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Gut Microbiota Affects Cardiovascular Diseases via Inflammatory Cytokines: a Bidirectional Two-sample Mendelian Randomization Study and Mediation Analysis

Authors

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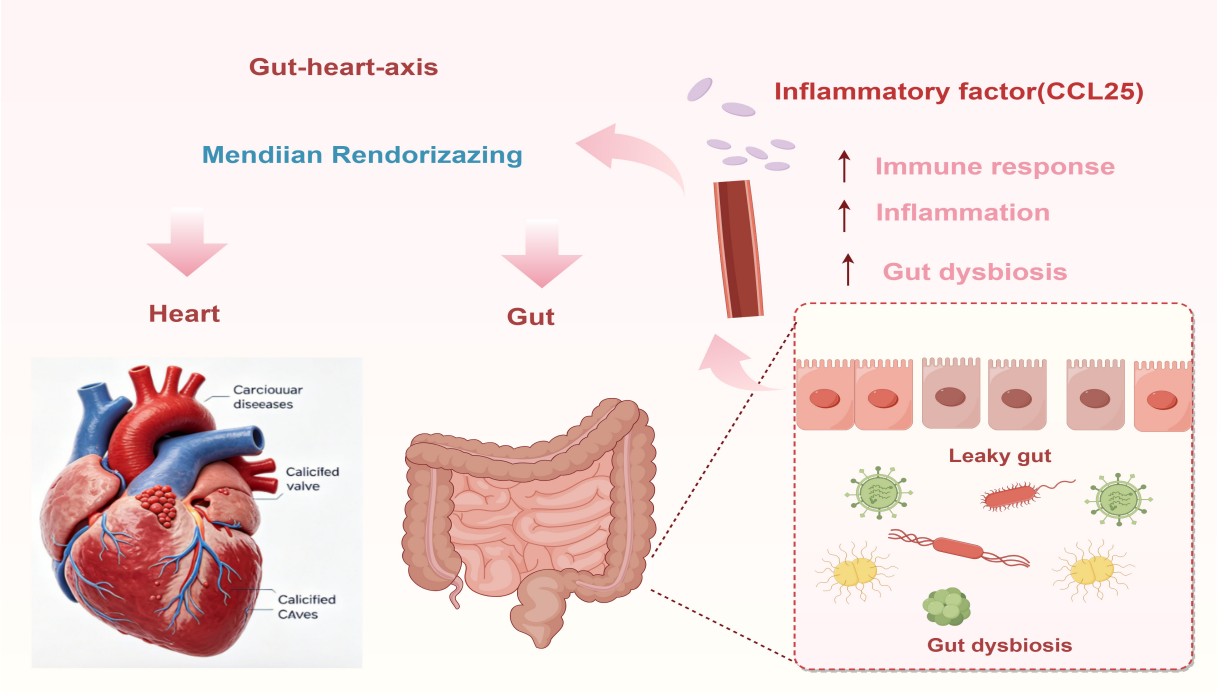
Correspondence

chuzefei@hebcm.edu.cn (Z. Chu)

Graphical Abstract



https://doi.org/10.71321/c18kt054

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Gut Microbiota Affects Cardiovascular Diseases via Inflammatory Cytokines: a Bidirectional Two-sample Mendelian Randomization Study and Mediation Analysis

Xuekun Hou [1] **,** Yunxiao Meng [1] , Yuxuan Zhang [1] , Xianghan Zheng [1] , Hongqi Guo [1] , Yichen Zhang [1] , Hongmei Yuan [1] , Zefei Chu [1*]

Received: 2026-03-11 | Accepted: 2026-04-10 | Published online: 2026-04-21

Abstract

Background: Cardiovascular diseases (CVDs) arise from complex interactions between genetic predisposition and environmental exposures, manifesting as persistent inflammatory disorders. Although accumulating evidence has implicated gut microbiota in the pathogenesis of CVDs, whether this association reflects a causal relationship remains to be firmly established. Therefore, the present study sought to examine the potential causal links between gut microbiota composition, circulating inflammatory cytokines, and susceptibility to CVDs.

Methods: Summary statistics for 196 gut microbiota taxa, 91 cytokines, and 8 CVD subtypes were extracted from the largest published genome-wide association studies (GWAS) to conduct bidirectional two-sample Mendelian randomization (MR) analysis. The inverse variance weighted (IVW) method was used as the primary statistical approach. We investigated the causal associations between gut microbiota and 8 CVD subtypes, including coronary artery disease (CAD), coronary atherosclerosis (CAS), heart failure (HF), stroke, atrial fibrillation (AF), angina pectoris (AP), calcific aortic valve stenosis (CAVS), and aortic aneurysm. Multivariable MR (MVMR) analysis was further performed to explore the potential mediating role of cytokines in the causal pathways from gut microbiota to CVDs.

Results: MR analysis identified causal associations between 58 gut microbiota taxa, 46 inflammatory cytokines, and CVDs. Specifically, 34 positive and 24 negative causal effects were observed between gut microbiota and CVDs, along with 16 positive and 30 negative causal effects between cytokines and CVDs. Additionally, mediation analysis identified C-C motif chemokine 25 (CCL25) level as a key mediator in the causal relationship from gut microbiota to CVDs.

Conclusions: Our findings support a causal link between gut microbiota, inflammatory cytokines, and CVDs, with CCL25 mediating the protective effect of specific gut taxa against CAVS. These identified biomarkers offer novel insights into the pathophysiological mechanisms of CVDs and may inform the development of novel strategies for CVD prevention, diagnosis, and treatment.

Keywords: Gut microbiota; Inflammatory Cytokines; Cardiovascular diseases; Mendelian randomization

Introduction

Cardiovascular diseases(CVDs) represent a significant global health burden and are a leading cause of mortality, prompting widespread public health concern [1]. Genomics and high-throughput sequencing analysis represent contemporary methodologies for investigating the role of gut microbiota in the development of CVDs [2]. The human intestine hosts a symbiotic community of 10 to 100 trillion microbial cells that coexist with the host [3]. The gut microbiota functions as a complex ecosystem, acting as virtual endocrine organs and playing a regulatory role in the occurrence and development of CVDs [4]. One such microbiota-dependent metabolite, trimethylamine N-oxide (TMAO), has been implicated in the pathologic processes of heart failure and may serve as an early warning marker for identifying individuals at risk of disease progression [5]. The relationship between gut microbiota and heart disease is elucidated through the gut-heart axis [6]. Dysbiosis of the gut microbiome has been associated with dysregulated immune activity, particularly the enhanced secretion of inflammatory cytokines implicated in the pathogenesis of cardiovascular diseases [7]. Despite the acknowledged role of inflammation in CVD progression, studies examining the interplay between gut microbiota and cytokine profiles among CVD patients remain scarce.

