Genome-wide association meta-analysis of 88,250 individuals highlights pleiotropic mechanisms of five ocular diseases in UK Biobank

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Summary

Background Ocular diseases may exhibit common clinical symptoms and epidemiological comorbidity. However, the extent of pleiotropic mechanisms across ocular diseases remains unclear. We aim to examine shared genetic etiology in age-related macular degeneration (AMD), diabetic retinopathy (DR), glaucoma, retinal detachment (RD), and myopia.

Methods We analyzed genome-wide association analyses for the five ocular diseases in 43,877 cases and 44,373 controls of European ancestry from UK Biobank, estimated their genetic relationships (LDSC, GNOVA, and Genomic SEM), and identified pleiotropic loci (ASSET and METASOFT).

Findings The genetic correlation of common SNPs revealed a meaningful genetic structure within these diseases, identifying genetic correlations between AMD, DR, and glaucoma. Cross-trait meta-analysis identified 23 pleiotropic loci associated with at least two ocular diseases and 14 loci unique to individual disorders (non-pleiotropic). We found that the genes associated with these shared genetic loci are involved in neuron differentiation ($P = 8.80 \times 10^{-6}$) and eye development systems ($P = 3.86 \times 10^{-5}$), and single cell RNA sequencing data reveals their heightened gene expression from multipotent progenitors to other differentiated retinal cells during retina developmental process.

Interpretation These results highlighted the potential common genetic architectures among these ocular diseases and can deepen the understanding of the molecular mechanisms underlying the related diseases.

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Keywords: Ocular diseases; Cross-disease genetics; GWAS; Genetic correlation; Pleiotropy; Retinal development

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Introduction

Approximately 295 million people with ocular diseases suffer moderate or severe vision impairment worldwide.[1] Clinical and epidemiological data have suggested associations among several common ocular diseases.[2–4] Longitudinal and retrospective cohort studies provided evidence that diabetic retinopathy (DR) is independently related with an increased risk of subsequent dry (HR = 1.24 ~ 3.89) and wet (HR = 1.68 ~ 3.42) age-related macular degeneration (AMD),[2,5] and both of wet AMD and diabetes/DR also increase the risk of open-angle glaucoma.[6,7] Additionally, retinal detachment (RD) refers to the separation of the neurosensory retina from the retinal pigment epithelium (RPE), and often occurs in AMD, DR and myopia patients.[8] As the most common ocular disorder, myopia with a global

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Research in context

Evidence before this study

Age-related macular degeneration (AMD), diabetic retinopathy (DR), glaucoma, retinal detachment (RD), and myopia are five common vision-threatening diseases, with extensive clinical associations among them. The substantial influence of genetic variation on risk for a broad range of these ocular diseases has been established by both twin and genome-wide association studies. In addition, the connection between RD and myopia has been explained through a significant genome-wide genetic correlation and the shared loci associated with both diseases. However, the genetic relationships and pleiotropic effects in these five ocular diseases remain unclear.

Added value of this study

Our study has identified genetic correlations between AMD, DR, and glaucoma, which are characterized by neurodegeneration. All three diseases showed positive genetic correlations with Type 2 diabetes and obesity. Cross-trait meta-analysis of the five ocular disorders detected 23 pleiotropic loci affecting at least two diseases, including three loci positively associated with all five diseases. Notably, we found the pleiotropic loci were involved in eye development systems and showed heightened expression during early retinal development. Ou3r results also suggest the important roles of Wnt signalling pathway and glucose metabolic process in the shared molecular mechanisms of ocular diseases.

Implications of all the available evidence

The shared genetic structure and pleiotropic mechanisms in ocular diseases interprets their clinical associations to some extent. Our results suggest that abnormalities in retinal development, Wnt signalling, and glucose metabolism may be the underlying mechanisms leading to susceptibility to multiple ocular diseases. These finds have important implications for risk prediction, clinical prevention, and drug development.

prevalence of 22.9%,[9] appeared to have a protective effect on AMD (odds ratio [OR], 0.45; 95% confidence interval [CI], 0.25-0.79) and DR (OR = 0.68, 95% CI, 0.46-0.98).[3] On the contrary, for every 1 dioptre (D) increase in myopia, the risks of open-angle glaucoma and RD increased by 20% and 30%, respectively.[4] These studies demonstrated that the coexistence of myopia and other ocular diseases is frequent. Some phenotypic and genetic overlap have been supported by recent evidence of shared molecular risk factors,[10,11] but the extent of these relationships remains unclear, given the small proportion of risk associated with individually identified variants.

One hypothesis to account for the similarity in symptoms for these diseases is due to a shared common genetic etiology. Genome-wide association studies (GWASs) have demonstrated the important roles of genetic factors in the pathogenesis of ocular diseases. For examples, the International AMD Genomics Consortium (IAMDGC) identified 52 common and rare variants at 34 loci associated with advanced AMD on the basis of 16,144 cases and 17,832 controls, accounting for 46.7% of variability.[12] A multi-trait analysis of glaucoma on UK Biobank (UKB) and International Glaucoma Genetics Consortium (IGGC) identified 107 loci and developed a powerful polygenic risk score for prediction.[13] In addition, a GWAS meta-analysis involving 542,934 European participants from UK Biobank, 23andMe, Genetic Epidemiology Research on Adult Health and Aging (GERA) cohort, and the Consortium for Refractive Error and Myopia (CREAM) found 438 loci associated with refractive error or myopia, explaining 18.4% of the heritability.[14] These GWASs show that we can test for shared genetics by looking for correlations in effect sizes across traits without measuring multiple traits per individual. Based on the GWASs, recent studies have found significant genetic correlations between RD and high myopia (r_g = 0.46, $P = 8.92\times10^{-9}$),[11] mean spherical equivalent (MSE; r_g = -0.45, $P = 1.3\times10^{-15}$) and intraocular pressure (IOP, one of the major risk factors for glaucoma; r_g = 0.28, $P = 1.6\times10^{-16}$).[15] To date, however, no studies have utilized pleiotropic meta-analytic techniques to comprehensively parse variance from AMD, DR, glaucoma, RD, and myopia focused GWASs that might pinpoint shared and differential biological mechanisms.

Here, we conducted a large-scale GWAS analysis for these five ocular diseases, based on 43,877 cases and 44,373 controls of European ancestry from UKB which is the largest and most complete European Biobank and provided a sufficient sample size and comprehensive eye health information. We then employed a pleiotropic meta-analytic approach, association analysis based on subsets (ASSET),[16] to explore genetic correlations and shared genetic components among these diseases. Subsequently, we performed a series of pathway and transcriptome-wide analyses to biologically characterize differential mechanisms underlying loci associated with risk for multiple disorders (pleiotropic loci) versus non-pleiotropic loci.

Methods

Study populations and quality control

UKB is a large-scale biomedical database and research resource, containing genetic and health information from half a million individuals aged 40 to 69 years in the United Kingdom.[17] There were 488,000 participants genotyped for 805,426 markers on the UK BiLEVE Axiom array and UK Biobank Axiom array. After standard quality control, the dataset was phased

and ~96M genotypes were imputed with the Haplotype Reference Consortium and UK10K haplotype resources. The imputed data has been aligned to the + strand of the reference and SNP positions are in GRCh37 coordinates.

We defined AMD, DR, glaucoma, and RD cases according to (i) ICD-10 diagnosis codes (AMD: H353; DR: H360; glaucoma: H401, H408, or H409; RD: H335); (ii) touchscreen question "Eye problems/disorders" (responded "macular degeneration" or "glaucoma"); and (iii) self-reported non-cancer illness (responded "macular degeneration", "glaucoma", or "retinal detachment") (Figure S1).[17] We identified 7,329 AMD cases, 2,281 DR cases, 10,154 glaucoma cases, and 4,192 RD cases and 82,473 controls without any ocular disease, history of eye surgery, or current infection. UKB measured refractive error of 130,494 participants by non-cycloplegic autorefraction using a TomeyRC - 5000 AutoRefractor Keratometer. We excluded unreliable results and calculated the spherical equivalent (SE) as spherical refractive error plus half the cylindrical error. We identified 38,289 myopia cases (participants with SE of both eyes ≤ -0.50D) and 49,029 controls (participants with SE of both eyes > -0.50D and didn't have any ocular disease) (Figure S1).

