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ARTICLE

Genome-wide meta-analysis identifies novel loci associated with age-related macular degeneration

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Abstract

Age-related macular degeneration (AMD) is the leading cause of irreversible blindness among the elderly population. To accelerate the understanding of the genetics of AMD, we conducted a meta-analysis of genome-wide association studies (GWAS) combining data from the International AMD Genomics Consortium AMD-2016 GWAS (16,144 advanced AMD cases and 17,832 controls), AMD-2013 GWAS (17,181 cases and 60,074 controls), and new data on 4017 AMD cases and 14,984 controls from Genetic Epidemiology Research on Aging study. We identified 12 novel AMD loci near or within *C4BPA—CD55*, *ZNF385B*, *ZBTB38*, *NFKB1*, *LINC00461*, *ADAM19*, *CPN1*, *ACSL5*, *CSK*, *RLBP1*, *CLUL1*, and *LBP*. We then replicated the associations of the novel loci in independent cohorts, UK Biobank (5860 cases and 126,726 controls) and FinnGen (1266 cases and 47,560 control). In general, the concordance in effect sizes was very high (correlation in effect size estimates 0.89), 11 of 12 novel loci were in the expected direction, 5 were associated with AMD at a nominal significance level, and rs3825991 (near gene *RLBP1*) after Bonferroni correction. We identified an additional 21 novel genes using a gene-based test. Most of the novel genes are expressed in retinal tissue and could be involved in the pathogenesis of AMD (i.e., complement, inflammation, and lipid pathways). These findings enhance our understanding of the genetic architecture of AMD and shed light on the biological process underlying AMD pathogenesis.

Introduction

Age-related macular degeneration (AMD), a degenerative disorder of the central retina, is the leading cause of central vision loss in the elderly population in the Western world [1–4]. AMD is classified as non-neovascular (dry AMD) and neovascular (wet) AMD. For the population aged over 45 years, the global prevalence of AMD is 8.69%, with a higher prevalence in Europeans (12.3%) [4]. It is estimated that the number of AMD patients will be 196 million in 2020, rising to 288 million in 2040 [4].

AMD is highly heritable with heritability estimates between 46 and 71% [5]. A recent genome-wide association study (GWAS) from the International AMD Genomics Consortium (IAMDGC) has identified 52 independent variants across 34 loci [6]. Understanding the genetic contributions for AMD is important to reveal insights into the biological mechanisms of AMD, and discover potential genetic variations for clinical diagnostic, predictive, and therapeutic targets [6, 7].

Recent statistical methodology and application studies have shown that multivariate GWAS can leverage multiple input summary statistics of the same trait or genetically correlated traits, and gain the power for identifying new genes [8, 9]. Compared with the traditional meta-analysis that assumes the input GWAS summary statistics are from the same trait (a genetic correlation close to one) and are

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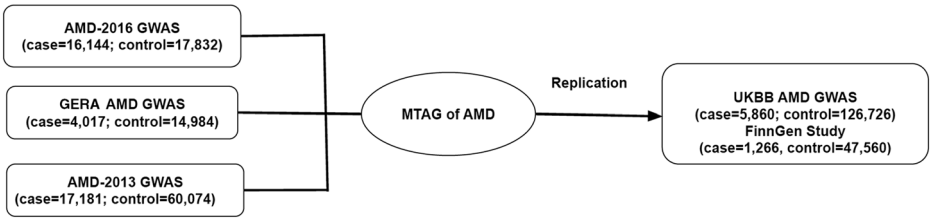

Fig. 1 Study design. The multivariate analysis of GWAS (MTAG), a method to jointly analyze summary statistics, was applied to three input summary statistics of AMD GWAS: AMD-2016 GWAS [6] and AMD-2013 GWAS [11] from the International AMD Genomics Consortium (IAMDGC), and AMD GWAS in Genetic Epidemiology Research on Aging (GERA) study. The novel loci were then replicated in the UK Biobank and FinnGen studies

sensitive to sample overlap [10], the multiple trait analysis of GWAS (MTAG) approach, a framework to generalize the standard inverse-variance meta-analysis method, can jointly analyze GWAS summary statistics from the same trait or multiple correlated phenotypes, with or without overlapping samples [8]. In this study, we identify novel AMD loci using the state-of-the-art multivariate GWAS method to combine several large AMD GWAS datasets.

