• Mendelian Randomization •

Assessment of causality between circulating inflammatory proteins and subtypes of diabetic retinopathy

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Abstract

● **AIM:** To explore the causal links among circulating inflammatory proteins (CIPs) and the varying severities of diabetic retinopathy (DR).

● **METHODS:** This research utilized a two sample Mendelian randomization (MR) approach to explore the causal relationships between 91 CIPs and various severities of DR: background DR (BDR) or non-proliferative DR (NPDR), and proliferative DR (PDR). Single-nucleotide polymorphisms (SNPs) related to the 91 CIPs as exposure factors were identified. These SNPs were selected from an extensive genome-wide association study (GWAS) analyzing large genomic datasets. Genetic variation data of various DR phenotypes provided by the FinnGen collaboration were utilized as outcomes. Inverse-variance weighting (IVW) was used as the main MR analysis. Robustness of study results was evaluated through a series of sensitivity analyses, employing the MR-pleiotropy-test and mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) to confirm the absence of pleiotropy.

● **RESULTS:** In a bidirectional MR analysis, we uncovered a complex relationship between CIPs and DR. Elevated levels of tumor necrosis factor ligand superfamily member 14 (TNFSF14), latency associated peptide transforming growth factors beta-1 (LAP-TGF-beta1), interleukin-10 (IL-10), and vascular endothelial growth factor A (VEGF-A) were associated with a reduced risk of NPDR. Conversely, elevated levels of fibroblast growth factor 23 (FGF-23) were associated with an increased risk of NPDR. Concentrations of adenosine deaminase (ADA), matrix metalloproteinase-10 (MMP-10), eotaxin, and IL-10 showed elevated levels and were linked to a reduced risk of NPDR. On the other hand, the levels of oncostatin-M, beta-nerve growth factor (β-NGF), and interleukin-7 (IL-7) were elevated and associated with an increased risk of SNPDR. Elevated levels of ADA, MMP-10, and macrophage colony-stimulating factor 1 (CSF1) were linked to a lower likelihood of PDR. Conversely, elevated levels of Caspase 8 and glial cell line-derived neurotrophic factor (GDNF) were associated with an increased risk of PDR. In reverse MR analysis, DR affected the expression of these factors.

● **CONCLUSION:** Our research demonstrates evidence supporting a potential causal link between key inflammatory factors and the risk and prognosis of various DR phenotypes. These findings emphasize the regulation of inflammatory factors responses as a strategic approach for preventing and managing DR. Altogether, our results validate the pathogenic role of inflammatory factors dysregulation in DR and support the rationale for exploring immunotherapeutic targets further.

● **KEYWORDS:** Mendelian randomization; circulating inflammatory proteins; background diabetic retinopathy; proliferative diabetic retinopathy

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INTRODUCTION

With the improvement of living standards, the incidence of blindness attributable to diabetic retinopathy (DR) has been rising annually. This trend severely impacts patient's life and increases the healthcare burden on society. Clinically, DR is categorized based on the presence of neovascularization

into background DR (BDR) or non-proliferative DR (NPDR), and proliferative DR (PDR)[1]. The NPDR stage is further subdivided into mild, moderate, severe NPDR. Approximately one-third of diabetic patients have DR, with over two-thirds exhibiting NPDR. Additionally, 1%-2% of type 2 diabetic patients may develop severe NPDR/PDR. In severe NPDR, inadequate blood supply due to dysfunctional microvasculature leads to oxidative stress and subsequent compensatory blood vessel growth, progressing to PDR. As fibrotic new blood vessels form, they extend along the retina's surface and potentially into the vitreous. They can result in tractional retinal detachment[2]. Hence, identifying potential risk factors for DR and implementing timely interventions is essential for its prevention and treatment.

DR is a complex disease involving multiple pathophysiological processes. Recent evidence increasingly suggests that DR is characterized by chronic, low-grade inflammatory damage, known as microinflammation. Cytokines, a group of proteins or peptides produced by immune cells, regulate immune and inflammatory responses. They play a significant role in the development and progression of DR by transmitting cellular signals through a complex immunoregulatory network[3-4]. In the context of DR, inflammation is not merely a response to retinal damage but a key event driving disease progression. Elevated levels of inflammatory mediators and activation of cellular inflammatory processes disrupt the blood retinal barrier (BRB), resulting in macular edema and damage to retinal neurons. Early leukocyte activation, triggered by metabolic disorders involving lipid and glucose metabolism, results in recurrent capillary occlusion and progressive retinal ischemia[5]. Inflammation can persist throughout DR, manifesting as increased expression of systemic and ocular inflammatory-related molecules including C-reactive protein (CRP), neutrophils, and intraocular inflammatory biomarkers[6-7]. Through vitreous proteomics analysis, researchers have identified variations in inflammatory factors within the aqueous humor or vitreous at different stages of DR[8]. In the blood serum of individuals with diabetes, inflammatory factors such as interleukin (IL)-6, IL-8, tumor necrosis factor (TNF)-α are markedly elevated, with their expression closely linked to the progression of DR[9]. These changes underscore the critical role of inflammatory factors in the progression of DR and provide fresh insights for its treatment and understanding. Nonetheless, these studies might not encompass the full scope, and clearly establishing a causal link between inflammatory factors and DR is difficult due to potential confounders or reverse causation.