We used the version 3 imputed genotypes data and only retained high quality variants with missingness < 0.05, Hardy-Weinberg equilibrium (HWE) test P-value $> 10^{-6}$, imputation quality (INFO) > 0.4, and minor allele frequency (MAF) > 0.01 on the basis of the combined case-control cohort. The Y chromosome and mitochondrial DNA were excluded from this study. We removed samples identified as outliers in heterozygosity and missing rates, participants with sex discrepancy, and individuals of non-Caucasian ancestry based on the sample QC provided by UKB (Figure S1). We estimated relatedness in each cohort by PLINK[18] and only kept one of any pair of individuals with relatedness ($\hat{\pi}$) > 0.2. In total, 88,250 Caucasian participants and 8,935,901 variants were included in this study. The sample overlap was showed in Table S1-S3.

Ethics

UK Biobank data has approval from the North West Multicentre Research Ethics Committee (MREC) (REC reference: 16/NW/0274). This research has been conducted with the UK Biobank Resource under project 45270.

Statistical analyses

Genome-wide association analyses. We performed GWAS analyses for each individual disease adjusting for age, sex and first ten principal components[19,20] using PLINK2,[21] SAIGE v0.44.5,[22] and fastGWA-GLMM v1.93.2,[23] respectively, and identified LD-independent loci using PLINK clumping function (parameters: -clump-p1 = 5×10^{-8}, -clump-p2 = 0.05, -clump-r2 = 0.4, -clump-kb = 500). The data of 88,250 samples was used as the reference panel for LD estimation. We searched each locus in the GWAS catalog (https://www.ebi.ac.uk/gwas, search date: March 15, 2022)[24] and GWAS literatures to identify which locus had been previously reported by other GWAS studies.

Heritability and genetic correlation. We performed linkage disequilibrium score regression (LDSC)[25,26] and GNOVA (genetic covariance analyzer)[27] analyses using the summary statistics of individual disease to estimate SNP-based heritability and genetic correlation. To explore the common risk factors of ocular diseases, we estimated genetic correlations between the five ocular diseases and 24 risk traits (Table S4).

Genomic structural equation modelling. To analyze the joint genetic architecture of five diseases, we used Genomic SEM[28] to fit structural equation models based on the GWAS summary statistics. An exploratory factor analysis (EFA) was performed with promax rotation and two factors using the factanal function (Table S5). We specified following genomic confirmatory factor models with two factors based on EFA results.

Cross-trait meta-analysis. To combine the association evidence and identify genomic loci shared across multiple ocular diseases, we performed a primary meta-analysis using a subset-based approach ASSET.[16] We used 2-sided ASSET for five ocular diseases, which allows subset search for positive and negative association and then combines association signals from two directions by chi-square test. Independent loci were determined via PLINK clumping (parameters: -clump-p1 = 5×10^{-8}, -clump-p2 = 0.05, -clump-r2 = 0.4, -clump-kb = 500), using the data of all 88,250 samples as LD reference panel. To confirm the independence of the index SNP in each locus, we performed conditional and joint analysis using GCTA-COJO.[29]

Next, we estimated posterior probabilities for each of the top loci identified from the meta-analysis to quantify disorder-specific using METASOFT[30–32] with random effects model (RE2).

Functional annotation and gene mapping. The SNPs in each locus that were in LD ($r^2 > 0.4$) with the index SNP and had $P < 0.05$ were defined as credible SNPs. For the index and credible SNPs in all loci, functional annotation and gene mapping were conducted using FUMA.[33]

Functional enrichment analysis. We conducted a gene-set enrichment analysis using Metascape[34] for Gene Ontology biological processes among the genes implicated in pleiotropic loci and non-pleiotropic loci separately. The pathways containing at least three candidate genes with $P < 0.01$ and enrichment score > 1.5 were defined as significantly enriched pathways. This online platform provided enrichment network using Cytoscape.[35]

Tissue enrichment analysis. Tissue specific expression enrichment was performed using RNA-seq data from Genotype-Tissue Expression v8 (GTEx, https://gtexportal.org/home/datasets)[36] and the Human Protein Atlas (HPA, https://www.proteinatlas.org/about/download),[37] respectively.

Expression analysis in human developing retina. To confirm the role of pleiotropic loci in retinal development, we plotted developmental expression trajectories for candidate genes using a gene expression data of the developing human retina (GSE98370),[38] which contains 21 samples obtained from embryonic and fetal retina, 3 samples of adult retina, and 8 whole embryonic eyes.

Single-cell RNA-seq analysis. Single cell expression profiles from the adult foveal retina, adult peripheral retina, and retinal organoids[39] were used to identify cell-type specificity of candidate genes. Expression values (transcripts per cell) were log-transformed and centred to the mean expression level for each cell. 73 genes (47 pleiotropic and 24 non-pleiotropic) were screened out with normalized expression ≥ 0.25 in at least one cell type of retinal organoids at 30 and 38 weeks. We compared the cell-type-specific expression of these candidate genes in foveal retina, peripheral retina, and retinal organoids at 30- and 38-week time points. Then we visualized the expression of these genes in cell types during the development of retinal organoids using scVis.[40]

Role of funding source

The funding sources of the study had no role in study design, data collection, data analysis, data interpretation, and writing of the report.

Results

Genome-wide association studies of individual ocular diseases

The single-phenotype GWAS of five ocular diseases were carried out on a total of 88,250 participants and 8,935,901 variants from UK Biobank after quality control (see "Methods"). For the analyses by PLINK, the quantile-quantile plot of the genome-wide meta-analysis revealed no evidence of inflation was found in AMD, DR, and RD, as their genomic inflation factor (λ) are close to one (Figure S2). Although the λ was 1.142 for glaucoma and 1.235 for myopia, the intercept (s.e.) from LDSC for glaucoma and myopia was 1.062 (0.008) and 1.065 (0.009), respectively (Table 1). Additionally, we reported no significant evidence for inflation of association statistics that would be expected in a study of λ_{1000} (Table 1). These indicated that the observed inflation of λ for glaucoma and myopia is mainly due to polygenic signals or asymmetric case/control sample sizes rather than population stratification. The LDSC SNP-based heritability (h^2) for AMD, DR, glaucoma, RD, and myopia were 0.073 (SE = 0.033), 0.173 (SE = 0.052), 0.167 (SE = 0.021), 0.071 (SE = 0.014), and 0.363 (SE = 0.024), respectively. We identified two genome-wide significant ($P < 5 \times 10^{-8}$) independent loci for AMD, three for DR, 18 for glaucoma, two for RD, and 61 for myopia (Figure 1a; Table 1; Table S6). Out of the independent loci reported here, we confirmed 83 loci that were previously known, and found three previously unreported loci, including rs139220415 for DR (11q22.3, $P = 2.10 \times 10^{-8}$), rs79807136 for glaucoma (14q23.2, $P = 3.88 \times 10^{-8}$), and rs58298352 for myopia (11q14.3, $P = 2.41 \times 10^{-8}$). The results of SAIGE and fastGWA models are highly consistent with PLINK (Pearson correlation coefficient ≈ 0.99; Figure 1a; Figure S3-S5).

