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Causal roles of immune cells in cardiovascular diseases: A Mendelian randomization (MR) study

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Abstract

Background: Despite being a major global cause of mortality, the exact underlying mechanisms of cardiovascular diseases (CVDs) remain uncertain. This study aimed to elucidate the possible pathological connection between circulating activated immune cell types and the advancement of CVD.

Methods: A two-sample Mendelian randomization analysis was performed on publicly available genetic databases to examine the potential causal relationships among 731 immune phenotypes and CVD risks. The study focused on four distinct immune signatures: relative cell counts (RC), absolute cell counts (AC), morphological parameters (MP), and median fluorescence intensities (MFI). A sensitivity analysis was performed to assess the findings' consistency, robustness, and potential pleiotropic effects.

Results: Significant associations between CVD and various immunophenotypes were observed in this study. Specifically, two phenotypes exhibited protective effects against CVD. The odds ratio (OR) for activated and secretory $CD4^+$ regulatory T-cells (Tregs) was 0.757 [95% confidence interval (CI): 0.628–0.913; $p = 0.004$], whereas that for B-cell activating factor receptor on IgD^-CD38^+ memory B-cells was 0.654 (95% CI: 0.468–0.915; $p = 0.013$). Conversely, three major immunophenotypes were linked to heightened risks of CVD: CD80 on myeloid dendritic cells (OR: 1.181; 95% CI: 1.015–1.376; $p = 0.032$), the proportion of $CD28^+$ $CD45RA^+$ $CD8^+$ T-cells in total T-cell population (OR: 1.064; 95% CI: 1.002–1.128; $p = 0.041$), and the proportion of $CD28^-CD45RA^+$ $CD8^+$ T-cells in total T-cell population (OR: 1.005; 95% CI: 1.000–1.011; $p = 0.045$).

Conclusion: This study underscores significant correlations between specific immune phenotypes and the risks associated with CVD onset, thus providing valuable perspectives for forthcoming clinical inquiries.

Keywords

Immune cells, cardiovascular diseases, Mendelian randomization

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Introduction

In developed nations, cardiovascular disease (CVD) is still the leading cause of death.[1] Between 1990 and 2010, CVD-associated global mortality rates were raised by one-third, and by 2015, it is estimated to reach a level where one in three deaths would be related to CVD.[2] Cardiovascular disease encompasses a spectrum of diseased conditions, including coronary and ischemic attacks that primarily affect the heart, brain vasculature, blood vessels, and deep vein arteries.[3] Both nonmodifiable (such as age, sex, and genetics) and modifiable (including hypertension, diabetes, dyslipidemia, smoking, unhealthy diet, and obesity) etiological factors have been linked to the onset of CVD.[4] Thus, living a healthy lifestyle and

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managing risk factors could be crucial in preventing or slowing down the progression of CVD. Notably, managing CVD risks typically involves three major stages: first, lifestyle and dietary habit improvement, especially in high-risk individuals; second, routine screening and early detection; and third, therapies targeting amelioration of chronic symptoms and improving quality of life (QoL).[5]

Physiologically, B and T lymphocytes play important roles in maintaining cardiac health and responding to harmful stressors. For instance, mice lacking PD-1, an essential factor in B-cell maturation, potentially develop dilated cardiomyopathy along with elevated levels of cardiomyocytes-targeting autoantibodies. Individuals with advanced-stage cardiac failure may also produce heightened levels of cytotoxic autoantibodies. During sudden decompensation and ischemia-reperfusion (I/R) injuries, B lymphocytes activate the complement cascade, leading to apoptosis. Studies on hypertensive mice have shown that depleting B-cells lessen the fibrotic load. Upon heart injury, B-cells interact with $CD4^+$ T-cells and monocytes via the interleukin-1 (IL-1) and myeloid differentiation protein 88 (MyD88) signaling pathway. IL-10, an anti-inflammatory factor predominantly secreted by B lymphocytes during the NF-κB/STAT3 signaling activation, is potentially bolstered by inhibiting T-cell stimulation.[6] Therefore, emerging studies on immune modulation in CVD models and clinical trials suggest a promising application of personalized regimens of immune modulators in treating these patients. Advancement in T-cell engineering also strongly supports potential clinical applications of immunomodulatory therapeutics.[6] Moreover, diverse types of immune cell populations have been shown to interact with resident cardiac cells, such as fibroblasts, endothelial cells, and myocardial cells in response to cardiac injuries.[7] Several cross-sectional and cohort studies have observed similar interactions in the context of CVD, however, they failed to reveal any mechanistic cross talk between immune cells and resident cardiomyocytes under the diseased condition.[8] Although randomized controlled trials (RCTs) can potentially establish the underlying causal relationship between different cell types during cardiac events, interventions that can elicit such interactions present practical and ethical challenges, making it difficult to draw definitive conclusions. Notably, Mendelian randomization (MR) methods can uncover significant cause-and-effect cross talk between immune cells and cardiomyocytes in CVD patients.[9] Mendelian randomization, a statistical method frequently employed in epidemiological investigations, is derived from the fundamental tenets of Mendelian independent assortment. Confirming a logical and well-founded causal sequence is critical in MR analysis.[10]