Changes in gut microbiota abundance are implicated in the onset of inflammation, a key factor in the pathophysiology of CVDs [8]. Epidemiological research has identified key interleukins as playing a role in the pathogenesis of CVDs [9]. It appears that both gut microbiota and cytokines can influence

1 Faculty of Integrated Traditional Chinese and Western Medicine, Hebei University of Chinese Medicine, Shijiazhuang, 050200, China

* Corresponding Author.

the progression of CVDs. It is hypothesized that cytokines may serve as mediators in the pathway linking gut microbiota to CVDs.

Randomized controlled trials (RCTs) have traditionally been utilized to establish causal relationships between gut microbiota or cytokines and CVDs. Nevertheless, ethical considerations and financial constraints restrict the widespread implementation of RCTs in clinical settings. Mendelian randomization (MR) analysis has emerged as a valuable tool for addressing issues of causality in epidemiological data by utilizing genetic variants as instrumental variables [10]. The advancement of Genome-wide Association Studies (GWASs) and increased accessibility to GWAS data have significantly enhanced the methodology of MR analysis in recent years. While numerous studies have identified protective and pathogenic relationships between gut microbiota and cardiovascular diseases (CVDs), the potential role of inflammatory cytokines as mediators in these relationships remains unexplored [11].

This study aimed to systematically evaluate the causal links between gut microbiota composition, inflammatory cytokines, and 8 CVD subtypes via bidirectional two-sample MR and MVMR analysis, with a secondary objective to explore the mediating role of inflammatory cytokines in the gut microbiota-CVD pathways.

Methods

Study design

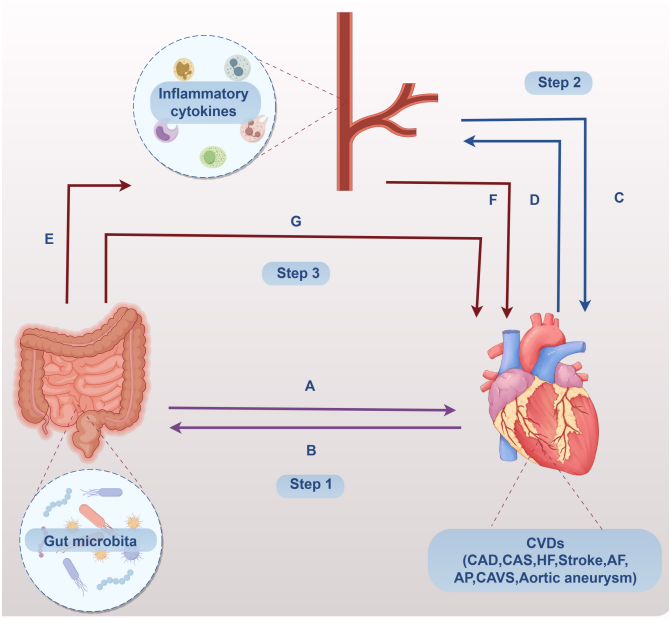
This study employed multiple single-nucleotide polymorphisms(SNPs) as instrumental variables under three core assumptions to evaluate the causal effects of gut microbiota and inflammatory cytokines on eight cardiovascular diseases: (i) the relevance assumption, which dictates that IVs must be highly associated with the exposure; (ii) the independence assumption, which stipulates that IVs are not influenced by confounding variables; and (iii) the exclusion restriction assumption, which posits that IVs impact the outcome solely through the exposure and not through alternative pathways [12]. In our study, we employed bidirectional two-sample MR analysis and MVMR analysis, following a structured approach illustrated in Figure 1. Specifically, Step 1 involved evaluating the bidirectional causal relationships between 196 gut microbiota and 8 cardiovascular diseases (CVDs); Step 2 entailed identifying bidirectional causality between 91 inflammatory cytokines and 8 CVDs; Step 3 focused on screening the 91 inflammatory cytokines as potential mediators in the pathways linking gut microbiota to CVDs. To estimate and quantify mediator effects, we utilized two-step MR and MVMR methodologies.

Of note, bidirectional MR analysis used exactly the same IV selection criteria, statistical methods (IVW as the primary approach), and significance threshold (P<0.05) as the forward causal analysis to ensure the comparability of forward and reverse results.

Data sources

This study utilized summary statistics obtained from publicly available data from Genome-Wide Association Studies (GWAS) conducted on populations of European ancestry. Detailed information regarding ethical approval and informed consent can be provided in the relevant publications.

Figure 1. Overview of the study design for the bidirectional two-sample Mendelian randomization (MR) and mediation analysis. The study included three core steps: Step 1, bidirectional two-sample MR analysis to assess the causal relationship between 196 gut microbiota taxa and 8 cardiovascular disease (CVD) subtypes; Step 2, bidirectional two-sample MR analysis to assess the causal relationship between 91 circulating inflammatory cytokines and 8 CVD subtypes; Step 3, two-step mediation MR and multivariate MR (MVMR) analysis to identify the mediating role of inflammatory cytokines in the causal pathway from gut microbiota to CVDs.



Genetic data for gut microbiota were obtained from previously published GWAS summary statistics curated by the MiBioGen consortium, which analyzed genome-wide genotypes and 16S fecal microbiome data from 18,340 individuals across 24 cohorts to assess host genetic influences on microbial composition [13]. The dataset encompassed 196 known bacterial taxa, spanning 9 phyla, 16 classes, 20 orders, 32 families, and 119 genera. Summary-level genetic data for inflammatory cytokines were derived from a separate study involving 8,293 participants, which profiled 91 cytokines and growth factors [14]. The data sources for CAD, CAS, HF, stroke, AF, AP, CAVS and aortic aneurysm used in this study are shown in Table 1.

Instrumental variables selection

Instrumental variable selection proceeded in two steps. First, SNPs significantly associated with gut microbiota were identified using a genome-wide significance threshold of $P < 1\times10^{-5}$. For inflammatory cytokines, a threshold of $P < 5\times10^{-6}$ was applied to retain a sufficient number of valid IVs, given the smaller sample size of the cytokine GWAS; this stricter threshold also mitigates the risk of weak instrument bias [15]. Second, to ensure SNP independence, linkage disequilibrium clumping was performed with an r^2 threshold of 0.001 within a 1000 kb window, using the 1000 Genomes Project European population as the reference panel [16]. To ensure that the effects of a SNPs on exposure and outcome were influenced by the same allele, we performed data harmonization; palindromic SNPs with ambiguous allele frequencies were excluded from the analysis to avoid bias from mismatched effect directions [17].