Mendelian randomization (MR) leverages genetic variations to determine how exposures impact outcomes, providing a robust methodological framework[10]. Using bidirectional MR, we explored the connections involving 91 circulating inflammatory proteins (CIPs) and various stages of DR. These findings not only reveal potential therapeutic targets for DR but also suggest the potential of personalized medical approaches. By examining the phenotypes and functions of patients' CIPs, we can predict individual responses to targeted therapies, thereby enabling customized treatment strategies.

MATERIALS AND METHODS

Study Design In this study, we adopted bidirectional two sample MR analysis to uncover the links among 91 CIPs and onset of DR, setting the stage for methods aimed at mitigating and treating diseases. To ensure the validity of the MR analysis, we adhered to three core assumptions[11]: 1) Relevance assumption: the selected genetic variants selected as instrumental variables (IVs) need strong links to the studied risk factors, namely CIPs; 2) Independence assumption: these IVs must be independent of any known or unknown confounding factors to ensure the purity of the results. To address this, Phenoscanner (https://www.phenoscanner.medschl.cam.ac.uk/) can be used to eliminate single-nucleotide polymorphisms (SNPs) associated with potential confounding variables[12]; 3) Exclusion restriction assumption: the impact of IVs on the disease outcome should only be through the risk factor itself, not any other direct causal pathways[13].

We conducted a rigorous selection of the latest 91 CIPs for comprehensive study to determine the genetic proxies as IVs. Using the summary statistics from the GWAS of Finnish NPDR, severe NPDR (SNPDR), and PDR, we performed MR analysis. Subsequently, with DR as the exposure and the 91 CIPs as the outcomes, we conducted reverse MR analysis to delve deeper into the causal aspects of various severities of DR and these inflammatory proteins. To reduce the potential bias caused by population stratification, the participants in the study were solely of European descent. Figure 1 illustrated the assumptions underlying the MR methodology employed in our study. Figure 2 presented the study design of MR used in our study.

Data Source for Inflammatory Proteins In this study, we utilized data from 14 824 participants primarily of European ancestry, drawn from 11 distinct cohorts. This data was acquired through the latest genome-wide association study (GWAS) summary statistics by Zhao *et al*[14], employing the Olink targeted inflammation immunoassay panel in conjunction with whole-genome genetic data and plasma proteomics to identify proteins associated with inflammation. Each cohort applied a GWAS analysis using an additive genetic association model based on linear regression. The effects of inflammation-related proteins were reported based on the impact of per-allele effect size on levels normalized through inverse-rank transformation. To manage population

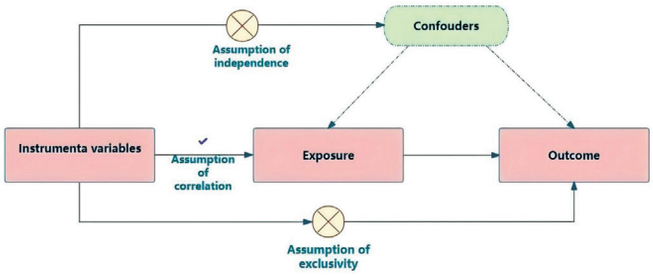

Figure 1 Foundations of Mendelian randomization for circulating inflammatory factors and diabetic retinopathy.

