Duo array (RS-I, RS-II), and the Illumina Infinium Human 610-Quad array (RS-I, RS-III). Samples with low call rate (<97.5%), with excess autosomal heterozygosity (>0.336), or with sex-mismatch were excluded, as were outliers identified by the identity-by-state clustering analysis (outliers were defined as being >3 s.d. from population mean or having identity-by-state probabilities >97%). A set of genotyped input SNPs with call rate >98%, MAF >0.1% and Hardy-Weinberg P-value >10^-6 was used for imputation. The Markov Chain Haplotyping (MACH) package version 1.0 software (49)(Rotterdam, The Netherlands; imputed to plus strand of NCBI build 37, 1000 Genomes phase 1 version 3) and minimac version 2012.8.6 was used for the imputation. Number of samples remained for RS are summarized in Table 1. Imputation quality (r^2) for rs121908120 was RSI=0.61, RSII=0.57 and RSIII=0.63 and for rs10189064 r^2 was above 0.99 in the three studies. Association analyses of rs121908120 and rs10189064 variants with CCT were performed using the ProbABEL package(50) using age, sex, the first 5 PCs and the technique of measurement (the latter only for RS-I) as covariates.
**Brisbane adolescent twin study and twin eye study in Tasmania**

*Samples*

Methodologies and recruitment of participants from the Brisbane adolescent twin study (BATS) and twin eye study in Tasmania (TEST) are described elsewhere(11, 12).

*Phenotype*

CCT was measured in this cohort using ultrasound pachymetry and recorded for both eyes. Measurements were performed using a Tomey SP 2000 (Tomey Corp., Nagoya, Japan).

*Genotyping and association*

Genotyping of 147 individuals from BATS and TEST with eye phenotype was performed using the Illumina HumanCoreExome array. Samples and SNPs with low call rate (<98%) were excluded, as well as variants with MAF <0.1% and Hardy-Weinberg P-value >10^-6. Association was performed using Merlin which effectively accounts for family structure(51). Age, sex and the first 3 principal components were used as covariates.

**Queensland twin registry**

*Samples*

Methodologies and recruitment of the Queensland Twin registry are described elsewhere (REF above). The unscreened controls for the keratoconus samples were a subset of the BATS and TEST projects. These controls were family members from BATS and TEST projects, selected to be unrelated to the 147 BATS and TEST individuals included in the CCT scan. The unscreened controls from were pruned to remove related individuals (typically both parents of a twin pair were used as controls).

*Genotyping*

Genotyping was carried out as described for the BATS and TEST CCT samples.

*Keratoconus cases*

*Sample*

Australian participants with keratoconus (n=621) were ascertained through the Department of Ophthalmology of Flinders Medical Centre, Adelaide, Australia; private optometry practices in Adelaide and Melbourne, Australia; the Royal Victorian Eye and Ear Hospital, Melbourne, Australia; and by Australia-wide mail out to members of Keratoconus Australia, a community-based support group for patients.
**Phenotypes**

The diagnosis of keratoconus was based on clinical examination and videokeratography pattern analysis. Clinical examination included slit lamp biomicroscopy, cycloplegic retinoscopy, and fundus evaluations. Slit lamp biomicroscopy was used to identify stromal corneal thinning, Vogt's striae, or a Fleischer ring. A retinoscopic examination was performed with a fully dilated pupil to determine the presence or absence of retroillumination signs of keratoconus, such as the oil droplet sign and scissoring of the red reflex. Videokeratography evaluation was performed on each eye by topographic modeling. Patients were considered as having keratoconus if they had at least one clinical sign of the disease and by confirmatory videokeratography map with an asymmetric bowtie pattern with skewed radial axis above and below the horizontal meridian (AB/SRAX). A history of penetrating keratoplasty performed because of keratoconus was also sufficient for inclusion.

**Genotyping**

Genotyping of rs121908120 in 621 individuals was completed using a pre-designed Taqman assay (Life Technologies), amplified in SensiFAST Probe No-ROX master mix (Bioline) on a LightCycler480 Real-time PCR Machine (Roche), according to manufacturer’s protocol. The genotyping cluster plot for rs121908120 genotyping is displayed in Supplementary Figure 2.

