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Sequence variation at *ANAPC1* accounts for 24% of the variability in corneal endothelial cell density

Erna V. Ivarsdottir^{1,2}, Stefania Benonisdottir¹, Gudmar Thorleifsson¹, Patrick Sulem¹, Asmundur Oddsson¹, Unnur Styrkarsdottir¹, Snaedis Kristmundsdottir¹, Gudny A. Arnadottir¹, Gudmundur Thorgeirsson^{1,3,4}, Ingileif Jonsdottir^{1,3,5}, Gunnar M. Zoega⁶, Unnur Thorsteinsdottir^{1,3}, Daniel F. Gudbjartsson^{1,2}, Fridbert Jonasson^{3,6}, Hilma Holm¹ & Kari Stefansson^{1,3}

The corneal endothelium is vital for transparency and proper hydration of the cornea. Here, we conduct a genome-wide association study of corneal endothelial cell density (cells/mm²), coefficient of cell size variation (CV), percentage of hexagonal cells (HEX) and central corneal thickness (CCT) in 6,125 Icelanders and find associations at 10 loci, including 7 novel. We assess the effects of these variants on various ocular biomechanics such as corneal hysteresis (CH), as well as eye diseases such as glaucoma and corneal dystrophies. Most notably, an intergenic variant close to *ANAPC1* (rs78658973[A], frequency = 28.3%) strongly associates with decreased cell density and accounts for 24% of the population variance in cell density ($\beta = -0.77$ SD, $P = 1.8 \times 10^{-314}$) and associates with increased CH ($\beta = 0.19$ SD, $P = 2.6 \times 10^{-19}$) without affecting risk of corneal diseases and glaucoma. Our findings indicate that despite correlations between cell density and eye diseases, low cell density does not increase the risk of disease.

¹deCODE genetics/Amgen, Reykjavik, Iceland. ²School of Engineering and Natural Sciences, University of Iceland, Reykjavik, Iceland. ³Faculty of Medicine, School of Health Sciences, University of Iceland, Reykjavik, Iceland. ⁴Division of Cardiology, Department of Internal Medicine, Landspítali, The National University Hospital of Iceland, Reykjavik, Iceland. ⁵Department of Immunology, Landspítali, The National University Hospital of Iceland, Reykjavik, Iceland. ⁶Department of Ophthalmology, Landspítali, The National University Hospital of Iceland, Reykjavik, Iceland. Correspondence and requests for materials should be addressed to H.H. (email: hilma.holm@decode.is) or to K.S. (email: kari.stefansson@decode.is)

Corneal diseases are among the most common causes of visual loss worldwide and endothelial cell failure is the leading indication for corneal transplantation¹. The corneal endothelium is the monolayer of cells at the innermost surface of the cornea. Through an ion pump function, the endothelium is responsible for balanced corneal hydration, thus maintaining transparency by preventing edema and disruption of lamellar spacing of the collagen fibrils in the corneal stroma². Maintaining proper function of the endothelium requires a minimum number of endothelial cells, around 400–500 cells/mm²³. The cells are generally thought to be incapable of mitosis after birth but are halted in the G1 phase of the cell cycle and their number decreases with age². The cell division is thought to be blocked by contact inhibition, high concentration of negative growth factors in the anterior chamber and by accumulation of reactive oxygen species promoting state of stress-induced senescence^{3,4}. The response to cell loss includes spreading and/or migration of adjacent cells which increase in size and become more variable in both cell size and shape^{3,5}.

Non-contact auto-tracking and focusing specular microscopy provides a non-invasive analysis of the structure of the corneal endothelium⁶. The equipment captures an image of the endothelial cell layer and provides measures of its structure including, cell density (cells/mm²), coefficient of cell size variation (CV), percentage of hexagonally shaped cells (HEX) and central corneal thickness (CCT). CCT has been studied extensively in large genome-wide association studies (GWAS) where the measurements are obtained using different types of instruments^{7–13}. Several CCT associating loci have been identified including *WNT10A*, *COL5A1*, and *ZNF469*¹¹. To our knowledge, however, there are no reports of sequence variants influencing other direct measures of endothelial structure such as cell density, CV and HEX.

The measures of endothelial structure are used to diagnose corneal diseases. Several corneal diseases including Fuchs endothelial corneal dystrophy (FECD) and macular corneal dystrophy (MCD) are known to have genetic components^{14,15}. FECD is a leading cause of corneal transplant surgery and is characterized by premature loss of endothelial cells resulting in increased variability in cell shape and size leading to corneal edema and visual loss^{16,17}. MCD is characterized by progressive spotted corneal opacities leading to severe visual impairment, caused by homozygous or compound heterozygous variants in the *CHST6* gene (OMIM: 605294). MCD is a rare condition worldwide, but because of the founder effect¹⁸, MCD is unusually common in Iceland where it accounts for approximately one-third of corneal transplantations¹⁹.

Cell density in the corneal endothelium may be reduced in glaucoma patients²⁰. Glaucoma is an ocular disease that affects

~3.5% of people over 40 years of age, and is a major cause of irreversible blindness worldwide^{21,22}. Commonly, elevated intraocular pressure (IOP) leads to progressive damage of the optic nerve causing visual loss and it has been postulated that elevated IOP affects the endothelium²³. The relationship between different corneal measures and glaucoma have been investigated, especially the role of IOP, CCT and more recently corneal hysteresis (CH) and corneal resistance factor (CRF)²⁴. CH and CRF are measures of corneal response to a rapid jet of air, where CH is a measure of the elasticity of the cornea and CRF is an overall indicator of the resistance of the cornea²⁵. Lower CH has been associated with faster rate of glaucoma progression^{26–28}.

Here we describe our search for sequence variants associating with measures of corneal structure, finding 10 sequence variants, nine common and one low-frequency (minor allele frequency (MAF) <5%), associating with CCT, cell density, CV, or HEX. Two of these variants satisfy thresholds for genome-wide significance for more than one trait. Seven of the associations are novel, two are represented by coding variants and one is located in a gene known to cause a Mendelian disorder (*ADAMTS17*, OMIM: 607511). We assess the effects on ocular biomechanics, including IOP and CH, and various eye diseases. We find a variant near *ANAPC1* that strongly associates with cell density but not with risk of eye disease, which indicates that low cell density alone does not affect disease development directly.

Results

Summary of the data. Endothelial images from a specular microscopy of 6125 Icelanders were used in the analysis, providing measures of cell density, CV, and HEX. The equipment also produced CCT measurements. These images were obtained as a part of a comprehensive phenotyping of a general population sample (the deCODE health study), currently including 6300 Icelanders that were between 18 and 94 years of age at the time of recruitment (44.6% men; mean age = 55.9, standard deviation (SD) = 15.1). We also measured several ocular biomechanics such as CH, CRF, Goldmann correlated intraocular pressure (IOPg), and corneal compensated intraocular pressure (IOPcc) (Supplementary Figures 1–6).

The number of endothelial cells declines with age and remaining cells enlarge to compensate for the cell loss. Consequently, cell density and HEX decrease with age while CV increases (Table 1, Fig. 1a–c and Supplementary Figure 7). Women have considerably lower HEX than men (50.6% vs 48.3%, $P = 8.4 \times 10^{-43}$; F test), while cell density and CV are slightly higher for women (2663 vs 2639 cells/mm², $P = 1.8 \times 10^{-3}$ (F test) and 30.3 vs 29.6, $P = 2.6 \times 10^{-6}$ (F test), respectively). To our knowledge, the gender differences of HEX, cell density and

Table 1 Summary of data							
	Mean (SD)	Men's mean (SD)	Women's mean (SD)	Sex effect	Sex P-value	Glaucoma effect [SD]	Glaucoma P-value
CD	2652 (296)	2639 (296)	2663 (294)	23.83	1.8×10^{-3}	−0.35	2.1×10^{-6}
CV	30.0 (5.9)	29.6 (6.7)	30.3 (5.1)	0.71	2.6×10^{-6}	0.13	0.069
CCT	563 (40)	565 (40)	562 (39.5)	−3.82	1.9×10^{-4}	−0.25	4.7×10^{-4}
HEX	49.3 (6.5)	50.6 (6.6)	48.3 (6.3)	−2.29	8.4×10^{-43}	−0.16	0.030
CH	10.4 (1.2)	10.2 (1.2)	10.5 (1.1)	0.28	5.7×10^{-22}	−0.37	3.8×10^{-7}
IOPg	14.7 (3.4)	14.6 (3.5)	14.7 (3.4)	0.13	0.15	0.18	0.014
IOPcc	15.3 (3.1)	15.4 (3.2)	15.2 (3.0)	−0.19	0.015	0.29	7.3×10^{-5}
CRF	13.1 (1.5)	13.0 (1.5)	13.3 (1.5)	0.29	5.7×10^{-14}	−0.07	0.34

The mean and standard deviation (SD) is shown for each corneal trait obtained from the specular microscopy equipment and the ocular response analyzer, overall and separately for each sex. The effect of sex and glaucoma status on each trait and the corresponding P-values (F test) are shown. The sample size was 6125 in total, 2733 men and 3392 women
CD cell density, CV coefficient of cell size variation, CCT central corneal thickness, HEX percentage of hexagonal cells, CH corneal hysteresis, IOPg Goldmann correlated intraocular pressure, IOPcc corneal compensated intraocular pressure, CRF corneal resistance factor

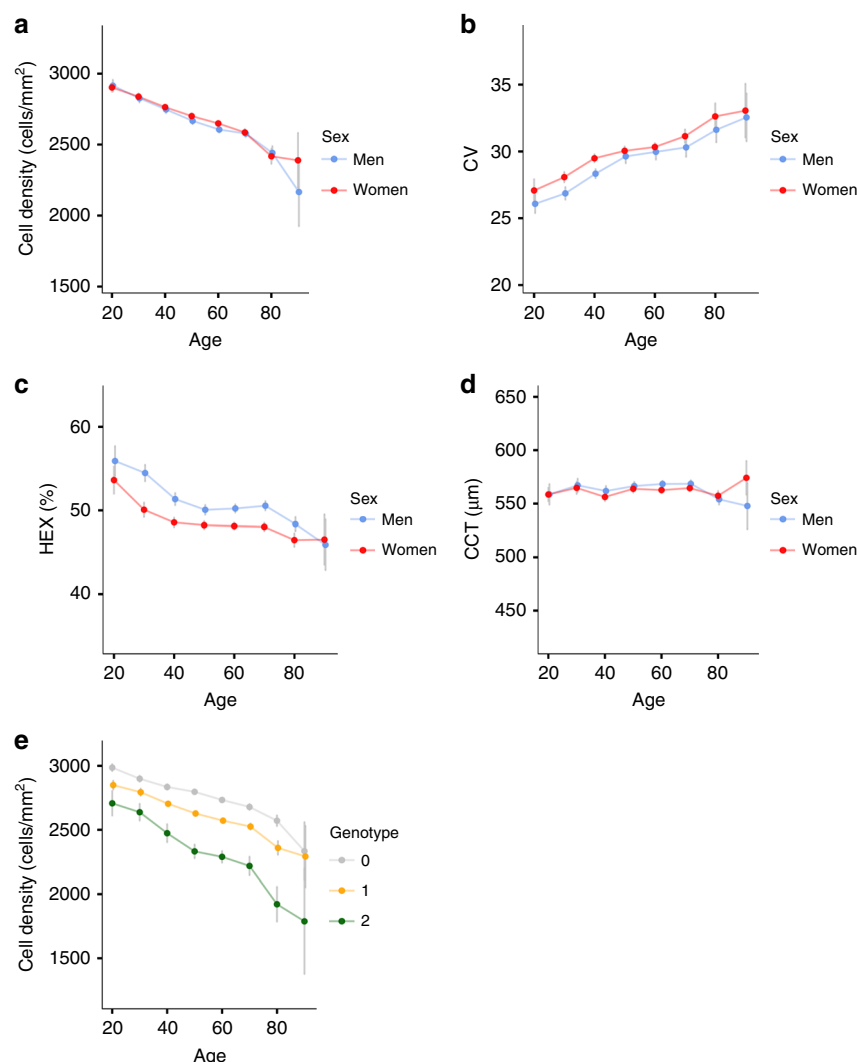


Fig. 1 Corneal structure measurements by age ($N = 6125$). The average **a** cell density, **b** HEX, **c** CV, and **d** CCT values for subjects belonging to a 10 year age group (e.g., age = 30 for individuals between 26 and 35) against age, for men and women. **e** The average cell density for subjects belonging to a 10 year age group against age for noncarriers, heterozygous, and homozygous carriers of the *ANAPC1* variant, rs78658973. The gray lines show the 95% confidence intervals

CV have not been reported before. Consistent with previous reports²⁹, CCT does not change with age and is higher for men than women (565.4 vs 561.5 μm , $P = 1.9 \times 10^{-4}$; F test) (Fig. 1d). The measurements of corneal structure are correlated, also after adjusting for sex and age (Supplementary Table 1). The strongest correlations are between CV and HEX ($r = -0.65$) and between CV and cell density ($r = -0.33$).

Study design. To search for sequence variants associating with corneal structure, we analyzed 35.2 million sequence variants identified through whole-genome sequencing of 28,075 Icelanders that were subsequently imputed into 155,250 chip-typed individuals, as well as their first- and second-degree relatives^{30,31} (Supplementary Figures 8–11). Ten sequence variants, satisfied our genome-wide significance thresholds that are dependent on sequence variant annotation³² (Table 2, Supplementary Table 2).

To determine whether the associating variants affect the risk of eye diseases we performed a meta-analysis of GWAS results from Iceland and the UK Biobank (Supplementary Table 3 and 4). We tested the variants for association with glaucoma (8432 cases and 641,353 controls) and the sub-categories: primary open-angle glaucoma (2296 cases and 705,937 controls), and primary angle

closure glaucoma (777 cases and 637,017 controls). We also tested the variants for association with disorders of cornea (ICD10 code H18, 756 cases and 663,218 controls) and the sub-categories; corneal degeneration (ICD10 code H18.4, 199 cases and 684,021 controls), hereditary corneal dystrophies (ICD10 code H18.5, 330 cases and 683,652 controls) and keratoconus (ICD10 code H18.6, 127 cases and 659,503 controls). We applied a Bonferroni corrected P -value threshold based on testing ten corneal structure variants for association with seven phenotypes ($P < 0.05/(7 \times 10) = 7.1 \times 10^{-4}$).

GWAS results. Two sequence variants associate with cell density (Fig. 2a, Table 2). The strongest association is represented by a common intergenic variant located 0.4 kb downstream of *ANAPC1*, rs78658973[A] (MAF = 28.3%), that associates with decreased cell density ($\beta = -0.77$ SD, $P = 1.8 \times 10^{-314}$; a likelihood-ratio test was performed in all genome-wide associations) (Fig. 1e, Supplementary Figure 12). rs78658973 is highly correlated with 113 variants ($r^2 > 0.8$) in the region, none of which is protein coding. The most highly correlated coding variants are two splice region variants in *ANAPC1*; rs201128688 and rs142711068 ($r^2 = 0.72$ and 0.73 , respectively). The effect of rs78658973[A] conditioning on the two splice region

Table 2 Association results										
Trait	Chr:Position	rs-name	Allele (min/maj)	MAF (%)	Gene/ [Locus]	Coding effect	LD class	P-value	β [SD] (95% CI)	Ref.
CD	2:111726948	rs78658973	(A/T)	28.3	[ANAPC1]	Intergenic	112	1.8×10^{-314}	-0.77 (-0.77, -0.77)	35
CD	18:55586154	—	CTG repeat > 33	6.1	TCF4		3	1.4×10^{-20}	-0.41 (-0.49, -0.32)	
CV	2:111726948	rs78658973	(A/T)	28.3	[ANAPC1]	Intergenic	112	2.8×10^{-28}	0.23 (0.19, 0.27)	
CV	17:14650919	rs2323458	(A/G)	36.1	17p12	Intergenic	47	6.9×10^{-13}	0.14 (0.10, 0.18)	13
CV	8:9943404	rs10094779	(G/A)	24.1	8p23.1	Intergenic	4	7.6×10^{-12}	0.15 (0.11, 0.19)	
CV	11:122029470	rs76561503	(C/T)	17.9	11q24.1	Intergenic	29	3.3×10^{-10}	0.16 (0.11, 0.21)	
CCT	16:88302168	rs12719930	(G/A)	39.4	[ZNF469]	Intergenic	21	1.9×10^{-14}	-0.15 (-0.19, -0.11)	9
CCT	2:218890289	rs121908120	(A/T)	2.6	WNT10A	Missense	6	4.5×10^{-11}	-0.39 (-0.51, -0.28)	
CCT	9:134545337	rs943423	(G/A)	27.2	[COL5A1]	Intergenic	0	6.1×10^{-11}	-0.14 (-0.19, -0.10)	
CCT	15:100152748	rs72755233	(A/G)	13.8	ADAMTS17	Missense	0	1.3×10^{-10}	0.18 (0.12, 0.23)	35
CCT	12:104015054	rs117801489	(C/T)	4.3	GLT8D2	Missense	2	3.9×10^{-10}	0.30 (0.20, 0.39)	
HEX	18:55586154	—	CTG repeat > 33	6.1	TCF4		3	5.9×10^{-18}	-0.37 (-0.45, -0.28)	
HEX	2:111726948	rs78658973	(A/T)	28.3	[ANAPC1]	Intergenic	112	2.8×10^{-13}	-0.16 (-0.20, -0.11)	

The 10 variants identified in the GWAS on cell density (CD), CV, HEX, and CCT. Effects are shown for the minor allele. Minor allele frequency in the Icelandic population is presented. The LD class column shows the number of highly correlated variants ($r^2 > 0.8$). The imputation information for all these variants is > 0.99

CD cell density, CV coefficient of cell size variation, CCT central corneal thickness, HEX percentage of hexagonal cells, MAF minor allele frequency, LD linkage disequilibrium, CI confidence interval

