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Shared genetic aetiology of Alzheimer's disease and age-related macular degeneration by APOC1 and APOE genes

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ABSTRACT

Background Alzheimer's disease (AD) and age-related macular degeneration (AMD) share similar pathological features, suggesting common genetic aetiologies between the two. Investigating gene associations between AD and AMD may provide useful insights into the underlying pathogenesis and inform integrated prevention and treatment for both diseases.

Methods A stratified quantile–quantile (QQ) plot was constructed to detect the pleiotropy among AD and AMD based on genome-wide association studies data from 17 008 patients with AD and 30 178 patients with AMD. A Bayesian conditional false discovery rate-based (cFDR) method was used to identify pleiotropic genes. UK Biobank was used to verify the pleiotropy analysis. Biological network and enrichment analysis were conducted to explain the biological reason for pleiotropy phenomena. A diagnostic test based on gene expression data was used to predict biomarkers for AD and AMD based on pleiotropic genes and their regulators.

Results Significant pleiotropy was found between AD and AMD (significant leftward shift on QQ plots). APOC1 and APOE were identified as pleiotropic genes for AD–AMD (cFDR <0.01). Network analysis revealed that APOC1 and APOE occupied borderline positions on the gene co-expression networks. Both APOC1 and APOE genes were enriched on the herpes simplex virus 1 infection pathway. Further, machine learning-based diagnostic tests identified that APOC1, APOE (areas under the curve (AUCs) >0.65) and their upstream regulators, especially ZNF131, ADNP2 and HINFP, could be potential biomarkers for both AD and AMD (AUCs >0.8).

Conclusion In this study, we confirmed the genetic pleiotropy between AD and AMD and identified APOC1 and APOE as pleiotropic genes. Further, the integration of multiomics data identified ZNF131, ADNP2 and HINFP as novel diagnostic biomarkers for AD and AMD.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Alzheimer's disease (AD) and age-related macular degeneration (AMD) exhibit overlapping pathological characteristics and are recognised as comorbid conditions in clinical practice.

WHAT THIS STUDY ADDS

⇒ This study confirmed the genetic pleiotropy between AD and AMD.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The findings from this study may promote the co-diagnosis/treatment for AD and AMD.

INTRODUCTION

Alzheimer's disease (AD) and age-related macular degeneration (AMD) are both common progressive neurodegenerative diseases associated with significant comorbidity. Both AD and AMD are common comorbidities in chronic diseases and represent major global public health challenges.[1 2] The clinical practice of AD has established several biomarkers, including MRI, Fluorodeoxyglucose-Positron Emission Tomography (FDG-PET), tau PET, cerebrospinal fluid measures of amyloid and tau, and plasma biomarkers, which are currently undergoing approval processes.[3] Some AMD biomarkers have been reported, such as complement factor H, age-related maculopathy susceptibility 2, high-density lipoprotein cholesterol and vascular endothelial growth factor.[4]

Past epidemiological studies have shown a substantial association between AD and AMD at the phenotype level.[5] In 1999, a study of 1438 patients diagnosed with both AD and AMD demonstrated that these two diseases may have common pathogenesis.[5] A meta-analysis consisting of 11 840 patients found that AD and AMD had a significant association.[6] Consistently with this, our recent study, based on 12 364 patients with eye and dementia tests from the UK Biobank, demonstrated that patients with existing AMD have an increased risk of dementia.[7]

Accumulating evidence shows that AD and AMD share similar pathological mechanisms.[8] For instance, ageing is a key risk factor for AD and AMD, and hypercholesterolaemia, hypertension, obesity, arteriosclerosis and smoking are common risk factors for them; deposition of amyloid beta plaques, other extracellular deposition, increased oxidative stress, and apolipoprotein and complement activation pathways have also all been implicated in the pathogenesis of both.[8–11] However, the nature of the relationship linking AD and AMD remains contentious.[5 12 13] Investigating gene associations between AD and AMD could provide mechanistic insights into the pathogenesis underlying these two diseases and their potential shared pathogenesis, promoting integrated prevention and treatment for AD and AMD. Due to the complexity of accurately diagnosing AD, the diagnosis of AMD could potentially enhance AD diagnosis, provided their pleiotropy is confirmed. Additionally, the identification of shared biomarkers for both AD and AMD would hold significant importance. Since 2005, genome-wide association studies (GWAS) have been widely used in the biomedical sphere to identify relationships between genetic variations—usually single nucleotide polymorphisms (SNPs). Logue *et al*[6] and Tan *et al*[14] independently explored the shared genetic aetiology between AD and AMD, and both teams suggested the genetic associations between these two diseases were significant. However, in their studies, further characterisation of the shared genetic mechanisms has been restricted by the relatively limited genetic information used for AMD and the lack of other validation data to independently verify their results.

To date, despite the substantial amount of data pointing towards the shared genetic aetiology of these two diseases, no previous studies have specifically analysed the pleiotropic genes implicated in AMD and AD on a biological network level. The proliferation of omics and complex network theory may herald a novel approach to investigating these associations. In the present study, we aimed to investigate the shared genetic aetiology between AD and AMD across genetic network, pathway and clinical levels. We used multiomics data to investigate the pleiotropy between AD and AMD. We further explored the pleiotropic genes on the genetic networks, pathways and tissues to identify specific common topological and biological features and potential diagnostic biomarkers for both diseases.

