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Retina

Exploring Therapeutic Targets for Age-Related Macular Degeneration From Circulating Proteins to Plasma Metabolites in the European Population

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Purpose: To explore the causal associations among circulating proteins, plasma metabolites, and age-related macular degeneration (AMD).

Methods: We employed Mendelian randomization (MR) analysis and colocalization analysis to discern the causal relationship between proteomes and AMD. This investigation utilized data from protein quantitative trait loci (pQTL) studies in deCODE and the UK Biobank. Additionally, plasma metabolite–related genome-wide association studies (GWAS) data and AMD-related GWAS data were incorporated.

Results: Our findings confirmed a potential causal relationship between cytoplasmic tryptophanyl-tRNA synthetase 1 (WARS1) and a higher risk of AMD. The observed causal impact of WARS1 on the two subtypes of AMD (dry and wet) align consistently with the aforementioned outcomes. Three plasma metabolites—*N*-acetyl-kynurenine, *N*-acetyltyrosine, and caproate (6:0)—were identified as mediators of the causal effect of WARS1 on AMD, and subgroup analysis revealed that *N*-acetyltyrosine is a specific negative metabolite associated with WARS1 and dry AMD, whereas X-16580 is a specific positive metabolite linked to WARS1 and wet AMD.

Conclusions: The outcomes of this study suggest a potential causal role of specific circulating proteins in AMD and identified the mediating role of plasma metabolites between WARS1 and AMD by integrating multiple genetic analyses. Nevertheless, further research is essential to validate and strengthen these conclusions.

Translational Relevance: This study establishes the causal role of specific circulating proteins in AMD and identified the mediating role of plasma metabolites between WARS1 and AMD.

Introduction

With the onset of the aging population era, illnesses associated with organ aging have garnered increased social attention. Among these, the deterioration of visual function stands out as it significantly impacts the quality of life for the elderly and contributes to a growing societal burden. age-related macular degeneration (AMD) is a condition that leads to central vision loss in individuals over the age of 55 due to macular degeneration.[1] Projections suggest that the global number of AMD patients will escalate to 288 million by the year 2040.[2] According to the international Beckmann AMD classification criteria and the Age-Related Eye Disease Study (AREDS) AMD staging criteria, AMD can be primarily classified into three stages: early AMD, intermediate AMD, and advanced AMD.[3,4] Advanced AMD can be further classified into two primary subtypes: dry AMD, identified by the presence of drusen, alterations in the retinal pigment epithelium, and the development of geographical atrophy; and wet AMD, distinguished by neovascularization. Among these subtypes, dry AMD makes up approximately 80% to 90% of all cases of AMD, whereas wet AMD represents a smaller proportion.[5] Clinically, patients with wet AMD experience more severe visual impairment, as neovascularization can cause significant damage to the retinal anatomical structure.[6] Currently, anti-vascular endothelial growth factor (VEGF) agents are extensively employed in the therapeutic intervention of wet AMD. However, the long-term vision gain effect in patients with wet AMD remains insufficient despite their widespread use.[7] Also, treatment methods for dry AMD remain relatively limited. The currently approved options predominantly encompass nutritional supplements endorsed by the AREDS, such as taurine and vitamins with antioxidant functions. However, these treatments fall short of preventing the progression of macular map atrophy.[8,9] Hence, there is an imminent necessity for the investigation of optimal drug targets for AMD and the subsequent development of corresponding pharmaceutical interventions.

The selective physiology of the blood–retinal barrier (BRB) generally restricts the passage of circulating proteins into the retina; however, in AMD, BRB integrity may be compromised due to chronic inflammation and oxidative stress, allowing systemic factors including proteins to affect retinal health.[10,11] Circulating proteins emerge as ideal drug targets due to their involvement in molecular pathways associated with various systemic diseases in the human body. Their non-invasive intervention advantage adds to their appeal. The connection between circulating proteins and AMD has been initially validated in several studies. For example, C-reactive protein levels were significantly elevated in AMD patients in the AREDS study,[12] and increased factor H-related protein 4 and CD200 levels have been observed in AMD patient plasma and serum.[13,14] Additionally, a retrospective case–control study identified a decrease in serum Beclin-1 levels among patients with AMD.[15] However, a population-based cross-sectional case-control study failed to establish an association between AMD and circulating inflammation-associated proteins.[16]

Plasma metabolites offer important insights into the metabolic state and underlying pathological conditions of patients with various diseases, serving as key mediators in numerous physiological pathways. Recent studies have increasingly indicated that distinct plasma metabolomic profiles exist among AMD patients, with the composition of plasma metabolites varying according to the severity of the disease.[17–21] It is crucial to note that the available evidence is derived from observational studies, whether in the form of controlled studies involving a specific number of individuals or basic research involving cells and animals. Although observational studies can reveal either positive or negative associations between a biomarker and a disease state, such associations do not imply causality. These studies are unable to definitively clarify the causal association between the multitude of circulating proteins and plasma metabolites in the human body and AMD. Moreover, they are susceptible to various confounding factors. The specific role of plasma metabolites in the pathogenesis of AMD remains largely underreported.

To address the limitations of observational studies, Mendelian randomization (MR) analysis has emerged as a powerful tool for inferring causal relationships. MR leverages genetic variants obtained from large genome-wide association study (GWAS) databases as instrumental variables (IVs) to mitigate confounding and reverse causation.[22–24] Single nucleotide polymorphisms (SNPs) are the most commonly employed genetic variations in MR analyses.[25] In this study, we employed MR analysis and colocalization analysis to explore the causal effects of circulating proteins on AMD and its subtypes (dry and wet AMD).[26] Some circulating proteins, initially screened as potentially related to the pathogenesis of AMD, including tryptophanyl-tRNA synthetase 1 (WARS1), collagen type X alpha 1 chain (COL10A1), protein tyrosine phosphatase non-receptor type 9 (PTPN9), lipopolysaccharide-binding protein (LBP), vitronectin (VTN), stromal cell-derived factor 2 (SDF2), CD46,

transforming growth factor beta 1 (TGF-β1), V-set domain containing T-cell activation inhibitor-1 (VTCN1), CD 300 antigen-like family member G (CD300LG), and colony-stimulating factor 2 (CSF2), among others, were further assessed for the strength of their causal association with AMD. Additionally, a two-step MR analysis was conducted to explore shared causal variation and identify potential influencing genes between specific circulating proteins (e.g., WARS1, with a high level of evidence) and plasma metabolites in the human body and AMD. By combining multiple genetic analyses, this study aimed to identify common pathogenic variants and explore potential influencing genes to provide insights into the biological pathways that link circulating proteins, plasma metabolites, and AMD.