Third, the genetic instrument's effectiveness was assessed through the F-statistic, and SNPs with an F-statistic greater than 10 were selected to prevent bias from a weak instrument [18].Formula for calculating F statistic for each SNP as

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

In the formula, R^2 refers to the variance explained by a model's fixed effects part, N indicates sample size. R^2 can be calculated as [19]

$$R^2 = \frac{\beta^2}{(\beta^2 + se^2 \times N)}$$

In this formula, β means effect size for the genetic variant of interest; se means a standard error for β.

MR analysis

Bidirectional causality analysis

We conducted two-sample MR analysis to estimate the bidirectional causal effects between gut microbiota abundance, inflammatory cytokines and CVDs, respectively step 1 and step 2 in Figure 1. We used the inverse variance weighted (IVW) method as a main method for MR analysis because it allows for robust causal estimation despite heterogeneity [20]. A pleiotropy-robust MR analysis was conducted using four MR methods (weighted median, weighted mode, MR-Egger, and MR-PRESSO) to investigate whether pleiotropy could contribute to MR estimation bias [21]. Mendelian randomization estimates are presented as odds ratios with 95% confidence intervals. A causal association was considered significant when the inverse-variance weighted method yielded $P < 0.05$ and the direction of effect was consistent with that obtained from MR-Egger regression.

Mediation analysis

A two-step MR design was used to test the causal mediation effect of inflammatory cytokines (mediator) on the relationship between the gut microbiota (exposure) and CVDs (outcome), as step 3 shown in Figure 1. A two-step approach has fewer biases than a multivariable approach [22]. In the first step of two-step MR, we perform two-sample MR to estimate the causal effect between exposure and mediator (Figure 1E), mediator and outcome (Figure 1F), exposure and outcome (Figure 1G) separately. In the second step, we conducted a MVMR between exposure and mediator to outcome, in order to explore whether inflammatory cytokines are confounding factors affecting the causal relationship between gut microbiota and CVDs. We evaluated mediation effects using the product of coefficients method ($\beta_E \times \beta_F$) with 95% CIs estimated via the delta method, which directly assessed the significance of the mediation effect that gut microbiota on CVDs through inflammatory cytokines. The total effect represents $\beta_G + \beta_E\beta_F$ [23]. Mediation effect percentage is measured by the mediated proportion = $(\beta_E \times \beta_F)/(\beta_G)$ [24].

Sensitivity analysis

Sensitivity analysis was conducted to assess the robustness of the causal associations. In the results, we used the cochran's Q test [25] to assess heterogeneity between SNPs. Scatter plots were used to visualize the SNP-to-exposure and SNP-to-outcome effects, at the same time, leave-one-out analyses was performed to determine whether the association was driven by a single SNP [26]. To assess horizontal pleiotropy, we employed the MR-Egger regression intercept and the MR-PRESSO global test [27]; outlier SNPs identified via MR-PRESSO were removed to correct for horizontal pleiotropy when necessary. Sensitivity analyses were also performed for all mediation pathways to validate the robustness of the mediation effects, with no significant heterogeneity or horizontal pleiotropy detected for the significant pathways [28].
All analyses were conducted using the TwoSampleMR (version 0.5.8) package and MRPRESSO (version 1.0) packages in R software (Version 4.3.2).

Results

Instrumental variable selection

First, we screened 2559 SNPs (Supplementary Table 1) from 196 bacterial taxa as IVs with a significance threshold of $P < 1 \times 10^{-5}$. To obtain more comprehensive information, we performed LD-clumping ($r^2 < 0.001$, distance=10,000kb) [29], harmonizing and testing pleiotropy for all the IVs to reduce correlations between the SNPs. All retained IVs had an F-statistic >10, indicating no risk of weak instrument bias. Second, we

Table 1. Summary of the GWAS data used in the MR analysis.

Data Source	GWAS ID	Trait	Sample size	Year	Population
IEU Open GWAS	ebi-a-GCST005195	Coronary artery disease (CAD)	122,733	2017	European
	ebi-a-GCST009541	Heart failure (HF)	977,323	2020	European
	ebi-a-GCST90018793	Angina pectoris (AP)	470,931	2021	European
	ebi-a-GCST90018783	Aortic aneurysm	479,194	2021	European
FINNGEN	finn-b-I9_CORATHER	Coronary atherosclerosis (CAS)	211,203	2021	European
	finn-b-C_STROKE	Stroke	180,862	2021	European
	finn-b-I9_AF	Atrial fibrillation (AF)	138,994	2021	European
	finngen_R9_I9_CAVS_OPERATED	Calcific aortic valve stenosis (CAVS)	377,277	2019	European

identified 1203 SNPs associated with 91 inflammatory cytokines by the threshold value of P < 5 × 10−6(Supplementary Table 2).

Causal effects of gut microbiota and cytokines on the risk of 8 types of CVD

CAD

The results for the primary analyses show 77 SNPs belong to six gut microbial taxa (including 3 families, 2 genus, and 1 order) were associated with CAD (Supplementary Table 3, Figure 2). The detailed information of the 77 SNPs is shown in Supplementary Table 4.

As presented in Figure 2, the results of the MR analyses suggested that 4 gut microbial taxa were causally associated with the risk of CAD. The family XI (OR=1.055, 95% CI=1.004 - 1.109, *P*=0.034), family Lachnospiraceae (OR=1.097, 95% CI=1.019 - 1.181, *P*=0.014) , family Veillonellaceae (OR=1.065, 95% CI=1.011 - 1.122, *P*=0.019), genus Oxalobacter (OR=1.062, 95% CI=1.019 - 1.106, *P*=0.004) increased risk for developing CAD. At the same time, Genetic prediction of two gut microbial taxa (genus Parabacteroides, order Lactobacillales) was associated with a significantly lower risk of CAD. The genus Parabacteroides (OR=0.866, 95% CI=0.784 - 0.956, *P*=0.005), order Lactobacillales (OR=0.866, 95% CI=0.784 - 0.956, *P*=0.005), decreased the risk of CAD.