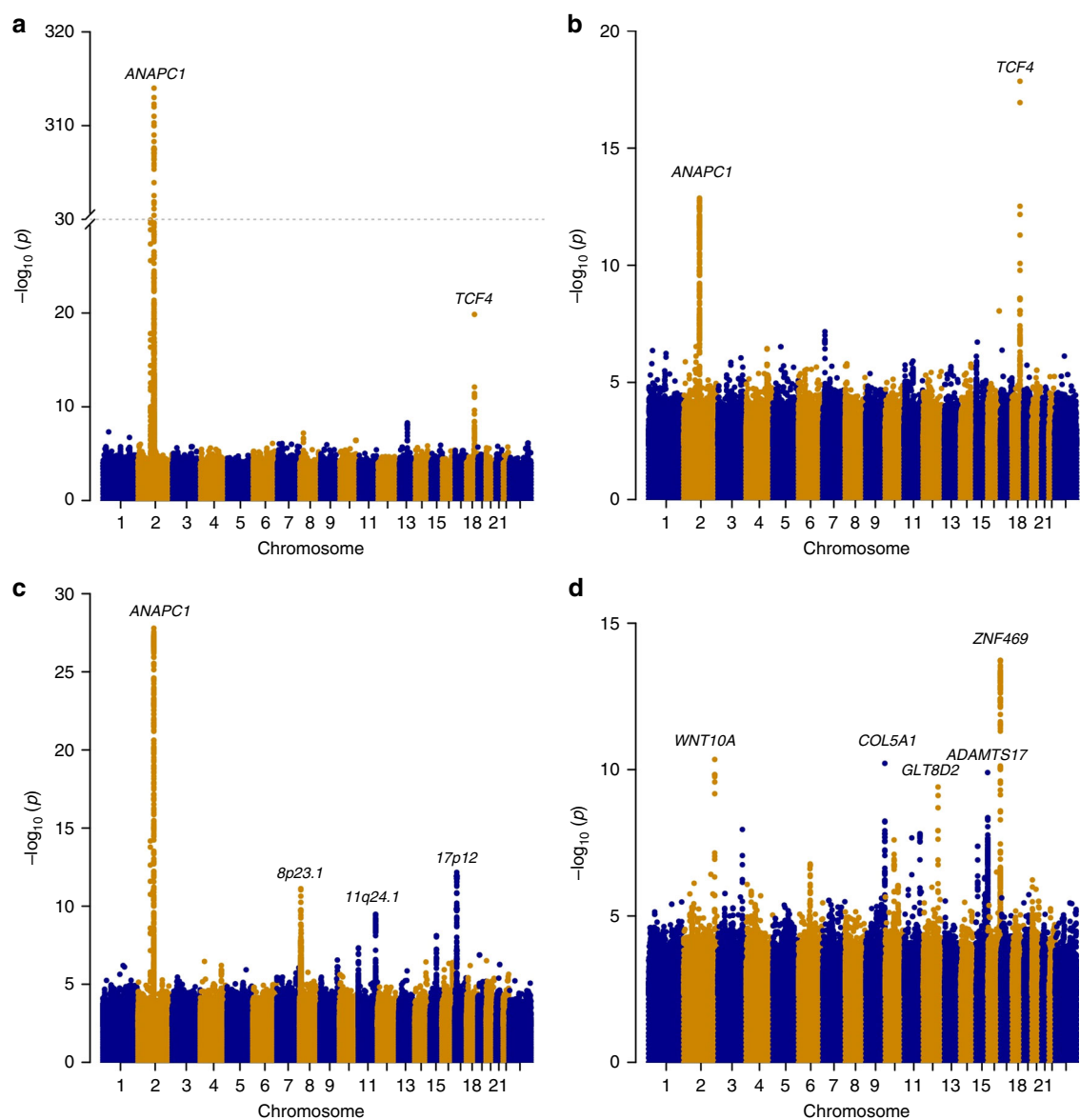


Fig. 2 Manhattan plots. Association results for the corneal measures ($N = 6125$) obtained from the specular microscopy; **a** cell density, **b** HEX, **c** CV, and **d** CCT. The $-\log_{10} P$ -values are plotted for each variant against their chromosomal position. A likelihood-ratio test was used when testing for association

variants is still significant ($\beta = -0.78$ SD, $P = 7.8 \times 10^{-85}$), while the effect of the splice region variants are completely explained by rs78658973. rs78658973[A] also associates with CV ($\beta = 0.23$ SD, $P = 2.8 \times 10^{-28}$) and HEX ($\beta = -0.16$ SD, $P = 2.6 \times 10^{-19}$), but this is largely driven by the strong effect on cell density (CV adjusted for cell density: $\beta = -0.04$ SD, $P = 0.049$; HEX adjusted for cell density: $\beta = -0.04$ SD, $P = 0.072$). Interestingly, rs78658973[A] also associates with CH ($\beta = 0.19$ SD, $P = 2.6 \times 10^{-19}$) (Supplementary Table 4, Fig. 3) which reflects the cornea's ability to absorb and dissipate energy^{25,33}. CH and cell density are only weakly correlated after adjusting for sex and age ($r = -0.03$) and the effect of rs78658973[A] on CH is not affected by adjustment for cell density ($\beta_{\text{adjusted}} = 0.21$ SD, $P_{\text{adjusted}} = 1.5 \times 10^{-18}$). CH is lower in patients with glaucoma or corneal disorders like keratoconus³⁴. However, rs78658973 does not associate with corneal diseases or glaucoma in our data ($P > 0.03$, Supplementary Table 4).

The other variant associating with cell density is a common allele of a microsatellite at *TCF4* (MAF = 6.1%), a CTG repeat of length ≥ 33 , corresponding to the expanded CTG 18.1 allele (OMIM: 602272, allelic variant 0.0007). This microsatellite is pathogenic according to Clinvar and has been reported to strongly predispose to autosomal dominant FECD^{35–38}, a disease of the corneal endothelium that affects roughly 4% of people over 40 years old (OMIM: 602272). Consistent with the characteristics of FECD, the expanded CTG 18.1 allele associates with lower cell density and HEX ($\beta = -0.38$ SD, $P = 1.6 \times 10^{-19}$ and $\beta = -0.37$ SD, $P = 5.9 \times 10^{-18}$, respectively). Interestingly, it also associates with decreased CH, CRF and IOPg ($\beta = -0.29$ SD, $P = 3.1 \times 10^{-12}$, $\beta = -0.30$ SD, $P = 7.9 \times 10^{-13}$ and $\beta = -0.18$ SD, $P = 1.7 \times 10^{-5}$, respectively) while not affecting glaucoma risk (OR = 0.92, CI = (0.82;1.03), $P = 0.15$). Consistent with previous reports, the expanded CTG 18.1 allele associates with hereditary corneal dystrophies in our data (OR = 7.7, $P = 3.3 \times 10^{-31}$, Supplementary Table 4).

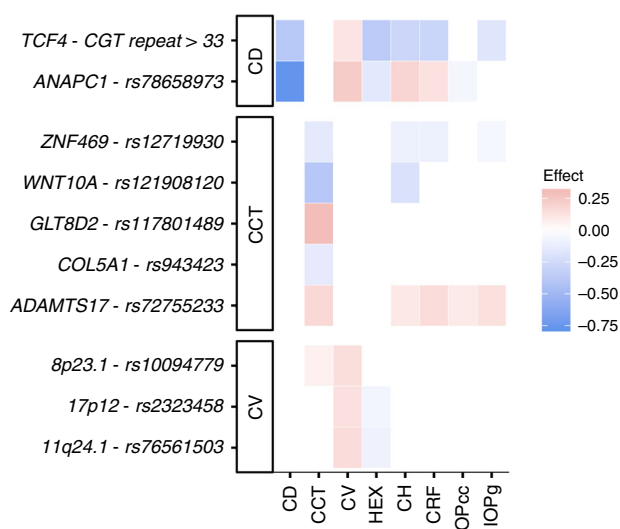


Fig. 3 Effects of the GWS corneal structure variants on different corneal measures. Each row shows the estimated effect of the minor allele on the corneal measures from the specular microscopy and the ocular response analyzer. The variants are annotated with their corresponding gene and grouped by their strongest associating trait. The effect is shown only for significant associations after adjusting for multiple testing with a false discovery rate procedure for each variant. Red color represents a positive effect on the corneal measures and blue color represents a negative effect. Non-significant associations are colored white

The GWAS on HEX revealed only the two variants at *ANAPC1* and *TCF4* (Table 2, Fig. 2b), both of which associate more strongly with cell density.

We identified three loci associating most strongly with CV (Fig. 2c, Table 2). At 17p12, an intergenic variant rs2323458[A] located ~300 kb downstream of *HS3ST3B1*, associates with increased CV (AF = 36.1%, $\beta = 0.14$ SD, $P = 6.9 \times 10^{-13}$). rs2323458 is in moderate linkage disequilibrium (LD) with rs2323457 ($r^2 = 0.61$) which has been reported to associate with CCT¹¹. In our data, rs2323457 also associates with CV ($\beta = 0.14$ SD, $P = 1.4 \times 10^{-10}$) but conditional analysis revealed that the effect is driven by rs2323458 ($\beta_{\text{adjusted}} = 0.043$ SD, $P_{\text{adjusted}} = 0.21$). We did not replicate the effect of rs2323457 on CCT ($\beta = -0.033$ SD, $P = 0.13$), even though the power for replication is 94% at a two-sided significance level of 0.05. Two more variants associate with CV: rs10094779[G] at 8p23.1 ~40 kb upstream of *MIR124-1* and rs76561503[C] at 11q24.1 ~60 kb downstream of *MIR100HG* (AF = 24.2%, $\beta = 0.15$ SD, $P = 7.6 \times 10^{-12}$ and AF = 17.9%, $\beta = 0.16$ SD, $P = 3.3 \times 10^{-10}$, respectively). Variants at 8p23.1, between *MIR124-1* and *MSRA*, have been reported to associate with high myopia³⁹. rs10094779 is only moderately correlated with the reported variants ($r^2 < 0.25$). Interestingly, a variant in *MIR100HG*, rs577948, has also been reported to associate with myopia⁴⁰. However, rs76561503 is not correlated with the reported variant ($r^2 = 0.03$).

Five variants associated with CCT in our data (Fig. 2d, Table 2). Three are at established CCT loci: *ZNF469*, *WNT10A*, and *COL5A1*^{8,9,41}. The two novel CCT associations are with the missense variants p.Thr446Ile in *ADAMTS17* (MAF = 13.8%, $\beta = 0.18$ SD, $P = 1.3 \times 10^{-10}$) and p.Tyr24Cys in *GLT8D2* (MAF = 4.3%, $\beta = 0.30$ SD, $P = 3.9 \times 10^{-10}$). P. Thr446Ile in *ADAMTS17* has been associated with decreased intraocular pressure⁴² and decreased height⁴³. P.Thr446Ile associates with decreased intraocular pressure in our data ($\beta = 0.14$ SD, $P = 5.8 \times 10^{-7}$), but after adjusting for CCT the association is much weaker ($\beta_{\text{adjusted}} = 0.06$ SD, $P_{\text{adjusted}} = 0.024$). Rare sequence variants in *ADAMTS17* cause autosomal recessive Weill-Marchesani syndrome, a rare connective tissue disorder with features including microspherophakia, severe myopia, glaucoma, cataract, and short stature (OMIM: 607511). Notably, p.Tyr24Cys in *GLT8D2* associates with increased height in a meta-analysis of the Icelandic and UK Biobank data ($\beta = 0.06$ SD, $P = 1.4 \times 10^{-11}$, $N = 490,381$). To understand the relationship between height and CCT, we evaluated the correlation between the effect on height in the Icelandic and UK Biobank data ($N = 490,381$) of 693 reported adult height variants⁴⁴ and their effects on CCT, but found no correlation ($r^2 = 0.006$; $P = 0.038$; F test) (Supplementary Figure 13). To validate the CCT association of the two novel variants, we tested them in a non-overlapping sample of 1459 Icelanders with CCT measurements from the Reykjavik Eye Study⁴⁵. At a significance threshold of $P < 0.05$, we replicated the associations for both p.Thr446Ile in *ADAMTS17* and p.Tyr24Cys in *GLT8D2* with CCT ($\beta = 0.25$ SD, $P = 7.3 \times 10^{-3}$ and $\beta = 0.11$ SD, $P = 0.045$, respectively) (Supplementary Table 5).

Gene expression. We examined the expression levels of the genes at the 10 associating loci using the publicly available Ocular Tissue Database⁴⁶. For non-coding variants we looked for all genes in a 500 kb region around the associating variant. We found that 13 of the 15 genes at the 10 loci are expressed in all ocular tissues (Supplementary Data 1). *MIR124-1* and *MIR100HG* were not found in the database, but previous studies have reported that *MIR124-1* is expressed in the human lens⁴⁷ and both *MIR124-1*

and *MIR100HG* are expressed in the human retina^{40,48}. Expression levels varied across tissues for some genes, e.g., *GLT8D2* is most highly expressed in the cornea, *TCF4* in the sclera and *ANAPC1* in the optic nerve head.

Disease variants affect corneal structure. Two different mutations in *CHST6* are known to cause MCD in Iceland; a missense variant p.Ala128Val (MAF = 0.66%) and a frameshift variant p.Val6MetfsTer106 (MAF = 0.07%)¹⁵. The prevalence of MCD in Iceland is ~1/13,000⁴⁹. We observed that 11 out of 16 homozygous carriers of p.Ala128Val, and two out of three homozygous carriers of p.Val6MetfsTer106, have been diagnosed with hereditary corneal dystrophies (ICD10 code H18.5). We investigated the effect of these known disease variants on the corneal measures from the specular microscopy. P.Val6MetfsTer106 associates with cell density and HEX ($\beta = -3.02$ SD, $P = 4.5 \times 10^{-4}$ and $\beta = -2.00$ SD, $P = 9.3 \times 10^{-3}$, respectively) under the recessive model. The association is due to two homozygote carriers showing extremely low values (Supplementary Table 6.a). No homozygote carriers of p.Ala128Val participated in the deCODE health study. We did not observe an effect of the two variants among heterozygous carriers (Supplementary Table 6b).

Out of the 6125 study participants, 194 (3.2%) had primary open-angle glaucoma (POAG). Among our corneal measures from the specular microscopy, POAG correlates most strongly with cell density (glaucoma patients have 104 cell/mm² lower cell density than controls, $P = 2.1 \times 10^{-6}$; F test). It also correlates with CH, IOPcc and CCT (Table 1).

Due to the correlation between various corneal measures and glaucoma status we assessed the effects of all 15 variants reported to associate with POAG⁵⁰ (Supplementary Data 2). First, we replicated the association of 11 out of the 15 variants with POAG ($P < 0.05$). The estimated replication power for the remaining four variants at *GMD2*, *ZFPM2*, *ATXN2*, and *PMM2* was > 99.7% at two-sided significance level of 0.05. We investigated the effects of the 11 replicated variants on different corneal measures (Fig. 4a). Five of the 11 variants associate with IOPg, where the POAG risk increasing allele associates with increased IOPg. Even though the strongest relationship of glaucoma is with CH (CH is lower in glaucoma patients), only two of the POAG variants associate with CH. Counter-intuitively, the *TMCO1* allele that increases glaucoma risk associates with lower CH but the *FND3B* allele that increases glaucoma risk associates with greater CH. The variant in *FOXC1* is the only POAG variant that also affects cell density. The correlation between the variants' effect on POAG and their effect on available corneal measures were not significant for any trait, controlling the false discovery rate at 0.05 (Supplementary Table 7).

Previously reported variants for CCT and IOP. GWASs have been published for two corneal traits, CCT and IOP. For CCT, 49 variants have been reported^{7,11} and we replicate 28 of them ($P < 0.05$) (Supplementary Data 3). The CCT effects of these variants, and the two novel CCT variants in *ADAMTS17* and *GLTD82*, correlate with their effects on CH, CRF, and IOPg (Supplementary Table 7, Supplementary Figure 14). The effects on different corneal measures by the CCT increasing allele is shown in Fig. 4b. A recent study using participants from the UK Biobank ($N = 115,486$) identified 209 variants at 175 novel loci associating with IOPg⁵¹. We replicate 56 out of these 209 variants in the much smaller Icelandic data ($P < 0.05$) (Supplementary Data 4). The effects on different corneal measures by the IOPg increasing allele is shown in Fig. 4c. The effects of the IOP variants on IOPg correlate with their effects on CH, CRF, IOPcc, and POAG (Supplementary Table 7, Supplementary Figure 15).

Discussion

Using measurements from a specular microscopy, we discovered seven novel variants associating with measurements of corneal structure, i.e., CCT, cell density, CV, and HEX. We further examined their effect on ocular biomechanics, such as CH, CRF, and IOP, as well as examining their effects on the risk of glaucoma and corneal diseases.

The most significant finding is the association of rs78658973 near *ANAPC1* with cell density. *ANAPC1* encodes the Anaphase Promoting Complex Subunit 1, a cell cycle-regulated E3 ubiquitin ligase that controls progression through mitosis and the G1 phase of the cell cycle⁵². The complex is composed of 15–17 subunits, which are highly conserved from yeast to humans. Mutations in the orthologous gene in the fruitfly, *shattered* (*shtd*), result in defective eye development⁵³ because of disruption of the G1 cell cycle arrest and progression through mitosis. Interestingly, rs78658973 also strongly increases CH, independently of its effect on cell density. CH measures ability of the corneal tissue to dampen pressure changes; such mechanical properties of most tissues are dominated by an extracellular matrix (ECM), in which the connective tissue fibers provide mechanical strength⁵⁴. Many rare connective tissue disorders are characterized by both skeletal and eye abnormalities, such as Marfan syndrome, Ehlers-Danlos syndrome, dermatosparaxis type and Weill-Marchesani syndrome (OMIM: 154700, 225410, and 607511, respectively). An intron variant in *ANAPC1*, rs17040773[G], in complete LD with rs78658973[A] ($r^2 = 1.00$), has been reported to associate with decreased bone mineral density⁵⁵ and *ANAPC1* is expressed in bone (Hs.436527). A possible mechanism explaining the association of rs78658973 with cell density in the corneal endothelium may be the role *ANAPC1* has in controlling proliferation of the developing corneal endothelial cells, and likewise, proliferation of bone cells influencing bone density. However, a direct relationship between rs78658973 and *ANAPC1*, or any other gene at the locus, remains to be shown. The estimated fraction of variance of cell density explained by rs78658973 is 24% which is extremely high for such a complex human trait. In comparison, no other variant listed in the GWAS catalog⁵⁶ explains higher fraction of variance of a quantitative phenotype available in the extensive deCODE database (Supplementary Table 8). This finding has clinical importance, since reduction in endothelial cell density along with more variable cell size and shape is usually the first sign of corneal endothelial diseases and cell density is important when assessing risk of corneal endothelial failure prior to intraocular surgery⁵⁷. Corneal graft survival studies found cell density lower than 1700 cells/mm², 6 months after graft surgery to be associated with increased risk of graft failure⁵⁸. Thirty-year-old homozygote carriers of the *ANAPC1* variant have on average 2637 endothelial cells per mm², which is less than the average 70-year old noncarriers (Fig. 1e). Further studies could assess if carriers of the variant are more likely to suffer endothelial decompensation after intraocular surgery or if their cornea is associated with increased risk of corneal graft failure when used as donors.

