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Family-based Genome-wide Association Study of South Indian Pedigrees Supports WNT7B as a Central Corneal Thickness Locus

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1 **Family-based Genome-wide Association Study of South Indian Pedigrees Supports**
2 ***WNT7B* as a Central Corneal Thickness Locus**

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1 **ABSTRACT**

2 Purpose: Central corneal thickness (CCT) is a highly heritable ocular quantitative trait related to

3 several eye diseases including keratoconus and glaucoma. The genetic risk factors contributing

4 to CCT as well as the average CCT values varies among populations. Genome-wide

5 association studies have not yet been completed for ocular quantitative traits in individuals from

6 South India, a population with a high prevalence of ocular disorders.

7 Methods: 195 individuals from 15 large consanguineous South Indian pedigrees were

8 genotyped using the Omni2.5 bead array. Family-based association, adjusting for age and sex,

9 was conducted using the score test in MERLIN to assess association between single nucleotide

10 polymorphisms (SNPs) and CCT.

11 Results: Genome-wide association analysis for CCT identified strongest association between

12 SNPs located on chromosome 22 in the first intron of *WNT7B* and CCT (top SNP rs9330813; β

13 =- 0.57, 95%CI: -0.78,-0.36; P= 1.7×10^{-7}). We further investigated rs9330813 in a Latino cohort

14 and 4 independent European cohorts. A meta-analysis of these datasets demonstrated

15 statistically significant association between rs9330813 and CCT (β = -3.94, 95%CI: -5.23,-2.66;

16 P= 1.7×10^{-9}). *WNT7B* SNPs located in the same genomic region that includes rs9330813 have

17 been associated with CCT in Latinos but with other ocular quantitative traits related to myopia

18 (corneal curvature and axial length) in a Japanese population (rs10453441 and rs200329677).

19 To evaluate the specificity of the observed *WNT7B* association with CCT in the South Indian

20 families we completed an ocular phenome-wide association study (PheWAS) for the top

21 *WNT7B* SNPs using 45 ocular traits measured in these same families including corneal

22 curvature and axial length. The ocular PheWAS results indicate that in the South Indian families

23 *WNT7B* SNPs are primarily associated with CCT.

1   Conclusion: Overall, we provide robust evidence for an association between *WNT7B* SNPs and

2   CCT, and suggest that *WNT7B* SNPs can have population-specific effects on ocular quantitative

3   traits.

INTRODUCTION

Ocular quantitative traits such as central corneal thickness (CCT), axial length and intraocular pressure are heritable intermediate phenotypes (endophenotypes) for common complex eye disorders such as keratoconus, myopia and glaucoma[1]. CCT is a highly heritable ocular quantitative trait with up to 95% of its phenotypic variance due to genetics[2]. Thin CCT is related to several diseases of the cornea, especially keratoconus[3] and brittle corneal syndrome[4]. Very thin corneas are a hallmark of Ehlers Danlos[5] and thicker than normal corneas are found in patients with aniridia[6]. Thinner than average CCT can influence development of primary open angle glaucoma[7,8] with more severe disease evident in people with thinner corneas[9-11].

Central corneal thickness varies among ethnic populations with individuals of African descent having lower values than European Caucasians and East Asians[2,12,13]. Genome-wide association studies in European Caucasians[14-16], Asians[14,17] and Hispanics[18] have identified *ZNF469*, *RXRA-COL5A1*, *COL8A2* and *FOXO1* among others as important loci contributing to CCT. *RXRA-COL5A1* and *ZNF469* have been associated with CCT in most populations studied while the associations of other loci (*COL8A2*, *FOXO1*) may be restricted to specific populations[19]. Recently, *WNT7B* SNPs have been associated with CCT in Latinos[20], and interestingly some of these same SNPs were associated with axial length and corneal curvature, traits influencing myopic refractive error, in a Japanese population.[21]

Few genetic studies of ocular quantitative traits have been completed in individuals from South India, a population with high prevalence of common ocular conditions, especially cataract and glaucoma[22-27]. In Indian populations CCT is thinner than the average values for Caucasians[22] suggesting that CCT could be an important factor in the development of CCT-related common ocular disorders in this population. To identify genetic risk loci for CCT in South Indians we completed a family-based association study using large pedigrees, many with

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consanguineous matings that are typical for this geographic region. For the top SNPs located in

the *WNT7B* region, we also completed a phenome-wide association study (PheWAS) to

examine the range of phenotypes associated with *WNT7B* SNPs in this South Indian population.