Disease	#Cases	#Controls	# of loci (# of loci reported)	Lambda	$Lambda_{1000}$	Intercept (SE)	Liability-based heritability (SE)
AMD	5,873	60,514	2 (2)	1.073	1.007	1.059 (0.007)	0.062 (0.030)
DR	1,652	60,577	3 (2)	1.051	1.016	1.027 (0.006)	0.173 (0.044)
Glaucoma	7,873	60,517	18 (17)	1.142	1.010	1.062 (0.008)	0.172 (0.018)
RD	3,449	60,562	2 (2)	1.079	1.012	1.031 (0.007)	0.071 (0.011)
Myopia	27,993	36,275	61 (60)	1.235	1.007	1.065 (0.009)	0.366 (0.022)

***Table 1*: GWAS results of each ocular disease in UKB cohort.**
The number of cases and controls used in the single-disease GWASs. LD score regression intercept and SNP heritability was estimated from the GWAS summary statistics using LDSC.

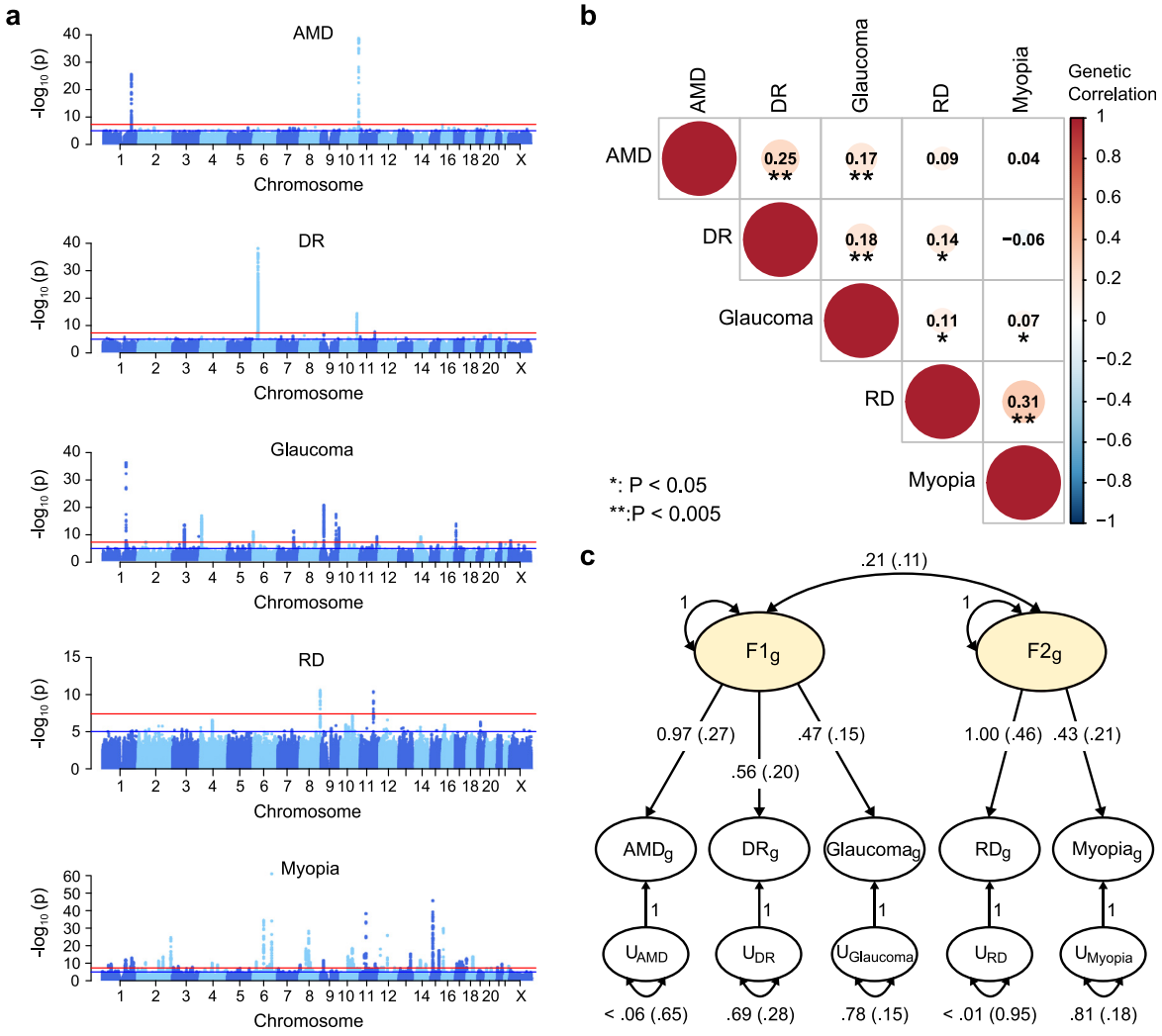

Figure 1. Genetic relationships across five ocular diseases. (a) Manhattan plots of GWAS results among five ocular diseases. The X-axis is the base-pair position, and the Y-axis is the -$\log_{10}$-transformed *P*-value for each SNP. The red line indicates genome-wide significance ($P < 5 \times 10^{-8}$), and the blue line represents a suggestive significance ($P < 1 \times 10^{-5}$). (b) SNP-based genetic correlations (r_g) were estimated between pairs of ocular diseases by GNOVA. The colour and size of each circle indicate the magnitude of the r_g. Asterisks indicate nominal significance ($P < 0.05$), and double asterisks represent statistical significance after Bonferroni correction ($P < 0.05/10$). (c) An exploratory factor analysis (EFA) and a confirmatory factor analysis (CFA) were conducted on the GWAS summary statistics using Genomic SEM. Here we showed the standardized estimates. $F1_g$ represents a shared genetic factor among AMD, DR, and glaucoma, while $F2_g$ represents a common genetic factor between RD and myopia. Arrows connecting the factors to the individual diseases represent regression coefficients of the genetic liability for the diseases on the common factor. The arrow connecting the two factors represents their correlation. Two-headed arrows linking the genetic components of the individual ocular diseases to themselves represent residual genetic variances, which can be interpreted as the proportion of heritable variation unexplained by the factors. SEs are shown in parentheses.

Genetic correlation among five ocular diseases

Based on the GWAS results, we first estimated pairwise genetic correlations among the five ocular diseases using LDSC.[25,26] In LDSC analysis, RD and myopia ($r_g = 0.47$, $P = 1.15 \times 10^{-13}$) were genetically correlated at a Bonferroni corrected significance threshold of $P < 5 \times 10^{-3}$ (Figure S6; Table S7), which was concordant with previous studies.[11,15] At a nominal threshold of $P < 0.05$, we observed glaucoma was correlated with AMD ($r_g = 0.37$, $P = 0.015$), DR ($r_g = 0.26$, $P = 0.022$), and RD ($r_g = 0.19$, $P = 0.010$). The highest degree of genetic correlation was observed for AMD and DR ($r_g = 0.61$, $P = 0.053$), though the correlation were not significant. Next, we applied GNOVA,[27] which is more powerful when genetic correlation is moderate, to dissect the genetic covariance among these diseases. We

found the relationships between four pairs of ocular diseases passed the Bonferroni correction threshold, including highest correlation between RD and myopia (r_g = 0.31, $P = 4.29 \times 10^{-15}$), followed by AMD and DR (r_g = 0.25, $P = 2.46 \times 10^{-4}$), glaucoma and DR (r_g = 0.18, $P = 8.26 \times 10^{-4}$), and glaucoma and AMD (r_g = 0.17, $P = 2.68 \times 10^{-4}$) (Figure 1b; Table S8).

