Journal of Translational Autoimmunity 9 (2024) 100259

Contents lists available at ScienceDirect

Journal of Translational Autoimmunity

journal homepage: www.sciencedirect.com/journal/journal-of-translational-autoimmunity

Autoimmune diseases and cardiovascular risk: Mendelian randomization analysis for the impact of 19 autoimmune diseases on 14 cardiovascular conditions

Yulin Bao [a], Lingfeng Gu [a], Jiayi Chen [a], Hao Wang [a], Zemu Wang [a], Huijuan Wang [b], Sibo Wang [a,1], Liansheng Wang [a,*,1]

[a] Department of Cardiology, Jiangsu Province Hospital and Nanjing Medical University First Affiliated Hospital, Nanjing, Jiangsu, 210029, China
[b] Department of Immunology, School of Basic Medical Science, Nanjing Medical University, Nanjing, Jiangsu, 211166, China

ARTICLE INFO

Handling Editor: Y Renaudineau

Keywords:
Autoimmune diseases
Cardiovascular risk
Mendelian randomization

ABSTRACT

Backgroud: Autoimmune diseases (AIDs) have been associated with various cardiovascular diseases (CVDs) in observational data. However, the causality of these associations remains uncertain. Therefore, a systematic assessment of the impact of AIDS on cardiovascular risk is required.

Results: We assessed the impact of 19 common AIDs on 14 CVDs using bidirectional Mendelian randomization (MR). Celiac disease (odds ratio [OR] = 2.949, 95 % confidence interval [CI]: 1.111–7.827, P = 0.030) and type 1 diabetes mellitus (T1DM) (OR = 1.044, 95 % CI: 1.021–1.068, P = 1.82e-4) were associated with an increased risk of peripheral arterial disease (PAD). Additionally, celiac disease was linked to an increased risk of arrhythmia (OR = 1.008, 95 % CI: 1.002–1.013, P = 0.004), multiple sclerosis to venous thromboembolism (OR = 1.001, 95 % CI: 1.000–1.001, P = 0.010), and psoriasis to heart failure (OR = 1.048, 95 % CI: 1.021–1.077, P = 0.001). Sensitivity analyses were conducted to enhance the robustness of these findings. Predominantly, immune response and inflammation-related pathways were enriched in the aforementioned associations. Mediation analysis identified human leukocyte antigen-DR positive myeloid dendritic cells as partially mediating the effect of T1DM on PAD, with a mediated proportion of 16.61 % (P = 0.028). Potential therapeutic agents, such as tumor necrosis factor-alpha inhibitors and interferon, may have efficacy in treating AID-related CVDs.

Conclusions: This study presents genetic evidence of certain AIDs impacting specific CVDs and identifies potential mediators and drugs.

1. Introduction

Autoimmune disorders (AIDs) impact around 10 % of the worldwide population [1]. Certain AIDs are associated with elevated cardiovascular morbidity and mortality [2]. Current guidelines provide only limited recommendations for preventing cardiovascular diseases (CVDs) in patients with AIDs [3]. These guidelines mainly focus on more prevalent autoimmune disorders such as systemic lupus erythematosus (SLE), inflammatory bowel disease (IBD), psoriasis, and rheumatoid arthritis (RA), and their impact on coronary heart disease and stroke [4]. A recent observational study indicated that patients with AIDs face a 1.4- to 3.6-fold higher risk of developing CVDs than those without AIDs [5]. These AIDs encompass not only common conditions, such as RA, SLE, and psoriasis, but also 16 other AIDs. The cardiovascular adverse events include not only typical atherosclerotic diseases but also infection-related heart diseases, cardiac inflammation, thromboembolism, and degenerative heart diseases. Nevertheless, patients with AIDs may have various CVDs risk and the study did not examine the relationship between each AID and CVD separately [6]. Moreover, observational studies are vulnerable to confounding factors and reverse causality, leaving the causal relationship between AIDs and CVDs uncertain. Further research is needed to determine the potential causality and the impact of AIDs on CVDs.

Mendelian randomization (MR) represents an effective method for exploring the impact of AIDs on cardiovascular risk. In recent years, extensive genome-wide association studies (GWAS) have uncovered

* Corresponding author.
E-mail address: drlswang@njmu.edu.cn (L. Wang).
[1] The authors were considered as corresponding authors.

https://doi.org/10.1016/j.jtauto.2024.100259
Received 3 October 2024; Received in revised form 28 October 2024; Accepted 30 October 2024
Available online 1 November 2024

numerous genetic variants linked to AIDs and CVDs, rendering MR designs feasible [7]. MR can evaluate the potentially causal relationship between exposures and outcomes by using genetic variations as instrumental variables (IVs). Since genetic variants are randomly allocated to offspring at conception and are independent of factors such as self-adopted lifestyle and environmental confounding, MR minimizes bias from confounding factors [8]. Furthermore, MR reduces the impact of reverse causality because genetic variations are fixed throughout an individual's lifetime and cannot be influenced by disease onset or progression [7]. Additionally, MR presents a strategy to overcome the time-consuming demands, considerable expenses, and ethical considerations typically associated with randomized controlled trials [7]. Previous MR studies have investigated the association of RA [9], SLE [10], ankylosing spondylitis (AS) [11], and multiple sclerosis (MS) [12] with a subset of CVDs. However, these studies did not utilize more robust methodologies to investigate the impact of AIDs on cardiovascular risk in a comprehensive manner.

This study is the first to separately investigate the causal relationships between a wide range of AIDs and CVDs utilizing extensive GWAS data and bidirectional, two-sample MR analysis. Moreover, the study aimed to provide genetic evidence indicating that AIDs contribute to the development of CVDs, thereby guiding the development of more comprehensive preventive approaches.

2. Methods

2.1. Study design

The MR design is depicted in Fig. 1. This research is a bidirectional two-sample MR study, based on three fundamental assumptions [13]: (1) the selected genetic variants are strongly linked to the exposure (association assumption). (2) the selected genetic variants are not affected by potential confounding factors (independence assumption). (3) the impact of the selected genetic variants on the outcome is exclusively mediated by the exposure (exclusivity assumption). In the forward MR analysis, AIDs are considered the exposure and CVDs the outcome, aiming to explore the effect of AIDs on CVD risk. In the reverse MR analysis, the roles are reversed. The datasets utilized in this research were acquired from publicly available databases and had obtained ethical approval before their use. Thus, no further ethical approval was necessary for this study. The study was conducted following the Strengthening the Reporting of Observational Studies in Epidemiology using MR (STROBE-MR) guidelines [14] (Table S9).

2.2. GWAS data for AIDs and CVDs

We included 19 of the most prevalent AIDs [5], including: AS, Addison's disease, polymyalgia rheumatica, RA, SLE, systemic sclerosis, Sjögren's syndrome, celiac disease, type 1 diabetes mellitus (T1DM), Graves' disease, Hashimoto's thyroiditis, IBD, MS, myasthenia gravis, psoriasis, primary biliary cirrhosis (PBC), pernicious anemia, vasculitis and vitiligo. To characterize the broad spectrum of CVDs [5], we included GWAS data on 14 CVDs: aortic aneurysm, atrial fibrillation and flutter, unspecified arrhythmias, conduction system disease, endocarditis, heart failure (HF), ischemic heart disease (IHD), myocarditis, peripheral arterial disease (PAD), pericarditis, all-cause stroke, valve disorders, venous thromboembolism (VTE), and pulmonary embolism (PE). Comprehensive details regarding the sources of the GWAS data are included in Table 1.

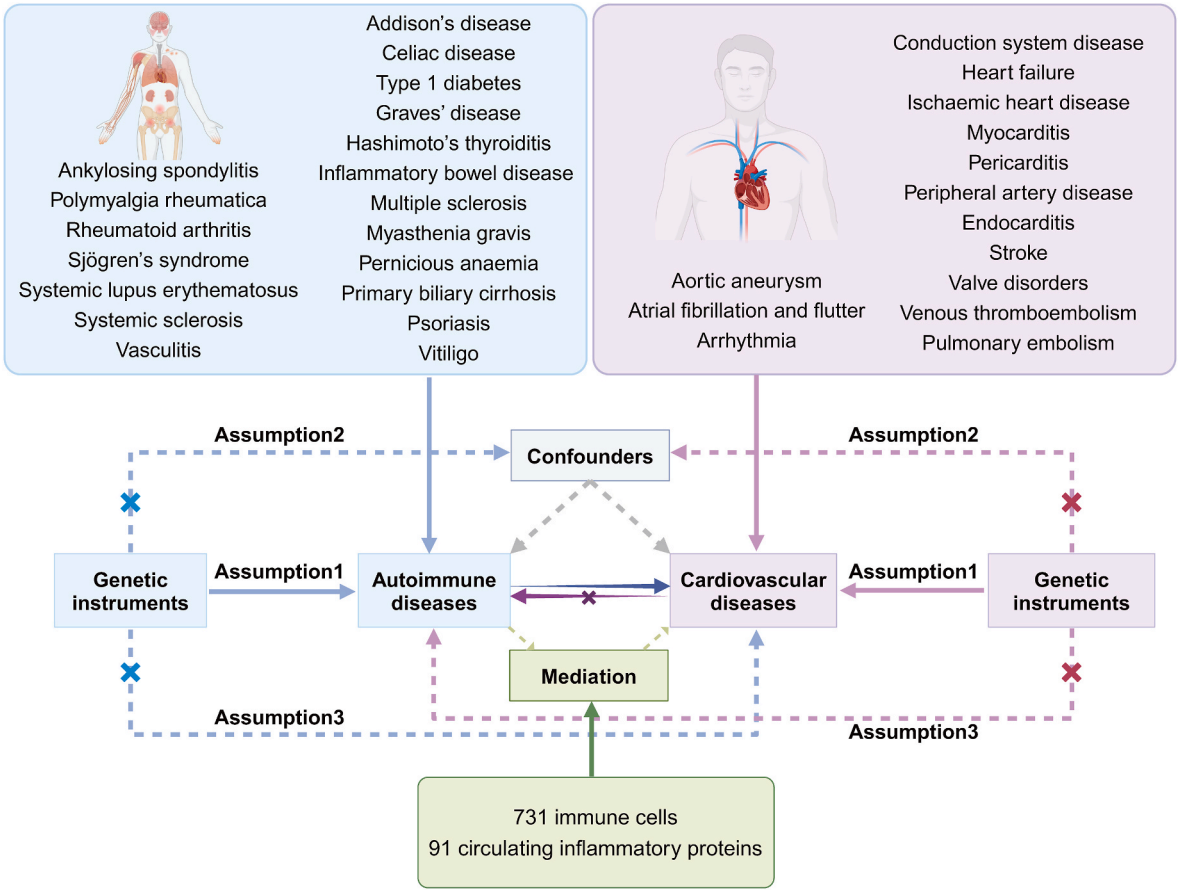


Fig. 1. An overview of the study design.

Table 1
Characteristics of the GWAS included in the MR study.

