• Mendelian Randomization •

Integrating plasma proteomics and genome-wide association data to identify therapeutic targets for retinal neurodegenerative diseases in Europeans

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Abstract

● **AIM:** To employ proteome-wide Mendelian randomization (MR) to explore novel protein and drug targets for retinal neurodegenerative diseases (RND) in individuals of European ancestry.

● **METHODS:** This study used summary data-based MR to analyze the correlation between plasma protein levels and three RND, with protein data derived from two independent large-scale proteomics datasets. Potential drug targets were identified using Bayesian colocalization, followed by MR analysis, sensitivity testing, and external validation. Drug prediction and molecular docking were conducted to evaluate the druggability of the target proteins.

● **RESULTS:** The study identified six promising protein targets, each successfully replicated at least twice. The results included three proteins related to diabetic retinopathy (ICAM1, GCKR, WARS), two proteins related to age-related macular degeneration (WARS, BRD2), and two proteins related to glaucoma (SVEP1, NPTXR). Additionally, drug prediction and molecular docking indicated that five drugs (fenofibrate, trofinetide, ticagrelor, lifitegrast, acetaminophen) effectively bound to the target proteins.

● **CONCLUSION:** This study identified six potential protein targets for RND and five existing drugs with therapeutic potential. By integrating plasma proteomics with genetic data, it provides a cost-effective framework for drug discovery.

● **KEYWORDS:** retinal diseases; Mendelian randomization analysis; proteomics; drug discovery

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INTRODUCTION

Retinal neurodegenerative diseases (RND) constitute a group of age-related ocular diseases characterized by damage to retinal neurons and photoreceptors, primarily including diabetic retinopathy (DR), age-related macular degeneration (AMD), and glaucoma[1-2]. RND are the leading cause of blindness and visual impairment worldwide[3]. As the global population ages, the number of patients with RND is growing, yet currently available treatments remain limited[4]. Intravitreal injections of anti-vascular endothelial growth factor (VEGF) drugs have been demonstrated to be effective in slowing the progression of DR and AMD[5-6]. However, these treatments are inadequate or even ineffective in approximately two-fifths of DR patients and one-third of those with wet AMD[7-8]. Repeated injections are reported to result in significant complications, including endophthalmitis, retinal tears, and retinal detachment[9]. In glaucoma, the primary treatment focus is on lowering intraocular pressure using drugs or surgery, but this does not completely prevent the loss of retinal ganglion cells[10]. Thus, there is an urgent need to explore new drug targets and develop targeted therapies.

Circulating proteins, which are involved in various key physiological and pathological processes, serve as biomarkers of inflammation, infection, and several diseases[11]. These proteins are also potential therapeutic targets due to their specific regions and binding sites for biologics[12]. Existing literature indicates that many proteins involved in the pathogenesis of various ocular diseases originate from plasma[13]. Plasma proteins play a crucial role in the monitoring of RND, providing valuable insights into ocular physiology[14-16]. The blood-retinal barrier (BRB) is crucial for maintaining retinal homeostasis, and its disruption is a major

contributing factor to RND. Compromise of the BRB can lead to the leakage of plasma proteins into the vitreous and retinal layers[17]. An increase in plasma thyroxine-binding globulin has been shown in patients with DR compared to patients with macular schisisis and diabetes without retinopathy[18]. C-C motif chemokine ligand (CCL), a soluble mediator of inflammation-associated chemotaxis and a characteristic mediator of AMD, has been reported to be elevated in patients with AMD compared to controls, whereas CCL3 and CCL5 concentrations are significantly lower[19]. Furthermore, research conducted by Wang *et al*[20] demonstrated that elevated plasma lactoferrin is significantly associated with more advanced stages of glaucoma and reduced retinal nerve fiber layer thickness. Although the etiology of different RND varies, chronic inflammation due to immune dysregulation associated with retinal aging is a common feature[21-22]. This immune dysregulation also leads to age-related lesions such as DR, AMD, and glaucoma[21,23]. Nevertheless, the exact mechanism remains unclear. Therefore, investigating the relationship between RND and plasma proteins could clarify the role of plasma proteins in the immune mechanisms and assist in identifying potential drug targets and corresponding targeted drugs.

Recent advancements in high-throughput proteome sequencing technology and large-scale genome-wide association studies (GWAS) of plasma proteins have identified thousands of protein quantitative trait loci (pQTL), providing a valuable resource for exploring biomarkers and novel therapeutic targets through genetic approaches[12,24]. Mendelian randomization (MR) is an emerging epidemiological method that uses genetic variation as an instrumental variable (IV) to infer causality. By using the principle of random allocation of gametes during meiosis to simulate randomized controlled experiments, MR minimizes the effects of confounding factors and reverse causation, thus overcoming the limitations of observational studies.

This study systematically integrated pQTL and GWAS data to conduct a proteome-wide MR analysis investigating causal relationships between circulating plasma proteins and RND. Furthermore, we performed comprehensive targeted drug prediction coupled with molecular docking simulations to prioritize therapeutic candidates targeting key pathogenic proteins. These findings provide novel mechanistic insights into RND pathophysiology while establishing a target-protein framework that lays the groundwork for developing precision therapeutic strategies.

MATERIALS AND METHODS

Ethical Approval This study was a secondary analysis based entirely on GWAS summary statistics obtained from publicly available repositories. All original studies providing GWAS datasets had received prior ethical approval from their respective institutional review boards, with written informed consent documented for all participants. According to the International Ethical Guidelines for Health-related Research Involving Humans, secondary analyses of publicly available, de-identified data may be exempt from additional ethical review[25].