MATERIALS and METHODS

Pedigrees and quantitative traits

This study adhered to the tenets of the Declaration of Helsinki and has been reviewed and

approved by the Institutional Review Boards of Massachusetts Eye and Ear Infirmary and

Medical Research Foundation, Sankara Nethralaya, Chennai, India. After obtaining written

informed consent, 197 individuals from 15 Indian pedigrees were recruited at Sankara

Nethralaya, Chennai, India. CCT was measured by an ultrasonic pachymeter in triplicate and

the average value was used. Methods to measure the other traits used in the PheWAS are

described in the Supplementary Methods. Collections of samples for replication cohorts are

described in the Supplementary Methods.

Genotyping and quality control

Genotyping for the South Indian families was performed at the Ocular Genomics Institute at the

Massachusetts Eye and Ear Infirmary using the Illumina HumanOmni2.5-8 Beadchip kit

(2,379,855 markers, Illumina, Inc., San Diego, CA). Genotypes were called using

GenomeStudio (v2011.1, Illumina, Inc.). The genetic sex of all individuals was consistent with

the reported sex. Two samples were removed because genotyping call rates were <99%. The

average call rate per sample was >99.8%. Quality control (QC) for 2,352,697 (98.9%) well-

clustered SNPs was performed with PLINK (v1.07)[28]. 25,088 (1.1%) SNPs with call frequency <

90% and 881,678 (37.5%) SNPs with minor allele frequency (MAF) < 0.01 were removed from

the analysis. 164,174 (7.0%) SNPs with Mendelian errors and 58,443 (2.5%) SNPs on

chromosome X or Y, or on the mitochondrial chromosome were also excluded. After QC,

1 1,223,314 SNPs were included in the final analysis. Genotyping for replication cohorts is

2 described in the Supplementary Methods.

3 **Statistical analysis**

4   The kinship coefficients for pairwise relationships across pedigrees were estimated from

5 the SNP data using the KING software[29]. The heritability for each trait was estimated with

6 restricted maximum likelihood–based linear modeling in the GCTA software[30], taking into

7 account all pedigree relationships simultaneously. Inverse-normal transformation of ranks was

8 applied to CCT measurements before analysis. Age and sex were included as covariates in the

9 association tests. The genome-wide association test was performed using the score test in

10 MERLIN (v1.1.2)[31,32], which incorporated genetic relatedness based on the family structure.

11 Because this program applies a restriction on pedigree size, 8 of the 15 pedigrees were split

12 into non-overlapping fragments of ≤18 bits using the PedSTR program[33], which breaks

13 inbreeding loops and identifies sub-pedigrees having the maximal total relationship between

14 individuals of interest, resulting in a total of 26 effective sub-pedigrees used in the final analysis.

15 To avoid an excess of false-positive results in regions of strong linkage, the likelihood-ratio test

16 was performed to accurately evaluate the SNPs with suggestive association. The regional SNP

17 association plot was generated using SNAP[34]. The variance in CCT explained by all the SNPs

18 in the Indian population was estimated using GCTA[30].

19   Meta-analysis using the inverse-variance weighting method was done using both fixed-

20 effects and random-effects models using Review Manager software (RevMan, version 5.3;

21 Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). The

22 heterogeneity between datasets was evaluated by heterogeneity index (I^2) and Cochran's Q

23 statistic[35]. Heterogeneity among datasets was further examined by evaluating differences in

24 ethnicity (Indians, Latinos, or Europeans), study design (family-based design or case-control

1 design), imputation quality score, age and sex in meta-regression models using the R package

2 "metafor"[36]. Forest plots were generated using the R package "metafor"[36].