As shown in Figure 3, Supplementary Table 4, the MR analysis results indicated that 9 cytokines may increase the incidence of CAD. The T-cell surface glycoprotein CD5 levels (OR=1.161, 95% CI=1.032 − 1.308, *P*=0.013), Hepatocyte growth factor levels (OR=1.121, 95% CI=1.007 − 1.247, *P*=0.036) significantly increased the risk of CAD. The Interleukin-5 levels (OR=0.889, 95% CI=0.813 − 0.973, *P*=0.011), Interleukin-8 levels (OR=0.942, 95% CI=0.888-1.000, *P*=0.049), Neurturin levels (OR=0.907, 95% CI 0.835–0.985, *P*=0.021) decreased the risk of CAD.

CAS

A total of 7 gut microbiota (including 1 class, 1 family, 1 genus, 2 orders and 2 phyla) were causally related to CAS (Supplementary Table 3, Figure 2). Details of related 76 SNPs are shown in Supplementary Table 4.

Figure 2 shows the genus Turicibacter (OR=1.906, 95% CI=1.014 − 1.184, *P*=0. 022)suggested to increase the risk of CAS by the IVW method. Six gut microbial taxa (class Gammaproteobacteria, family Bifidobacteriaceae, order Bifidobacteriales, order Burkholderiales, phylum Actinobacteria, phylum Firmicutes) has an decreased risk of CAS. The class Gammaproteobacteria (OR=0.829, 95% CI=0.727 − 0.945, *P*=0. 005) and phylum Firmicutes (OR=0.876, 95% CI=0.786 − 0.976, *P*=0. 017) significantly decreased the risk of CAS.

Figure 3 shows that C-C motif chemokine 4 levels, Fibroblast growth factor 5 levels and Interleukin-22 receptor subunit alpha-1 levels seemed to be risk factors to CAS. The Interleukin-22 receptor subunit alpha-1 levels significantly increased the incidence of CAS. The Leukemia inhibitory factor receptor levels was a protective factor for CAS.

HF

A total of 9 gut microbiota (including 2 class, 4 genus and 3 orders 2 phylums) were causally associated with HF (Supplementary Table 3, Figure 2). Details of related 91 SNPs are shown in Supplementary Table 4.

As Figure 2 shows that five gut microbial taxa (class Negativicutes, genus Eubacterium eligens group, genus Eubacterium oxidoreducens group, genus Flavonifractor, order Selenomonadales) seemed to be risk factors to HF. The genus Eubacterium eligens group (OR=1.126, 95% CI=1.017 − 1.247, *P*=0. 023) and genus Flavonifractor(OR=1.144, 95% CI=1.031 − 1.270, *P*=0. 012) considerably elevated risk for HF. Four gut microbial taxa (class Bacteroidia, genus Anaerostipes, order Bacillales and order Bacteroidales) seemed to be protective factors to HF. The genus Anaerostipes (OR=0.899, 95% CI=0.825 − 0.978, *P*=0. 014) significantly reduced the risk for HF.

In Figure 3, four cytokines (T-cell surface glycoprotein CD5 levels, Macrophage colony-stimulating factor 1 levels, Interleukin-2 levels, Tumor necrosis factor levels) were associated with a increased risk of HF. The T-cell surface glycoprotein CD5 levels (OR=1.100, 95% CI=1.016 − 1.191, *P*=0. 019) and Tumor necrosis factor levels (OR=1.110, 95% CI=1.029 − 1.199, *P*=0. 007) significantly increased the risk of HF. The Interleukin-17C levels (OR=0.907, 95% CI=0.832 − 0.989, *P*=0. 027), Leukemia inhibitory factor receptor levels (OR=0.913, 95% CI=0.849 − 0.981, *P*=0. 014) and Sulfotransferase 1A1 levels (OR=0.950, 95% CI=0.905 − 0.998, *P*=0. 041) decreased the risk of HF.

Stroke

A total of 10 gut microbiota (including 4 family, 5 genus, 1 order) had a causal relationship with stroke (Supplementary Table 3, Figure 2). Details of related 97 SNPs are shown in Supplementary Table 4.

As shown in Figure 2, the family Bacteroidales S24-7 group (OR=1.109, 95% CI=1.025 − 1.200, *P*=0. 010), genus Holdemanella (OR=1.080, 95% CI=1.014 − 1.151, *P*=0. 017) and genus Marvinbryantia (OR=1.111, 95% CI=1.002 − 1.231, *P*=0. 046) suggested to be risk factors for stroke. Seven gut microbial taxa (family Alcaligenaceae, family Defluviitaleaceae, family Desulfovibrionaceae, genus Eggerthella, genus Faecalibacterium, genus Ruminococcus torques group) may be protective factors for stroke. The family Desulfovibrionaceae (OR=0.875, 95% CI=0.783 − 0.979, *P*=0. 020) and genus Ruminococcus torques group (OR=0.769, 95% CI=0.631 − 0.936, *P*=0. 009) were significantly lower risk for stroke.

Figure 3 shows that C-X-C motif chemokine 11 levels (OR=1.096, 95% CI=1.012 − 1.187, *P*=0. 025), FGF5 (OR=1.053, 95% CI=1.022 − 1.085, *P*=0. 001), Interleukin-2 receptor subunit beta levels (OR=1.123, 95% CI=1.026 − 1.230, *P*=0. 012) seemed to be risk factors to stroke. The Cystatin D levels (OR=0.963, 95% CI=0.935 − 0.992, *P*=0. 014) and Stem cell factor levels (OR=0.954, 95% CI=0.910 − 1.000, *P*=0. 048) were protective factors to stroke.

AF

The results suggest a causal relationship between 9 gut microbiota (including 1 family, 5 genus, 1 order and 2 phylum) and AF (Supplementary Table 3, Figure 2). Details of related 106 SNPs are shown in Supplementary Table 4.

As shown in Figure 2, the family Pasteurellaceae (OR=1.107 , 95% CI=1.021 - 1.199, *P*=0. 013), genus Ruminiclostridium5(OR=1.142, 95% CI=1.017 - 1.283, *P*=0. 025) and order Pasteurellales(OR=1.107 , 95% CI=1.021 - 1.199, *P*=0. 013) suggest to be risk factors to AF. Six gut microbial taxa (genus Bifidobacterium, genus Eggerthella, genus Howardella, genus

Figure 2. Mendelian randomization results of the causal effects between gut microbiota taxa and 8 CVD subtypes, estimated using the inverse variance weighted (IVW) method. Results are presented as odds ratios (ORs) with 95% confidence intervals (CIs). Red squares indicate a significant positive causal association (increased CVD risk, $P < 0.05$), and blue squares indicate a significant negative causal association (decreased CVD risk, $P < 0.05$).