Methods

Study overview

Our study design is displayed in Fig. 1. We conducted a meta-analysis of GWASs based on the MTAG approach [8], which generalizes the standard inverse-variance meta-analysis method to jointly analyze GWAS summary statistics with overlapping samples. We applied MTAG to three input summary statistics: AMD-2016 GWAS [6] and AMD-2013 GWAS [11] from the IAMDGC, and AMD GWAS in Genetic Epidemiology Research on Aging (GERA) study [12]. We then replicated the novel AMD loci in independent datasets from the UKBB and FinnGen studies.

International Age-related Macular Degeneration Genomics Consortium

GWAS summary statistics: AMD-2016 GWAS and AMD-2013 GWAS

We downloaded two publicly available AMD summary statistics from the IAMDGC: AMD-2016 GWAS and AMD-2013 GWAS (web resources in the supplement) [6, 11]. In the AMD-2016 GWAS, there are 16,144 cases and 17,832 controls of European descent with *P* values and directions available in the summary statistics. We used the same method as mentioned in Burgess et al.'s study to derive the beta coefficients and standard errors (SEs) for all SNPs [13]. Briefly, the *P* values and directions of associations from the summary statistics were used to calculate *z*-scores. With the assumption that SE multiplied by $\sqrt{\mathrm{MAF} \times (1 - \mathrm{MAF})}$ should be a constant, where "MAF" is the minor allele frequency, we estimated the constant using the average estimations from 34 genome-wide significant variants from Fritsche et al.'s study [6, 13]. The constant was further used to calculate SEs and beta coefficients for other variants. The validity of the method was also fully assessed in Burgess et al.'s study [13].

For the AMD-2013 GWAS, the GWAS summary statistics from 17,181 AMD cases and 60,074 controls were used. We used the same method above to calculate the beta coefficients and SEs for all variants.

Genetic Epidemiology Research on Aging (GERA) study

The GERA cohort is a substudy of the longitudinal cohort enrolled in the Kaiser Permanente Research Program on Genes, Environment, and Health. The detailed description of the study design can be found in the database of Genotypes and Phenotypes (study accession: phs000674.v1.p1) [12, 14]. In our authorized access data, 78,486 participants have both phenotype and genotype data. We only included self-reported whites for the following analysis.

We performed genotype quality control using PLINK software (version 1.90 beta) [15]. For samples, we removed individuals with >3% missing genotypes. For markers, SNPs with call rate <95%, MAF < 0.01, and Hardy–Weinberg equilibrium $P < 1 \times 10^{-6}$ were discarded. For relatives, we calculated identity by descent using autosomal SNPs and only kept one of any pair of individuals with pi-hat > 0.2 for analysis. Michigan Imputation Server was used for imputation (parameters: HRC reference panel, version r1.1 2016; phasing, ShapeIT; population, EUR) [16]. SNPs with imputation quality score > 0.3 and MAF > 0.01 were retained for association analysis.

Macular degeneration cases were recorded in electronic health record (EHR) system as International Classification of Diseases, Ninth Revision (ICD-9) diagnosis codes (362.5, 362.50, 362.51, 362.52, and 362.57). Finally, we

reported a GWAS on 4017 macular degeneration cases and 14,984 controls from the GERA cohort.

Replication datasets: UK Biobank and FinnGen study age-related macular degeneration data

The UK Biobank project is a large-scale prospective cohort study of half a million participants across the United Kingdom, aged between 40 and 69 at the time of recruitment (2006–2010) [17]. In our analysis, we only included participants with written consent and of white-British ancestry based on self-reported ethnicity and genetic principal components [17, 18]. To control relatedness between samples, we used a pruning method in PLINK software (version 1.90 beta) to keep one of any pair of individuals with pi-hat > 0.2. We identified 5860 AMD cases using the following criteria: (1) ICD-9 or ICD-10 diagnosis codes (3625 and H353); (2) responded "Macular degeneration" in "eye problems/disorders" (Field 6148); (3) responded "macular degeneration" in self-reported non-cancer illness (Field 20002). We selected 126,726 "healthy" controls who did not have serious eye diseases (Field 6148). The UKBB genotype data and quality control procedures were described previously [17]. In our association analysis, we only included SNPs with MAF > 0.01 and imputation quality score > 0.3.