Phenotype	Sery ID	Sample size (ncase/ncontrol)	Ethnicity	Year of publication	PMID
Cardiovascular disease					
Aortic aneurysm	GCST90018783	479194 (3230/475964)	73.3 % European	2021	34594039
Atrial fibrillation and flutter	GCST90043977	456348 (8404/447944)	European	2021	34737426
Arrhythmia	GCST90038611	484598 (7207/477391)	European	2021	33959723
Conduction system disease	ukb-d-I9_CONDUCTIO	361194 (1055/360139)	European	2018	30305743
Heart failure	HERMES	977323 (47309/930014)	European	2020	31919418
Ischaemic heart disease	CARDIoGRAMplusC4D	1165690 (181522/984168)	European	2022	36474045
Myocarditis	GCST90018882	427911 (633/427278)	73.3 % European	2021	34594039
Pericarditis	GCST90018896	455165 (1795/453370)	73.3 % European	2021	34594039
Peripheral artery disease	GCST90018890	483078 (7114/475964)	73.3 % European	2021	34594039
Endocarditis	ieu-b-4972	486484 (1080/485404)	European	2021	30305743
Stroke	MEGASTROKE	446696 (40585/406111)	European	2018	29531354
Valve disorders	GCST90038612	484598 (3742/480856)	European	2021	33959723
Venous thromboembolism	GCST90038607	484598 (12240/472358)	European	2021	33959723
Pulmonary embolism	GCST90038614	484598 (3940/480658)	European	2021	33959723
Autoimmune disease					
Ankylosing spondylitis	finn-b-M13_ANKYLOSPON	166144 (1462/164682)	European	2021	36653562
Polymyalgia rheumatica	finn-b-M13_POLYMYALGIA	214668 (1523/213145)	European	2021	36653562
Rheumatoid arthritis	GCST90132223	97173 (22350/74823)	European	2022	36333501
Sjögren's syndrome	finn-b-M13_SJOGREN	214435 (1290/213145)	European	2023	36653562
Systemic lupus erythematosus	GCST003156	14267 (5201/9066)	European	2015	26502338
Systemic sclerosis	finn-b-M13_SYSTSLCE	213447 (302/213145)	European	2023	36653562
Vasculitis	finn-b-L12_VASCULITISNAS	207744 (262/207482)	European	2023	36653562
Addison's disease	GCST90011871	5320 (1223/4097)	European	2021	33574239
Coeliac disease	GCST90014443	325334 (1260/324074)	European	2021	34278373
Type 1 diabetes	finn-b-T1D	185258 (2685/182573)	European	2023	36653562
Graves' disease	finn-b-E4_THYTOXGOITDIF	190034 (2350/187684)	European	2023	36653562
Hashimoto's thyroiditis	finn-b-E4_THYROIDITAUTOIM	187928 (244/187684)	European	2023	36653562
Inflammatory bowel disease	GCST90225550	335453 (4859/330594)	European	2022	36446896
Multiple sclerosis	MSGC	115803 (47429/68374)	European	2019	31604244
Myasthenia gravis	finn-b-G6_MYASTHENIA	217288 (232/217056)	European	2023	36653562
Pernicious anaemia	GCST90014451	324497 (423/324074)	European	2021	34278373
Primary biliary cirrhosis	GCST90061440	24510 (8021/16489)	European	2021	34033851
Psoriasis	finn-b-L12_PSORIASIS	216752 (4510/212242)	European	2023	36653562
Vitiligo	GCST004785	40258 (2853/37405)	European	2016	27723757

2.3. Selection

Stringent filtering steps were conducted to ensure the quality of IVs, guided by three fundamental assumptions: (1) The genome-wide association significance threshold was set to a P-value $<$ 5e-8 and considered independent with a linkage disequilibrium (LD) threshold of $r^2 < 0.001$ within a 10,000 kilobase (kb) window. If the MR analysis included fewer than four SNPs, the thresholds were adjusted to a P-value $<$ 5e-6 and $r^2 < 0.01$. (2) For palindromic SNPs with intermediate allele frequencies, strand alignment was attempted to ensure accurate analysis. (3) The LDtrait tool (https://ldlink.nih.gov/?tab=ldtrait) was used to assess all known phenotypes related to the genetic instruments and to exclude SNPs associated with potential confounders at a genome-wide significance level (P-value $<$ 5e-8), thereby minimizing the effect of confounders. (4) For each SNP, an F-statistic $>$10 was considered indicative of sufficient strength for the selected IVs. The instrument's strength was determined by calculating the F-statistic utilizing specified formulas: $F = \frac{R^2(N-2)}{(1-R^2)}$ and $R^2 = \frac{2\times beta^2 \times eaf\times(1-eaf)}{2\times beta^2\times eaf\times(1-eaf)+2\times N\times eaf\times(1-eaf)\times se^2}$, where *eaf* represents the frequency of the effect allele, *beta* denotes the estimated impact of the genetic effect on the outcome, and *N* represents the sample size of the GWAS data [15]. (5) The outlier (MR pleiotropy residual sum and outlier [MR-PRESSO]) test was employed to identify and remove potential outlier SNPs. The IVs obtained through the 5 rigorous selection steps mentioned above are presented in Table S4.

2.4. Forward MR analysis

Three analytical methods were utilized: random-effects inverse variance weighted (IVW), weighted median, and MR-Egger methods. The IVW is regarded as the most robust MR approach because it presumes all genetic variants are valid and adheres strictly to the three fundamental assumptions [16]. The weighted median method requires that the genetic variants contributing at least half of the weight are valid. The MR-Egger method operates under the assumption that over half of the genetic variants are invalid, making it more applicable to scenarios where horizontal pleiotropy is widespread. Consequently, the IVW was employed as the principal analytical approach. If the outcome of the IVW method is statistically significant, it can be considered a positive result, even if the outcomes of other methods are not statistically significant, provided that the beta values of the other methods align in the same direction. Given the strong pleiotropy of SNPs in the MHC region, we performed a secondary analysis excluding SNPs in the MHC region (CRCh37 coordinates, chr6: 28,477,797–33,448,354) [17] and repeated the MR analysis on the significant results from the previous forward MR analysis.

Multiple sensitivity analyses were performed in our study to confirm the reliability of the results: (1) The intercepts of MR-Egger and MR-PRESSO were utilized to evaluate the presence of uncorrelated horizontal pleiotropy. (2) MR-PRESSO was supplemented with leave-one-out analysis to detect outlier SNPs. If outliers were present, they were excluded and re-analyzed using MR analysis. (3) Cochran's Q test for MR-Egger and IVW were used to detect heterogeneity, and a funnel plot was utilized as a visual aid to assess heterogeneity. (4) Although MR-Egger and MR-PRESSO can address uncorrelated pleiotropy (where horizontal effects on the outcome are not related to effects on the exposure), correlated pleiotropy (where horizontal effects on the outcome are linked with effects on the exposure) can still produce many false-positive results [18]. Therefore, the causal analysis using summary effect estimates (CAUSE) approach was employed to detect correlated pleiotropy and minimize false-positive results when examining causal relationships. This method incorporates information from all variants and calculates the discrepancy in the expected log pointwise posterior density (ΔELPD) to evaluate the fit of the sharing and the causal model

[18].

2.5. Reverse MR analysis

Although MR is less susceptible to bias from reverse causation, recent studies have suggested that it cannot be entirely immune to such bias [19]. Reverse causation can still influence MR analyses in three ways: the strength of genetic association between the genetic variant and exposure or outcome, the presence of a feedback loop between genetic variant, exposure, and outcome, and the cross-generational effects [19]. Moreover, if both forward and reverse MR analyses found causality at the same time, the results of this bidirectional MR may reflect the possible presence of a common etiological pathway between the exposure and outcome, rather than a true causal effect in both directions [20,21]. To eliminate the interference of reverse causality on the previously obtained significant results, we performed reverse MR analyses using the IVW method, treating CVD as the exposure and AID as the outcome. Additionally, we applied the weighted median and MR-Egger methods.

2.6. Pathway and gene enrichment analysis

To perform the enrichment analysis and explore the function of IVs, we identify potential genes within a 100 kb range around the SNP, we used the "vaultils" package (version 0.1.0) in R, referencing Human Genome version 19. The genes corresponding to the SNPs were enriched using Metascape (https://metascape.org/). The results of the Gene Ontology (GO) analysis were visualized in semantic space by REVIGO (http://revigo.irb.hr/) as clustered representations, illustrating the remaining terms after reducing redundancy.

2.7. Mediation analysis

To further elucidate the mediating effects within the statistically significant causal relationships identified, we performed mediation analyses. The "total" effect of the exposure on the outcome encompasses both the "direct" effect and any "indirect" effects mediated by one or more mediators. Specifically, the effect of the exposure on the mediator is represented as β1, while the effect of the mediator on the outcome is denoted as β2. The mediating effect is quantified as β1 × β2. In this study, the total effect (β3) was obtained through standard MR analysis. The proportion of the total effect mediated by mediators was calculated by dividing product of the mediating effect (β1 × β2) by the total effect (β3). The confidence interval (CI) and standard error (SE) for this proportion were estimated using the bootstrap method.

2.8. Processing and analysing data from single-cell RNA sequencing (scRNA-seq)

Peripheral blood scRNA-seq data from patients with T1DM were obtained from public datasets (Synapse accession code: syn53641849). We used the "Seurat" package (version 4.3.0) in R for subsequent processing. After integrating the data, we extracted dendritic cells (DCs) and presented the expression of myeloid dendritic cell (mDC) markers (C-type lectin domain containing 9A [CLEC9A] and cell adhesion molecule 1 [CADM1]) in both T1DM and healthy groups using the "Dotplot(2)" function. Then, the mDCs were extracted separately, and the expression of human leukocyte antigen-DR (HLA-DR) molecules was compared between the two groups using the "Vlnplot(2)" function.

2.9. Potential drug prediction

To more accurately annotate GWAS variants to genes and predict potential drugs, we first used MAGMA [22] (Multi-marker Analysis of GenoMic Annotation), which uses a multiple regression approach to appropriately incorporate linkage disequilibrium between markers and detect multi-marker effects. We then used 2 large pharmacogenetic databases, DGIdb [23] and PharmGKB [24], respectively, for deriving drugs matching candidate genes and taking the intersection of the results obtained from both databases.

2.10. Statistical analysis

MR estimates were reported as β coefficients, odds ratios (OR), and their corresponding 95 % CIs. To address multiple testing issues, we implemented a Bonferroni-corrected significance threshold of $P < 0.05/(19 \times 14)$ ($P < 1.88e\text{-}4$) for identifying significant causal relationships. Meanwhile, P values between 1.88e-4 to 0.05 were considered to indicate suggestive causal associations. In the CAUSE method, the P-value threshold for statistical significance was set at 0.05. All analyses were conducted using the "CAUSE" package (version 1.2.0.0335) and the "TwoSampleMR" package (version 0.6.2) in R (version 4.3.1).

3. Results

3.1. Selection of IVs

After a series of rigorous screenings, we obtained the IVs for 19 AIDs and 14 CVDs. Each individual IV exhibited an F-statistic over 10, showing that they were not impacted by weak instrument bias. Additionally, outlier SNPs identified by the MR-PRESSO assay, as well as SNPs potentially related to confounding factors, were excluded. Comprehensive details on all IVs utilized for forward, secondary forward and reverse MR analysis is available in Table S4.

3.2. Forward MR analysis unraveled the impact of AIDs on CVDs

The IVW method identified 38 causal associations (Fig. 2A). Subsequently, we excluded associations demonstrating horizontal pleiotropy, as indicated by the Egger regression intercept or MR-PRESSO global test, and those where the directional results of the other two MR methods (weight median and MR-Egger) was not consistent. Finally, 29 significant preliminary results were identified: 24 positive and 5 negative (Fig. 2B and Table S5). This finding partially aligns with those of previous observational studies suggesting that AIDs elevate the risk of CVDs.

