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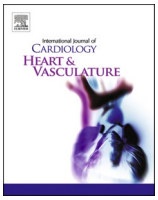

Dissecting causal relationships between immune cells, blood metabolites, and aortic dissection: A mediation Mendelian randomization study

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ABSTRACT

Background: There exists a robust correlation between the infiltration of immune cells and the pathogenesis of aortic dissection (AD). Moreover, blood metabolites serve as immunomodulatory agents within the organism, influencing the immune system's response and potentially playing a role in the development of AD. Nevertheless, the intricate genetic causal nexus between specific immune cells, blood metabolites, and AD remains partially elucidated.

Objectives: This study aims to elucidate the causal relationships between specific immune cell types and the risk of developing AD, mediated by blood metabolites, using Mendelian Randomization (MR) methods.

Methods: We undertook a comprehensive investigation of 731 immune cell types through the analysis of published genome-wide association studies (GWAS). Our methodology hinged on the application of two-sample Mendelian randomization (MR) and mediator MR analyses, prioritizing blood metabolites as potential intermediary factors and AD as the principal outcome of interest. The primary statistical method employed was inverse variance-weighted estimation, complemented by a variety of sensitivity analyses to reinforce our conclusions. The entirety of our statistical analyses was executed on the R software platform.

Results: Our analyses elucidated that three immune cell types exhibited a positive correlation with the incidence of AD, whereas two immune cell types were inversely associated with AD risk. Significantly, our mediation Mendelian randomization (MR) findings identified Benzoate as a pivotal mediator in the influence of CD19 on IgD − CD38br cells on AD, with a mediation proportion of 5.38 %. Additionally, N-acetylproline was determined to mediate the effect of CD24 on IgD- CD38- cells on AD, accounting for a mediation proportion of 13.70 %. Furthermore, Carnitine C5:1 was found to mediate the effect of CD28 on secreting T regulatory (Treg) cells on AD, with a mediation proportion of 17.80 %.

Conclusions: These findings offer a nuanced understanding of the pathophysiological mechanisms underlying AD, thereby advancing the precision medicine paradigm in the clinical management of AD.

Abbreviations: AD: aortic dissection; AA: aortic aneurysm; GWAS: genome-wide association study; MR: Mendelian randomization; TSMR: two-step Mendelian randomization; Treg: secreting T regulatory cell; VSMC: vascular smooth muscle cell; MMP: matrix metalloproteinase; ROS: reactive oxygen species; IV: instrumental variable; SNP: single-nucleotide polymorphism; IVW: inverse variance weighted; LDSC: linkage disequilibrium score regression; OR: odds ratio; CI: confidence interval; LD: linkage disequilibrium; AC: absolute cell; MFI: median fluorescence intensity; MP: morphological parameter; RC: relative cell; CLSA: Canadian Longitudinal Study of Aging; Lp(a): Lipoprotein a; OxPL: oxidised phospholipid; NMDAR: N-methyl-d-aspartate glutamate receptor; STROBE-MR: Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian Randomization.

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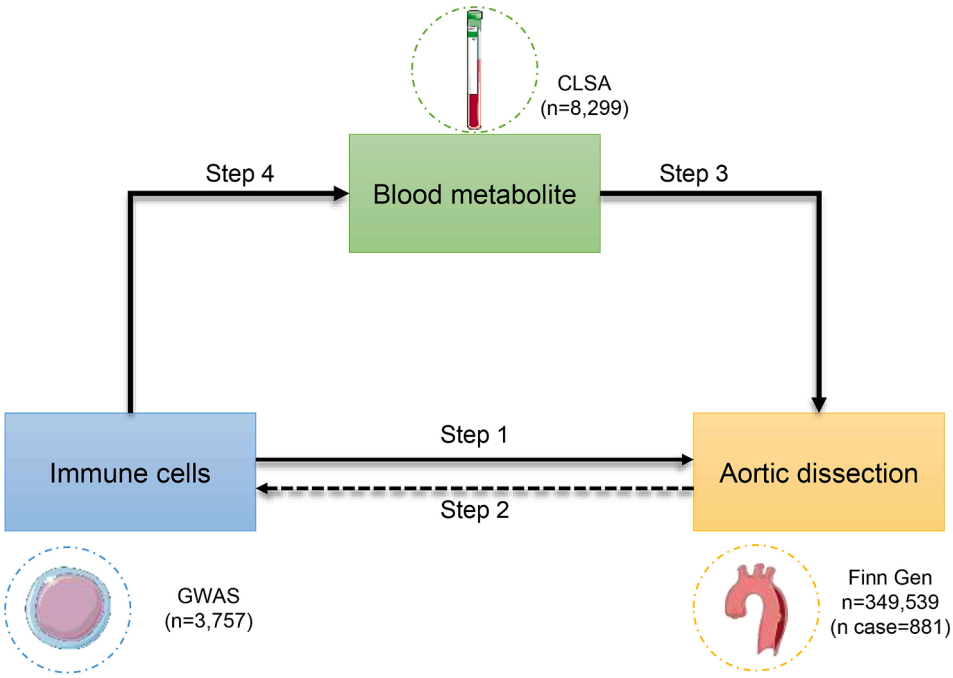

Fig. 1. The flow diagram of the mediate MR analysis. (Step1) Mendelian randomization analyses were initiated by assessing a comprehensive set of 731 types of immune cells to elucidate those causally associated with aortic dissection (AD). (Step2) Subsequently, reverse Mendelian randomization was implemented, treating these identified immune cells as exposure, with AD serving as outcome. (Step3) Further, a Mendelian randomization approach was undertaken with a dataset comprising 1,400 blood metabolites to identify any causal associations with AD. (Step4) The investigation proceeded to perform Mendelian randomization with the previously screened immune cells designated as the exposure and the positive metabolites identified as the outcome. GWAS genome-wide association study, CLSA Canadian Longitudinal Study of Aging, FinnGen is a research project in genomics and personalized medicine.

Table 1
MR analysis result: between Immune cells (731 types) and Aortic Dissection(Pivw < 0.01).(pval: p – value; se: Standard Error; lo_ci: lower confidence interval; up_ci: upper confidence interval; or_lci95: odds ratio lower confidence interval at 95 %; or_uci95: odds ratio upper confidence interval at 95 %.).

exposure	outcome	method	nsnp	b	se	pval	lo_ci	up_ci	or	or_lci95	or_uci95
CD19 on IgD- CD38br	Aortic Dissection	Inverse variance weighted	17	−0.3062	0.1089	0.00493	−0.5196	−0.0927	0.73627	0.59476	0.91144
CD19 on IgD- CD38dim	Aortic Dissection	Inverse variance weighted	28	0.29182	0.07727	0.00016	0.14037	0.44327	1.33886	1.1507	1.55779
CD24 on IgD- CD38-	Aortic Dissection	Inverse variance weighted	29	0.15058	0.04956	0.00238	0.05344	0.24771	1.16251	1.0549	1.28109
CD24 on sw mem	Aortic Dissection	Inverse variance weighted	38	0.15592	0.05031	0.00194	0.05732	0.25452	1.16873	1.05899	1.28985
CD3 on HLA DR + CD8br	Aortic Dissection	Inverse variance weighted	26	0.21077	0.0783	0.00711	0.05729	0.36424	1.23462	1.05897	1.43942
CD28 on secreting Treg	Aortic Dissection	Inverse variance weighted	17	−0.1399	0.0535	0.00892	−0.2448	−0.035	0.86944	0.78289	0.96556
CD4 on CD4 Treg	Aortic Dissection	Inverse variance weighted	28	0.15374	0.05926	0.00948	0.03758	0.2699	1.16619	1.0383	1.30983
CD4 on activated Treg	Aortic Dissection	Inverse variance weighted	24	0.15025	0.0522	0.004	0.04794	0.25256	1.16213	1.04911	1.28732
SSC-A on HLA DR + CD4+	Aortic Dissection	Inverse variance weighted	21	0.25427	0.08757	0.00369	0.08264	0.4259	1.28952	1.08615	1.53096
CD8 on CD39 + CD8br	Aortic Dissection	Inverse variance weighted	24	−0.2549	0.09279	0.00602	−0.4367	−0.073	0.77501	0.64613	0.9296