The FinnGen study (https://www.finngen.fi/en) is a nation-wide study launched in Finland in 2017. The FinnGen study combines both genetic information and health care data to improve personalized health care. We downloaded the available summary statistics from the public release of FinnGen data freeze 2 results for 1266 AMD cases (wet or dry macular degeneration) and 47,560 controls. The UKBB and FinnGen AMD results were meta-analyzed as the replication sample.

The Blue Mountains Eye Study

The Blue Mountains Eye Study (BMES) is a population-based cohort study investigating the etiology of common ocular diseases among suburban residents aged 49 years or older, in Australia [3]. The full description of the study design, phenotype definition, and genetic data were described previously [3, 6, 19, 20]. In brief, retinal photographs were assessed for AMD lesions following the Wisconsin Age-Related Maculopathy Grading System for late AMD cases [21]. The late AMD cases were defined as presence of neovascular AMD or pure geographic atrophy. The controls were defined as no soft (distinct or indistinct) or intermediate drusen, any retinal pigment abnormalities (either depigmentation or increased pigment), and no signs of early or late AMD. DNA samples were obtained during the 5-year follow-up and ancillary surveys, which were performed between 1997 and 2000. Participants were genotyped with Human610-Quad arrays (Illumina, San Diego, CA, USA). Genotype data were imputed in Michigan Imputation Server. We included 100 late AMD cases and 2136 controls of European descent with genetic information in our analysis.

Statistical analysis

For both of GERA and UKBB AMD GWASs, we conducted logistic regression models under an additive genetic model adjusting for sex, age, and the first ten genetic principal components in PLINK software (version 2.0) [15]. Bivariate LD score regression was used to estimate the genetic correlation between pairs of AMD datasets [22]. We then used the MTAG software (version 1.0.8) to meta-analyze the GWAS summary statistics from AMD-2016 GWAS, AMD-2013 GWAS, and AMD GWAS in GERA study (Fig. 1) [8]. The default quality control procedures in MTAG were used to filter SNPs with MAF > 0.01. We then used a stepwise model selection procedure in the GCTA-COJO software (1.91.7beta) to identify lead independent genome-wide significant SNPs (both conditional and unconditional P value $< 5 \times 10^{-8}$) [18, 23]. The lead SNPs were looked up in the Eye Genotype Expression (EyeGEx) database of retinal tissue to identify retina-specific expression quantitative trait loci (eQTL) and expression-trait associations from transcriptome-wide association study (TWAS) summary results [24]. We applied summary data-based Mendelian randomization (SMR) and heterogeneity in dependent instruments (HEIDI) tests based on AMD meta-analysis summary statistics and the EyeGEx eQTL data [25]. We conducted gene-based and pathway analysis in MAGMA (v1.06) as implemented in FUMA platform (version 1.3.4) [26, 27].

To derive a polygenic risk score (PRS), we selected the lead independent genome-wide significant SNPs, and the PRS was weighted based on the estimated AMD log odds ratios from the MTAG analysis. The "pROC" package was used to calculate the area under the curve (AUC) [28]. All general analyses were performed with R (version 3.4.1).

Results

Meta-analysis of AMD GWASs identifies 12 novel loci

We conducted a meta-analysis based on MTAG method to combine three AMD GWAS summary statistics: AMD-2016 GWAS and AMD-2013 GWAS from the IAMDGC, and AMD GWAS in the GERA cohort (Fig. 1). The genetic correlations between the AMD input datasets were very high based on the LD score regression method