We also found association of variants at known disease loci, *TCF4* and *CHST6* (FCED and MCD, respectively), with cell density and HEX, demonstrating how these structural measurements are affected by corneal diseases. The *TCF4* variant also strongly associates with CH and it is interesting to note that the *TCF4* and *ANAPC1* variants control almost the same quantitative corneal traits (Fig. 3) but have very different associations with corneal disease. Furthermore, glaucoma risk is strongly associated with lower cell density, while for example the *ANAPC1* variant that controls a quarter of the cell density variance does not associate directly with POAG or PACG. These findings suggest that the pathogenic processes that cause corneal diseases and

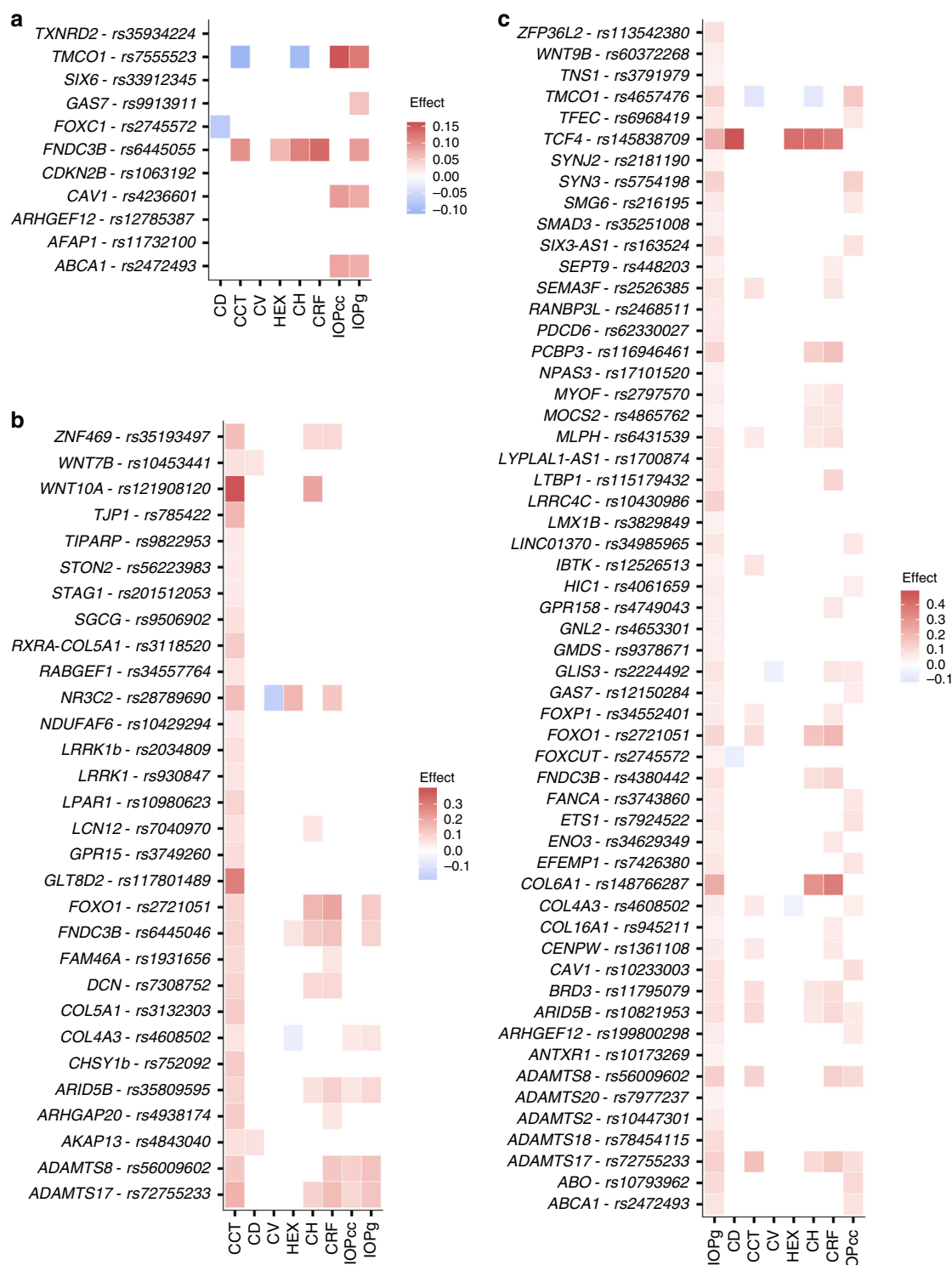


Fig. 4 The effect of reported POAG, CCT, and IOP variants on corneal measures. Red color represents a positive effect on the corneal measures and blue color represents a negative effect. **a** Effect of previously reported POAG variants on corneal traits for the POAG risk increasing allele. Effects on the traits are shown for significant associations after adjusting for multiple testing with a false discovery rate procedure for each variant. **b** Effect of previously reported CCT variants that replicate in our data ($P < 0.05$) and novel CCT variants on corneal measures for the CCT increasing allele. Effects on other traits are shown for significant associations after adjusting for multiple testing with a false discovery rate procedure for each variant. **c** Effect of previously reported IOPg variants that replicate in our data ($P < 0.05$) on corneal measures for the IOPg increasing allele. Effects on other traits are shown for significant associations after adjusting for multiple testing with a false discovery rate procedure for each variant

glaucoma may also lower the cell density, but low cell density in and of itself does not increase risk of disease. In addition, we evaluated the effects of reported POAG variants on corneal structure to further explore the relationship between these traits. Even though CH, cell density, and CCT are lower in POAG patients, the effects of POAG associating variants on POAG risk do not correlate with their effects on these traits in our data. This suggests that these variants do not confer risk of POAG through their effect on these corneal metrics.

Healthy corneal endothelium is necessary for visual perception and its dysfunction is the most common reason for corneal transplantation. Our understanding of the structure and function of corneal endothelial cells and how they relate to diseases is limited. The work presented here constitutes a contribution toward shedding a light on this.

Methods

Study Subjects. Endothelial images from non-contact auto-tracking and -focusing Konan CellCheck SL specular microscopy (Konan Medical USA Inc., Irvine, CA) and measures of ocular biomechanics using the Reichert ocular response analyzer^R (ORA G3, Reichert Technologies, Depew, NY, USA) were obtained for 6266 Icelanders as a part of the deCODE health study. Participation in the deCODE health study also includes an online questionnaire and verbal interviews about health and lifestyle, a number of physical measurements, blood sample collection, and permission to access health-related information from a range of registries and records, including hospital data. We defined subjects in the study to have glaucoma if they reported history of glaucoma or had a hospital discharge diagnosis of primary open-angle glaucoma (ICD10 code H40.1). CCT associations were replicated in a previously described dataset from the Reykjavik Eye Study⁵⁹. Testing variants for association with ocular diseases, we defined the glaucoma and corneal disorder populations based on six different ICD diagnoses; glaucoma information was obtained from participants in the Reykjavik Eye Study and from Icelandic ophthalmologists as described previously⁶⁰ (ICD10 H40.1, 4004 cases and 237,214 controls), primary open-angle glaucoma (ICD10 code H40.1, 1261 cases and 303,388 controls), primary angle-closure glaucoma (ICD10 code H40.2, 78 cases and 229,149 controls), disorders of cornea (ICD10 code H18, 133 cases and 255,274 controls), corneal degeneration (ICD10 code H18.4, 81 cases and 287,394 controls), and hereditary corneal dystrophies (ICD code H18.5, 119 cases and 301,665 controls). Written informed consent was obtained from all participants, in accordance with the Declaration of Helsinki, the study was approved by the Icelandic Data Protection Authority and the National Bioethics Committee (VSNb2015120006/03.01 with amendments).

The UK Biobank study is a large prospective cohort study of ~ 500,000 individuals from across UK, aged between 40 and 69 at recruitment⁶¹. Extensive phenotypic and genotypic information has been collected for the participants, including ICD coded diagnoses from inpatient and outpatient hospital episodes. In this study, we defined the glaucoma and corneal disorder populations based on six different ICD diagnoses; glaucoma (ICD10 code H40, 4428 cases and 404,139 controls), primary open-angle glaucoma (ICD10 code H40.1, 1035 cases and 402,449 controls), primary angle-closure glaucoma (ICD10 code H40.2, 699 cases and 407,868 controls), disorders of cornea (ICD10 code H18, 623 cases and 407,868 controls), corneal degeneration (ICD10 code H18.4, 118 cases and 396,627 controls), and hereditary corneal dystrophies (ICD code H18.5, 211 cases and 378,987 controls). Diagnoses were obtained from primary or secondary diagnoses codes a participant had recorded across all their episodes in hospital. Self-reported diagnoses were excluded from our analysis and we only included individuals determined to be of white British ancestry⁶². We did not exclude related individuals from the analysis but used LD score regression⁶³ to account for inflation in test statistics due to relatedness. In addition, height measurements were available for 407,825 individuals. UK Biobank's scientific protocol and operational procedures were reviewed and approved by the North West Research Ethics Committee (REC Reference Number: 06/MRE08/65), and informed consent was obtained from all participants.

Images from Konan CellCheck SL specular microscopy. The specular microscopy images were taken by specially trained nurses. One image was taken per eye. The nurse made a visual assessment of the image and selected automated analysis with auto trace for all normal images. Before automatic analysis, the size of cells (S, M, L, or XL) was determined and the cell border lines were compared with the cell borders visually.

All automated analysis were reviewed by a cornea specialist (G.M.Z.) and if found acceptable the automated analysis was used.

Images with any abnormalities, e.g., black areas, highly irregular cell size or shape, or poor quality images, were marked for manual analysis. These images were reviewed and analysed with the Center Method or in a few cases with the Flex Center Method. Images, where no cell structure was seen, were marked ungradable. All analysis was done by a cornea specialist (G.M.Z.).

We excluded 415 low-quality images for 276 subjects prior to the analysis by regressing cell count against estimated cell density and removed images where the residuals from the fitted model where < -15 . Thus, the sample size reduced from $N = 6266$ to $N = 6125$.

Whole-genome sequencing. The process used to whole-genome sequence the 28,075 Icelanders, and the subsequent imputation has been described in a recent publication^{30,31}. In summary, we sequenced the whole genomes of 28,075 Icelanders using Illumina technology to a mean depth of at least $10 \times$ (median $32 \times$). SNPs and indels were identified and their genotypes called using joint calling with GraphTyper⁶⁴. In total, 155,250 Icelanders were genotyped using Illumina SNP chips and their genotypes were phased using long-range phasing⁶⁵. All sequenced individuals were also chip-typed and long-range phased, which provided information about haplotype sharing that was used to improve genotype calls. Genotypes of the 37.6 million high quality sequence variants were imputed into all chip-typed Icelanders. Using genealogic information, the sequence variants were also imputed into relatives of the chip-typed to further increase the sample size for association analysis and increased the power to detect associations. All of the variants that were tested had imputation information over 0.8.

In UK Biobank genotyping was performed using a custom-made Affymetrix chip, UK BiLEVE Axiom in the first 50,000 individuals⁶⁶, and with Affymetrix UK Biobank Axiom array in the remaining participants⁶⁷; 95% of the signals overlap in both chips. Imputation was performed by Wellcome Trust Centre for Human Genetics using a combination of 1000Genomes phase 3⁶⁸, UK10K⁶⁹ and HRC reference panels⁷⁰, for up to 92,693,895 SNPs⁶².

Association analysis. All quantitative ocular measurements were averaged for both eyes, rank-based inverse normal transformed to a standard normal distribution separately for each sex and adjusted for age using a generalized additive model⁷¹. For each sequence variant, a linear regression model, using the genotype as an additive covariate, the transformed quantitative trait as a response and assuming the variance-covariance matrix to be proportional to the kinship matrix, was used to test for association.

We used LD score regression to account for distribution inflation in the dataset due to cryptic relatedness and population stratification⁶³. With a set of 1.1 million variants we regressed the χ^2 statistics from our GWASs against LD score and used the intercepts as a correction factors. The estimated correction factors were 1.05, 1.03, 1.03, 1.06, 1.06, 1.05, 1.05, and 1.05 for cell density, CV, HEX, CCT, CH, CRF, IOPg, and IOPcc, respectively.

We used logistic regression to test for association between sequence variants and binary traits, regressing trait status against expected genotype count. In the Icelandic data, we adjusted for sex, age or age at death, county of birth, blood sample availability and an indicator function for the overlap of the subject's lifetime with the time span of phenotype collection, by including these variables in the logistic regression model. In the UK Biobank data, we adjusted for sex and age, as well as 40 principle components in order to adjust for population stratification.

For the meta-analysis we used a fixed-effects inverse variance method⁷² based on effect estimates and standard errors from deCODE and the UK Biobank study. Sequence variants from deCODE and the UK Biobank imputation were matched on position and alleles.

Significance thresholds. The thresholds for genome-wide significance were estimated from the Icelandic data and corrected for multiple testing with a weighted Bonferroni adjustment using the enrichment of variant classes as weights with predicted functional impact among association signals³². With 37.6 million sequence variants in the Icelandic data, the weights given in Sveinbjornsson et al. were rescaled to control the family-wise error rate. This resulted in significance thresholds of 2.5×10^{-7} for loss-of-function variants, 5.0×10^{-8} for moderate-impact variants, 4.5×10^{-9} for low-impact variants, 2.3×10^{-9} for other variants within DHS sites, and 7.5×10^{-10} for remaining variants. We evaluated false discovery rate, assessed with the q -value package in R. The P value cutoff of 5.0×10^{-8} corresponded to q -values of 0.0014 for cell density, 0.0035 for CV, 0.0117 for CCT, and 0.0116 for HEX, which add up to 3.4%.

When assessing, if associating variants have an effect on other corneal trait, we used the Benjamini-Hochberg false discovery rate (FDR) procedure controlling the FDR at 0.05 at each variant to account for multiple testing.

Correlation between effect sizes. We assessed the relationship between the effects of sequence variant on any two different traits by fitting a weighted linear regression model where the effect sizes for trait 1 was regressed on effect sizes for trait 2 and each variant was weighted by $f(1-f)$ where f is the minor allele frequency of the variants, so that rare variants have less weight in the computation than common variants. For binary traits we used log(OR) as effect size.

Fraction of variance explained. The fraction of variance explained is calculated using the formula $2f(1-f)a^2$ where f is the minor allele frequency and a is the additive effect⁷³. Calculating the fraction of variance explained for variants in the GWAS catalog, we estimated the effects of published variants with corresponding

phenotypes available in the deCODE data and calculated the fraction of variance explained using f and a obtained from the Icelandic population.

Code availability

We used the following publicly available software for the whole-genome sequencing process: for BWA 0.7.10 mem, see <https://github.com/lh3/bwa>; for Picard tools 1.117, see <https://broadinstitute.github.io/picard/>; for SAMtools 1.3, see <http://samtools.github.io/>; for Bedtools v2.25.0-76-g5e7c696z, see <https://github.com/arq5x/bedtools2/>; for GraphTyper 1.3, see <https://github.com/DecodeGenetics/graph typer>; for Variant Effect Predictor, see <https://github.com/Ensembl/ensembl-vep>.

Data availability

The sequence variants from the Icelandic population whole-genome sequence data have been deposited at the European Variant Archive under accession [PRJEB15197](https://www.eva.io/variant/PRJEB15197), GWAS summary statistics for association with $P < 1 \times 10^{-6}$ are available in Supplementary Data 5. The authors declare that the data supporting the findings of this study are available within the article, its supplementary files, and upon request.

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Author contributions

E.V.I., S.B., G.Thorleifsson, P.S., A.O., U.S., G.M.Z., U.T., D.F.G., F.J., H.H. and K.S. designed the study and interpreted the results. E.V.I., S.B., S.K., G.Thorleifsson, G.A.A. and D.F.G. analyzed the data. E.V.I., H.H., G.Thorgeirsson, I.J., G.Thorleifsson, G.A.A., G.M.Z. and F.J. performed recruitment and phenotyping. The manuscript was drafted by E.V.I., S.B., P.S., D.F.G., F.J., H.H. and K.S. All authors contributed to the final version of the manuscript.

Additional information

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Competing interests: The authors E.V.I., S.B., G.Thorleifsson, P.S., A.O., U.S., S.K., G.A.A., G.Thorgeirsson, I.J., U.T., D.F.G., H.H., and K.S. declare competing financial interests as employees of deCODE genetics/Amgen, Inc. G.M.Z. and F.J. declare no competing interests.

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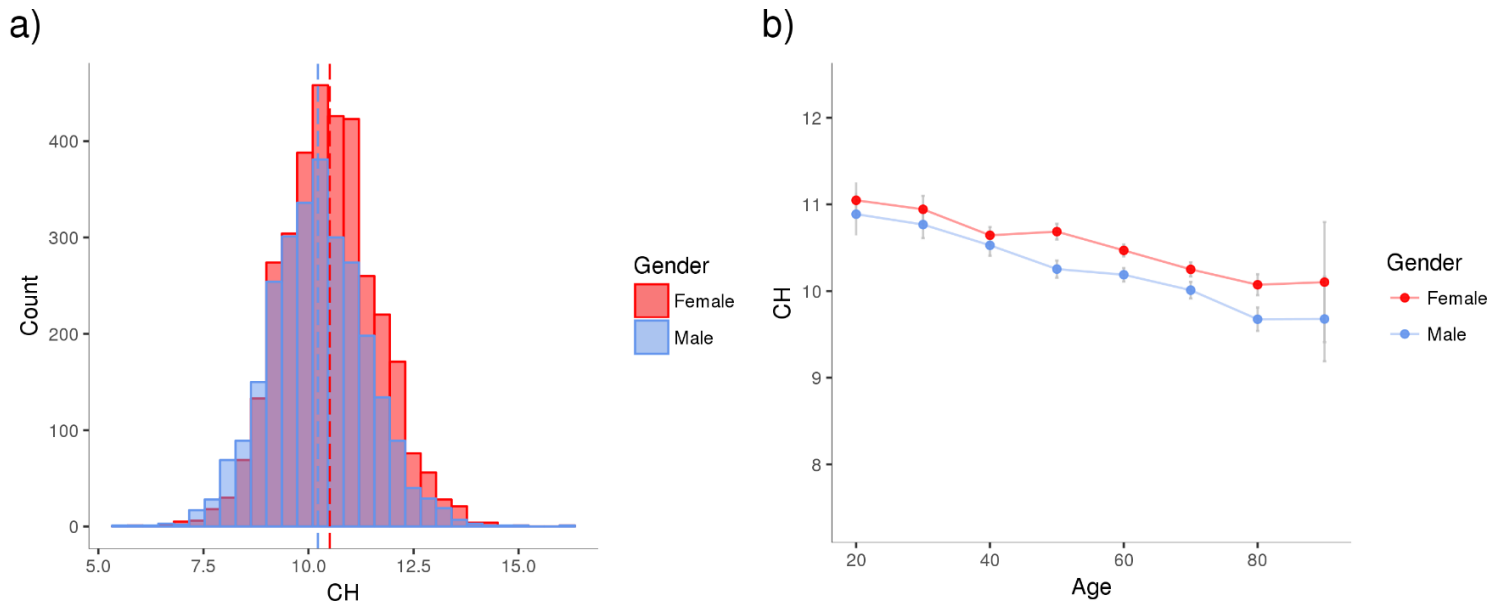
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Supplementary Information

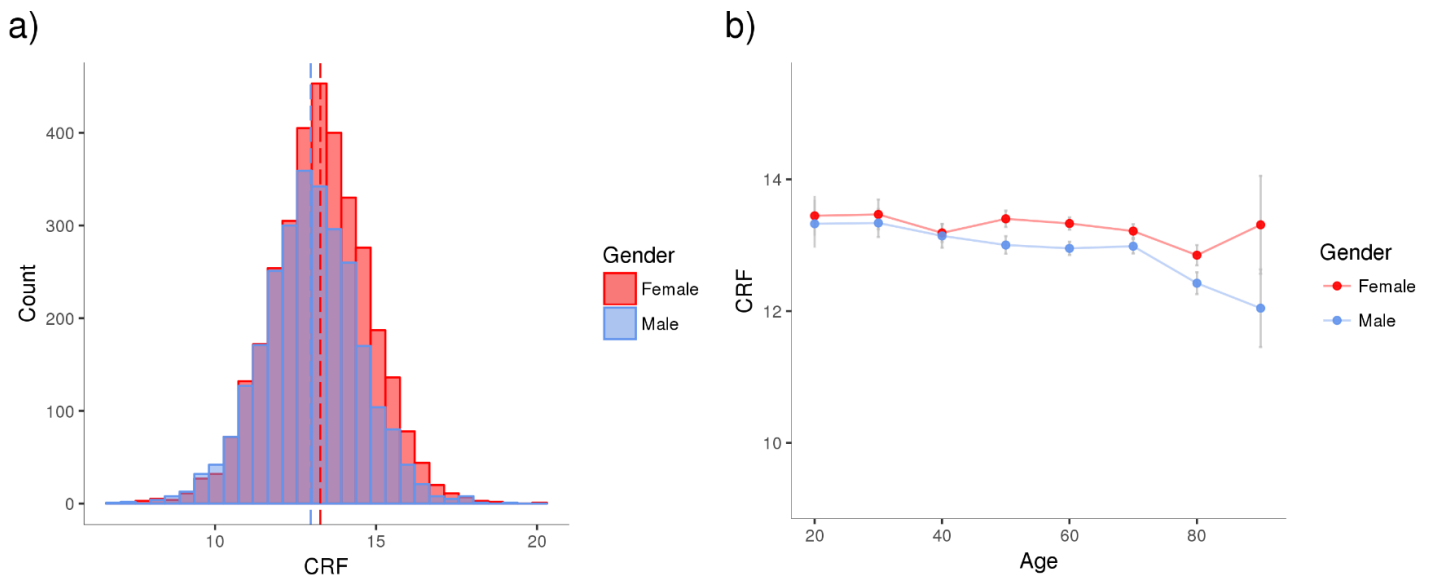
Sequence variation at *ANAPC1* accounts for 24% of the variability in corneal endothelial cell density

Ivarsdottir et al.