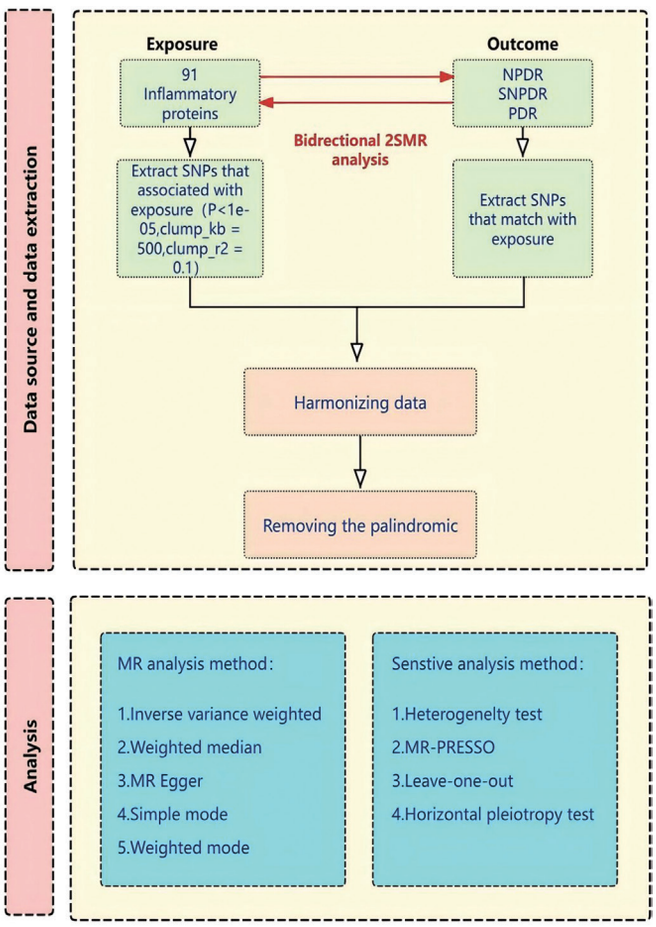

Figure 2 Flowchart of the mendelian randomization analysis for 91 circulating inflammatory proteins and diabetic retinopathy SNPs: Single nucleotide polymorphisms; NPDR: Non-proliferative diabetic retinopathy; SNPDR: Severe non-proliferative diabetic retinopathy; PDR: Proliferative diabetic retinopathy.

substructure and minimize the impact of potential confounders, genetic principal components were employed for adjustments. Additionally, the model accounted for covariates such as age and gender. Detailed quality control measures and data source information are documented in the original literature. The comprehensive summary statistics for each protein analyzed in the GWAS are publicly accessible on designated websites and the EBI GWAS Catalog (GCST90274758 to GCST90274848)[14].

Data Sources for Diabetic Retinopathy The DR GWAS data was obtained from the FinnGen Research Project (https://www.finngen.fi/en/access_results)[15]. Genetic research benefits from population isolates, such as those found in Finland, due to historical bottlenecks concentrating deleterious alleles in low-frequency variants. In this study, we selected three DR phenotypes with varying degrees of severity as outcomes: BDR (4011, 344569, Finngen_R9_H7_RETINOPATHYDIAB_BKG), SNPDR (816, 344569, Finngen_R9_H7_RETINOPATHYDIAB_BKG_SE-VERE) and PDR (2468, 344569, Finngen_R9_H7_RETINOPATHYDIAB_PROLIF). The analysis adjusted for potential confounding factors, including sex and age, genotyping batch, genetic relatedness, duration of diabetes, history of hypertension, cardiovascular disease, and the average daily dose of hypoglycemic drugs after cohort entry. The FinnGen study, utilizing data from nine Finnish biobanks, is a comprehensive nationwide GWAS analysis. Due to minimal overlap with the UK Biobank GWAS, which involves various UK centers, we believe the risk of bias is reduced by the limited sample overlap between inflammatory factors and DR data.

Single Nucleotide Polymorphisms Selection In conducting MR analysis, it is crucial to select IVs for SNPs that are highly relevant to exposure. To this end, we have adopted the following strategy: First, we set a rigorous statistical threshold ($P<5\times10^{-8}$) to pinpoint SNPs linked to outcomes and 91 CIPs. However, we noted that at this threshold, the number of available SNPs for some inflammatory proteins was relatively low. Therefore, to increase the number of positive SNPs, we adjusted the threshold to 1e-05. Subsequently, we clustered the SNPs to eliminate linkage disequilibrium (setting the range to 500 kb, with an r^2 of 0.1)[16]. During this process, we excluded SNPs that were inconsistent with intermediate allele frequencies or constituted palindromes. Finally, F-statistic was utilized to evaluate the instrumental strength of each SNP, determined by the formula:

$$F=\frac{R^2\bullet(N-2)}{1-R^2}$$

where R^2 stands for the amount of variance explained, where N respects the sample size. To ensure accurate results, we excluded SNPs with an F-statistic below 10, thereby eliminating unreliable IVs from the analysis[17].

Mendelian Randomization and Sensitive Analysis We used five distinct approaches to assess the impact of the exposure on the outcome variables in this study: the random effects inverse-variance weighting (IVW)[18], MR-Egger regression, weighted median (WM)[19], weighted mode, and the simple mode method. Among these, IVW is considered the most effective and unbiased method for detecting causal relationships in two-sample MR analysis[20].

To ensure the stability and reliability of our MR results, we employed three different sensitivity analysis methods to