Study Design The overall study design was illustrated in Figure 1. We conducted summary data-based Mendelian randomization (SMR) analyses on pQTL data from a large-scale European cohort to explore the relationship between plasma protein abundance and RND risk. Bayesian colocalization analysis was performed to elucidate whether genetic variants were shared between plasma proteins and RND. To validate our findings, we performed additional two-sample MR analyses and used pQTL data and GWAS data from different sources to confirm the relationships found using SMR and MR analyses. Furthermore, drug prediction and molecular docking analyses were conducted on the candidate proteins to identify potential therapeutic drugs against RND targets and their associated pharmacological activities.

The MR study design relies on three basic assumptions: 1) genetic variation is strongly associated with exposure; 2) genetic variation is independent of any confounding factors; 3) genetic variation acts on outcomes only through exposure[26]. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology using MR reporting guidelines[27]. Full details of the sample sizes, population characteristics, and original publications of the included studies were presented in Figure 2.

Data Sources The discovery phase of the plasma protein pQTL data was derived from a study of 35 559 Icelanders conducted by Ferkingstad *et al*[28]. They detected and analyzed 4907 plasma proteins using a multi-analyte modifier aptamer binding assay, and analyzed the results using SOMAscan version 4 and found 18 084 sequence variants associated with plasma proteins. Summary statistics for DR, AMD, and glaucoma were all obtained from version R9 of the FinnGen database (https://r9.finngen.fi/), which included 10 413 DR cases and 308 633 controls, 8913 AMD cases and 348 936 controls, and 18 902 glaucoma cases and 348 936 controls. The three diseases under investigation (DR, AMD, and glaucoma) were defined in accordance with the International Classification of Diseases 10th Revision. DR included both non-proliferative DR and proliferative DR; AMD included both wet and dry forms; and glaucoma encompassed various types, including but not limited to primary open angle glaucoma, primary angle-closure glaucoma, and secondary glaucoma (due to inflammation or trauma). For the validation phase, pQTL data were obtained from the UK Biobank Pharmaceutical Proteomics Project (UKBPPP), which comprises 2923 plasma

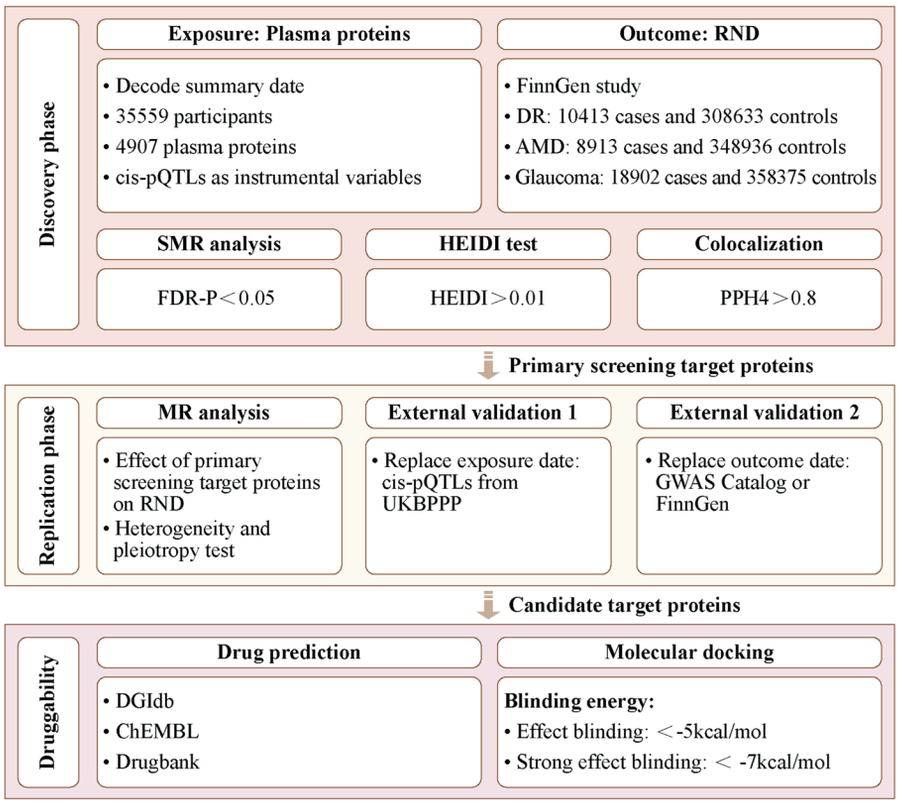

Figure 1 Overview of the study design, outlining the key methodological steps, including GWAS data sources, analytical methods, and reference thresholds used in both the discovery and validation phases, and the databases utilized for drug prediction and the key parameters of molecular docking analysis The figure was created using Office PowerPoint 2021 (Microsoft Corporation). AMD: Age-related macular degeneration; DR: Diabetic retinopathy; FDR: False discovery rate; HEIDI: Heterogeneity in Dependent Instruments; MR: Mendelian randomization; PP: Posterior probability; pQTL: Protein quantitative trait loci; RND: Retinal neurodegenerative diseases; SMR: Summary data-based Mendelian randomization.