METHODS

Data collection

We downloaded genetic data from GWAS summary statistics, RNA sequencing (RNA-seq) and microarray to conduct pleiotropy analysis and biomarker identification (download date: January 2022). A detailed description of included datasets was presented in table 1.

GWAS data

The AD dataset, downloaded from the GRASP database,[15] consisted of 17 008 AD cases with approximately 7 million SNPs. This dataset was derived from a meta-analysis by Lambert *et al*.[16]

We downloaded two AMD datasets from two separate meta-analyses: (1) a discovery dataset containing 16 144 patients of GWAS data integrated from 26 studies[17]; (2) a replication AMD dataset including 14 034 patients from 11 studies for verification.[18]

The association of pleiotropic genes derived from the GWAS with phenotypes was validated in the UK Biobank.[19] We included 2943 patients with AD and 7308 patients with AMD (86 with both AD and AMD) in the analysis.

The quality control and overall genetic statistics of these datasets have been described in detail elsewhere.[16–19] In order to expand the collection of AD and AMD-related SNPs, a nominal p value threshold of 1×10^{-05} was

Table 1 Descriptive characteristics of datasets included in this study

Disease	Sample size (cases/controls)	Mean age (years)	Gender (% female)	Source	Phase	Reference
AD	17 008/37 154	63.6–82.1	57.6–74.5	GWAS	Discovery	Lambert *et al*[16]
AMD	16 144/17 832	73.33	58	GWAS	Discovery	Fritsche *et al*[17]
AMD	14 034/91 214	47.5–77.2	NA	GWAS	Replication	Winkler *et al*[18]
AD	2943/502 854	56.5	54.4	GWAS	Verification	UK Biobank[19]
AMD	7308/174 957	57.2	54.4	GWAS	Verification	UK Biobank[19]
AD–AMD	86/174 957	57.2	54.4	GWAS	Verification	UK Biobank[19]
Healthy	838	NA	32.9	RNA-seq	Verification	GTEx[25]
AD	26/62	93.1	56.3	Microarray	Discovery	Hokama *et al*[21]
AD	97/98	85.02	48	Microarray	Replication	Piras *et al*[22]
AMD	8/3	76.6	37.5	Microarray	Discovery	Strunnikova *et al*[23]
AMD	5/5	84	80	RNA-seq	Replication	Wang *et al*[24]

AD, Alzheimer's disease; AMD, age-related macular degeneration; GTEx, Genotype-Tissue Expression; GWAS, genome-wide association studies; NA, not available; RNA-seq, RNA sequencing.