Thus, this study utilized a detailed two-sample MR analysis to investigate any potential correlative associations between diverse immune cell populations and CVD onset and progression. The findings of this study will be aimed at advancing treatment strategies, improving patient outcomes, and paving the way for personalized management of CVD by integrating targeted immune modulation with effective conventional therapies.

Materials and methods

Study design

Here, we examined the causal relationship among 731 immune cell types and CVD using a two-sample MR analysis. In MR, genetic variations serve as stand-ins for potential hazards, and credible instrumental variables (IVs) must meet three vital presumptions: (1) genetic variation is immediately related to the exposure; (2) genetic variation is unrelated to possible confounding factors among the exposure and outcome; and (3) genetic variation fails to impact this result through channels unrelated to the exposure. This study protocol was approved by the institutional ethics committee, and all participants provided written informed consent (see Figure 1).

Sources of genome-wide association study data on immunity

The genome-wide association study (GWAS) catalog offers public access to GWAS data sources for studying a broad spectrum of immune features for accession numbers ranging from GCST0001391 to GCST0002121.[11] Using CVD-related keywords, we discovered immune traits such as ebi-a-GCST90086056 (CVD) in GWAS resources (https://gwas.mrcieu.ac.uk/). This index served as a valuable repository of information on genetic variations and their associations with a broad array of traits and diseases to aid researchers and healthcare professionals in unraveling the genetic underpinnings of complex CVD cases. Using the CVD ID, we accessed the GWAS data online and conducted a specific analysis on the connections between immune cells and CVD development in a cohort of 56,637 individuals (comprising 15,009 patients and 41,628 control participants) based on their ID of immune traits (https://www.ebi.ac.uk/gwas/).

Instrumental variable selection

Since we noticed a significant proportion of single-nucleotide polymorphisms (SNPs) in GWAS analysis ($p < 5 \times 10^{-8}$) for immune cell characteristics, we implemented a stricter selection threshold ($p < 5 \times 10^{-9}$) level for genetic IVs.[11] These IVs were identified by grouping according to the Linkage Disequilibrium (LD) reference panel, obtained from the 1000 genetic sequencing program, with an LD threshold of $R^2 < 0.001$ within a