PheWAS

4 45 quantitative traits (including CCT) (Supplementary Table 1) were analyzed for association as

5 described above. Methods for measuring each trait are described in the Supplementary

6 Methods. The average value for each trait for both eyes was used for analysis. Age and sex

7 were included as covariates in the association tests. The association tests were performed

8 using the likelihood-ratio test in MERLIN (v1.1.2)[31,32]. The PheWAS plots were generated using

9 the R package ggplot2[37]. Phenotypes were grouped along the x-axis by categorization of ocular

10 measures (Biometric traits, Corneal traits, Optic nerve traits, Refractive error traits). Each point

11 in the plot represents the $-\log_{10}(P)$ value of a trait measure in association analysis. The lower

12 grey dashed line indicates P = 0.05. The upper black dashed line indicates a single-SNP

13 Bonferroni correction P = 0.001 (0.05/45).

Power analysis

15 Power analysis was performed using the Genetic Power Calculator[38]. The total proportion of

16 trait variance was derived from the estimated heritability of these ocular traits in the South

17 Indian pedigrees. For CCT, axial length (AXL) and corneal curvature heritability was 0.54, 0.84

18 and 0.82, respectively. The QTL increaser allele frequency was set to the same as the marker

19 allele frequency. Linkage disequilibrium between the QTL and the marker was set at D' = 1.0.

20 The sample size was set as 26 because a total of 26 effective sub-pedigrees were used in the

21 final analysis. The sibling correlation was set as 0.5. The sibship size was set as 2. An additive

22 effects only (1 df) test was used to calculate the power at the type I error rate of 5×10^{-8} for

23 GWAS or 0.001 (0.05/45 traits) for PheWAS. Power results for all traits are listed in

24 Supplementary Table 1.

RESULTS

Study sample

195 individuals from 15 pedigrees (Supplementary Figure 1) were recruited at Sankara Nethralaya Eye Hospital, Chennai, India for a family-based genetic association study. These pedigrees were unrelated to each other; the maximum kinship coefficient estimated from the SNP data across pedigrees was 0.0344. The pedigree size ranged from 2 to 26 members. 10 of the pedigrees included at least one consanguineous mating. 58% of the subjects were female and 42% male. The average age was 44.9 (±15.0) years and the age ranged from 16-85 years. These families were not ascertained on specific eye conditions. CCT was measured by an ultrasonic pachymeter in triplicate for each eye (Supplementary methods) and the average value for both eyes was used (516.2 (±30.2) μm average; 433 to 608 μm range; Supplementary Table 1).

Genome-wide association results for CCT

After quality control, 1,223,314 SNPs were included in the genome-wide CCT analysis. The results for the family-based association test are shown in Supplementary Figure 2. The genomic inflation factor of 1.05 (QQ plot, Supplementary Figure 3) suggested that population substructure or other confounding factors were not significant. Six SNPs located on chromosomes 6, 13, 18 and 22 showed suggestive evidence of association with CCT ($P <$ $1.0×10^{-5}$; Table 1), with the top SNP (rs9330813, P=$1.7×10^{-7}$, β = -0.57, 95%CI: -0.78,-0.36 [A]) located in the first intron of *WNT7B* on chromosome 22 (Figure 1). CCT association with rs9330813 was two orders of magnitude greater than any other SNP (Table 1) and accounted for 17% of the phenotypic variance in the South Indian families. *WNT7B* SNPs have previously only been associated with CCT in a Latino population (MAGGS, Mexican American Glaucoma Genetics Study)[20] and the top SNP in the Latino study (rs10453441), is 422 bp from rs9330813.