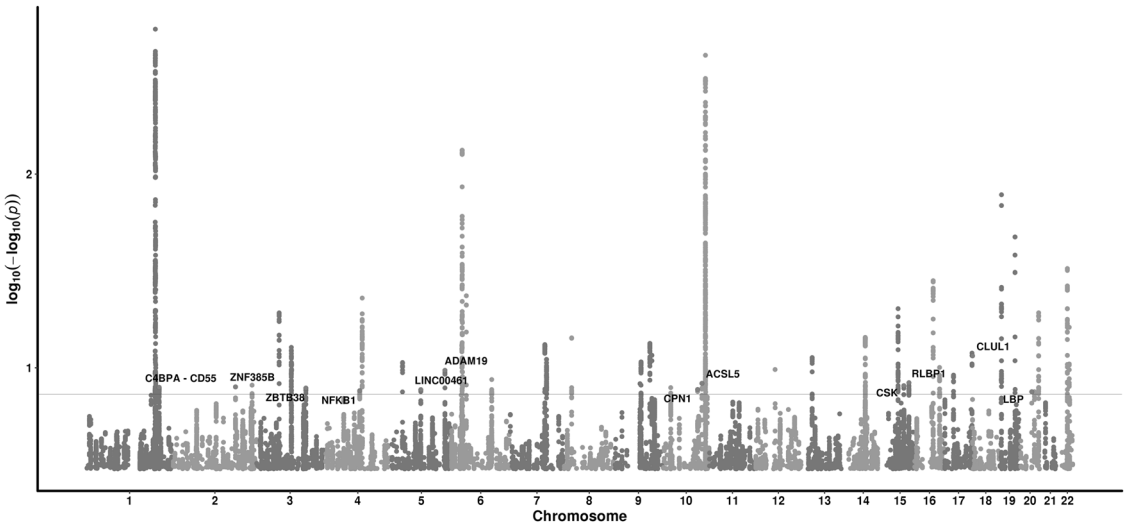

Fig. 2 Manhattan plot of the meta-analysis of genome-wide association studies for AMD. Novel loci are highlighted in red dots, with the nearest gene names in black text. The red line is the genome-wide significance level at 5×10^{-8} (color figure online)

(Supplementary Table S1). We then investigated the MTAG output summary statistics, and found no evidence of genomic inflation (lambda genomic control 1.18, LD score regression intercept 1.03, Supplementary Fig. S1). There is also no evidence of inflation due to violation of the homogeneity assumption in MTAG (max False Discovery Rate 0.0016). From the MTAG GWAS output, we identified 69 lead independent genome-wide significant SNPs (12 of them are novel loci, Fig. 2, Supplementary Fig. S2, and Supplementary Table S2).

We then replicated the 12 novel AMD loci in the UKBB and FinnGen AMD studies. The concordance of SNP effect sizes between the MTAG discovery cohorts and replication datasets (UKBB and FinnGen) was high (Pearson's correlation coefficient 0.89, P value 1.2×10^{-24}, Fig. 3). Of the 12 novel loci, the effect sizes of 11 loci were in the expected direction direction (binomial test P value $= 6.3 \times 10^{-3}$), 5 were associated with AMD at a nominal significance level (P value < 0.05), and 1 (rs3825991 in gene *RLBP1*, P value $= 1.6 \times 10^{-3}$) after Bonferroni correction (Table 1). We also built a PRS using the 12 novel SNPs, and the score was strongly associated with AMD status in UKBB ($P = 2.4 \times 10^{-4}$).

Most of the novel genes are expressed in retinal tissue and could be involved in the pathogenesis of AMD (Box 1). For instance, *C4BPA–CD55* loci are involved in the regulation of complement activation [29, 30], and *NFKB1* and *LBP* are important factors for inflammatory response pathways [31, 32]. *LINC00461* was identified as the most significant loci associated with macular thickness [33]. *RLBP1* is associated with multiple Mendelian retinal dystrophy [34, 35], and also one of the strongest AMD-associated candidate genes from a recent transcriptome-wide