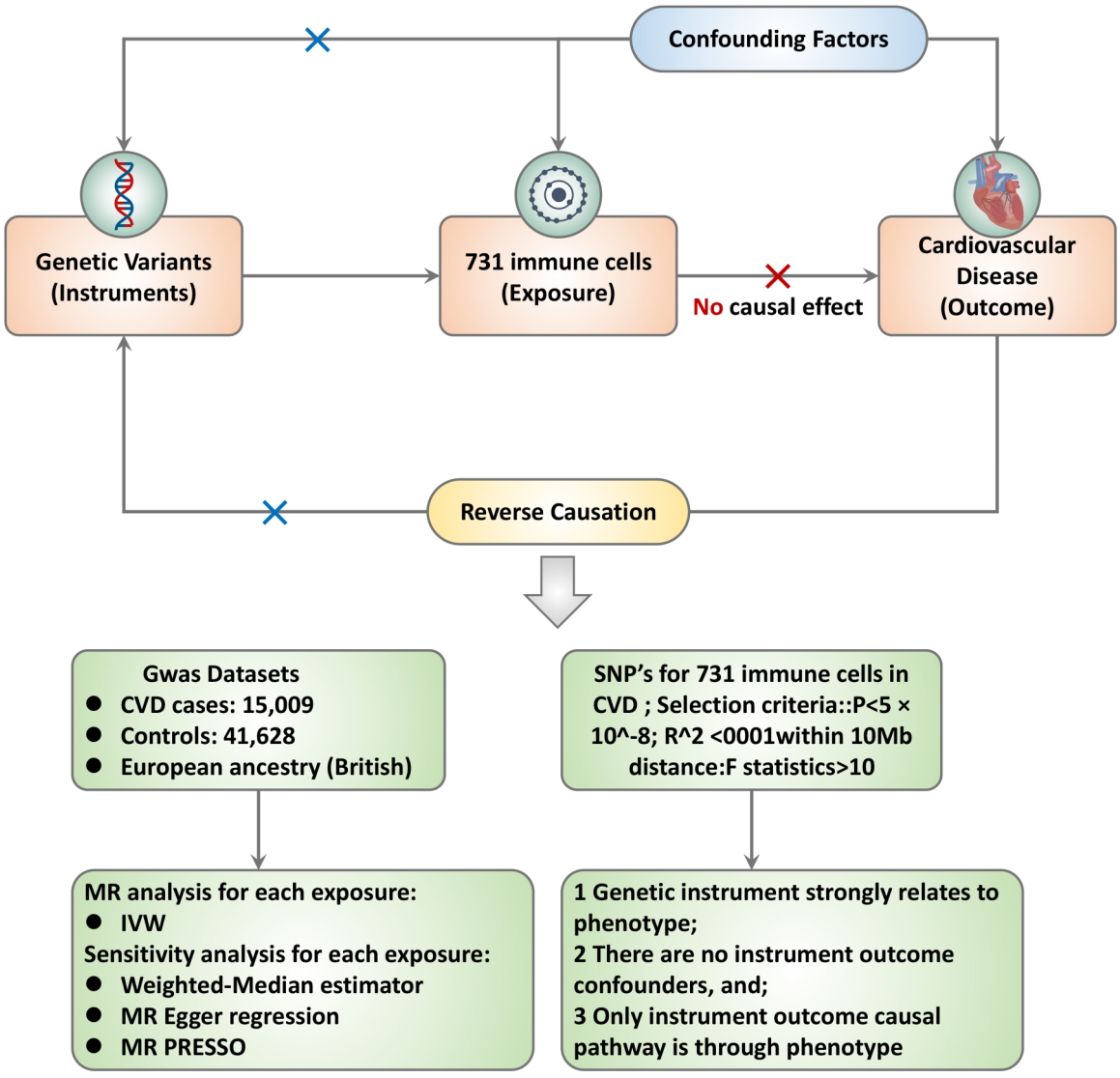

Figure 1. The flowchart of the study design.

1000-kilobase (kb) radius. Given a relatively limited scale of GWAS data for immune cells, we adopted a marginally relaxed p-value threshold of 5×10^{-5} along with a corresponding LD clustering threshold of $R^2 < 0.001$ across 1000 kb.[12] To ensure the dependability of our instruments, we prioritized IVs with F-test exceeding 10, which indicated their strength for further analysis. These IVs were selected from the summary data on CVD outcomes, excluding any that showed potential pleiotropic effects on CVD ($p < 10^{-5}$), following an established research protocol.[13] For reproducibility, we identified SNPs across the treatment and outcome datasets to maintain uniform effect measurements for identical alleles. Furthermore, SNPs with moderate allele frequencies (EAFs > 0.42) but incompatible with the allele of interest were omitted from this analysis.[12]

Statistical analysis

The analysis was conducted using R v3.5.3 software (http://www.Rproject.org). Different techniques including the inverse variance weighting (IVW) method, weighted median, and mode were utilized to evaluate the causal relationship between 731 immunophenotypes and CVD using the MR v0.4.3 software. We then assessed heterogeneity between the chosen IVs using Cochran's Q test and associated p-values. If the zero hypothesis was rejected, the random-effect IVW method was utilized rather than fixed-effect IVW. The MR-Egger method was employed to tackle horizontal pleiotropy, and the MR-PRESSO method was applied to detect and eliminate possible horizontal pleiotropic outliers that could impact the outcomes. Scatter diagrams, funnel plots, and leave-one-out analyses were employed to assess the data, indicating that the

findings were not influenced by outliers, thus demonstrating the strength of the association without any observed variability.[14]

Results

In this genetic causal inference study, we evaluated the impact of five immune cell markers on CVD risks. Among these markers, two exhibited protective effects, while the remaining three were identified as risk factors for CVD. Notably, activated and secretory $CD4^+$ regulatory T-cells showed a significant protective effect against CVD risk [odds ratio (OR): 0.757; 95% confidence interval (CI): 0.628–0.913; $p = 0.004$]. Similarly, the expression of B-cell activating factor receptor (BAFF-R) on IgD^- $CD38^+$ B-cells demonstrated a protective influence against CVD with a notable association (OR: 0.654; 95% CI: 0.468–0.915; $p = 0.013$). Conversely, CD80 on myeloid dendritic cells emerged as a risk factor for CVD, indicating a positive causal effect (OR: 1.181; 95% CI: 1.015–1.376; $p = 0.032$). Additionally, the proportions of $CD28^+$ $CD45RA^+$ $CD8^+$ T-cells (OR: 1.064; 95% CI: 1.002–1.128; $p = 0.041$) and $CD45RA^+$ $CD28^-$ $CD8^+$ T-cells (OR: 1.005; 95% CI: 1.000–1.011; $p = 0.045$) in the total T-cell population were recognized as risk factors for CVD (Figure 2). These findings suggest an intricate interplay between the immune cell markers and the risk of CVD, with specific immune cell subsets offering protection while others pose risks. Further exploration of these associations could provide valuable insights into the CVD pathomechanism and potential therapeutic targets. Both the MR-Egger intercept examination and Cochran's Q statistic showed no significant signs of pleiotropy or heterogeneity ($p > 0.05$) (Tables 1 and 2). Furthermore, scatter plots (Figure 3), funnel plots (Figure 4), and leave-one-out analysis (Figure 5) were employed to thoroughly examine the data by conclusively eliminating the impact of outliers and horizontal pleiotropy on the identified key immune cells.