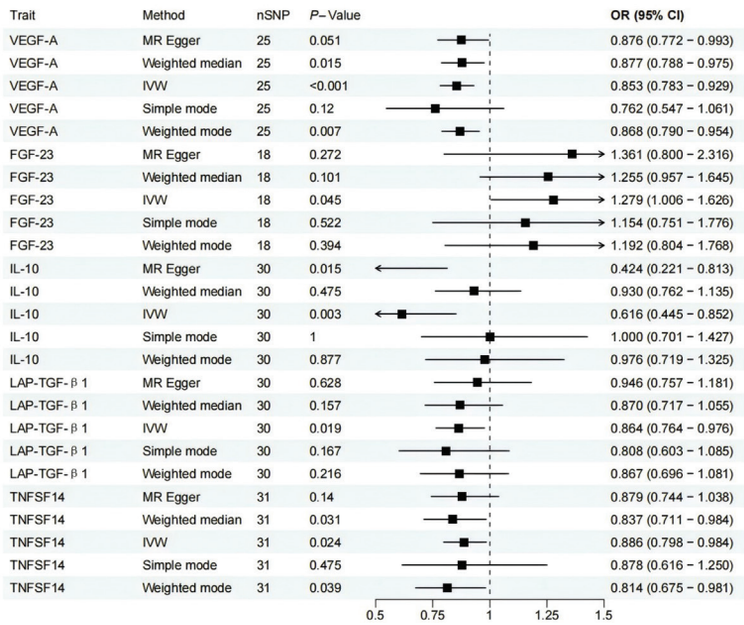

Figure 3 A forest plot displaying Mendelian randomization results highlighting the causal associations of five circulating inflammatory proteins with non-proliferative diabetic retinopathy VEGF-A: Vascular endothelial growth factor A; FGF23: Fibroblast growth factor 23; IL-10: Interleukin-10; LAP-TGF-beta1: Latency-associated peptide transforming growth factor beta 1; TNFSF14: Factor ligand superfamily member 14; OR: Odds ratio; CI: Confidence interval; IVW: Inverse-variance weighting; MR: Mendelian randomization.

evaluate the robustness of our findings, such as heterogeneity, pleiotropy, and Leave-One-Out tests. Initially, we employed the Cochran Q statistic to access heterogeneity, where a *P*-value under 0.05 suggested heterogeneity. which usually has higher statistical power when a larger number of studies are included[21]. Second, we assessed genetic pleiotropy using the intercept term from MR-Egger and mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO). A *P*-value below 0.05 implies horizontal pleiotropy[22]. Global MR-PRESSO detects outliers and removes them upon discovery. After outlier removal, MR analysis is conducted again. Lastly, the Leave-One-Out method involved sequentially excluding individual SNPs, recalculating casual effect estimates using the remaining ones to evaluate the impact of each SNP on the regression coefficient[23]. If pleiotropy is detected, Radial MR will be employed to remove outliers and reanalyze the data[24]. The aforementioned statistical analyses were conducted using the Two-Sample-MR package within the R software environment.

RESULTS

Influence of 91 Inflammatory Proteins on NPDR By employed the IVW method, we assessed the relationship between 91 CIPs and NPDR. As shown in Figure 3, elevated levels of tumor necrosis factor ligand superfamily member 14 (TNFSF14), latency associated peptide transforming growth factors beta-1 (LAP-TGF-beta1), IL-10, and vascular endothelial growth factor A (VEGF-A) were associated with a reduced risk of NPDR [odds ratio (OR): 0.886, 95% confidence interval (CI): 0.798-0.984, *P*=0.024; OR: 0.864, 95%CI: 0.764-0.976, *P*=0.019; OR: 0.616, 95%CI: 0.445-0.852, *P*=0.003; OR: 0.853, 95%CI: 0.783-0.929, *P*<0.001]. Conversely, elevated levels of fibroblast growth factor 23 (FGF-23) were associated with an increased risk of NPDR (OR: 1.279, 95%CI: 1.006-1.626, *P*=0.045).

Influence of 91 Circulating Inflammatory Proteins on SNPDR To assess the relationships between SNPDR and 91 CIPs, the IVW method was utilized. The findings are illustrated in Figure 4. Concentrations of adenosine deaminase (ADA), matrix metalloproteinase-10 (MMP-10), eotaxin, and IL-10 showed elevated levels and were linked to a reduced risk of NPDR (OR: 0.766, 95%CI: 0.617-0.949, *P*=0.015; OR: 0.629, 95%CI: 0.483-0.819, *P*=0.001; OR: 0.686, 95%CI: 0.522-0.901, *P*=0.007; OR: 0.459, 95%CI: 0.306-0.687, *P*<0.001).

On the other hand, the levels of oncostatin-M, beta-nerve growth factor (β-NGF), and IL-7 were elevated and associated with an increased risk of SNPDR (OR: 1.486, 95%CI: 1.065-2.072, *P*=0.02; OR: 1.545, 95%CI: 1.130-2.112, *P*=0.006; OR: 1.529, 95%CI: 1.008-2.318, *P*=0.046).

Influence of 91 Circulating Inflammatory Proteins on PDR Employing the IVW method, we assessed the relationships among 91 CIPs and PDR. The findings were illustrated in Figure 5. Elevated levels of ADA, MMP-10, and macrophage colony-stimulating factor 1 (CSF1) were linked to a lower likelihood of PDR (OR: 0.842, 95%CI: 0.744-0.954, *P*=0.007; OR: 0.848, 95%CI: 0.728-0.987, *P*=0.033; OR: 0.831, 95%CI: 0.691-0.998, *P*=0.048). Conversely, elevated levels of Caspase 8 and glial cell line-derived neurotrophic factor (GDNF) were associated with an increased risk of PDR (OR: 1.378, 95%CI:

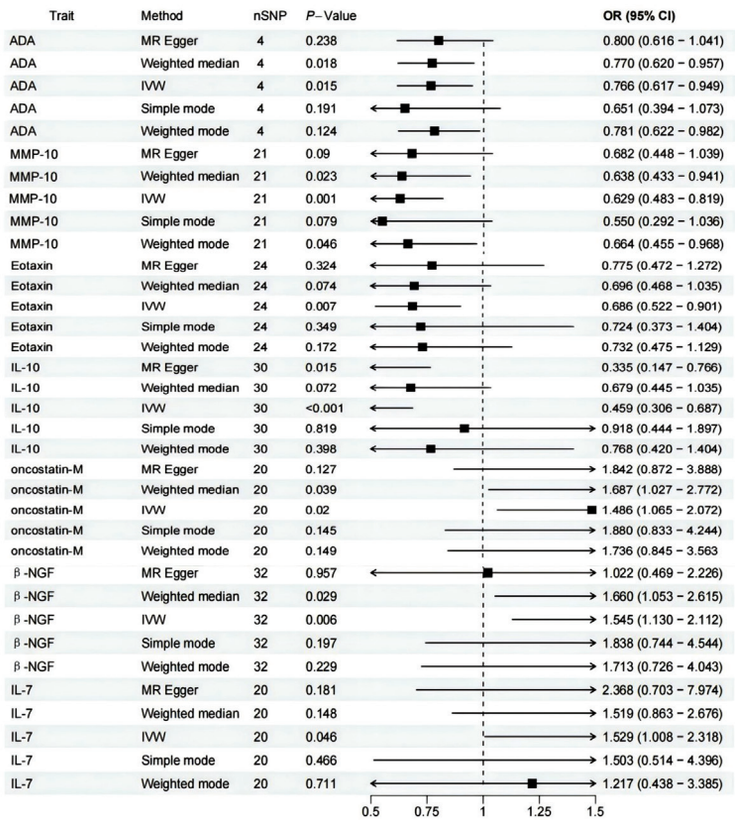

Figure 4 A forest plot displaying Mendelian randomization results highlighting the causal associations of seven circulating inflammatory proteins with non-proliferative diabetic retinopathy ADA: Adenosine deaminase; MMP-10: Matrix metalloproteinase-10; IL-10: Interleukin-10; β-NGF: Beta-nerve growth factor; IL-7: Interleukin-7; OR: Odds ratio; CI: Confidence interval; IVW: Inverse-variance weighting; MR: Mendelian randomization.

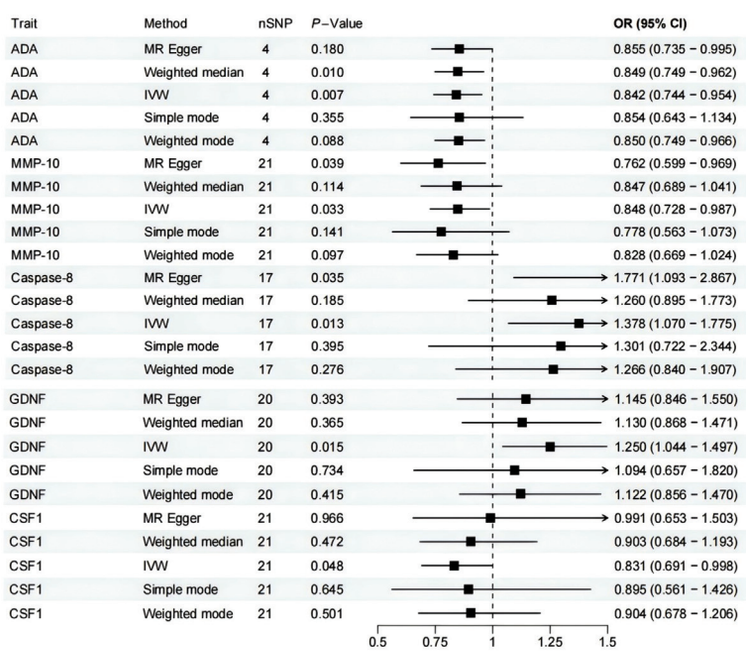

Figure 5 A forest plot displaying Mendelian randomization results highlighting the causal associations of five circulating inflammatory proteins with proliferative diabetic retinopathy ADA: Adenosine deaminase; MMP-10: Matrix metalloproteinase-10; GDNF: Glial cell line-derived neurotrophic factor; CSF1: Macrophage colony-stimulating factor 1; OR: Odds ratio; CI: Confidence interval; IVW: Inverse-variance weighting; MR: Mendelian randomization.

1.070-1.775, *P*=0.013; OR: 1.250, 95%CI: 1.044-1.497, *P*=0.015).