Traits	Sample sizes (Case/Control)	Population	Consortium/Reference	GWAS ID/Download link
AMD (discovery phase)	357849 (8913/348936)	European ancestry	PMID: 36829046	finngen_R9_H7_AMD
DR (discovery phase)	319046 (10413/308633)	European ancestry	PMID: 36829046	finngen_R9_DM_RETINOPATHY_EXMORE
Glaucoma (discovery phase)	377277 (18902/358375)	European ancestry	PMID: 36829046	finngen_R9_H7_GLAUCOMA
AMD (external validation)	56637 (3685/52952)	European ancestry	PMID: 33893285	GCST90086108
DR (external validation)	351387 (6818/344569)	European ancestry	PMID: 36829046	finngen_R9_H7_RETINOPATHYDIAB
Glaucoma (external validation)	456348 (1190/455158)	European ancestry	PMID: 34737426	GCST90043783
plasma proteins (Icelander)	35559	European ancestry	PMID: 34857953	https://www.decode.com/summarydata/
plasma proteins (UKB-PPP)	54219	European ancestry	PMID: 37794186	http://ukb-ppp.gwas.eu

Figure 2 Details of the plasma proteins and RND data sources The figure was created using Office Word 2021 (Microsoft Corporation). AMD: Age-related macular degeneration; DR: Diabetic retinopathy; RND: Retinal neurodegenerative diseases.

proteins and revealed 14 287 significant genetic associations[24]. In addition, the GWAS summary data for the three RND used in the validation phase were obtained from three different data sources. Further details regarding the data sources are provided in Figure 2.

SMR Analysis We first used SMR analysis to explore the association between plasma protein abundance and RND occurrence by combining pQTL and GWAS datasets[29]. The pQTL included in the study during the discovery and validation phases were subjected to the same screening criteria: 1) to be the single most significantly associated cis-pQTL; 2) to be within a 1000-kb window of the corresponding protein-coding sequence; 3) to have demonstrated genome-wide significance ($P<5\times10^{-8}$); 4) to exclude any paired datasets between single single-nucleotide polymorphisms (SNPs) with allele frequency differences greater than 0.2[30]. SMR analyses were conducted using SMR software version 1.3.1. To control for multiple testing, the Benjamini-Hochberg method was applied after completing MR analyses for all plasma proteins within a single dataset for a specific disease outcome, ensuring that the false discovery rate (FDR) remained below 0.05. This approach accounts for the multiple comparisons performed within each dataset while assessing the association between plasma proteins and the given disease outcome. Furthermore, the results were subjected to the Heterogeneity in Dependent Instruments (HEIDI) test. If *P*-HEIDI was <0.01, this indicated that the observed associations between proteins and RND could be attributed to linkage disequilibrium (LD) and horizontal

pleiotropy[29,31]. Subsequently, Bayesian colocalization analyses were conducted for proteins that satisfied both the FDR<0.05 and *P*-HEIDI>0.01 criteria.

Bayesian Colocalization The coloc package (version 5.2.3) was employed to conduct colocalization analyses, aiming to estimate the posterior probability (PP) that plasma proteins and RND risk share causal variants within the same genomic region. To define the genomic region for colocalization analysis, we considered a 1 Mb window centered around the lead cis-pQTL of each protein, ensuring that the selected region captures relevant genetic variation while minimizing the inclusion of unrelated signals. This approach effectively controls for linkage disequilibrium, a potential confounder[32]. The colocalization analyses evaluated five mutually exclusive hypotheses: H0, the variant is not associated with either the protein or RND occurrence; H1, it is associated with the protein only; H2, it is associated with RND occurrence only; H3, it is associated with both the protein and RND occurrence but through distinct causal variants; H4, it is associated with both the protein and RND occurrence and shares a single causal variant. The posterior probability of each hypothesis was computed, with PPH4>80% considered strong evidence for a shared genetic variant[33].

MR Analysis and External Validation Since SMR relies on a single SNP as an IV for causal inference, its results may be influenced by horizontal pleiotropy and LD. To enhance the reliability of our findings, we conducted a two-sample MR analysis with external validation, which offers two key advantages: 1) incorporating multiple SNPs as IVs improves statistical power and strengthens the robustness of causal inference; 2) MR sensitivity analyses can detect potential horizontal pleiotropy, ensuring the validity of causal estimates. In the validation phase, target proteins whose association direction was inconsistent with that observed in the discovery phase were deemed to be non-robust and thus excluded. Furthermore, statistical significance was determined using a nominal *P*-value threshold of <0.05.

MR analysis and sensitivity analysis Two-sample MR analyses were conducted using the "TwoSampleMR" package (version 0.5.8), combining discovery-stage primary-screening target proteins as exposure and three FinnGen-origin RND occurrence as the outcome. The MR analyses served to reinforce the evidence derived from the primary analyses. In conducting MR studies on plasma proteins, the selection of IVs must adhere to the following criteria: 1) the variants must be cis-pQTL, meaning they are located in close proximity to the gene encoding the target protein; 2) they should exhibit a genome-wide significant association with plasma protein levels ($P<5\times10^{-8}$); 3) they must be independent, with minimal LD clustering ($r^2<0.001$), thereby reducing potential bias due to genetic correlation[34-35]. Causal effects were evaluated using the Wald ratio method where plasma proteins exhibited (SNPs), whereas when the number of SNPs was ≥2, analyses were conducted using the inverse-variance weighted (IVW), MR-Egger, maximum likelihood, and the weighted median methods. The IVW method was the primary method employed[36-37]. The IVW method demonstrates superior statistical power and the ability to explain heterogeneity; however, it relies on the assumption that "all SNPs are valid IVs". As a result, its estimates are susceptible to horizontal pleiotropy[38]. In contrast, other methods exhibit stronger resistance to horizontal pleiotropy. The weighted median method assumes that at least 50% of the included SNPs are valid IVs[39]. The MR-Egger method operates under the assumption of instrument strength independent of direct effect, allowing for the possibility that all IVs may be invalid[40]. Additionally, sensitivity analyses were performed to assess potential heterogeneity and horizontal pleiotropy, further strengthening the robustness of the findings. Cochran's *Q* test, implemented using both the MR-Egger and IVW methods, was conducted to evaluate heterogeneity among IVs. A *P*-value >0.05 indicated no significant heterogeneity, warranting the use of a fixed-effects IVW model, whereas a *P*-value ≤0.05 suggested substantial heterogeneity, necessitating the application of a random-effects IVW model[41-42]. To detect horizontal pleiotropy, MR-Egger regression analysis was employed, with a *P*-value <0.05 denoting significant pleiotropic effects[43-44]. Subsequently, we also calculated the statistical power of the MR analysis. Statistical power, which is based on the sample size, effect size, and the variance explained by the IVs, represents the probability of correctly rejecting the null hypothesis when it is false[45]. This ensures the study is adequately powered to detect a true causal effect, and only results with a power value greater than 0.8 were retained.