We modelled the joint genetic architecture of the five ocular diseases using an exploratory factor analysis (EFA) and a confirmatory factor analysis (CFA) by Genomic SEM.[28] Genomic SEM identified two correlated factors, which together explained 52.6% of the genetic variation in the five ocular diseases (Table S5). The first factor consisted of three age-related neurodegenerative diseases of the retina[41–43]: AMD, DR, and glaucoma. The second factor was characterized by axial elongation,[44,45] specifically RD and myopia (Figure 1c; Figure S7a; Table S9). Similar to the Genomic SEM results, hierarchical clustering analyses also identified two groups among the five diseases (Figure S7b). To examine the genetic correlational pattern between F1 group (AMD, DR, and glaucoma) and F2 group (RD and myopia), we extended our genetic correlation analysis to other traits and confirmed two clusters among these diseases (Figure S8; Table S10). The r_g values of AMD and DR strongly mirrored each other (the Pearson correlation coefficient between their r_g values was r = 0.80; $P = 2.94 \times 10^{-6}$; Table S11). As F1-grouped diseases, AMD, DR, and glaucoma were both positively correlated with hypertension, type 2 diabetes, BMI, insomnia, smoking behaviour, and time spent in watching television, while negatively correlated with education years and several physical activities. However, the correlational patterns for F1-grouped and F2-grouped diseases were markedly different and sometimes in opposite directions. For example, myopia was positively associated with intelligence and education attainment, while negatively correlated with BMI, smoking behaviour, and television time. Together, these findings confirmed the strong genetic correlation between RD and myopia, and identified genetic relationships between AMD, DR, and glaucoma.

Cross-trait meta-analysis

Given the strong genetic relationships, we performed a primary cross-trait meta-analysis to detect the loci shared by at least two ocular diseases using ASSET.[16] Although the genomic inflation factor λ was 1.226, the λ_{1000} was close to one, suggesting no inflation of test statistics due to confounding (λ_{1000} = 1.005; Figure 2a). We identified 1,667 genome-wide significant association ($P_{ASSET} < 5 \times 10^{-8}$) variants map to 37 independent loci (Figure 2b; Figure S9; Table S12). All the 37 index SNPs were confirmed to be independent by conditional analysis using GCTA-COJO.[29] Of all the index SNPs, four were in exonic regions, 21 were intronic, and 12 were in inter-genic regions (Table S13). Among these index SNPs, rs5442 ($P_{ASSET} = 8.51 \times 10^{-11}$) on 12p13.31 was a missense variant of *GNB3* with the highest combined annotation-dependent depletion (CADD)[46] score (26.5), leading to a glycine-to-serine change.[47] The product of *GNB3* modulates cone transducin function and bipolar cell signalling, associated with congenital stationary night blindness.[48] This SNP shows a significant association with myopia in single-trait GWAS model (Table S6). Of all the 3,718 index and credible SNPs (SNPs with $P_{ASSET} < 0.05$ and in high linkage disequilibrium with the independent index SNPs, see "Methods"), 32 were in exonic regions (0.9%), 2,093 were in intronic regions (56.3%), 1,234 were in intergenic regions (33.2%), and 49.9% were annotated as potentially having a regulatory function (Figure 2c). Partitioned heritability analysis[49] of meta-analysis results using LDSC showed significant enrichment for h^2 of SNP located in conserved regions (enrichment = 16.66, $P = 2.10 \times 10^{-6}$), super-enhancer (enrichment = 1.96, $P = 5.61 \times 10^{-5}$), intron (enrichment = 1.40, $P = 1.85 \times 10^{-3}$), and acetylated lysine 27 on histone H3 (H3K27ac; enrichment = 1.78, $P = 3.28 \times 10^{-5}$) (Figure 2d). Our results suggest evolutionarily conserved and regulatory regions may harbour variants with pleiotropic effects on many ocular diseases.

Decoding cross-trait pleiotropic associations

To quantify the best-fit model of cross-disorder genotype-phenotype relationships, we used METASOFT[30,31] to estimate the posterior probability (m-value) of association with each disease. M-value > 0.9 indicated that a particular variant was associated with a given disease, while m-value < 0.1 was predicted that there is no effect between genotype and phenotype. The plots of the *P*-value, beta, and m-value for each index SNP are shown in Figure S10. We finally identified 23 pleiotropic loci (i.e., associated with more than one ocular disease) and 14 non-pleiotropic loci (Table 2; Table S12). Of these 23 pleiotropic loci, 4 had not been identified in our GWAS of individual disorders, and their lead SNPs are located in the genomic regions of 10q26.3 (rs12570944, $P_{ASSET} = 1.00 \times 10^{-8}$), 11q14.2 (rs9667489, $P_{ASSET} = 2.01 \times 10^{-8}$), 5q13.2 (rs10036789, $P_{ASSET} = 4.43 \times 10^{-10}$), and 2q31.1 (rs62181740, $P_{ASSET} = 4.79 \times 10^{-8}$) (Table S12). In addition, all pleiotropic loci had same directional effects on their associated ocular diseases, including 14 susceptible loci and nine protective loci (Figure S10).

We found three pleiotropic loci that were positively associated with all five diseases (Figure 3). The first locus covered *FGF5*, *C4orf22*, *BMP3*, and *PRKG2* on 4q21.21(index SNP rs7678123, $P_{ASSET} = 3.99 \times 10^{-13}$), which has been previously reported in RD and myopia GWASs.[11,50] The same eQTL in multiple GTEx tissues for *BMP3* (bone morphogenetic protein 3) and *PRKG2* (protein kinase cGMP-dependent 2) also colocalized