1   rs10453441 is in moderate linkage disequilibrium with rs9330813 in the South Indian dataset (r^2

2   = 0.55) and was nominally associated with CCT in the South Indian pedigrees (P = 5.85 x10^{-4},

3   Supplementary Table 2).

4   To provide further support for the association of *WNT7B* with CCT in the South Indian

5   pedigrees, we investigated the association of rs9330813 in the Latino study cohort as well as in

6   4 independent European datasets (Figure 2). In addition we investigated association of

7   rs10453441 with CCT in an independent Singaporean Indian cohort, and 5 independent

8   European datasets (Supplementary Figure 4). The *WNT7B* SNPs were imputed from previous

9   genotype data for the European cohorts. For both rs9330813 and rs10453441 association with

10   CCT was evident with consistent direction of effects observed in all datasets with the exception

11   of one European cohort for rs10453441 (Supplemental Figure 4). For both SNPs, strongest

12   association was observed for the South Indian and MAGGS (Latinos) datasets, with smaller

13   effects in European cohorts (Figure 2, Supplemental Figure 4). Significant heterogeneity was

14   detected among datasets, due to imputation quality and study design (meta-regression P =

15   0.0001 and P=0.02 respectively). Limiting the meta-analysis to datasets with imputation scores

16   > 0.7 for each SNP reduced but did not completely eliminate heterogeneity (Figure 2,

17   Supplemental Figure 4). Because of the residual heterogeneity reverse inverse weighted meta-

18   analyses were completed using both fixed and random effects and investigated separately the

19   datasets with imputation scores > 0.7 for each SNP. Using the fixed effects model, significant

20   association was observed for CCT and rs9330813 [A] (P= 1.7 x10^{-9}, β = -3.94, 95%CI: -5.23,-

21   2.66; Figure 2), and rs10453441 [G] (P=2.20x10^{-11}, β= -3.11, 95%CI: -4.02, -2.02, Supplemental

22   Figure 4). Evidence for association improved using only the datasets with imputation scores >

23   0.7: rs9330813[A] (P= 5.0x10^{-12}, β = -5.59, 95%CI: -7.17,- 4.00; Figure 2), and rs10453441 [G]

24   (P=5.3x10^{-12}, β= -3.43, 95%CI: -4.40, -2.45, Supplemental Figure 4). Reduced but consistent

25   association was observed using the random effects model for both SNPs: rs9330813 [A]

1   (P=7.0x10^{-3}, β = -8.00, 95%CI: -13.85, -2.15); rs10453441 [G] (P=1.0x10^{-4}, β= -3.44, 95%CI: -

2   5.21,-1.68).

3   The top *WNT7B* SNP, rs9330813 is in strong equilibrium with rs9723267, (r^2 = 0.96 and

4   1.0 in the South Indian dataset and 1000 Genomes, Haploreg v.4.1, respectively), that disrupts

5   a Rad21 binding motif and a CTCF (CCCTC-binding factor) binding site as well as other

6   transcription factor binding sites (RegulomeDB, Supplementary Figure 5) suggesting a role in

7   regulation of gene expression. The region of intron 1 that includes the *WNT7B* SNPs associated

8   with CCT contains multiple DNaseI hypersensitivity sites and features of enhancers as

9   annotated by ENCODE in multiple cell types (Supplementary Figure 5).

10   In the South Indian family dataset we also replicated association (*P*<0.005) with a

11   number of loci previously associated with CCT including *RXRA-COL5A1*[16], *ZNF469*[15], *GPR15*[13]

12   and *GLT8D2*[13], although none of these associations were as significant as those observed for

13   the *WNT7B* SNPs in this population (Supplementary Table 3). It was estimated that 53.8% of

14   the variance in CCT was explained by all the CCT-associated SNPs in this Indian population.

15   We also investigated the association of the *WNT7B* SNPs associated with CCT in this

16   study with primary open angle glaucoma (POAG) in our NEIGHBORHOOD European

17   Caucasian dataset of 3853 cases and 33480 controls[39]. However, similar to other studies[14] we

18   did not find evidence for association of these SNPs with POAG (P>0.05).