1. Introduction

Aortic dissection (AD) is a hyperacute cardiovascular disease that seriously jeopardizes the physical and mental health of human beings and brings heavy costs to individuals, families, and society [1]. Usually, aortic coarctation is initially triggered by an intimal tear, which leads to blood flow into the middle layer of the aorta, creating a false lumen, which in turn leads to separation of the layers within the aortic wall [2]. The main typical symptoms include severe chest pain, hypotension, or syncope, similar to acute myocardial infarction or pulmonary embolism. There is a 1 % increase in mortality per hour after the onset of symptoms in untreated patients [3], as the aortic epicardium can rupture and bleed to death at any time. In conclusion, aortic coarctation is characterized by the following three main features: early onset of symptoms, rapid onset and progression, and extremely high morbidity and mortality [4]. Hypertension, atherosclerosis, Marfan syndrome, and smoking are all associated risk factors for AD [5–7]. Among them, about 2/3 of patients with entrapment have hypertension, 50 % of AD patients under 40 years of age have Marfan syndrome, and about 20 % have thoracic aortic aneurysm (AA) or a family history of AD [8].

In addition to genetics, lifestyle habits, and background diseases, it is important to focus on the cellular and molecular mechanisms of AD,

Table 2
reverse MR analysis result: between Aortic Dissection and Immune cells (10 types) (Pivw > 0.05). (pval: p – value; se: Standard Error; lo_ci: lower confidence interval; up_ci: upper confidence interval; or_lci95: odds ratio lower confidence interval at 95 %; or_uci95: odds ratio upper confidence interval at 95 %; revPvalue: reversed p − value.).

exposure	outcome	method	nsnp	b	se	pval	lo_ci	up_ci	or	or_lci95	or_uci95	revPvalue
Aortic Dissection	CD19 on IgD- CD38br	Inverse variance weighted	17	−0.3062	0.1089	0.00493	−0.5196	−0.0927	0.73627	0.59476	0.91144	0.377444
Aortic Dissection	CD19 on IgD- CD38dim	Inverse variance weighted	28	0.29182	0.07727	0.00016	0.14037	0.44327	1.33886	1.1507	1.55779	0.867269
Aortic Dissection	CD24 on IgD- CD38-	Inverse variance weighted	29	0.15058	0.04956	0.00238	0.05344	0.24771	1.16251	1.0549	1.28109	0.219569
Aortic Dissection	CD24 on sw mem	Inverse variance weighted	38	0.15592	0.05031	0.00194	0.05732	0.25452	1.16873	1.05899	1.28985	0.568707
Aortic Dissection	CD3 on HLA DR + CD8br	Inverse variance weighted	26	0.21077	0.0783	0.00711	0.05729	0.36424	1.23462	1.05897	1.43942	0.490866
Aortic Dissection	CD28 on secreting Treg	Inverse variance weighted	17	−0.1399	0.0535	0.00892	−0.2448	−0.035	0.86944	0.78289	0.96556	0.474752
Aortic Dissection	CD4 on CD4 Treg	Inverse variance weighted	28	0.15374	0.05926	0.00948	0.03758	0.2699	1.16619	1.0383	1.30983	0.817207
Aortic Dissection	CD4 on activated Treg	Inverse variance weighted	24	0.15025	0.0522	0.004	0.04794	0.25256	1.16213	1.04911	1.28732	0.670536
Aortic Dissection	SSC-A on HLA DR + CD4+	Inverse variance weighted	21	0.25427	0.08757	0.00369	0.08264	0.4259	1.28952	1.08615	1.53096	0.431726
Aortic Dissection	CD8 on CD39 + CD8br	Inverse variance weighted	24	−0.2549	0.09279	0.00602	−0.4367	−0.073	0.77501	0.64613	0.9296	0.43419

with immune and metabolic abnormalities being two important aspects of the onset and development of AD [9–11]. Recent clinical and basic studies have shown that extravascular matrix degradation and vascular smooth muscle cell (VSMC) apoptosis are exacerbated with increasing levels of inflammation [12,13]. This finding suggests that the inflammatory response may play an important role in the early onset of AD and can activate a variety of pathologic processes that further contribute to the onset of AD. In addition, immune cells such as macrophages, lymphocytes, and neutrophils are often detected in the media and outer membrane of AD tissues [14,15]. Immunometabolism has gradually become a new research hotspot in the field of medicine and has been widely used in the study of diabetes, cancer, and cardiovascular diseases. It is crucial to choose more rational strategies to regulate immune metabolism and maintain natural immune homeostasis. Infiltrating immune cells not only promotes the secretion of matrix metalloproteinases (MMPs) and adhesion molecules but also releases reactive oxygen species (ROS), leading to changes in microenvironmental metabolism and VSMC apoptosis, which ultimately lead to the development of AD [16–18]. On the contrary, alterations in immune cells and parenchymal cell metabolism alter the immune microenvironment and immune cell function, which are associated with the development of AD [19,20]. Therefore, exploring the causality of AD development from the immune and metabolic perspectives may provide new perspectives for the comprehensive perception, diagnosis, and treatment of AD.

It is well known that both confounders and reverse causality may influence the results of current observational epidemiologic studies, making causal inference difficult. Mendelian randomization (MR) methods using genetic variation as an instrumental variable (IV) in epidemiological investigations have been generally accepted for estimating the causal effect of exposure on disease [21]. Due to this natural randomization of allele assignment, MR inherently mitigates the effects of confounding environmental factors and precludes reverse causation [22,23]. Thus, MR provides a powerful mechanism for deriving causal inferences from observational data. In this study, causal relationships between immune cells and plasma metabolites and AD were explored by two-sample MR analysis using pooled data from genome-wide association studies (GWASs).

2. Methods

2.1. Study design

Fig. 1 shows the study design diagram. In addition, we have highlighted the three assumptions necessary for a causal interpretation of MR estimates [24]. These assumptions require that the genetic variants used as IVs, known as single nucleotide polymorphisms (SNPs), (1) strongly predict the exposures, (2) only associate with the outcome through the exposures, and (3) are not associated with any confounder of the exposure-outcome association. The observational study completed the STROBE-MR (Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian Randomization) checklist (Appendix_1) according to the guidelines.

Hypothesis and design of bidirectional and mediated Mendelian randomization (MR) analyses. The first step involved a two-sample Mendelian randomization analysis to investigate the causal relationship between blood immune cells (exposure) and aortic dissection (outcome). In the second step, reverse Mendelian randomization was performed to study aortic dissection (exposure) with positive immune cells obtained in the first step (outcome). Finally, a two-step Mendelian randomization analysis was conducted to identify potential mediating metabolites. In step 3, the effect of 1400 blood metabolites on aortic dissection was examined, screening for positive metabolites. In step 4, the effect of positive immune cells on the identified metabolites was investigated. Images of immune cells, blood metabolites, and aortic dissection were adapted from smart.servier.com under the terms of the non-commercial use license.