Methods

GWAS Data Sources

In order to alleviate confounding bias arising from ethnic disparities, the IVs chosen for data extraction in connection with MR analysis were exclusively sourced from individuals of European ancestry. Protein quantitative trait loci (pQTL) data for circulating proteins were sourced from two large-scale studies. One set of pQTL data encompassed 2940 circulating proteins, drawing from protein genomics profiles obtained from 54,219 participants in the UK Biobank.[27] The second set of pQTL data was gathered from the deCODE database, incorporating plasma protein genomes from 4907 Icelanders (35,559 plasma samples).[28] The GWAS data on 1400 plasma metabolites were primarily derived from a Canadian cohort consisting of over 8000 participants of European ancestry.[29] To enhance the credibility of the study results and extend the exploration of the genetic association between circulating proteins and plasma metabolites in the human body and AMD on a broader scale, AMD-related GWAS data were selected from two distinct sources. One set of data was derived from a meta-analysis led by Winkler et al.[30] and included in the IEU OpenGWAS project, which included information from patients in the early stage of AMD (GWAS ID: ebi-a-GCST010723; 14,034 cases and 91,214 controls), and all diagnoses of early AMD were confirmed by color fundus photography. The other AMD-related GWAS data were extracted from FinnGen data freeze 9, which encompasses 8913 cases and 348,936 controls (GWAS ID: finngen_R9_H7_AMD). IVs related to two AMD subtypes were also obtained from FinnGen data freeze 9; the analysis associated with dry AMD included 6065 cases and 251042 controls (GWAS ID: finngen_R9_DRY_AMD), and the wet AMD–related analysis consisted of 4848 cases and 252,277 controls (GWAS ID: finngen_R9_WET_AMD). The diagnostic classification of AMD in the FinnGen database is based on the International Classification of Diseases, 10th Revision (ICD-10; code H7) and ICD-9 (code 362.52). The data presented in this study are available at https://www.jianguoyun.com/p/Da6663cQuaiFChjlsswFIAA.

Mendelian Randomization Analysis Design and Genetic Instrument Extraction

We employed the summary-data-based MR (SMR) method and Wald ratio/inverse variance weighted MR (IVW-MR) to collectively deduce the causal impact of circulating proteins on AMD. The IVs used in this analysis were restricted to the *cis*-pQTL located within 1 Mb on both sides of each circulating protein. SMR is specifically used to integrate gene expression and trait association data, allowing us to infer the causal effects of the population based on the effects of genetic variation in the population.[31] In this study, the SMR method was preliminarily utilized to screen circulating proteins with potential causal associations with AMD, and the significant related proteins based on deCODE or UK Biobank were considered as potential research objects in the next step. In the SMR analysis, additional thresholds were applied to identify SNPs significantly associated with each circulating protein, with criteria including a minor allele frequency > 0.01 and $P < 5 \times 10^{-8}$. Complementing the SMR results, a traditional two-sample MR analysis method based on generalized aggregated data was employed to further estimate the effect size of the association between circulating proteins and AMD and enhance the interpretation of our results. Similarly, a threshold of 5×10^{-8} was applied for SNP extraction. It is important to note that, because the threshold of 5×10^{-8} is too stringent for extracting plasma metabolite-related IVs, we further adjusted the threshold to 1×10^{-5} in order to capture a broader range of plasma metabolite-related genetic variables. To ensure independence in linkage disequilibrium analysis, $r^2 < 0.001$ and kb = 10,000 were set. When the number of extracted SNPs was 1, the Wald ratio method was employed to estimate the causal effect. If the number of SNPs was greater than or equal to 2, the IVW method was utilized. The IVW method is a widely used instrumental variable approach that provides a pooled

estimate of causal effects by weighting the effect of each SNP.[32] Two-step MR analysis was conducted to investigate the mediating role of plasma metabolites between circulating proteins and AMD. Moreover, in order to explore more possibilities regarding the causal effect of circulating proteins on plasma metabolites and AMD, $r^2 < 0.3$ and kb = 100 were further set when extracting pQTL for specific circulating proteins.[33] MR-Egger regression, weighted median and weighted mode models were further developed to supplement the IVW results in two-step MR analysis. This drug target–related MR study adhered to the Strengthening the Reporting of Observational Studies in Epidemiology Using Mendelian Randomization (STROBE-MR) guidelines. It satisfies the assumptions crucial for MR analysis: SNPs exhibited a close association with the exposed factors; excluded any confounding factors influencing the exposure–outcome correlation; and ensured that SNPs affected outcome factors solely via exposure factors (Fig. 1).[34,35] To avoid bias arising from weak IVs, we retained IVs with an *F*-statistic greater than 10.[36] The *F*-statistic is directly estimated using β-exposure and standard error (SE) exposure.[37]

Colocalization Analysis Design

Bayesian colocalization analysis was conducted to assess whether circulating proteins and AMD share a common genetic loci at a given locus. By identifying loci in which genetic variants affect both traits, Bayesian colocalization helps to strengthen the evidence of a causal relationship suggested by MR via excluding confounding due to linkage imbalance.[38] The selection of circulating proteins included those not incorporated in the MR analysis, broadening the exploration of common genetic variability between circulating proteins and AMD at the genetic level. The Bayesian colocalization analysis involved calculating the posterior probabilities for five distinct hypotheses: (1) P_{H1}, the probability that neither circulating proteins nor AMD are associated; (2) P_{H2}, the probability associated only with circulating proteins; (3) P_{H3}, the probability associated only with AMD; (4) P_{H4}, the probability is correlated with both circulating proteins and AMD, but there is a significant probability of causal variation; and (5) P_{H5}, circulating proteins and AMD are correlated, and circulating proteins and AMD have the same probability of causal variation.[39] The probability that the SNP is independently associated with trait 1 (P_1) and trait 2 (P_2) was set as 1×10^{-4}, whereas the probability that the SNP is associated with both trait 1 and trait 2 (P_{12}) was set as 1×10^{-5}. $P_{H4} \geq 0.8$ was considered high-grade colocalization evidence, $0.5 \leq P_{H4} < 0.8$ was considered low-grade colocalization evidence, and $P_{H4} < 0.5$ indicates a lack of support for colocalization between the two traits.[40]

Evidence Hierarchy Establishment

Combined with the outcomes of MR analysis and colocalization analysis, the causal effects of circulating protein on AMD and its subtypes were divided into the following four levels (Fig. 1): In level 1, the results of SMR and Wald ratio/IVW MR both supported a significant causal association between the two traits. The evidence level of colocalization analysis was high grade, and the Heterogeneity in Dependent Instruments (HEIDI) result did not indicate the existence of pleiotropy. All results were verified simultaneously in deCODE and UK Biobank databases. In level 2, the results of SMR and Wald ratio/IVW MR both supported a significant causal association between the two traits. The evidence level of colocalization analysis was high grade, and the HEIDI result did not indicate the existence of pleiotropy. All results were only verified in one of the two databases. In level 3, the results of SMR and Wald ratio/IVW MR both supported a significant causal association between the two traits. The evidence level of colocalization analysis was low grade, and the HEIDI result did not indicate the existence of pleiotropy. All results were only verified in one of the two databases. Any remaining results not meeting the above criteria did not support a causal association of circulating proteins with AMD.