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A full set of summary association statistics are available at gump.qimr.edu.au/general/gabrieC/CCT”

Conflicts of interest

The authors declare that there are no conflicts of interest.
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**Figure 1.** Quantile-Quantile plots of single SNP association (a), gene-based association (b) and pathway-based (c) results in the discovery cohort (Raine study). Each dot represents an observed statistic (-log10P) versus the corresponding expected statistic. The black line corresponds to the null distribution. Dotted lines show the significance threshold based on a Bonferroni correction for multiple testing.

**Figure 2.** Manhattan plot of association results for central corneal thickness in the discovery cohort (Raine study). The plot shows -log10–transformed p-values for all single nucleotide polymorphisms. The dotted horizontal line represents the threshold of genome-wide significance (p-value < 5.0 x 10^-8).
Table 1. Descriptive statistics of the samples.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>CCT (SD) (μm)</th>
<th>Age (SD) (years)</th>
<th>Males (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raine</td>
<td>1,029</td>
<td>538.2 (32.3)</td>
<td>20.1 (0.4)</td>
<td>48%</td>
</tr>
<tr>
<td>BATS/TEST</td>
<td>147</td>
<td>554.5 (33.8)</td>
<td>22.6 (12.2)</td>
<td>54%</td>
</tr>
<tr>
<td>RS-I</td>
<td>873</td>
<td>544.4 (33.9)</td>
<td>76.3 (6.7)</td>
<td>48%</td>
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<tr>
<td>RS-II</td>
<td>1,215</td>
<td>547.7 (34.2)</td>
<td>72.6 (5.3)</td>
<td>47%</td>
</tr>
<tr>
<td>RS-III</td>
<td>2,391</td>
<td>550.3 (33.9)</td>
<td>62.3 (5.8)</td>
<td>43%</td>
</tr>
</tbody>
</table>

Abbreviations: CCT: Central corneal thickness; BATS/TEST: Brisbane Adolescent Twin Study / Twin Eye Study in Tasmania; RS-I, RS-II, RS-III: Rotterdam Study cohorts; SD = standard deviation.
Table 2. The results of the genome-wide association study with central corneal thickness (CCT) as outcome. Only the genome-wide significant single nucleotide polymorphism (SNP) is showed (rs121908120), together with a previously known associated SNP with CCT (rs10189064). Both SNPs were conditioned for the other SNP. Beta = effect size on central corneal thickness (μm) based on the minor allele.

<table>
<thead>
<tr>
<th>SNP Minor / Major allele</th>
<th>Discovery</th>
<th>Replication</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raine</td>
<td>BATS/TEST</td>
</tr>
<tr>
<td></td>
<td>Beta ± s.e</td>
<td>P-value</td>
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<tr>
<td>rs121908120 A/T</td>
<td>-23.8 ± 3.9</td>
<td>6.63E-10</td>
</tr>
<tr>
<td>rs10189064 A/G</td>
<td>-14.6 ± 4.0</td>
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<td>rs121908120 a.f. rs10189064</td>
<td>-23.8 ± 5.3</td>
<td>9.28E-06</td>
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<tr>
<td>rs10189064 a.f. rs121908120</td>
<td>0.44 ± 5.2</td>
<td>9.31E-02</td>
</tr>
</tbody>
</table>

Table 3. Top 10 results from the gene-based association with central corneal thickness (CCT) performed using the SKAT-O approach in the Raine cohort.

<table>
<thead>
<tr>
<th>Gene</th>
<th>P-value</th>
<th>#SNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>WNT10A</td>
<td>1.65E-10</td>
<td>3</td>
</tr>
<tr>
<td>SH3BGR</td>
<td>4.07E-05</td>
<td>4</td>
</tr>
<tr>
<td>ANKR6</td>
<td>4.94E-05</td>
<td>6</td>
</tr>
<tr>
<td>STEAP1B</td>
<td>1.38E-04</td>
<td>1</td>
</tr>
<tr>
<td>ATPBD4</td>
<td>2.28E-04</td>
<td>1</td>
</tr>
<tr>
<td>TAF11</td>
<td>2.78E-04</td>
<td>1</td>
</tr>
<tr>
<td>EFCAB7</td>
<td>4.10E-04</td>
<td>6</td>
</tr>
<tr>
<td>PRRG2</td>
<td>4.32E-04</td>
<td>3</td>
</tr>
<tr>
<td>CROCC</td>
<td>5.56E-04</td>
<td>1</td>
</tr>
<tr>
<td>C6orf1</td>
<td>5.88E-04</td>
<td>1</td>
</tr>
</tbody>
</table>