19   **PheWAS**

20   Recently SNPs also located in this same region of the first intron of *WNT7B* have been

21   associated with two other ocular quantitative traits, corneal curvature and axial length, in a

22   GWAS using a Japanese population[21]. The lead SNP in the Japanese study, rs10453441 is the

23   same SNP associated with CCT in the Latino study[20] located 422 bp from rs9330813, the lead

24   SNP in the South Indian pedigrees (Supplementary Figure 5). To determine if the *WNT7B*

1   association in our dataset was specific for CCT we performed an age- and sex-adjusted

2   PheWAS (Phenotype-wide association study) using association data for 45 ocular quantitative

3   traits measured in the same families used for the CCT analysis (see Supplementary Table 1 for

4   complete list of traits), including axial length and corneal curvature, the two traits associated

5   with the *WNT7B* SNP rs10453441 in the Japanese study[21]. For the PheWAS, we investigated

6   the top 3 *WNT7B* SNPs (rs9330813, rs9723267 and rs75159625) from our data (Supplementary

7   Table 2) and also the top 2 SNPs in the Japanese study (rs10453441 and rs200329677). Four

8   of these SNPs are preferentially associated with CCT in the South Indian sample (the remaining

9   SNP, rs200329677, was not significantly associated with CCT or any other trait in this dataset)

10   (Figure 3, Supplementary Figure 6). In the South Indian dataset, the PheWAS data did not

11   support significant association of any *WNT7B* SNP with any trait other than CCT (P>0.001)

12   including axial length or corneal curvature as was observed in the Japanese study (Figure 3,

13   Supplementary Figure 6) despite having sufficient power (>99.9%) for axial length and corneal

14   curvature to detect the associations previously described (Supplementary Table 1).

15   **DISCUSSION**

16   This is the first GWAS for CCT in individuals residing in Southern India, a population at

17   increased risk for blinding ocular disorders[27,40]. In this family-based study that included large

18   consanguineous pedigrees we identified association of CCT with *WNT7B* SNPs located in an

19   apparent regulatory region likely to impact gene expression. Pedigrees with consanguineous

20   matings are known to have added power for genetic studies of recessive traits. In this study we

21   have shown that consanguineous families can also provide genetic insights leading to discovery

22   of loci for quantitative traits. The CCT boxplot for three genotypes of top SNP rs9330813 was

23   consistent with an additive model in this South Indian dataset (Supplementary Figure 7). We

24   estimated that we had at least 82% power to detect the associations between these *WNT7B*

25   SNPs and CCT in this South Indian dataset.

1   *WNT7B* codes for a member of the Wnt family of proteins that have critical roles in cell

2   growth, patterning and differentiation of multiple tissues and organs[41]. The canonical WNT

3   signaling pathway that includes WNT7b (the product of *WNT7B*) is known to contribute to stem

4   cell proliferation in development[42]. In the eye *WNT7B* has been shown to have increased

5   expression in the central cornea and may also be necessary for corneal limbal stem cell

6   development[43]. Interestingly a rare exonic variant in another WNT family member, *WNT10A*,

7   has also been associated with central corneal thickness in a quantitative trait study of European

8   Caucasians[44].

9   The *WNT7B* SNPs associated with CCT are located in the first intron of the gene in a

10   region with multiple DNaseI hypersensitivity sites and enhancers as annotated by ENCODE.

11   The top SNP is in strong linkage disequilibrium with rs9723267 that impacts Rad21 and CTCF

12   (CCCTC-binding factor) binding sites. Rad21 is one of the subunits of the cohesin complex that

13   together with CTCF associates with active enhancers and promoters forming long-range

14   interactions important for gene regulation[45]. Rad21 and CTCF activity is highest when a general

15   transcription factor (TBP) binding site is also nearby[46] as is the case in the *WNT7B* region

16   associated with CCT (Supplementary Figure 5), suggesting that genetic variants in this region

17   could impact gene expression.