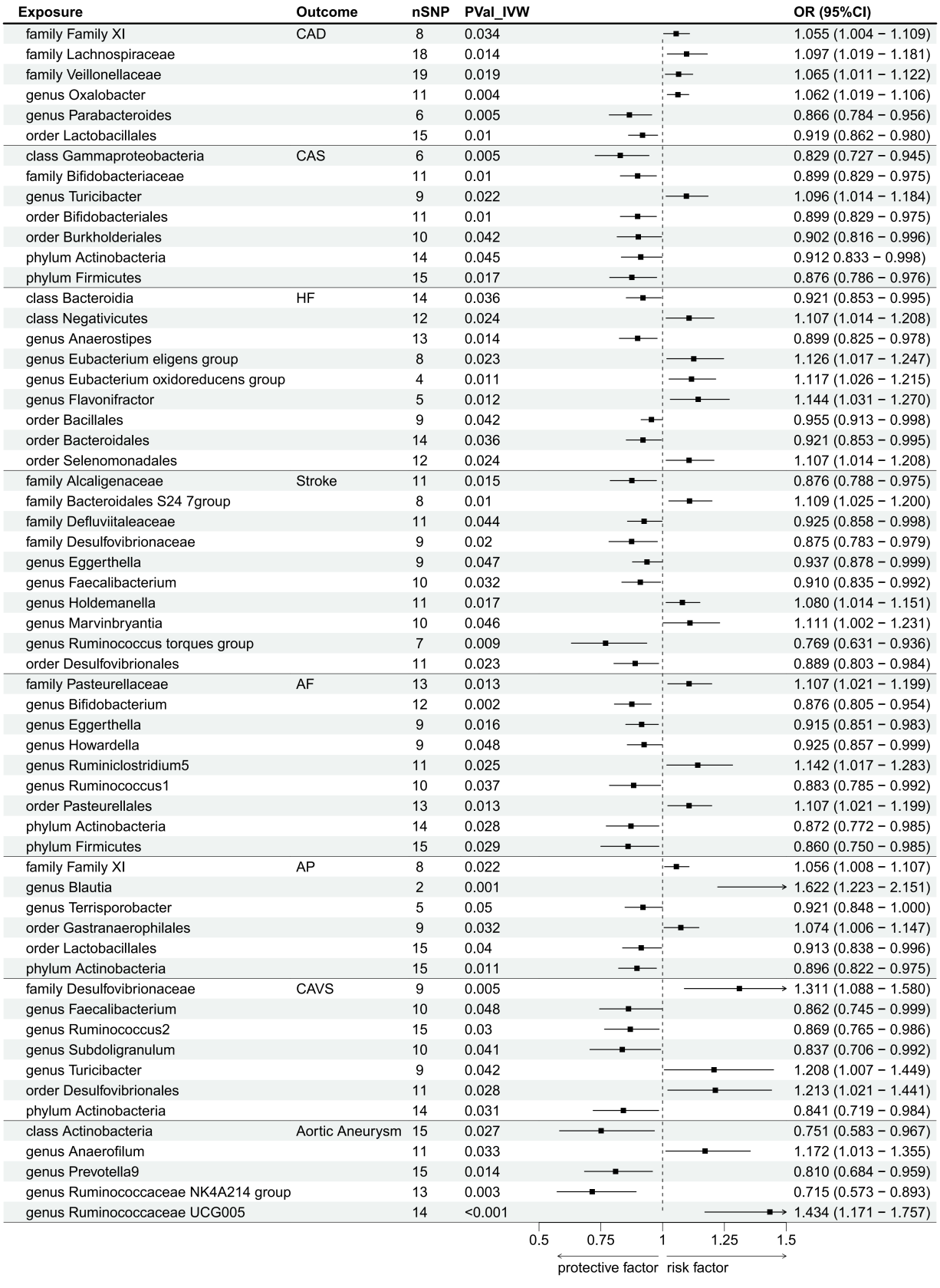


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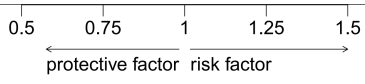
Figure 3. Mendelian randomization results of the causal effects between circulating inflammatory cytokines and 8 CVD subtypes, estimated using the inverse variance weighted (IVW) method. Results are presented as odds ratios (ORs) with 95% confidence intervals (CIs). Red squares indicate a significant positive causal association (increased CVD risk, $P < 0.05$), and blue squares indicate a significant negative causal association (decreased CVD risk, $P < 0.05$).