Discussion

Leveraging publicly available genetic databases, we conducted a thorough MR-based examination of the relationship between 731 immune cell characteristics and CVD risks or pathology in respective patients. Here, we uncovered five key immune cells, including BAFF-R on IgD^-CD38^+ B-cells, $CD80^+$ myeloid-derived dendritic cells (MDDCs), activated and secretory $CD4^+$ regulatory T cells (Tregs), $CD28^+$ $CD45RA^+$ $CD8^+$ T-cells, and $CD45RA^+$ $CD28^-CD8^+$ T-cells. Among these, $CD80^+$ MDDCs, $CD28^+$ $CD45RA^+$ $CD8^+$ T-cells, and $CD45RA^+$ $CD28^-CD8^+$ T-cells were recognized as the major risk factors for CVD onset, while BAFF-R on IgD^-CD38^+ B-cells and activated $CD4^+$ Tregs exhibited protective effects against CVD.

CD4 is a surface protein found on immune cells, particularly on Tregs, and plays an essential role in mediating immune responses by promoting interactions among diverse immune cell types, such as antigen-presenting cells (APCs).[15] Activated $CD4^+$ Tregs represent a specialized subset of T-cells that are activated to carry out regulatory functions within the immune system. These cells contribute to immune homeostasis by suppressing tissue-damaging immune reactions as well as autoimmune responses.[16] On the other hand, secretory $CD4^+$ Tregs belong to another subset of regulatory T-cells capable of producing and releasing anti-inflammatory cytokines such as IL-10 and transforming growth factor-beta. These cytokines play a key role in dampening inflammatory processes and fostering immune tolerance in the host body, thus indicating the importance of secretory $CD4^+$ Tregs in modulating immune signaling.[17,18] A study has shown that personalized immune therapies targeting the activated $CD4^+$ Tregs can effectively protect the ischemic heart from fibrotic damage and dysfunction.[19] Furthermore, $CD4^+$ Tregs may protect the heart against hypertension by reducing oxidative stress, enhancing endothelial functionality, decreasing inflammation, and attenuating cardiac issues.[20] Interestingly, our research uncovered a significant association between activated and secretory $CD4^+$ Tregs and protecting the heart from severe CVDs.

B-cell activating factor receptor is a receptor protein that is expressed on the B-cell surface. Within the context of IgD^-CD38^+ B-cells, BAFF-R plays a crucial role in supporting B-cell survival, activation, and differentiation into antibody-secreting plasma cells, through its interaction with the BAFF ligand.[21] The interaction between BAFF-R and BAFF is essential for maintaining the immune function and coordinating antibody production in response to infections or immune challenges.[22] Notably,

Exposure	Method	No.of SNP	OR(95% CI)	P
BAFF−R on IgD− CD38+ B cell	Inverse variance weighted	3	0.654 (0.468 to 0.915)	0.013
CD80 on myeloid Dendritic Cell	Inverse variance weighted	4	1.181 (1.015 to 1.376)	0.032
CD4 on activated & secreting CD4 regulatory T cell	Inverse variance weighted	3	0.757 (0.628 to 0.913)	0.004
CD28+ CD45RA+ CD8+ T cell %T cell	Inverse variance weighted	28	1.064 (1.002 to 1.128)	0.041
CD45RA+ CD28− CD8+ T cell %T cell	Inverse variance weighted	54	1.005 (1.000 to 1.011)	0.045

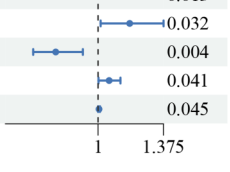

Figure 2. The causal role of immune cells and cardiovascular disease.

Table 1. Heterogeneity of immune cells in cardiovascular disease.