Table 3
MR analysis result: between Blood metabolites (1400 types) and Aortic Dissection (Pivw < 0.01). (pval: p – value; se: Standard Error; lo_ci: lower confidence interval; up_ci: upper confidence interval; or_lci95: odds ratio lower confidence interval at 95 %; or_uci95: odds ratio upper confidence interval at 95 %.).

exposure	outcome	method	nsnp	b	se	pval	lo_ci	up_ci	or	or_lci95	or_uci95
Benzoate levels	Aortic Dissection	Inverse variance weighted	18	0.31488	0.11992	0.00865	0.07983	0.54994	1.3701	1.08311	1.73314
N-acetylproline levels	Aortic Dissection	Inverse variance weighted	19	−0.6192	0.18882	0.00104	−0.9893	−0.2491	0.53836	0.37184	0.77947
Carnitine C5:1 levels	Aortic Dissection	Inverse variance weighted	26	−0.6282	0.17273	0.00028	−0.9668	−0.2896	0.53355	0.38032	0.74854
Tridecenedioate (C13:1-DC) levels	Aortic Dissection	Inverse variance weighted	6	0.86865	0.33474	0.00946	0.21255	1.52474	2.38368	1.23683	4.59395
N1-methyladenosine levels	Aortic Dissection	Inverse variance weighted	33	−0.5177	0.16625	0.00184	−0.8436	−0.1919	0.59587	0.43017	0.82541

2.2. Genetic studies to clarify causality

We initially performed bidirectional Mendelian Randomization (MR) analyses to investigate the causal relationship between immune cells and AD. To demonstrate the genetic correlation between immune cells and AD, we conducted bivariate linkage disequilibrium score regression (LDSC) using GWAS summary statistics [25]. The inverse-variance-weighted (IVW) method, a conventional MR approach, was employed for effect estimation. The continuous outcomes were reported as beta (β) values with standard errors, while binary outcomes were presented as odds ratios (OR) with 95 % confidence intervals (CI). A P-value of less than 0.05 was considered nominally significant. The bivariate LDSC method operates on the principle that genetic variants in linkage disequilibrium (LD) are co-inherited and have a higher likelihood of being associated with a trait or disease compared to non-LD variants. In summary, the IVW method *meta*-analyzed SNP-specific Wald estimates (calculated as the SNP outcome estimate divided by the SNP exposure estimate) using random effects to derive a final estimate of the causal effect [26]. This method estimates the genetic correlation between two traits by regressing the LD score of each SNP against the effect sizes of both traits simultaneously.

2.3. GWAS dataset

All genome-wide association study (GWAS) data included in this study are restricted to populations of European origin. The FinnGen project (DATA FREEZE 9, https://www.finngen.fi/en) was used to obtain aggregated GWAS statistics for AD. The FinnGen project is a large-scale genetic research program exploring the relationship between genomic information and health characteristics in the Finnish population (Europeans) about genomic information and health characteristics. The data includes GWAS information and health characteristics records for 377,277 individuals. The GWAS data for AD were obtained by submitting an approval request to the researchers of the FinnGen study. The dataset included 881 patients with AD and 349,539 controls. Genetic association results for exposure factors were obtained from two datasets: the immune cell GWAS dataset and the blood metabolite GWAS dataset. GWAS summary statistics for each immune cell are publicly available from the GWAS Catalog (accession numbers from GCST0001391 to GCST0002121)(https://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics/). A total of 731 immunophenotypes including absolute cell (AC) counts (n = 118), median fluorescence intensities (MFI) reflecting surface antigen levels (n = 389), morphological parameters (MP) (n = 32) and relative cell (RC) counts (n = 192) were included. Specifically, the MFI, AC, and RC features contain B cells, CDCs, mature stages of T cells, monocytes, myeloid cells, TBNK (T cells, B cells, natural killer cells), and Treg panels, while the MP feature contains CDC and TBNK panels [27]. The metabolite database was obtained from the Canadian Longitudinal Study of Aging (CLSA) [28]. The GWAS sample was collected from 8,299 unrelated European subjects, and the associated analysis was performed on 15.4 million SNPs from this population. After quality control, 1,091 metabolites were available for GWAS analysis. These metabolites were divided into 850 known and 241 unknown metabolites. The 850 known metabolites were divided into 8 biochemical groups(lipid, amino acid, xenobiotics, nucleotide, cofactor, and vitamins, carbohydrate, peptide, and energy). The blood metabolites in the entire GWAS data were from the Metabolomics GWAS server (https://metabolomics.helmholtz-muench en.de/gwas/). Detailed cohort characteristics can be found in the published CLSA cohort profile [29].

As the GWAS datasets are all publicly available and were previously certified by the appropriate ethics review committees, additional ethics approval was not required for the analysis conducted in this study.

2.4. Selection of instrumental variables (IVs)

For MR, it is important for the genetic variants used to be representative of the Immune cell's features, thus we selected SNPs associated with the Immune cells at a more suggestive P-value of less than 1 × e-5 (linkage disequilibrium [LD] r2 threshold < 0.001 within 10000 kb distance), as used in previous MR studies [27]. We selected SNPs associated with AD and blood metabolites at conventional GWAS thresholds (P < 1 × e-5). Independent SNPs were then clumped to a linkage disequilibrium (LD) threshold of r2 < 0.001. We excluded SNPs whose F-statistic was < 10 (a measure of the strength of these IVs) to avoid weak instrumental bias [30].

2.5. Mediation analyses link "immune cells–blood metabolites–aortic dissection"

The mediation approach we adopted is two-step Mendelian randomization (TSMR) [31], to decompose the direct and indirect effects of the immune cells and blood metabolites on AD. The TSMR assumes no interaction between exposure and mediator. In addition to the basic effect estimates of immune cells on blood metabolites (β1) obtained from the univariate MR analyses, two more estimates were calculated: (1) the causal effect of the mediator (blood metabolites) on AD (β2), and (2) the causal effect of the exposure (significant immune cells on AD in primary MR analysis) on the AD(β_all). Step 1: immune cells to AD MR (get the total effect, β_all).Step 2: AD to immune cells (make sure it can be mediated). Step 3: blood metabolites to AD MR (get β2). Step 4: immune cells to blood metabolites MR (get β1). Mediated effect: β1_2 = β1*β2. Direct effect: β_dir = β_all-β1_2.

2.6. Sensitivity analyses

The primary analysis method employed in this study to assess the significant causal relationship between blood metabolites, immune cells, and AD was the inverse variance weighted (IVW) method. Additionally, up to four MR methods (MR-Egger, weighted median, simple mode, and weighted mode) that make differing pleiotropy assumptions have been used to generate effect estimates as sensitivity analyses [22,32]. We assessed horizontal pleiotropy using the MR-Egger method,

Table 4
MR analysis result: between Immune cells (10 types) and Blood metabolites (5 types) (Pivw < 0.05). (pval: p – value; se: Standard Error; lo_ci: lower confidence interval; up_ci: upper confidence interval; or_lci95: odds ratio lower confidence interval at 95 %; or_uci95: odds ratio upper confidence interval at 95 %.).