Statistical Analysis

All MR analyses were conducted using R 4.3.1 (R Foundation for Statistical Computing) and SMR 1.3.1 software. The Wald ratio/IVW-MR analysis results were obtained using a specialized R package.[25,41] The R package *coloc* was utilized to evaluate whether the linkage disequilibrium had an impact on the association between circulating proteins and AMD.[42] Cochran's *Q* test was utilized to identify heterogeneity among the extracted IVs.[43] The results of this test guided the application of either the fixed-effects IVW model (in the absence of heterogeneity) or the multiplicative random-effects IVW model (when heterogeneity was detected).[32,44] The HEIDI test was employed to identify linkage disequilibrium between circulating proteins and AMD in SMR.[45] The MR-Egger intercept test and the Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) global test were further performed to find potential

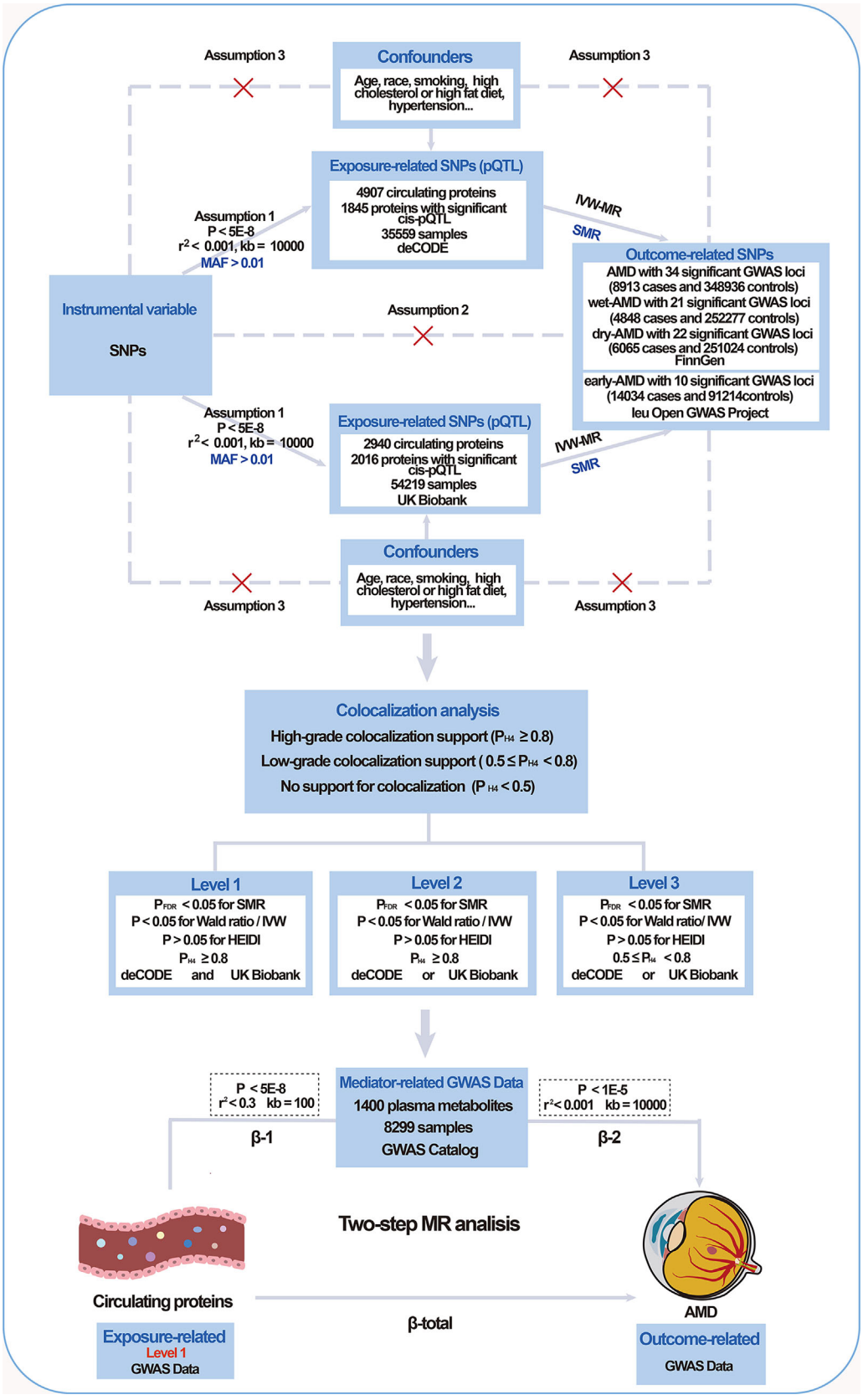

Figure 1. Schematic diagram of study design and three key assumptions satisfied by MR analysis.

pleiotropy in two-step MR analyses. The robustness of the results in the two-step MR analyses was evaluated by conducting leave-one-out analyses. The P values obtained from the multiple comparisons in SMR and the Wald ratio/IVW-MR were subjected to further conversion to the false discovery rate (FDR) using the Benjamin–Hochberg multiple tests method. A significance threshold of $P < 0.05$ was considered statistically significant. The computational methods involved in the mediation analysis are presented in the Supplementary Materials.

Results

MR Analysis of Circulating Proteins With Risk of AMD

In the SMR analysis of the two groups of proteome-wide gene variables and AMD gene variables (including various subtypes), the available protein-related pQTL were extracted as far as possible to assess the causal impact of specific circulating proteins on AMD. After excluding results with insignificant causal effect ($P_{FDR} > 0.05$) and possible pleiotropy ($P_{HEIDI} < 0.05$), 22 circulating proteins were found to have potential causal associations with AMD or its subtypes based on SMR results, all of which are depicted in Figure 2.

In the MR analysis based on deCODE, the expression levels of WARS1, COL10A1, and LBP were potentially causally correlated with the increased risk of AMD, whereas the expression levels of ribosomal protein S10 (RPS10), lymphotoxin alpha (LTA), PTPN9, and C-terminal Src kinase (CSK) were potentially causally correlated with the decreased risk of AMD. SMR results based on LTA and RPS10 were not supported by the Wald ratio/IVW MR method. In the subgroup analyses, WARS1 and COL10A1 may act as harmful factors for dry AMD and wet AMD, RPS10 and LTA may be specific protective factors for dry AMD, and PTPN9 may be a specific protective factor for wet AMD. Complement C3 (C3), LBP, VTN and SDF2 may be specific harmful factors for wet AMD. TNF superfamily member 14 (TNFSF14) may serve as a protective factor for wet AMD and early AMD, which has not been verified by traditional MR analysis methods. Complement factor B (CFB), mast cell expressed membrane protein 1 (MCEMP1), and CD46 may be protective against early AMD (Table 1).

In the MR analysis based on the UK Biobank, the expression levels of WARS1, alpha-1,3-*N*-acetylgalactosaminyltransferase and alpha-1,3-galactosyltransferase (ABO), dipeptidase 2 (DPEP2), interleukin 20 receptor subunit beta (IL20RB), and VTCN1 were potentially causally correlated with the increased risk of AMD, in addition to TGF-β1 being considered as a possible protective factor for AMD. In the subgroup analyses, the roles of WARS1, IL20RB, and DPEP2 in AMD were also shown in dry AMD. The functions of WARS, TGF-β1, and ABO in wet AMD are consistent with their roles in AMD. Mesothelin (MSLN) and CD300LG may be specific harmful factors in dry AMD, whereas LBP may be specific harmful factors in wet AMD. C3 may be causatively linked to the heightened risk of early AMD. The SMR results of causal effects of IL20RB and CSF2 on AMD and its subtype were not supported by the Wald ratio/IVW MR method (Table 2).

Colocalization Analysis of Circulating Proteins With AMD

To further investigate shared genetic architectures between circulating proteins and AMD, we performed a colocalization analysis. High-confidence colocalization evidence was found for WARS1, TGF-β1, IL20RB, and VTCN1 with AMD, whereas LBP demonstrated moderate colocalization evidence. COL10A1, PTPN9, CSK, and ABO exhibited only low levels of colocalization evidence with AMD. Subgroup colocalization analysis revealed the following associations: (1) dry AMD—high colocalization evidence for WARS1, IL20RB, MSLN, and CD300LG; (2) wet AMD—high colocalization evidence for WARS1, LBP, VTN, SDF2, TGF-β1, and CSF2 and moderate colocalization for COL10A1 and PTPN9; (3) early AMD—high colocalization evidence for CFB and MCEMP1, moderate colocalization for CD46, and low colocalization for ABO and C3. For the remaining proteins, no robust genetic colocalization was observed (Figs. 3A and 3B).