18   In addition to the association between *WNT7B* and CCT we also confirmed association

19   with several other loci previously associated with CCT in other populations, in particular *ZNF469*

20   and *RXRA-COL5A1*. Genomic association studies have now been completed for CCT in a

21   variety of ethnic populations including European Caucasians[13-16], Asians[17] and Latinos[18,20].

22   Evidence for association of CCT with *ZNF469* and *RXRA-COL5A1* has been found in most

23   populations, while other CCT loci such as *COL8A2*, significantly associated in Asians[17], may be

24   restricted to specific populations[19]. Our study suggests that *WNT7B* is an important locus for

25   CCT in the South Indian population.

12

WNT7B SNPs may also contribute to other ocular phenotypes. In a study conducted in Japanese, SNPs in the same genomic region associated with CCT in our study were associated with AXL and corneal curvature, ocular quantitative traits related to refractive error and myopia[21]. We have previously measured 45 quantitative traits in the collection of Indian pedigrees used for this study including AXL, corneal curvature and refractive error. This collection of quantitative trait data made it possible to complete an ocular PheWAS for the WNT7B SNPs associated with CCT in our study and the WNT7B SNPs associated with AXL and corneal curvature in the Japanese study. Understanding the range of phenotypic consequences of DNA sequence variants may provide insights into the mechanisms by which a variant or gene leads to disease. The PheWAS approach can test the association of a disease-associated variant with a broad range of phenotypes.[48-50] We found that in the South Indian population the WNT7B SNPs are specifically associated with CCT and did not show evidence of association with any other traits, including those related to myopia and refractive error. While the Japanese study did not specifically interrogate association with CCT, it appears that the WNT7B SNPs can be associated with additional or different traits in the Japanese population. The opportunity to complete a PheWAS to evaluate the association of the WNT7B SNPs with a broad range of ocular phenotypes was a strength of our study.

In summary our family-based association analysis using South Indian pedigrees has identified WNT7B as a locus for CCT in this population and an ocular PheWAS conducted in the same dataset showed that the WNT7B association is specific for this trait in these South Indian pedigrees. WNT7B is known to be associated with CCT in a Latino population[20] but has not been previously shown to be a CCT locus in Asians or European Caucasians suggesting that genomic studies in specific ethnic populations can uncover new loci for complex traits that provide additional insights into the underlying genetic architecture of these common conditions.

Acknowledgements

3   **Web Resources**

4   1000 Genomes Project Phase I v3 haplotypes

5   http://csg.sph.umich.edu/abecasis/MACH/download/1000G.2012-03-14.html

6   GCTA

7   http://gcta.freeforums.net/

8   Genetic Power Calculator

9   http://pngu.mgh.harvard.edu/~purcell/gpc/

10   HaploReg

11   http://www.broadinstitute.org/mammals/haploreg/haploreg.php

12   KING

13   http://people.virginia.edu/~wc9c/KING/index.html

14   MERLIN

15   https://csg.sph.umich.edu/abecasis/Merlin/

16   PedSTR

17   http://mga.bionet.nsc.ru/soft/PedStr/PedStr.tar.gz

18   PLINK

19   http://pngu.mgh.harvard.edu/~purcell/plink/

20   R

21   https://www.r-project.org/

22   RegulomeDB

23   http://regulome.stanford.edu/

24   SNAP

25   http://archive.broadinstitute.org/mpg/snap/

Conflict of Interest Statement:

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Figure Legends

Figure 1. Regional SNP association plot for the 22q13 region. A region of 408 kb around the top SNP (rs9330813) is displayed. The degree of LD between the top SNP and any SNP tested is indicated by red shading. The recombination rate is displayed by a blue line with scale on the right-hand axis. Characterized genes in the region are represented with a green bar. The *P* value for rs9330813 (1.71×10^{-7}) is shown as a red diamond.