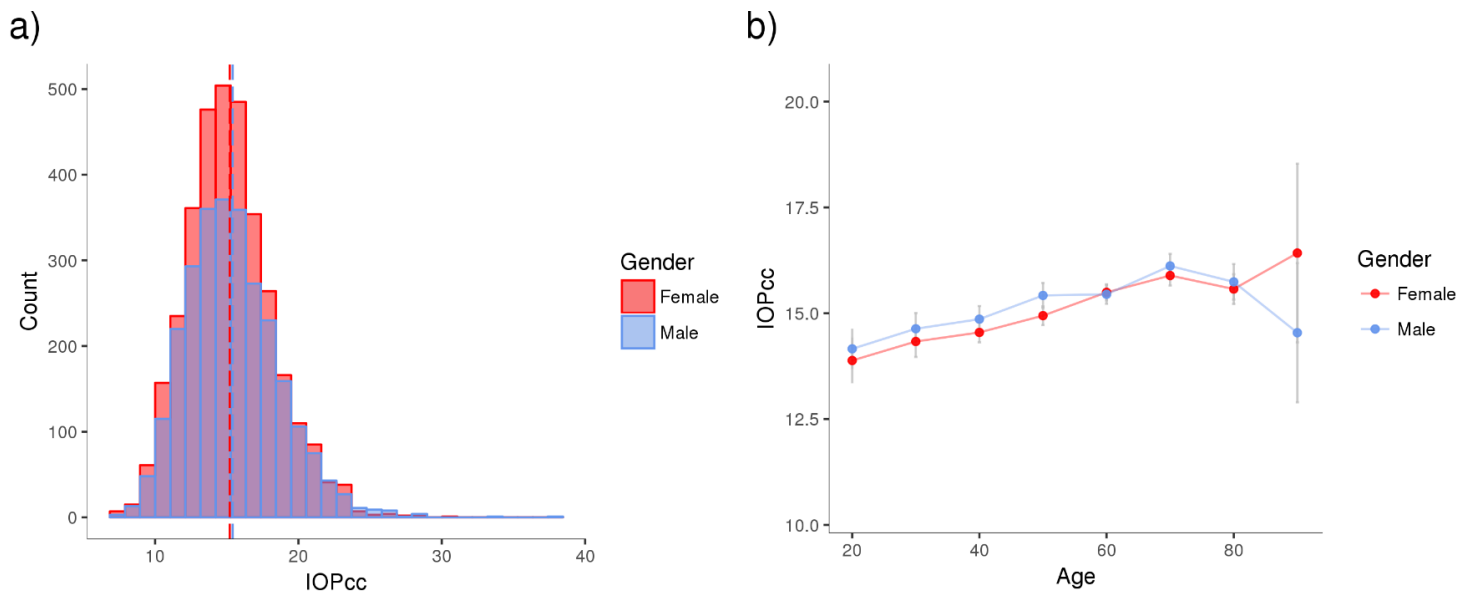
Supplementary Figures



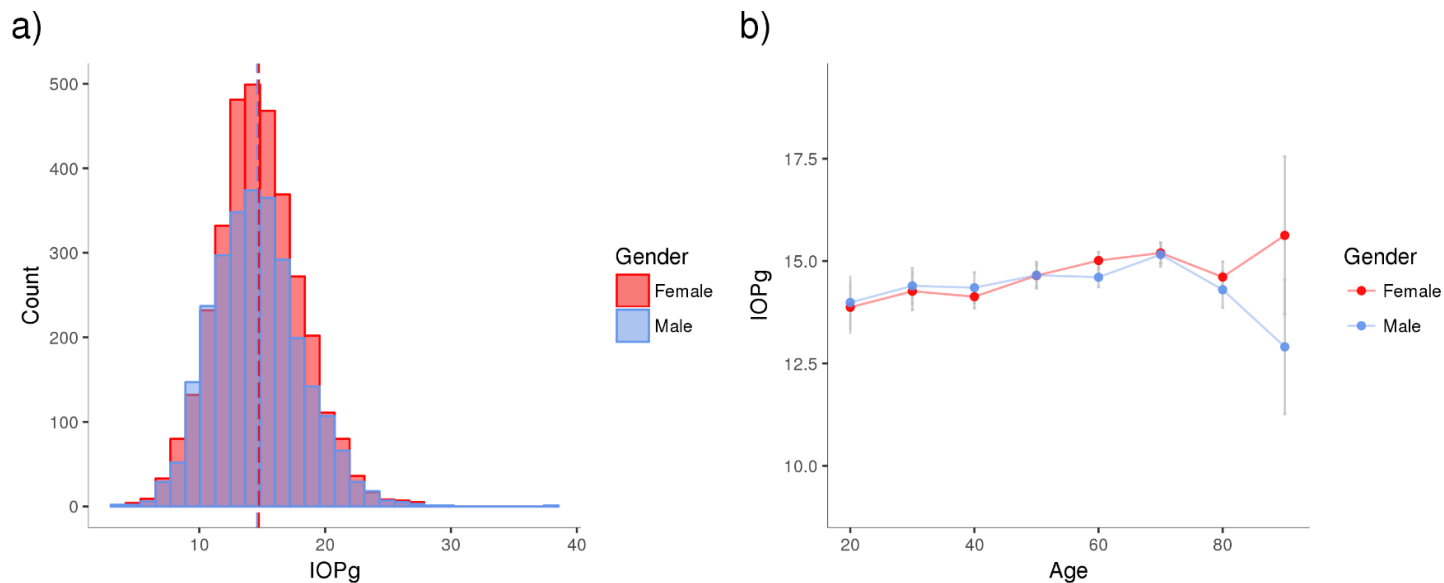
Supplementary Figure 1. Corneal hysteresis (CH) by age and sex (N=6,125). **a)** Histograms showing the distribution of CH measurements for each sex. **b)** The average CH values for subjects belonging to a 10 year age group (e.g. Age=30 for individuals between 26 and 35) against age, for men and women. The grey lines show the 95% confidence intervals.



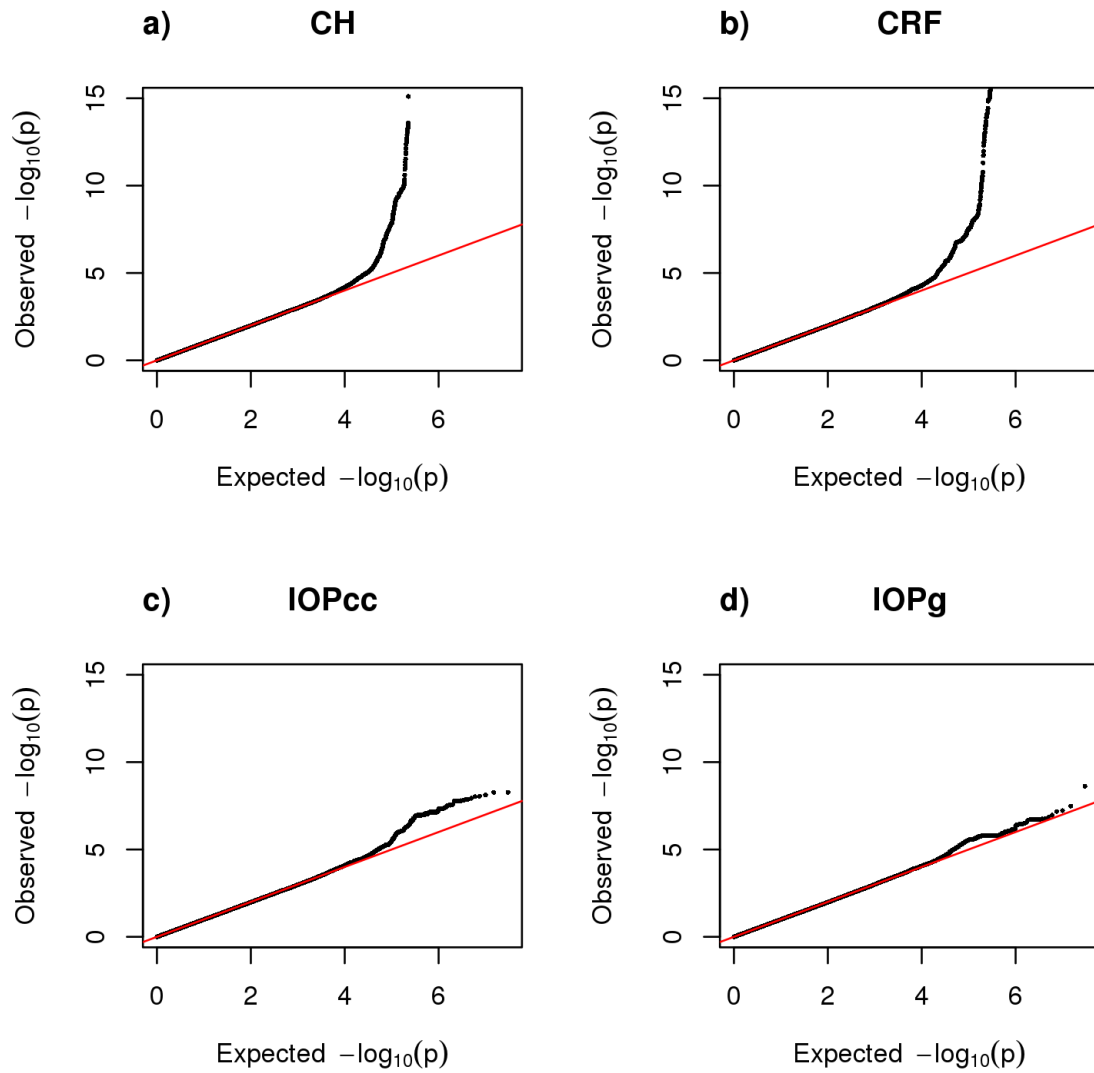
Supplementary Figure 2. Corneal resistance factor (CRF) by age and sex (N=6,125). a) Histograms showing the distribution of CRF measurements for each sex. **b)** The average CRF values for subjects belonging to a 10 year age group (e.g. Age=30 for individuals between 26 and 35) against age, for men and women. The grey lines show the 95% confidence intervals.



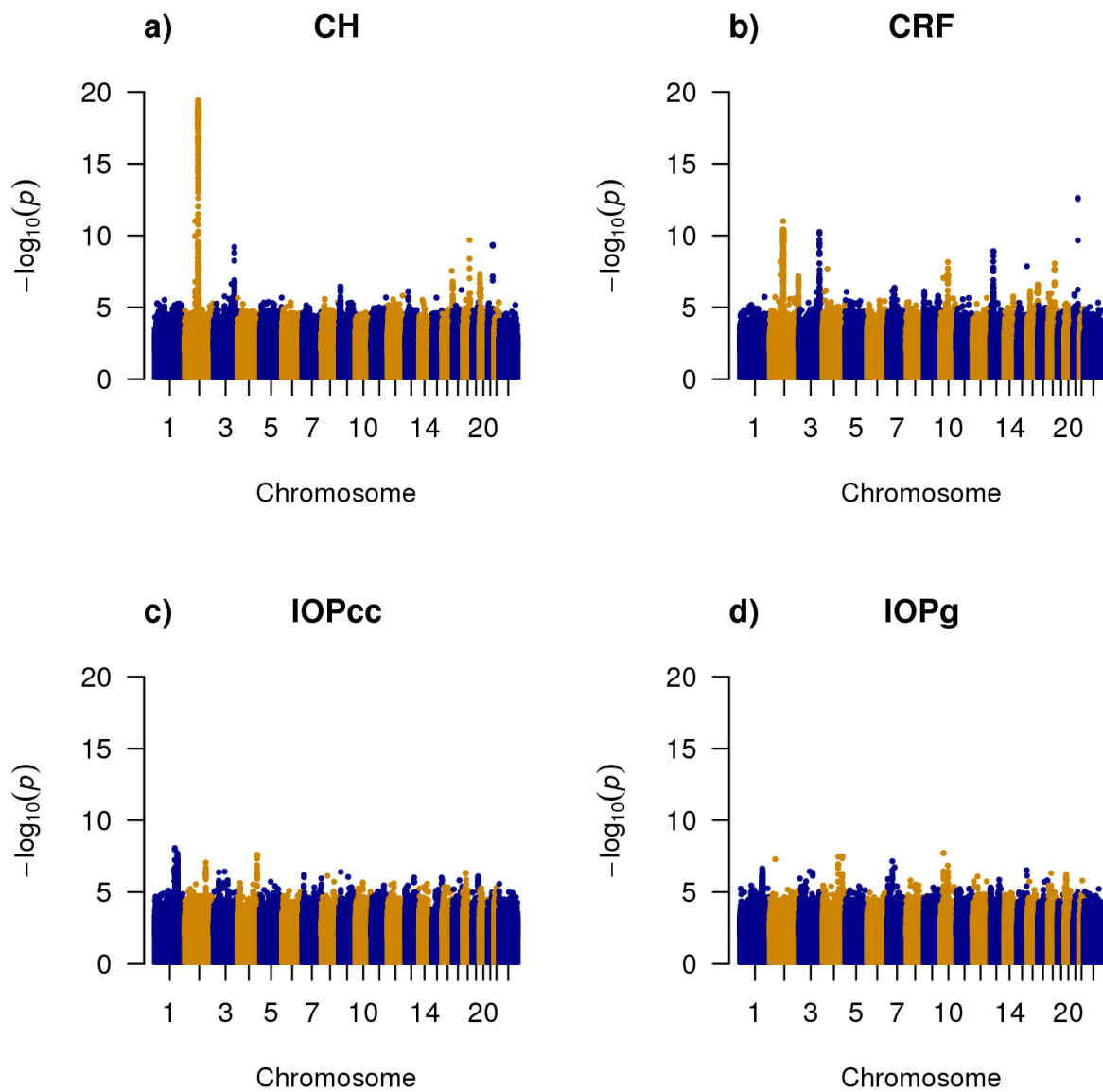
Supplementary Figure 3. Corneal compensated intraocular pressure (IOPcc) by age and sex (N=6,125). **a)** Histograms showing the distribution of IOPcc measurements for each sex. **b)** The average IOPcc values for subjects belonging to a 10 year age group (e.g. Age=30 for individuals between 26 and 35) against age, for men and women. The grey lines show the 95% confidence intervals.



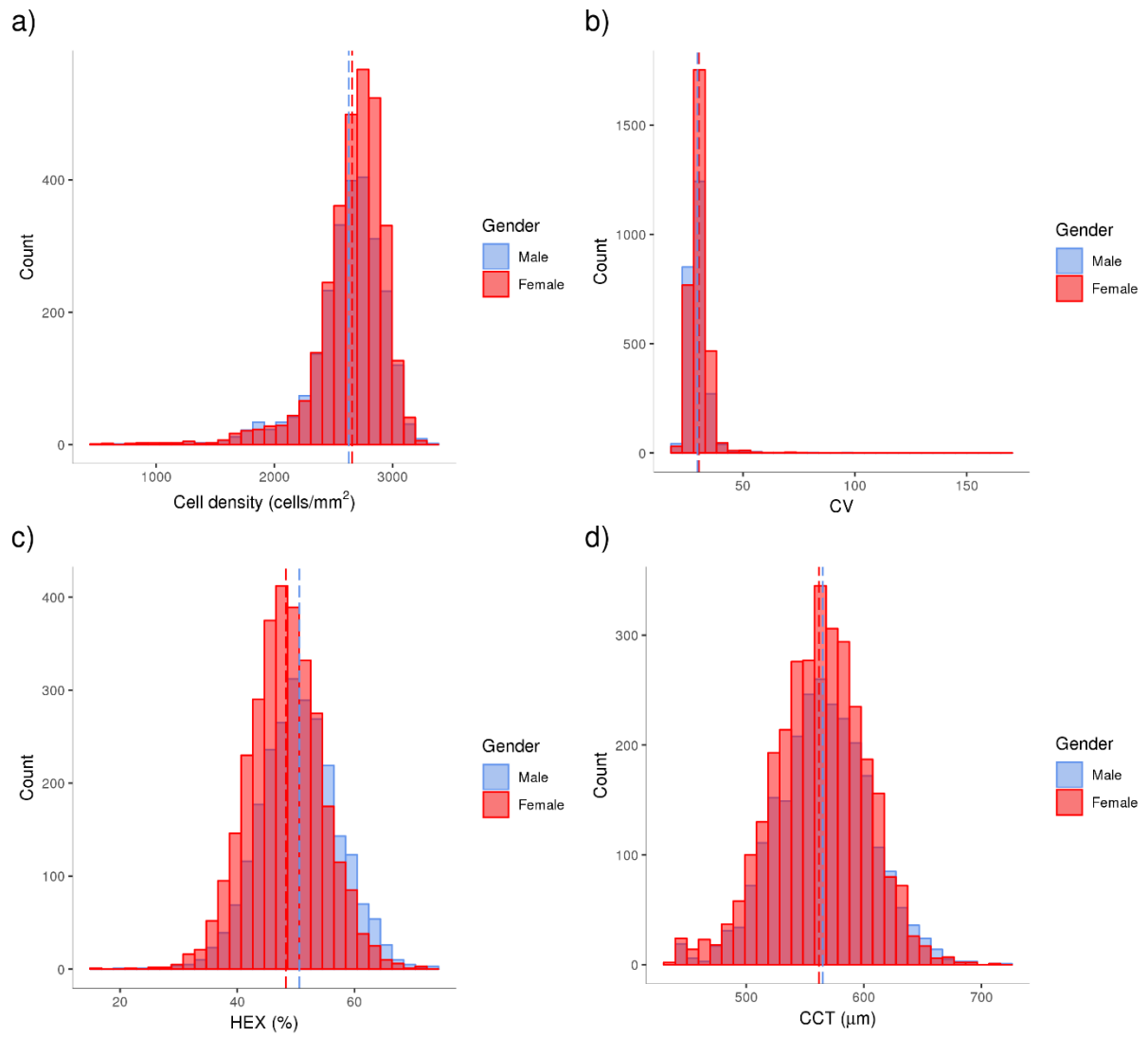
Supplementary Figure 4. Goldmann correlated intraocular pressure (IOPg) by age and sex (N=6,125). a) Histograms showing the distribution of IOPg measurements for each sex. **b)** The average IOPg values for subjects belonging to a 10 year age group (e.g. Age=30 for individuals between 26 and 35) against age, for men and women. The grey lines show the 95% confidence intervals.