Influence of NPDR on 91 Circulating Inflammatory Proteins To investigate reverse causality, we thoroughly

examined SNPs exhibiting robust and independent associations with various DR phenotypes. We used the IVW method to evaluate the relationship between NPDR and 91 CIPs. Increased NPDR risk was potentially associated with increased concentrations of Axin-1, natural killer cell receptor 2B4 (CD244), C-X-C motif chemokine 11 (CXCL11), IL-12 subunit beta (IL-12B), IL-15 receptor subunit alpha (IL-15RA), IL-17C, IL-1α), IL-6, MMP-10, programmed cell death 1 ligand 1 (PD-L1), SIR2-like protein 2 (SIRT2), tumor necrosis factor receptor superfamily member 9 (TNFRSF9), and TNF related activation induced cytokine (TRAIL; OR: 1.027, 95%CI: 1.008-1.046, *P*=0.005; OR: 1.022, 95%CI: 1.003-1.041, *P*=0.019; OR: 1.021, 95%CI: 1.000-1.042, *P*=0.044; OR: 1.022, 95%CI: 1.000-1.044, *P*=0.011; OR: 1.026, 95%CI: 1.007-1.045, *P*=0.007; OR: 1.031, 95%CI: 1.008-1.055, *P*=0.009; OR: 1.041, 95%CI:1.016-1.066, *P*=0.001; OR: 1.019, 95%CI: 1.006-1.036, *P*=0.024; OR: 1.027, 95%CI: 1.007-1.047, *P*=0.024; OR: 1.022, 95%CI: 1.005-1.039, *P*=0.010; OR: 1.020, 95%CI: 1.004-1.037, *P*=0.015; OR: 1.036, 95%CI: 1.015-1.057, *P*=0.001; OR: 1.032, 95%CI: 1.016-1.050, *P*<0.001).

Influence of SNPDR on 91 Circulating Inflammatory Proteins The IVW method was utilized to estimate the relationship between SNPDR and 91 CIPs. there could be a connection the among increased risk of SNPDR and the elevated levels of MMP-10 (OR: 1.033, 95%CI: 1.011-1.057, *P*=0.004). There may the increased risk of SNPDR and the decreased levels of C-C motif chemokine (CCL) and osteoprotegerin (OPG; OR: 0.943, 95%CI: 0.912-0.976, *P*=0.001; OR: 0.977, 95%CI: 0.961-0.994, *P*=0.007).

Influence of PDR on 91 Circulating Inflammatory Proteins We employed the IVW to determine the association between SNPDR and 91 CIPs. A heightened risk of PDR could be associated increased concentrations of IL-12B, IL-15RA, and MMP-10 (OR: 1.035, 95%CI: 1.017-1.054, *P*=0.0001; OR: 1.026, 95%CI: 1.004-1.049, *P*=0.020; OR: 1.032, 95%CI: 1.009-1.056, *P*=0.007). In Summary. In the results of reverse analysis, DR may promote or inhibit the expression of some inflammatory factors (Figure 6).

Sensitive Analysis After conducting the Cochran Q test, we found that the results of using inflammatory factors as either exposure factors or outcome variables mostly show no heterogeneity, as detected by the MR-Egger and IVW methods, indicating that the findings across different studies are consistent. If MR-PRESSO identifies pleiotropy, we will use Radial MR to remove outliers and then reanalyze the data[24].

DISCUSSION

Recognition of the influence of circulating inflammatory cytokines on the progression of DR at various stages has

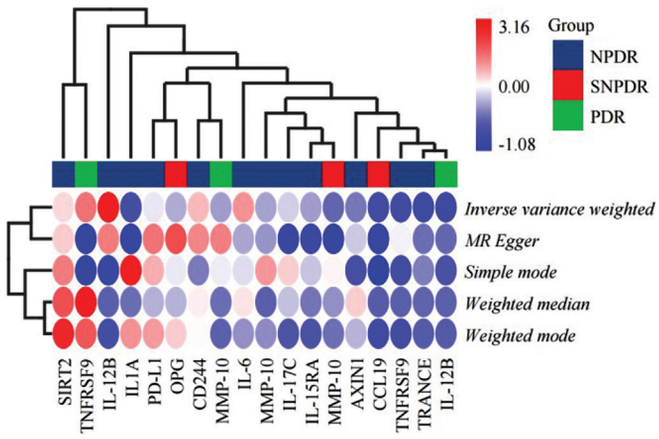

Figure 6 In reverse Mendelian randomization analysis, diabetic retinopathy affects the expression of these factors SNPs: Single nucleotide polymorphisms; NPDR: Non-proliferative diabetic retinopathy; SNPDR: Severe non-proliferative diabetic retinopathy; PDR: Proliferative diabetic retinopathy; SIRT2: SIR2-like protein 2; TNFRSF9: Tumor necrosis factor receptor superfamily member 9; TNFSF14: Tumor necrosis factor ligand superfamily member 14; IL-11: Interleukin-11; IL-1A: Interleukin-1-alpha; PD-L1: Programmed cell death 1 ligand 1; OPG: Osteoprotegerin; CD244: Natural killer cell receptor 2B4; MMP-10: Matrix metalloproteinase-10; IL-6: Interleukin-6; MMP-7: Matrix metalloproteinase-7; IL-8: Interleukin-8; IL-17C: Interleukin-17C; MMP-12: Matrix metalloproteinase-12; IL-18RA: Interleukin-18 receptor subunit alpha; AXIN1: Axis inhibitor 1; IL-19: Interleukin-19; CCL19: C-C motif chemokine 19; TRANCE: TNF-related activation-induced cytokine; IL-12B: Interleukin-12 subunit beta.