Determination of the Final Results Grade

By integrating MR and colocalization findings, we assigned evidence levels to circulating proteins associated with AMD risk as follows:

- Level 1 (highest confidence)—WARS1 was validated in both proteome-wide datasets. deCODE had odds ratio $[OR]_{SMR} = 1.57$; 95% confidence interval [CI], 1.34–1.83; $P_{FDR} = 1.49E-$

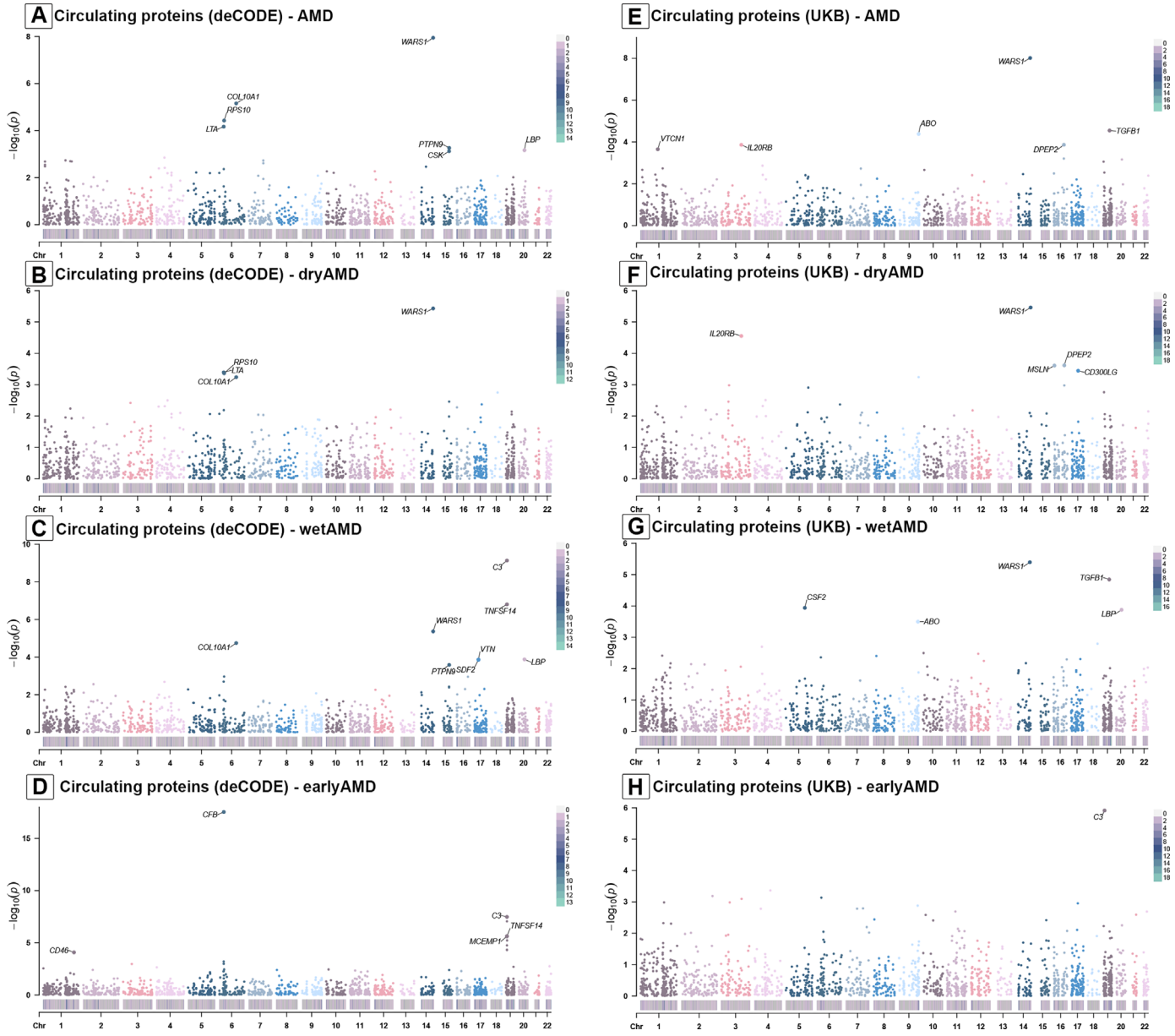

Figure 2. Manhattan plots for the associations of genetically predicted circulating protein levels with AMD and its subtypes in SMR analysis. (**A**–**D**) deCODE associations of genetically predicted circulating proteins levels with various types of AMD: AMD (**A**), dry AMD (**B**), wet AMD (**C**), and early AMD (**D**). (**E**–**F**) UK Biobank associations of genetically predicted circulating proteins levels with various types of AMD: AMD (**E**), dry AMD (**F**), wet AMD (**G**), and early AMD (**H**).

06. UK Biobank had OR_{SMR} = 1.56; 95% CI, 1.34–1.82; P_{FDR} = 1.94E-06.

- Level 2 (moderate confidence)—AMD, TGF-β1 (UK Biobank) and VTCN1 (UK Biobank); wet AMD, LBP (deCODE), VTN (deCODE), SDF2 (deCODE), and TGF-β1 (UK Biobank); dry AMD, CD300LG (UK Biobank).
- Level 3 (lower confidence)—Wet AMD, COL10A1, PTPN9; early AMD, CD46.

Proteins not included in these categories did not exhibit strong causal associations with AMD (Tables 1 and 2).

Mediating Role of Plasma Metabolites Between WARS1 and AMD

WARS1, the highest confidence protein from the genetic analysis, was included in a two-step

Table 1. MR Analysis and Colocalization of Circulating Proteins With AMD and Its Subtypes (deCODE)