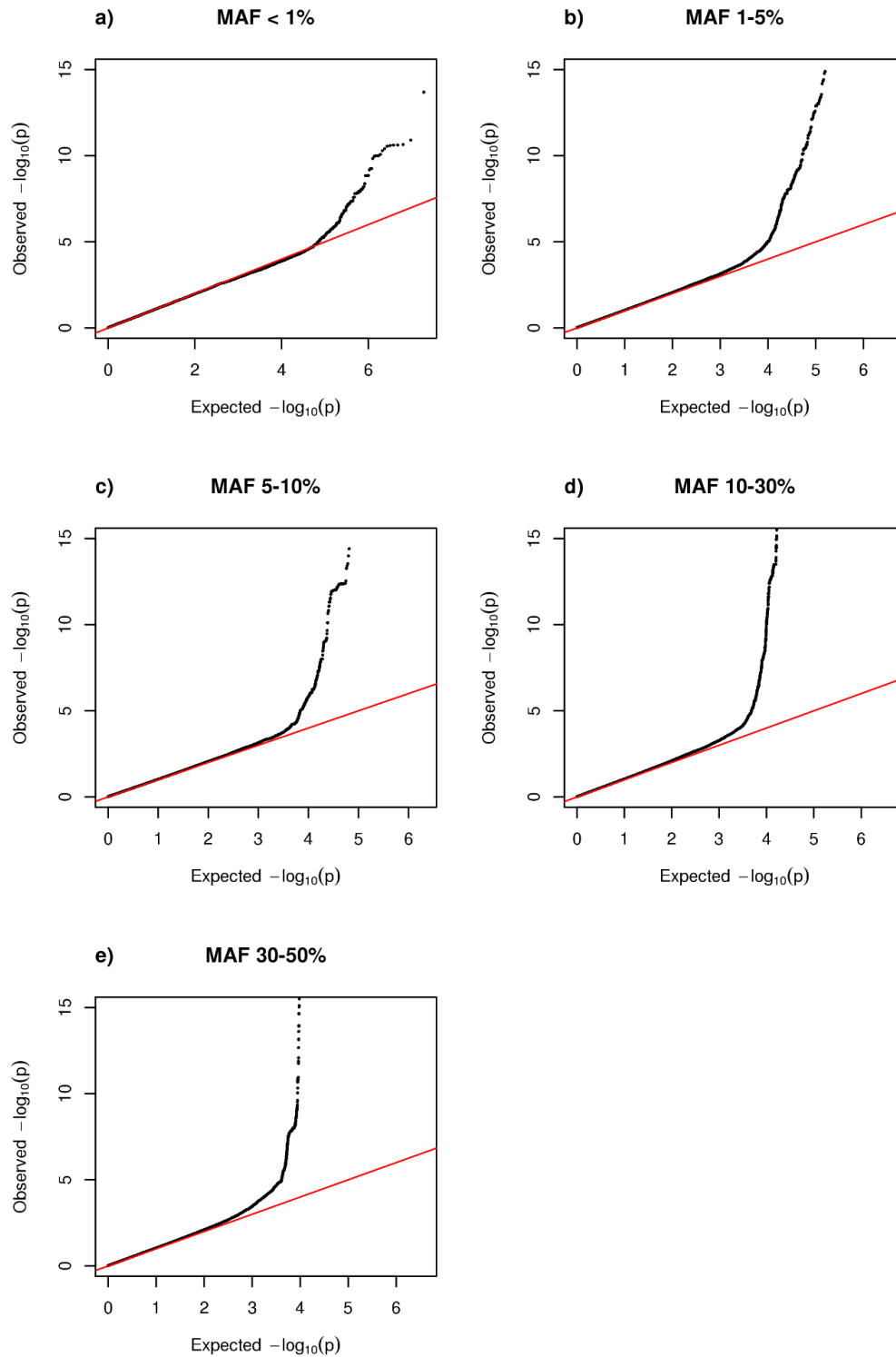
Supplementary Figure 5. Q-Q plot for GWAS results for ocular biomechanics; CH, CRF, IOPcc and IOPg. The y-axis shows the observed $-\log_{10}P$ values after adjustment using LD score regression. The observed values were ranked and for each rank the expected $-\log_{10}P$ value was determined. The correction factors are a) 1.06, b) 1.05, c) 1.05 and d) 1.05.



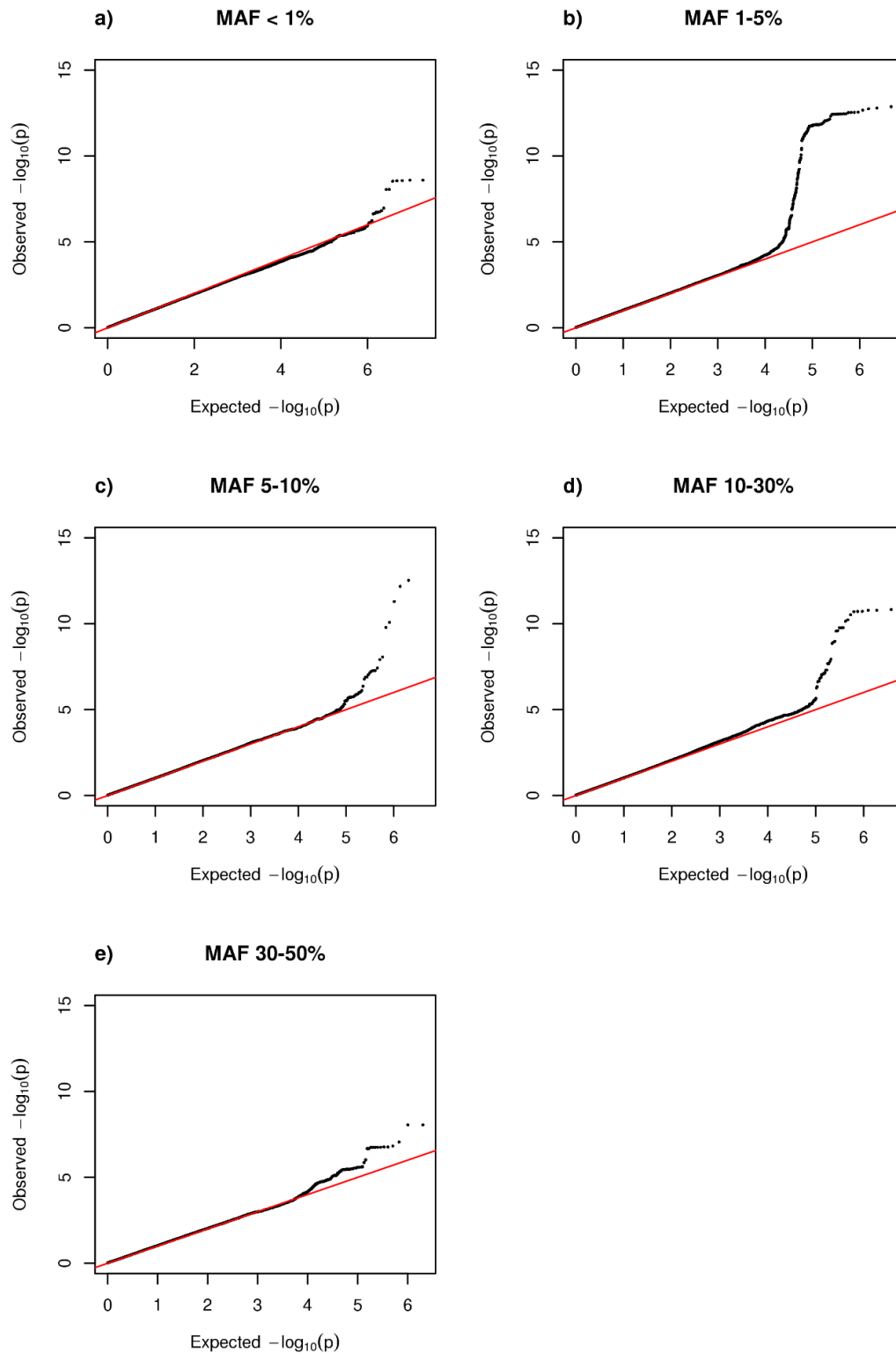
Supplementary Figure 6. Manhattan plots for the GWAS (N=6,125) on ocular biomechanics obtained from the ocular response analyzer. a) CH, b) CRF, c) IOPcc and d) IOPg. The $-\log_{10} P$ -values are plotted for each variant against their chromosomal position.



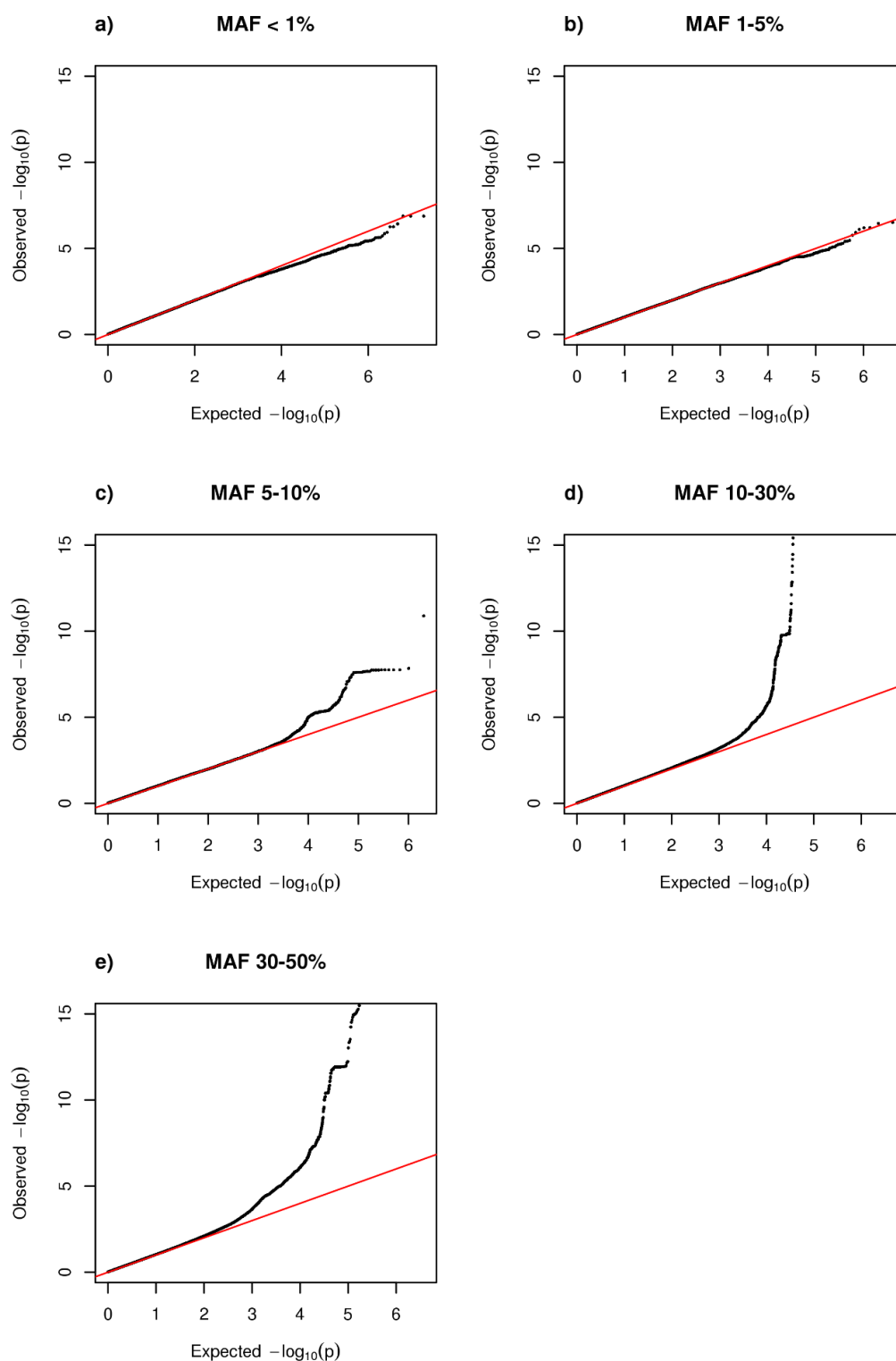
Supplementary Figure 7. Distribution plots for structural corneal measures. Histograms showing the distribution of **a)** cell density, **b)** coefficient of cell size variation (CV), **c)** percentage of hexagonal cells (HEX) and **d)** central corneal thickness (CCT) measurements for each sex.



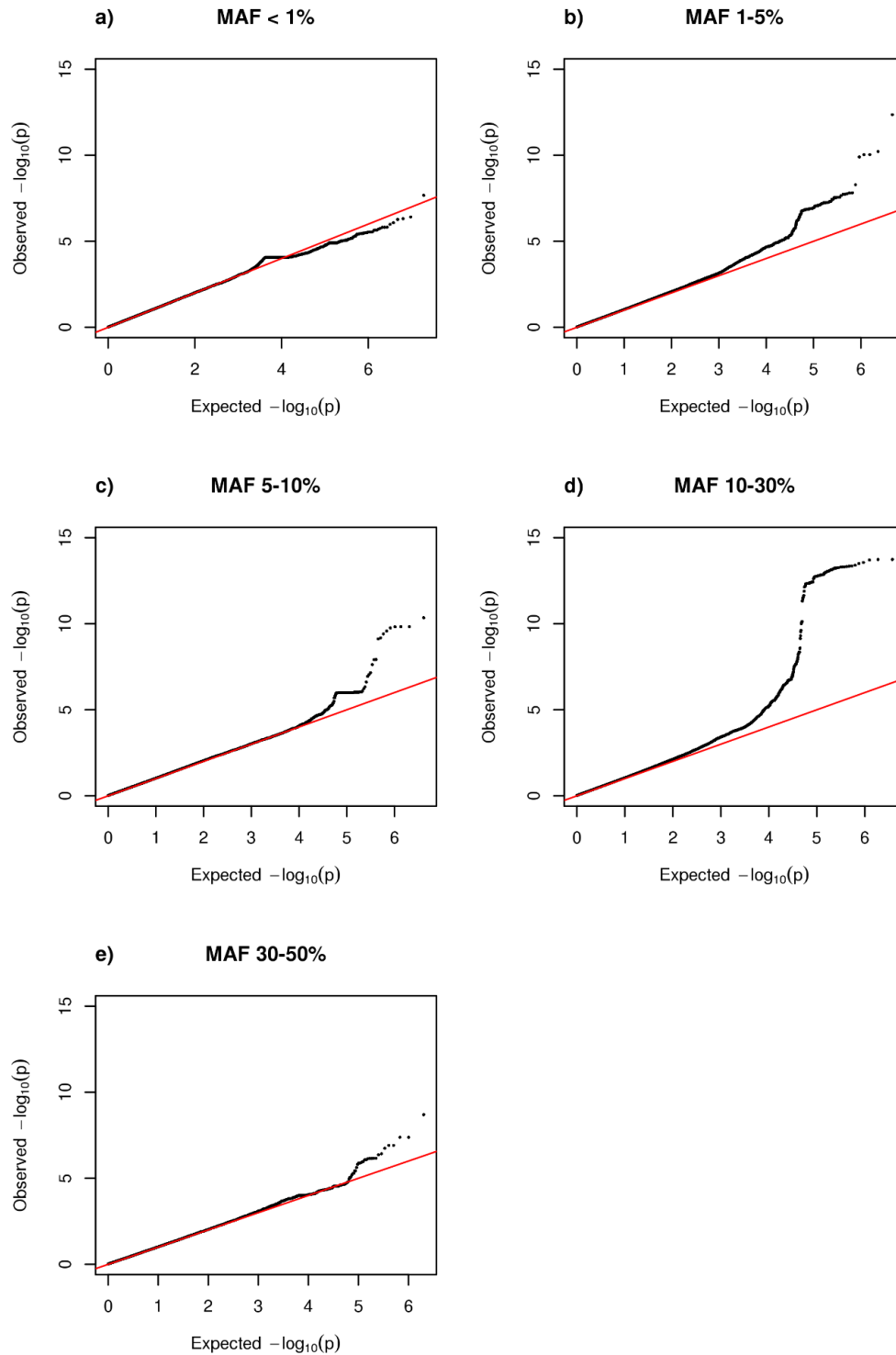
Supplementary Figure 8. Q-Q plot for GWAS results for cell density stratified by allele frequency. The y-axis shows the observed $-\log_{10}P$ values after adjustment using LD score regression (correction factor = 1.05). The observed values were ranked and for each rank the expected $-\log_{10}P$ value was determined.



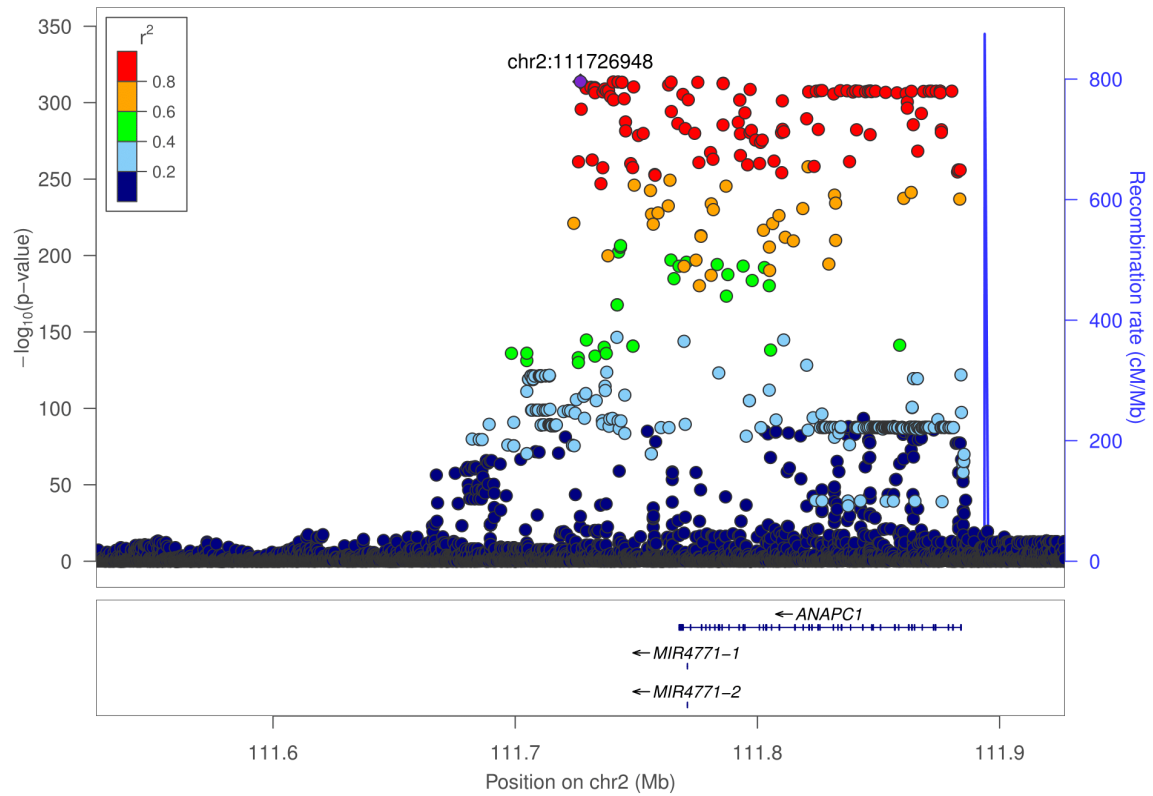
Supplementary Figure 9. Q-Q plot for GWAS results for HEX stratified by allele frequency. The y-axis shows the observed $-\log_{10}P$ values after adjustment using LD score regression (correction factor = 1.03). The observed values were ranked and for each rank the expected $-\log_{10}P$ value was determined.