External validation In the external validation phase, an alternative independent proteomics data set (UKBPPP) was employed for SMR analyses, allowing for a reassessment of the associations obtained in the discovery phase. Subsequently, we changed the GWAS data source for RND to verify the relationship between the identified plasma proteins and RND occurrence in different sources of RND data.

Drug Prediction and Molecular Docking We analyzed the target proteins for druggability. Three drug characterization databases (DGIdb[46], ChEMBL[47], and DrugBank[48]) were initially searched to identify potential drugs against the target proteins. Additionally, the stage of drug development and the range of disease applications of the developed drugs were also retrieved. Next, we employed molecular docking techniques to model the interaction pattern of the drug with the target protein

and to calculate the binding energy. The tertiary structure of the target protein was obtained from the Protein Data Bank (http://www.rcsb.org/)[49]. Further, the structure of the drug was obtained from the PubChem Compound Database (https://pubchem.ncbi.nlm.nih.gov/)[50]. AutoDock (Linux, version 4.2.6) was used to molecularly dock the drugs with their corresponding target proteins[51]. Lower binding energies indicate more stable conformations. We considered a binding energy of less than -5 kcal/mol to indicate effective drug-target protein binding, and a binding energy of less than -7 kcal/mol to indicate strong binding[52]. Finally, the molecular docking results were visualized using PyMol version 2.5.7[53].

Classification Hierarchy of Proteins as Candidate Target Proteins Candidate target proteins were stratified according to the strength of evidence to clarify the priority of future studies of these drug targets. On the premise that *P*-HEIDI>0.01, colocalized PPH4>80%, and the direction of effect of all proteins in the validation and discovery phases is consistent must be met, the following criteria were set: 1) Proteins were validated by MR and had no horizontal pleiotropy. 2) Proteins were validated in the UKBPPP dataset. 3) Results were replicated in other GWAS data on RND. 4) The predicted drug had been approved for clinical application. 5) Drug and target protein showed strong binding in molecular docking analyses. Proteins that met four or more of the specified criteria were classified as class 1 target proteins. Those that met three of the criteria were classified as class 2 target proteins, and those that met two of the criteria were classified as class 3 target proteins.

RESULTS

In our study, we initially conducted SMR analyses to investigate the potential relationship between plasma protein abundance and the three RND (DR, AMD, and glaucoma). Next, colocalization analyses were performed on proteins that met both the FDR<0.05 and *P*-HEIDI>0.01 criteria. Proteins with robust shared genetic variants in colocalization analyses (PPH4>80%) were identified as our primary-screening target proteins. Then, MR and external validation were conducted, and positive results reinforced the evidence of an association between the initial target proteins and RND occurrence. This, in turn, facilitated the screening of more robust candidate proteins from among them. Subsequently, drug prediction and molecular docking analyses were performed on the candidate target proteins to ascertain the druggability value of the drug targets. Ultimately, the target proteins were classified according to the aforementioned strength of evidence, thereby providing a reference value for prioritizing studies on these drug targets in the future.

Screening the Associated Proteins Following the FDR correction, SMR identified 38, 21, and 6 plasma proteins that were associated with DR, AMD, and glaucoma risk, respectively. Following the elimination of proteins with the potential for pleiotropy (*P*-HEIDI<0.01), 18, 6, and 3 proteins remained, respectively. A colocalization analysis was conducted to identify plasma proteins with robust evidence of colocalization (PPH4>80%) as primary-screening target proteins. Ultimately, seven primary screening target proteins were obtained (Figure 3), including WARS, ICAM1, CFHR, and GCKR associated with DR; WARS and BRD2 associated with AMD; and SVEP1 and NPTXR associated with glaucoma. Specifically, the four plasma proteins genetically predicted to be associated with DR (WARS, ICAM1, CFHR3, GCKR) were all found to be positively associated with DR risk. In particular, WARS demonstrated consistent associations with DR and AMD. Furthermore, WARS was identified as a risk factor for AMD, while BRD2 was found to reduce the risk of AMD. For glaucoma, elevated SVEP1 levels may be associated with an increased risk of developing the condition. However, NPTXR has been identified as a protective factor, reducing the likelihood of developing glaucoma.

MR Analysis and External Validation

MR analysis and sensitivity analysis Two-sample MR analyses were performed for the seven primary-screening target proteins identified during the discovery phase as exposure factors and the three FinnGen-sources of RND occurrence as outcomes. The main findings indicated that the direction of the estimates derived from the MR analyses was consistent with the SMR for all seven proteins. Except for ICAM1 (*P*=0.29), for which the causality was not statistically significant, the remaining six proteins showed significant causality in the MR method Figure 4. MR Egger, maximum likelihood, and weighted median methods showed directional estimates consistent with the IVW results, which confirms the reliability of the MR study. In addition, sensitivity analyses did not detect significant levels of pleiotropy or heterogeneity.