Exposure	Method	Q	Q_df	Q_pval
BAFF-R on IgD- $CD38^+$ B cell	Inverse variance weighted	0.26572896	2	0.875583743
BAFF-R on memory B cell	MR Egger	5.96284782	3	0.113431816
CD80 on myeloid Dendritic Cell	MR Egger	2.193095093	2	0.334022292
CD80 on myeloid Dendritic Cell	Inverse variance weighted	3.502671288	3	0.320415829
CD4 on activated & secreting CD4 regulatory T cell	MR Egger	0.219270483	1	0.639596337
CD4 on activated & secreting CD4 regulatory T cell	Inverse variance weighted	0.454524562	2	0.796711792
$CD28^+$ $CD45RA^+$ $CD8^+$ T cell %T cell	Inverse variance weighted	14.45030782	27	0.976457056
$CD28^+$ $CD45RA^+$ $CD8^+$ T cell %T cell	MR Egger	14.24155443	26	0.96967846
$CD45RA^+$ $CD28^-CD8^+$ T cell %T cell	Inverse variance weighted	44.55929227	53	0.788780679
$CD45RA^+$ $CD28^-CD8^+$ T cell %T cell	MR Egger	44.18114909	52	0.771141114

Table 2. Pleiotropy of immune cells in cardiovascular disease.

Exposure	egger_intercept	se	pval
BAFF-R on IgD- $CD38^+$ B cell	−0.033261379	0.071292063	0.722095018
CD80 on myeloid Dendritic Cell	0.082002352	0.075036861	0.388543319
CD4 on activated & secreting CD4 regulatory T cell	0.056217807	0.11590584	0.712502492
$CD28^+$ $CD45RA^+CD8^+$ T cell %T cell	−0.177257786	0.387961433	0.651540721
$CD45RA^+CD28^-CD8^+$ T cell %T cell	−0.104401458	0.169776833	0.541280412

the findings from our study suggest that BAFF-R signaling might play a role in protecting cardiac cells from CVD, especially hypertension-induced vascular inflammation.[23] Additionally, emerging studies highlight the potential of B-cells in preventing secondary cardiovascular events in individuals with atherosclerosis.[24] Nevertheless, our study revealed a significant correlation between the presence of BAFF-R on IgD^-CD38^+ memory B-cells and the protective effect of this subset of B-cells against CVDs.

CD80, also known as B7-1, functions as a costimulatory effector molecule in MDDCs and transforms MDDCs into specialized APCs, which is crucial for initiating a Th1-polarized immune response. In response to pathogens, MDDCs undergo maturation through enhanced expressions of CD80, major histocompatibility class II, and other costimulatory molecules. $CD80^+$ MDDCs are important for activating the antigen-specific naive T-cells. Elevated CD80 expression is also believed to be a determining factor for the migration of MDDCs into the lymph nodes. The regulation of CD80 expression and its function on MDDCs can be influenced by several environmental factors and the stage of innate immunity in the body.[25] A study demonstrating elevated levels of CD80 expression on MDDCs in Group B Coxsackievirus (CVB3)-infected mice suggests CD80 overexpression as a potential risk factor for immune dysregulation and disease pathogenesis in myocarditis, highlighting the importance of CD80 signaling pathway in modulating immune responses and treatment outcomes.[26] In this regard, this study demonstrated a significant correlation between the CD80 positivity on MDDCs and its potential role in risk stratification for CVD onset.

$CD28^+$ $CD45RA^+$ $CD8^+$ T-cells are a subset of cytotoxic T-cells distinguished by their unique cell surface markers CD28 and CD45RA. CD28 functions as a crucial costimulatory molecule and is essential for the activation and proliferation of T lymphocytes, while CD45RA is a surface marker that is typically associated with unresponsive T lymphocytes. The concurrent expression of CD28 and CD45RA on this cell type indicates their potential for activation and responsiveness to antigen stimulation.[27] During the immune activation, $CD28^+$ $CD45RA^+$ $CD8^+$ T-cells may contribute to pathogen surveillance and defense against abnormal or foreign cell bodies, representing a diverse T-cell population capable of mounting specific immune responses as required.[28] Furthermore, $CD8^+$ T-cells are important predictors of atherosclerosis. A study has reported increased populations of $CD8^+$ T-cells in a mouse model of advanced atherosclerosis and patients with severe CVDs, suggesting a possible association of $CD8^+$ T-cells with advanced stages of atherosclerosis.[29] Moreover, this study uncovered a notable association between the presence of $CD28^+$ $CD45RA^+$ $CD8^+$ and $CD28^-CD45RA^+$ $CD8^+$ T-cells and elevated risks of CVD.