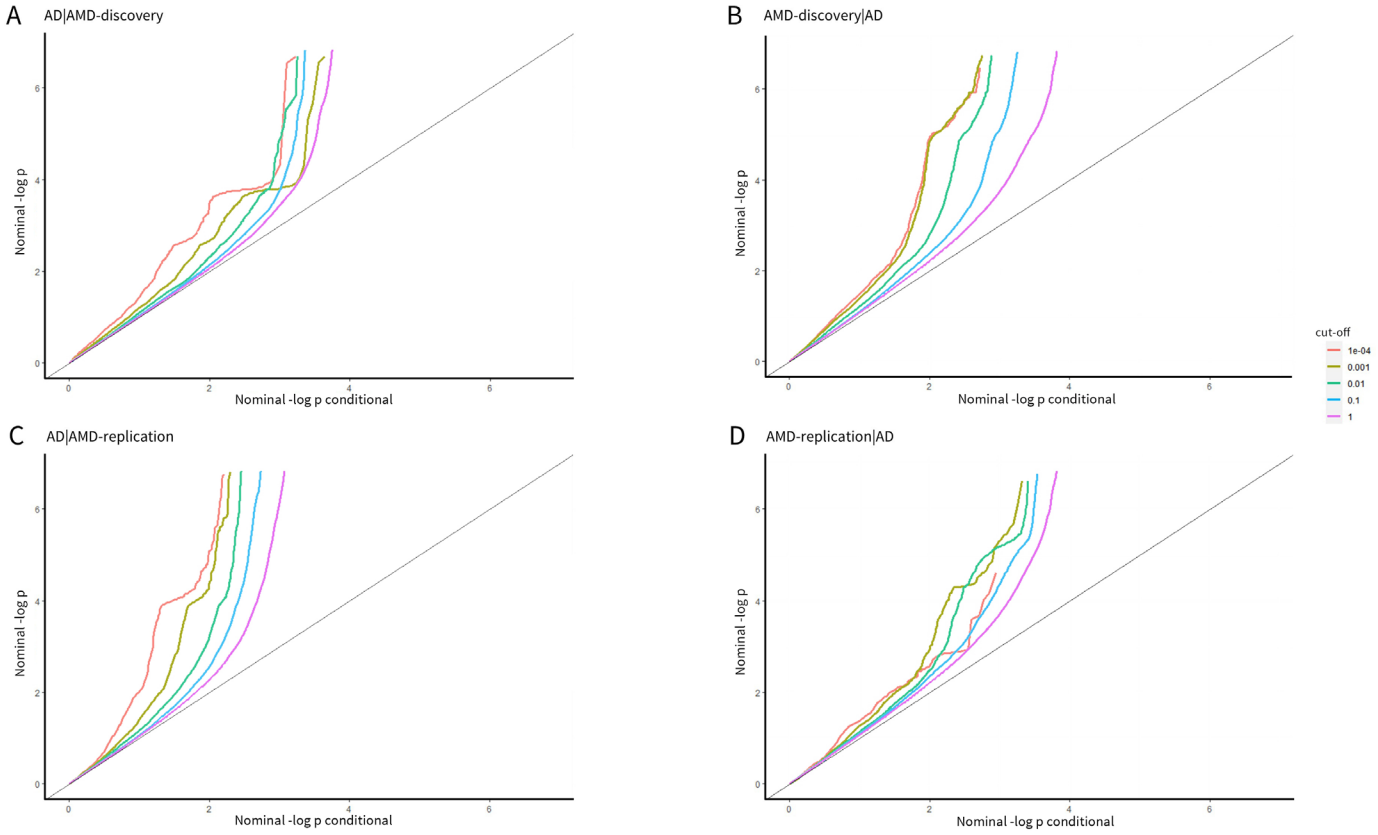


Figure 1 (A) Conditional QQ plot for AD|AMD discovery. The x-axis is $-\log_{10}$ (p value of AD SNP), and the y-axis is $-\log_{10}$ (p value of AMD SNP). Different curves represent different cut-offs for the AMD p value. A significant left deviation was found among all the curves, indicating obvious pleiotropy for AD|AMD. (B) Conditional QQ plot for AMD discovery|AD. (C) Conditional QQ plot for AD|AMD replication. (D) Conditional QQ plot for AMD replication|AD. AD, Alzheimer's disease; AMD, age-related macular degeneration; QQ, quantile–quantile; SNP, single nucleotide polymorphism.

considered to be of statistical significance for relationships between SNPs and diseases.

Gene expression data

Gene expression datasets for the biomarker selection from the pleiotropic genes were downloaded from the GEO database.[20] We included discovery datasets and replication datasets for AD and AMD separately. The AD discovery dataset (GSE36980) comprised expression data from the frontal cortex, temporal cortex and hippocampus from 26 patients with AD and 62 healthy controls.[21] The AD replication dataset (GSE132903) included expression data of the middle temporal gyrus from 97 patients with AD and 98 healthy controls.[22]

The AMD discovery dataset (GSE1719) consisted of 36 samples (comprising 18 patients and 18 controls) from 8 patients with AMD and 3 controls,[23] and the replication dataset (GSE99287) included 26 early/late AMD samples and 19 normal samples of the retina and retinal pigment epithelium from 5 patients with AMD and 5 controls.[24]

The Genotype-Tissue Expression database provided the gene expression data from RNA-seq for 54 non-diseased tissue sites of 838 volunteers,[25] to observe the expression situation of pleiotropic genes in different tissues and cells.

Pleiotropy analysis

A stratified quantile–quantile (QQ) plot with $-\log_{10}$(p value-exposure) as the x-axis and $-\log_{10}$(p value-outcome) as the y-axis was constructed to detect the pleiotropy between AD|AMD (AD to AMD) and AMD|AD (AMD to AD). Different p value cut-off thresholds for exposure were set to delineate individual curves. The assessment of pleiotropy was based on the level of the leftward shift from the null lines expected.

In 2015, a Bayesian conditional false discovery rate-based (cFDR) method was created to detect the pleiotropy between two diseases,[26–28] which has since been applied successfully in the discovery of a series of pleiotropic SNPs.[29–32] The false discovery rate (FDR) is a statistical approach for the correction in multiple hypothesis testing.[33 34] In pleiotropy analysis, FDR is used to reflect the possibility of non-pleiotropy for an SNP.

$$FDR(p_i) = Pr\left(H_o^{(i)} | P_i \leq p_i\right)$$

Where the P_i is the random variable of p value for a trait i among all SNPs, and p_i is the instance of P_i to a specific SNP. $H_0^{(i)}$ represents the null hypothesis that the specific SNP is not associated with trait i. Detection of pleiotropy

between two diseases can be enhanced with cFDR, an extension of FDR.

$$cFDR\left(p_i|p_j\right) = Pr\left(H_o^{(i)}|P_i \leq p_i,\ P_j \leq p_j\right)$$

Where p_i is the association of a specific SNP with the principal disease, and p_j is with the conditional disease.

To find the pleiotropic SNPs both significant in AD|AMD and AMD|AD, a conjunction-cFDR (ccFDR)[31] was developed. In this study, we calculate cFDR from AD to AMD and AMD to AD separately, then select the larger as ccFDR.

$$ccFDR_{i\&j} = max\ (cFDR_{i|j}, cFDR_{j|i})$$

where $ccFDR_{i\&j}$ is the max value of cFDR (AD|AMD) and cFDR (AMD|AD). The threshold of <0.01 for ccFDR was designated as the threshold for significance for pleiotropic SNP.

We selected the bigger ccFDR value among the discovery and replication GWAS datasets and defined it as MccFDR (max ccFDR).

The *KehaoWu/GWAScFDR* package in R (V.4.1.0) was used to conduct the cFDR analysis. The pleiotropic SNPs were mapped to the corresponding genes from the information in the AD dataset.[16]

Linkage disequilibrium analysis

Linkage disequilibrium (LD) analysis was conducted using LDlink and R^2 was used to measure the LD level between SNPs. R^2 ranked among 0–1, and 1 implies the SNPs provide exactly the same information.