CD19 on IgD- CD38br on Benzoate levels											
exposure	outcome	method	nsnp	b	se	pval	lo_ci	up_ci	or	or_lci95	or_uci95
CD19 on IgD-CD38br	Benzoate levels	MR Egger	15	−0.0594	0.03999	0.16136	−0.1378	0.01899	0.94233	0.87129	1.01917
CD19 on IgD-CD38br	Benzoate levels	Weighted median	15	−0.0497	0.03846	0.19632	−0.1251	0.02568	0.95153	0.88244	1.02602
CD19 on IgD-CD38br	Benzoate levels	Inverse variance weighted	15	−0.0523	0.02623	0.0461	−0.1037	−0.0009	0.94903	0.90147	0.9991
CD19 on IgD-CD38br	Benzoate levels	Simple mode	15	−0.0511	0.05865	0.39855	−0.166	0.06389	0.95021	0.84701	1.06597
CD19 on IgD-CD38br	Benzoate levels	Weighted mode	15	−0.0485	0.03793	0.22156	−0.1229	0.02581	0.95263	0.88439	1.02615
CD24 on IgD- CD38- on N-acetylproline levels											
exposure	outcome	method	nsnp	b	se	pval	lo_ci	up_ci	or	or_lci95	or_uci95
CD24 on IgD-CD38-	N-acetylproline levels	MR Egger	28	−0.0368	0.0228	0.11851	−0.0815	0.00788	0.96386	0.92173	1.00791
CD24 on IgD-CD38-	N-acetylproline levels	Weighted median	28	−0.0415	0.0228	0.06896	−0.0862	0.00322	0.95938	0.91744	1.00323
CD24 on IgD-CD38-	N-acetylproline levels	Inverse variance weighted	28	−0.0334	0.0161	0.03801	−0.0649	−0.0018	0.96715	0.93712	0.99815
CD24 on IgD-CD38-	N-acetylproline levels	Simple mode	28	−0.049	0.03927	0.22267	−0.126	0.02795	0.95217	0.88163	1.02834
CD24 on IgD-CD38-	N-acetylproline levels	Weighted mode	28	−0.0477	0.02463	0.06323	−0.096	0.00056	0.9534	0.90847	1.00056
CD28 on secreting Treg on Carnitine C5:1 levels											
exposure	outcome	method	nsnp	b	se	pval	lo_ci	up_ci	or	or_lci95	or_uci95
CD28 on secreting Treg	Carnitine C5:1 levels	MR Egger	17	0.02787	0.0205	0.19401	−0.0123	0.06804	1.02826	0.98777	1.07041
CD28 on secreting Treg	Carnitine C5:1 levels	Weighted median	17	0.03896	0.02061	0.05875	−0.0014	0.07936	1.03973	0.99856	1.0826
CD28 on secreting Treg	Carnitine C5:1 levels	Inverse variance weighted	17	0.03955	0.01618	0.0145	0.00784	0.07126	1.04034	1.00787	1.07386
CD28 on secreting Treg	Carnitine C5:1 levels	Simple mode	17	0.01729	0.02864	0.55457	−0.0388	0.07342	1.01744	0.9619	1.07618
CD28 on secreting Treg	Carnitine C5:1 levels	Weighted mode	17	0.03925	0.01921	0.05783	0.0016	0.07689	1.04003	1.00161	1.07993
CD4 on CD4 Treg on Tridecenedioate (C13:1-DC) levels											
exposure	outcome	method	nsnp	b	se	pval	lo_ci	up_ci	or	or_lci95	or_uci95
CD4 on CD4 Treg	Tridecenedioate (C13:1-DC) levels	MR Egger	29	−0.0311	0.01814	0.0979	−0.0667	0.00445	0.96937	0.93551	1.00446
CD4 on CD4 Treg	Tridecenedioate (C13:1-DC) levels	Weighted median	29	−0.0257	0.01945	0.1858	−0.0639	0.01239	0.97459	0.93813	1.01247
CD4 on CD4 Treg	Tridecenedioate (C13:1-DC) levels	Inverse variance weighted	29	−0.0304	0.01344	0.02391	−0.0567	−0.004	0.9701	0.94489	0.996
CD4 on CD4 Treg	Tridecenedioate (C13:1-DC) levels	Simple mode	29	−0.02	0.02911	0.49865	−0.077	0.0371	0.98024	0.92588	1.0378
CD4 on CD4 Treg	Tridecenedioate (C13:1-DC) levels	Weighted mode	29	−0.0294	0.01894	0.13153	−0.0665	0.0077	0.97101	0.93563	1.00773
SSC-A on HLA DR + CD4 + on Tridecenedioate (C13:1-DC) levels											
exposure	outcome	method	nsnp	b	se	pval	lo_ci	up_ci	or	or_lci95	or_uci95
SSC-A on HLA DR + CD4+	Tridecenedioate (C13:1-DC) levels	MR Egger	21	−0.0325	0.03124	0.31085	−0.0938	0.02871	0.96799	0.91049	1.02912
SSC-A on HLA DR + CD4+	Tridecenedioate (C13:1-DC) levels	Weighted median	21	−0.0805	0.03079	0.00897	−0.1408	−0.0201	0.9227	0.86867	0.98009
SSC-A on HLA DR + CD4+	Tridecenedioate (C13:1-DC) levels	Inverse variance weighted	21	−0.0588	0.02048	0.00406	−0.099	−0.0187	0.94286	0.90577	0.98147
SSC-A on HLA DR + CD4+	Tridecenedioate (C13:1-DC) levels	Simple mode	21	−0.1075	0.05023	0.0449	−0.2059	−0.009	0.8981	0.8139	0.99102
SSC-A on HLA DR + CD4+	Tridecenedioate (C13:1-DC) levels	Weighted mode	21	−0.0894	0.03926	0.03385	−0.1664	−0.0125	0.91445	0.84673	0.98759

which performs weighted linear regression with the intercept unconstrained [22]. The intercept represents the average pleiotropic effect across the genetic variants (the average direct effect of a variant with the outcome). If the intercept differed from zero (MR-Egger intercept P-value < 0.05), there was evidence of horizontal pleiotropy. We also used leave-one-out analyses to detect SNP outliers.

2.7. Pleiotropy and heterogeneity analysis

We initiated our analysis using the MR-PRESSO approach to identify outliers and proceeded with a re-analysis after their exclusion[33]. Furthermore, to discern horizontal pleiotropy in MR analysis, the MR-Egger regression test was employed, emphasizing the statistical significance of the intercept term [34]. Finally, we computed the Cochran Q statistic to detect heterogeneity, setting the significance of the threshold at P = 0.05 [35]. All statistical analyses were conducted in R, version 4.2.3, with the MR and MR-PRESSO packages.

All MR analyses were conducted in R (version 4.1.2; R Foundation for Statistical Computing, Vienna, Austria) using the "TwoSampleMR," "tidyverse," "ggplot2," "purrr," and "data. table" packages.

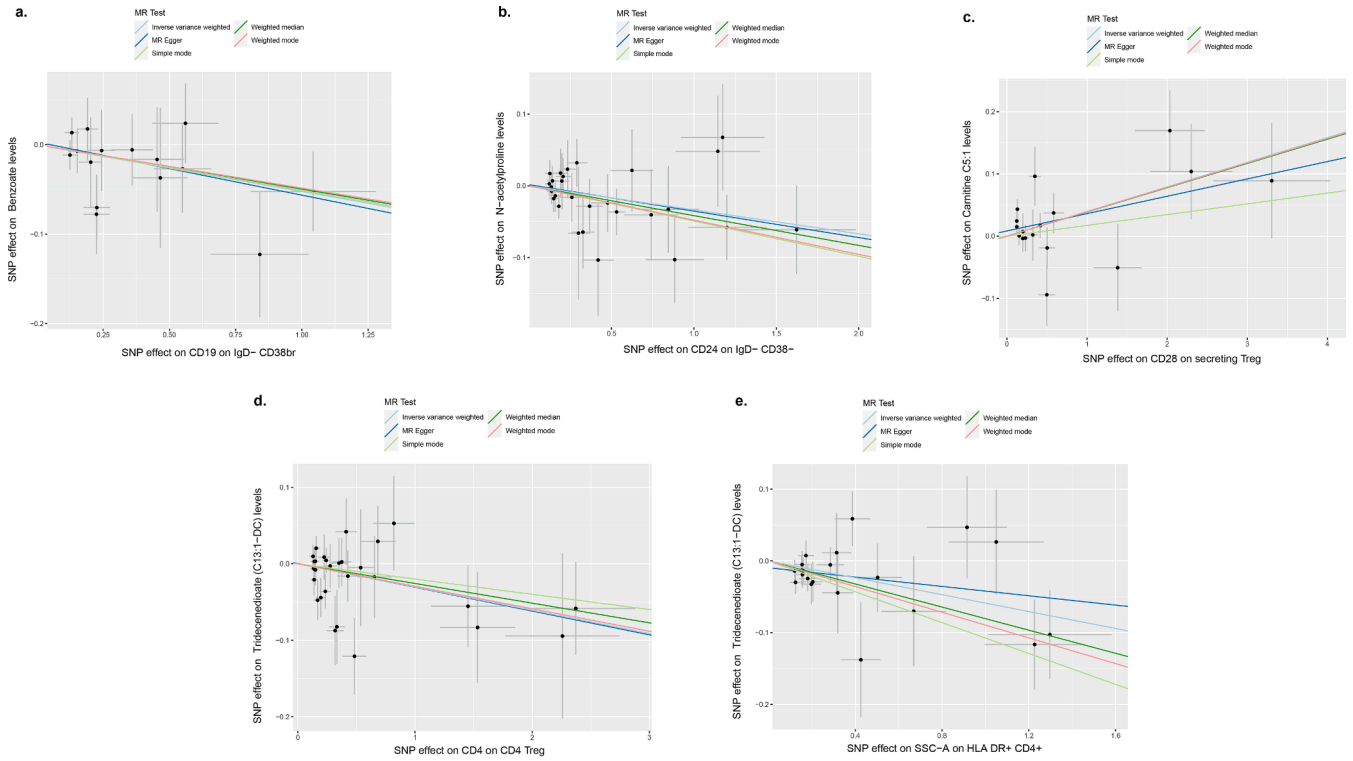

Fig. 2. Extensive Mendelian randomization analysis yielded five pairs of positive results. (a) Causal relationships between CD19 on IgD − CD38br and Benzoate in scatter plots. (b) CD24 on IgD − CD38 − and N − acetylproline. (c) CD28 on secreting Treg and Carnitine C5:1. (d) CD4 on CD4 Treg and Tridecenedioate (C13:1 − DC). (e) SSC − A on HLA DR + CD4 + and Tridecenedioate (C13:1 − DC). SNPs single-nucleotide polymorphisms, MR Mendelian randomization.