	SMR			Wald Ratio/IVW MR			HEIDI			
Exposure	OR (95%CI)	*P*	FDR	nSNP	OR (95% CI)	*P*	*P*	P_{H4}	Level	Functions
AMD Outcome										
WARS1	1.57 (1.34, 1.83)	1.13E-08	1.49E-06	2	1.51 (1.30, 1.75)	5.16E-08	6.54E-01	9.90E-01	1	Innate immune activator with proinflammatory effects[47]
COL10A1	1.43 (1.22, 1.67)	6.96E-06	7.56E-04	3	1.35 (1.17, 1.56)	3.80E-05	1.67E-01	3.60E-01		—
RPS10	0.33 (0.19, 0.55)	3.75E-05	3.64E-03	—	—	—	2.44E-01	2.40E-02		—
LTA	0.07 (0.02, 0.25)	6.81E-05	6.28E-03	—	—	—	6.61E-02	8.00E-02		—
PTPN9	0.38 (0.22, 0.66)	5.47E-04	3.74E-02	1	0.38 (0.24, 0.63)	1.25E-04	4.33E-01	3.16E-01		—
LBP	1.08 (1.03, 1.13)	6.83E-04	4.50E-02	8	1.06 (0.98, 1.14)	1.33E-01	7.36E-01	6.80E-01		—
CSK	0.39 (0.22, 0.67)	7.56E-04	4.81E-02	1	0.39 (0.24, 0.63)	1.62E-04	7.28E-02	3.46E-01		—
Dry AMD Outcome										
WARS1	1.54 (1.28, 1.85)	3.71E-06	4.28E-04	2	1.48 (1.24, 1.76)	1.50E-05	8.05E-01	8.60E-01	1	Innate immune activator with proinflammatory effects[47]
LTA	0.08 (0.02, 0.32)	4.04E-04	3.39E-02	1	0.08 (0.03, 0.29)	4.23E-06	1.05E-01	8.00E-02		—
RPS10	0.32 (0.17, 0.60)	4.34E-04	3.48E-02	—	—	—	4.51E-01	2.36E-02		—
COL10A1	1.38 (1.15, 1.66)	5.75E-04	4.25E-02	3	1.32 (1.11, 1.57)	1.46E-03	6.07E-02	1.05E-01		—
Wet AMD Outcome										
C3	0.15 (0.08, 0.27)	7.35E-10	1.23E-07	1	1.88 (0.98, 3.59)	5.64E-02	1.97E-01	3.48E-08		—
TNFSF14	0.06 (0.02, 0.17)	1.57E-07	2.42E-05	—	—	—	8.33E-02	3.50E-12		—
WARS1	1.61 (1.31, 1.97)	4.31E-06	4.97E-04	2	1.54 (1.26, 1.87)	1.91E-05	6.33E-01	9.90E-01	1	Innate immune activator with proinflammatory effects[47]
COL10A1	1.57 (1.28, 1.94)	1.79E-05	1.94E-03	3	1.47 (1.22, 1.78)	7.08E-05	2.12E-01	7.76E-01	3	Neovascularization, EMT induction, ECM modulation[79,80]
LBP	1.12 (1.06, 1.19)	1.32E-04	1.22E-02	8	1.15 (1.04, 1.27)	6.59E-03	7.72E-02	8.83E-01	2	Acute-phase protein that binds lipopolysaccharides and participates in immune responses[81]
VTN	1.07 (1.03, 1.10)	1.37E-04	1.20E-02	11	1.10 (1.03, 1.18)	4.53E-03	2.41E-01	8.60E-01	2	ECM glycoprotein involved in cell adhesion and migration, impacts tissue remodeling[82]
SDF2	1.41 (1.18, 1.69)	1.55E-04	1.30E-02	3	1.36 (1.14, 1.61)	5.20E-04	1.78E-01	8.58E-01	2	Cytokine involved in angiogenesis that regulates endothelial cell migration and vascular repair[66]
PTPN9	0.26 (0.13, 0.53)	2.61E-04	2.09E-02	1	0.26 (0.14, 0.49)	3.98E-05	6.54E-01	7.86E-01	3	Modulates cell signaling and regulates immune responses[83]
Early AMD Outcome										
CFB	0.68 (0.62, 0.74)	2.98E-18	1.10E-15	4	1.03 (0.89, 1.19)	6.76E-01	9.03E-02	9.90E-01		—
C3	3.15 (2.10, 4.73)	3.29E-08	6.75E-06	2	3.68 (2.22, 6.09)	3.98E-07	8.24E-01	1.77E-01		—
TNFSF14	0.21 (0.11, 0.40)	2.35E-06	3.61E-04	1	0.83 (0.66, 1.03)	9.12E-02	4.60E-01	1.14E-06		—
MCEMP1	0.17 (0.08, 0.37)	5.92E-06	8.41E-04	—	—	—	3.32E-01	9.90E-01		—
CD46	0.78 (0.69, 0.88)	8.25E-05	8.46E-03	2	0.76 (0.57, 0.99)	4.78E-02	3.02E-01	5.15E-01	3	Membrane protein involved in regulating complement activation and immune modulation[84]

ECM, extracellular matrix; EMT, epithelial–mesenchymal transition; nSNP, single nucleotide polymorphisms.

Table 2. MR Analysis and Colocalization of Circulating Proteins With AMD and Its Subtypes (UK Biobank)

	SMR			Wald Ratio/IVW MR			HEIDI			
Exposure	OR (95% CI)	*P*	FDR	nSNP	OR (95% CI)	*P*	*P*	P_{H4}	Level	Functions
AMD Outcome										
WARS1	1.56 (1.34, 1.82)	9.60E-09	1.94E-06	1	1.57 (1.36, 1.81)	3.60E-10	0.20	0.99	1	Innate immune activator with proinflammatory effects[47]
TGF-β1	0.75 (0.65, 0.86)	2.84E-05	4.77E-03	2	0.77 (0.68, 0.87)	2.42E-05	0.74	0.98	2	Growth factor that regulates cell differentiation, immune response, and fibrosis[85]
ABO	1.08 (1.04, 1.12)	4.14E-05	5.96E-03	5	1.07 (0.99, 1.16)	1.03E-01	0.32	0.37		—
DPEP2	1.64 (1.26, 2.14)	1.37E-04	1.63E-02	2	1.32 (0.79, 2.20)	2.88E-01	0.27	0.01		—
IL20RB	1.76 (1.32, 2.36)	1.38E-04	1.55E-02	—	—	—	0.18	0.82		—
VTCN1	2.72 (1.60, 4.63)	2.23E-04	2.25E-02	1	2.72 (1.66, 4.46)	7.27E-05	0.10	0.96	2	Immune checkpoint protein that inhibits T-cell activation and regulates immune tolerance[54]
Dry AMD Outcome										
WARS1	1.53 (1.28, 1.84)	3.45E-06	6.33E-04	2	1.54 (1.30, 1.82)	5.00E-07	0.12	0.98	1	Innate immune activator with proinflammatory effects[47]
IL20RB	2.11 (1.49, 3.00)	2.81E-05	4.35E-03	—	—	—	0.13	0.92		—
DPEP2	1.64 (1.26, 2.14)	2.42E-04	3.26E-02	2	1.42 (0.87, 2.30)	1.60E-01	0.71	0.04		—
MSLN	1.13 (1.06, 1.20)	2.46E-04	3.10E-02	5	1.13 (0.97, 1.31)	1.15E-01	0.83	0.83		—
CD300LG	1.21 (1.09, 1.34)	3.59E-04	4.26E-02	3	1.20 (1.08, 1.33)	1.04E-03	0.59	0.87	2	Immune receptor that regulates immune responses and inflammation[60]
Wet AMD Outcome										
WARS1	1.60 (1.31, 1.96)	4.02E-06	9.00E-04	2	1.62 (1.34, 1.95)	4.16E-07	0.96	0.99	1	Innate immune activator with proinflammatory effects[47]
TGF-β1	0.67 (0.56, 0.80)	1.43E-05	2.62E-03	2	0.70 (0.60, 0.82)	1.16E-05	0.15	0.99	2	Growth factor that regulates cell differentiation, immune response, and fibrosis[85]
CSF2	1.43 (1.19, 1.72)	1.15E-04	1.93E-02	1	0.70 (0.59, 0.82)	9.62E-05	0.07	0.87	2	Hematopoietic growth factor that stimulates granulocyte and macrophage production[86]
LBP	1.11 (1.05, 1.17)	1.33E-04	2.06E-02	3	1.02 (0.78, 1.32)	9.01E-01	0.12	0.89		—
ABO	1.09 (1.04, 1.14)	3.17E-04	4.26E-02	5	1.05 (0.94, 1.17)	3.75E-01	0.31	0.26		—
Early AMD Outcome										
C3	1.94 (1.48, 2.53)	1.22E-06	3.07E-04	1	1.93 (1.50, 2.49)	2.81E-07	0.37	0.42		—

