Diabetology & Metabolic Syndrome

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Causal influence of immune factors on the risk of diabetic retinopathy: a mendelian randomization study

Yuanyuan Li[1,2†], Ying Xiang[2,3†], Bo Mou[1,2] and Xiusheng Song[1,2,3*]

Abstract

Objectives Diabetic retinopathy (DR) is a prevalent microvascular complication in diabetic patients. Various mechanisms have been implicated in the pathogenesis of DR. Previous studies have observed the relationship between immune factors and DR, but the causal relationship has not been determined.

Methods We conducted a two-sample Mendelian randomization (MR) analysis of 731 immune cells and DR, using publicly available genome-wide association study (GWAS) summary statistics, to evaluate potential causal relationships between them. Four types of immune traits were included in the analysis through flow cytometry. GWAS statistics for DR were obtained from the Finngen database, which performed GWAS on 190,594 European individuals (Ncase = 14,584, Ncontrol = 176,010) to assess genetically predicted DR. The primary method used to perform causality analysis was inverse variance weighting (IVW).

Results Following false discovery rate (FDR) correction, 11MFI-DR, 5AC-DR, 5RC-DR, and 1MP-DR reached a significant causal association level ($P_{FDR} < 0.05$). Notably, all AC traits exhibited potential associations with a decreased risk of DR(OR < 1), while a majority of MFI traits, along with the singular MP trait, exhibited potential associations with an increased risk of DR (OR > 1). The highest proportion of T-cell subsets in the final results.

Conclusion This study elucidates that the progression of DR is intricately influenced by immune responses, thereby confirming the immunological susceptibility of DR. Our findings may offer new targets for diagnosing and treating DR, as well as aid in developing therapeutic strategies from an immunological standpoint.

Keywords Immune cells, Mendelian randomization, Diabetic retinopathy, Genome wide association study, T cell subsets

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Introduction

Diabetic retinopathy (DR) is a condition resulting from prolonged damage to the retina caused by chronic hyperglycemia. The initial sign is the breakdown of the blood-retinal barrier (BRB), primarily due to vascular endothelial growth factor (VEGF) [1]. Current research indicates that the intricate mechanisms underlying DR are closely associated with oxidative stress, activation of protein kinase C, elevated levels of advanced glycation end-products [2], and inflammatory injury [3], among various factors. Pertinent risk factors encompass prolonged diabetic duration, pregnancy, suboptimal glycemic and blood pressure control [4]. Studies propose that the global burden of DR is projected to remain substantial until 2045 [5]. Nevertheless, to date, satisfactory therapeutic strategies for DR have yet to be established.

In a healthy state, the retina is immunoprivileged to prevent damage from immune stimuli. The BRB is important to limit the entry of immune cells into the eye. However, chronic inflammation-induced BRB destruction can threaten the immune privilege state, leading to retinal destruction [6]. Immunodysregulation is acknowledged as a pivotal factor in the pathophysiology of DR [7]. A growing body of evidence suggests that the activation of the immune system plays a crucial role in the progression of DR. Microglial cells, resident immune cells in the retina, assume a significant role in the development of diabetic retinal lesions. When exposed to elevated glucose levels, these microglial cells undergo activation and can mediate inflammatory responses leading to visual deterioration [8]. It has been suggested that T cells may promote inflammatory damage in the DR vasculature by releasing cytokines and cytotoxic factors, resulting in retinal inflammation, angiogenesis, and vascular leakage [9]. A study discovered increased levels of B-cell-generated antibodies in the vitreous fluid of type 2 diabetic patients with DR, indicating that a B-cell-mediated immune response could be a factor in the advancement of the disease [10]. Overall, the involvement of the immune system in the inflammatory and angiogenic processes of DR has been postulated, yet a definitive causal impact of immune cells on the risk of DR remains inconclusive [11]. Activation of these immune cells and their role in DR pathology will open up new possibilities for clinical applications and treatment strategies.

Mendelian Randomization (MR) studies serve as effective tools in mitigating biases arising from confounding factors and reverse causation [12]. Leveraging naturally occurring genetic variations as Instrumental Variables (IVs), MR enables a robust assessment of causal relationships between exposures and outcomes [13]. In this study, immune cells were chosen as the exposure variable, and DR as the outcome variable, to explore the causal relationship between immune cells and the occurrence and progression of DR. Not only did the study yield results similar to existing research analyses, but it also uncovered some previously unexplored immune factors that may be causally related to the onset of DR. This contributes novel perspectives for the diagnosis and treatment of DR in the future. It is important to note that this analysis is based on genetic data, which can provide evidence for causal inference. It is designed to generate causal hypotheses rather than confirm causal relationships.

Materials and methods

Study design

Based on aggregated summary-level data from a large-scale genome-wide association study (GWAS), we employed a two-sample Mendelian randomization approach to assess the causal relationship between a myriad of immune cells (731 immune cells within 7 immune panels) and the risk of DR. To enhance the precision of our results, single nucleotide polymorphisms (SNPs) were selected as IVs. Simultaneously, adherence to three fundamental assumptions (Fig. 1) was imperative: (1) genetic variations are directly associated with the exposure (the relevance assumption); (2) genetic variations are unrelated to potential confounders between the exposure and the outcome, ensuring that the results are not influenced by confounding factors (the independence assumption); (3) genetic variations do not affect the outcome through pathways other than the exposure (the exclusivity assumption). All studies included in the utilized dataset have obtained approval from the relevant institutional review boards.