Epidemiological verification for pleiotropic genes in AD and AMD

The logistic regression model evaluated ORs and 95% CIs for pleiotropic SNPs with AD and AMD. We used two logistical models in this analysis: model 1 was adjusted for age and gender, and model 2 was adjusted for model 1 plus ethnicity, education and income.

Identification of upstream regulators

The TF2DNA database[35] and the Kyoto Encyclopaedia of Genes and Genomes (KEGG)[36] database were used to search the upstream regulators for AD–AMD pleiotropy genes.

Biological network analysis

The CEMiTool database[37] was used to conduct the gene co-expression network (GCN) analysis for AD (GSE36980, GSE132903) and AMD (GSE1719, GSE99287) gene expression data.

AD–AMD pleiotropic genes along with their co-expressed genes were mapped on the human protein–protein interaction (PPI) network from the String database.[38] Cytoscape V.3.7.2 was used to calculate the network topology features of these genes. In this study, we used two popular network indicators to describe the positions and features of pleiotropic genes. 'Degree' indicated the number of neighbours that directly connect to the specified node. 'Average shortest path length' was the average shortest distance (number of nodes on the way) of a random node connecting to other nodes on the network.

$$C\left(v\right) = \frac{\sum_w d\left(v, w\right)}{n-1}$$

Where d (v, w) is the distance between nodes v and w, and n is the number of nodes on the network. We also calculated the average values and SD in whole network models of degree and average shortest path length.

Functional analysis

The KEGG pathway enrichment analysis and Gene Ontology annotation were conducted for the functional analysis of these pleiotropic genes, which was performed by the String database.[38]

Discovery of novel biomarkers

Diagnostic logistic regression tests were used to discover new biomarkers from pleiotropic genes and their regulators for AD and AMD. The gene expression was used as the predictor for the diagnostic test. The receiver operating characteristic curve was used to evaluate biomarker performance, and a value of greater than 0.6 for the area under the curve (AUC) was designated as the cut-off for an adequate biomarker. Python toolbox *sklearn.metrics* was used to perform this test. The DeLong test was used to statistically compare the AUCs for different tests. P<0.05 was considered as with significant differences among diagnostic tests.

RESULTS

Genetic overlap of AD and AMD

Conditional QQ plots were plotted to identify the pleiotropy between AD and AMD (figure 1). Both discovery datasets showed significant leftward shift, indicating high pleiotropy among AD and AMD.

The combined AD–AMD Manhattan plot of discovery and replication GWAS datasets were presented in figure 2A, where the MccFDR and chromosomal for SNPs were presented. 62 significant pleiotropic SNPs were found in the discovery dataset, of which 5 (rs429358, rs12721051, rs10414043, rs12721046 and rs7412) were verified on the replication data (figure 2A and online supplemental tables 1–3). The significant SNPs were all mapped on chromosome 19. In particular, SNPs rs12721051, rs12721046 and rs10414043 were mapped to the APOC1 gene, and rs429358 and rs7412 were mapped to APOE.

We have checked the LD states among the five pleiotropic SNPs (figure 2B), and the three SNPs within APOC1 showed moderate LD (R^2=0.213 for rs12721051 and rs10414043; R^2=0.668 for rs12721051 and rs12721046; R^2=0.340 for rs10414043 and rs12721046). rs7412 is independent from SNPs within APOE, with the highest R^2=0.014; rs429358 demonstrates moderate LD with rs12721051, with an R^2=0.4. Thus, rs12721051 was