Exposure	Outcome	nSNP	PVal_IVW	OR (95%CI)
C−C motif chemokine 4 levels	CAD	13	0.01	1.051 (1.012 − 1.092)
T−cell surface glycoprotein CD5 levels		15	0.013	1.161 (1.032 − 1.308)
Macrophage colony−stimulating factor 1 levels		11	0.039	1.072 (1.003 − 1.145)
C−X−C motif chemokine 10 levels		20	0.05	1.093 (1.000 − 1.194)
Fibroblast growth factor 23 levels		8	0.029	1.112 (1.011 − 1.222)
Fibroblast growth factor 5 levels		18	0.014	1.085 (1.016 − 1.158)
Fms−related tyrosine kinase 3 ligand levels		21	0.001	1.066 (1.025 − 1.108)
Hepatocyte growth factor levels		11	0.036	1.121 (1.007 − 1.247)
Interleukin−17A levels		10	0.026	1.088 (1.010 − 1.172)
Interleukin−5 levels		8	0.011	0.889 (0.813 − 0.973)
Interleukin−8 levels		14	0.049	0.942 (0.888 − 1.000)
Neurturin levels		7	0.021	0.907 (0.835 − 0.985)
C−C motif chemokine 4 levels	CAS	18	0.043	1.031 (1.001 − 1.063)
Fibroblast growth factor 5 levels		23	0.026	1.056 (1.007 − 1.107)
Interleukin−22 receptor subunit alpha−1 levels		11	0.001	1.158 (1.060 − 1.265)
Leukemia inhibitory factor receptor levels		15	0.036	0.918 (0.847 − 0.994)
T−cell surface glycoprotein CD5 levels	HF	19	0.019	1.100 (1.016 − 1.191)
Macrophage colony−stimulating factor 1 levels		13	0.019	1.077 (1.012 − 1.147)
Interleukin−17C levels		13	0.027	0.907 (0.832 − 0.989)
Interleukin−2 levels		11	0.047	1.080 (1.001 − 1.165)
Leukemia inhibitory factor receptor levels		15	0.014	0.913 (0.849 − 0.981)
Sulfotransferase 1A1 levels		16	0.041	0.950 (0.905 − 0.998)
Tumor necrosis factor levels		11	0.007	1.110 (1.029 − 1.199)
Cystatin D levels	Stroke	30	0.014	0.963 (0.935 − 0.992)
C−X−C motif chemokine 11 levels		22	0.025	1.096 (1.012 − 1.187)
Fibroblast growth factor 5 levels		23	0.001	1.053 (1.022 − 1.085)
Interleukin−2 receptor subunit beta levels		11	0.012	1.123 (1.026 − 1.230)
Stem cell factor levels		32	0.048	0.954 (0.910 − 1.000)
Eotaxin levels	AF	19	0.03	1.069 (1.006 − 1.135)
C−C motif chemokine 25 levels		24	0.045	0.960 (0.923 − 0.999)
CD40L receptor levels		15	0.001	0.933 (0.896 − 0.971)
C−X−C motif chemokine 1 levels		8	0.034	1.072 (1.005 − 1.143)
C−X−C motif chemokine 11 levels		22	0.007	1.122 (1.032 − 1.220)
Fibroblast growth factor 5 levels		23	<0.001	1.094 (1.055 − 1.135)
Interleukin−2 receptor subunit beta levels		11	0.045	1.131 (1.003 − 1.275)
Leukemia inhibitory factor receptor levels		15	<0.001	0.871 (0.812 − 0.935)
Matrix metalloproteinase−10 levels		14	0.037	0.929 (0.866 − 0.995)
TNF−beta levels		22	0.003	1.053 (1.018 − 1.090)
T−cell surface glycoprotein CD5 levels	AP	22	0.031	1.099 (1.009 − 1.198)
Macrophage colony−stimulating factor 1 levels		17	0.028	1.069 (1.007 − 1.135)
Fibroblast growth factor 5 levels		24	0.005	1.085 (1.025 − 1.148)
Interleukin−10 receptor subunit beta levels		18	0.041	1.037 (1.002 − 1.074)
Interleukin−17A levels		14	0.027	1.108 (1.012 − 1.214)
Interleukin−1−alpha levels		14	0.04	0.943 (0.891 − 0.997)
Interleukin−6 levels		8	0.012	0.856 (0.758 − 0.967)
Leukemia inhibitory factor receptor levels		18	0.023	0.931 (0.875 − 0.990)
C−C motif chemokine 19 levels	CAVS	20	0.007	1.111 (1.029 − 1.200)
C−C motif chemokine 25 levels		24	0.007	0.941 (0.901 − 0.983)
Hepatocyte growth factor levels		18	0.006	1.202 (1.054 − 1.372)
Interleukin−10 levels		18	0.048	0.877 (0.770 − 0.999)
Interleukin−8 levels		17	0.048	1.131 (1.001 − 1.277)
TNF−beta levels		22	0.005	1.084 (1.025 − 1.146)
C−C motif chemokine 25 levels	Aortic Aneurysm	23	0.001	0.894 (0.836 − 0.956)
Fractalkine levels		23	0.023	1.228 (1.029 − 1.465)
C−X−C motif chemokine 6 levels		15	0.038	1.230 (1.011 − 1.497)
Fibroblast growth factor 5 levels		24	0.002	1.156 (1.056 − 1.265)
Interleukin−20 receptor subunit alpha levels		12	0.035	1.251 (1.015 − 1.541)
Stem cell factor levels		33	0.015	0.870 (0.778 − 0.973)



Ruminococcus 1, phylum Actinobacteria, phylum Firmicutes) may be protective factors for AF. The phylum Firmicutes (OR=0.860, 95% CI=0.750 - 0.985, *P*=0. 029) significantly decreased the incidence of AF.
Figure 3 shows that six inflammatory cytokines (Eotaxin levels, C-X-C motif chemokine 1 levels, C-X-C motif chemokine 11 levels, FGF5 levels, Interleukin-2 receptor subunit beta levels, TNF-beta levels) seemed to be risk factors for AF. The Interleukin-2 receptor subunit beta levels (OR=1.131, 95% CI=1.003 - 1.275, *P*=0. 045) significantly increased the risk of AF. Four inflammatory cytokines (C-C motif chemokine 25 levels, CD40L receptor levels, Leukemia inhibitory factor receptor levels, Matrix metalloproteinase-10 levels) suggested to be protective factors to AF. Among them, the Leukemia inhibitory factor receptor levels (OR=0.871, 95% CI=0.812 - 0.935, *P*<0.001) significantly decreased the risk of AF.

AP

The IVW method revealed 52 causal associations of gut microbiota on AP, including 1 family,1 genus,2 orders and 1 phylum (Figure 2, Supplementary Table 3). Details of related 52 SNPs are shown in Supplementary Table 4. The risk of AP may be increased by three gut microbiota traits, family XI (OR=1.056 , 95% CI=1.008 - 1.107, *P*=0.022), genus Blautia (OR=1.622 , 95% CI=1.223 - 2.151, *P*=0.010), order Gastranaerophilales (OR=1.074 , 95% CI=1.006 - 1.147, *P*=0.032).The genus Terrisporobacter (OR=0.921 , 95% CI=0.848 - 1.000, *P*=0.050), order Lactobacillales (OR=0.913, 95% CI=0.838 - 0.996, *P*=0.040), phylum Actinobacteria (OR=0.896 , 95% CI=0.822 - 0.975, *P*=0.011) may reduce the risk of AP.
Described in Figure 3, five inflammatory cytokines (T-cell surface glycoprotein CD5 levels, Macrophage colony-stimulating factor 1 levels, FGF5 levels, Interleukin-10 receptor subunit beta levels, Interleukin-17A levels) may increase the risk of AP (Supplementary Table 3). Among them, the Interleukin-17A levels (OR=1.108, 95% CI=1.012 - 1.214, *P*=0.027) significantly increased the risk of AP. At the same time, the Interleukin-1-alpha levels (OR=0.943, 95% CI=0.891 - 0.997, *P*=0.040), Interleukin-6 levels (OR=0.856, 95% CI=0.758 - 0.967, *P*=0.012), Leukemia inhibitory factor receptor levels (OR=0.931, 95% CI=0.875 - 0.990, *P*=0.023) may decreased the risk of AP.