Data sources for exposure and outcome

The summary statistics of 731 immune traits are publicly available in the GWAS Catalog (https://www.ebi.ac.uk/gwas/), accession numbers from GCST90001391 to GCST90002121 [14], encompassing a total of 3,757 Sardinian samples (57% female). All included data in the analysis were derived through flow cytometry, involving the examination of approximately 22 million SNPs. These data are categorized based on trait type, distinguishing absolute cell (AC) counts ($n=118$), median fluorescence intensity (MFI) reflecting surface antigen levels ($n=389$), morphological parameters (MP) ($n=32$), and relative cell (RC) counts ($n=192$). The GWAS summary statistics pertaining to diabetic retinopathy (GWAS ID: finngen_R9_DM_RETINOPATHY_EXMORE) were extracted from the Finngen research project (https://r9.finngen.fi/) [15], a large-scale case-control study. This GWAS incorporated 14,584 cases of DR and 176,010 controls of European ancestry, involving a total of 16,380,347 SNPs. Rigorous quality checks were conducted on SNPs to ensure data robustness and result accuracy, meeting the requirements for IVs.

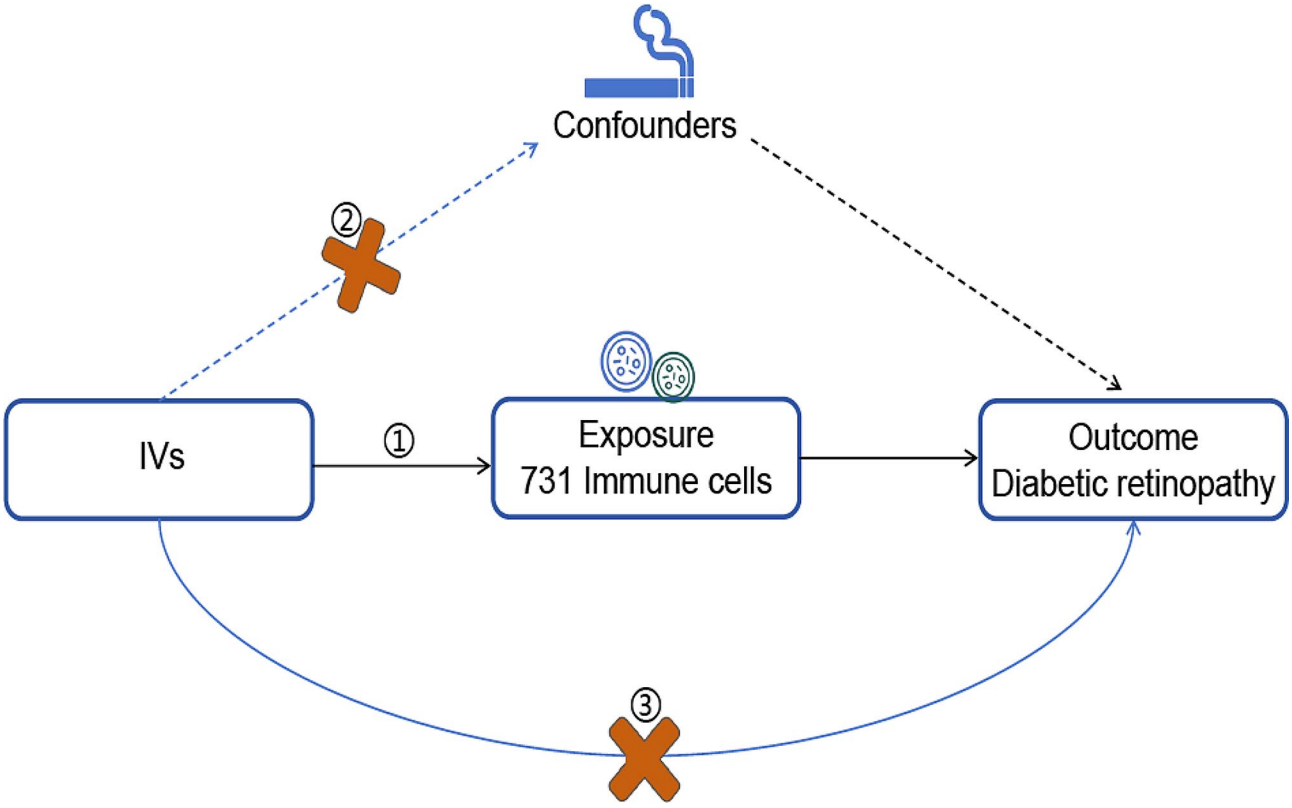

Fig. 1 Three basic assumptions of Mendelian randomization analysis. ①the relevance assumption; ②the independence assumption; ③the exclusivity assumption. IVS, instr-umental variables