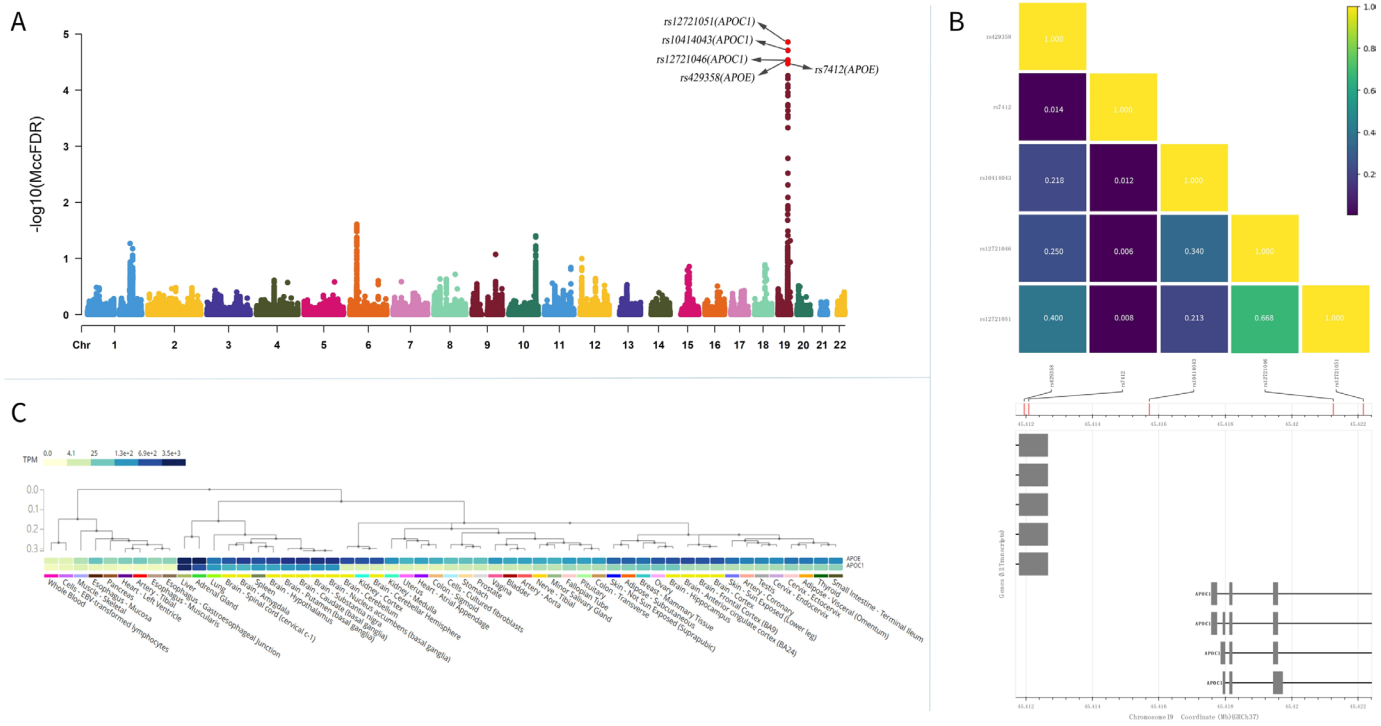


Figure 2 (A) AD–AMD combined Manhattan plot. MccFDR was set as y-axis, which indicated the max ccFDR value among discovery and replication datasets. Five SNPs (rs429358, rs12721051, rs10414043, rs12721046 and rs7412) were identified as shared SNPs for AD and AMD, and they are all located on chromosome 19. Their corresponding genes were APOC1 and APOE. (B) LD analysis results; rs12721051 was removed because of significant LD level. (C) RNA-seq expression heatmap for APOC1 and APOE. Both APOE and APOC1 expressed significantly high in the liver, adrenal gland and brain. AD, Alzheimer's disease; AMD, age-related macular degeneration; ccFDR, conjunction-conditional false discovery rate; EBV, Epstein-Barr virus; LD, linkage disequilibrium; SNPs, single nucleotide polymorphisms.

removed, in order to keep the remaining SNPs more independent.

Phenotypical verification of the association between APOC1/APOE to AD and AMD

Using the UK Biobank dataset, we examined the associations between the pleiotropic genes (APOC1 and APOE) with AD and AMD phenotypes (online supplemental table 4). APOC1 and APOE showed significant associations with AD, AMD and their comorbidities ($p<0.05$).

Genomic verification for APOC1 and APOE in healthy controls

Gene expression analysis in 54 tissues of 838 healthy controls for these pleiotropic genes was expressed on the heatmap in figure 2C and online supplemental figure 1. The expression of APOE and APOC1 was significantly higher in the liver, adrenal gland and brain. APOE was also highly expressed in the skin.

Biological network analysis for pleiotropic genes

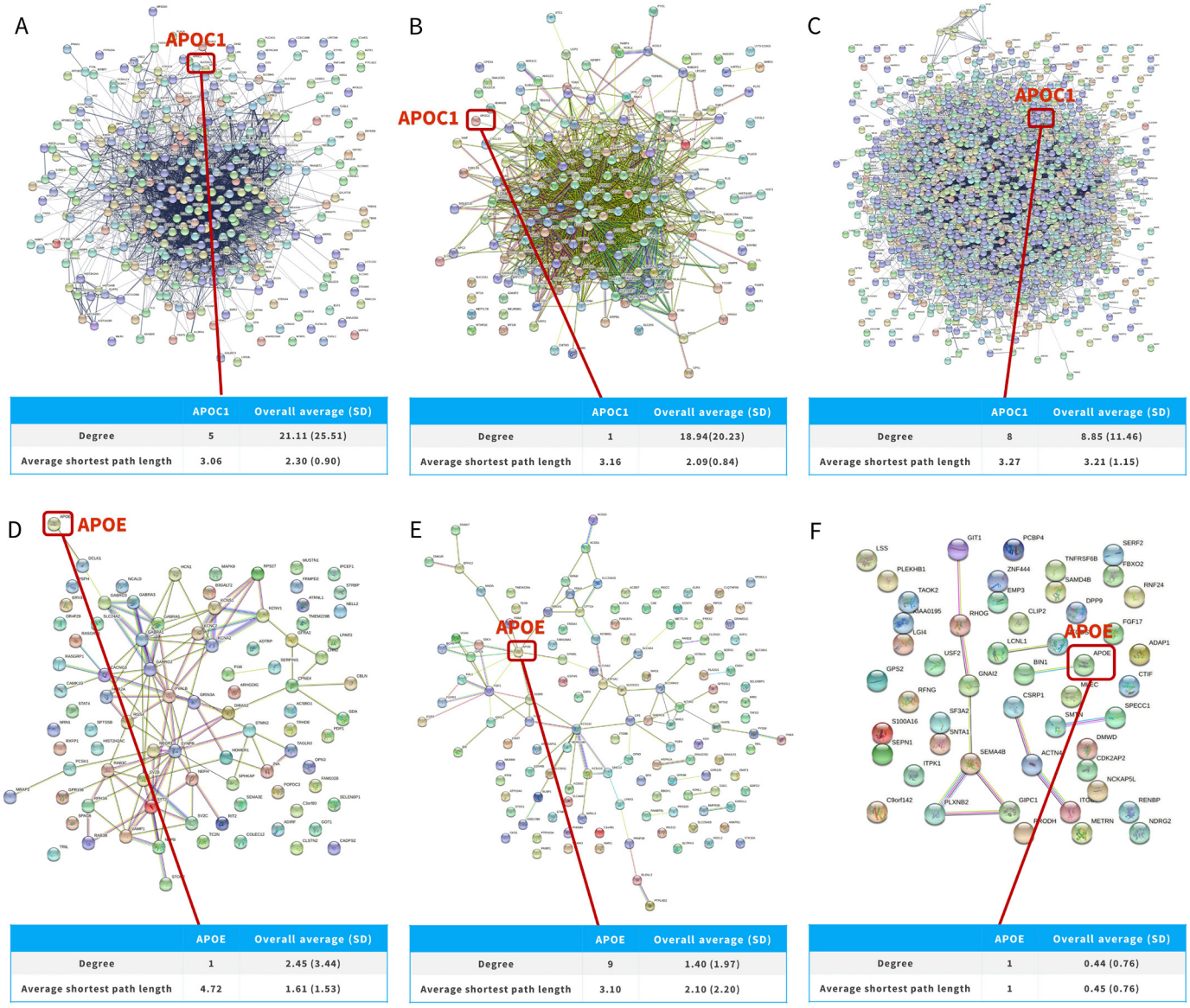
Gene expression datasets were used to construct the GCNs (figure 3 and online supplemental figure 2). We observed that APOC1 and APOE expression was assessed on three datasets. The GCNs for APOE and APOC1 were presented in figure 3A–C and figure 3D–F, separately. Overall, APOC1-related network models were larger in size and exhibited stronger compactness. However, no significant differences were found between patients and controls (online supplemental figure 1). Further, APOC1 and APOE were not identified as hubs on the GCNs, with degrees less than 10 in all models. The relatively medium average shortest path lengths (~3 in most models) indicated that the connections of APOC1 and APOE with other genes on the GCNs were not discrete. These features suggested that APOC1 and APOE were situated at peripheral positions on the GCNs but maintained moderate interaction with other genes.