External validation To enhance the robustness of the findings, the analyses were repeated using alternative data sources. First, an alternative independent proteomics data set (UKBPPP) was employed for SMR analyses, which demonstrated in Figure 5 and that, in accordance with the discovery phase results, ICAM1 was a risk factor for DR, WARS elevated the risk of DR and AMD, and NPTXR diminished the risk of glaucoma. The remaining proteins were not available in this dataset. Subsequently, GWAS RND data from other sources were analyzed for SMR. The results illustrated that our previous WARS, ICAM1, GCKR, BRD2, and SVEP1 findings were successfully confirmed in Figure 6. However, the association between WARS and AMD was not validated, and CFHR3 was excluded from further consideration because the direction of the effect was reversed in the validation and discovery phases.

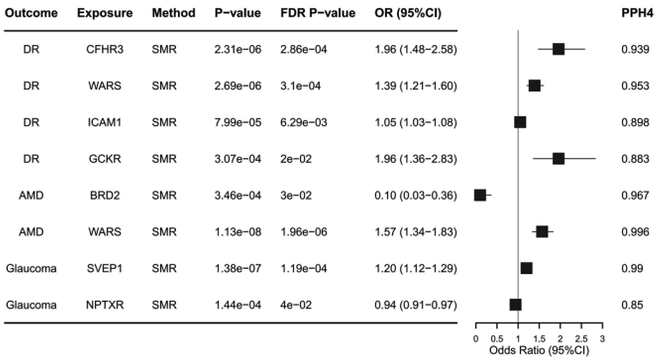

Figure 3 Forest plot illustrating the association between genetically predicted plasma protein levels and the risk of RND in the discovery phase, analyzed using the SMR method Proteins passing the statistical significance threshold (FDR<0.05 and PPH4>0.8) are highlighted as potential candidates for further validation. Forest plot was performed using the ggplot2 package (version 3.1.3), and the forestplot package (version 3.5.0) in the R programme (version 4.3.2). AMD: Age-related macular degeneration; CI: Confidence interval; DR: Diabetic retinopathy; FDR: False discovery rate; OR: Odds ratio; PP: Posterior probability; SMR: Summary data-based Mendelian randomization; RND: Retinal neurodegenerative diseases.

Drug Prediction and Molecular Docking We evaluated six candidate target proteins (WARS, ICAM1, GCKR, BRD2, SVEP1, NPTXR) for their druggability. Except for WARS, the relevant drugs for the remaining five candidate proteins (ICAM1, GCKR, BRD2, SVEP1, NPTXR) have been identified, which are also approved for clinical use. Lifitegrast, a drug that targets ICAM1, is an integrin antagonist that is primarily used for the treatment of dry eye. The utilization of micronized fenofibrate, a drug targeting GCKR, a potent lipid-lowering agent, has been demonstrated to be an effective strategy for the reduction of DR progression. The antiplatelet agent ticagrelor, a drug targeting SVEP1, has been demonstrated to improve endothelial cell function and is therefore employed in the treatment of cardiovascular conditions. The drug targeting BRD2 is a well-known non-steroidal anti-inflammatory drug acetaminophen. The neuroprotective drug Trofinetide is a potential drug targeting NPTXR and has recently been approved by the Food and Drug Administration as the first drug for treatment of Rett syndrome. To date, no drugs targeting WARS have been identified. Subsequently, molecular docking was performed using Autodock, and the corresponding drug IDs and protein structure data can be obtained from Figure 7. The results of the molecular docking (Figures 7 and 8) demonstrated that all drug-proteins exhibited effective binding (with binding energies below -5 kcal/mol) and that each drug successfully occupied the binding pocket of the candidate protein. Among them, fenofibrate and lifitegrast demonstrated the strongest binding activity, with binding energies of less than -7 kcal/mol to their respective proteins. In conclusion, five existing drugs were identified as having effective binding activity with the target proteins.

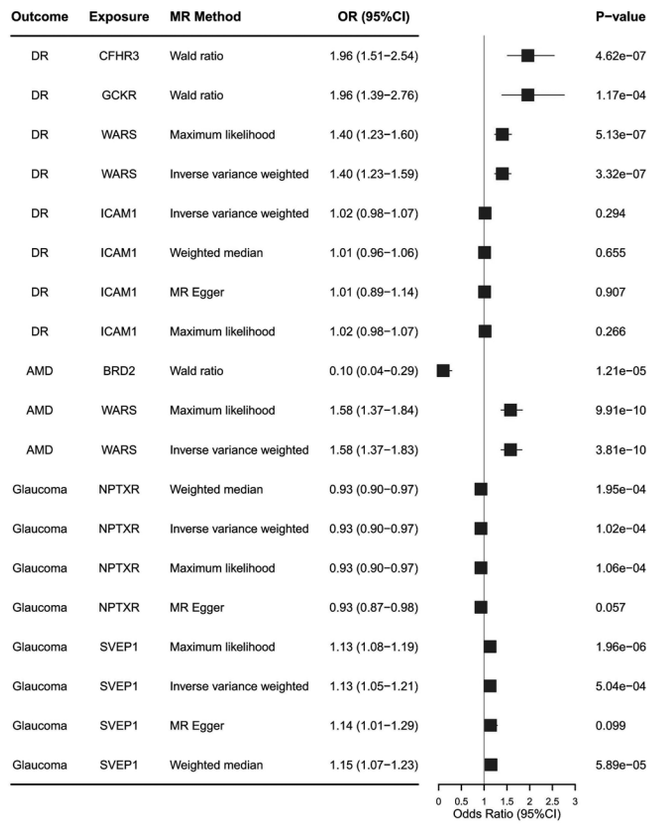

Figure 4 Forest plots displaying the validation results of the relationship between genetically predicted plasma protein levels and RND risk using additional MR methods This results aims to confirm the robustness and consistency of the findings from the discovery phase. Forest plot was performed using the ggplot2 package (version 3.1.3), and the forestplot package (version 3.5.0) in the R programme (version 4.3.2). AMD: Age-related macular degeneration; CI: Confidence interval; DR: Diabetic retinopathy; MR: Mendelian randomization; OR: Odds ratio; RND: Retinal neurodegenerative diseases.