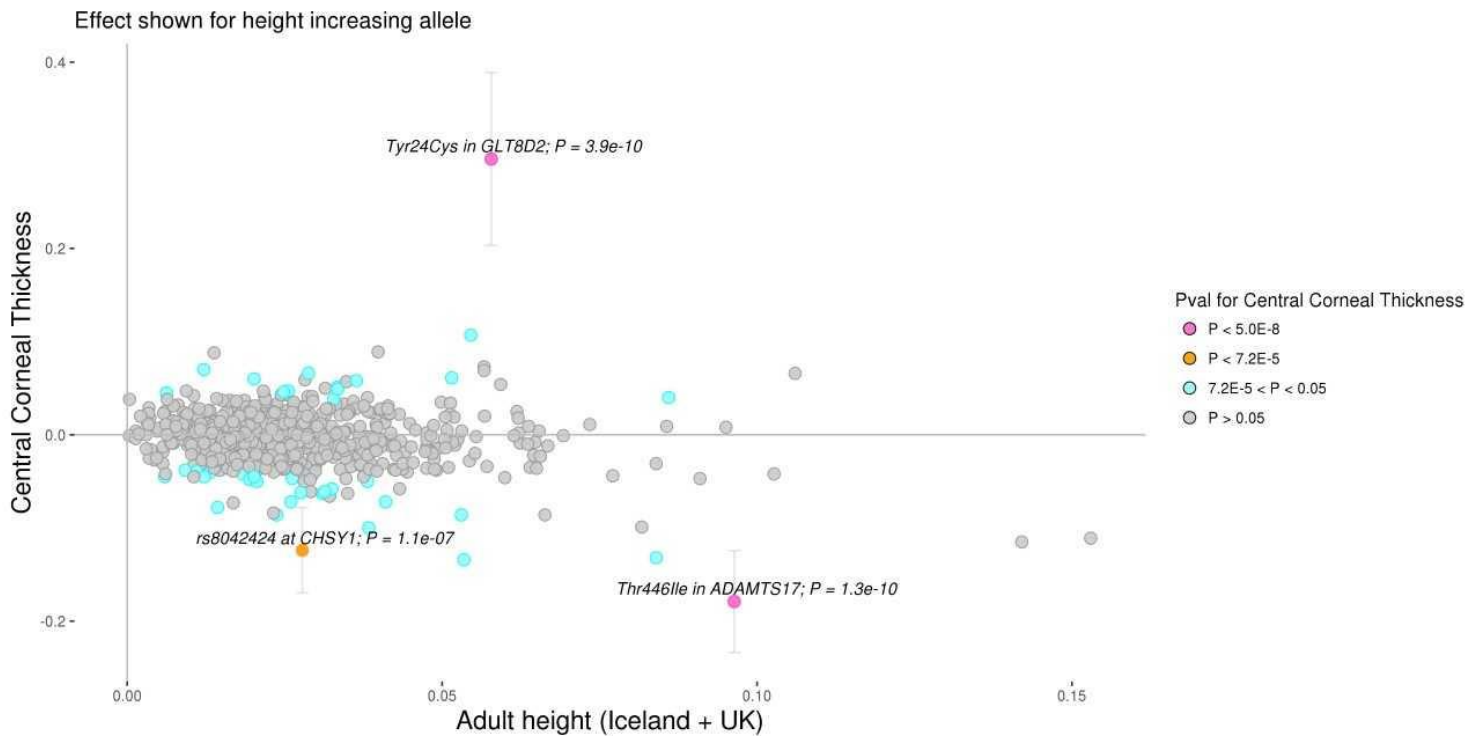
Supplementary Figure 10. Q-Q plot for GWAS results for CV stratified by allele frequency. The y-axis shows the observed $-\log_{10}P$ values after adjustment using LD score regression (correction factor = 1.03). The observed values were ranked and for each rank the expected $-\log_{10}P$ value was determined.



Supplementary Figure 11. Q-Q plot for GWAS results for CCT stratified by allele frequency. The y-axis shows the observed $-\log_{10}P$ values after adjustment using LD score regression (correction factor = 1.06). The observed values were ranked and for each rank the expected $-\log_{10}P$ value was determined.

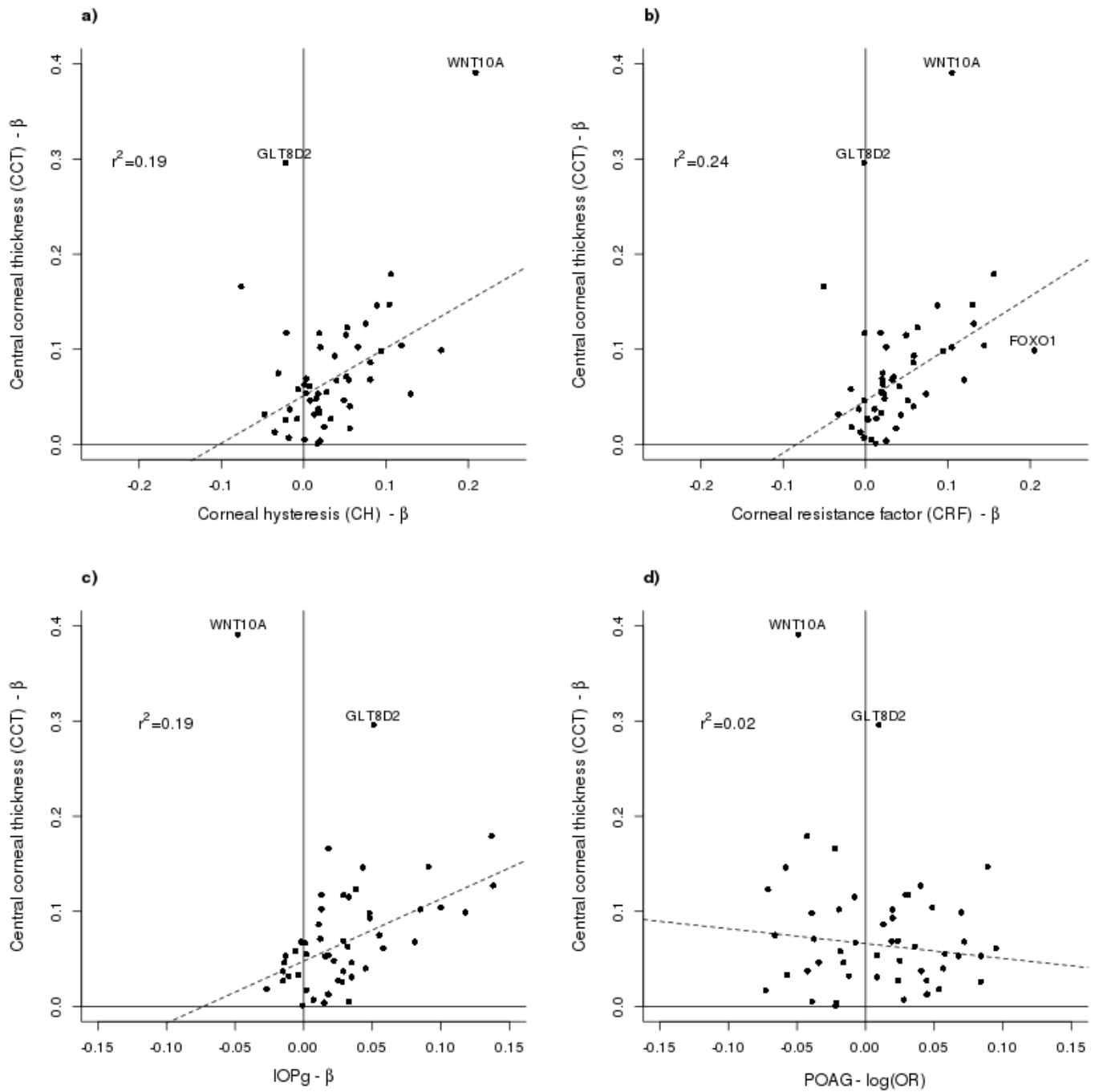


Supplementary Figure 12. Locus-plot showing variants at the *ANAPC1* locus (hg38) associating with cell density. The leading variant, rs78658973[A], is labelled as a purple diamond, other variants are colored according to correlation (r^2) with the leading marker. $-\log_{10}$ P -values are shown on the left y-axis and correspond to the variants depicted in the plot. The right y-axis shows calculated recombination rates at the chromosomal location, plotted as a blue line.

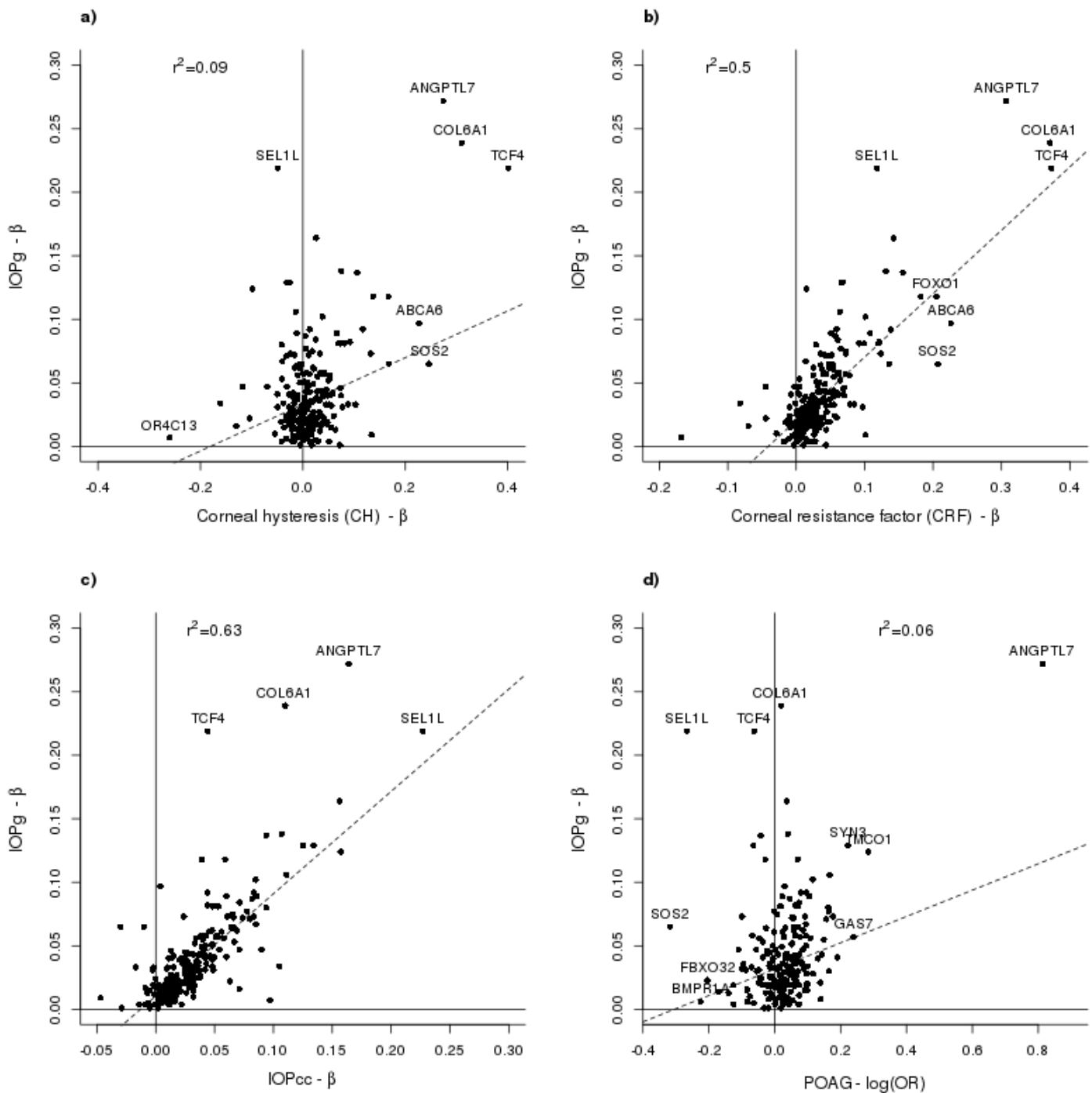


Supplementary Figure 13. The effect on height vs. effect on central corneal thickness (CCT) for known height variants.

Scatter plot showing the effect of 693 variants reported by GIANT¹, as well as the two novel CCT variants in *GLT8D2* and *ADAMTS17*, on adult height against their effect on CCT in our data. The effects are shown for the allele that is associated with increased height in our data. Different colors of points represent their P -values for their association with CCT. The grey lines show the 95% confidence intervals.



Supplementary Figure 14. Reported central corneal thickness (CCT) variants and their effect on CCT vs their effect on corneal hysteresis (CH), corneal resistance factor (CRF), Goldmann correlated intraocular pressure (IOPg) and primary open angle glaucoma (POAG). Scatter plot showing the effect of 48 variants reported to associate with CCT^{2,3}, as well as the two novel CCT variants in *GLT8D2* and *ADAMTS17*, on CCT against their effect on a) CH, b) CRF, c) IOPg and d) POAG in our data. Effects are given for the allele that increases CCT in our data.



Supplementary Figure 15. Reported intraocular pressure variants and their effect on Goldmann correlated intraocular pressure (IOPg) vs their effect on hysteresis (CH), corneal resistance factor (CRF), corneal compensated intraocular pressure (IOPcc) and primary open angle glaucoma (POAG). Scatter plot showing the effect of 175 variants reported to associate with IOP⁴ on IOPg against their effect on a) CH, b) CRF, c) IOPcc and d) POAG in our data. Effects are given for the allele that increases IOPg in our data.

Supplementary Tables

Supplementary Table 1. Correlation matrix for corneal measures. Pierson's correlation coefficients (r) and corresponding *P*-values are shown for all corneal traits obtained from the specular microscopy equipment and the ocular response analyzer. Correlation coefficients were computed after standardization and adjusting the measurements for age and sex. Correlation coefficients are shown in bold for significant correlations after adjusting for multiple testing with a false discovery rate procedure.

	CD		CV		CT		HEX		CH		IOPg		IOPcc	
	r	<i>P</i> -value	r	<i>P</i> -value	r	<i>P</i> -value	r	<i>P</i> -value	r	<i>P</i> -value	r	<i>P</i> -value	r	<i>P</i> -value
CV	-0.33	1.5E-155												
CT	0.10	8.0E-14	0.04	5.3E-03										
HEX	0.15	1.3E-30	-0.65	<1E-300	0.04	2.3E-03								
CH	-0.03	1.0E-02	0.08	2.2E-09	0.53	<1E-300	-0.01	6.6E-01						
IOPg	-0.03	9.6E-03	0.02	8.3E-02	0.46	<1E-300	0.06	2.2E-06	0.14	4.2E-26				
IOPcc	-0.01	3.0E-01	-0.01	3.3E-01	0.23	4.4E-73	0.06	1.3E-06	-0.27	3.9E-103	0.91	<1E-300		
CRF	-0.04	2.3E-03	0.07	5.5E-08	0.63	<1E-300	0.03	1.9E-02	0.71	<1E-300	0.75	<1E-300	0.44	2.3E-289

Supplementary Table 2. Significance thresholds for each variant category. The thresholds were estimated from the Icelandic data as previously described⁵.

Category	Variants in category	Broad DHS	Significance threshold
High impact	Splice donor variant, splice acceptor variant, stop gained, frameshift variant, stop lost, initiator codon variant.	-	2.5×10^{-7}
Moderate impact	Inframe indels, missense, splice region variants, stop retained variants.	-	5.0×10^{-8}
Low impact	Synonymous variants, 5' UTR variant, 3' UTR variant, up- and downstream variants, coding sequence variants.	-	4.5×10^{-9}
Other	Intronic, intergenic variants.	Yes	2.3×10^{-9}
		No	7.5×10^{-10}

DHS = Dnase I hypersensitivity sites

Supplementary Table 3. Meta-analysis. Number of cases and controls for each phenotype we tested in a meta-analysis of GWAS results from Iceland and the UK Biobank.

Categorical Trait	ICD 10 code	Iceland		UK Biobank		Total	
		N cases	N controls	N cases	N controls	N cases	N controls
Glaucoma	H40	4004	237214	4428	404139	8432	641353
Primary open angle glaucoma (POAG)	H40.1	1261	303388	1035	402549	2296	705937
Primary angle closure glaucoma (PACG)	H40.2	78	229149	699	407868	777	637017
Disorders of cornea	H18	613	328012	623	407944	1236	663218
Corneal degeneration	H18.4	81	287394	118	396627	199	684021
Hereditary corneal dystrophies	H18.5	119	304665	211	378987	330	683652
Keratoconus	H18.6	46	251017	81	408486	127	659503
Quantitative Trait		N		N		N	
Height		82556		407825		490381	

Supplementary Table 4. The association of corneal structure variants on all corneal measures and ocular diseases. Effects are given for the minor allele. Effects and *P*-values are shown in bold for genome-wide significant associations.