Our study has several limitations that should be noted during the interpretation of the study findings. First, despite conducting multiple sensitivity analyses, the assessment of horizontal pleiotropy remained incomplete. Second, the lack of individual-level data hindered further stratified analyses within the population. Third, the

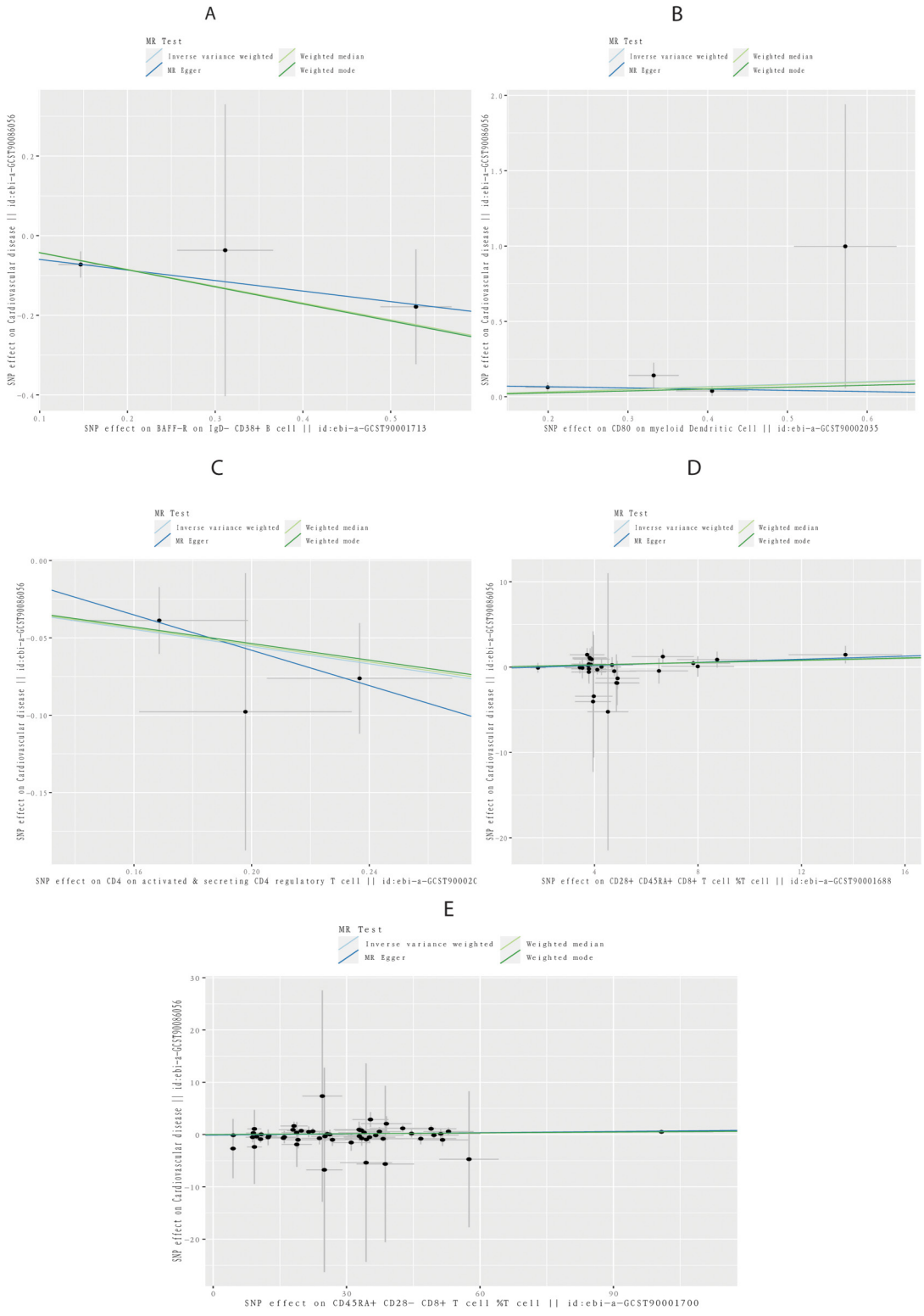

Figure 3. The scatter plot demonstrating the genetic associations of five immune cells and CVD. (A) BAFF-R on IgD-CD38^{+} memory B-cells in CVD, (B) CD80 on myeloid dendritic cells in CVD, (C) activated and secretory CD4^{+} regulatory T-cells (Tregs) in CVD, (D) CD28^{+}CD45RA^{+}CD8^{+} T-cells, (E) CD45RA^{+}CD28^{-}CD8^{+} T-cells in CVD.