grown significantly. Under physiological conditions, retinal immune cells maintain immune homeostasis within the retina. However, in a hyperglycemic environment, this balance is disrupted, leading to the triggering of innate immune response and the sustainment of a chronic inflammatory state. This persistent, low-level inflammation is observed across various stages of DR[25-26], triggering the increased secretion and release of various inflammatory cytokines. This cascade of inflammatory responses promotes the onset and progression of DR. Prolonged and excessive inflammatory reactions result in retinal tissue remodeling and functional loss, causing irreversible damage to the patient's vision[27-29]. Throughout the advancement of DR, several pathological changes indicative of chronic retinal inflammation have been documented in both DR patients and animal models. These modifications encompass heightened retinal blood flow, irregular leukocytosis, tissue swelling, increased vascular permeability, elevated cytokine levels, and the stimulation of the complement system and microglia, along with the infiltration of neutrophils and macrophages[30].

However, previous studies have predominantly focused on inflammatory cytokines within specific ocular tissues. As DR is a complication arising from diabetes, a systemic disease,

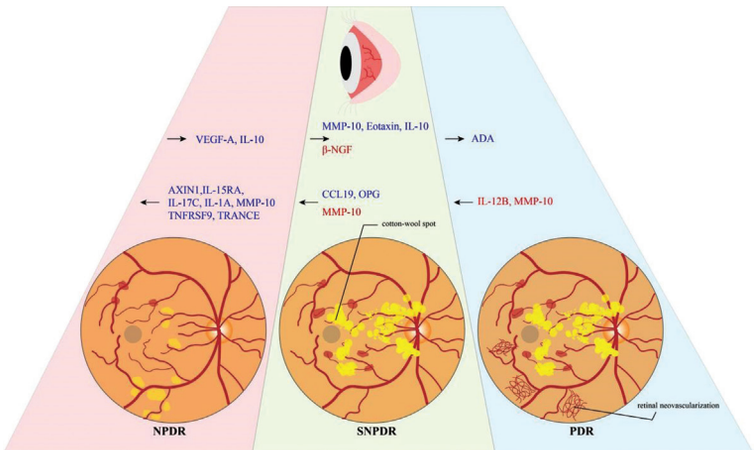

Figure 7 Schematic diagram of the interaction between circulating inflammatory factors and diabetic retinopathy subtypes Right-pointing arrows indicate forward analysis with inflammatory factors as the exposure and diabetic retinopathy as the outcome: red indicates that increased exposure is associated with higher diabetic retinopathy risk, blue indicates that increased exposure is associated with lower diabetic retinopathy risk; left-pointing arrows denote reverse analysis with diabetic retinopathy as the exposure and inflammatory factors as the outcome: red shows higher diabetic retinopathy severity linked to increased factor levels, blue represents higher diabetic retinopathy severity associated with decreased factor levels. NPDR: Non-proliferative diabetic retinopathy; SNPDR: Severe non-proliferative diabetic retinopathy; PDR: Proliferative diabetic retinopathy; VEGF-A: Vascular endothelial growth factor A; IL-10: Interleukin-10; MMP-10: Matrix metalloproteinase-10; β-NGF: Beta-nerve growth factor; ADA: Adenosine deaminase; AXIN1: Axis inhibitor 1; IL-15RA: Interleukin-15 receptor subunit alpha; IL-17C: Interleukin-17C; IL-1A: Interleukin-1-alpha; TNFRSF9: Tumor necrosis factor receptor superfamily member 9; TRANCE: TNF-related activation-induced cytokine; CCL19: C-C motif chemokine 19; OPG: Osteoprotegerin; IL-12B: Interleukin-12 subunit beta.

the role of systemic inflammatory cytokines in DR should not be underestimated. Given the constrains of these studies, the precise genetic causal relationships remain ambiguous. In this preliminary analysis, we comprehensively explored the potential bidirectional connections between 91 CIPs and different DR phenotypes using MR analysis. This research seeks to offer more dependable evidence to inform clinical decisions. Initially, we explored the potential causal links between 91 CIPs as exposures and different phenotypes of DR as outcomes. The results indicated that some inflammatory cytokines might either promote or inhibit DR, and conversely, DR could increase the expression of specific inflammatory cytokines. This suggests that the bidirectional effects between inflammatory cytokines and DR may lead to a vicious cycle.