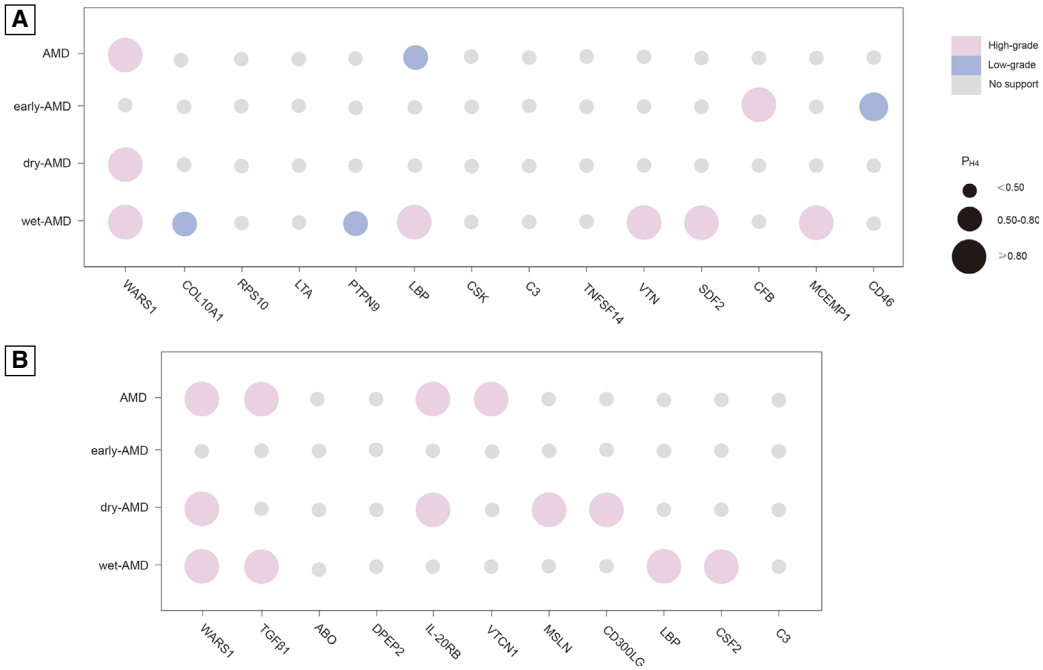

Figure 3. Classification of colocalization evidence between circulating proteins and AMD. (**A**) Colocalization evidence based on deCODE data. (**B**) Colocalization evidence based on UK Biobank data. P_{H4} represents the posterior probability of a shared causal variant between the selected SNP and the two phenotypes (circulating proteins and AMD). *High-grade* indicates that strong evidence of colocalization exists between the circulating proteins and AMD. *Low-grade* indicates that limited evidence of colocalization exists between the circulating proteins and AMD. *No support* indicates that no colocalization evidence exists between the circulating proteins and AMD.

MR analysis to explore the potential mediation of plasma metabolites between WARS1 and AMD. First, WARS1 was set as the exposure and plasma metabolites as the outcome. Second, plasma metabolites were analyzed as the exposure with AMD as the outcome. The initial MR analysis confirmed the association between WARS1 and AMD, even with an increased number of WARS1-associated SNPs due to relaxed r^2 and distance thresholds (Supplementary Tables S11, S12). Five plasma metabolites (*N*-acetyl-kynurenine [2], *N*-acetyltyrosine, caproate [6:0], X-23587, and fructosyllysine) showed significant results in both MR steps (Fig. 4), with three (*N*-acetyl-kynurenine [2], *N*-acetyltyrosine, and caproate [6:0]) validated in deCODE and the UK Biobank. After excluding pleiotropic effects and unstable results (Supplementary Tables S11, S13–S15; Supplementary Figs. S1–S3), mediation analysis suggested that WARS1 causally reduces *N*-acetyl-kynurenine, and its decrease may increase AMD risk. *N*-acetyltyrosine, similar to *N*-acetyl-kynurenine, is also a negative mediator. Caproate (6:0) mediates a potential increase in AMD risk (Fig. 4, Table 3). Subgroup analyses revealed that *N*-acetyltyrosine is a specific negative mediator for dry AMD, and X-16580 is a specific positive mediator for wet AMD (Fig. 5, Table 3).

Discussion

Main Interpretation

AMD stands as a primary ocular ailment leading to vision impairment and, in severe cases, blindness among the elderly.[1] Although anti-VEGF therapy is widely used for retinal neovascularization,[46] the available treatment options for AMD remain limited,[8] and new drug development is essential for AMD management. Using proteome-wide GWAS data from the deCODE database and UK Biobank, we employed MR and colocalization analyses to investigate the causal effect of circulating proteins on AMD. We identified 22 candidate proteins through MR analysis, with eight reaching strong colocalization evidence. WARS1 demonstrated a consistent positive causal effect on AMD risk. Additionally, LBP, VTN, and SDF2 were associated with wet AMD, whereas TGF-β1, VTCN1, and CD300LG showed potential links to AMD subtypes.

WARS1, an enzyme consisting of 417 amino acids, facilitates tryptophan-tRNA binding for protein synthesis and functions as an innate immune activator.[47,48] It promotes inflammation by activating Syk and Akt phosphorylation in the TREM signaling