CAVS

Based on the IVW method, the results suggested 78 causal relationships between gut microbiota and CAVS, including 1 family, 4 genus, 1 order and 1 phylum (Figure 2, Supplementary Table 3). Details of related 78 SNPs are shown in Supplementary Table 4. The family Desulfovibrionaceae (OR=1.311, 95% CI=1.088 - 1.580, *P*=0.005), genus Turicibacter (OR=1.208, 95% CI=1.007 - 1.449, *P*=0.042), order Desulfovibrionales (OR=1.213, 95% CI=1.021 - 1.441, *P*=0.028) seemed to be risk factors to CAVS. There were four gut microbiota (genus Faecalibacterium, genus Ruminococcus 2, genus Subdoligranulum, phylum Actinobacteria) was associated with an decreased risk of CAVS. The phylum Actinobacteria (OR=0.841, 95% CI=0.719 - 0.984, *P*=0.031) significantly decreased risk of CAVS.
As shown in Figure 3, four inflammatory cytokines (C-C motif chemokine 19 levels, Hepatocyte growth factor levels, Interleukin-8 levels, TNF-beta levels) seemed to increase the risk of CAVS. The Hepatocyte growth factor levels (OR=1.202, 95% CI=1.054 - 1.372, *P*=0.006) significantly increased risk of CAVS. The C-C motif chemokine 25 levels (OR=0.941 , 95% CI=0.901 - 0.983, *P*=0.007) and Interleukin-10 levels(OR=0.877 , 95% CI=0.770 - 0.999, *P*=0.048) decreased the risk of CAVS.

Aortic aneurysm

Genetic prediction of five gut microbiota (including 1 class and 4 genus) was associated with a causal effect of Aortic aneurysm (Figure 2, Supplementary Table 3). Details of related 68 SNPs are shown in Supplementary Table 4. The genus Anaerofilum(OR=1.172, 95% CI=1.013 - 1.355, *P*=0.033) and genus Ruminococcaceae UCG005(OR=1.434, 95% CI=1.171 - 1.757, *P*<0.001) may increase the risk of Aortic aneurysm. The class Actinobacteria (OR=0.751, 95% CI=0.583 - 0.967, *P*=0.027), genus Prevotella 9 (OR=0.810, 95% CI=0.684 - 0.959, *P*=0.014), genus Ruminococcaceae NK4A214 group (OR=0.715, 95% CI=0.573 - 0.893, *P*=0.003) may be protective factors to Aortic aneurysm.
A total of six inflammatory cytokines were associated with Aortic aneurysm (Figure 3, Supplementary Table 4). Four inflammatory cytokines (Fractalkine levels, C-X-C motif chemokine 6 levels, FGF5 levels, Interleukin-20 receptor subunit alpha levels) were risk factors for aortic aneurysm, the Interleukin-20 receptor subunit alpha levels (OR=1.251, 95% CI=1.015 - 1.541, *P*=0.035) significantly increased risk of aortic aneurysm. The C-C motif chemokine 25 levels (OR=0.894, 95% CI=0.836 - 0.956, *P*=0.001) and Stem cell factor levels (OR=0.870, 95% CI=0.778 - 0.973, *P*=0.015) had a protective causal effect on Aortic aneurysm.

Bi-directional causal effects of CVDs on gut microbiota and cytokines

CAD

When assessing the bi-directional causal effect of CAD on gut microbiota and cytokines, we found evidence suggesting a genetic predisposition to CAD was linked to specific gut microbiota, with lower abundance in one family, five genus and one order, and higher abundance in one family, four genus and 1 order (Supplementary Table 7). The most significant result in lower abundance was the genus Actinomyces (OR=0.926, 95% CI=0.872 - 0.983, *P*=0.011), and in higher abundance was the genus Gordonibacter (OR=1.098, 95% CI=1.010 - 1.193, *P*=0.027). CAD was associated with a lower abundance in 8 cytokines (Supplementary Table 8), the most significant result was the TNF-beta levels (OR=0.943, 95% CI=0.894 - 0.996, *P*=0.034).

CAS

CAS has a causal relationship on nine gut microbiota(Supplementary Table 7), with lower abundance in two genus(-genus Dialister (OR=0.956, 95% CI=0.915 - 1.000, *P*=0.050), genus Family XIII UCG001 (OR=0.927, 95% CI=0.887 - 0.968, *P*=0.001)),and with higher abundance in one class ,one family, four genus and one order, the most significant result was the genus Veillonella (OR=1.083, 95% CI=1.030 - 1.140, P=0.002). CAS was associated with a lower abundance in 4 cytokines (Supplementary Table 8), the most significant result was the T-cell surface glycoprotein CD5 levels (OR=0.953, 95% CI=0.910 - 0.999, P=0.046).

HF

HF was associated with lower abundance in one class, two

family, three genus and one order (Supplementary Table 7), the most significant result was the genus Victivallis (OR=0.787, 95% CI=0.635 - 0.974, *P*=0.028). HF has a causal relationship with higher abundance in one family, six genus and one order, the most significant result was the genus Lachnospiraceae UCG001 (OR=1.157, 95% CI=1.027 - 1.303, *P*=0.016). HF has a causal association with Interleukin-24 levels(OR=1.094, 95% CI=1.005 - 1.191, *P*=0.038).

Stroke

Stroke has a causal relationship with lower abundance in two genus (genus Marvinbryantia (OR=0.902, 95% CI=0.816 - 0.998, *P*=0.045), genus Prevotella 9 (OR=0.888, 95% CI=0.805 - 0.979, *P*=0.017)), and with higher abundance in two class (Supplementary Table 7), three genus, the most significant result was the genus Lactococcus (OR=1.230, 95% CI=1.050 - 1.440, *P*=0.01). As shown in Supplementary Table 8, there was no reverse effect between stroke and cytokines.

AF

AF was associated with lower abundance in 1 family and 7 genus (Supplementary Table 7), the most significant result was the genus Prevotella7 (OR=0.943, 95% CI=0.890-0.998, *P*=0.044), and with high abundance in genus Butyricimonas (OR=1.035, 95% CI=1.001-1.069, *P*=0.041) and genus Intestinibacter (OR=1.134, 95% CI=1.003-1.066, *P*=0.034). As shown in Supplementary Table 8, there was no reverse effect between AF and cytokines.