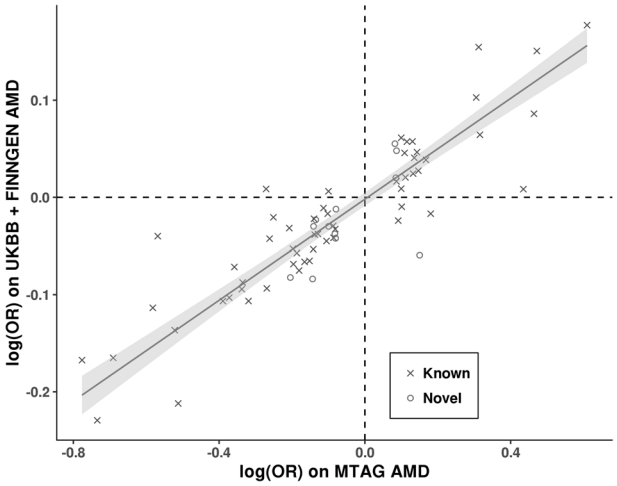

Fig. 3 Comparison of the effect sizes for 69 genome-wide significant independent SNPs identified from meta-analysis of AMD GWASs versus those in UK Biobank and FinnGen AMD GWAS. Pearson's correlation coefficient is 0.89 (P value $= 1.2 \times 10^{-24}$). The red line is the best fit line, with the 95% confidence interval region in gray. Novel AMD SNPs are highlighted in circle (red) and known SNPs in cross of oblique line (purple) (color figure online)

association analysis [24]. These findings are important for our understanding of the pathogenesis of AMD development, and could potentially constitute therapeutic targets for AMD [36].

Gene-based and pathway analysis

We then conducted a genome-wide gene-based association analysis and identified an additional 21 novel genes (defined as no genome-wide significant SNPs within the region of a gene, Supplementary Fig. S3 and Table S3). For example,

Table 1 List of 12 novel AMD loci from the meta-analysis of genome-wide association studies

SNP	CHR	BP	Nearest gene	EA	NEA	FREQ	*P* (2016)	*P* (2013)	*P* (GERA)	BETA (MTAG)	*P* (MTAG)	*P* (replication)[a]	*P* (eQTL)[b]	*P* (TWAS)[c]
rs11120691	1	207486475	*C4BPA–CD55*	G	T	0.44	2.3×10^{-5}	8.2×10^{-5}	0.1	−0.08	1.2×10^{-8}	0.02	0.006	0.47
rs259842	2	180738840	*ZNF385B*	C	T	0.62	5.6×10^{-6}	2.4×10^{-3}	0.01	−0.08	1.1×10^{-8}	0.04	0.10	0.43
rs2011092	3	141124607	*ZBTB38*	C	T	0.35	4.4×10^{-6}	4.6×10^{-4}	0.22	0.09	1.4×10^{-8}	0.27	$\mathbf{6.2\times10^{-15}}$	9.4×10^{-5}
rs1005819	4	103504305	*NFKB1*	T	C	0.42	8.6×10^{-6}	4.0×10^{-4}	0.23	−0.08	2.4×10^{-8}	0.49	0.007	0.08
rs17421410	5	87836307	*LINC00461*	G	A	0.07	9.8×10^{-7}	3.4×10^{-3}	0.29	0.15	2.0×10^{-8}	0.08	0.004	0.48
rs6899205	5	156943285	*ADAM19*	A	G	0.28	5.8×10^{-7}	1.2×10^{-6}	0.98	−0.10	2.0×10^{-10}	0.13	6.7×10^{-4}	0.04
rs7896471	10	101788308	*CPN1*	T	G	0.04	1.5×10^{-7}	0.01	0.57	−0.20	1.9×10^{-8}	0.07	0.02	0.004
rs1926564	10	114139896	*ACSL5*	A	G	0.90	3.4×10^{-7}	5.1×10^{-4}	0.79	−0.13	4.9×10^{-9}	0.39	0.007	0.39
rs1378940	15	75083494	*CSK*	A	C	0.68	3.5×10^{-6}	1.8×10^{-4}	0.36	0.09	8.3×10^{-9}	9.6×10^{-3}	$\mathbf{1.6\times10^{-9}}$	0.25
rs3825991	15	89761664	*RLBP1*	A	C	0.48	1.9×10^{-7}	3.7×10^{-3}	0.26	0.08	4.3×10^{-9}	$\mathbf{1.6\times10^{-3}}$	$\mathbf{1.7\times10^{-20}}$	1.0×10^{-6}
rs9973159	18	597950	*CLUL1*	T	C	0.15	9.4×10^{-8}	4.3×10^{-6}	0.04	−0.14	2.9×10^{-12}	0.22	$\mathbf{5.9\times10^{-18}}$	0.001
rs2232613	20	36997655	*LBP*	T	C	0.08	4.3×10^{-7}	0.06	0.03	−0.14	3.0×10^{-8}	7.0×10^{-3}	–	0.06