Regulators of APOC1 and APOE

16 and 14 regulators for APOC1 and APOE were identified, respectively. Five were replications (ZBTB48, MZF1, ZNF131, ZNF319 and ZNF273) for both APOC1 and APOE (figure 4A). The locations and binding strengths for these regulators were presented in online supplemental figure 3A,B. Most of APOC1, APOE and their regulators had no interaction with each other on the PPI network (online supplemental figure 4). The reason for no interaction may be the discovery of PPI is not complete.

Biological function analysis results for pleiotropic genes and their regulators

Regulation of the cellular biosynthetic process, binding and intracellular membrane-bounded organelle was annotated by Gene Ontology analysis for APOC1, APOE and their regulators (online supplemental tables 5–7). In



	APOC1	Overall average (SD)
Degree	5	21.11 (25.51)
Average shortest path length	3.06	2.30 (0.90)

	APOC1	Overall average (SD)
Degree	1	18.94(20.23)
Average shortest path length	3.16	2.09(0.84)

	APOC1	Overall average (SD)
Degree	8	8.85 (11.46)
Average shortest path length	3.27	3.21 (1.15)

	APOE	Overall average (SD)
Degree	1	2.45 (3.44)
Average shortest path length	4.72	1.61 (1.53)

	APOE	Overall average (SD)
Degree	9	1.40 (1.97)
Average shortest path length	3.10	2.10 (2.20)

	APOE	Overall average (SD)
Degree	1	0.44 (0.76)
Average shortest path length	1	0.45 (0.76)

Figure 3 GCNs for APOC1 and APOE in different datasets. The degree and average shortest path length for APOC1 and APOE and average in whole network models were presented. (A) AD discovery-frontal cortex-APOC1 model. (B) AD discovery-temporal cortex-APOC1 model. (C) AD replication-APOC1 model. (D) AD discovery-frontal cortex-APOE model. (E) AD discovery-temporal cortex-APOE model. (F) AD replication-APOE model. AD, Alzheimer's disease; GCNs, gene co-expression networks.

KEGG pathway analysis, six genes were mapped on the herpes simplex virus 1 (HSV1) infection (online supplemental figure 5 and online supplemental table 8).

Notably, we found several APOE–APOC1-specific enriched pathways, including very low-density lipoprotein particle clearance, chylomicron remnant clearance and positive regulation of cholesterol esterification on the biological process level, phosphatidylcholine-sterol o-acyltransferase activator activity on the molecular function level and chylomicron on the cellular component level (online supplemental tables 6–8). We also found that APOE was mapped on the AD pathway, regulated by amyloid beta, which was further regulated by the APP gene through C99 (online supplemental figure 6).

Novel biomarker discovery for AD and AMD

Diagnostic test results for APOC1, APOE and their regulators have been presented in figure 4B. APOC1 demonstrated good performance in six datasets (AUC: 0.71, 0.74, 0.72, 0.84, 0.66, 0.69). APOE only performed well on two datasets (AMD replication-retinal pigment epithelium: AUC=0.90; AD discovery-hippocampus: AUC=0.65). 23 of 25 regulators showed good performance in the diagnostic test (average AUCs in all datasets >0.6). Among the five shared regulators for APOC1 and APOE, ZNF131 showed the best performance in all datasets (all AUCs >0.6, four AUCs >0.8). ADNP2 was the best-performing regulator for APOC1, which showed good diagnostic values in three datasets (AUCs >0.85).