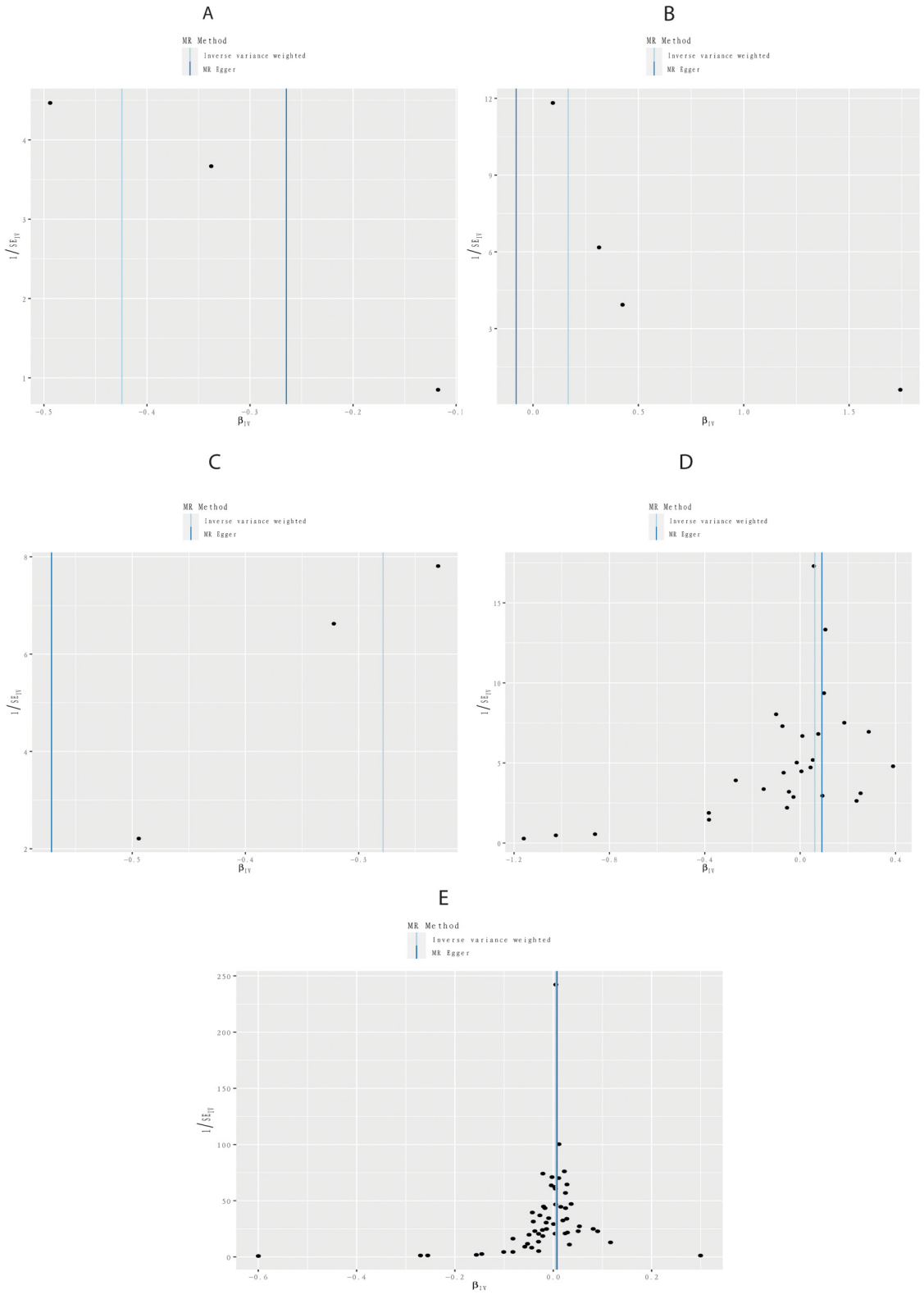

Figure 4. The funnel plot represents IVs for each significant causal relation between immune cells and CVD. (A) BAFF-R on IgD-CD38^{+} memory B-cells in CVD, (B) CD80 on myeloid dendritic cells in CVD, (C) activated and secretory CD4^{+} regulatory T-cells (Tregs) in CVD, (D) CD28^{+}CD45RA^{+}CD8^{+} T-cells, (E) CD45RA^{+}CD28^{-}CD8^{+} T-cells in CVD.