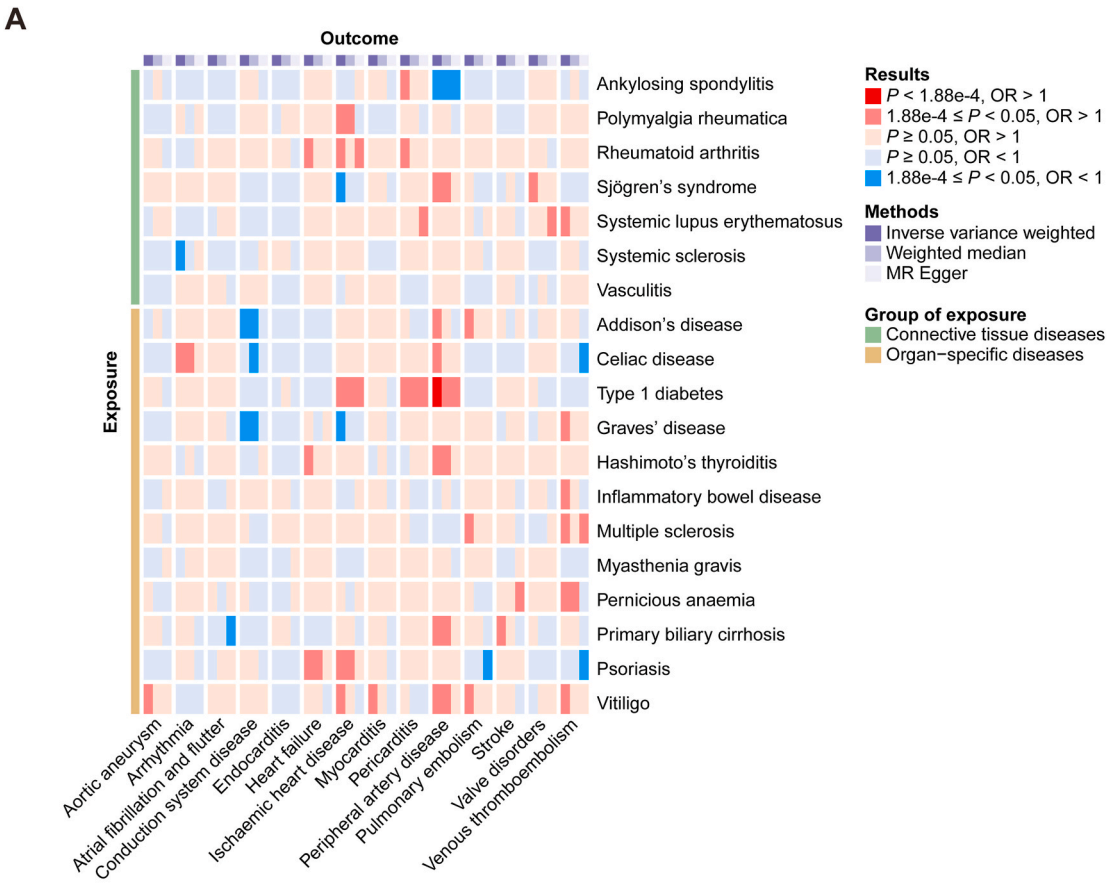
In the context of PAD, the study found several AIDs that may significantly increase the risk. These include celiac disease (OR = 2.949, 95 % CI: 1.111–7.827, P = 0.030), Hashimoto's thyroiditis (OR = 1.048, 95 % CI: 1.014–1.084, P = 0.006), Sjögren's syndrome (OR = 1.047, 95 % CI: 1.016–1.079, P = 0.003), vitiligo (OR = 1.038, 95 % CI: 1.016–1.060, P = 0.001), and PBC (OR = 1.027, 95 % CI: 1.001–1.053, P = 0.039). T1DM (OR = 1.044, 95 % CI: 1.021–1.068, P = 1.82e-4) shows a significant positive association with PAD. Conversely, AS showed a suggestive negative association with the risk of PAD (OR = 0.974, 95 % CI: 0.951–0.996, P = 0.024).

Regarding IHD, RA (OR = 1.016, 95 % CI: 1.002–1.029, P = 0.022), and T1DM (OR = 1.015, 95 % CI: 1.007–1.023, P = 2.27e-4) showed suggestive positive associations. In contrast, Graves' disease (OR = 0.986, 95 % CI: 0.975–0.998, P = 0.022) and Sjögren's syndrome (OR = 0.989, 95 % CI: 0.977–1.000, P = 0.045) showed suggestive negative associations.

For HF, Hashimoto's thyroiditis (OR = 1.021, 95 % CI: 1.004–1.039, P = 0.017), psoriasis (OR = 1.048, 95 % CI: 1.021–1.077, P = 0.001), and RA (OR = 1.023, 95 % CI: 1.005–1.042, P = 0.012) are suggestive associated with an increased risk.

Regarding pericarditis, AS (OR = 1.065, 95 % CI: 1.003–1.130, P = 0.039), RA (OR = 1.074, 95 % CI: 1.008–1.145, P = 0.028), and T1DM (OR = 1.060, 95 % CI: 1.011–1.111, P = 0.015) showed suggestive positive associations.

Celiac disease demonstrated a suggestive positive association with the risk of arrhythmia (OR = 1.008, 95 % CI: 1.002–1.013, P = 0.004). Vitiligo showed a suggestively positive association with aortic aneurysm



B

Exposure	Outcome	nSNP	P value	OR(95%CI)	Pleiotropy	Heterogeneity
Coeliac_disease	Peripheral_artery_disease	10	0.030	2.949 (1.111 to 7.827)	0.678 / 0.058	0.044
Rheumatoid_arthritis	Pericarditis	62	0.028	1.074 (1.008 to 1.145)	0.314 / 0.872	0.868
Ankylosing_spondylitis	Pericarditis	14	0.039	1.065 (1.003 to 1.130)	0.743 / 0.867	0.919
Type_1_diabetes	Pericarditis	23	0.015	1.060 (1.011 to 1.111)	0.440 / 0.880	0.854
Hashimoto_thyroiditis	Peripheral_artery_disease	9	0.006	1.048 (1.014 to 1.084)	0.756 / 0.641	0.591
Psoriasis	Heart_failure	26	0.001	1.048 (1.021 to 1.077)	0.378 / 0.192	0.171
Sjögren_syndrome	Peripheral_artery_disease	34	0.003	1.047 (1.016 to 1.079)	0.909 / 0.285	0.288
Type_1_diabetes	Peripheral_artery_disease	26	0.000	1.044 (1.021 to 1.068)	0.885 / 0.161	0.117
Vitiligo	Peripheral_artery_disease	38	0.001	1.038 (1.016 to 1.060)	0.963 / 0.587	0.584
Vitiligo	Aortic_aneurysm	34	0.047	1.037 (1.000 to 1.075)	0.809 / 0.495	0.497
Primary_biliary_cirrhosis	Peripheral_artery_disease	42	0.039	1.027 (1.001 to 1.053)	0.697 / 0.143	0.138
Rheumatoid_arthritis	Heart_failure	60	0.012	1.023 (1.005 to 1.042)	0.417 / 0.102	0.096
Hashimoto_thyroiditis	Heart_failure	7	0.017	1.021 (1.004 to 1.039)	0.757 / 0.508	0.529
Rheumatoid_arthritis	Ischaemic_heart_disease	57	0.022	1.016 (1.002 to 1.029)	0.319 / 0.052	0.048
Type_1_diabetes	Ischaemic_heart_disease	20	0.000	1.015 (1.007 to 1.023)	0.241 / 0.463	0.418
Coeliac_disease	Arrhythmia	13	0.004	1.008 (1.002 to 1.013)	0.902 / 0.572	0.534
Graves_disease	Venous_thromboembolism	23	0.025	1.001 (1.000 to 1.001)	0.481 / 0.538	0.558
Multiple_sclerosis	Venous_thromboembolism	61	0.010	1.001 (1.000 to 1.001)	0.614 / 0.173	0.188
Vitiligo	Venous_thromboembolism	33	0.047	1.001 (1.000 to 1.001)	0.767 / 0.122	0.109
Systemic_lupus_erythematosus	Venous_thromboembolism	40	0.041	1.000 (1.000 to 1.001)	0.534 / 0.160	0.163
Sjögren_syndrome	Valve_disorders	30	0.023	1.000 (1.000 to 1.001)	0.871 / 0.758	0.727
Vitiligo	Pulmonary_embolism	34	0.020	1.000 (1.000 to 1.001)	0.759 / 0.232	0.225
Multiple_sclerosis	Pulmonary_embolism	63	0.040	1.000 (1.000 to 1.001)	0.816 / 0.815	0.809
Addison_disease	Pulmonary_embolism	13	0.017	1.000 (1.000 to 1.000)	0.138 / 0.421	0.296
Addison_disease	Conduction_system_disease	14	0.036	1.000 (1.000 to 1.000)	0.370 / 0.370	0.294
Graves_disease	Conduction_system_disease	22	0.001	1.000 (0.999 to 1.000)	0.998 / 0.901	0.891
Sjögren_syndrome	Ischaemic_heart_disease	32	0.045	0.989 (0.977 to 1.000)	0.970 / 0.185	0.168
Graves_disease	Ischaemic_heart_disease	23	0.022	0.986 (0.975 to 0.998)	0.591 / 0.624	0.574
Ankylosing_spondylitis	Peripheral_artery_disease	17	0.024	0.974 (0.951 to 0.996)	0.114 / 0.436	0.403

Pleiotropy test: Egger Regression Intercept (pvalue) / MR PRESSO Global Test (pvalue)
Heterogeneity test: Cochran's Q Test (pvalue)

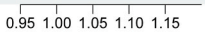


Fig. 2. Causal effects of 19 AIDs on 14 CVDs. A. Heatmap depicting 798 causal relationships obtained using three methods (IVW, weighted median, and MR-Egger). B. Forest plot showing 29 potentially causal relationships.

(OR = 1.037, 95 % CI: 1.000–1.075, P = 0.047).

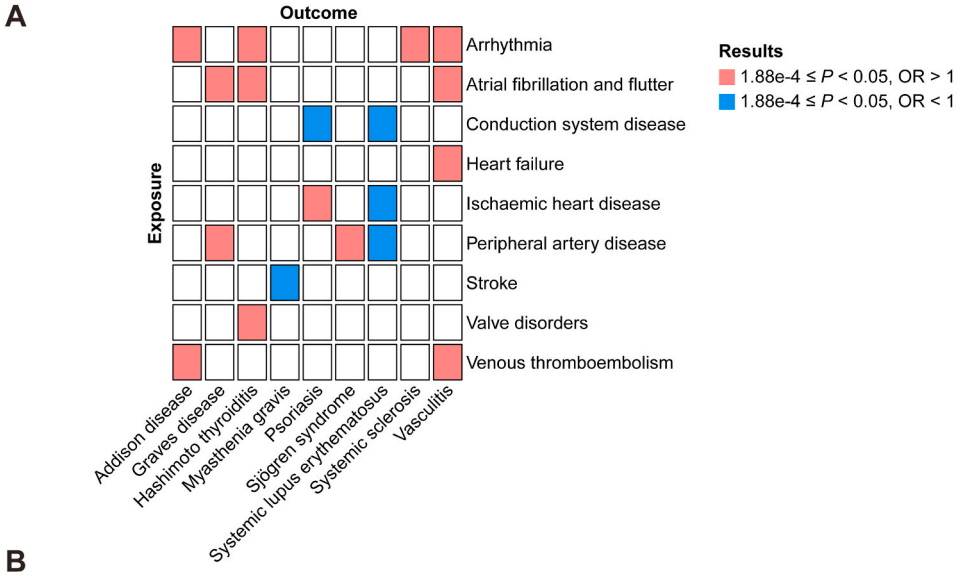
Additionally, some results exhibit suggestive causal relationships, albeit with relatively modest ORs (Fig. 2B and Table S1). In the context of VTE, Grave's disease (P = 0.025), MS (P = 0.010), SLE (P = 0.041), and vitiligo (P = 0.047) demonstrated suggestive positive associations. For PE, Addison disease (P = 0.017), MS (P = 0.040), and vitiligo (P = 0.020) showed suggestive positive associations. For conduction system disease, Addison disease (P = 0.036), and Grave's disease (P = 0.001) show suggestive negative associations. Sjögren's syndrome is suggestive associated with an increased risk of valve disorders (P = 0.023).

Most associations showed no heterogeneity according to Cochran's Q statistics, with exceptions observed for RA with IHD (P = 0.048), and celiac disease with PAD (P = 0.044) (Fig. 2B). Funnel plots, forest plots, scatter plots, and leave-one-out analyses illustrated the single SNP effect sizes, which are presented in Fig. S1 and Table S1.

The secondary MR reanalysis examined the 29 causal relationships identified by the forward MR analysis after excluding SNPs in the MHC region. Fourteen of the 29 significant results remained statistically significant (Fig. S2 and Table S5). Details of all secondary MR results, as well as the sensitivity analysis, are presented in Table S2.