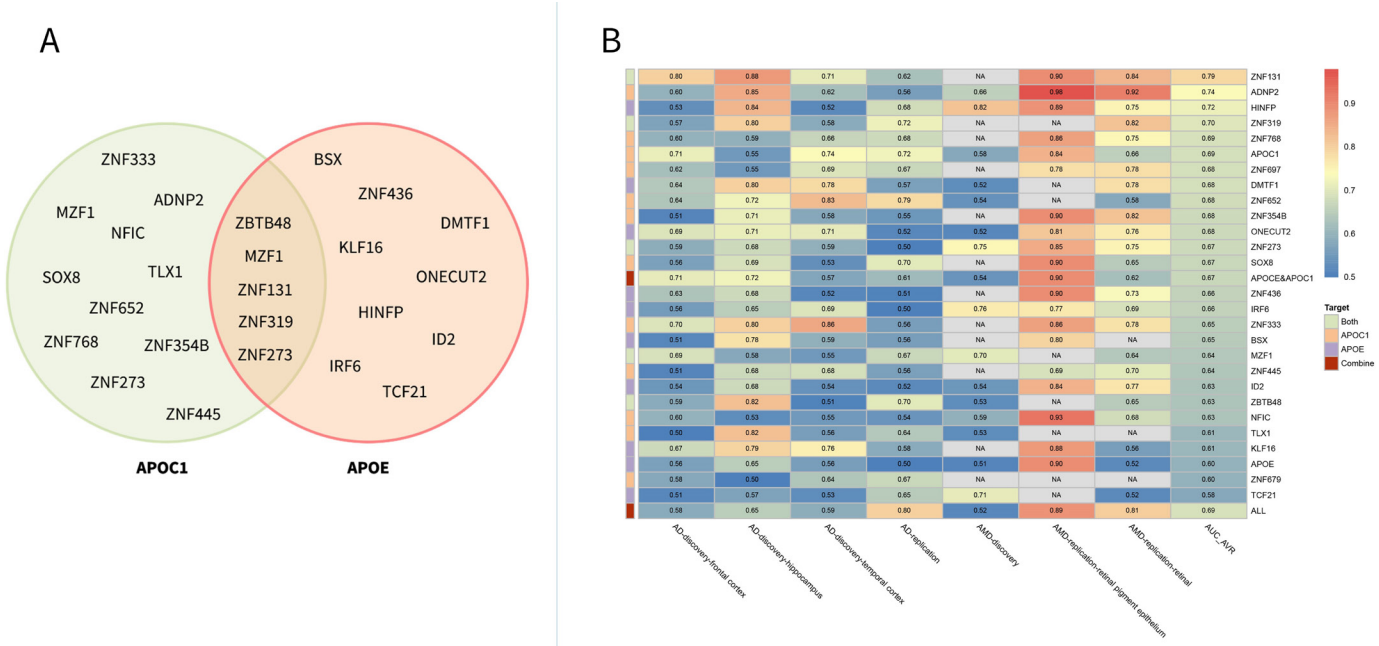


Figure 4 (A) Regulators for APOC1 and APOE. Five shared regulators were found for APOC1 and APOE. (B) Heatmap of diagnostic performance (y-axis: AUC) for APOC1, APOE and their regulators in different expression datasets (x-axis). Average AUCs were calculated and displayed in the last row (average). APOC1 demonstrated good performance in six datasets (AUC: 0.71, 0.74, 0.72, 0.84, 0.66, 0.69) except on two datasets (AD discovery-hippocampus and AMD discovery). APOE only performed well on two datasets (AMD replication-retinal pigment epithelium: AUC=0.90; and AD discovery-hippocampus: AUC=0.65). 23 of 25 regulators showed good performance in the diagnostic test (average AUCs in all datasets >0.6). Among the five shared regulators for APOC1 and APOE, ZNF131 showed the best performance in all datasets (all AUCs >0.6, AUC=0.8 in AD discovery-frontal cortex; 0.88 in AD discovery-hippocampus (AUC: 0.88), AMD replication-retinal pigment epithelium (AUC:0.90) and AMD replication-retina (AUC: 0.84)). ADNP2 was the best-performed regulator for APOC1, which showed good diagnostic values in AD discovery-hippocampus (AUC: 0.85), AMD replication-retinal pigment epithelium (AUC:0.98) and AMD replication-retina (AUC: 0.92). Among the regulators of APOE, HINFP performed best (average AUC=0.72). We have combined APOC1 with APOE, and got AUCs of 0.71, 0.72, 0.57, 0.61, 0.54, 0.90, 0.62 and 0.67 in different groups, which showed significant improvements compared with APOC1 or APOE separately. Meanwhile, we also combined APOC1, APOE and their regulators, and did not find significant improvements. AD, Alzheimer's disease; AMD, age-related macular degeneration; AUC, area under the curve.

Among the regulators of APOE, HINFP performed best (average AUC=0.72).

The DeLong test was used to statistically compare the AUCs for the diagnostic tests among APOC1 and APOE (online supplemental table 9). Except in AD replication group (p=0.0000009173), all the AUCs showed no differences (p>0.05).

In order to detect the performance of combined biomarkers, we combined APOC1 and APOE together and got AUCs of 0.71, 0.72, 0.57, 0.61, 0.54, 0.90, 0.62 and 0.67 in different groups, which showed significant improvements compared with APOC1 or APOE separately. Meanwhile, we also combined APOC1, APOE and their regulators, and did not find significant improvements (figure 4B).