#SNP = number of single nucleotide polymorphisms used for the gene-based test.
Table 4. Top 10 results from the pathway-based association with central corneal thickness (CCT) performed using the SKAT-O approach in the Raine cohort

<table>
<thead>
<tr>
<th>Pathway</th>
<th>P-value</th>
<th>#S NP</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>GO:2000096</td>
<td>2.57E-04</td>
<td>11</td>
<td>Positive regulation of Wnt receptor signaling pathway</td>
</tr>
<tr>
<td>GO:0030145</td>
<td>2.68E-04</td>
<td>76</td>
<td>Manganese ion binding</td>
</tr>
<tr>
<td>GO:0038180</td>
<td>2.84E-04</td>
<td>19</td>
<td>Nerve growth factor signaling pathway</td>
</tr>
<tr>
<td>GO:1990090</td>
<td>4.39E-04</td>
<td>18</td>
<td>Cellular response to nerve growth factor stimulus</td>
</tr>
<tr>
<td>GO:0035249</td>
<td>5.18E-04</td>
<td>46</td>
<td>Synaptic transmission, glutamatergic</td>
</tr>
<tr>
<td>GO:0048406</td>
<td>6.45E-04</td>
<td>16</td>
<td>Nerve growth factor binding</td>
</tr>
<tr>
<td>GO:0007608</td>
<td>7.25E-04</td>
<td>175</td>
<td>Sensory perception of smell</td>
</tr>
<tr>
<td>GO:0003730</td>
<td>7.36E-04</td>
<td>20</td>
<td>mRNA 3'-UTR binding</td>
</tr>
<tr>
<td>GO:0007520</td>
<td>8.15E-04</td>
<td>53</td>
<td>Myoblast fusion</td>
</tr>
<tr>
<td>GO:0048172</td>
<td>8.41E-04</td>
<td>17</td>
<td>Regulation of short-term neuronal synaptic plasticity</td>
</tr>
</tbody>
</table>

#SNP = number of single nucleotide polymorphisms used for the pathway-based test.
Table 5. Results of *WNT* genes available from the gene-based association with central corneal thickness (CCT) performed using the SKAT-O approach in the Raine cohort.

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Position</th>
<th>Gene</th>
<th>P-value</th>
<th>#SNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>219745254</td>
<td><em>WNT10A</em></td>
<td>1.65E-10</td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td>102222811</td>
<td><em>WNT8B</em></td>
<td>0.06</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>75897369</td>
<td><em>WNT11</em></td>
<td>0.13</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>120969089</td>
<td><em>WNT16</em></td>
<td>0.2</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>116916685</td>
<td><em>WNT2</em></td>
<td>0.2</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>228109164</td>
<td><em>WNT9A</em></td>
<td>0.24</td>
<td>1</td>
</tr>
<tr>
<td>17</td>
<td>44928967</td>
<td><em>WNT9B</em></td>
<td>0.48</td>
<td>1</td>
</tr>
<tr>
<td>17</td>
<td>44839871</td>
<td><em>WNT3</em></td>
<td>0.54</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>49359122</td>
<td><em>WNT10B</em></td>
<td>0.85</td>
<td>1</td>
</tr>
</tbody>
</table>

#SNP = number of single nucleotide polymorphisms used for the gene-based test.
List of abbreviations

BATS = Brisbane Adolescent Twins Study
BMES = Blue Mountains Eye Study
CCT = central corneal thickness
GWAS = genome-wide association study
OR = Odds Ratio
RS = Rotterdam Study
SD = standard deviation
SE = standard error
SNP = single nucleotide polymorphism
TEST = Twins Eye Study in Tasmania