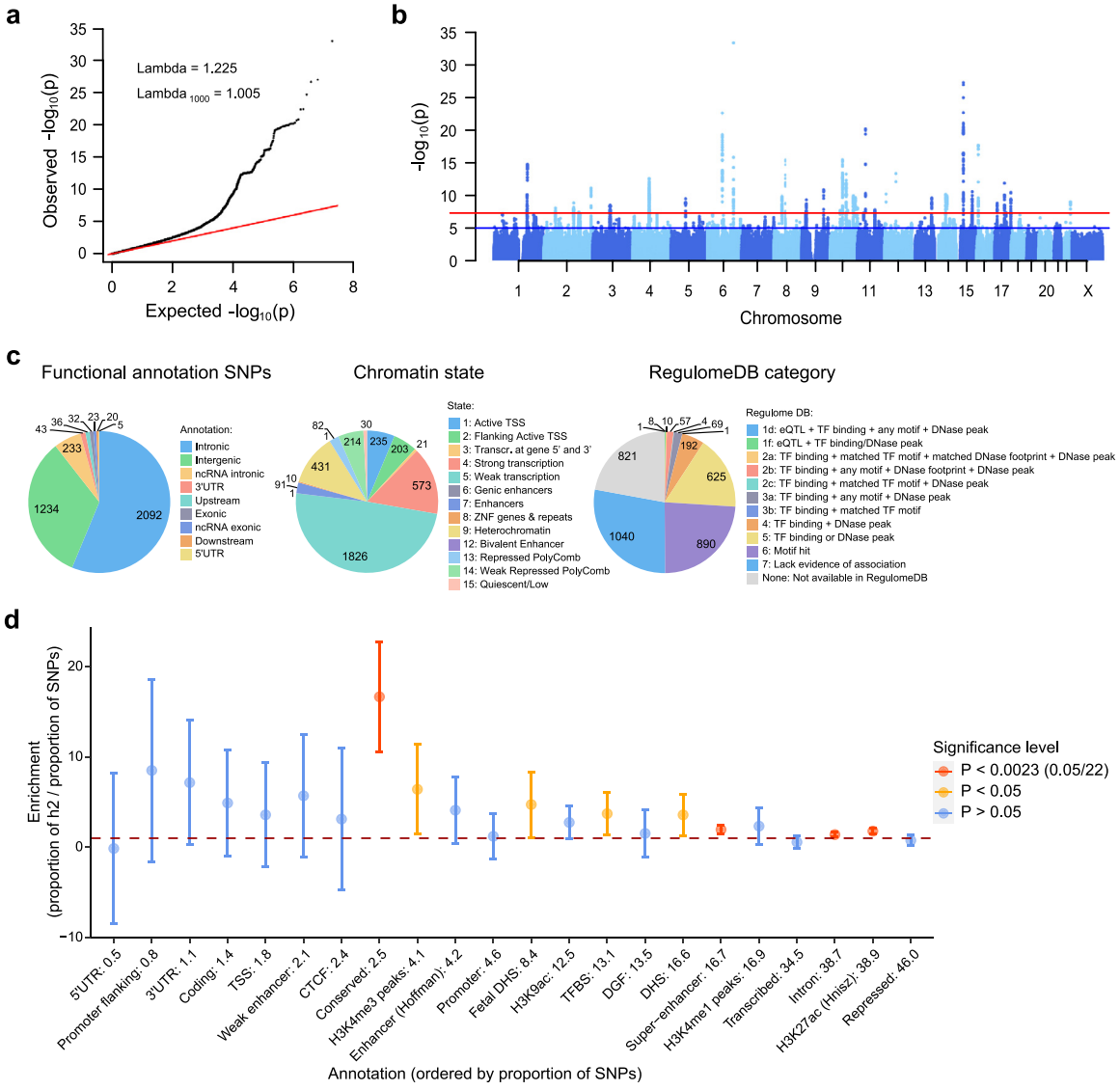

Figure 2. Results of cross-traits meta-analysis by ASSET based on 88,250 individuals. (a) Quantile-quantile (QQ) plot of the meta-analysis displaying the observed significance versus the expected significance for each variant. (b) Manhattan plot of the meta-analysis with the X-axis showing genomic position and the Y-axis showing the significance on a -$\log_{10}$ scale for each SNP. The red and blue lines represent the thresholds for genome-wide significance ($P_{ASSET} = 5 \times 10^{-8}$) and suggestive associations ($P_{ASSET} = 1 \times 10^{-5}$), respectively. (c) Distribution of index SNPs and credible SNPs in functional consequences, minimum chromatin state across 127 tissue and cell types, and RegulomeDB score (The lower the score, the more likely the SNP is to have a regulatory function). (d) Heritability enrichment of 22 functional SNP annotations by stratified LDSC. The X-axis shows the proportion of SNPs in each region, and the Y-axis displays the enrichment, estimated as the proportion of heritability / the proportion of SNPs. The dashed line represents enrichment of 1. Error bars show 95% confidence intervals. TSS, transcription start site; CTCF, CCCTC binding factor; DHS, DNase I hypersensitivity site; TFBS, transcription factor binding site; DGF, digital genomic footprint.

with this signal (eQTL association FDR < 0.05), supporting them as plausible candidate genes (Figure S11; Table S14). Gene expression data revealed that *PRKG2* is highly expressed in embryonic retina from 4.7 to 7 post-conception weeks (PCW), and then lowly expressed in retina from 7.8 PCW to adult, while BMP3 is mainly expressed from 9 to 17 PCW (Figure S14a). We found this pattern was not in brain development (Figure S14b). The second pleiotropic locus associated with all five diseases was on 10q26.3 (index SNP rs12570944, $P_{ASSET} = 1.00 \times 10^{-8}$), which has been previously reported in glaucoma.[51] This locus has mapped to *DPYSL4* (dihydropyrimidinase like 4) with significant cis-eQTL associations in multiple GTEx tissues (Figure

SNP	CHR	POS	Locus (hg19)	AMD	DR	Glaucoma	RD	Myopia	m>0.9
rs7678123	4	81372405	chr4:81209680-82037207	0.998	0.981	0.94	1	1	5
rs12570944	10	134139057	chr10:134116354-134216014	0.975	0.961	0.995	0.976	1	5
rs9667489	11	86314579	chr11:86297189-86403375	0.996	0.971	1	0.987	0.998	5
rs10036789	5	71695918	chr5:71683885-71743322	0.998	0.908	1	0.886	1	4
rs138650617	10	60335073	chr10:60229260-60374898	1	0.617	1	0.994	1	4
rs2738265	14	54422399	chr14:54411057-54431575	0.924	0.916	0.003	0.999	1	4
rs12950511	17	47320938	chr17:47260130-47461433	0.832	0.977	0.998	0.966	1	4
1:164194417:TA:T	1	164194417	chr1:164073168-164250081	0.306	0.958	0.948	0.512	1	3
rs62181740	2	172540401	chr2:172527238-172932333	0.907	0.746	0.742	0.997	1	3
rs13118211	4	82400811	chr4:82390465-82423807	0.729	0.879	0.984	0.994	1	3
rs7744813	6	73643289	chr6:73569159-73648822	0.998	0.064	0	0.998	1	3
rs10887262	10	86009171	chr10:86004238-86016892	0.903	0.329	0.376	0.982	1	3
rs36090025	10	114774433	chr10:114746580-114818754	0.08	1	0.949	0.897	0.964	3
rs112115087	14	60807865	chr14:60789176-61186263	0.069	0.469	1	0.983	1	3
rs9330814	22	46364191	chr22:46362822-46394928	0.075	0.974	1	0.225	1	3
rs41393947	2	56011517	chr2:55991004-56116193	0.023	0.603	0.632	0.993	1	2
2:146913702:CCTCT:C	2	146913702	chr2:146664218-147026854	0.811	0.282	0.089	0.969	1	2
rs12193446	6	129820038	chr6:129732674-129858150	0	0	0	0.993	1	2
rs10824539	10	79157133	chr10:79030744-79162267	0.028	0.575	0.738	0.985	1	2
rs3138142	12	56115585	chr12:56115585-56213297	0	0.072	0	1	1	2
rs7184522	16	7460699	chr16:7457972-7462074	0	0.019	0	1	1	2
rs113941606	17	11437291	chr17:11395143-11487165	0.818	0.8	0.897	0.954	1	2
rs7405453	17	79615572	chr17:79526821-79686552	0.079	0.252	0.046	0.998	1	2

***Table 2*: Summary of 23 pleiotropic loci.**
SNP ID, location, locus, disorder-specific m-values for 23 pleiotropic loci. The number of disorders with high confidence association (m-values > 0.9) is shown in the last column.

S12; Table S14). *DPYSL4* has high expression in all stages of retinal development (Figure S14a) and is highly expressed in multiple cell types of mature retina, including ganglion cells (GC), cones, amacrine cell (AC), OFF bipolar cells (HBC), and horizontal cell (HC) (Figure S15). The third locus is located in an intron of *ME3* (malic enzyme 3) on 11q14.2 (index SNP rs9667489, $P_{ASSET} = 2.01 \times 10^{-8}$). *ME3* was also a