DISCUSSION

This study demonstrated that AD and AMD shared a common genetic aetiology, consistent with previous findings of Logue *et al*[6] and Tan *et al*.[14] The identification of pleiotropy between AD and AMD holds significant clinical significance. It is widely recognised that the diagnosis and prevention of AD pose global challenges. Confirming the shared genetic aetiology between AD and AMD could enhance AD prevention and diagnosis through accurate and timely detection of AMD. Advancing from these studies, our study investigates the overall association between AD and AMD GWAS data using cFDR, a robust Bayesian algorithm that greatly facilitated pleiotropic gene identification. Further, for both diseases, we included both discovery and replication datasets and identified the identical SNPs, lending further credence to the generalisability of our analysis. In addition, the application of network topology analysis enabled the observation of the position of pleiotropic genes on the AD–AMD GCNs. We found that although APOC1 and APOE were not hubs on the GCNs, they present an 'average shortest path lengths' of around three, indicating that pleiotropic genes may have a moderate ability to interact with other genes on GCN (figure 3). This finding may prompt the identification and verification of further pleiotropic genes on networks.

Our study verified that both APOC1 and APOE were pleiotropic genes for both AD and AMD. The protein encoded by APOE serves as a major lipid carrier and has been confirmed as a key risk factor for AD and AMD.[34 39] APOC1, as a close neighbour of APOE (online supplemental figure 4), also encodes a protein in the apolipoprotein C family that plays an essential role in lipoprotein metabolism. APOC1 has been considered to be a risk factor for the development of AD.[40] However, no previous studies reported the association between APOC1 and AMD. Our study, verified with the UK Biobank dataset, confirms that both APOE and APOC1 significantly contribute to the development of AD and AMD, particularly the comorbidity of AD and AMD (online supplemental table 4). We also conducted a genomic analysis to understand the pleiotropy of AD and AMD, by located gene expression level of their pleiotropic genes on tissues, to further understand the pleiotropy aetiology in phenotype level. Since both are neurodegenerative diseases that are closely related to neuronal tissue, their pleiotropy exhibits a substantially high expression in the brain (figure 2B).

Our study identified several upstream regulators for both APOC1 and APOE and a subsequent biological function analysis to detect the pleiotropic genes. Regulators are genes with the ability to control the expression of other genes and are instrumental to the maintenance of healthy biological processes. The investigation of upstream regulators for pleiotropic genes is critical to the understanding of the pleiotropy of AD–AMD. We found significant pathways (online supplemental figure 5 and online supplemental tables 5–8) that may be essential for AD–AMD pathogenesis and may guide future pleiotropic gene discovery. We found that most of the enriched pathways are key pathways for the basic biological process like metabolic and binding-related pathways (online supplemental tables 5–8). Interestingly, as these genes are enriched on the HSV1 infection pathway (online supplemental figure 5), HSV1 infection is a risk factor for AD,[41] but its association with AMD remains unknown. Our study may inspire future research to investigate the association between HSV1 with AMD or AD–AMD comorbidity. According to our pathway enrichment finding, we postulate that the pleiotropy of AD–AMD comorbidity may be mediated by basic biological processes and may occur even at a molecular level.

Our study used pleiotropic genes and their regulators to identify multifunctional biomarkers for AD, AMD and AD–AMD comorbidity. Our investigation, using gene expression data for multifunctional AD–AMD biomarkers detection among pleiotropic genes, suggests that APOC1 has good diagnostic potential for both AD and AMD while APOE might more focus on the AMD diagnosis from the retinal pigment epithelium. Further, most of the regulators of APOC1 and APOE showed good diagnostic values, especially ZNF131, ADNP2 and HINFP. ZNF131 is a protein-coding gene, which conducts function in the adult central nervous system.[42] ADNP2 is also a protein-coding gene, which has been reportedly related to autosomal dominant non-syndromic intellectual disability.[43] HINFP is the final link in the cyclinE/CDK2/p220NPAT/HINFP pathway, playing a key role in the G1/S phase transition, which has been identified as a biomarker for colorectal cancer[44] and type 2 diabetes.[45]

Our study has several limitations. First, we did not analyse the subtypes of AD and AMD as the GWAS datasets did not specify this information. The subtypes of these diseases may be important information for clinical diagnosis and treatment. Second, the sample sizes in microarray datasets for diagnosis tests were relatively small, limiting our potential further identification of multifunctional AD–AMD biomarkers among pleiotropic genes. Third, we were able to examine the diagnostic value of the identified biomarkers in the patients with either AD or AMD but not in patients with both AD and AMD due to data availability. Fourth, we did not take into consideration the onset time and duration of diseases of AD and AMD in our analyses. Fifth, our consideration of only two network features in our network analysis leaves room for further analysis of additional network features in future studies.

CONCLUSIONS

In this study, we constructed the genetic pleiotropy between AD and AMD and identified APOC1 and APOE to be pleiotropic genes for both diseases. These results support existing clinical and biochemical evidence that demonstrates common features in the pathophysiological pathways leading to both AD and AMD. Further, the biological network and pathway analysis in our study characterise the network topology features and pathways for pleiotropic genes for AD–AMD. The integration of multiomics data identified ZNF131, ADNP2 and HINFP as novel diagnostic biomarkers for AD and AMD.

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