3.3. Reverse MR analysis unraveled the impact of CVDs on AIDs

To mitigate the influence of reverse causality on our results, we conducted the reverse MR analysis on 14 CVDs and 19 AIDs (Fig. S3). Using the IVM method, after excluding instances with horizontal pleiotropy that did not meet MR assumptions, we identified 20 potential reverse causal pairs (Fig. 3A, Tables S3 and S5). Among the 29



B

Exposure	Outcome	nSNP	P value	OR(95%CI)	Pleiotropy	Heterogeneity
Peripheral_artery_disease	Sjögren_syndrome	12	0.035	1.220 (1.014 to 1.467)	0.057 / 0.568	0.550
Aortic_aneurysm	Vitiligo	21	0.056	1.116 (0.997 to 1.249)	0.131 / 0.088	0.078
Peripheral_artery_disease	Coeliac_disease	11	0.077	1.006 (0.999 to 1.012)	0.100 / 0.633	0.865
Peripheral_artery_disease	Hashimoto_thyroiditis	14	0.079	1.495 (0.954 to 2.343)	0.879 / 0.065	0.066
Peripheral_artery_disease	Type_1_diabetes	11	0.158	1.139 (0.951 to 1.366)	0.501 / 0.223	0.183
Ischaemic_heart_disease	Type_1_diabetes	58	0.306	1.063 (0.946 to 1.193)	0.622 / 0.104	0.112
Pericarditis	Ankylosing_spondylitis	11	0.316	1.038 (0.965 to 1.116)	0.128 / 0.386	0.470
Conduction_system_disease	Addison_disease	6	0.327	0.000 (0.000 to 1.458e+20)	0.963 / 0.131	0.097
Pulmonary_embolism	Vitiligo	5	0.382	0.000 (0.000 to 1.489e+4)	0.782 / 0.386	0.371
Venous_thromboembolism	Multiple_sclerosis	25	0.439	4.642 (0.095 to 225.848)	0.511 / 0.074	0.063
Pulmonary_embolism	Addison_disease	7	0.443	2.825e+3 (0.000 to 1.824e+12)	0.243 / 0.256	0.238
Ischaemic_heart_disease	Rheumatoid_arthritis	58	0.448	0.971 (0.899 to 1.048)	0.823 / 0.136	0.131
Venous_thromboembolism	Systemic_lupus_erythematosus	27	0.458	6.095 (0.052 to 719.648)	0.128 / 0.120	0.091
Pericarditis	Rheumatoid_arthritis	11	0.459	0.980 (0.928 to 1.035)	0.144 / 0.287	0.331
Valve_disorders	Sjögren_syndrome	8	0.469	2.815e+4 (0.000 to 3.078e+16)	0.241 / 0.057	0.029
Pulmonary_embolism	Multiple_sclerosis	4	0.518	35.458 (0.001 to 1.755e+6)	0.428 / 0.552	0.529
Ischaemic_heart_disease	Graves_disease	61	0.594	0.971 (0.871 to 1.083)	0.583 / 0.033	0.035
Venous_thromboembolism	Vitiligo	27	0.603	5.352 (0.010 to 2.982e+3)	0.879 / 0.068	0.071
Arrhythmia	Coeliac_disease	9	0.622	0.891 (0.563 to 1.410)	0.756 / 0.420	0.277
Pericarditis	Type_1_diabetes	11	0.659	0.980 (0.898 to 1.070)	0.496 / 0.085	0.043
Heart_failure	Hashimoto_thyroiditis	9	0.664	1.196 (0.534 to 2.676)	0.753 / 0.959	0.953
Conduction_system_disease	Graves_disease	14	0.708	0.028 (0.000 to 3.472e+6)	0.986 / 0.325	0.296
Heart_failure	Rheumatoid_arthritis	9	0.774	1.031 (0.836 to 1.272)	0.044 / 0.219	0.192
Venous_thromboembolism	Graves_disease	30	0.838	0.733 (0.037 to 14.399)	0.463 / 0.578	0.536
Peripheral_artery_disease	Vitiligo	13	0.900	0.986 (0.796 to 1.222)	0.064 / 0.138	0.105
Peripheral_artery_disease	Primary_biliary_cirrhosis	8	0.934	0.999 (0.981 to 1.018)	0.375 / 0.497	0.000
Peripheral_artery_disease	Ankylosing_spondylitis	13	0.947	0.994 (0.836 to 1.182)	0.761 / 0.270	0.236
Heart_failure	Psoriasis	9	0.957	0.995 (0.826 to 1.199)	0.321 / 0.872	0.870
Ischaemic_heart_disease	Sjögren_syndrome	63	0.980	0.998 (0.882 to 1.130)	0.843 / 0.428	0.416

Pleiotropy test: Egger Regression Intercept (pvalue) / MR PRESSO Global Test (pvalue)
Heterogeneity test: Cochran's Q Test (pvalue)

Fig. 3. Reverse causal effects of 14 CVDs on 19 AIDs. A. Heatmap illustrating 20 potential reverse causal pairs identified through reverse MR analysis. B. Forest plot demonstrating whether 25 positive causal pairs are affected by reverse causality.

significant forward causal pairs previously identified (Fig. 2B), only the relationship between PAD and Sjögren's syndrome showed evidence of reverse causality (OR = 1.220, 95 % CI: 1.014–1.467, P = 0.035) (Fig. 3B). Consequently, this pair was excluded from subsequent analyses to prevent bias from reverse causality.

3.4. Cause analysis

To avoid the influence of correlated horizontal pleiotropy and false positives on the results ensuring more robust results, we performed the CAUSE analysis (Table S6). By calculating the ΔELPD, we found that among the 29 causal relationships examined, five were more consistent with the causal models rather than the sharing models. These included celiac disease with arrhythmia (ΔELPD = −4.781, P = 0.011) or PAD (ΔELPD = −6.055, P = 0.001); T1DM with PAD (ΔELPD = −7.403, P = 6.74e-7); MS with VTE (ΔELPD = −4.242, P = 0.031); and psoriasis with HF (ΔELPD = −3.743, P = 0.034) (Fig. 4).

3.5. Gene enrichment analysis

We annotated GWAS variants to genes by proximity and obtained a total of 687 genes from 5 credible results as described above (Fig. S4). We employed Metascape for gene enrichment analysis, followed by the use of Revigo to analyze the semantics and hierarchy of the Metascape results, presenting all these non-redundant terms (Fig. 5A). Based on the dot plot weighted by p-values (Fig. 5B), we found the following results: In the causal association between celiac disease and arrhythmia, pathways such as "MHC protein complex assembly", "positive regulation of immune response", and "regulation of leukocyte proliferation" were prioritized. In the association between celiac disease and PAD, "MHC protein complex assembly", "antigen processing and presentation", and "positive regulation of T cell activation" were prominent. For the association between T1DM and PAD, "antigen processing and presentation" and "positive regulation of immune response" were significant. In the association between MS and VTE, "lymphocyte activation", "regulation of adaptive immune response", and "cellular response to cytokine stimulus" were prioritized. In the association between psoriasis and HF, pathways like "regulation of leukocyte-mediated cytotoxicity" and "MHC class I via ER pathway, TAP-independent" were prominent. Considering the significant pathways across these five aforementioned causal associations, we concluded that immune response and inflammation-related pathways were enriched. Specific pathways can be found in Table S7.

3.6. Mediation analysis

To further explore how AIDs increase the risk of CVDs in the five causal pairs described above, we performed the two-step mediation analysis. Our above results suggest that inflammation and immune response may play an important role in the development of AIDS-related CVDs, and growing body of evidence indicating that inflammation plays a key role in CVDs, we employed 731 immune cells [25] and 91 circulating inflammatory proteins [26] as mediators in our analysis. Consequently, we discovered that HLA-DR$^+$ DCs (GCST90002106) (mediation proportion = 17.95 %, P = 0.020) play a significant mediating role in T1DM affecting PAD (Fig. 6A). The primary mediator was HLA-DR$^+$ mDCs (GCST90002104) (mediation proportion = 16.61 %, P = 0.028) (Fig. 6B), while no significant mediating effect was observed for HLA-DR$^+$ plasmacytoid DCs (Table S8). The remaining four significant causal pairs were not mediated by the 822 factors. These IVs of T1DM were primarily associated with antigen processing and presentation, major histocompatibility complex (MHC) protein complex assembly, and positive regulation of the immune response (Fig. 5B). We further analyzed peripheral blood scRNA-seq data from T1DM and healthy controls (Fig. 6C) [27]. By extracting DCs individually, we discovered that the percentage of cells with high expression of mDC markers (CLEC9A and CADM1) was higher in patients with T1DM than in healthy individuals (Fig. 6D). Furthermore, the expression of HLA-DRA and HLA-DRB1 in mDCs of T1DM was significantly higher compared to those in healthy individuals (Fig. 6E). Taken together, these results suggest that high levels of HLA-DR$^+$ mDCs in T1DM increase the risk of PAD by participating in antigen presentation. This cell type could be valuable for

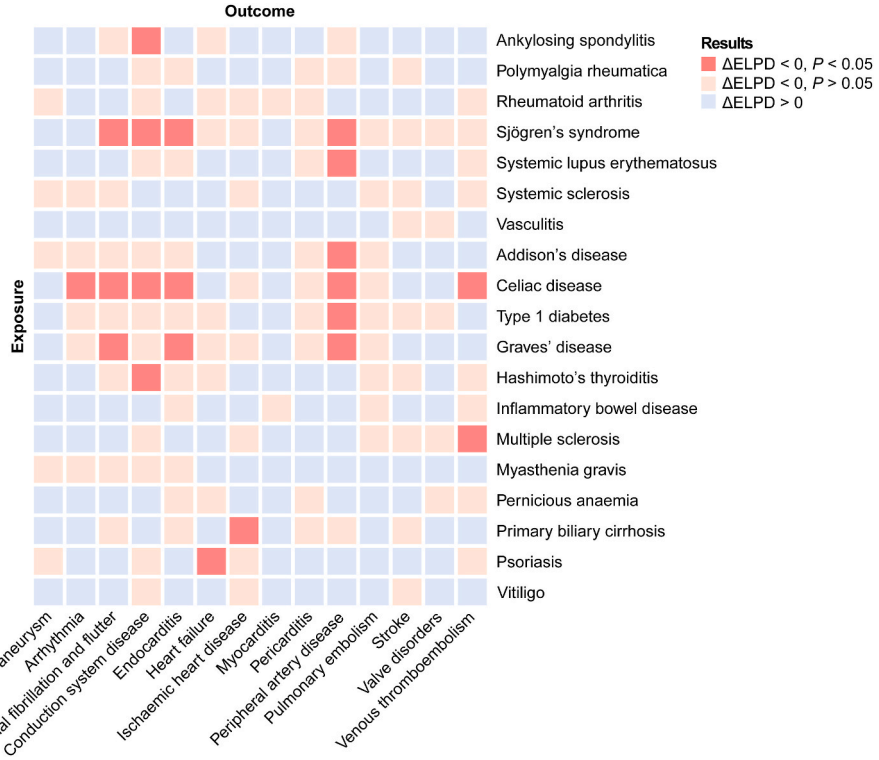


Fig. 4. CAUSE test for19 AIDs on 14 CVDs.