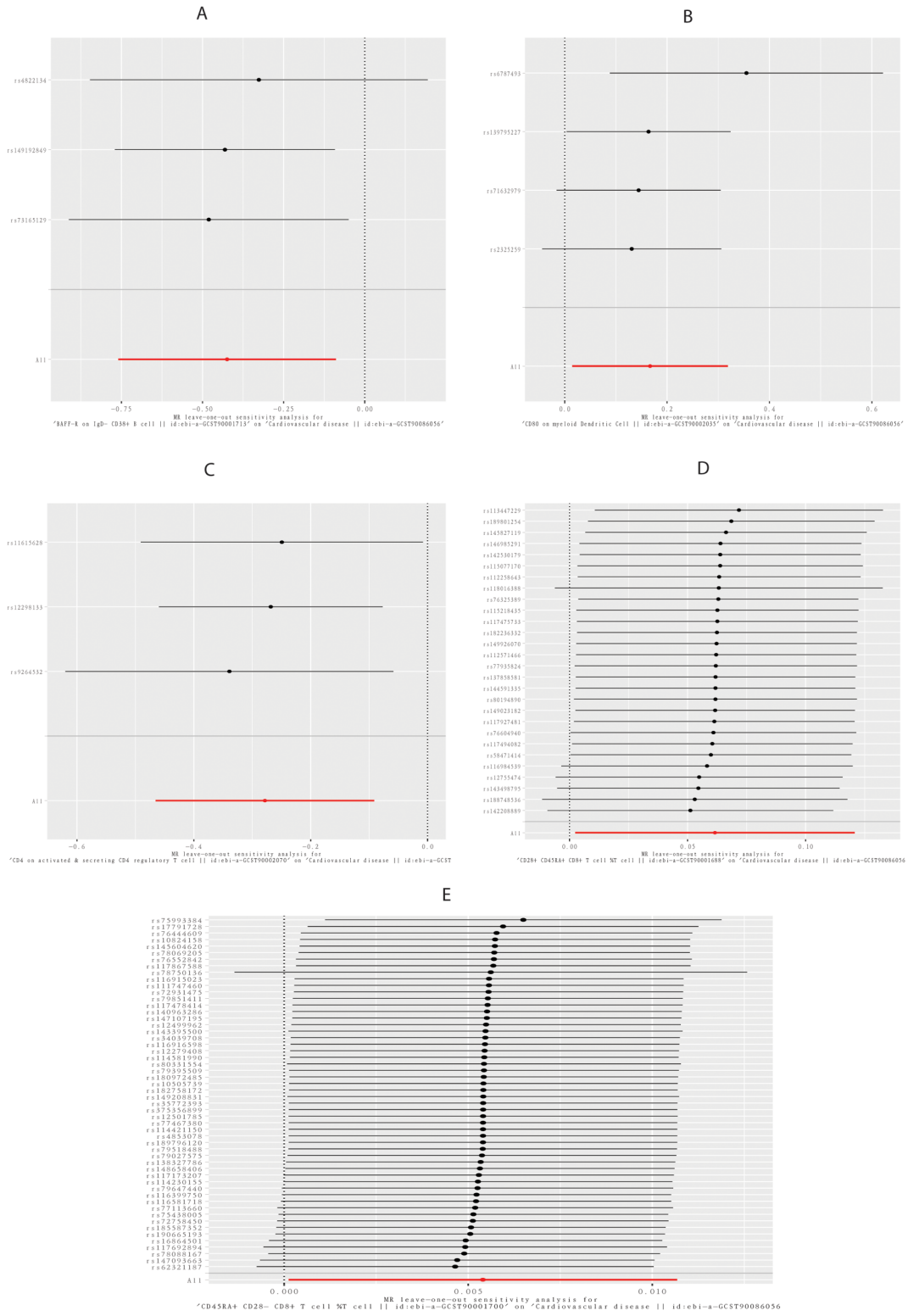

Figure 5. Leave-one-out showed causal relation between immune cells and CVD. (A) BAFF-R on IgD-CD38^{+} memory B-cells in CVD, (B) CD80 on myeloid dendritic cells in CVD, (C) activated and secretory CD4^{+} regulatory T-cells (Tregs) in CVD, (D) CD28^{+} CD45RA^{+} CD8^{+} T-cells, (E) CD45RA^{+} CD28^{-}CD8^{+} T-cells in CVD.

study's dependency on a European database limits the generalizability of our conclusions to other ethnic groups. Finally, the use of a less stringent threshold for result evaluation might have introduced false positives in our results. However, this study enabled us to conduct a more thorough exploration of the powerful connection between immune cell profiling and CVD risks.

Conclusions

Our exhaustive dual-direction MR analysis unveils causal relationships between diverse immune cell phenotypes and risks of CVD. Notably, rigorous consideration of confounding variables and the mitigation of reverse causality have enhanced the reliability of our results. As a result, this research paves the way for exploring the pathomechanism of CVD and provides opportunities for early interventions and improved therapeutic strategies.

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Authors' contribution

Vicheth Virak and Chongbin Zhong, acquisition of data, analyzing, interpretation of data, and drafting the article; Pingzhen Yang designing, revising, and guiding the study. The authors read and approved.

Data availability

All the data for this article can be found on GWAS database.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Informed consent

All the authors of the article agreed to be published in the journal.

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