Selection of IVs

In this study, immune cells were utilized as the exposure variable, and DR was employed as the outcome variable to assess the role of immune cells in DR. To satisfy the three assumptions of the two-sample MR analysis, several quality control steps were implemented to select IVs closely associated with immune cells and meeting the criteria. (1) The paucity of significant IVs below the stringent threshold of $P<5\times10^{-8}$ compromises the statistical power of subsequent analyses. Upon careful investigation, we noted that a threshold of $P<1\times10^{-5}$ is commonly used in studies utilizing this dataset with 731 immune cells for MR analyses [16–19]. After careful consideration, and in line with recent research trends, we adopted a more relaxed significance threshold of $P<1\times10^{-5}$ for our IVs selection. While this threshold is more lenient than traditional standards, it represents a pragmatic compromise given the current limitations of available genetic variations within the study domain. (2) Additionally, the Clump function was employed to conduct linkage disequilibrium testing (setting criteria as $r^2<0.001$, kb=10000). (3) The F-statistic was employed to gauge IVs strength [20]. We computed the F-statistic and retained IVs with F>10 to prevent bias introduced by weak IVs. (4) To meet the independence assumption, potential confounding factors correlated with the outcome were identified and removed using PhenoScanner (http://www.phenoscanner.medschl.cam.ac.uk/) [21] for comprehensive cross-validation. (5) To ensure that IVs corresponded to the same allelic genes in the same order for exposure and outcome, we adjusted for non-palindromic IVs chains and removed IVs containing palindromic sequences, thereby harmonizing the exposure and outcome datasets. In summary, subsequent MR analysis was conducted using the IVs that remained after a rigorous multistep selection process.

Statistical analysis

Recent MR analyses have confirmed that risk factors like Body mass index [22], obesity [23], waist circumference [23], Waist hip ratio [24], and smoking [24] are causally linked to DR. Through a comprehensive search on the Phenoscanner website, we identified 96 SNPs (Supplementary Table S1) that displayed suggestive associations ($P<1\times10^{-5}$) with these risk factors and subsequently excluded them to effectively minimize potential confounding influences. To prevent outliers from causing horizontal pleiotropy in subsequent analyses, we utilized MR-PRESSO to detect and remove outliers while performing MR analyses [25]. 13 outliers (Supplementary Table S2) with Distortion test *P*-values<0.05 (indicating a difference in results before and after correction) were

removed from the IVs that were found to potentially cause horizontal pleiotropy legacy. Subsequently, the filtered set of 18,468 IVs (Supplementary Table S3) underwent a renewed MR analysis (MR workflow in Fig. 2). This study used inverse variance-weighted (IVW), weighted median, simple mode, Weighted mode, and MR-Egger methods to assess potential causal effects (Supplementary Table S4), and we gave priority to positive results from IVW methods. We opted for the random-effects IVW method due to acknowledging the presence of heterogeneity between studies. Random-effects method enhances the consideration of heterogeneity. However, we are also aware that even with the use of random-effects model, there remains some uncertainty and the results may be influenced by heterogeneity. We conduct the sensitivity analysis to confirm the reliability of our results.

To mitigate potential over-bias, sensitivity analyses were conducted. Heterogeneity was evaluated using Cochran's Q test [26]. Horizontal pleiotropy tests were conducted for MR-PRESSO with MR-Egger regression (commonly denoted by the intercept term). If the intercept term of the MR-Egger regression differed significantly from 0 (P-value < 0.05), it indicated the presence of horizontal pleiotropy [27]. Furthermore, the results were re-calculated using the Leave-one-out(LOO) method after stepwise removal of individual SNPs. The optimal outcome would be minimal changes in the results after sequentially removing each SNP. Causal effects were presented in terms of odds ratios (OR) and their 95% confidence intervals (CI). The direction and magnitude of the OR values are crucial indicators for evaluating causal relationships. Furthermore, to guard against Type I errors in multiple hypothesis testing, the false discovery rate (FDR) was applied to correct P-values obtained from the IVW method. In the field of MR analysis, it is common and advisable to use FDR as a means of controlling false positive rates [28]. Discoveries that yield adjusted P-values below the conventional 0.05 threshold are considered statistically significant [29, 30]. The aforementioned statistical analyses were primarily executed using the R software (version 4.3.2) and the "TwoSampleMR" package (version 0.5.8), along with the "MR-PRESSO" package (version 1.0) [31].

Results

Overview

After rigorous quality checks, a two-sample Mendelian randomization analysis excluded 96 confounder-associated IVs and 13 outliers, resulting in 33 trait pairs. To account for potential false positives, we applied the FDR method to adjust the P values obtained from the IVW method. Following this correction, 11 trait pairs (5AC-DR, 5RC-DR, and 1MFI-DR) were identified with P_{FDR} >0.05. As the goal of the study was to investigate immunological factors causally linked to DR using Mendelian randomization, these 11 pairs were excluded from the final analysis. The sensitivity analyses results for the remaining 22 trait pairs (11 MFI-DR, 5 AC-DR, 5 RC-DR,