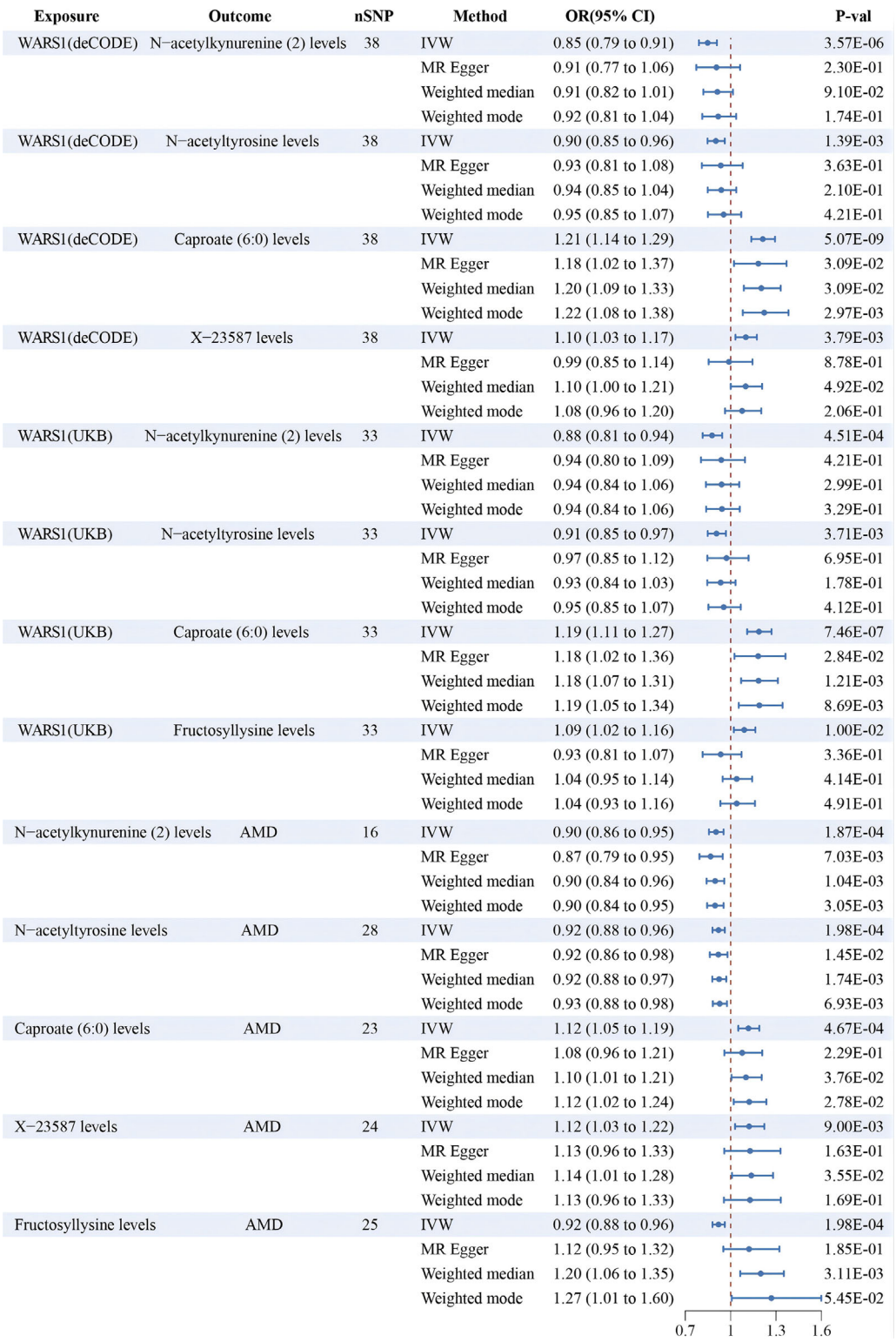

Figure 4. Forest plot for causal associations between WARS1 and plasma metabolites and between plasma metabolites and AMD.

cascade, triggering key inflammatory pathways (p38 mitogen-activated protein kinase [MAPK], nuclear factor-κB [NF-κB], extracellular signal-regulated kinase [ERK]), which are implicated in AMD pathogenesis.[49] The Akt/mTOR pathway contributes to retinal pigment epithelium (RPE) dysfunction and choroidal neovascularization, WHEREAS p38 MAPK, NF-κB, and ERK regulate oxidative stress,

Table 3. Two-Step Mendelian Randomization Analysis for Exploring the Mediating Role of Plasma Metabolites Between WARS1 and AMD

WARS1 Source	Mediator	Outcome	Beta-Mediation	SE	*Z*	*P*	Indirect Effect (%), OR (95% CI)
deCODE	*N*-acetyl-kynurenine (2) levels	AMD	0.017	0.007	2.58	9.81E-03	4.09 (0.99, 7.19)
	X-23587 levels		0.011	0.006	1.84	6.57E-02	2.65 (−0.17, 5.47)
	N-acetyltyrosine levels		0.009	0.004	2.56	2.41E-02	2.10 (0.58, 3.92)
	Caproate (6:0) levels		0.021	0.007	2.95	3.22E-03	5.17 (1.73, 8.62)
	N-acetyltyrosine levels	Dry AMD	0.010	0.004	2.40	1.66E-02	2.62 (0.48, 4.76)
	Glutamine degradant levels	Wet AMD	0.019	0.011	1.69	9.10E-02	5.33 (−0.85, 11.52)
	X-16580 levels		0.036	0.015	2.45	1.43E-02	10.30 (2.06, 18.54)
UK Biobank	*N*-acetyl-kynurenine (2) levels	AMD	0.013	0.006	2.37	1.79E-02	2.81 (0.48, 5.14)
	N-acetyltyrosine levels		0.008	0.004	2.15	3.17E-02	1.73 (0.15, 3.33)
	Caproate (6:0) levels		0.019	0.007	2.76	5.80E-03	3.98 (1.15, 6.81)
	Fructosyllysine levels		0.010	0.006	1.77	7.74E-02	2.14 (−0.24, 4.52)
	N-acetyltyrosine levels	Dry AMD	0.010	0.004	2.28	2.25E-02	2.01 (0.28, 3.74)
	Glutamine degradant levels	Wet AMD	0.017	0.011	1.54	1.24E-01	3.83 (−1.04, 8.70)
	X-16580 levels		0.034	0.015	2.32	2.02E-02	7.71 (1.21, 14.20)

inflammation, and angiogenesis—key drivers of AMD.[50–53] Although WARS1 itself has not been directly studied in AMD, its expression in immune cells involved in retinal inflammation suggests a potential role. Given the pro-inflammatory and immune-modulatory properties of WARS1 and its potential interactions with AMD-related pathways, our findings suggest that WARS1 may play a role in the pathogenesis of AMD. However, the specific cell types and retinal microenvironment in which WARS1 exerts its effects are not well understood. Further investigation of the spatial and temporal expression of WARS1 in retinal and choroidal tissues, as well as its interaction with AMD-related pathways, is warranted.

Other identified proteins also exhibit potential relevance to AMD. VTCN1 is associated with autoimmune regulation, and its inhibition has been linked to slowing age-related diseases.[54,55]. TGF-β1 regulates cellular signaling and apoptosis, with microRNA changes observed in AMD patients and intraocular administration reducing retinal damage in animal models.[56–58] CD300LG, a capillary endothelial sialomucin, may influence inflammation, although its role in dry AMD is unclear.[59,60] VTN, involved in extracellular matrix regulation, has been implicated in retinal neovascularization.[61,62] In addition, VTN is also particularly relevant to lipid metabolism. VTN is a component of drusen, the hallmark deposits in AMD, and has been implicated in lipid transport and extracellular matrix remodeling in the retina.[63] LBP and SDF2 are linked to vascular dysfunction and neovascular diseases, suggesting their potential involvement in AMD progression.[64–66] It should be noted that, referring to previous GWAS studies and proteomic studies, a variety of proteins such as CFH, C2/CFB, CFI, and C3 have been identified as playing important roles in the pathogenesis of AMD,[67] and CFB and C3 have also been implicated in lipid dysregulation and inflammation in AMD pathogenesis.[21] Although we found a potential role for CFB and C3 in early AMD in some genetic analyses based on circulating proteins and early AMD, our study did not find strong evidence linking these complement-associated proteins to AMD in the analyzed datasets, based on pre-established evidence classification criteria. Discrepancies may arise from differences in study design, sample characteristics, or genetic tools used. Moreover, some proteins identified in this study had not been previously reported, indicating the need for further research using complementary approaches such as targeted proteomics and functional validation experiments.