Figure 2. Meta-analysis for rs9330813 and CCT. Forest plot showing effect estimates for the South Indian pedigree, as well as for the replication effort. Pooled estimates for β and 95% confidence interval (95% CI) were calculated by fixed-effects, inverse variance weighting meta-analysis. Reduced evidence of association but with similar effects was observed if the meta-analysis was calculated using random effects: $P=7.0 \times 10^{-3}$, β = -8.00, 95%CI: -13.85, -3.15. Individual dataset results are indicated by black squares and summary values are indicated by

1   black diamonds. Abbreviations: MAGGS, Mexican American Glaucoma Genetic Study;

2   ORCADES, Orkney Complex Disease Study; TwinsUK, UK Twin Study.

3

4   **Figure 3. PheWAS plot for the top SNP associated with CCT in the South Indian**

5   **population (rs9330813).** The association results for each measured trait (Supplementary

6   Table 1) for this SNP were plotted with the phenotypes (ocular traits) grouped along the x-axis

7   and the –log10(P) value for association analysis on the y-axis. The phenotype group is

8   indicated by the color of the graph point as indicated by the side panel. The lower grey dashed

9   line indicates P = 0.05. The upper black dashed line indicates a single-SNP Bonferroni

10   correction for 45 traits, P = 0.001 (0.05/45). Abbreviations: CCT, central corneal thickness;

11   IOPg , intraocular pressure measured by Goldman applanation ; AXL, axial length; CRF,

12   corneal resistance factor; K_H, corneal curvature, horizontal; K_V, corneal curvature, vertical;

13   RNFL_VC, retinal nerve fiber layer curvature as measured by the (Heidelberg Retina

14   Tomograph and analyzed by using Glaucoma Probability Score (GPS). Other traits were not

15   labeled in these figures due to limited space. Categories are grouped according to

16   Supplementary Table 1.

Table 1. SNPs with $P < 1.0{\times}10^{-5}$ for association with CCT in South Indian pedigrees
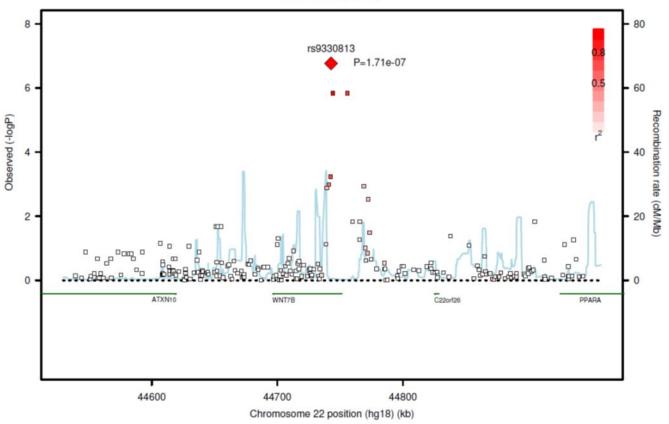
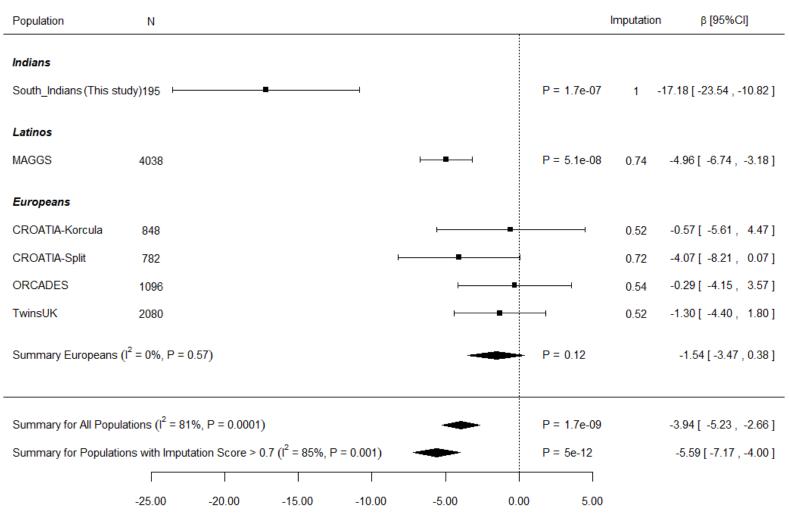| SNP	Chr	Position[a]	Gene	A1/A2[b]	MAF[c]	β[d]	s.e.	p
rs77747357	6	151377143	MTHFD1L	G/A	0.247	0.605	0.133	$1.97{\times}10^{-6}$
rs67580603	13	90875539	LINC00559-MIR622	A/G	0.082	0.875	0.195	$3.83{\times}10^{-6}$
rs10084050	18	28657553	DSC2	C/T	0.013	2.012	0.451	$5.91{\times}10^{-6}$
rs9330813	22	46364161	WNT7B	A/G	0.495	-0.570	0.107	$1.71{\times}10^{-7}$
rs9723267	22	46365557	WNT7B	T/G	0.495	-0.530	0.107	$1.45{\times}10^{-6}$
rs75159625	22	46377008	WNT7B	C/A	0.497	-0.530	0.107	$1.46{\times}10^{-6}$