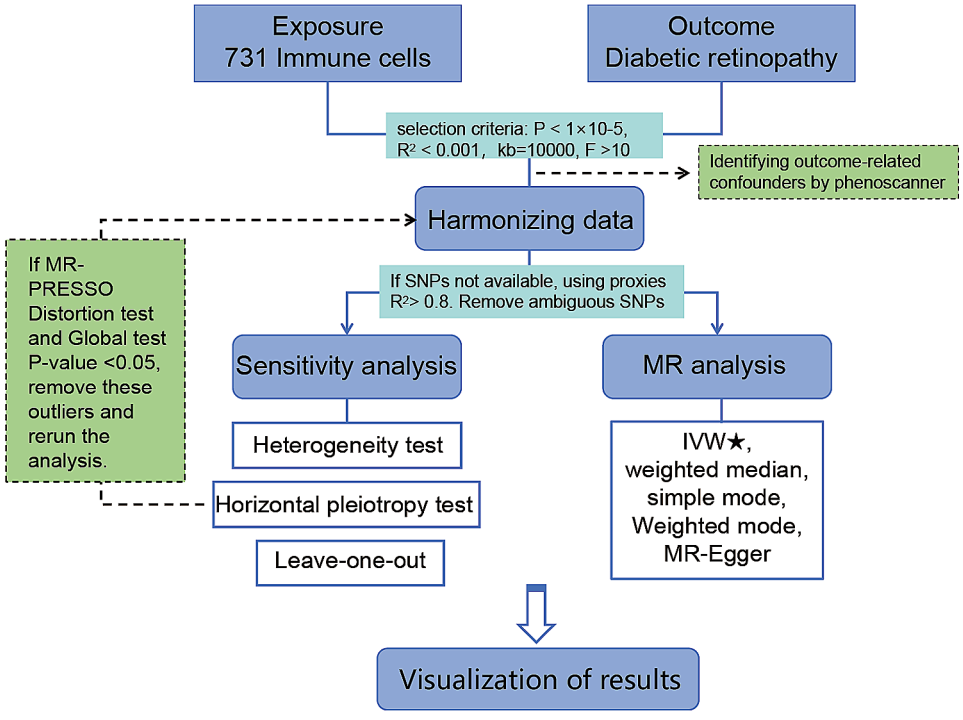

Fig. 2 Flowchart of MR design. MR, Mendelian randomization; IVW, inverse variance weighted

and 1 MP-DR) indicated no horizontal pleiotropy, and four trait pairs showed potential heterogeneity. As previously mentioned, we utilized a random-effects IVW method that effectively addresses heterogeneity [32], so the results are acceptable [33]. In all results, the F-statistic was >19, ensuring the strength of the IVs. When the 22 traits were classified into seven sets of panel types, most were found to be linked to T-cell subsets (Supplementary Table 6). Furthermore, upon classifying based on OR values, there were 9 trait pairs with OR<1 (5 AC-DR, 2 RC-DR, 2 MFI-DR), and 13 trait pairs with OR>1 (9 MFI-DR, 3 RC-DR, 1 MP-DR). We found that all AC traits among these 22 traits exhibited a potential association with a decreased risk of DR, while most MFI traits and the sole MP trait may be associated with an increased risk of DR.

Immune traits with OR values < 1

Figure 3 clearly shows 9 immune traits with ORs<1, and none of their 95% CIs crossed 1. This indicates that our results are statistically significant. After FDR correction, the results belonging to the AC trait all presented the possibility of reducing the risk of DR [CD11c+monocyte AC(OR:0.962, 95% CI: 0.929–0.998, P_{FDR}=0.0475), CD14+CD16- monocyte AC(OR: 0.974, 95% CI. 0.954–0.994, P_{FDR}=0.0396), CD4+CD8dim AC(OR:0.958, 95% CI: 0.922–0.996, P_{FDR}=0.0443), CD45RA-CD4+AC(OR:0.978, 95% CI: 0.959–0.996, P_{FDR}=0.0410), EM DN(CD4-CD8-) AC (OR:0.946, 95% CI: 0.904–0.991, P_{FDR}=0.0400)]. We further established the causal link between these five AC traits and the risk of DR through the scatter plot in Fig. 4. It's worth noting that CD25 on IgD- CD24- within the B-cell group showed a more substantial causal connection with the risk of developing DR (OR: 0.943, 95% CI: 0.901–0.987, P_{FDR}=0.0369). This suggests that this particular subpopulation of immune cells might have a significant impact on reducing the risk of DR. Furthermore, CD14+CD16+monocyte %monocyte, CD39+secreting Treg %CD4 Treg and HVEM on TD CD4+may all act as protective factors for DR. Up to four of these nine immune traits are linked to membership in the T-cell group.

Immune traits with OR values > 1

The LOO plots for the 9 MFI traits with OR values>1 are depicted in Fig. 5, illustrating the robustness of the results. This suggests a causal relationship between the MFI features of these cells and the risk of DR. Among them, the Myeloid cell CD45 on CD33- HLA DR- (OR: 1.050, 95% CI: 1.010–1.092, P_{FDR}=0.0377) exhibits a relatively significant positive causal relationship with the risk of DR. FSC-A on CD14+monocyte (OR: 1.033, 95% CI: 1.004–1.063, P_{FDR}=0.0432) is the only MP trait in the analysis results. Additionally, it is noteworthy that from Fig. 3, Activated Treg %CD4 (OR: 1.040, 95% CI: 1.000-1.081, P_{FDR}=0.0495), and CD3 on NKT (OR: 1.059, 95%