	ANAPC1		TCF4		17p12		11q24.1		8p23.1		ZNF469		WNT10A		COL5A1		ADAMTS17		GLT8D2	
	P-value	Effect	P-value	Effect	P-value	Effect	P-value	Effect	P-value	Effect	P-value	Effect	P-value	Effect	P-value	Effect	P-value	Effect	P-value	Effect
Cell density	1.8E-314	-0.77	1.6E-19	-0.39	9.3E-02	-0.03	1.0E-02	-0.07	6.5E-01	-0.01	2.2E-02	-0.05	9.4E-02	-0.10	4.7E-01	-0.02	8.3E-01	-0.01	4.5E-01	-0.04
CV	2.8E-28	0.23	4.1E-03	0.12	6.9E-13	0.14	3.3E-10	0.16	7.6E-12	0.15	8.5E-01	0.00	2.2E-01	-0.07	6.0E-01	-0.01	9.8E-01	0.00	7.6E-01	0.01
CCT	1.2E-01	-0.03	5.4E-01	-0.03	6.7E-02	-0.04	1.6E-02	-0.06	2.6E-03	0.07	1.9E-14	-0.15	4.5E-11	-0.39	6.1E-11	-0.14	1.3E-10	0.18	3.9E-10	0.30
HEX	2.8E-13	-0.16	5.9E-18	-0.37	5.4E-04	-0.07	5.4E-04	-0.09	1.5E-02	-0.05	3.2E-01	0.02	2.8E-02	0.13	6.4E-01	-0.01	1.2E-01	-0.04	9.3E-01	0.00
CH	2.6E-19	0.19	3.1E-12	-0.29	3.9E-02	0.04	1.8E-01	-0.03	1.1E-01	0.04	2.4E-07	-0.10	3.5E-04	-0.21	1.7E-01	-0.03	1.1E-04	0.11	6.4E-01	-0.02
IOPg	3.6E-01	0.02	1.7E-05	-0.18	9.4E-01	0.00	7.6E-02	-0.04	4.0E-01	-0.02	5.1E-03	-0.06	4.1E-01	0.05	4.9E-01	-0.02	5.8E-07	0.14	2.7E-01	0.05
IOPcc	4.5E-03	-0.06	1.9E-01	-0.05	4.5E-01	-0.02	2.1E-01	-0.03	1.2E-01	-0.03	4.4E-01	-0.02	1.8E-02	0.14	7.8E-01	-0.01	5.8E-04	0.09	2.1E-01	0.06
CRF	9.9E-11	0.14	7.9E-13	-0.30	1.8E-01	0.03	1.2E-01	-0.04	6.2E-01	0.01	3.2E-07	-0.10	7.2E-02	-0.11	1.5E-01	-0.03	1.4E-08	0.16	9.7E-01	0.00
Disorders of cornea (H18)	4.3E-01	0.95	1.4E-02	1.78*	8.9E-01	0.99	4.7E-02	1.13	3.2E-01	0.94	8.0E-03	1.35	1.6E-01	1.55	3.1E-01	0.94	1.7E-01	0.89	7.4E-01	0.94
Corneal degeneration (H18.4)	8.6E-01	0.98	9.9E-09	3.98*	5.5E-01	1.07	5.9E-03	1.39	9.2E-01	0.99	6.8E-02	0.97	9.3E-01	1.01	4.5E-01	1.09	8.2E-01	0.96	6.4E-01	1.15
Hereditary corneal dystrophies (H18.5)	3.0E-02	0.81	3.3E-31	7.77*	2.0E-01	0.90	5.6E-01	0.94	2.1E-01	0.89	3.7E-01	1.08	6.2E-01	1.13	5.1E-01	0.94	1.4E-01	0.82	8.2E-01	0.94
Keratoconus (H18.6)	5.1E-01	0.88	5.1E-01	1.35*	8.8E-01	0.98	3.3E-01	1.16	1.4E-01	0.80	2.7E-02	0.73	4.3E-01	1.35	6.2E-03	1.47	1.3E-01	0.71	6.6E-01	0.82
Glaucoma (H40)	4.8E-02	1.04	1.5E-01	0.92*	5.8E-01	0.99	4.2E-01	1.02	2.9E-01	1.02	3.4E-01	1.05	4.0E-01	1.14	7.9E-01	0.99	8.3E-01	1.01	6.7E-01	1.02
POAG (H40.1)	2.4E-01	0.96	5.1E-01	0.94*	5.4E-01	0.98	3.4E-01	1.04	3.4E-01	1.03	8.7E-02	0.95	6.2E-01	1.05	6.9E-01	1.02	3.7E-01	0.96	9.2E-01	1.01
PACG (H40.2)	1.9E-01	0.92	2.4E-01	0.61*	6.9E-01	0.98	7.9E-01	1.02	7.1E-01	0.98	7.5E-01	0.98	9.9E-01	1.00	7.4E-01	0.98	6.3E-01	1.04	7.1E-01	0.93

*Effect and P-value was estimated with Icelandic data only.

Supplementary Table 5. Association of novel CCT variants with CCT in the Reykjavik Eye Study (N=1,459). Effects are given for the minor allele.

Chr	Pos	rs-name	Allele (min/maj)	MAF (%)	Gene	Coding effect	P-value	Effect
15	100152748	rs72755233	(A/G)	13.8	ADAMTS17	missense	1.21E-02	0.145
12	104015054	rs117801489	(C/T)	4.3	GLT8D2	missense	7.89E-03	0.258

Supplementary Table 6. Mutations in *CHST6* known to cause macular corneal dystrophy (MCD) under the recessive model. a) The standardized values of corneal measures for two homozygote carriers of the frameshift mutation p.Val6MetfsTer106 in *CHST6*. b) The association of MCD variants in *CHST6* with corneal measures under the additive model.

a)	Subject	Sex	Age	CD (SD)	HEX (SD)	CV (SD)	CCT (SD)	CH (SD)	IOPcc (SD)	IOPg (SD)	CRF (SD)
	1	Male	44	-3.38	-0.81	-1.12	0.76	-0.78	1.88	1.72	1.78
	2	Male	51	-2.65	-3.18	2.97	2.31	-1.54	0.18	-0.51	-2.32

b)			CCT		CD		CV		HEX	
	Variant	MAF (%)	P-value	Effect	P-value	Effect	P-value	Effect	P-value	Effect
	p.Ala128Val	0.662	0.42	-0.10	0.54	-0.07	0.05	0.23	0.21	-0.15
	p.Val6MetfsTer106	0.072	0.29	0.34	0.01	-0.83	0.08	0.54	0.07	-0.56

CD = cell density; HEX = percentage of hexagonal cells; CV = coefficient of cell size variation; CCT = central corneal thickness; CH= corneal hysteresis; IOPg = Goldmann correlated intraocular pressure; IOPcc = corneal compensated intraocular pressure; CRF = corneal resistance factor.

Supplementary Table 7. Correlation between effect sizes. For known primary open angle glaucoma (POAG), central corneal thickness (CCT) and intraocular pressure (IOP) variants, the table shows the correlations between their effects of POAG, CCT and IOP, respectively, and their effect on different corneal measures. *P*-values are shown in bold for significant correlations after controlling the false discovery rate at 0.05 using Benjamin-Hochberg procedure.

	POAG variants			CCT variants			IOP variants		
	Effect	P-value	R ²	Effect	P-value	R ²	Effect	P-value	R ²
POAG	-	-	-	-0.16	3.3E-01	0.02	0.10	2.4E-04	0.06
CD	-0.32	0.49	0.03	0.35	1.6E-01	0.04	-0.16	3.4E-03	0.04
CCT	-0.17	0.58	0.02	-	-	-	0.14	2.6E-03	0.04
CV	-0.65	0.27	0.09	0.11	5.4E-01	0.01	-0.04	5.3E-01	0.00
HEX	0.35	0.57	0.02	-0.09	6.4E-01	0.00	0.05	3.7E-01	0.00
CH	-0.20	0.51	0.03	0.50	1.4E-03	0.19	0.18	1.5E-05	0.09
CRF	0.18	0.63	0.02	0.55	3.6E-04	0.24	0.50	4.1E-33	0.50
IOPcc	0.94	0.03	0.31	0.34	1.5E-01	0.04	0.81	8.4E-48	0.63
IOPg	0.83	0.06	0.22	0.65	1.6E-03	0.19	-	-	-

POAG = primary open angle glaucoma; CD = cell density; CCT = central corneal thickness; CV = coefficient of cell size variation; HEX = percentage of hexagonal cells; CH= corneal hysteresis; CRF = corneal resistance factor; IOPcc = corneal compensated intraocular pressure; IOPg = Goldmann correlated intraocular pressure.

Supplementary Table 8. Fraction of variance explained. Fraction of variance explained was calculated for all variants in GWAS catalog⁶ that associated with a quantitative trait where corresponding measurements were available in deCODE data, using the Icelandic effects and MAF. The table shows the variant explaining highest fraction of variance for the top 10 traits.

Chr	Pos	Gene	Quantitative trait	Fraction of variance explained
6	160589086	LPA	Lp (a) levels	19.5%
2	233763993	UGT1A1	Bilirubin levels	6.6%
3	133758857	TF	Iron status biomarkers (transferrin levels)	4.1%
5	177415473	F12	Activated partial thromboplastin time	2.6%
19	48703160	FUT2	Vitamin B12 levels	2.5%
2	43847292	ABCG8	Sitosterol	2.5%
19	44909976	APOC1	LDL cholesterol	2.4%
16	56961324	CETP	HDL cholesterol	2.2%
4	9920543	SLC2A9	Uric acid levels	1.9%
12	121779004	TMEM120B	Mean platelet volume	1.9%

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3. Lu, Y. *et al.* Genome-wide association analyses identify multiple loci associated with central corneal thickness and keratoconus. *Nat. Genet.* **45**, 155–163 (2013).
4. Gao, X. R., Huang, H., Nannini, D. R., Fan, F. & Kim, H. Genome-Wide Association Analyses Identify New Loci Influencing Intraocular Pressure. *Hum. Mol. Genet.* **0**, 1–9 (2018).
5. Sveinbjornsson, G. *et al.* Weighting sequence variants based on their annotation increases power of whole-genome association studies. *Nat. Genet.* **48**, 314–317 (2016).
6. MacArthur, J. *et al.* The new NHGRI-EBI Catalog of published genome-wide association studies (GWAS Catalog). *Nucleic Acids Res.* **45**, D896–D901 (2017).

Reviewer #1 (Remarks to the Author):

Key results

Ivarsdottir et al. performed genome-wide association analyses on the following four corneal structure measurements in 6,125 Icelanders: corneal endothelial cell density (CD), coefficient of cell size variation (CV), percentage of hexagonal cells (HEX) and central corneal thickness (CCT). Some of the explored measurements show high or moderate degrees of correlation. A total of ten independent risk variants were found to be associated with at least one of the four traits. Six of the 10 variants were stated to be novel in regard to the analyzed traits.

Follow up analyses included meta-analyses of ocular disease case control studies (e.g. glaucoma, corneal disorders) of the larger Iceland and the UK Biobank cohort. In addition, the authors explored the index variants and their effect on ocular biomechanics like corneal hysteresis, two intraocular pressure measures and the corneal resistance factor as well as investigated expression of implicated genes in ocular tissues of publicly available data.

Originality and significance

The presented analyses focused on several correlated ocular traits whose genetic factors were not extensively studied in genome-wide analyses before (except CCT). Six associations were stated to be novel for these traits, however the provided explorations revealed that none of these “novel” variants were directly relevant for any of the studied complex ocular disorders. Subjectively, the presented results might not be overly exciting for people who work in statistical, human or ocular genetics.

Data & methodology

The sampling mechanism for the 6,266 Icelanders of the discovery study is unclear. Overall, a well-established GWAS analysis strategy was applied. While correction factors for population stratification based on 1.1 million variants were provided for the four main traits, no QQ plots were included in the presented material. Providing QQ plots stratified by allele frequency would give peers a better sense about the applied adjustment for population stratification and about the behaviour of the test statistics across all frequency bins.

Generally, no analysis software packages were cited or stated in the manuscript thus making it hard to comprehend the performed analyses and its quality.

Appropriate use of statistics and treatment of uncertainties

Overall the authors provide confidence intervals and/or standard deviations for most of the reported analyses, but no confidence intervals are given for correlation analyses (e.g. Table 1).

The case control ratios of the analysed ocular traits (glaucoma traits and disorders of cornea; Supplementary Table 2) are extremely unbalanced and could potentially result in inflated test statistics if not addressed properly (Ma et al. Genet. Epidemiol 37, 539–550 (2013)). Without further

explanation or adjustment of these analyses, their appropriateness could be questioned because the case control ratios range from 59 to even >3500 controls per case.

The reported odds ratios of >140 for two rarer variants for hereditary corneal dystrophies in an autosomal recessive model (page 5) might be problematic and suffer from partial or complete separation (see Heinze and Schemper, Stat Med. 2002 Aug 30;21(16):2409-19).

The correction for multiple testing seems to be inconsistently applied throughout the manuscript: for discovery studies a correction for the number of independent variants was applied but not for the number of traits, for replication studies of known risk variants a correction for the number of traits but not for the number of variants was applied (bottom page 5, Figure 4, Supplementary Table 7,9,10).

Conclusions

The conclusion for the ANAPC1 variant that “no other variant reported in the GWAS catalog explains a higher fraction of variance of its associating quantitative phenotypes” needs to be taken with a grain of salt. First, the applied method warrants further explanation or references, secondly, the analysis was limited to measurements that were also available in deCODE data, and thirdly, the association might not be generalisable because only Icelanders were analysed.

Suggested improvements

The analyses on corneal hysteresis, two intraocular pressure measures and the corneal resistance factor seem to be doable genome-wide and consequently could be adjusted for population stratification. Presenting the corresponding Manhattan and QQ would help evaluate the quality of the obtained association results.

References

No references are given for some of the applied methods, e.g. correlation of between effect sizes or fraction of variance explained. The latter was probably described by Ju-Hyun Park et al. (Nature Genetics volume 42, pages 570–575 (2010); <https://www.nature.com/articles/ng.610>).

Clarity and context

The abstract and conclusions provided sufficient clarity and context. However, two paragraphs of the introduction and parts of the manuscript appear to focus on additional traits (MCD, FECD, IOP, CH, CRF). The main result section is interspersed with findings or literature from these additional phenotypes and appears disrupted in terms of flow, thereby affecting the clarity of the presented story.

Additional comments

1. Ordering Table 2 by trait instead of by p-value across all traits might improve its readability.
2. In general, no explanations are given for the bolded text in the tables (e.g. Table 1 and Supplement Tables 3 & 7-10).
3. The description of HEX and CCT and their corresponding Figure 1 in the second result paragraph seems incorrect/swapped.
4. The samples sizes are inconsistent throughout the manuscript: 6,123 (Table 1), 6,125 (results, Figure 2), 6,266 in methods. Understanding the applied exclusions would help understanding of these inconsistencies.
5. No imputation accuracy measures are given for the analysed variants; however, imputation information filter might have been applied (as indicated in the legend of Figure 2).
6. Figure 2 (especially 2A) would benefit from an axis break and labeled association peaks (locus name or rsID). Chromosome 23 should read X.
7. Figure 3,4 report gene names or chromosome bands while they are actually reporting single variants. Adding the variants reference IDs would be more appropriate and provide more clarity.
8. Effect sizes from linear and logistic regression analyses are mixed in Supplementary Table 3.
9. Gene name (CHST6) is missing for the two missense variants associated with MCD (center of page 5).
10. Gene names are not always given in italics (Table 2, Supplementary Tables)

Reviewer #3 (Remarks to the Author):

Ivarsdottir and co-authors present a genome-wide association study of 6,125 Icelanders on four corneal traits. These traits are:

- a) Corneal endothelial cell density
- b) Coefficient of cell size variation
- c) Percentage of hexagonal cells, and
- d) Central corneal thickness.

They identified multiple genetic loci showing strong evidence of association for the four corneal traits studied. Overall, it was a great pleasure reading the manuscript. Very interestingly, many of the loci showed pleiotropic effects across the four traits. There are both important positives which were emphasized, as well as important negatives (i.e. lack of association) which were also discussed

in the paper. The statistical analysis were beautifully controlled for, and appropriate adjustments using genomic control to account for dispersion of test statistics were undertaken.

I have some minor suggestions which could help in improving the presentation of the data to the general reader of Nature Communications.

1. The authors note clearly that glaucoma risk is strongly associated with decreased cell density ($P = 2.1 \times 10^{-6}$). However, whereas the index ANAPC1 SNP showed overwhelming association with cell density (as well as CV, HEX, and CH), it does not show any association at all with POAG and PACG. The authors should consider discussing this, as it could be very interesting to the general reader (a genetic variant which controls 24% of trait variance does not associate directly with the disease it is correlated with).

2. Strong evidence of association was observed between TCF4 and cell density as well as HEX, CH, and CRF. Very strikingly, TCF4 was also shown to be very strongly associated with hereditary corneal dystrophies, such as Fuch's. Apart from mentioning the association statistics, the authors should consider discussing this very interesting observation with a view of helping the general reader of the journal (as well as eye specialists) appreciate the results. In the opinion of the authors, why is the association between TCF4 and ANAPC1 so different with regards to disease? This is very interesting because they control almost the same corneal quantitative traits. In this regard, it is also curious that ZNF469, which the authors and others show to be very strongly associated with CCT and a gene for brittle cornea disease, showed almost no association at all with cell density.

3. Do any of the variants associate with Keratoconus?

Reviewer #1

Originality and significance

The presented analyses focused on several correlated ocular traits whose genetic factors were not extensively studied in genome-wide analyses before (except CCT). Six associations were stated to be novel for these traits, however the provided explorations revealed that none of these “novel” variants were directly relevant for any of the studied complex ocular disorders. Subjectively, the presented results might not be overly exciting for people who work in statistical, human or ocular genetics.

Response:

In our opinion, it is of great interest to reveal the causal relationship between correlated traits and diseases. Genetics is very useful in exploring the causal relations of phenotypes. One way of assessing the relationship between a biomarker/risk factor and disease, is by identifying a sequence variant that associates with the biomarker/risk factor of a disease of interest and testing if this variant associates directly with the disease. This method utilizes the fact that genotypes are randomly assigned at meiosis, and not affected by environmental risk factors or other related disease processes, and is thus independent of many confounders that are problematic in epidemiological studies. In this study we examine quantitative traits used clinically to diagnose corneal diseases and we see that endothelial diseases and glaucoma are associated with lower cell density. We find a sequence variant that associates highly significantly with cell density with large effect, accounting for a quarter of the population variance of cell density, but it has no association with disease. This effectively shows that low cell density in and of itself does not increase the risk of disease. However, further studies could assess if carriers of this variant are more likely to suffer endothelial decompensation after intraocular surgery or if their cornea is associated with increased risk of corneal graft failure when used as donors. We have now emphasized the clinical relevance of our findings in the discussion:

“This finding has clinical importance, since reduction in endothelial cell density along with more variable cell size and shape is usually the first sign of corneal endothelial diseases and cell density is important when assessing risk of corneal endothelial failure prior to intraocular surgery⁵⁷. Corneal graft survival studies found cell density lower than 1700 cells/mm² six months after graft surgery to be associated with increased risk of graft failure⁵⁸. Thirty year old homozygote carriers of the *ANAPC1* variant have on average 2.637 endothelial cells per mm² which is less than the average seventy year old non-carriers (Figure 1.e). Further studies could assess if carriers of the variant are more likely to suffer endothelial

decompensation after intraocular surgery or if their cornea is associated with increased risk of corneal graft failure when used as donors.

We also found association of variants at known disease loci, *TCF4* and *CHST6* (FCED and MCD, respectively), with cell density and HEX, demonstrating how these structural measurements are affected by corneal diseases. The *TCF4* variant also strongly associates with CH and it is interesting to note that the *TCF4* and *ANAPC1* variants control almost the same quantitative corneal traits (Figure 3) but have very different associations with corneal disease. Furthermore, glaucoma risk is strongly associated with lower cell density, while for example the *ANAPC1* variant that controls a quarter of the cell density variance does not associate directly with POAG or PACG. These findings suggest that disease processes that cause corneal diseases and glaucoma may also lower cell density, but low cell density in and of itself does not increase risk of disease.”

Data & methodology

The sampling mechanism for the 6,266 Icelanders of the discovery study is unclear.

Response:

We added to the main text (p. 4) that the deCODE health study is “a comprehensive phenotyping of a general population sample”.

Overall, a well-established GWAS analysis strategy was applied. While correction factors for population stratification based on 1.1 million variants were provided for the four main traits, no QQ plots were included in the presented material. Providing QQ plots stratified by allele frequency would give peers a better sense about the applied adjustment for population stratification and about the behaviour of the test statistics across all frequency bins.