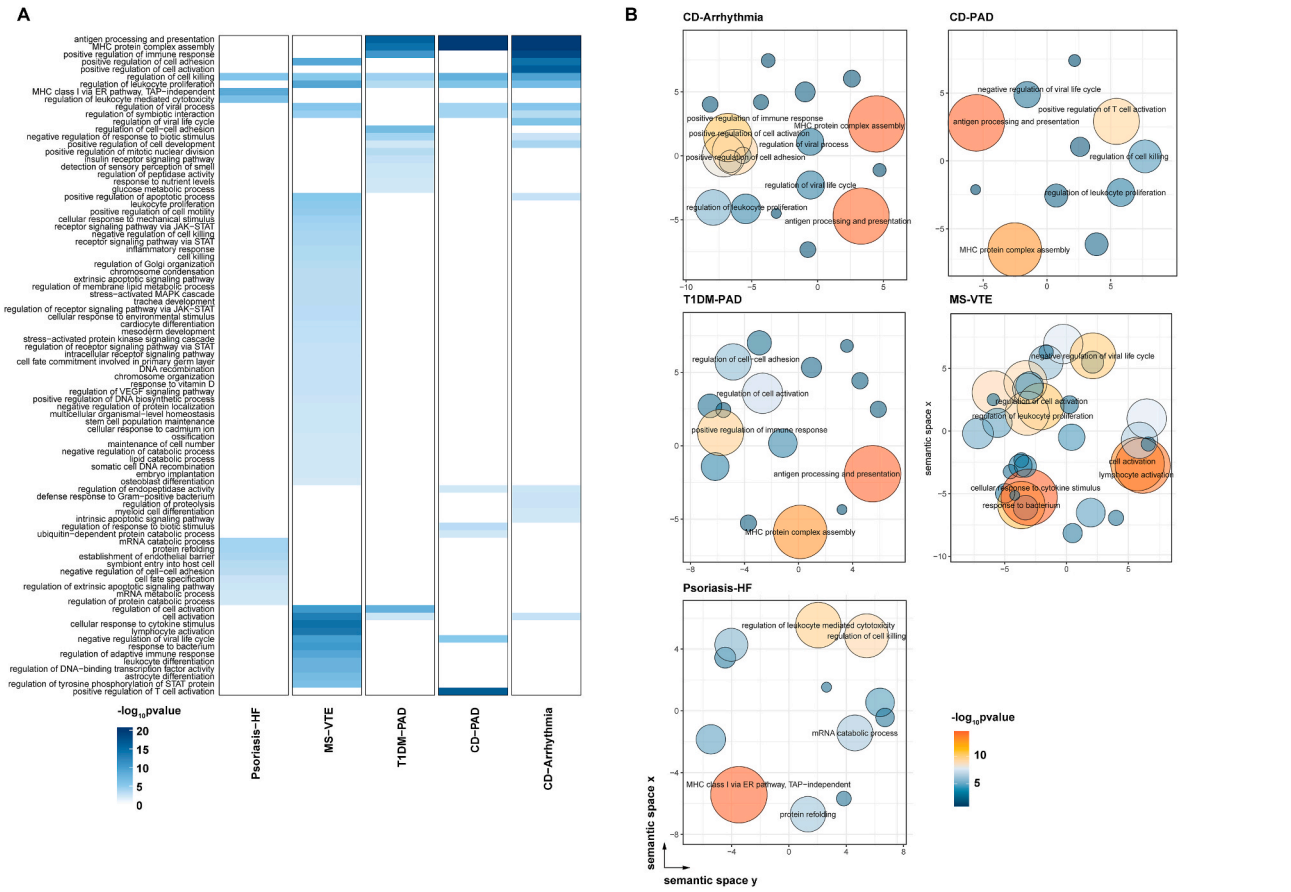


Fig. 5. Gene enrichment analysis of IV-related genes. A. Gene ontology analysis of genes annotated from IVs by proximity. Heatmap illustrating all non-redundant terms. B. Dot plot show the top term. The color and proportional size of the circles indicate the log10 (P value). P values were obtained using Metascape. CD: celiac disease, PAD: peripheral arterial disease, MS: multiple sclerosis, VTE: venous thromboembolism, HF: heart failure.

predicting the development of PAD in patients with T1DM.

3.7. Druggability evaluation

In order to more accurately infer the genes corresponding to IVs, we used MAGMA to find 66 genes (Fig. 7A). We then employed DGIdb and PharmGKB to infer candidate drugs independently, taking the intersection of the two to identify 29 candidate drugs (Fig. 7B). We found that these drugs mainly focus on TNF-α inhibitors (etanercept, infliximab and adalimumab), nonsteroidal antiinflammatory drugs (NSAIDs) (aspirin and acetaminophen), interferon β-1a/b,and glatiramer. Some of these drugs mentioned above have been initiated for the treatment of patients with AIDs [28,29], and our evidence may support the expanded application of these agents for the treatment of comorbid conditions associated with AIDS.

4. Discussion

To our knowledge, this is the first MR study to systematically explore the impact of a variety of AIDs on the cardiovascular risk. This study found that celiac disease and T1DM increase the risk of PAD, celiac disease is associated with a higher risk of arrhythmia, MS increases the risk of VTE, and psoriasis is linked to an increased risk of HF. Moreover, we identified that HLA-DR$^+$ mDCs might have a significant role in the increased risk of PAD development in patients with T1DM.

The assumption that AIDs may increase the risk of CVD is under increasing scrutiny, as numerous observational studies are investigating this association. Our results validate or complement the findings of these observational studies from a genetic perspective. A study indicated that patients with celiac disease exhibit increased intima-media thickness (IMT) and reduced endothelium-dependent dilatation [30]. These findings suggest an elevated risk of early arteriosclerosis in these individuals. Additionally, patients with celiac disease exhibit significantly higher QT dispersion values and Tp-e interval compared to healthy controls [31]. A meta-analysis on T1DM indicated that patients with T1DM exhibited higher IMT and pulse wave velocity than healthy controls, suggesting a high risk of PAD [32]. A nationwide cohort research showed that psoriasis is related to increased rates of HF, with a hazard ratio (HR) of 1.53 (95 % CI: 1.34–1.74) for severe cases and 1.22 (95 % CI: 1.16–1.29) for mild cases [33]. A cohort study found the risk of VTE was higher in patients with MS (HR = 2.6, 95 % CI: 2.06–3.20), compared to the comparison group [34]. Our findings, in conjunction with those of previous observational studies, indicate that certain AIDs may increase the likelihood of developing specific CVDs. Consequently, it is imperative to incorporate strategies for reducing the risk of CVDs into the standard management plan for patients diagnosed with the aforementioned AIDs. Early intervention is crucial to improving the patient's prognosis.

Some findings from previous observational studies did not yield causal evidence in this MR analysis. An observational study based on Clinical Practice Research Datalink GOLD and Aurum datasets showed that patients with AIDs exhibited a higher rate of cardiovascular morbidity than patients without AIDs (HR = 1.56, 95 % CI: 1.52–1.59) [5]. Nevertheless, the aforementioned study could not isolate the effect of specific AIDs on the risk of specific CVDs, because the primary findings were derived from a composite outcome that encompassed all these diseases. A meta-analysis of demonstrated that SLE is linked to a higher risk of stroke (relative risk [RR] = 2.30, 95 % CI: 1.52–3.50), peripheral

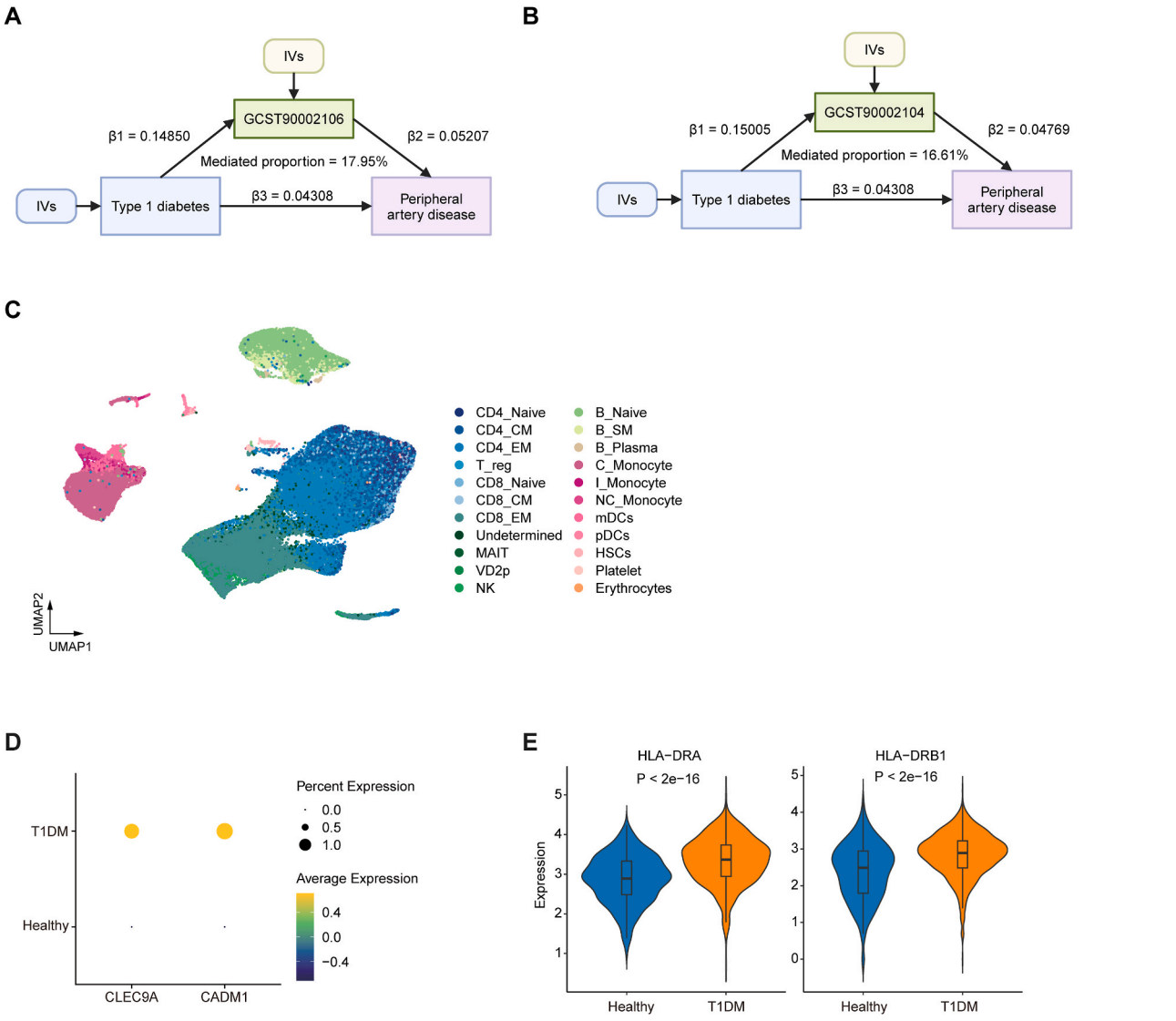


Fig. 6. Mediation effect analysis. A. The effect of T1DM on PAD is mediated by HLA DR + DCs (GCST90002106). B. The effect of T1DM on PAD is mediated by HLA DR + DCs (GCST90002104). C. Peripheral blood scRNA-seq data from T1DM and healthy controls, visualized through UMAP. D. Visualization of the changes in the expression of CLEC9A and CADM1 in T1DM and healthy controls. The size of the dots indicates the percentage of cells in the group, and the color indicates the average expression level of all cells in the group. E. Expression levels of HLA-DRA and HLA-DRB1 in mDCs from patients with T1DM and healthy controls were quantified and compared using the Wilcoxon rank sum test.