exposure	nsnp	method	P_{IVW}	OR(95% CI)	P_{FDR}
Activated Treg %CD4	19	Inverse variance weighted	**0.0480**	1.040 (1.000 to 1.081)	**0.0495**
CD11c+ monocyte AC	16	Inverse variance weighted	**0.0360**	0.962 (0.929 to 0.998)	**0.0475**
CD14+ CD16− monocyte AC	26	Inverse variance weighted	**0.0108**	0.974 (0.954 to 0.994)	**0.0396**
CD14+ CD16+ monocyte %monocyte	25	Inverse variance weighted	**0.0269**	0.952 (0.911 to 0.994)	**0.0445**
CD24 on transitional	20	Inverse variance weighted	**0.0105**	1.061 (1.014 to 1.110)	**0.0433**
CD25 on IgD− CD24−	25	Inverse variance weighted	**0.0112**	0.943 (0.901 to 0.987)	**0.0369**
CD28 on CD28+ CD45RA+ CD8br	15	Inverse variance weighted	**0.0407**	1.043 (1.002 to 1.087)	**0.0480**
CD28 on CD45RA+ CD4+	13	Inverse variance weighted	**0.0144**	1.068 (1.013 to 1.126)	**0.0397**
CD3 on NKT	16	Inverse variance weighted	**0.0495**	1.059 (1.000 to 1.122)	**0.0495**
CD39+ secreting Treg %CD4 Treg	20	Inverse variance weighted	**0.0327**	0.979 (0.960 to 0.998)	**0.0470**
CD4+ CD8dim AC	16	Inverse variance weighted	**0.0296**	0.958 (0.922 to 0.996)	**0.0443**
CD45 on CD33− HLA DR−	14	Inverse variance weighted	**0.0148**	1.050 (1.010 to 1.092)	**0.0377**
CD45 on NKT	22	Inverse variance weighted	**0.0365**	1.032 (1.002 to 1.063)	**0.0464**
CD45RA− CD4+ AC	15	Inverse variance weighted	**0.0174**	0.978 (0.959 to 0.996)	**0.0410**
CD62L on monocyte	25	Inverse variance weighted	**0.0233**	1.025 (1.003 to 1.047)	**0.0427**
CD8 on CD39+ CD8br	24	Inverse variance weighted	**0.0206**	1.072 (1.011 to 1.137)	**0.0425**
EM DN (CD4−CD8−) AC	21	Inverse variance weighted	**0.0182**	0.946 (0.904 to 0.991)	**0.0400**
FSC−A on CD14+ monocyte	24	Inverse variance weighted	**0.0275**	1.033 (1.004 to 1.063)	**0.0432**
HVEM on EM CD8br	18	Inverse variance weighted	**0.0250**	1.040 (1.005 to 1.077)	**0.0433**
HVEM on TD CD4+	26	Inverse variance weighted	**0.0103**	0.965 (0.939 to 0.992)	**0.0483**
IgD− CD38− %B cell	12	Inverse variance weighted	**0.0131**	1.077 (1.016 to 1.142)	**0.0393**
Naive DN (CD4−CD8−) %DN	21	Inverse variance weighted	**0.0214**	1.054 (1.008 to 1.103)	**0.0416**

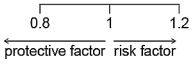

Fig. 3 The forest plot illustrating the causal impact of immune cells on the risk of DR, derived using the Inverse Variance Weighted method. OR, odds ratios; CI, confidence intervals; P_{IVW}, *P*-value of the IVW method; P_{FDR}, FDR-corrected *P*-value