2.8. Ethical approval and consent to participate

This study is based on publicly available data. Individual studies within each Genome-Wide Association Study (GWAS) received approval from the relevant Institutional Review Board, and informed consent was obtained from the participants or a caregiver, legal guardian, or other proxy.

3. Results

3.1. Instrument variables included in the analysis

Following the elimination of single nucleotide polymorphisms (SNPs) deemed non-significant for both exposure and outcome, as delineated in the Methods section, the resultant SNP sets were amalgamated to derive instrumental variables for utilization in Mendelian randomization analysis (Sup. Table S1-3). All SNPs included in our analysis possessed an F-value exceeding 10.

3.2. Causal effects of immune cells and AD

To explore the causal effect of immune cells on AD, two-sample MR analysis and sensitivity analysis were performed. The IVW method was used as the primary analysis method (5 methods in total), and the IVW results of the Mendelian randomization analysis were filtered according to a p-value less than 0.01. Next, immune cells with ORs in the same direction for the five analysis methods and with a p-value greater than 0.05 in the multiplicity analysis were extracted. A total of 10 immune cells were screened, of which CD19 on IgD- CD38br, CD28 on secreting Treg and CD8 on CD39 + CD8br were negatively correlated with the development of aortic dissection, while CD19 on IgD- CD38dim, CD24 on IgD- CD38-, CD24 on sw mem, CD3 on HLA DR + CD8br, CD4 on CD4 Treg, CD4 on activated Treg and SSC-A on HLA DR + CD4 + were positively associated with the development of aortic dissection, as (Table 1). Among them, CD19 on IgD- CD38dim had the strongest effect on the risk of AD (OR = 1.339,95 % CI:1.151–1.558; P = 0.00015).

To further evaluate the causal impact of aortic dissection on immune cells, a reverse MR analysis was performed. The results showed no significant correlation between aortic dissection and any of the ten immune cells mentioned above in the random-effects IVW analysis(Table 2).

3.3. Mediation analyses of potential blood metabolites

First, the causal effects of blood metabolites on AD were explored. The Methods section examined the causal association between these 1091 blood metabolites and aortic dissection using the IVW method. A total of 5 blood metabolites were found to be significantly associated with aortic dissection for multiple tests (Pivw < 0.01) (Table 3). Of these metabolites, 2 increased the risk of aortic dissection, including benzoate (OR = 1.3581, 95 % CI: 1.1115—1.6595, Pivw = 0.0028), tridecenedioate (C13:1-DC) (OR = 1.3701, 95 % CI: 1.0831–1.7331, Pivw = 0.0086). The remaining three metabolites were associated with a reduced risk of aortic dissection, such as N-acetylproline (OR = 0.5384, 95 % CI: 0.3718—0. 7795, Pivw = 0.0010), carnitine C5:1 (OR = 0.5336, 95 % CI: 0.3803—0.7485, Pivw = 0.0003) and N1-methyladenosine (OR = 0.5959, 95 % CI: 0.4302—0.8254, Pivw = 0.0018).

Second, two-sample Mendelian randomization analyses were performed using the five metabolites and 10 immune cells described above as having a strong causal association with AD, with immune cells as the exposure and metabolites as the outcome. Again, the IVW method was used as the primary analysis method (five methods in total), and the IVW results from the Mendelian randomization analysis were filtered based on p-values less than 0.01. Next, results with ORs in the same direction and p-values greater than 0.05 in the test of multiplicity were extracted for all five analysis methods. However, there was no single positive result when filtering the results based on Pivw less than 0.01. Therefore, after adjusting the filtering condition to Pivw less than 0.05, a significant

a.

exposure	outcome	nsnp	method	pval	OR(95% CI)
		15	MR Egger	0.161	0.942 (0.871 to 1.019)
		15	Weighted median	0.196	0.952 (0.882 to 1.026)
CD19 on IgD− CD38br	Benzoate levels	15	Inverse variance weighted	**0.046**	0.949 (0.901 to 0.999)
		15	Simple mode	0.399	0.950 (0.847 to 1.066)
		15	Weighted mode	0.222	0.953 (0.884 to 1.026)
		18	MR Egger	0.070	1.359 (0.997 to 1.852)
		18	Weighted median	0.109	1.296 (0.944 to 1.779)
Benzoate levels	Aortic dissection	18	Inverse variance weighted	**0.009**	1.370 (1.083 to 1.733)
		18	Simple mode	0.317	1.352 (0.762 to 2.396)
		18	Weighted mode	0.110	1.270 (0.962 to 1.676)
		17	MR Egger	0.126	0.751 (0.532 to 1.061)
		17	Weighted median	0.436	0.891 (0.666 to 1.192)
CD19 on IgD− CD38br	Aortic dissection	17	Inverse variance weighted	**0.005**	0.736 (0.595 to 0.911)
		17	Simple mode	0.431	0.829 (0.525 to 1.308)
		17	Weighted mode	0.377	0.866 (0.635 to 1.181)

b.

exposure	outcome	nsnp	method	pval	OR(95% CI)
		28	MR Egger	0.119	0.964 (0.922 to 1.008)
		28	Weighted median	0.069	0.959 (0.917 to 1.003)
CD24 on IgD− CD38−	N−acetylproline levels	28	Inverse variance weighted	**0.038**	0.967 (0.937 to 0.998)
		28	Simple mode	0.223	0.952 (0.882 to 1.028)
		28	Weighted mode	0.063	0.953 (0.908 to 1.001)
		19	MR Egger	0.123	0.499 (0.216 to 1.155)
		19	Weighted median	**0.042**	0.596 (0.361 to 0.982)
N−acetylproline levels	Aortic dissection	19	Inverse variance weighted	**0.001**	0.538 (0.372 to 0.779)
		19	Simple mode	0.672	0.809 (0.308 to 2.126)
		19	Weighted mode	0.442	0.702 (0.291 to 1.695)
		29	MR Egger	**0.009**	1.211 (1.059 to 1.386)
		29	Weighted median	**0.017**	1.188 (1.031 to 1.369)
CD24 on IgD− CD38−	Aortic dissection	29	Inverse variance weighted	**0.002**	1.163 (1.055 to 1.281)
		29	Simple mode	0.744	1.037 (0.837 to 1.285)
		29	Weighted mode	**0.012**	1.181 (1.046 to 1.333)

c.

exposure	outcome	nsnp	method	pval	OR(95% CI)
		17	MR Egger	0.194	1.028 (0.988 to 1.070)
		17	Weighted median	0.059	1.040 (0.999 to 1.083)
CD28 on secreting Treg	Carnitine C5:1 levels	17	Inverse variance weighted	**0.015**	1.040 (1.008 to 1.074)
		17	Simple mode	0.555	1.017 (0.962 to 1.076)
		17	Weighted mode	0.058	1.040 (1.002 to 1.080)
		26	MR Egger	**0.022**	0.334 (0.139 to 0.803)
		26	Weighted median	**0.022**	0.576 (0.359 to 0.924)
Carnitine C5:1 levels	Aortic dissection	26	Inverse variance weighted	**<0.001**	0.534 (0.380 to 0.749)
		26	Simple mode	0.291	0.597 (0.234 to 1.524)
		26	Weighted mode	0.230	0.574 (0.237 to 1.391)
		17	MR Egger	0.050	0.870 (0.765 to 0.989)
		17	Weighted median	0.069	0.878 (0.764 to 1.010)
CD28 on secreting Treg	Aortic dissection	17	Inverse variance weighted	**0.009**	0.869 (0.783 to 0.966)
		17	Simple mode	0.315	0.896 (0.728 to 1.103)
		17	Weighted mode	0.083	0.887 (0.781 to 1.007)

Fig. 3. The results of the Mendelian randomisation analysis indicate a causal relationship between immune cells mediated by blood metabolites and the risk of aortic dissection (AD) ($P < 0.05$). (a) CD19 on IgD − CD38br − Benzoate levels − Aortic dissection. (b) CD24 on IgD − CD38− − N − acetylproline levels − Aortic dissection. (c) CD28 on secreting Treg − Carnitine C5:1 levels − Aortic dissection. SNPs single nucleotide polymorphisms, OR odds ratio, CI confidence interval.