vascular disease (RR = 2.56, 95 % CI: 1.07–6.09), myocardial infarction (RR = 2.66, 95 % CI: 1.97–3.59), and HF (RR = 2.89, 95 % CI: 1.63–5.13) [35]. For MS, the disease increases the risk of developing overall stroke (incidence rate ratios [IRRs] = 1.96, 95 % CI: 1.42–2.71), HF (IRR = 1.92, 95 % CI: 1.27–2.90) [36]. Patients with Sjögren's syndrome exhibit higher IMT values (mean difference = 0.07 mm, 95 % CI: 0.04–0.11) and ankle-brachial index (OR = 5.78, 95 % CI: 2.23–14.99) [37], indicating a heightened susceptibility to PAD compared to the healthy population. RA increases the risk of IHD (RR = 1.68, 95 % CI: 1.40–2.03) [38], atrial fibrillation (RR = 1.29, 95 % CI: 1.05–1.59) [39], all-cause stroke (RR = 1.91, 95 % CI: 1.73–2.12) [39], and HF (HR = 1.22, 95 % CI: 1.09–1.37) [40]. Systemic sclerosis increases the risk of CVD (HR = 2.12, 95 % CI: 1.36–3.30), stroke (HR = 1.64, 95 % CI: 1.35–2.01), PVD (HR = 5.23, 95 % CI: 4.25–6.45), and VTE (HR = 2.75, 95 % CI: 1.7–4.28) [41]. IBD significantly increases the risk of VTE (HR = 3.4, 95 % CI: 2.7–4.3) [42] and PE (HR = 6.4, 95 % CI: 2.0–20.3) [43]. T1DM increases the RR of HF to 4.29 (95 % CI: 3.54–5.19), stroke to 4.08 (95 % CI: 3.42–4.86), atrial fibrillation to 1.36 (95 % CI: 1.17–1.59), and IHD to 9.38 (95 % CI: 5.56–15.82) [44]. PBC may not increase the risk of stroke (HR = 0.98, 95 % CI: 0.73–1.31) or myocardial infarction (HR = 1.04, 95 % CI: 0.67–1.62) [45,46]. Psoriasis increases the risk of IHD (OR = 1.5, 95 % CI: 1.2–1.9) and PAD (OR = 1.5, 95 % CI: 1.2–1.8) [47]. Although certain findings from our MR analysis exhibited statistical significance (Fig. 2A), such as RA, T1DM, and psoriasis increasing the risk of IHD, RA was found to increase the risk of HF, and Sjögren's syndrome was linked to an increased risk of PAD. However, these results did not yield robust evidence through the CAUSE test or reverse MR analysis, suggesting that these causal relationships might be influenced by correlated horizontal pleiotropy, reverse causation, or confounding factors, resulting in false positive results. Therefore, it is advisable to approach these findings with caution. Some of the aforementioned results did not exhibit any discernible relationships in our MR analysis. The discrepancy between observational evidence and causal evidence can be explained by several factors: First, the combined treatment of AIDs with multiple drugs is often common [48]. In these observational studies, almost all patients received medication (steroids, antirheumatic drugs, or non-steroidal anti-inflammatory drugs), and variations in drug combinations and

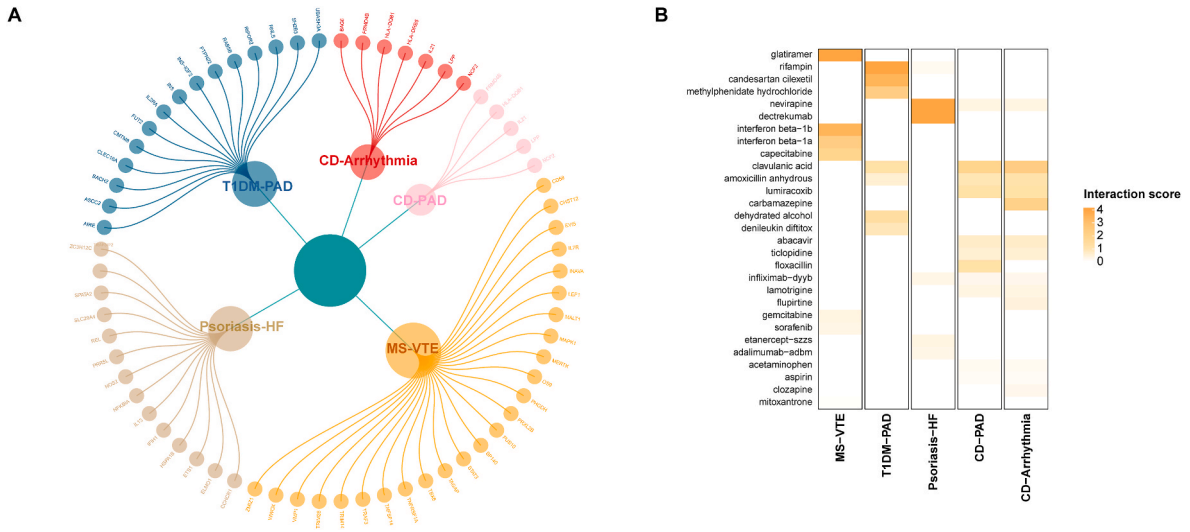


Fig. 7. Druggability evaluation. A. Circular dendrogram presenting all gene identified by MAGMA. B. Heatmap illustrating the candidate drug predicted by DGIdb and PharmGKB. CD: celiac disease, PAD: peripheral arterial disease, MS: multiple sclerosis, VTE: venous thromboembolism, HF: heart failure.

treatment duration may have caused interference. Second, physical activity, dietary intake, severe infections, and other adverse events can confound the results of observational studies. Third, MR studies consider lifelong effects from genetics rather than short-term effects. Fourth, observational studies cannot rule out the impact of reverse causation. In addition to the common AIDs involved in our study, other AIDs have also been reported to potentially affect CVDs. For instance, antiphospholipid syndrome (APS) is a rare autoimmune disease characterized by thrombosis in different locations and obstetric events related to the persistent presence of antiphospholipid antibodies [49]. Paschalis et al. found that patients with APS and antiphospholipid antibody-positive subjects have an increased risk of subclinical atherosclerosis [50]. Stanley et al. reported an increased risk of valvular heart disease in patients with APS [51]. Currently, there is limited GWAS data with suitable conditions available for rare AIDs [52]. To further explore the genetic evidence of these rare AIDs on CVDs, more GWAS studies are needed.

Previous MR analyses have explored the impact of certain AIDs on the risk of developing specific CVDs. Some of the causal relationships they derived were broadly in line with our 29 preliminary results (Fig. 2B). RA was linked to increased IHD risk (OR = 1.0006, 95 % CI: 1.000244–1.00104), independent of atrial fibrillation and arrhythmias [53], which our results confirmed. Additionally, our research suggests RA might be related to HF and pericarditis. SLE was shown to elevate the risk of HF, VTE, and ischemic stroke [10], whereas SLE was found to only slightly increase the risk of VTE (Fig. 1A), potentially due to different study populations. Celiac disease has not been associated with major CVDs [54], which is consistent with our findings. In addition, celiac disease could increase arrhythmias and PAD risks. Hashimoto's thyroiditis has been found to increase the risk of IHD (OR = 1.07, 95 % CI: 1.01–1.13) [55]. However, our study did not yield the similar outcome, which may be due to the different data used. T1DM has been linked to an increased risk of PAD (OR = 1.06, 95 % CI: 1.02–1.10), consistent with our findings. Previous studies did not establish a significant causal relationship between T1DM and HF, IHD, AF, MI, and stroke [56]. However, our research suggests that T1DM may enhance the risk of developing IHD, possibly due to differences in the selection of exposure data or the smaller sample sizes used in earlier GWAS compared to the larger CARDIoGRAMplusC4D dataset we utilized, which enhances reliability. Additionally, our study found the potential impact of T1DM on pericarditis. IBD has not been associated with IHD or stroke, consistent with our findings [57]. MS has been linked to an increased risk of IHD, HF, and all-cause stroke [12]. However, we only found that MS may increase the risk of VTE and PE. This discrepancy may be related to the screening threshold of instrumental variables (IVs), differences in the outcome data selection, or the fact that they used fixed-effect IVW [12], whereas we used random-effect IVW. No causal relationship has been observed between MG and stroke, consistent with our research findings [58]. Although the aforementioned results are roughly consistent with our preliminary results, some of the CAUSE tests failed to achieve statistical significance, suggesting the possibility of false positives. Therefore, we need to further expand our datasets and increase sample sizes to validate the reliability of these results.

The pathophysiologic mechanisms underlying CVDs induced by AIDs are currently unclear. One of the most significant contributing factors appears to be inflammation [2,59]. Systemic inflammatory factors, such as cytokines, chemokines, proteases, autoantibodies, adhesion receptors, and pro-inflammatory lymphocytes and stromal cells produced by AIDs, may increase the risk of CVDs by damaging the vasculature, myocardium, or pericardium [60]. This is consistent with our findings. Our enrichment analysis of IV-related genes also revealed a major focus on Immune response and inflammation (Fig. 5A and B). To further elucidate the inflammatory mechanisms through which AIDs increase the risk of CVDs, we conducted mediation analyses using 731 immune cells and 91 circulating inflammatory proteins. We found that only HLA-DR^{+} mDCs are significant in the association between T1DM and PAD. Through scRNA-seq, we discovered that these cells are significantly increased in the peripheral blood of patients with T1DM. According to previous studies [61], the percentage of mDCs in femoral artery plaques is significantly higher than in controls. mDCs may be recruited into atherosclerotic plaques. Close monitoring of changes in the number of mDCs during the course of T1DM may assist in predicting the risk of developing PAD in patients. For the other causal relationships identified in our study where no mediator was found, it is possible that inflammatory factors other than those mentioned above may play a mediating role. Furthermore, other potential contributing factors, such as metabolic disorders [62] and the gut microbiome [63], warrant further investigation in future studies.

Several studies have shown that anti-inflammatory therapy can reduce major adverse cardiovascular events and mortality. For example, canakinumab (Interleukin-1 beta [IL-1β] antibody) [64], colchicine [65], and adalimumab (tumor necrosis factor-alpha [TNF-α] antibody) [66] have generally yielded favorable results. Our drug prediction analysis identified several candidate drugs, including TNF-α antibodies [29], NSAIDs [67], and interferon [68], that are already used in the treatment of AIDs. Previous animal studies have shown that the TNF-α

antibody, infliximab, inhibits JNK pathway activation, improves arterial eNOS expression and vasorelaxation, and has potential therapeutic significance in early vascular lesions in T1DM [69]. However, there is no definitive clinical and future studies for their therapeutic potential on AIDs related CVDs complications are still needed.

SNPs in the MHC region are associated with a complex LD structure, which may lead to pleiotropy and introduce bias into the MR results [70]. However, it may not be appropriate to directly exclude all SNPs in the MHC region. MHC genes provide the strongest genetic contribution to AIDs [71,72]. In most MR studies where AIDs are considered as exposures, SNPs in the MHC region were not excluded [56,73,74]. To examine the effects of the MHC region on the identified associations, we conducted a secondary analysis excluding MHC SNPs. Among the 29 significant results from the forward MR analysis, only 14 remained statistically significant. The remaining associations did not persist after removing the genetic instruments in the MHC region, suggesting that these associations may be partially driven by the shared pleiotropic effects of MHC genes on AIDs [75]. This suggests that AIDs and CVDs may share a genetic etiology related to immune. Although this may violate the third assumption of MR analysis, it may play a potential role in explaining the mechanisms by which AIDs affect CVDs and suggesting clinical complications [75]. Additionally, removing the MHC-related region directly may lead to a significantly reduced variance explained by the IVs, which could, in turn, result in insufficient power for the MR analysis [75,76].

The present study exhibits several significant advantages. First, compared with previous observational and MR studies, this is the first study to provide a more comprehensive and systematic assessment of the impact of AIDs on the cardiovascular risk. Second, this study employs multiple MR analysis approaches, various sensitivity analysis methods, and bidirectional MR analysis to minimize biases caused by confounding factors and reverse causality, hence augmenting the dependability of the findings. Third, we primarily utilized populations of European origin to minimize demographic bias.