In our study, we found that VEGF, IL-10, AXIN1, IL-15RA, IL-17C, IL-1α, PD-L1, TNFRSF9, and TRAIL are strongly associated with NPDR. Additionally, MMP-10, CCL11, IL-10, β-NGF, CCL19, and OPG show strong correlations with SNPDR. Furthermore, ADA, IL-12B, and MMP-10 are significantly associated with PDR, as shown in Figure 7. Previous studies have identified VEGF-A plays a significant role in the progression of DR, being involved in multiple pathways triggered by ischemia and inflammation, making it a primary clinical target for the treatment of DR[31]. Interestingly, MR analysis suggests that VEGF may inhibit the onset of NPDR. Nonetheless, a significant rise in VEGF levels is noted as the condition and advances to PDR[9]. In 38% of PDR patients, intraocular VEGF levels are low and not significantly different from non-diabetic controls, suggesting these patients may not respond to anti-VEGF therapy[32]. Analysis from the DRCR.net Protocol T indicates that 30% to 66% of patients continue to experience persistent macular edema after 24wk of standard anti-VEGF treatment[33]. This suggests that pathways other than VEGF, particularly those related to inflammation, could also contribute to the development of DR. The link between DR and VEGF warrants additional research. MMPs play a crucial role in the breakdown of the extracellular matrix and play a crucial role in retinal pathology. They increase vascular permeability by degrading connexins, occludins. According to Toni *et al*[34], the lack of MMP-10 has been shown to inhibit the development of DR in streptozotocin-induced diabetic mouse models, which is supported by our analysis. Abu El-Asrar *et al*[35] investigated the expression levels of OPG, TRAIL, in patients with PDR, other factors and the vitreous fluid are analyzed. Their study identified significantly elevated OPG and reduced TRAIL levels within the vitreous fluid of individuals with PDR. The imbalance in the OPG/RANKL/RANK pathway and TRAIL may contribute to both angiogenesis and inflammation in PDR.

This study employs MR to estimate the potential causative impacts between different DR phenotypes and 91 CIPs were thoroughly explored in this study. Our findings reveal strong correlations between certain rarely studied inflammatory factors and DR, filling a significant gap in the existing

literature. Because genetic variations are random and immutable, MR analysis reduces biases from confounding factors and reverse causation, offering stronger evidence of causality. Unlike traditional observational researches, our method has clear benefits. Current study on the connection between these inflammatory factors and DR is limited and mostly observational, often confounded by various factors. Our study overcomes these challenges by using genetic variants as IVs. The potential biological mechanisms involve these cytokines promoting angiogenesis and inflammatory responses, thereby altering the retinal microenvironment and accelerating the progression of DR. The findings from our MR analysis offer novel insights for the treatment and prevention and to establish the groundwork for further drug development. However, this analytical method has certain limitations. The validity of MR depends on whether the selected instrumental variables meet key assumptions, including independence from confounders related to the outcome and influencing the outcome solely through the exposure. MR analysis is based on genetic determinism, but not all biological processes have a genetic basis, which may limit the use of MR in exploring determinative connections among certain environmental factors and diseases. The results of MR may be influenced by genetic heterogeneity and population specificity. Considering that the FinnGen study is composed exclusively of Finnish participants, the generalizability of the findings may be limited. Therefore, further validation in diverse ethnic groups is necessary to transform genetic associations into treatments that can be applied in clinical settings. Future researches are required to delve deeper into the causative links among the various phenotypes of DR and 91 CIPs. To bridge the critical gap between genetic evidence and clinical translation, a prospective investigation is being undertaken to procure peripheral blood and vitreous biospecimens from DR patients spanning all severity spectrums, including NPDR, SNPDR, and PDR stages. These biological samples will undergo quantitative analysis for key inflammatory proteins identified *via* MR, such as IL-10, MMP-10, and VEGF-A, with the primary objective of validating associations between genetic predictions and in vivo protein expression profiles across diverse populations. Initial analytical efforts will focus on establishing correlations between protein concentrations and disease progression trajectories—specifically, the transition from NPDR to PDR—whereas subsequent phases will integrate multi-omics datasets to elucidate their mechanistic roles in retinal inflammatory cascades and microvascular remodeling pathways.

By contextualizing genetic associations within direct biological measurements, this methodological approach not only addresses inherent limitations of MR—such as population specificity and mechanistic ambiguity—but also generates robust clinical evidence to inform the development of targeted diagnostic biomarkers and therapeutic interventions anchored in inflammatory protein networks. Such endeavors represent a pivotal advancement toward precision medicine for DR, wherein individualized treatment strategies may be tailored to patient-specific inflammatory phenotypes, potentially improving risk stratification and therapeutic outcomes.

Given the current cohort's reliance on Finnish genetic isolates, however, corroborative studies in ethnically diverse populations are imperative to generalize these findings and translate genetic insights into universally applicable clinical protocols. Future investigations should prioritize deepening our understanding of causal relationships between DR phenotypic heterogeneity and the 91 CIPs identified herein, ensuring that research outcomes contribute meaningfully to evidence-based strategies for prevention, prognostic staging, and mechanistic therapy of this leading cause of irreversible blindness.

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Data Availability Statement: All the data utilized in this study can be accessed from the public repository (EBI GWAS Catalog https://www.ebi.ac.uk/gwas/ numbers GCST90274758-GCST90274848, accessed on 10 May 2024; FinnGen database https://www.finngen.fi/en, accessed on 10 May 2024).

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