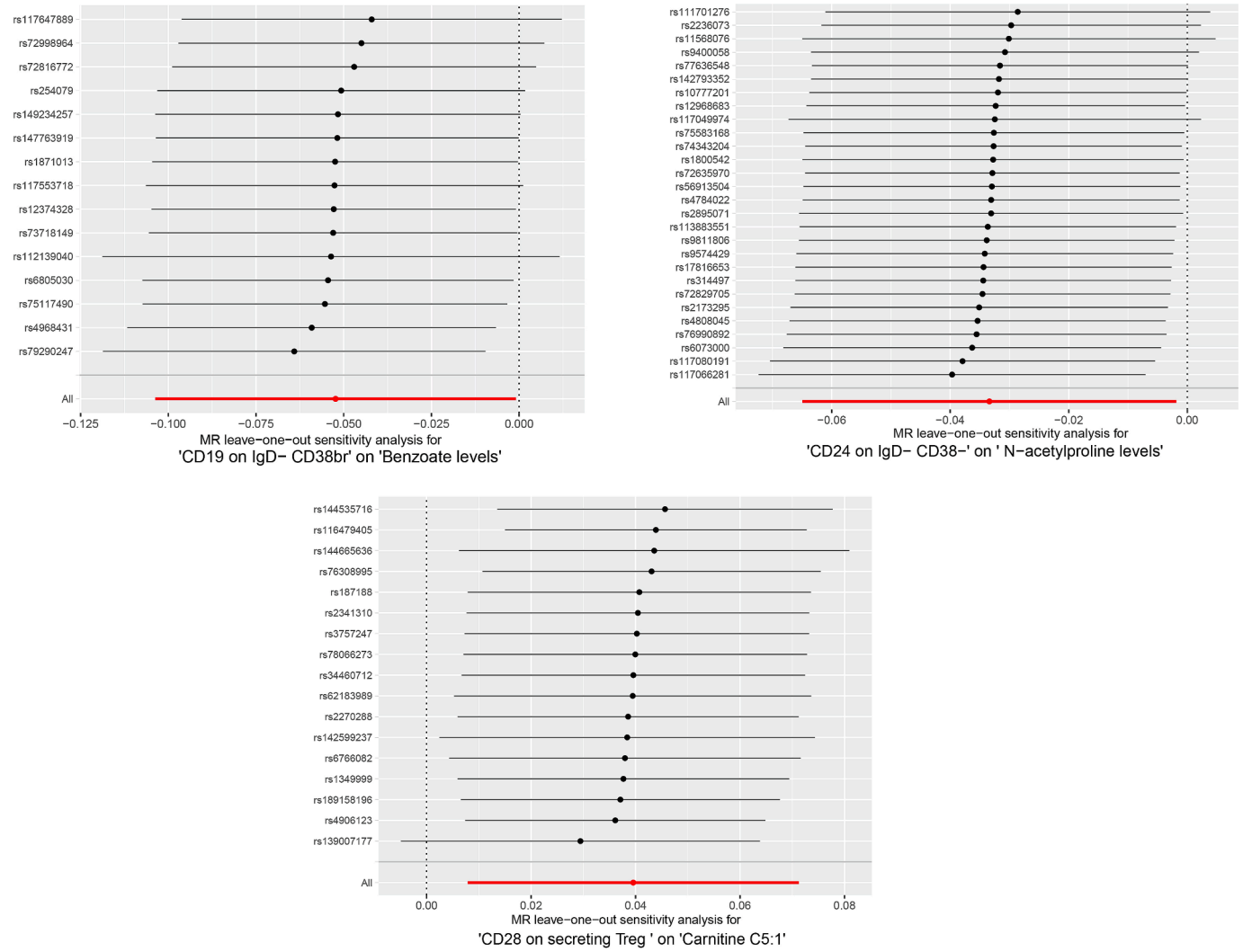

Fig. 4. Leave-one-out sensitivity analysis was conducted on immune cells, blood metabolites, and aortic dissection (AD) in three pairs of positive results. (4a) Immune cells and blood metabolites. (4b) Blood metabolites and AD. (4c) Immune cells and AD. MR Mendelian randomization.

causal relationship was obtained between five pairs of immune cells and metabolites (which have a close causal relationship with AD) (Table 4).

The first pair was CD19 on IgD- CD38br and Benzoate with negative correlation (OR = 0.9490, 95 % CI: 0.9015—0.9991, Pivw = 0.0461); the second pair was CD24 on IgD- CD38 − and N-acetylproline with negative correlation (OR = 0.9672, 95 % CI: 0.9371—0.9982, Pivw = 0.0380); the third pair was CD28 on secreting Treg and Carnitine C5:1 with positive correlation (OR = 1.0403, 95 % CI: 1.0079—1.0739, Pivw = 0.0145); the fourth pair was CD4 on CD4 Treg and Tridecenedioate (C13:1-DC) with negative correlation (OR = 0.9701, 95 % CI: 0.9449—0.9960, Pivw = 0.0239); the fifth pair was SSC-A on HLA DR + CD4 + and Tridecenedioate (C13:1-DC) with a negative correlation (OR = 0.9429, 95 % CI: 0.9058——0.9815, Pivw = 0.0041)(Fig. 2).

In summary, immune cells CD19 on IgD- CD38br can mediate protection against aortic dissection via the metabolite Benzoate [mediation effect: (b = -0.0165, 95 %CI: −0.0329 ~ -6.28e-05); proportion mediation = 5.38 % (0.0205–10.7 %);pvalue = 0.049]. Immune cells CD24 on IgD- CD38- can mediate the detrimental effects on aortic dissection through the metabolite N-acetylproline [mediation effect: (b = 0.0207, 95 %CI: 0.00112–0.0402); proportion mediation = 13.70 % (0.741–26.70 %),pvalue = 0.038]. Immune cells CD28 on secreting Treg can mediate the protective effects on aortic dissection through the metabolite Carnitine C5:1[mediation effect: (b = -0.0248, 95 %CI:-0.0448 ~ -0.00489); proportion mediation = 17.80 % (3.49–32.00 %), pvalue = 0.015] (Fig. 3). Among the identified relationships, as proportional mediation is less than 0, the causal linkage between two immune cell phenotypes, CD4 on CD4 T regulatory (Treg) cell [mediation effect: (b = -0.0264, 95 %CI:-0.0493 ~ -0.00347); proportion mediation = -17.1 % (–32, −2.26 %),pvalue = 0.024] and SSC-A on HLA-DR + CD4 + cell [mediation effect: (b = -0.0511, 95 %CI: −0.0861 ~ -0.0162); proportion mediation = -20.1 % (–33.8, −6.63 %), pvalue = 0.004], in relation to aortic dissection (AD), with Tridecenedioate (C13:1-DC) acting as a mediator, eludes a straightforward explanation (Supplementary Figure S1). This anomaly may suggest that our experimental design did not account for certain unidentified blood metabolites that could play a significant role in influencing the onset and progression of AD. This oversight indicates a potential gap in our understanding, underscoring the necessity for further research to explore and identify these unknown metabolites, thereby enriching our comprehension of the complex biological processes contributing to AD.