Nonetheless, our study has several limitations. Firstly, unrecognized confounders may still exist [77], potentially biasing our results, despite efforts to minimize horizontal pleiotropy in MR studies [56]. Secondly, the results of this study should be cautiously interpreted, because the OR values were relatively low. Thirdly, sample overlap bias may exist. However, two-sample MR methods (except MR-Egger) can be confidently utilized when overlapping samples originate from extensive biobanks, and the robustness of IVs (i.e., the F statistic is considerably larger than 10) is likely to minimize sample overlap bias [78]. Fourthly, this study investigated the impact of AIDs on CVDs. However, the reverse MR analysis revealed that some CVDs might affect AIDs. This aspect of the study requires further investigation. Fifthly, some of the associations were influenced by SNPs in the MHC region, which may be affected by pleiotropy. However, this also indicates a shared genetic etiology about immune and suggests potential clinical complications. We set strict thresholds to minimize LD and conducted MR-Egger, MR-PRESSO, and CAUSE to mitigate the impact of pleiotropy and to ensure the robustness of our results. Sixth, the secondary MR analysis excluding MHC SNPs may lack adequate power caused by a greatly reduced variance.

5. Conclusion

This MR study comprehensively assessed the impact of AIDs on cardiovascular risk separately. Our study suggests an increased risk of PAD in patients with celiac disease or T1DM, a higher likelihood of arrhythmia in those with celiac disease, an elevated risk of VTE in those with MS, and an increased susceptibility to HF among those with psoriasis. Immune response and inflammation-related pathways were found to be significant in the aforementioned pairs. HLA-DR^{+} mDCs play a crucial role in the induction of PAD by T1DM. TNF-α inhibitors and Interferon, among others, may have potential to treat certain cardiovascular comorbidities of AIDs. In the management of these patients, implementing early and close monitoring, as well as preventive measures for CVDs, is crucial to enhancing their quality of life.

CRediT authorship contribution statement

Yulin Bao: Writing – original draft, Visualization, Software, Methodology, Formal analysis, Conceptualization. **Lingfeng Gu:** Validation, Methodology, Formal analysis. **Jiayi Chen:** Visualization, Validation. **Hao Wang:** Visualization, Investigation. **Zemu Wang:** Validation. **Huijuan Wang:** Supervision, Methodology, Conceptualization. **Sibo Wang:** Writing – review & editing, Conceptualization. **Liansheng Wang:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization.

Availability of data and material

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Abbreviations

AIDs	autoimmune diseases
AS	ankylosing spondylitis
CADM1	cell adhesion molecule 1
CAUSE	causal analysis using summary effect estimates
CLEC9A	C-type lectin domain containing 9A
CVDs	cardiovascular diseases
DCs	dendritic cells
ELPD	expected log pointwise posterior density
GWAS	genome-wide association studies
HF	heart failure
HLA-DR	human leukocyte antigen-DR
HR	hazard ratio
IBD	inflammatory bowel disease
IHD	ischemic heart disease
IL-1β	Interleukin-1 beta
IRRs	incidence rate ratios
IVs	instrumental variables
IVW	inverse variance weighted
MAGMA	Multi-marker Analysis of GenoMic Annotation
mDC	myeloid dendritic cell
MHC	major histocompatibility complex
MR	mendelian randomization
MR-PRESSO	MR pleiotropy residual sum and outlier
MS	multiple sclerosis
NSAIDs	nonsteroidal antiinflammatory drugs
OR	odds ratios
PAD	peripheral arterial disease
PBC	primary biliary cirrhosis
PE	pulmonary embolism
RA	rheumatoid arthritis
RR	relative risk
scRNA-seq	single-cell RNA sequencing
SLE	systemic lupus erythematosus
STROBE-MR	Strengthening the Reporting of Observational Studies in Epidemiology using MR
T1DM	type 1 diabetes mellitus
TNF-α	tumor necrosis factor-alpha
VTE	venous thromboembolism

Funding

This work is supported by grants from the National Natural Science Foundation of China (NO. 82370344) and the Postgraduate Research & Practice Innovation Program of Jiangsu Province (No. SJCX23_0703).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We sincerely thank all the consortia and investigators for making their GWAS summary data publicly available.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtauto.2024.100259.

Data availability

Data will be made available on request.

References

[1] N. Conrad, S. Misra, J.Y. Verbakel, G. Verbeke, G. Molenberghs, P.N. Taylor, et al., Incidence, prevalence, and co-occurrence of autoimmune disorders over time and by age, sex, and socioeconomic status: a population-based cohort study of 22 million individuals in the UK, Lancet 401 (2023) 1878–1890.
[2] J.M. Gelfand, S. Wang, M.S. Garshick, Autoimmune diseases and cardiovascular risk, Trends Mol. Med. 28 (2022) 1025–1027.
[3] P. Karakasis, D. Patoulias, P. Stachteas, E. Lefkou, T. Dimitroulas, N. Fragakis, Accelerated atherosclerosis and management of cardiovascular risk in autoimmune rheumatic diseases: an updated review, Curr. Probl. Cardiol. 48 (2023) 101999.
[4] F.L.J. Visseren, F. Mach, Y.M. Smulders, D. Carballo, K.C. Koskinas, M. Bäck, et al., ESC Guidelines on cardiovascular disease prevention in clinical practice, Eur. Heart J. 42 (2021) 3227–3337, 2021.
[5] N. Conrad, G. Verbeke, G. Molenberghs, L. Goetschalckx, T. Callender, G. Cambridge, et al., Autoimmune diseases and cardiovascular risk: a population-based study on 19 autoimmune diseases and 12 cardiovascular diseases in 22 million individuals in the UK, Lancet 400 (2022) 733–743.
[6] S. Funada, Y. Luo, N. Nishioka, T. Yoshioka, Cardiovascular risk in systemic autoimmune diseases, Lancet 401 (2023) 21.
[7] S.C. Larsson, A.S. Butterworth, S. Burgess, Mendelian randomization for cardiovascular diseases: principles and applications, Eur. Heart J. 44 (2023) 4913–4924.
[8] E. Sanderson, M.M. Glymour, M.V. Holmes, H. Kang, J. Morrison, M.R. Munafò, et al., Mendelian randomization, Nat Rev Methods Primers 2 (2022).
[9] S. Yuan, P. Carter, A.M. Mason, F. Yang, S. Burgess, S.C. Larsson, Genetic liability to rheumatoid arthritis in relation to coronary artery disease and stroke risk, Arthritis Rheumatol. 74 (2022) 1638–1647.
[10] N. Gao, M. Kong, X. Li, D. Wei, X. Zhu, Z. Hong, et al., Systemic lupus erythematosus and cardiovascular disease: a mendelian randomization study, Front. Immunol. 13 (2022) 908831.
[11] Y. Zhong, Y. Chen, X. Zhang, W. Cai, C. Zhao, W. Zhao, No evidence of a causal relationship between ankylosing spondylitis and cardiovascular disease: a two-sample Mendelian randomization study, Front Cardiovasc Med 10 (2023) 1243867.
[12] F. Yang, T. Hu, K. He, J. Ying, H. Cui, Multiple sclerosis and the risk of cardiovascular diseases: a mendelian randomization study, Front. Immunol. 13 (2022) 861885.
[13] C.A. Emdin, A.V. Khera, S. Kathiresan, Mendelian randomization, JAMA 318 (2017) 1925–1926.
[14] V.W. Skrivankova, R.C. Richmond, B.A.R. Woolf, J. Yarmolinsky, N.M. Davies, S. A. Swanson, et al., Strengthening the reporting of observational studies in Epidemiology using mendelian randomization: the STROBE-MR statement, JAMA 326 (2021) 1614–1621.
[15] N. Papadimitriou, N. Dimou, K.K. Tsilidis, B. Banbury, R.M. Martin, S.J. Lewis, et al., Physical activity and risks of breast and colorectal cancer: a Mendelian randomisation analysis, Nat. Commun. 11 (2020) 597.
[16] F.P. Hartwig, G. Davey Smith, J. Bowden, Robust inference in summary data Mendelian randomization via the zero modal pleiotropy assumption, Int. J. Epidemiol. 46 (2017) 1985–1998.
[17] C. Ning, M. Jin, Y. Cai, L. Fan, K. Hu, Z. Lu, et al., Genetic architectures of the human hippocampus and those involved in neuropsychiatric traits, BMC Med. 22 (2024) 456.
[18] J. Morrison, N. Knoblauch, J.H. Marcus, M. Stephens, X. He, Mendelian randomization accounting for correlated and uncorrelated pleiotropic effects using genome-wide summary statistics, Nat. Genet. 52 (2020) 740–747.
[19] S. Burgess, S.A. Swanson, J.A. Labrecque, Are Mendelian randomization investigations immune from bias due to reverse causation? Eur. J. Epidemiol. 36 (2021) 253–257.
[20] S. Burgess, J.A. Chirinos, S.M. Damrauer, D. Gill, Genetically predicted pulse pressure and risk of abdominal aortic aneurysm: a mendelian randomization analysis, Circ Genom Precis Med 15 (2022) e003575.
[21] S. Burgess, G. Davey Smith, N.M. Davies, F. Dudbridge, D. Gill, M.M. Glymour, et al., Guidelines for performing Mendelian randomization investigations: update for summer 2023, Wellcome Open Res 4 (2019) 186.
[22] C.A. de Leeuw, J.M. Mooij, T. Heskes, D. Posthuma, MAGMA: generalized gene-set analysis of GWAS data, PLoS Comput. Biol. 11 (2015) e1004219.
[23] M. Cannon, J. Stevenson, K. Stahl, R. Basu, A. Coffman, S. Kiwala, et al., DGIdb 5.0: rebuilding the drug-gene interaction database for precision medicine and drug discovery platforms, Nucleic Acids Res. 52 (2024) D1227, d35.
[24] J.M. Barbarino, M. Whirl-Carrillo, R.B. Altman, T.E. Klein, PharmGKB: a worldwide resource for pharmacogenomic information, Wiley Interdiscip Rev Syst Biol Med 10 (2018) e1417.
[25] V. Orrù, M. Steri, C. Sidore, M. Marongiu, V. Serra, S. Olla, et al., Complex genetic signatures in immune cells underlie autoimmunity and inform therapy, Nat. Genet. 52 (2020) 1036–1045.
[26] J.H. Zhao, D. Stacey, N. Eriksson, E. Macdonald-Dunlop, K. HedmanÅ, A. Kalnapenkis, et al., Genetics of circulating inflammatory proteins identifies drivers of immune-mediated disease risk and therapeutic targets, Nat. Immunol. 24 (2023) 1540–1551.
[27] M.A. Honardoost, A. Adinatha, F. Schmidt, B. Ranjan, M. Ghaeidamini, N. Arul Rayan, et al., Systematic immune cell dysregulation and molecular subtypes revealed by single-cell RNA-seq of subjects with type 1 diabetes, Genome Med. 16 (2024) 45.
[28] J. Hirt, K. Dembowska, T. Woelfle, C. Axfors, C. Granziera, J. Kuhle, et al., Clinical trial evidence of quality-of-life effects of disease-modifying therapies for multiple sclerosis: a systematic analysis, J. Neurol. 271 (2024) 3131–3141.
[29] S.W. Syversen, K.K. Jørgensen, G.L. Goll, M.K. Brun, Ø. Sandanger, K.H. Bjørlykke, et al., Effect of therapeutic drug monitoring vs standard therapy during maintenance infliximab therapy on disease control in patients with immune-mediated inflammatory diseases: a randomized clinical trial, JAMA 326 (2021) 2375–2384.
[30] S. De Marchi, G. Chiarioni, M. Prior, E. Arosio, Young adults with coeliac disease may be at increased risk of early atherosclerosis, Aliment. Pharmacol. Ther. 38 (2013) 162–169.
[31] H. Birinci, C. Yolcu, G. Dogan, M.K. Basaran, M. Elevli, Are Tp-e interval and QT dispersion values important in children with coeliac disease? Cardiol. Young 33 (2023) 1853–1858.
[32] E.Z. Giannopoulou, I. Doundoulakis, C. Antza, A. Christoforidis, A.B. Haidich, V. Kotsis, et al., Subclinical arterial damage in children and adolescents with type 1 diabetes: a systematic review and meta-analysis, Pediatr. Diabetes 20 (2019) 668–677.
[33] U. Khalid, O. Ahlehoff, G.H. Gislason, S.L. Kristensen, L. Skov, C. Torp-Pedersen, et al., Psoriasis and risk of heart failure: a nationwide cohort study, Eur. J. Heart Fail. 16 (2014) 743–748.
[34] P.J. Peeters, M.T. Bazelier, B.M. Uitdehaag, H.G. Leufkens, M.L. De Bruin, F. de Vries, The risk of venous thromboembolism in patients with multiple sclerosis: the Clinical Practice Research Datalink, J Thromb Haemost 12 (2014) 444–451.
[35] X. Lu, Y. Wang, J. Zhang, D. Pu, N. Hu, J. Luo, et al., Patients with systemic lupus erythematosus face a high risk of cardiovascular disease: a systematic review and Meta-analysis, Int Immunopharmacol 94 (2021) 107466.
[36] C.F. Christiansen, S. Christensen, D.K. Farkas, M. Miret, H.T. Sørensen, L. Pedersen, Risk of arterial cardiovascular diseases in patients with multiple sclerosis: a population-based cohort study, Neuroepidemiology 35 (2010) 267–274.
[37] P. Karakasis, D. Patoulias, K. Pamporis, P. Stachteas, E. Lefkou, K.I. Bougioukas, et al., Risk of subclinical atherosclerosis in primary Sjogren's syndrome: a systematic review and meta-analysis, Eur. J. Intern. Med. 122 (2024) 93–101.
[38] J.A. Avina-Zubieta, J. Thomas, M. Sadatsafavi, A.J. Lehman, D. Lacaille, Risk of incident cardiovascular events in patients with rheumatoid arthritis: a meta-analysis of observational studies, Ann. Rheum. Dis. 71 (2012) 1524–1529.
[39] A.G. Semb, E. Ikdahl, G. Wibetoe, C. Crowson, S. Rollefstad, Atherosclerotic cardiovascular disease prevention in rheumatoid arthritis, Nat. Rev. Rheumatol. 16 (2020) 361–379.
[40] Ä. Mantel, M. Holmqvist, D.C. Andersson, L.H. Lund, J. Askling, Association between rheumatoid arthritis and risk of ischemic and nonischemic heart failure, J. Am. Coll. Cardiol. 69 (2017) 1275–1285.
[41] I.W. Chen, W.T. Wang, Y.C. Lai, C.M. Lin, P.H. Liu, S.Z. Wu, et al., Association between systemic sclerosis and risk of cerebrovascular and cardiovascular disease: a meta-analysis, Sci. Rep. 14 (2024) 6445.
[42] M.J. Grainge, J. West, T.R. Card, Venous thromboembolism during active disease and remission in inflammatory bowel disease: a cohort study, Lancet 375 (2010) 657–663.
[43] M.D. Kappelman, E. Horvath-Puho, R.S. Sandler, D.T. Rubin, T.A. Ullman, L. Pedersen, et al., Thromboembolic risk among Danish children and adults with inflammatory bowel diseases: a population-based nationwide study, Gut 60 (2011) 937–943.
[44] X. Cai, J. Li, W. Cai, C. Chen, J. Ma, Z. Xie, et al., Meta-analysis of type 1 diabetes mellitus and risk of cardiovascular disease, J Diabetes Complications 35 (2021) 107833.
[45] M. Solaymani-Dodaran, G.P. Aithal, T. Card, J. West, Risk of cardiovascular and cerebrovascular events in primary biliary cirrhosis: a population-based cohort study, Am. J. Gastroenterol. 103 (2008) 2784–2788.
[46] D. Suraweera, C. Fanous, M. Jimenez, M.J. Tong, S. Saab, Risk of cardiovascular events in patients with primary biliary cholangitis - systematic review, J Clin Transl Hepatol 6 (2018) 119–126.
[47] I.M. Miller, C. Ellervik, S. Yazdanyar, G.B. Jemec, Meta-analysis of psoriasis, cardiovascular disease, and associated risk factors, J. Am. Acad. Dermatol. 69 (2013) 1014–1024.
[48] S.J. Li, Y.L. Wu, J.H. Chen, S.Y. Shen, J. Duan, H.E. Xu, Autoimmune diseases: targets, biology, and drug discovery, Acta Pharmacol. Sin. 45 (2024) 674–685.
[49] D. Taieb, Q. Moyon, R. Lhote, I. Annesi-Maesano, J. Haroche, R. Cervera, et al., Phenotypes in antiphospholipid syndrome: a hierarchical cluster analysis based on two independent databases, J. Autoimmun. 144 (2024) 103173.