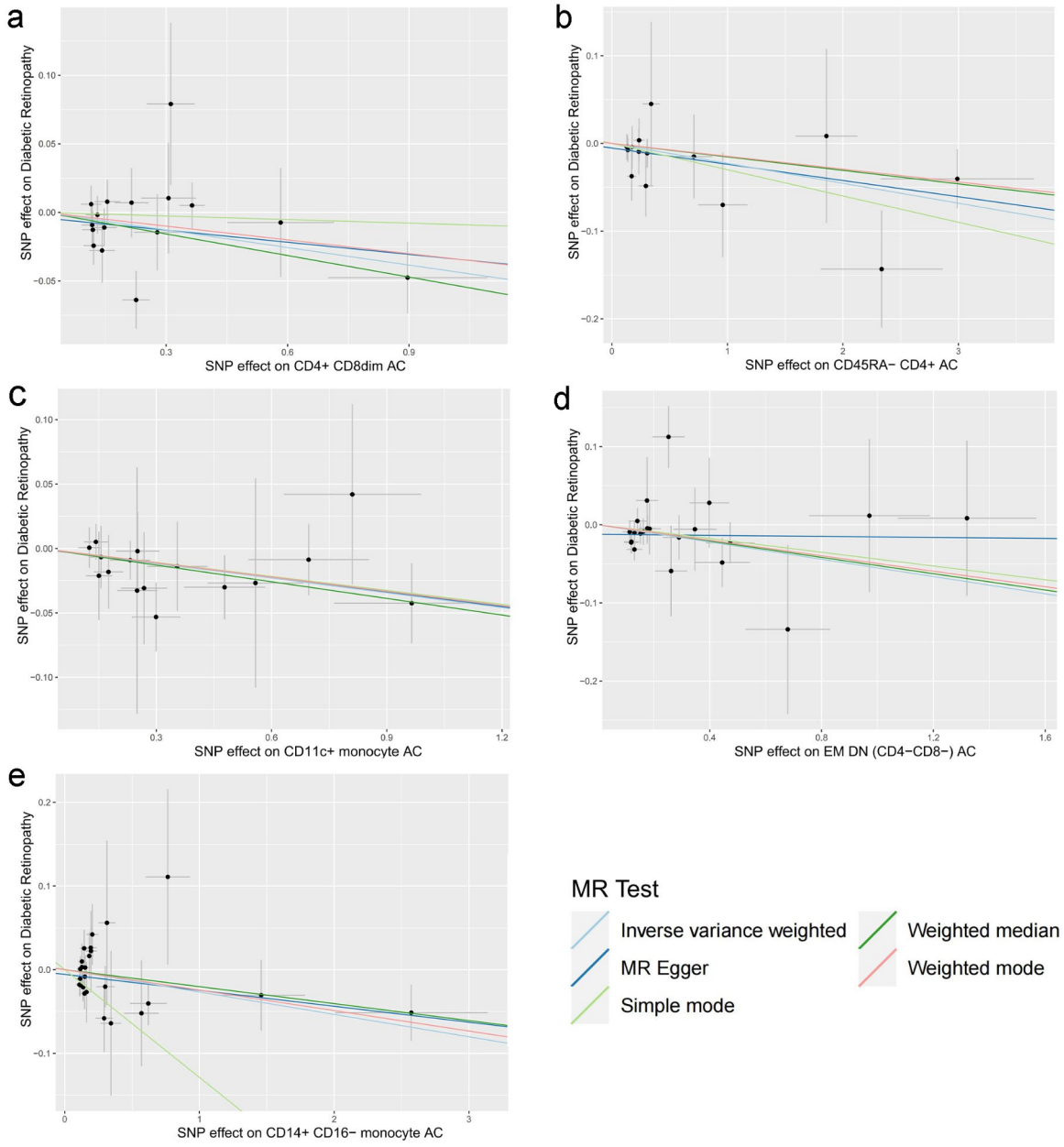

Fig. 4 The scatter plot depicting the risk of DR associated with 5 AC traits with OR < 1 (primarily focusing on the IVW method). DR, diabetic retinopathy; AC, absolute cell; OR, odds ratios; IVW, inverse variance-weighted

CI: 1.000-1.122, P_{FDR}=0.0495) exhibit distinct characteristics. The 95% CI for both traits covered 1.000, indicating that we cannot dismiss the possibility of a null effect, and thus this may necessitate further in-depth study and understanding. Consistent with earlier findings, immune traits with OR > 1 showed the most significant associations for T cell subset characteristics compared to other groups. This underscores the significance of T cells in regulating the immune response and offers clues for further exploration of the potential role of these T cell subsets in the mechanisms of DR occurrence.

Discussion

Earlier studies have hinted at the role of immune factors in DR development, but this study is the first to comprehensively examine the causal link between multiple immune traits and DR using MR analysis. To ensure the reliability of causality, we minimized the impact of potential confounders and outliers. The number of causal relationships between MFI traits and DR was the highest compared to the other three. Our results show that CD25 on lgD- CD24- (belonging to the B cell) with MFI traits demonstrated a significant negative causal association