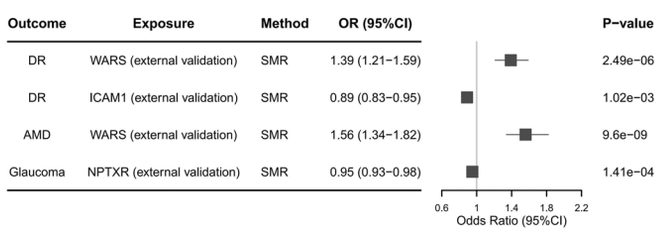

Figure 5 Forest plots from the external validation phase, where associations between plasma protein levels from the UKBPPP dataset and RND risk were re-evaluated using the SMR method This phase aimed to validate the findings using an independent proteomic dataset, ensuring the robustness and reproducibility of the observed associations. Forest plot was performed using the ggplot2 package (version 3.1.3), and the forestplot package (version 3.5.0) in the R programme (version 4.3.2). AMD: Age-related macular degeneration; CI: Confidence interval; DR: Diabetic retinopathy; OR: Odds ratio; SMR: Summary data-based Mendelian randomization; RND: Retinal neurodegenerative diseases.

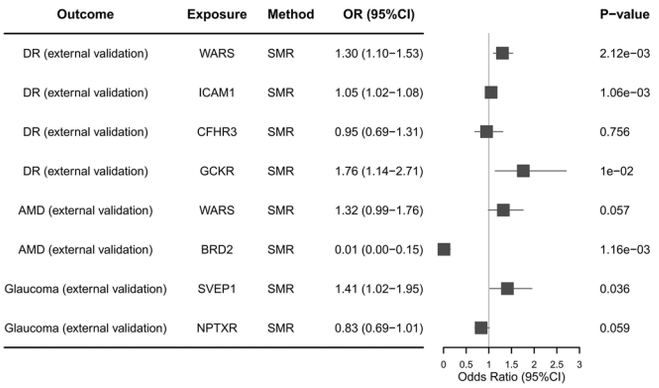

Figure 6 During the external validation phase, forest plots illustrate the reassessment of the relationship between genetically predicted plasma protein levels and the risk of RND using the SMR approach with independent RND GWAS datasets from other sources This phase specifically aimed to validate the findings using additional independent RND GWAS datasets, further reinforcing the robustness and generalizability of the observed associations. Forest plot was performed using the ggplot2 package (version 3.1.3), and the forestplot package (version 3.5.0) in the R programme (version 4.3.2). AMD: Age-related macular degeneration; CI: Confidence interval; DR: Diabetic retinopathy; OR: Odds ratio; SMR: Summary data-based Mendelian randomization; RND: Retinal neurodegenerative diseases.

Classification Hierarchy of Proteins as Candidate Target Proteins For DR, both ICAM1 and GCKR are class 1 target proteins, whereas WARS is a class 2 target protein. For AMD, BRD2 and WARS are classified as class 2 and class 3 target proteins, respectively. Finally, SVEP1 and NPTXR are class 2 target proteins for glaucoma (Figure 9).

DISCUSSION

To the best of our knowledge, this study is the first to explore the associations between plasma protein abundance and three RND (DR, AMD, and glaucoma) using proteome-wide SMR. We applied multiple methods to minimize bias and identify more robust and reliable target proteins for potential therapeutic targets for RND. This study was divided into three main phases. In the first phase, we identified seven primary target proteins through SMR and colocalization analyses. In the second phase, we performed a series of validations, including MR analysis, sensitivity analysis, and external validation, to identify six robust candidate target proteins: WARS, ICAM1, GCKR, BRD2, SVEP1, NPTXR. In the third phase, we searched through multiple drug databases to gain a comprehensive understanding of the existing drugs acting on the candidate proteins and used molecular docking techniques to confirm the interactions and affinities between the drugs and the target proteins. Subsequently, the candidate proteins were ranked according to the strength of the evidence, as shown in Figure 9.

ICAM1, an immunoglobulin and adhesion receptor primarily expressed by leukocytes and endothelial cells[54]. It binds to leukocyte function-associated antigen-1 (LFA-1) *via* its D1 domain, mediating the dynamic "rolling-activation-firm adhesion" cascade[55-56]. This interaction activates the endothelial RhoA/ROCK pathway, inducing actomyosin contraction, disrupting tight junction proteins, and compromising the integrity of the BRB, leading to vascular leakage[57-58]. Adherent leukocytes release matrix metalloproteinase-9, which further degrades components of the basement membrane[59]. Infiltrating inflammatory cells in the retina secrete tumor necrosis factor (TNF)-α and interleukin (IL)-1β, which upregulate ICAM1 *via* nuclear factor kappa-B (NF-κB)-mediated positive feedback and activate the complement system, sustaining a proinflammatory microenvironment[60-61]. This milieu promotes monocyte-derived VEGF-A secretion, driving pathological neovascularization. The newly formed endothelial cells overexpress ICAM1, recruiting additional inflammatory cells and perpetuating a vicious cycle of inflammation, vascular leakage, and hypoxia. ICAM1 antibodies have been effective in reducing leukostasis and ameliorating endothelial cell injury, cell death, and vascular leakage[62]. Furthermore, lifitegrast, an ICAM1/LFA-1 antagonist, has been approved as a targeted drug for treating dry eye[63]. In light of this evidence, we suggest that primarily targeting the initiating link of early DR *via* inhibition of the ICAM-1/LFA-1 signaling may impede DR progression.