[a]Genomic positions are based on NCBI Build 37/hg19.
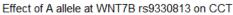[b]A1/A2, minor allele/common allele.
[c]MAF, minor allele frequency.
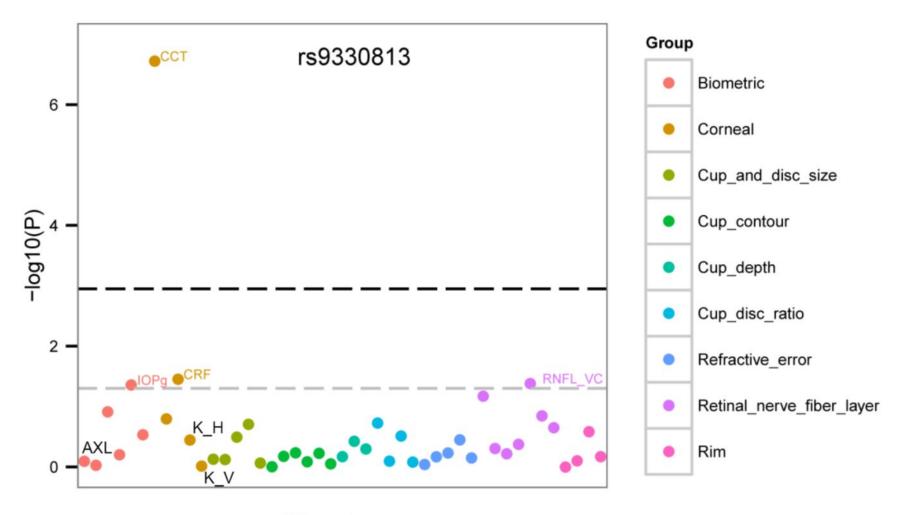[d]β models the expected change in mean CCT per increase of one A1 allele.
Chr, chromosome; s.e, standard error.

Population	N		Imputation	β [95%CI]
Indians				
South_Indians (This study)	195	P = 1.7e-07	1	-17.18 [-23.54 , -10.82]
Latinos				
MAGGS	4038	P = 5.1e-08	0.74	-4.96 [-6.74 , -3.18]
Europeans				
CROATIA-Korcula	848		0.52	-0.57 [-5.61 , 4.47]
CROATIA-Split	782		0.72	-4.07 [-8.21 , 0.07]
ORCADES	1096		0.54	-0.29 [-4.15 , 3.57]
TwinsUK	2080		0.52	-1.30 [-4.40 , 1.80]
Summary Europeans (I^2 = 0%, P = 0.57)		P = 0.12		-1.54 [-3.47 , 0.38]
Summary for All Populations (I^2 = 81%, P = 0.0001)		P = 1.7e-09		-3.94 [-5.23 , -2.66]
Summary for Populations with Imputation Score > 0.7 (I^2 = 85%, P = 0.001)		P = 5e-12		-5.59 [-7.17 , -4.00]

Effect of A allele at WNT7B rs9330813 on CCT