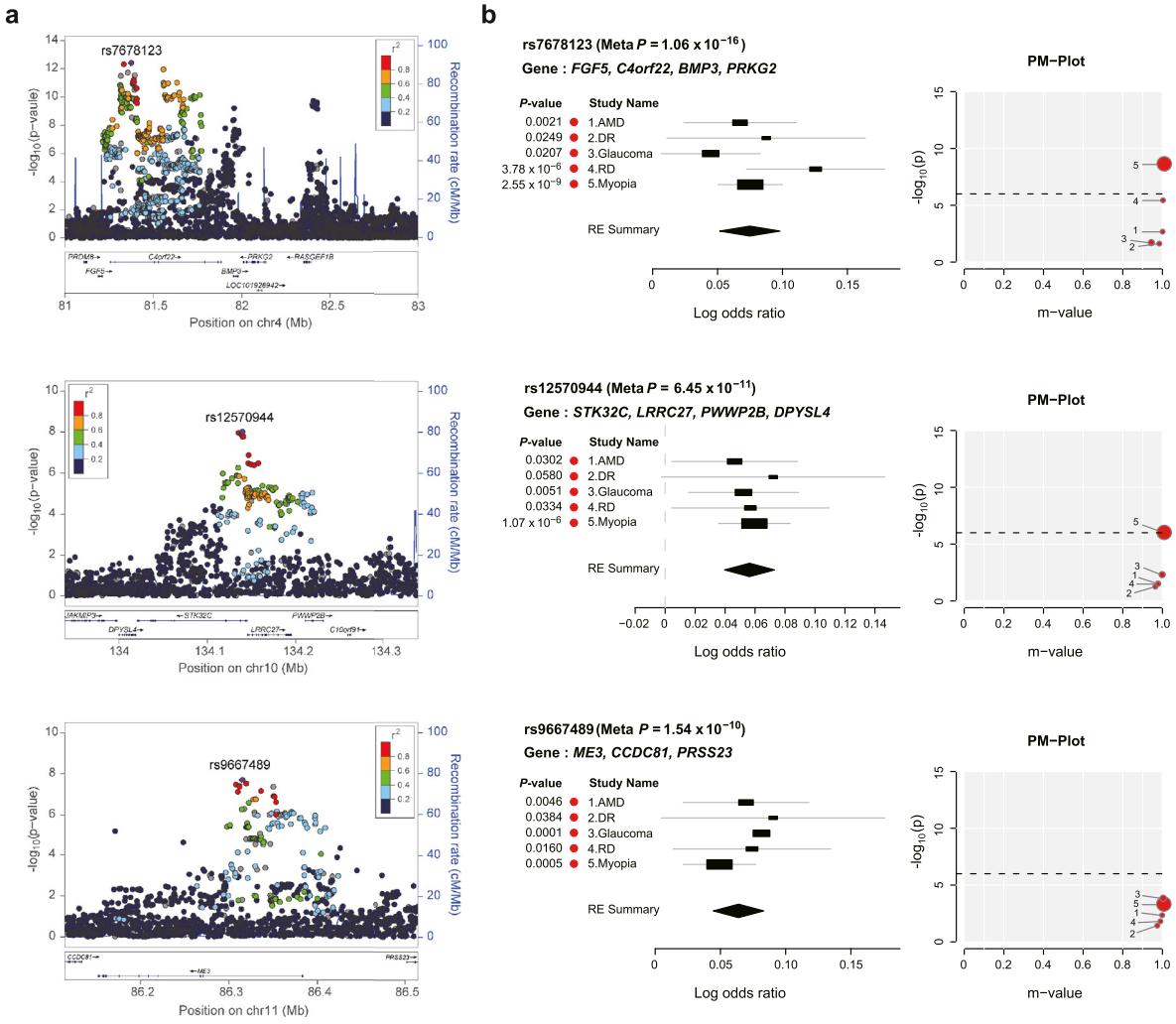

Figure 3. Three most pleiotropic loci associated with all five ocular diseases. (a) Regional association plots of the three loci: 4q21.21 (index SNP rs7678123), 10q26.3 (index SNP rs12570944), and 11q14.2 (index SNP rs9667489). (b) Forest plots with PM-plots show disease-specific effects of the index SNP in each locus. Forest plots display the *P*-value in METASOFT meta-analysis (Meta *P*) and the *P*-value, log(OR) and its standard error of the SNP in the GWAS of individual diseases. PM-plots visualize the posterior probability (m-value, X-axis) of the SNP in each study with disease-specific association significance as $-\log_{10}(P)$ (Y-axis). M-values > 0.9 (coloured in red) suggests that the SNP does have an effect on the disease. The dot size represents the GWAS sample size estimated from summary statistics.

significant retina eQTL target gene of this locus (eQTL association FDR = 1.07×10^{-6}; Figure S13), with a high expression during retinal development (Figure S14). *PRSS23* (serine protease 23), another target gene detected by eQTL colocalization in multiple GTEx tissues (Figure S13), may also be involved in retinal development. Its expression in retina is highest in 4.7 PCW, rapidly decreases after 16 PCW, and is lowest in adulthood (Figure S14a), when it is mainly expressed in endothelial cells (END) (Figure S15).

Functional dissection of pleiotropic and non-pleiotropic loci

To investigate characteristic features between pleiotropic loci and non-pleiotropic loc, we first used three strategies to link our SNP results to genes by FUMA[33]: positional mapping, expression quantitative trait locus (eQTL) mapping, and chromatin interaction mapping (Figure 4a; Figure S16; Methods). Finally, a total of 163 genes were mapped from the 37 loci, including 84 genes implicated through positional mapping, 78 implicated

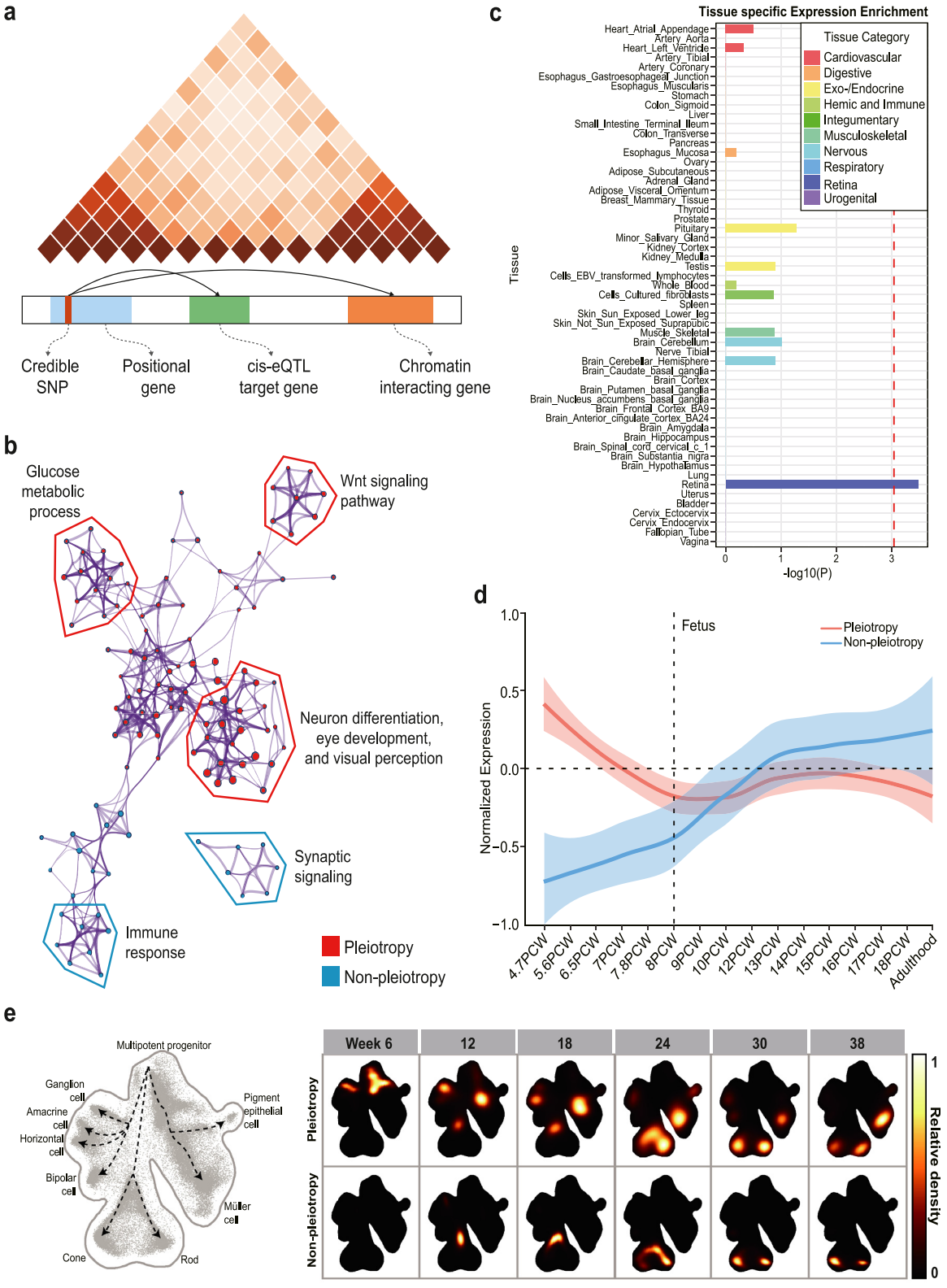

Figure 4. Gene mapping of meta-analysis results. (a) Three gene mapping strategies for the index SNP and credible SNPs of each locus. We mapped these SNPs to the protein-coding genes within 10 kb, mapped cis-eQTL markers to their target genes, and

through eQTL mapping, and 95 implicated through chromatin interaction mapping (Figure S17; Table S14). Of these, 23 were implicated by all three methods, of which seven had chromatin interaction and eQTL associations in the same tissue. Gene overlap between the three strategies was significant in hypergeometric tests ($P < 1.67 \times 10^{-2}$; Figure S17). 113 genes were mapped from the pleiotropic loci and the other 50 genes were implicated in the non-pleiotropic loci.