Chromosomal position is based on the NCBI RefSeq hg19 human genome reference assembly

BETA beta coefficient, *CHR* chromosome, *EA* effect allele, *eQTL* expression quantitative trait loci, *FREQ* allele frequency of effect allele, *MTAG* multiple trait analysis of GWAS, *NEA* non-effect allele, *P P* values, *SNP* single nucleotide polymorphism, *TWAS* transcriptome-wide association analysis, *UKBB* UK Biobank data, 2016 AMD-2016 GWAS, 2013 AMD-2013 GWAS, *GERA* Genetic Epidemiology Research on Aging study

[a]Novel genes passing multiple testing correction ($P < 0.05/12$) in replication datasets (meta-analysis of UK biobank and FinnGen study) are highlighted in bold font

[b]eQTL passing gene-level multiple testing correction are highlighted in bold font

[c]TWAS *P* values look up from Ratnapriya et al. [24]. For loci rs7896471 and rs2232613 are based on genes *DNMBP* and *KIAA1755*, respectively

the novel gene *PDGFB* encodes platelet-derived growth factor subunit B, which is a member of the protein family comprised of both platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF), and could provide genetic insight into the development of VEGF and PDGF inhibition for neovascular AMD [37, 38]. Pathway analysis of 10,678 gene sets (MsigDB v6.2, curated gene sets: 4761, Gene Ontology terms: 5917) resulted in 19 significant gene sets after FDR correction, which include complement cascade, high-density lipoprotein particle remodeling, cholesterol transporter activity, and negative regulation of macrophage-derived foam cell differentiation (Supplementary Table S4).

eQTL and transcriptome-wide association analysis

We also looked up the 69 genome-wide significant SNPs in retina from the EyeGEx database to identify eQTL [24]. We found that 12 genome-wide significant SNPs were significant eQTL for 25 SNP-gene pairs (*cis*-eQTLs) after gene-level multiple testing correction across the genome (Table 1 and Supplementary Table S5). Five SNP-gene *cis*-eQTLs were from our novel AMD SNPs: rs2011092 (*cis*-eQTL target gene *ZBTB38*), rs1378940 (*MPI*), rs3825991 (*RLBP1*), rs9973159 (*CLUL1*), and rs9973159 (*RP11-806L2.2*). Of the 12 genome-wide significant SNPs we identify here, one was study-wide significant in a previous TWAS based on earlier AMD GWAS summary statistics [24] (Table 1).

To test the effects of genetic variants on AMD risk that is mediated by gene expression levels, we also conducted SMR and HEIDI tests [25]. SMR investigates the relationships between gene expression levels (exposure) and phenotype (outcome) using genetic variants as instrumental variables. We identified 19 genes after multiple testing correction ($P_{SMR} < 0.05/5075 = 9.85\times10^{-6}$, Supplementary Table S6). We further used the HEIDI method to test the null hypothesis that there is a single causal variant affecting both gene expression levels and AMD risk. We identified two genes that passed the HEIDI test ($P_{HEIDI} \geq 0.05$), *PMS2P1* and *BLOC1S1*, which are associated with AMD risk underlying the GWAS hits suggesting that these genes are good candidates of prioritizing for functional follow-up studies.