Response:

We have added QQ-plots stratified by allele frequency as supplementary figures for all the traits (Supplementary Figures 8-11).

Generally, no analysis software packages were cited or stated in the manuscript thus making it hard to comprehend the performed analyses and its quality.

Response:

The software packages used for the association testing were developed in-house at deCODE and we describe our methods in detail. Regarding the methods used for sequencing the subjects and the subsequent imputation, we have now added more detailed information in methods:

“The process used to whole-genome sequence the 28,075 Icelanders, and the subsequent imputation has been described in a recent publication^{30,31}. We sequenced the whole genomes of 28,075 Icelanders using Illumina technology to a mean depth of at least 10X (median 32X). SNPs and indels were identified and their genotypes called using joint calling with the Genome Analysis Toolkit HaplotypeCaller (GATK version 3.4.07)⁶⁴. Genotype calls were improved by using information about haplotype sharing, taking advantage of the fact that all the sequenced individuals had also been chip-typed and long range phased. The 37.6 million variants passed the high quality threshold were then imputed into 155,250 Icelanders who had been genotyped with various Illumina SNP chips and their genotypes phased using long-range phasing⁶⁵. Using genealogic information, the sequence variants were imputed into relatives of the chip-typed to further increase the sample size for association analysis and increased the power to detect associations. All of the variants that were tested had imputation information over 0.8.”

Appropriate use of statistics and treatment of uncertainties

Overall the authors provide confidence intervals and/or standard deviations for most of the reported analyses, but no confidence intervals are given for correlation analyses (e.g. Table 1).

Response:

We have added a supplementary table 1 with p-values for the correlation coefficients shown in Table 1.

The case control ratios of the analysed ocular traits (glaucoma traits and disorders of cornea; Supplementary Table 2) are extremely unbalanced and could potentially result in inflated test statistics if not addressed properly (Ma et al. Genet. Epidemiol 37, 539–550 (2013)). Without further explanation or

adjustment of these analyses, their appropriateness could be questioned because the case control ratios range from 59 to even >3500 controls per case.

Response:

As we describe in methods, in our case-control association testing we use likelihood ratio tests. The paper the reviewer refers to states that for high count variants the likelihood ratio test is well-calibrated, even in unbalanced studies. However, they also state that for low-count variants, the likelihood ratio test can be slightly anti-conservative for some MACs (minor allele counts). We find this statement to be in paradox with Figures 1.C and 2.A and B in the paper, which actually show that the likelihood ratio test is performing well for unbalanced studies (see snap-shot from paper below). They also state that all test perform well for variants with $MAC > 400$ and suggest using that as a threshold. The variants that we are testing in our study all have high MAC. The least frequent variant (at *GLT8D2*) has $MAC > 13000$. We therefore are not worried that our performed tests are anti-conservative. Also, all of the case-control analysis were performed on the genome-wide level and we used LD score regression to account for inflation of test statistics.

Figure 1:

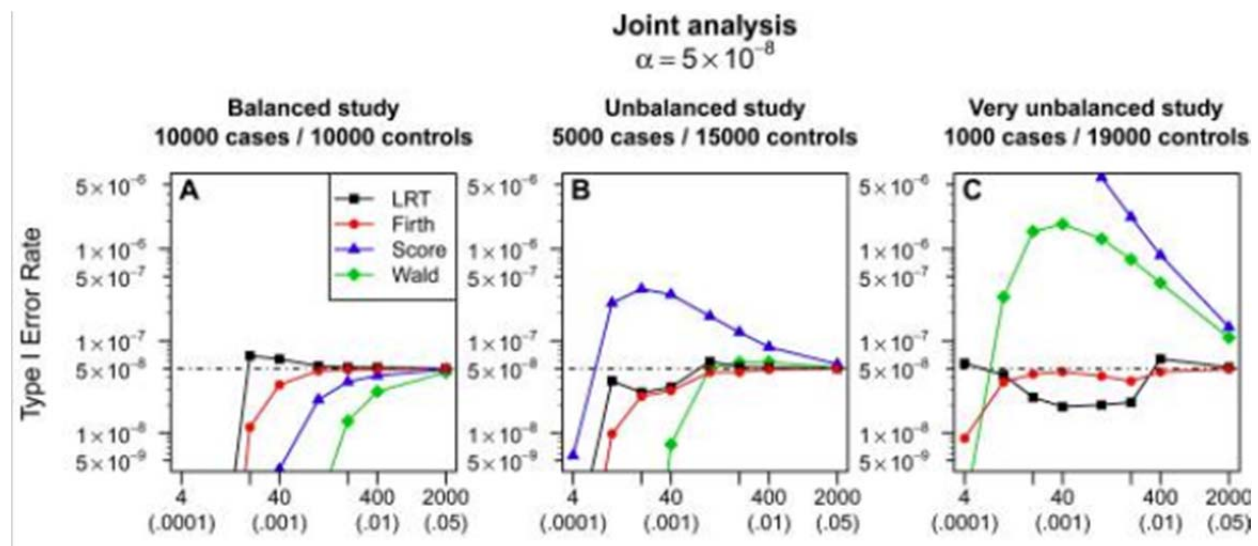
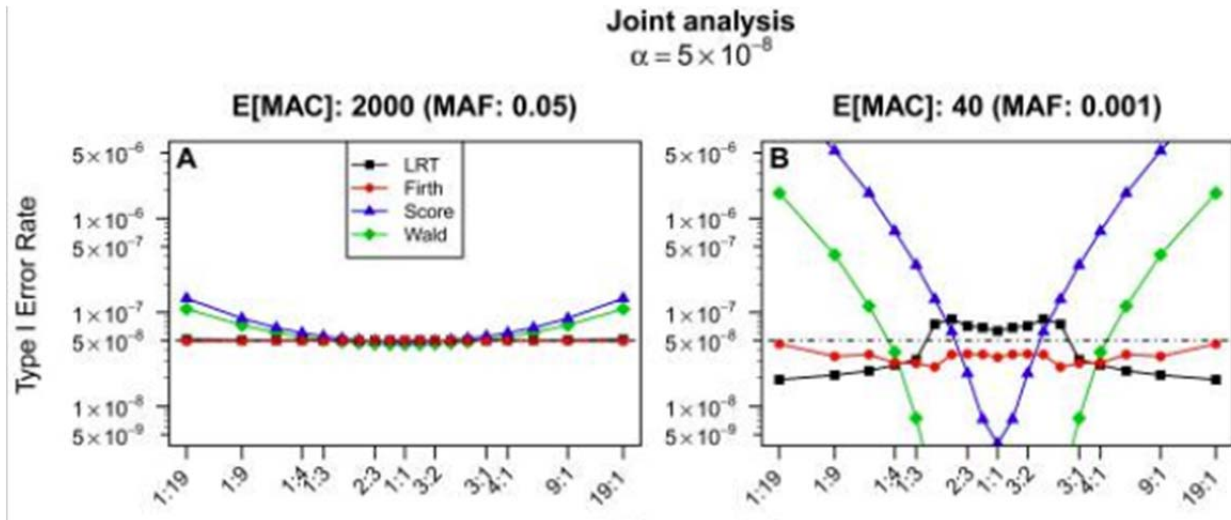


Figure 2:



The reported odds ratios of >140 for two rarer variants for hereditary corneal dystrophies in an autosomal recessive model (page 5) might be problematic and suffer from partial or complete separation (see Heinze and Schemper, Stat Med. 2002 Aug 30;21(16):2409-19).

Response:

We agree that odds ratios for such rare variants causing a disease are not meaningful. We have thus decided to report instead the number of homozygotes for each variant that has been diagnosed with the disease:

“Two different mutations in *CHST6* are known to cause MCD in Iceland; a missense variant p.Ala128Val (MAF=0.66%) and a frameshift variant p.Val6MetfsTer106 (MAF=0.07%)¹⁶. The prevalence of MCD in Iceland is $\sim 1/13,000$ ⁴⁹. We observed that 11 out of 16 homozygous carriers of p.Ala128Val, and two out of three homozygous carriers of p.Val6MetfsTer106, have been diagnosed with hereditary corneal dystrophies (ICD10 code H18.5).”

The correction for multiple testing seems to be inconsistently applied throughout the manuscript: for discovery studies a correction for the number of independent variants was applied but not for the number

of traits, for replication studies of known risk variants a correction for the number of traits but not for the number of variants was applied (bottom page 5, Figure 4, Supplementary Table 7,9,10).

Response:

In the discovery studies, we think it is not appropriate to correct for the number of traits studies. The number of significant findings in each phenotype is substantial which translates into a false discovery rate of 0.0014 for cell density, 0.0035 for CV, 0.0117 for CCT and 0.0116 for HEX at the 5.0×10^{-8} significance threshold. These false discovery rates, which add up to 3.4%, suggest that our multiple testing procedure is overall conservative. FDR approach using 5% discovery rate has been suggested robust in a previous genome-wide association publication (Nelson, C.P., et.al., Nature Genetics volume 49, pages 1385–1391 (2017)). We have added a statement describing this to the text of the manuscript: “We evaluated false discovery rate, assessed with the qvalue package in R. The P value cutoff of 5.0×10^{-8} corresponded to q-values of 0.0014 for cell density, 0.0035 for CV, 0.0117 for CCT and 0.0116 for HEX, which add up to 3.4%.”

When assessing if any of the 10 GWS variants associate with an ocular disease we adjust for the number of tests performed by adjusting for the number of phenotypes and number of variants.

In the heatmap–figures, where we are exploring how the detected variants associate with different corneal measures we think it is not necessary to adjust for the number of variants. We are more exploring association patterns than claiming novel associations (unless they are genome-wide significant). But instead of using Bonferroni correction procedure we have now switched to using the Benjamini-Hochberg false discovery rate (FDR) procedure controlling the FDR at 0.05 at each variant to account for multiple testing. We added to methods:

“When assessing if associating variants have an effect on other corneal traits, we used the Benjamini-Hochberg false discovery rate (FDR) procedure controlling the FDR at 0.05 at each variant to account for multiple testing.”

Conclusions

The conclusion for the ANAPC1 variant that “no other variant reported in the GWAS catalog explains a higher fraction of variance of its associating quantitative phenotypes” needs to be taken with a grain of salt. First, the applied method warrants further explanation or references, secondly, the analysis was limited to measurements that were also available in deCODE data, and thirdly, the association might not be generalisable because only Icelanders were analysed.

Response:

We have changed the sentence to “In comparison, no other variant listed in the GWAS catalog⁵⁶ explains higher fraction of variance of a quantitative phenotype available in the extensive deCODE database“ and we have added reference in the methods for the formula used to compute the fraction of variance explained.

We can’t replicate the association between the *ANAPC1* variant and cell density abroad due to lack of data. To the best of our knowledge, quantitative measures from a specular microscopy have never been collected before for a genetic study. However, Icelanders have proven a reasonably good animal model for *Homo sapiens*. Also, the UK Biobank has CH measures and the association we see between *ANAPC1* and CH in our data (rs78658973; $\beta=0.19$, $P=2.6 \times 10^{-19}$) replicates well in the UK Biobank data (rs1044864; $\beta=0.12$, $P=2.6 \times 10^{-100}$, $R^2=1.0$ between rs1044864 and rs78658973, <https://biobankengine.stanford.edu/variant/2-112526866>). We therefore believe that the cell density association is also not restricted to Icelanders.

Suggested improvements

The analyses on corneal hysteresis, two intraocular pressure measures and the corneal resistance factor seem to be doable genome-wide and consequently could be adjusted for population stratification. Presenting the corresponding Manhattan and QQ would help evaluate the quality of the obtained association results.

Response:

The analysis of CH, CRF, IOPg and IOPcc were performed genome-wide and reported p-values have been adjusted for population stratification. We have now added QQ and Manhattan plots in the supplement (Supplementary Figures 5-6) as well as this information in the methods: “The estimated correction factors were 1.05, 1.03, 1.03, 1.06, 1.06, 1.05, 1.05 and 1.05 for cell density, CV, HEX, CCT, CH, CRF, IOPg and IOPcc, respectively.”

References

No references are given for some of the applied methods, e.g. correlation of between effect sizes or fraction of variance explained. The latter was probably described by Ju-Hyun Park et al. (Nature Genetics volume 42, pages 570–575 (2010); <https://www.nature.com/articles/ng.610>).

Response:

We have added a reference for the fraction of variance explained method (Gudbjartsson, D. F. et al. Many sequence variants affecting diversity of adult human height. Nat. Genet. 40, 609–615 (2008)).

A simple weighted linear regression (Mandel, J., The Statistical Analysis of Experimental Data. (1964)) is used to calculate the correlation between effect sizes which is a general method that we describe in the methods chapter. For clarity we added a more detailed explanation for the chosen weights:

“..., so that rare variants have less weight in the computation than common variants”

Clarity and context

The abstract and conclusions provided sufficient clarity and context. However, two paragraphs of the introduction and parts of the manuscript appear to focus on additional traits (MCD, FECD, IOP, CH, CRF). The main result section is interspersed with findings or literature from these additional phenotypes and appears disrupted in terms of flow, thereby affecting the clarity of the presented story.

Response:

We have added and rephrased sentences in the introduction in the attempt to make the flow better and we have added subtitles in the result section to make the presented story clearer.

“The measures of endothelial structure are clinically used to diagnose corneal diseases. Several corneal diseases including...

Cell density in the corneal endothelium may be reduced in glaucoma patients²¹. Glaucoma is an ocular disease...”

Additional comments

1. Ordering Table 2 by trait instead of by p-value across all traits might improve its readability.

Response:

We agree and table 2 is now ordered by trait first and then p-value.

2. In general, no explanations are given for the bolded text in the tables (e.g. Table 1 and Supplement Tables 3 & 7-10).

Response:

Explanations for bolded text has now been added in table legends.

3. The description of HEX and CCT and their corresponding Figure 1 in the second result paragraph seems incorrect/swapped.

Response:

This has now been fixed.

4. The samples sizes are inconsistent throughout the manuscript: 6,123 (Table 1), 6,125 (results, Figure 2), 6,266 in methods. Understanding the applied exclusions would help understanding of these inconsistencies.

Response:

There was a typing error in table 1, the sample size is 6,125. In methods we describe how we exclude low-quality images for 415 eyes in 276 individuals. For clarity we added in methods on page 15: “Thus, the sample size reduced from N=6,266 to N=6,125.”

5. No imputation accuracy measures are given for the analysed variants; however, imputation information filter might have been applied (as indicated in the legend of Figure 2).

Response: We have added a sentence in the legend of table 2:

“The imputation information for all the detected variants is >0.99 .”

6. *Figure 2 (especially 2A) would benefit from an axis break and labeled association peaks (locus name or rsID). Chromosome 23 should read X.*

Response: The manhattan figures now have labelled peaks by locus name and figure 2A has axis break.

7. *Figure 3,4 report gene names or chromosome bands while they are actually reporting single variants. Adding the variants reference IDs would be more appropriate and provide more clarity.*

Response:

We have now added rs-names in the figures.

8. *Effect sizes from linear and logistic regression analyses are mixed in Supplementary Table 3.*

Response:

This has been fixed.

9. *Gene name (CHST6) is missing for the two missense variants associated with MCD (center of page 5).*

Response:

This has been fixed.

10. *Gene names are not always given in italics (Table 2, Supplementary Tables)*

Response:

This has been fixed.

Reviewer #3 (Remarks to the Author):

1. The authors note clearly that glaucoma risk is strongly associated with decreased cell density ($P = 2.1 \times 10^{-6}$). However, whereas the index ANAPC1 SNP showed overwhelming association with cell density (as well as CV, HEX, and CH), it does not show any association at all with POAG and PACG. The authors should consider discussing this, as it could be very interesting to the general reader (a genetic variant which controls 24% of trait variance does not associate directly with the disease it is correlated with).

2. Strong evidence of association was observed between TCF4 and cell density as well as HEX, CH, and CRF. Very strikingly, TCF4 was also shown to be very strongly associated with hereditary corneal dystrophies, such as Fuch's. Apart from mentioning the association statistics, the authors should consider discussing this very interesting observation with a view of helping the general reader of the journal (as well as eye specialists) appreciate the results. In the opinion of the authors, why is the association between TCF4 and ANAPC1 so different with regards to disease? This is very interesting because they control almost the same corneal quantitative traits. In this regard, it is also curious that ZNF469, which the authors and others show to be very strongly associated with CCT and a gene for brittle cornea disease, showed almost no association at all with cell density.

Response to 1 and 2:

We have emphasized the points made by the reviewer in the discussion:

“The *TCF4* variant also strongly associates with CH and it is interesting to note that the *TCF4* and *ANAPC1* variants control almost the same quantitative corneal traits (Figure 3) but have very different associations with corneal disease. Furthermore, glaucoma risk is strongly associated with lower cell density, while for example the *ANAPC1* variant that controls a quarter of the cell density variance does not associate directly with POAG or PACG. These findings suggest that disease processes that cause corneal diseases and glaucoma may also lower cell density, but low cell density in and of itself does not increase risk of disease.”

3. Do any of the variants associate with Keratoconus?

Response:

We did not include keratoconus before in our analysis due small number of cases (127 cases combined in Iceland and UK Biobank). But we have now performed the meta-analysis and found that none of the variants associate with keratoconus. We have added these results to Supplementary Tables 3 and 4.

Reviewer #3 (Remarks to the Author):

The authors have been very responsive to the previous round of review. The statistical analysis of the genetic data is transparently presented, both in the main text as well as in the Supplementary Tables and Figures.

The addition of data on keratoconus patients and controls is helpful despite the small number (N=127 cases). This is because as the authors now show in Supplementary Table 4, they again observe the paradoxical effect first reported in 2013 (Lu Y et al., Nat Genet 45:155-163) whereby the largest effect CCT locus at ZNF469 which decreases CCT confers decreased susceptibility to keratoconus. One would expect a gene locus which **decreases** CCT to **increase** risk of keratoconus, so this was unexpected. The authors here report the same trend (OR = 0.73, P = 0.027). This kind of supportive evidence provided in the context of previously 'controversial' findings helps clarify the literature and guides future work in this field.

I have no further concerns.

Reviewer #4 (Remarks to the Author):

I think the authors have addressed the reviewers comments adequately.

Description of Additional Supplementary Files

Supplementary Data 1

Tissue expression of genes at the associating loci according to the Ocular Tissue Database. In the Ocular Tissue Database (OTDB), the gene expression is indicated as Probe Logarithmic Intensity (PLIER) normalized value.

Supplementary Data 2

Previously reported POAG variants and their effect on corneal measures. POAG replication *P*-values are shown in bold for nominal significant associations ($P < 0.05$).

Supplementary Data 3

Previously reported CCT variants and their effect on corneal measures. CCT replication *P*-values are shown in bold for nominal significant associations ($P < 0.05$).

Supplementary Data 4

Previously reported IOP variants and their effect on corneal measures. IOP replication *P*-values are shown in bold for nominal significant associations ($P < 0.05$). The variants and their reported effects were obtained from Gao, X.R. et al. (2018).

Supplementary Data 5

GWAS summary statistics for cell density, CV, HEX and CCT are provided for all associations with *P*-value below $1 \cdot 10^{-6}$.