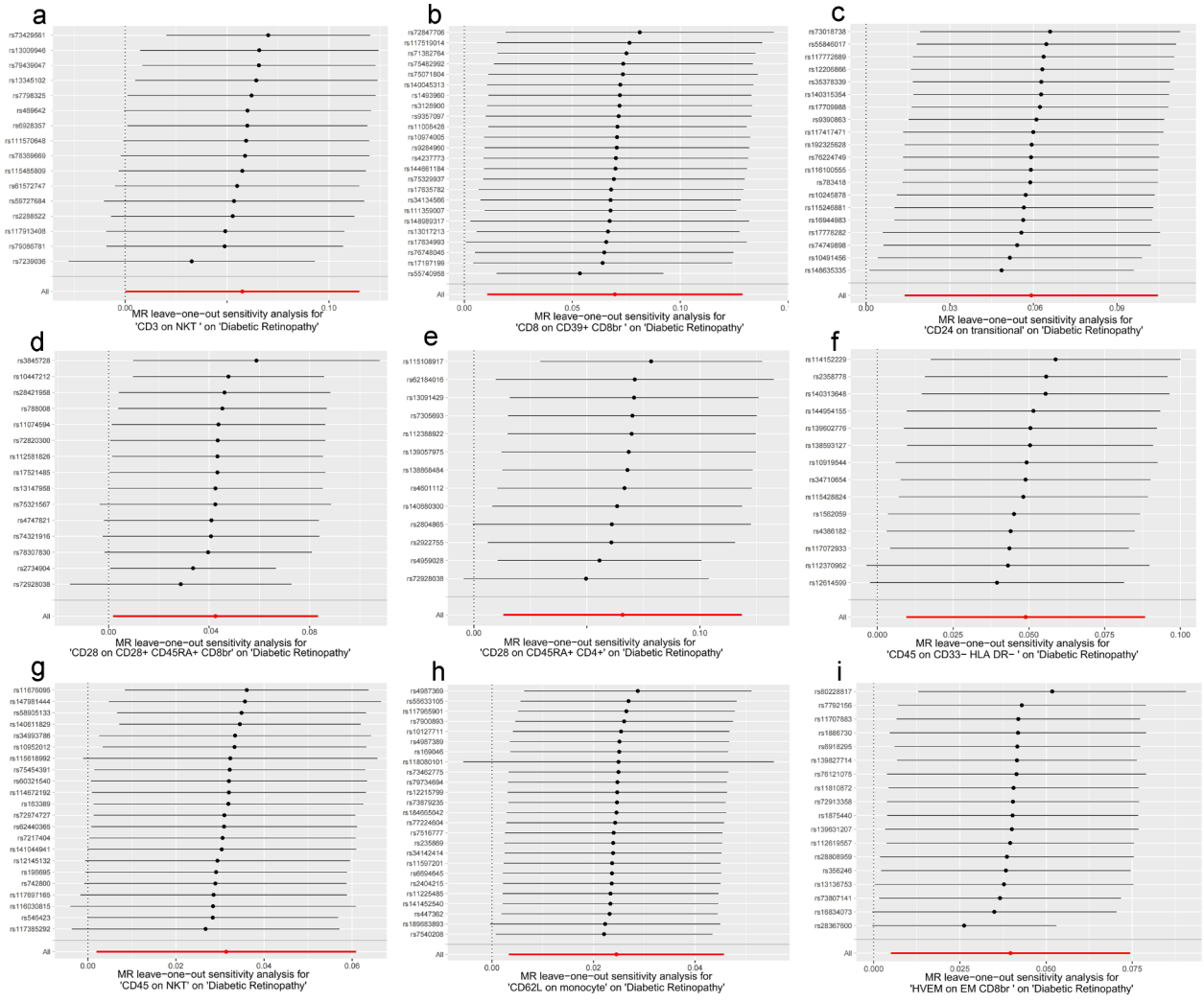

Fig. 5 The LOO forest plot illustrating the OR > 1 for 9 MFI traits in relation to DR. LOO, leave-one-out; OR, odds ratios; MFI, median fluorescence intensity; DR, diabetic retinopathy

with the risk of DR. CD25 has long been known to have a negative regulatory role in the immune system, primarily by modulating the immune response through inhibition of free Interleukin-2 (IL-2), which helps prevent excessive immune responses [34]. However, past literature has reported that CD25 is typically associated with regulatory T cells (Treg) [35]. Research on the specific role of CD25 in B cells and its effects on immune responses [36] is relatively limited and requires further exploration and investigation.

The AC and RC traits serve as valuable parameters for the analysis of specific immune cell subsets, allowing for the examination of both the quantity and relative proportions. Notably, both the percentage of CD14+CD16+monocytes and the AC of CD14+CD16- monocytes demonstrate potential negative correlations with the risk of DR. The CD14+CD16+monocytes, recognized as intermediate monocytes, constitute the third major subpopulation of monocytes alongside classical (CD14+CD16-) and nonclassical (CD14 CD16-) monocytes. This unique subpopulation exhibits a distinct gene expression profile that has been linked to various diseases [37]. CD14+CD16- monocytes make up about 80% of all monocytes and play a role in phagocytosis, defense against pathogens, homeostasis, and tissue repair [38]. Previous research has linked CD11c+monocytes with atherosclerosis [39], intestinal inflammation [40], and the promotion of chronic inflammatory responses [41], making them a focal point in studies of immune system function and disease pathogenesis. Contrary to expectations, our findings suggest that CD11c+monocytes with AC features may be negatively linked to DR risk, warranting further experiments and studies for verification.

Additionally, it is of significance to highlight that, mirroring previous research trends, a majority of the immune cells identified to have a causal association

with the risk of DR in this study belong to the T-cell category. As early as 1995, Tang et al. [42]. made a seminal observation that T lymphocytes, in conjunction with interleukin-2 (IL-2), a growth-promoting factor for various immune cells, infiltrated the retinal tissue of individuals with DR. Furthermore, in the fibrovascular membranes (FVM) of proliferative diabetic retinopathy (PDR) patients, researchers noted a significant elevation in the densities of both CD4+T lymphocytes and CD8+T lymphocytes [43]. Several T cell subsets associated with CD4 or CD8 surface markers were also identified in our findings, and their heightened risk of DR demonstrated a positive and causal correlation. Notably, both CD28 on CD28+CD45RA+CD8br and CD28 on CD45RA+CD4+encompass a subpopulation of T cells expressing CD28, with an additional association with CD45RA expression. Previous literature has hinted at a potential link between CD28 expression in T cells and the pathogenesis of DR [44]. Regarding CD8 on CD39+CD8br, upon thorough examination, we found that CD39 serves as an extracellular nucleotidase that, in conjunction with CD73, catalyzes the conversion of extracellular ATP to adenosine, contributing to the immunosuppressive adenosine pathway. Research has indicated that the CD39+T cell population exhibits robust anti-tumor cytotoxicity across various cancer types [45]. In line with our current investigation, an increased level of HVEM on EM CD8br, belonging to the Maturation stages of T cells, is identified as a potential contributor to an elevated risk of DR. The HVEM signaling pathway is postulated to play a regulatory role in influencing the function of the CD8 T cell subpopulation [46]. Furthermore, our analysis has brought attention to the involvement of double-negative T (DNT) cells. DNT cells, inclusive of naive subpopulations, have been a focal point of research [47] in the realms of autoimmune diseases, inflammation, and cancer [48]. However, additional investigations may be warranted to comprehensively understand the impact of these distinctly expressed T cell subsets on DR within diverse physiological and pathological contexts.