Prediction value of AMD polygenic risk score

We constructed an AMD PRS from 69 lead SNPs ($PRS_{69\text{-}SNP}$), and then evaluated the prediction performance in 100 late AMD cases and 2136 controls from the BMES. The AUC of the $PRS_{69\text{-}SNP}$ was 0.76 (95% confidence interval [CI]: 0.72–0.80). To assess the improvement of our new PRS compared with previous AMD PRS, we derived a PRS

Box 1 Biology annotations of 12 novel AMD loci

Nearest genes	Gene function
C4BPA–CD55	Complement component 4 binding protein alpha (C4BPA) and decay accelerating factor for complement (CD55) are involved in the regulation of complement activation [30, 31]. Previous functional studies proposed that the expression of CD55 in retinal pigment epithelium cells could be a potential therapeutic target for AMD [37]. *CD55* was also reported to be associated with myopia [46]
ZNF385B	Zinc finger protein 385B (ZNF385B) is highly expressed in retinal tissue [47]. Patient with a 2q31.2-32.3 deletion presented microphthalmia and retinal coloboma [48]
ZBTB38	*ZBTB38* encodes zinc finger and BTB domain containing 38, a zinc finger transcriptional activator that binds methylated DNA. Its function in eye is uncharacterized
NFKB1	Nuclear factor kappa B subunit 1 (NFKB1) is related to many biological processes such as inflammation, immunity, differentiation, cell growth, tumorigenesis, and apoptosis. The activation of NF-κB is an important pathway to the development of AMD [33, 49]
LINC00461	LINC00461 is a long noncoding RNA and expressed predominantly in the brain and visual cortex. It is the most significant loci associated with macular thickness [34]. It is also associated with retinal vascular caliber [42, 43], and macular telangiectasia type 2 [44]
ADAM19	Disintegrin and metalloproteinase domain-containing protein 19 (ADAM19) is a member of the ADAM (a disintegrin and metalloprotease domain) family. It is associated with Alzheimer's disease and could play an important role in retinal degeneration diseases [50, 51]
CPN1	Carboxypeptidase N subunit 1 (CPN1) plays a central role in regulating the biologic activity of peptides such as kinins and anaphylatoxins. It could be involved in choroid development [52], and a recently Bayesian functional association study also showed that *CPN1* is associated with AMD
ACSL5	ACSL5 plays a key role in lipid biosynthesis and fatty acid degradation [45]
CSK	C-terminal Src kinase (CSK) plays an important role in T-cell activation and the phosphorylation of C-terminal tyrosine residues. It is expressed in retinal vascular endothelial cells [53]
RLBP1	Retinaldehyde binding protein 1 (RLBP1) is related to multiple Mendelian retinal dystrophy [24, 35]. A recent study showed this gene could increase AMD risk by the interaction effect between the nuclear and mitochondrial genome [54]. A transcriptome-wide association study also identified this gene associated with AMD [36]
CLUL1	*CLUL1* encodes retinal clusterin-like protein. Clusterin is expressed in many eye tissues, such as retinal pigment epithelium, ganglion cells, and photoreceptor cells. Although candidate gene study found no pathogenic variants [55], a recent study showed an interaction effect of gene age for AMD risk [55]
LBP	Lipopolysaccharide binding protein (LBP) is involved in inflammatory response through NF-kB and MAPK signaling. It protects human retinal pigment epithelial cells against oxidative stress-induced apoptosis, which contributes to the pathogenesis of AMD development [32]

from previously published 52 SNPs ($PRS_{52\text{-}SNP}$) for BMES. The prediction ability of our new $PRS_{69\text{-}SNP}$ was better than that based on previously published SNPs $PRS_{52\text{-}SNP}$ ($AUC_{52\text{-}SNP} = 0.74$, 95% CI: 0.70–0.79), although the AUC improvement was not significant ($P = 0.21$).

Discussion

We have conducted a large meta-analysis of GWAS for AMD and identified 69 genome-wide significant SNPs (12 novel). We found that most of the novel genes are expressed in retina and could be involved in AMD pathogenesis. Through genome-wide gene-based association analysis, we identified an additional 21 novel genes. Pathway analysis indicated complement cascade, high-density lipoprotein particle remodeling, cholesterol transporter activity, and negative regulation of macrophage-derived foam cell differentiation are involved in the biological process underlying AMD risk.