GCKR, a regulatory protein that affects lipid and glucose homeostasis, is significantly associated with the susceptibility to type 2 diabetes mellitus (T2DM)[64-65]. GCKR is a key physiological inhibitor of glucokinase, suppressing it translocation to the cytoplasm, thereby reducing hepatic glucose phosphorylation, insulin secretion, and glycogen synthesis[66-67]. Genetic variation in GCKR, particularly the rs780094 variant, is significantly associated with T2DM[68]. A cohort study in Danish subjects demonstrated that the minor A allele of rs780094 correlates with elevated fasting serum triglycerides, impaired insulin secretion during fasting and oral glucose tolerance tests, and reduced HOMA-IR scores[67]. Thus, targeting GCKR inhibition may represent a potential therapeutic strategy for T2DM and its complications. Moreover, GCKR variants increase circulating free fatty acid levels, promoting lipotoxicity in retinal endothelial cells, and inducing cytokine production. This combined dysregulation of glucose and lipid metabolism synergistically disrupts the BRB and drives pathological neovascularization. Micronized fenofibrate, a lipid-lowering drug, represents a potential target for GCKR. Currently, a substantial number of randomized controlled trials are underway to ascertain its efficacy in patients with DR. Initial results indicate that fenofibrate can delay the progression of DR by improving retinal leukostasis,

Target	PDB ID	Drug	PubChem ID	Binding energy
GCKR	4BB9	Fenofibrate	3339	-8.6
NPTXR	AF-O95502-F1	Trofinetide	11318905	-5.2
SVEP1	-	Ticagrelor	9871419	-5.4
ICAM1	1IAM	Lifitegrast	11965427	-7.2
BRD2	5IG6	Acetaminophen	1983	-5.8

Figure 7 Docking results of potential target proteins with drug molecules The table presents the PDB ID of each protein, the PubChem ID of each drug, and the corresponding binding energy for each protein-drug pair. The figure was created using Office Word 2021 (Microsoft Corporation).

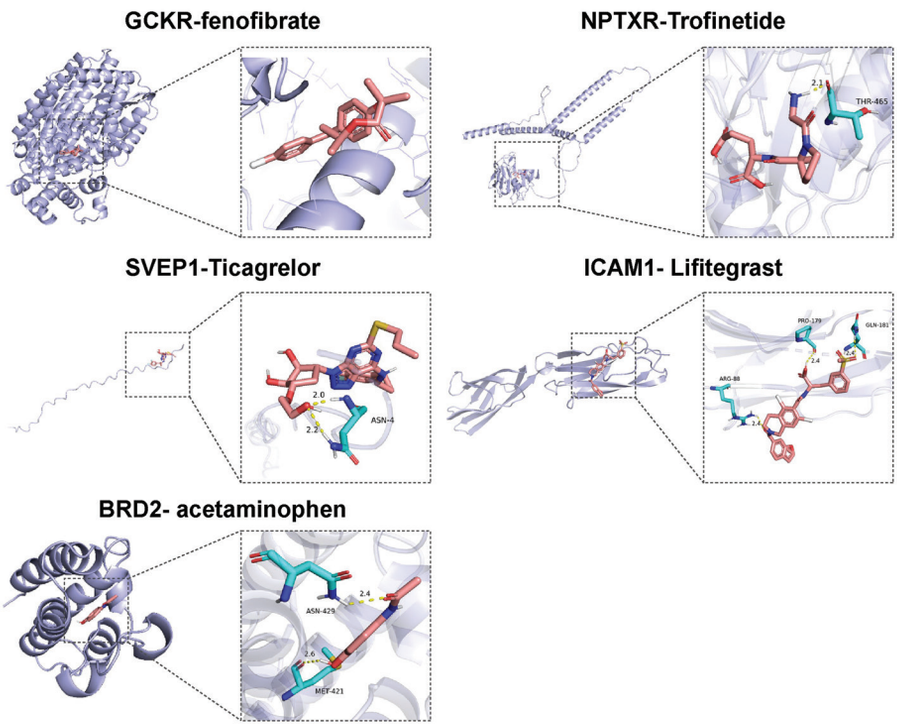

Figure 8 Molecular docking analysis of five candidate drugs with their respective potential target proteins identified in the study This figure provides structural insights into the protein-drug interactions, suggesting potential therapeutic relevance of the identified plasma proteins for RND treatment. The molecular docking results were visualized using PyMol version 2.5.7. RND: Retinal neurodegenerative diseases.

Candidate proteins	Outcome	MR analysis	UKBPPP	Replace RND date	Existing drugs	Strong blinding	Catagory
ICAM1	DR	×	√	√	√	√	Class 1
GCKR	DR	√	×	√	√	√	Class 1
WARS	DR	√	√	√	×	×	Class 2
BRD2	AMD	√	×	√	√	×	Class 2
WARS	AMD	√	√	×	×	×	Class 3
NPTXR	Glaucoma	√	√	×	√	×	Class 2
SVEP1	Glaucoma	√	×	√	√	×	Class 2

Figure 9 Classification hierarchy of candidate target proteins This table categorizes candidate target proteins based on their association with RND and key validation criteria. The figure was created using Office Word 2021 (Microsoft Corporation). AMD: Age-related macular degeneration; DR: Diabetic retinopathy; MR: Mendelian randomization; RND: Retinal neurodegenerative diseases.

vascular leakage, and reducing endothelial cell damage[69-71]. This evidence further underscores involvement of the GCKR protein in DR, although the precise mechanism remains to be elucidated.