Among the T cell subsets associated with CD4 or CD8 surface markers, CD39+secreting Treg %CD4 Treg showed a negative causal association with DR risk in this analysis. This observation implies a pivotal role for this specific T cell subset in the regulation of immune responses and the maintenance of immune homeostasis. Additionally, three Maturation stages of T cells, namely CD45RA- CD4+, EM DN(CD4-CD8-) AC, and HVEM on TD CD4+, were also found to exhibit a negative association, potentially contributing to the regulation of T cell function [49, 50]. However, their relevance to the mechanism underlying the occurrence of DR remains a subject that warrants further, in-depth investigation.

In summary, it is evident that there is a dearth of studies focusing on the segmentation and elucidation of clear causality within these cell subpopulations. Our analysis, however, has produced more nuanced results, offering suggestive evidence for subsequent investigations. Nevertheless, our study is not without limitations. Firstly, the reliance on a European database restricts the applicability of our conclusions to other racial groups, thus limiting the generalizability of the results in terms of demographic aspects. Secondly, despite the execution of multiple sensitivity analyses, a comprehensive evaluation of horizontal pleiotropy remains challenging. Thirdly, all conclusions are derived from exploratory analyses based on existing data and require further validation. Even though our results are statistically significant with FDR<0.05, they exceed 0.036, which may indicate weaker associations. Further analysis is needed once stronger and more suitable data become accessible. Fourthly, relaxing the selection criterion from 5×10^{-8} to 1×10^{-5} represents a trade-off aimed at improving statistical power, but also carries the risk of increased false-positive rates. A stringent threshold ($p<5\times10^{-8}$) is generally favorable for obtaining robust MR results. However, MR analysis requires a trade-off between statistical power and risk of bias, based on study objectives and sensitivity analyses [51]. In exceptional cases, such as when the exposure lacks sufficiently potent IVs resulting in inadequate statistical power, a moderate relaxation of the standards may be warranted. Indeed, Wootton et al. [52]. adopted the relaxed threshold of 1×10^{-5} in their 2018 MR study. The complexity of immune system regulation requires us to not only focus on highly significant genetic signals but also to explore genetic variants that exhibit multiple coincident associations with various immune phenotypes and disease states. These variants may be crucial for understanding disease mechanisms, but their effects may appear statistically weaker due to multifactorial influences. Therefore, we choose to moderately relax the threshold to avoid overlooking these potentially key genetic markers, while rigorously validating their authenticity and effect size through subsequent analyses. While relaxing the significance threshold, we are fully aware of the necessity to control the risk of false positives. Our strategies include but are not limited to implementing sensitivity analyses, using *P*-value correction methods to ensure the biological plausibility of the selected IVs. These supplementary measures aim to seek a reasonable balance between statistical power and biological significance. Bowden et al. [27]. suggested using the MR Egger method to provide unbiased estimates of the overall causal effect and to quantify potential systematic biases when IVs may not meet all necessary assumptions, thereby assisting researchers in assessing IVs efficacy and providing more reliable causal inferences.

Our application of the MR Egger method revealed no evidence of horizontal pleiotropy, suggesting that the relaxed criteria still yield effective IVs. We recognize that relaxing the threshold is applicable to the exploratory research phase, aimed at discovering new genetic clues and hypotheses. Like other studies that have also relaxed thresholds, our goal is to open up new research directions rather than reaching final conclusions. Future analyses should be conducted when larger genetic studies become available to bolster the selection of IVs. Furthermore, we call upon the scientific community to collaboratively explore more systematic approaches to threshold selection, particularly in devising reasonable compromises when confronted with data limitations. Future studies should strive to uncover additional genetic variants closely tied to exposure variables, thereby enhancing the precision of MR analyses. This concerted effort will not only strengthen the validity of our findings but also pave the way for a more nuanced understanding of complex disease etiologies and the intricate interplay between genetics and phenotype. In conclusion, utilizing MR as an exploratory tool, this study is a significant advancement in understanding the potential causal link between immune cells and DR. However, limitations in IVs emphasize the importance of interpreting findings cautiously and call for future studies to enhance genetic marker data and sample size. These enhancements are crucial for creating stronger and more widely applicable scientific insights.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13098-024-01441-6.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Acknowledgements

We want to acknowledge the participants and investigators of the FinnGen study. Thanks to the Genome-Wide Association Research Alliance for making these summary statistics publicly available. Without them, this study would have been impossible.

Author contributions

Y.L. and Y.X. collected and analysed the data, and wrote the main manuscript text. X.S. designed the project. B.M. drafted the manuscript. All authors have reviewed and approved the fnal manuscript.

Funding

This work was supported by the Enshi Science and Technology Program, D20220052.

Data availability

The GWAS data on 731 immune traits originated from the GWAS Catalog (GCST0001391 to GCST0002121), https://www.ebi.ac.uk/gwas/. The GWAS summary statistics pertaining to diabetic retinopathy (GWAS ID: finngen_R9_DM_RETINOPATHY_EXMORE) were extracted from the Finngen research project, https://r9.finngen.fi/. Use PhenoScanner to remove confounders, http://www.phenoscanner.medschl.cam.ac.uk/.

Declarations

Ethics approval and consent to participate

Since the data adopted in this MR analysis were all publicly available data from the Finngen database and GWAS Catalog, all data-related studies were approved by their respective ethical review committees and received written informed consent from patients. Therefore, this study does not need additional ethics approval.

Competing interests

The authors declare no competing interests.

Received: 4 April 2024 / Accepted: 6 August 2024
Published online: 12 August 2024

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