Given the high level of evidence linking WARS1 to a potential increased risk of AMD in this study,

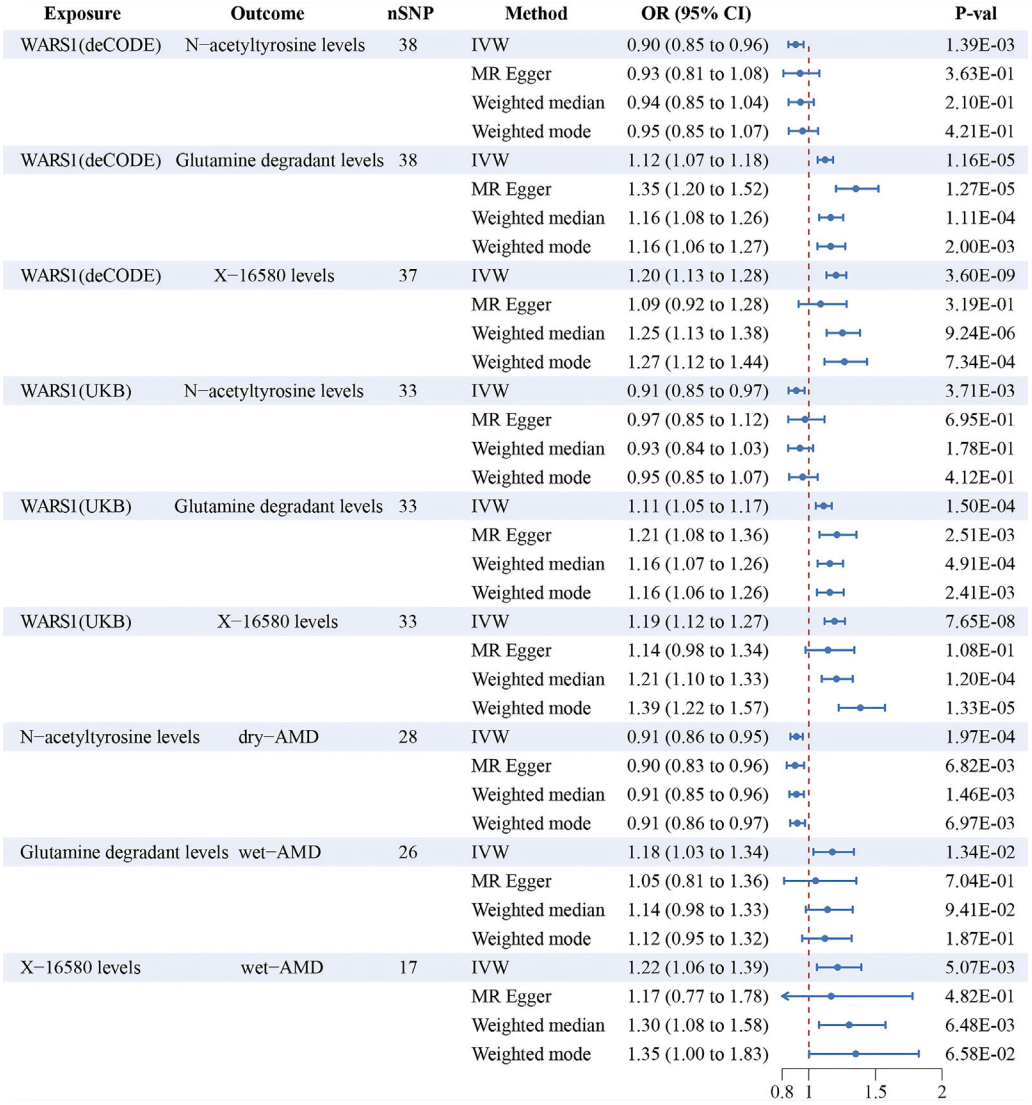

Figure 5. Forest plot for causal associations between WARS1 and plasma metabolites and between plasma metabolites and diverse AMD subtypes.

we further focused on WARS1 to explore the potential mechanism in AMD pathogenesis. We identified *N*-acetyl-kynurenine, *N*-acetyltyrosine, and caproate (6:0) as potential mediators, with *N*-acetyltyrosine being specifically associated with dry AMD and X-16580 with wet AMD. Importantly, all subgroup analyses in our study revealed that the circulating proteins and plasma metabolites influencing dry AMD and wet AMD are not identical. Existing evidence has shown that the primary AMD pathogenesis is mainly linked to genetic factors, and the genetic association signals of dry AMD and wet AMD are not exactly the same.[68] Therefore, our results offer valuable insights that could guide the exploration of potential drug targets for AMD.

An increasing number of investigations have confirmed the biochemical pathway continuity of circulating proteins and plasma metabolites, highlighting their significant role in the pathogenesis of various human diseases.[69–71] Despite this, there has been no previous research on the potential interaction between WARS1 and plasma metabolites. Our mediation analysis not only supports the genetic association between circulating proteins and AMD but also represents the first identification of specific plasma metabolites mediating the relationship between WARS1 and AMD. *N*-acetyltyrosine, an endogenous trigger of reactive oxygen species in animal mitochondria, regulates the expression of antioxidant enzymes and plays a crucial role in maintaining oxidative stress balance.[72] It has

been shown that *N*-acetyltyrosine pretreatment can significantly reduce lipid peroxidation in the serum of mice.[29] Given that oxidative stress in the RPE is a major trigger for AMD pathogenesis,[73] and lipid peroxidation is known to promote AMD progression through ferroptosis.[74] As we have previously stated, WARS1 has been shown to be an important regulator of protein synthesis and a pro-inflammatory factor in the human body, and there is a strong association between inflammation and oxidative stress.[75] Thus, we speculate that the mediating effect of *N*-acetyltyrosine between WARS1 and AMD is most likely achieved by influencing the oxidative stress pathway. Caproate, an important short-chain fatty acid, has received limited attention in disease mechanisms.[76] Although some studies have linked increased serum levels of caproate to immune diseases, the underlying mechanisms remain underexplored.[77] The roles of *N*-acetylkynurenine and X-16580 in AMD and their mechanisms remain largely unknown, warranting further investigation.

Limitations

Some circulating proteins identified in this study showed significant associations in SMR analysis but lacked support from Wald ratio/IVW MR methods or colocalization analysis, classifying them as lower level evidence (level 3). Despite this, they still offer valuable insights for potential AMD drug targets. As GWAS databases expand, the number of relevant instrumental variables will increase, likely strengthening these findings. However, several limitations still existed in the current MR analysis: (1) Although evidence suggests that gender may play a role in AMD, limitation of our data mining prevented us from stratifying SNPs further based on gender.[78] (2) Because the GWAS data associated with early AMD were partially derived from the UK Biobank, there was a small sample overlap when conducting MR analyses, which could have introduced bias into the overall results. (3) The study was limited to the European population, restricting generalizability to other ethnic groups. (4) Although GWAS data capture the genetic variation of the whole locus, it is not limited to the variation affecting the subsequent exons; therefore, GWAS-based data and MR studies can only infer the potential causal relationship, and the results of this study must be verified by additional experimental or clinical studies. (5) Imbalances in case–control proportions, particularly for AMD subtypes, limited the statistical power of this study. Future studies with more balanced datasets are needed to enhance result reliability.

Conclusions

This study represents a pioneering effort in investigating potential causal associations among genome-wide proteomes, plasma metabolites, and AMD at a genetic level. Our study provides further confirmation of the causal role of specific circulating proteins in AMD and identified the mediating role of plasma metabolites between WARS1 and AMD by integrating multiple genetic analyses, which shed new light on the clinical value of circulating proteins and metabolites in AMD treatment. Although our findings indicate a potential association between specific circulating proteins and AMD risk, as well as the mediating role of plasma metabolites in this relationship, the current evidence does not establish a definitive mechanistic link to AMD pathogenesis. These results should be viewed as a basis for generating new hypotheses and guiding future research. Further experimental research and functional analyses are necessary to confirm the role of circulating proteins and plasma metabolites in AMD pathogenesis and to clarify whether they directly contribute to AMD development or reflect secondary effects of the disease.

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