[50] P. Karakasis, E. Lefkou, K. Pamporis, V. Nevras, K.I. Bougioukas, A.B. Haidich, et al., Risk of subclinical atherosclerosis in patients with antiphospholipid syndrome and subjects with antiphospholipid antibody positivity: a systematic review and meta-analysis, Curr. Probl. Cardiol. 48 (2023) 101672.
[51] S. Niznik, M.J. Rapoport, O. Avnery, M. Kidon, R. Shavit, M.H. Ellis, et al., Heart valve disease in primary antiphospholipid syndrome, Rheumatology 63 (2024) 1397–1402.
[52] M. Sugiura-Ogasawara, Y. Omae, M. Kawashima, L. Toyo-Oka, S.S. Khor, H. Sawai, et al., The first genome-wide association study identifying new susceptibility loci for obstetric antiphospholipid syndrome, J. Hum. Genet. 62 (2017) 831–838.
[53] M. Wang, C. Chao, K. Mei, D. Di, Y. Qian, B. Wang, et al., Relationship between rheumatoid arthritis and cardiovascular comorbidity, causation or co-occurrence: a Mendelian randomization study, Front Cardiovasc Med 10 (2023) 1099861.
[54] J. Huang, Assessment of the causal association between celiac disease and cardiovascular diseases, Front Cardiovasc Med 9 (2022) 1017209.
[55] E. Marouli, A. Kus, M.F. Del Greco, L. Chaker, R. Peeters, A. Teumer, et al., Thyroid function affects the risk of stroke via atrial fibrillation: a mendelian randomization study, J. Clin. Endocrinol. Metab. 105 (2020) 2634–2641.
[56] Z. Liu, H. Wang, Z. Yang, Y. Lu, C. Zou, Causal associations between type 1 diabetes mellitus and cardiovascular diseases: a Mendelian randomization study, Cardiovasc. Diabetol. 22 (2023) 236.
[57] B. Liu, Z. Qin, Z. Cai, Z. Liu, Y.L. Chen, X. Yin, et al., Evaluating the causal association between inflammatory bowel disease and risk of atherosclerotic cardiovascular disease: univariable and multivariable mendelian randomization study, Biomedicines 11 (2023).
[58] C. Liu, C. Mao, S. Li, Y. Su, H. Liu, X. Wang, et al., Myasthenia gravis and ischemic stroke: a bidirectional mendelian randomization study, Curr Neurovasc Res 20 (2023) 270–279.
[59] A.S. Manolis, A.G. Tzioufas, Cardio-rheumatology: cardiovascular complications in systemic autoimmune rheumatic diseases/is inflammation the common link and target? Curr. Vasc. Pharmacol. 18 (2020) 425–430.
[60] M. Noack, P. Miossec, Importance of lymphocyte-stromal cell interactions in autoimmune and inflammatory rheumatic diseases, Nat. Rev. Rheumatol. 17 (2021) 550–564.
[61] D. Kretzschmar, I. Rohm, S. Schäller, S. Betge, R. Pistulli, Y. Atiskova, et al., Decrease in circulating dendritic cell precursors in patients with peripheral artery disease, Mediators Inflamm 2015 (2015) 450957.
[62] A. Afeltra, A. Abbate, G. Valentini, R. Giacomelli, Inflammation and dysmetabolism in systemic autoimmune diseases, J Immunol Res 2019 (2019) 5438287.
[63] J.C. Clemente, J. Manasson, J.U. Scher, The role of the gut microbiome in systemic inflammatory disease, Bmj 360 (2018) j5145.
[64] A. Abbate, S. Toldo, C. Marchetti, J. Kron, B.W. Van Tassell, C.A. Dinarello, Interleukin-1 and the inflammasome as therapeutic targets in cardiovascular disease, Circ. Res. 126 (2020) 1260–1280.
[65] A. Aimo, D.A. Pascual-Figal, A. Barison, G. Cediel, H. Vicente Á, L.F. Saccaro, et al., Colchicine for the treatment of coronary artery disease, Trends Cardiovasc. Med. 31 (2021) 497–504.
[66] A. González-Cantero, D. Ortega-Quijano, N. Álvarez-Díaz, M.A. Ballester, N. Jimenez-Gomez, P. Jaen, et al., Impact of biological agents on imaging and biomarkers of cardiovascular disease in patients with psoriasis: a systematic review and meta-analysis of randomized placebo-controlled trials, J. Invest. Dermatol. 141 (2021) 2402–2411.
[67] R. Ben Mrid, N. Bouchmaa, H. Ainani, R. El Fatimy, G. Malka, L. Mazini, Anti-rheumatoid drugs advancements: new insights into the molecular treatment of rheumatoid arthritis, Biomed. Pharmacother. 151 (2022) 113126.
[68] L. Kappos, M.S. Freedman, C.H. Polman, G. Edan, H.P. Hartung, D.H. Miller, et al., Long-term effect of early treatment with interferon beta-1b after a first clinical event suggestive of multiple sclerosis: 5-year active treatment extension of the phase 3 BENEFIT trial, Lancet Neurol. 8 (2009) 987–997.
[69] C. Nacci, V. Leo, L. De Benedictis, M.A. Potenza, L. Sgarra, M.A. De Salvia, et al., Infliximab therapy restores adiponectin expression in perivascular adipose tissue and improves endothelial nitric oxide-mediated vasodilation in mice with type 1 diabetes, Vascul Pharmacol 87 (2016) 83–91.
[70] P. Alipour, K. Senkevich, J.P. Ross, D. Spiegelman, D. Manousaki, P.A. Dion, et al., Investigation of the causal relationship between ALS and autoimmune disorders: a Mendelian randomization study, BMC Med. 20 (2022) 382.
[71] S.B. Kanaan, O. Sensoy, Z. Yan, V.K. Gadi, M.L. Richardson, J.L. Nelson, Immunogenicity of a rheumatoid arthritis protective sequence when acquired through microchimerism, Proc Natl Acad Sci U S A 116 (2019) 19600–19608.
[72] L.A. Zenewicz, C. Abraham, R.A. Flavell, J.H. Cho, Unraveling the genetics of autoimmunity, Cell 140 (2010) 791–797.
[73] B. Wang, Y. Xiong, R. Li, S. Zhang, Potential role of SNP rs2071475 in rheumatoid arthritis and inflammatory bowel disease in the East Asian population: a Mendelian randomization study, Inflammopharmacology 32 (2024) 683–692.
[74] C.H.C. Yeung, S.L. Au Yeung, C.M. Schooling, Association of autoimmune diseases with Alzheimer's disease: a mendelian randomization study, J. Psychiatr. Res. 155 (2022) 550–558.
[75] S. Yuan, F. Jiang, J. Chen, B. Lebwohl, P.H.R. Green, D. Leffler, et al., Phenome-wide Mendelian randomization analysis reveals multiple health comorbidities of coeliac disease, EBioMedicine 101 (2024) 105033.
[76] Y. He, X. Zhang, M. Timofeeva, S.M. Farrington, X. Li, W. Xu, et al., Bidirectional Mendelian randomisation analysis of the relationship between circulating vitamin D concentration and colorectal cancer risk, Int. J. Cancer 150 (2022) 303–307.
[77] M. Verbanck, C.Y. Chen, B. Neale, R. Do, Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases, Nat. Genet. 50 (2018) 693–698.
[78] B.L. Pierce, S. Burgess, Efficient design for Mendelian randomization studies: subsample and 2-sample instrumental variable estimators, Am. J. Epidemiol. 178 (2013) 1177–1184.