3.4. Sensitivity analysis

Several sensitivity analyses were employed to scrutinize and adjust for the potential influence of pleiotropy on our causal estimates. The application of Cochran's Q-test and the examination of funnel plots

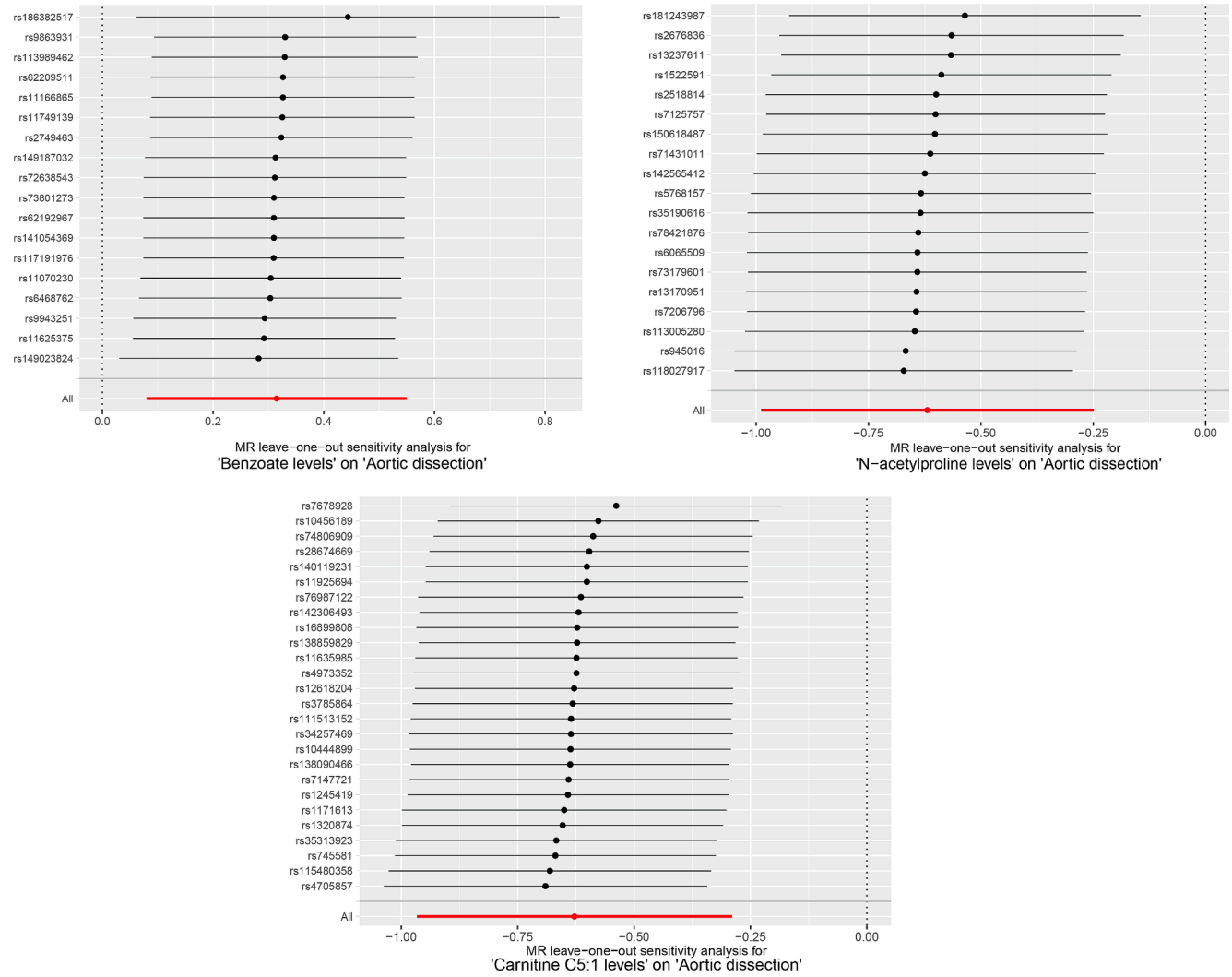

Fig. 4. (*continued*).

revealed no significant evidence of heterogeneity or asymmetry among the single nucleotide polymorphisms (SNPs) implicated in the causal pathways (refer to Sup. Table S4-6). The MR-PRESSO global test did not reveal any potential horizontal pleiotropy, further substantiating the robustness of our findings (Sup. Table S4-6). The integrity of each SNP's contribution to the overall causal inference was rigorously validated through a leave-one-out analysis (illustrated in Fig. 4 a-c). By methodically re-evaluating the MR analysis upon the sequential exclusion of each SNP, we ascertained the consistency of our results, affirming that the collective contribution of all SNPs was integral to establishing the significance of the causal relationship.

4. Discussion

To investigate the relationship between immune cells, blood metabolites, and aortic dissection, we conducted the first multiple bidirectional two-sample MR studies and mediation studies to investigate the causal relationship between immune cells and aortic dissection via blood metabolites. We found that three types of immune cells can be genetically associated with aortic dissection via three blood metabolites. One is a risk factor for aortic dissection and two are protective factors. Reverse MR analysis showed that aortic dissection was not genetically correlated to these types of immune cells. All sensitivity analyses consistently supported the results of our primary analysis, demonstrating the reliability and stability of this MR analysis. To our knowledge, this is the first large-scale genetic correlation analysis of immune cells, blood metabolites, and aortic dissection. Because we used GWAS genetic data, the results are not susceptible to environmental confounders.

Immune cells and parenchymal cells need to constantly regulate their own metabolism to perform their corresponding functions in AD. During this process, the production of both cytokines and metabolites alters the microenvironment, further affecting metabolism; for instance, Lipoprotein a (Lp(a)) triggers a proinflammatory response via oxidised phospholipid (OxPL), which is identified as a danger-associated molecular pattern by pattern recognition receptors on innate immune cells. Lp (a) induces monocyte recruitment to induce inflammation in the arterial wall [36].

It has been reported that monocytes are a heterogeneous cell population that can be classified into three subpopulations based on their phenotypic and functional properties: 'classical' ($CD14^{++}CD16^{-}$), 'intermediate' ($CD14^{++}CD16^{+}$), and 'nonclassical' ($CD14^{+}CD16^{+}$). In addition, AAD is associated with a significant increase in classical monocytes and a significant decrease in intermediate monocytes [37]. Research has shown that T cells/Th cells may contribute to the development of AD by inducing VSMC apoptosis and MMP synthesis [38]. Clinical studies have shown that T cell activation is involved in the development of AD, as evidenced by high levels of CD3+, CD4+, CD8+, and CD45 + T cells in the aortic tissue of AD patients [39]. Under different conditions, CD4 + T cells can differentiate into various