We tested several characteristics related to genomic function between pleiotropic and non-pleiotropic loci. More than 22% and 26% of the genes associated with pleiotropic and non-pleiotropic were intolerant of loss of function mutations (pLI score $\geq$ 0.9), but 12% and 8% of the genes associated with pleiotropic and non-pleiotropic were intolerant of missense changes (missense z-score, mis_z $\geq$ 3.09). These overlap between pleiotropic and constrained genes is unlikely to occur by chance. When considering subsets of genes at increasing thresholds of gene constraint using the probability of pLI and mis_z, we found the relationship of increasing odds ratio in pleiotropic genes with increasing gene constraint (Figure S18).

Gene Ontology (GO) pathway enrichment analysis revealed functional differences between pleiotropic and non-pleiotropic loci. The pleiotropic loci showed the most significant enrichment of genes involved in regulation of neuron differentiation ($P = 8.80 \times 10^{-6}$), eye development ($P = 3.86 \times 10^{-5}$), and visual perception ($P = 1.59 \times 10^{-4}$; Figure 4b; Table S15), as well as enriched in canonical Wnt signalling pathway ($P = 9.26 \times 10^{-4}$) and glucose metabolic process ($P = 7.28 \times 10^{-4}$). Enrichment of these gene-sets was not seen for the non-pleiotropic loci, however, they were significantly enriched in immune response ($P = 2.26 \times 10^{-3}$) and synaptic signalling pathway ($P = 5.57 \times 10^{-3}$).

Spatiotemporal gene expression of pleiotropic and non-pleiotropic loci

To understand whether the 37 identified loci are enriched for expression in retina, we performed a tissue-specific expression analysis using the Genotype Tissue Expression (GTEx) pilot data.[36] GTEx tissue-specific enrichment analysis showed that the genes mapped from all 37 loci were significantly enriched in the retina (OR = 3.45, $P_{adjust} = 0.02$), but not in the other tissues (Figure 4c; Table S16), and the enrichment score was significantly higher than that obtained under random simulations (Figure S19). We repeated the analysis using Human Protein Atlas (HPA)[37] data and observed a similar enrichment for the genes in retina-specific categories ($P_{adjust} = 2.10 \times 10^{-5}$; Figure S20; Table S17).

The results of retina enrichment and eye development pathway enrichment prompted our hypothesis that the pleiotropic loci may play a role in early development of retinogenesis. Therefore, we compared the gene expression patterns of the pleiotropic risk loci and the non-pleiotropic loci during human retinal development[38] and preformed a t-statistic that assesses the relative prenatal versus postnatal expression bias for each gene. Of all the 163 genes, 52 genes (34 pleiotropic and 18 non-pleiotropic) with significant dynamic expression during human retinal development were screened by linear regression. The pleiotropic genes display a marked embryo (< 8 PCW) bias ($P = 1.50 \times 10^{-5}$, Wilcoxon test; Figure S21a), reaching peak expression in the retina at early embryo development (Figure 4d), whereas the non-pleiotropic genes show fetus bias ($P = 5.00 \times 10^{-10}$, Wilcoxon test; Figure S21a), having their highest expression in early midfetal (13 $\leq$ Age $\leq$ 18 PCW; Figure 4d). Additionally, to enhance temporal gene expression resolution, we selected genes that were expressed in embryo at a significantly higher level than fetus and adult; specifically, log2 fold change of 0.5 or more and FDR of less than 0.05 (t-test). The gene expression heatmap also showed that most pleiotropic gene were expressed in embryo development stage (Figure S21b).

Next, we compared the gene expression of pleiotropic and non-pleiotropic loci in the single-cell data of adult foveal retina, peripheral retina, and retinal organoids.[39] We selected 71 genes (47 pleiotropic and 24 non-pleiotropic) with normalized expression values greater than 0.25 in at least one cell type of mature retinal

mapped the SNPs in the genomic regions interacting with gene promoter regions to corresponding genes. (b) GO pathway enrichment for pleiotropic versus non-pleiotropic loci. This network shows the terms with $P < 0.01$, a minimum gene count of 3, and an enrichment factor > 1.5. The nodes sizes are scaled with *P*-value. The genes implicated in pleiotropic loci are most significantly enriched in eye development and neuron differentiation. (c) GTEx tissue-specific enrichment results for 37 loci associated with at least one ocular disease. 55 GTEx tissues were classified as 10 categories, and the retina were coloured in dark blue. The red dotted line represents the *P*-value threshold after Bonferroni correction ($P = 0.05/55 = 9.09 \times 10^{-4}$). Genomic risk loci show significant enrichment in genes specifically expressed in retina. (d) Retinal development RNA-seq data from 4.7 PCW to adult was used to plot the normalized expression of 34 genes of pleiotropic loci and 18 genes of non-pleiotropic loci with significant expression variation during development. (e) Left: cell classes marked on scVis map. Arrows represents developmental trajectories. Right: ScVis map displays the expression trajectories of candidate genes during the development of retinal organoids. The genes implicated in pleiotropic loci highly expressed in multipotent progenitors, GCs, HCs, cones, rods, MCs, and RPEs, successively, whereas the genes mapped to non-pleiotropic loci only had high expression in cones and rods.

organoids (week 30 and 38). In the adult retina and mature retinal organoids, the genes implicated in pleiotropic loci were expressed in most cell types, including RPE, MC, GC, cones, and rods, while the non-pleiotropic loci showed the highest gene expression in cones and rods (Figure S22). During the development of retinal organoids, we found genes mapped from pleiotropic loci were expressed as retinal development progressed, expressed in retinal progenitor cells (RPCs) by 6 week and involved in photoreceptors and RPE/MC fate determination by 12 and 18 week (Figure 4e).

Discussion

In the large cross-trait GWAS meta-analysis of ocular diseases, comprising 88,250 individuals, we have shown robust genetic relationships between five clinically related ocular diseases, as well as identified 23 loci that affected at least two diseases. Furthermore, we found that the pleiotropic loci played important roles in the development and differentiation process of various cell types in the retina. Our study provided multiple lines of evidence for a shared genetic basis of ocular diseases and generated new insights into ocular diseases susceptibility.

We conducted GWAS for five ocular diseases respectively and identified three previously unknown loci: 11q22.3 (nearest genes: *GRIA4, CASP1*, and *CARD16*) for DR, 14q23.2 (nearest genes: *KCNH5*) for glaucoma, and 11q14.3 (nearest genes: *CCDC91*) for myopia. The activity of caspase-1 (product of *CASP1*) is increased in retinas of diabetic patients, and inhibiting hyperglycemia-induced caspase-1 activity can prevent retinal capillary degeneration.[52] *KCNH5* encodes a member of voltage-gated potassium channels, which regulate neurotransmitter release and neuronal excitability.[53] *CCDC91* is involved in Golgi to lysosome transport and lysosomal enzyme maturation.[54]

Our results show molecular evidence of the sharing of genetic risk factors across key ocular disorders, especially across AMD, DR, and glaucoma. Modelling of genetic correlations using Genomic SEM and hierarchical clustering identified two groups of diseases with shared genetic factors. The first group comprised three diseases characterized by age-related retinal degenerative changes,[41–43] including AMD, DR, and glaucoma. These three diseases were both positively associated with type 2 diabetes. Close genetic relationship between AMD and DR is also reflected in their similar genetic correlations with several risk factors such as fat, insomnia, and lack of physical activity. The second group contained RD and myopia, with the highest genetic correlation estimate, have characterized by axial elongation.[44,45] Overall, these results suggest significant pairwise genetic correlations among multiple ocular disorders and a higher level of genetic architecture that points to broader domains that underlie genetic risk for ocular pathology.