NPTXR, a membrane protein involved in synaptic maturation and transmission[72-73], is regarded as a promising biomarker for synaptic dysfunction[74]. Reduced expression of NPTXR has been observed in the brains of patients with Alzheimer's disease, which may potentially be attributed to synaptic damage[75-76]. Although direct evidence linking glaucoma and NPTXR is lacking, the loss of dendritic and axonal synaptic terminals is characteristic of retinal ganglion cells degeneration[77]. Therefore, NPTXR has the potential to serve as a biomarker for glaucoma. Trofinetide is a neuroprotective drug that targets NPTXR and is approved by the Food and Drug Administration for treating Rett syndrome[78]. It is suggested

that trofinetide may regulate synapse formation, maturation, and neuroplasticity by regulating insulin like growth factor (IGF)-1 signaling in neurons and glia[79]. Moreover, trofinetide also increases the activation of transcription factor 3, thereby reducing the expression of inflammatory cytokine genes[80]. Considering research on neuroprotective targets is an important direction in the treatment of glaucoma[81], future studies should focus on investigating the potential of the target protein NPTXR and the drug trofinetide in the context of glaucoma.

SVEP1, an immune and age-related extracellular matrix protein, promotes cell adhesion by binding to integrin α9β1 with high affinity and amplify pro-inflammatory signaling[82]. It is also suggested as a promising therapeutic target for glaucoma[83], with genome-wide meta-analysis indicating SVEP1 as an important risk locus for primary open angle glaucoma[83]. Furthermore, Elenbaas *et al*[84]. have proposed that elevated plasma SVEP1 may serve as a risk factor for open angle glaucoma. Besides its pro-inflammatory properties, a few studies have postulated that the involvement of SEVP1 in open angle glaucoma may be due to the abnormal accumulation of extracellular matrix components, which increases the resistance to aqueous outflow in the trabecular pathway and raises intraocular pressure[85]. Activation of the phosphoinositide 3-kinase (PI3K)-protein kinase B (AKT) pathway plays a crucial role downstream of SVEP1[86-87]. PI3K-AKT-mediated extracellular matrix remodeling is associated with trabecular meshwork dysfunction, as it induces the secretion of fibronectin, laminin, and the matrix metalloproteinase inhibitor tissue inhibitor of metalloprotease (TIMP)-1[88-89]. Additionally, PI3K-AKT activation leads to mitochondrial dysfunction, oxidative stress, and premature cellular senescence, collectively contributing to increased intraocular pressure[90]. Ticagrelor, an antiplatelet drug that targets SVEP1, may prevent glaucoma resulting from blood vessel obstruction[91-92].

Currently, only a few studies exist on the role of WARS and BRD2 proteins in retina-related tissues, which suggests that these proteins may play a role in retinal inflammation. WARS is involved in various immune responses[93] and can directly bind to the Toll-like receptor 4-myeloid differentiation factor 2 complex on macrophages. This interaction promotes the production of a range of inflammatory and chemotactic factors, enhancing the infiltration of immune cells[94]. BRD2 is a significant member of the bromodomain protein family. Its malfunction alters acetylation levels associated with inflammatory processes[95-96]. Acetaminophen, a BRD2-target drug, is one of the most well-known non-steroidal anti-inflammatory drug[97], which is widely used in ophthalmology, particularly for AMD[98].

Despite providing useful insights, our study has a few limitations. First, the pQTL and GWAS data utilized in the discovery and validation phases were exclusively derived from European populations, which, while reducing demographic bias, limits the generalizability of findings to other populations. We feel, further validation in diverse ethnic cohorts is required. Second, another limitation of this study is the stringent criteria applied for selecting IVs for plasma proteins. While this approach helps reduce bias caused by weak IVs, it also results in the exclusion of certain plasma proteins that do not have eligible SNPs meeting the predefined thresholds. Consequently, this restriction limits the number of candidate proteins available for analysis, potentially omitting biologically relevant associations. Third, our MR analysis treated each RND category as a unitary entity, while clinically distinct subtypes (*e.g.*, proliferative *vs* non-proliferative DR) represent divergent pathophysiological stages with potentially discordant biomarker profiles. This aggregation may obscure meaningful differences between early neurodegenerative changes and late-stage vascular pathology, for example, VEGF-driven angiogenesis in advanced AMD versus complement-mediated inflammation in early dry AMD. Such phenotypic amalgamation may attenuate causal estimates *via* two key mechanisms: 1) dilution of stage-specific protein signals by different biological processes across disease phases, 2) misclassification of biomarkers acting primarily in subtype-specific pathways. Future proteomic investigations should adopt stratified analyses based on clinically different disease stages[16]. Fourth, the pQTL data used in this study were derived from plasma proteins, which may not fully capture the molecular changes specific to retinal tissue, aqueous humor, or vitreous fluid. Protein expression and regulation can differ significantly across biological compartments, meaning that the associations identified in plasma may predominantly reflect systemic effects rather than direct retinal pathophysiology. Consequently, some observed associations may be indirect rather than indicative of proteins acting within the retina itself. Future studies should incorporate eye-specific proteomic datasets to validate these findings and refine potential therapeutic targets. The integration of retinal tissue, aqueous humor, or vitreous-based pQTL data could enhance the specificity and translational relevance of MR analyses in ophthalmology. Finally, although we used databases to identify drug targeting of candidate proteins and their application in ophthalmology and other diseases in detail, molecular docking technology was used to explore the interactions and affinities between drugs and target proteins, guiding RND drug development priorities. We suggest that future studies should extensively employ *in vitro* and *in vivo* experiments, along with clinical studies, to verify the feasibility of our approach and findings.

In conclusion, our study identified six plasma proteins—ICAM1, GCKR, WARS, BRD2, SVEP1, and NPTXR—as potential drug targets for RND. In addition, we assessed the therapeutic potential of these targets through drug prediction and molecular docking, highlighting five approved drugs that could be used to treat RND. Prioritizing clinical trials on these identified target proteins and existing drugs will advance the development of RND treatment.

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