In this study, we conducted a multivariate GWAS (based on MTAG method) rather than a traditional inverse-variance meta-analysis. Traditional meta-analysis assumes that the input GWAS summary statistics are derived for the same trait (a genetic correlation close to one) [10]. In practice, the heterogeneity of the case phenotype would lead to a lower genetic correlation (<1) even for the same trait. Recent statistical methodology studies showed that multivariate GWAS can leverage multiple input summary statistics of the same trait with different measures or even different traits with a high genetic correlation [8, 9]. Our input files AMD-2016 GWAS and AMD-2013 GWAS are summary statistics for advanced AMD, and the AMD cases in GERA are identified using EHRs, which could include both of advanced and early or intermediate AMD cases. The MTAG approach is able to handle this issue by leveraging

the high genetic correlation between the input summary statistics and maximize the statistical power to detect genetic associations for advanced AMD (our index input AMD-2016 GWAS, which has the highest power). More importantly, the MTAG approach can handle sample overlap between the input GWAS summary statistics [8]. In our multivariate GWAS, there is some sample overlap between the AMD-2016 GWAS and the AMD-2013 GWAS. In this scenario, MTAG framework is an ideal method for taking full advantage of the large public available GWAS summary statistics.

The gene discovery from our MTAG GWAS will contribute toward the understanding of the biology mechanisms and the etiology of AMD. As we presented in Box 1, most of the novel loci are potentially involved in the biological process of AMD. For instance, macular thickness is an important quantitative trait for AMD [39]. A recent first macular thickness GWAS identified 139 loci, and some of them are known AMD genes, such as *RDH5*, *NPLOC4*, *RAD51B*, and *SLC16A8* [33]. Our meta-analysis of GWASs identified a novel AMD loci *LINC00461*, which is the most significant signal from the macular thickness GWAS [33]. *LINC00461* is a long noncoding RNA and expressed predominantly in the brain and visual cortex [40]. Previous GWAS also indicated that *LINC00461* is associated with retinal vascular caliber [41, 42], a risk factor of AMD, and macular telangiectasia type 2 [43], a rare neurovascular degenerative retinal disease. Our meta-analysis of GWASs also identified novel genes involved in the regulation of complement activation [29, 30], lipid biosynthesis [44], inflammatory response [31, 32], and Mendelian retinal diseases [34, 35]. All together, these gene findings help us have a better understanding of the pathogenesis of AMD.

In this study, we conducted a meta-analysis of GWASs for individuals of European ancestry, hence the generalizability of the novel AMD genes to other populations still needs further replication. Besides, our replication dataset of UKBB has a relatively small sample size and young participants (40–69 years old), and the AMD cases were identified using both hospital health records and self-reported cases. Although the concordance of SNP effect sizes between the MTAG discovery cohorts and replication cohorts was high and most of them were in the expected direction, replication datasets with larger sample size of clinical diagnosis cases would improve the power to replicate our novel genes. Moreover, although we looked up the eQTL and TWAS results in retinal tissue and further literature search indicated that most of these genes are probably involved in the pathogenesis of AMD, additional functional studies are warranted to investigate the underlying biological mechanisms of the novel genes. Finally, using BMES samples we evaluated the prediction value of a PRS based on (1) 69 lead SNPs identified here and (2) 52 previously published SNPs. Both PRSs were derived using the AMD consortium data which included a subset of the BMES samples used here to test the PRS and in theory this could induce slight over-fitting due to sample overlap. In practice this would have a negligible effect on our results because (1) the sample overlap is very small (~0.5% of cases) and (2) we only used a small number of SNPs in our PRS. Although our new PRS improved the prediction AUC (from 0.74 previously, to 0.76 here), the increase was not statistically significant, possibly due to the limited number of advanced AMD cases in BMES or the small effect sizes of the additional GWAS signals. We performed an exploratory analysis using the PRS in the UKBB cohort although the AUC values were substantially lower (data not shown), reflecting the fact that due to their relatively young age, most UKBB cases did not have advanced AMD.

In conclusion, we conducted a meta-analysis GWAS for AMD and identified 12 novel loci. Most of the novel genes are expressed in retinal tissue and could be involved in the pathogenesis development of AMD. These findings enhance our understanding of the disease mechanisms of AMD.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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