The cross-trait meta-analysis supported the existence of pleiotropy in variant level. We identified 23 pleiotropic loci and 14 non-pleiotropic (disease-specific) loci by using a fixed-effects-based method for these ocular diseases. Of these pleiotropic loci, three with particularly extensive pleiotropy were associated with all five ocular diseases. The potential candidate genes mapped in these pleiotropic regions plays an important role in retinal development. *DPYSL4* has high expression during retinal development (Figure S14a) and in adult retina,[55] involved in cell migration, neuronal growth cone collapse, and axon guidance,[56] and participates in nervous system development and neuron death pathway.[57] *PRKG2* (also named *cGKII*), *PRSS23, ME3*, and *BMP3* are highly expressed at specific time points in retinal development (Figure S14a). A prior study found that nitric oxide-mediated *PRKG2* signalling may control the neuronal cell viability during early retinal development.[58] *PRKG2* knockdown prevented nitric oxide-induced cell death in six-day-old chick retina and cell survival in eight-day-old chick retina.[58] *Bmp3* is involved in Zebrafish ocular development by regulating Smad3 phosphorylation in neural crest cells.[59] Some candidate genes have been reported in the pathogenesis of multiple ocular diseases. The product of *FGF5* plays a role in the angiogenesis in AMD[60] and ganglion cell injury in DR.[61] *ME3* encodes the mitochondrial NADP(+)-dependent isoform of malic enzyme, which catalyzes the oxidative decarboxylation of malate to pyruvate,[62] supporting the contribution of mitochondrial dysfunction to the pathology of AMD, DR, and glaucoma.[63,64]

Genetic correlations have been estimated across five ocular diseases, functional analyses for pleiotropic loci can be constructed that could improve power to describe the shared biological etiology of five ocular diseases. Compared to non-pleiotropic loci, we found extensive evidence that involvement of pleiotropic loci in eye development underlies the cross-diseases genetics of ocular diseases. The gene-set enrichment analysis of GO pathway indicated that pleiotropic loci were distinguished from non-pleiotropic loci in biological function. The genes implicated in pleiotropic loci are significantly enriched in neuron differentiation and eye development. In addition, the retinal developmental expression trajectory showed the genes mapped from pleiotropic loci are on average expressed at higher levels in the early stages of retinal development, while the expression of genes related to disease-specific (mainly myopia) loci peaked in adulthood (Figure 4d). During development, the genes of pleiotropic loci were highly expressed in multipotent progenitors, GC, HC, RPE, MC, cones, and rods successively (Figure 4e). In contrast, the genes implicated in single-disorder loci only had high expression in photoreceptors (cones and rods) and their precursors in mid-late stage of development. As we know, pleiotropy occurs when a single mutation or one gene influences more than one trait, contributing to genetic

correlations among traits,[65] quite simply, pleiotropy sometimes refer to the breadth of expression across tissues and time points.[66] Pleiotropic gene have multiple roles in distinct cell types[67]; thus, any genetic change that alters expression or function of pleiotropic gene can potentially have wide-ranging effects in a variety of tissues.

The functional enrichment analysis also suggested canonical Wnt signalling pathway is related to multiple ocular diseases. Wnt signalling pathway is divided into two types: the canonical Wnt/β-catenin signalling pathway which acts through β-catenin as a transcriptional coactivator and the non-canonical Wnt signalling pathway that does not depend on β-catenin.[68] The candidate genes we identified by meta-GWAS analysis are mainly enriched in the canonical Wnt signalling pathway, which is a key regulatory system that coordinates the behaviour of endothelial cells to control vascular morphogenesis.[69] Aberrantly activated Wnt signalling have been reported as one of the pathogenic factors in AMD and DR,[70] and suppression of canonical Wnt signalling can prevent neovascularization in murine choroidal neovascularization models[71] and diabetic models.[72] Wnt/β-catenin pathway also regulates the RPE response to oxidative stress, which suggests its pathogenic role in dry AMD.[73] In addition to vascular ocular diseases, canonical Wnt signalling can regulate the outflow of aqueous humor and IOP,[74] associated with glaucoma pathogenesis. In the murine myopia model, the inhibition of canonical Wnt signalling by niclosamide significantly reduced the growth of lens thickness, vitreous chamber depth and axial length, thereby inhibiting myopia.[75]

These results should be interpreted in consideration of several limitations. First, we used summary statistics from GWAS of large cohorts, which we screened for overlapping samples. However, some overlap may persist across the five ocular diseases owing to the comorbidity of these phenotypes. In addition, GNOVA and LDSC are robust approaches for the estimation of genetic correlation that are not biased by sample overlap[26,27] and we controlled for sample overlap applied ASSET in meta-analysis. Second, our sample size of single-trait GWAS is not as large as the published studies, which may lead to some missing genetic correlation. The genetic correlation between glaucoma and myopia is little in our analysis, but significant when use public glaucoma GWAS[76] (LDSC: r_g = 0.16, $P = 8.86 \times 10^{-6}$; GNOVA: r_g = 0.12, $P = 3.12 \times 10^{-7}$). Third, there is an imbalance in sample size among individual diseases, which may limit our detection of pleiotropic loci for diseases with small sample sizes, especially a minimum of 1,652 cases for DR. The availability of more samples in the future will improve power for detection of shared risk effects. Fourth, due to the limited number of cases, we only focused on the five ocular diseases with relatively large samples. Thus, non-pleiotropic loci we identified may have additional effects on other ocular diseases that were not included in this study. Lastly, we restricted our analyses to individuals of European ancestry to avoid potential confounding due to ancestral heterogeneity across distinct disorder studies. Further analyses are needed to explore the pleiotropic mechanism in other populations.

In summary, we report SNP-based heritabilities that are significantly greater than zero for all five disorders studied. We have used the currently available large-scale ocular genome-wide association studies in the UKB data sets, and our results provide evidence of substantial sharing of the genetic risk variants tagged by SNPs between AMD, DR, and glaucoma; RD and myopia. All of the 23 genomic loci with pleiotropic effects showed same directional effects on two or more ocular diseases. These results highlight further GWAS and rare variant studies will be needed to account more completely for shared genetic contributions across disorders. In particular, alterations in eye development, Wnt signalling pathway and glucose metabolic process could represent a fundamental mechanism contributing to a broad vulnerability to ocular pathology. Our results can also provide theoretical support for the occurrence of comorbidity of ocular diseases in clinical practice and remind doctors and patients to prevent it. Furthermore, they will encourage investigations into shared biological etiology across disorders, including potential clarification of common therapeutic mechanisms.

Contributors

The study was conceived, designed, and supervised by H.C., J.S., J.Q., J.Y., and Z.X. Analysis of data was performed by Z.X., J.Y., F.C., Y.Y., S.X., X.Y., K.L., C.W., and J.B. The manuscript was written by Z.X., J.Y., J.S., and H.C. Z.X., J.Y., and J.S. have verified the underlying data and take responsibility for the accuracy of the data analysis. All authors read and approved the final manuscript.

Data sharing statement

UK Biobank data are available via application at https://www.ukbiobank.ac.uk. This research was conducted under the project of UK Biobank Application Number 45270. GWAS summary statistics for individual ocular diseases and meta-analysis have been returned to the UK Biobank. All the code used is publicly available at https://github.com/xuezhengbo/Pleiotropy.

Declaration of interests

The authors declare no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2022.104161.

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