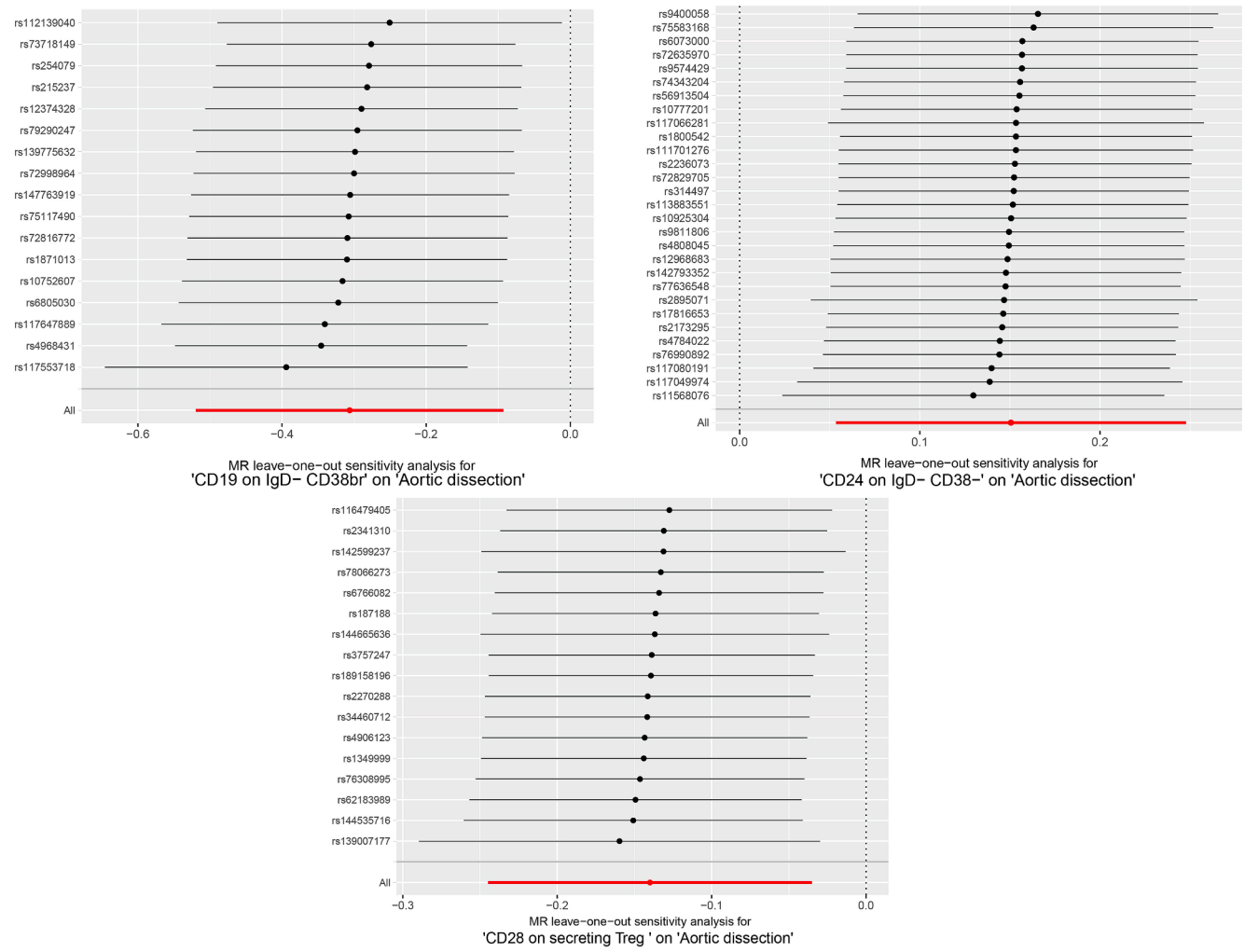

Fig. 4. (*continued*).

subpopulations, including T helper 1 (Th1), Th2, Th17, and T regulatory (Treg) cells [40]. Studies have confirmed that Treg cells can play both anti-inflammatory and pro-inflammatory roles, which may be attributed to the heterogeneity of Treg cells. For example, Treg cells that produce IL-10 may safeguard against aortic wall rupture by exerting anti-inflammatory effects on AAD. Conversely, CD25 + Treg cells are elevated and promote inflammation in patients with symptomatic carotid stenosis [41]. Previous studies have demonstrated that Treg cells inhibit the development of AD [42], and our findings support this view. Our study discovered that CD28 on secreting Treg may have a protective effect on the development of AD by influencing the levels of the metabolite Carnitine C5:1, which in turn plays a protective role in the development of AD.

There is an increasing amount of data indicating that abnormalities in lipid metabolism are closely linked to the development of aortic dissection. Two observational studies conducted in China have demonstrated significant changes in blood lysophospholipids and sphingolipids in patients with aortic dissection [43]. In the present systematic MR study, we confirmed the lipid molecules (Tridecenedioate (C13:1 − DC)) involved in the pathogenesis of aortic dissection from a genetic perspective.

Furthermore, the significant role of amino acid metabolism in aortic dissection has become increasingly apparent. For instance, Hao et al. (2022) discovered that concentrations of dimethylglycine were notably elevated in patients with type A AD, with the highest area under the curve values. This finding suggests that dimethylglycine could serve as a potential biomarker for AD [44]. The kynurenine pathway is the primary metabolic pathway for tryptophan and is linked to inflammation and immune response. Previous studies have shown that elevated levels of circulating kynurenine are associated with an increased risk of cardiovascular disease [45]. In contrast, our study has identified a new amino acid, N-acetylproline, which has a positive causal effect on aortic dissection, a finding that has not been reported previously. A metabolite molecule, specifically carnitine C5:1, was identified as a risk factor for aortic dissection in our study. These findings suggest that blood metabolic biomarkers could be potential targets for preventing and treating aortic dissection. However, the exact underlying mechanisms are unknown and require further investigation.

Recently, it has been discovered that Benzoate may have therapeutic benefits for depression. The primary mechanism of action for these drugs is the modulation of the N-methyl-d-aspartate glutamate receptor (NMDAR) and the reduction of inflammation in the brain [46]. However, in this case, it was found that Benzoate acts as a mediator of the immune cell CD19 on IgD- CD38br, which is one of the risk factors for aortic dissection.

Our mediation analyses provide genetic evidence of an association between immune cells and blood metabolites. Previous studies have not directly linked AD-related immune cells to AD-related metabolites. However, several studies have assessed the independence and interaction of immunity and metabolism in aortic dissection. Abnormalities in carbohydrate, lipid, and amino acid metabolism have received increasing attention in recent years. Immune metabolism has become a

significant area of medical research, with potential clinical applications in fields such as cancer and cardiovascular diseases. Therefore, it is crucial to adopt rational strategies to regulate immune metabolism and maintain natural immunity homeostasis [47].

The present study has several strengths. Firstly, it utilized the largest and latest GWASs of summary data for immune cells, blood metabolites, and aortic dissection, which ensured the statistical power of the findings. Secondly, we used a two-sample MR design to minimize confounding factors that are inevitable in observational studies. Thirdly, sensitivity analyses provided no evidence of horizontal pleiotropy, which reinforces the robustness of our MR estimates. However, our study has several limitations. Firstly, our study population was limited to individuals of European ancestry, which restricts the generalizability of our results to other populations. Future studies in other populations are warranted. Secondly, the smaller number of cases in AD is in the GWAS dataset of AD, and it is hoped that larger GWAS data will be available for validation in the future. Thirdly, although this study covered a wide range of blood metabolites, there are still many unknown metabolites that were not included, and the roles and mechanisms of many of these metabolites in the disease are not fully understood.

5. Conclusions

In conclusion, this research represents a pioneering application of mediated Mendelian randomization (MR) analysis leveraging genome-wide data to elucidate the causal interplay among immune cells, blood metabolites, and aortic dissection (AD). It delineates genetic causality links between specific immune cells and AD, accentuating the instrumental role of blood metabolites as mediators. Furthermore, the study unveils potential metabolic pathways through which these metabolites might influence the pathogenesis of AD. By offering profound insights into the etiological factors and progression mechanisms of aortic dissection, this study contributes significantly to the body of knowledge in the field, paving the way for novel diagnostic and therapeutic strategies.

Declarations

Ethics approval and consent to participate

Given that the GWAS data set is publicly available and we merely carry out secondary organization and analysis of the data, the analysis conducted in this research does not necessitate additional ethical approval.

Consent for publication

All authors have read and approved the final manuscript. Additionally, all individuals who participated in the study have provided their consent for the publication of the data and results derived from their contributions. Written informed consent was obtained from all participants prior to their inclusion in the study.

Availability of data and materials

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Authors' contributions

LA, ZZW, FZA, FSH, MZX, and ZHY designed the research. LA, FZA, FSH, and MZX collected the data. LA and FZA were involved in analyzing and interpreting the data. LA, FSH, and MZX searched the literature. LA and FSH drafted the article. Supervision, funding, manuscript reviewing, and editing were performed by ZHY, FZA, and MZX. All authors were involved in writing the paper. LA and ZHY mainly completed the manuscript. All authors read and approved the final manuscript.

CRediT authorship contribution statement

Ao Li: . ZiAn Feng: . ShiHao Fu: . ZhenXiao Ma: . HaiYang Zhang: . ZhiWei Zhao: Funding acquisition, Formal analysis.

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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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcha.2024.101530.

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