AP

AP has a causal relationship with lower abundance in 7 gut microbiota(including 1 class,1 family,4 genus and 1 order), the most significant result was the genus Allisonella (OR=0.891, 95% CI=0.803-0.989, *P*=0.030). AP was associated with higher abundance in 1 class, 1 family, 3 genus and 1 order, the most significant result was the genus Veillonella (OR=1.097, 95% CI=1.038-1.159, *P*=0.001). AP was associated with a lower abundance in 8 cytokines (Supplementary Table 8), the most significant result was the T-cell surface glycoprotein CD5 levels (OR=0.913, 95% CI=0.879-0.948, *P*=0.001).

CAVS

CAVS was associated with lower abundance in 7 gut microbiota(including 1 class, 1 family, 3 genus, 1 order and 1 phylum, Supplementary Table 7), the most significant result was the genus Ruminococcus gnavus group (OR=0.947, 95% CI=0.896-1, *P*=0.005), and with high abundance in family XI (OR=1.140, 95% CI=1.027-1.264, *P*=0.014). As shown in Supplementary Table 8, there was no reverse effect between CAVS and cytokines.

Aortic aneurysm

Aortic aneurysm was associated with lower abundance in 13 gut microbiota(including 2 class, 4 family, 4 genus, 2 order and 1 phylum, Supplementary Table 7), the most significant result was the genus Eubacterium nodatum group (OR=0.906, 95% CI=0.827-0.993, *P*=0.035), and with high abundance in the genus Catenibacterium (OR=1.119, 95% CI=1.004-1.246, *P*=0.041). Aortic aneurysm was associated with a lower abundance in 4 cytokines (Supplementary Table 8), the most significant result was the Leukemia inhibitory factor levels (OR=0.958, 95% CI=0.932-0.986, *P*=0.003), and with high abundance in Interleukin-18 levels (OR=1.031, 95% CI=1.004-1.058, *P*=0.024).

Sensitivity analyses

For the robustness of the estimates, we further conducted a sensitivity and pleiotropy analysis to avoid horizontal pleiotropy, with all intercept *P* values >0.05 and global test *P* values >0.05, indicating no significant horizontal pleiotropy for all identified causal associations. Cochran's Q tests demonstrated no significant heterogeneity (*P* > 0.05, Supplementary Table 9).

Mediation analysis of gut microbiota, inflammatory cytokines, and CVDs

To explore the underlying potential mechanisms involving in the occurrence and development of CVDs, we performed mediation analysis to identify the causal pathway from gut microbiota to CVDs mediated by cytokines. Initially, the results of two-sample MR indicate that gut microbiota has causal effects on cytokines. We identified 22 associations of gut microbiota to cytokines (CAD,3; CAS,3; HF,4; Stroke,1; AF,5; AP,1; CAVS,3; Aortic Aneurysm,2) (Supplementary Table 10). Secondly, MVMR was applied to explore the significant causal association between cytokines with CVDs after correcting for the gut microbiota. The reliability of results was verified by sensitivity analysis, indicating that no heterogeneity and horizontal pleiotropy existed (Supplementary Table 9).

Notably, the majority of tested pathways showed only a non-significant trend of mediation effect without statistical support, and thus were not considered as valid mediating pathways. In summary, we identified 2 mediating relationships, including 2 gut microbiota–CAVS causal pathways mediated by 1 cytokine (Table 2). The mediation analysis reveals that C-C motif chemokine 25 levels exhibit significant negative mediation effects to CAVS, respectively on genus Subdoligranulum (beta = -0.017, 95% CI [-0.035, -0.002], *P*=0.034) with 9.55% proportion, and phylum Actinobacteria (beta = -0.018, 95% CI [-0.035, -0.005], *P*=0.024) with 10.41% proportion.

Discussion

Emerging evidence has established strong associations between gut microbiota-derived metabolites [30]—particularly trimethylamine-N-oxide and short-chain fatty acids—and the pathogenesis of cardiovascular diseases [31], including ischemic heart disease [32], heart failure, and hypertension [33]. Beyond metabolic pathways, the gut microbiota also exerts profound effects on host immune responses, modulating key cellular processes such as immunoregulation, apoptosis, and inflammatory signaling [34-36]. Chronic inflammation and autoimmune mechanisms have likewise been implicated in cardiovascular pathology [37-38]. Of particular interest, recent studies have identified autoantibodies directed against C-X-C motif chemokine receptor 3, a G-protein-coupled receptor known to participate in atherosclerotic progression [39]. Nevertheless, the intricate nature of cardiovascular diseases and the diverse composition of gut microbiota present challenges in comprehensively elucidating the impact of gut microbiota on cardiovascular conditions through observational investiga-

tions. Furthermore, the potential mediating role of inflammatory factors in signaling pathways remains uncertain.

This study utilized MR analysis to assess the causal relationship between gut microbiota, inflammatory cytokines and CVDs. The investigation examined potential causal associations between 196 gut microbiota abundance variables and 8 types of CVDs (CAD, HF, AP, Aortic aneurysm, CAS, AF, CAVS), identifying a potential causal relationship involving 58 gut microbiota, 46 inflammatory cytokines, and 8 types of CVDs. Furthermore, mediation analysis results provided support for the involvement of altered inflammatory cytokines in the pathogenesis of CVDs.

Our research findings indicate a potential causal relationship between specific gut microbiota and various cardiovascular diseases, with certain gut microbiota exhibiting a consistent causal role across different types of cardiovascular diseases. Specifically, our results suggest that a high abundance of the phylum Actinobacteria may serve as a protective factor against four cardiovascular diseases (coronary artery stenosis, atrial fibrillation, aortic aneurysm, and calcific aortic valve stenosis). A clinical trial conducted in northwestern China further supported this notion, demonstrating elevated levels of Actinobacteria in the fecal samples of healthy individuals compared to those with hypertension [40]. Collinsella, a dominant taxon of the family Coriobacteriaceae within the phylum Actinobacteria [41], has been found to have elevated abundance in patients with type 2 diabetes and symptomatic carotid atherosclerotic stenosis [42-43].

Additionally, our research indicates that FGF5 levels are a key risk factor for stroke, atrial fibrillation, angina pectoris, and aortic aneurysm. Previous studies from the 1990s have demonstrated the active role of fibroblast growth factors (FGFs) in regulating endothelial cells (EC) and smooth muscle cells (SMC) [44]. Moreover, numerous studies have demonstrated the significance of inflammatory responses in the vascular system as key contributors to atherosclerosis, with basic Fibroblast Growth Factor (bFGF) playing a role in vascular inflammation [45]. FGF5, a member of the FGF family, has been linked to vascular remodeling and hypertension in previous clinical studies, which aligns with our finding that FGF5 increases the risk of multiple CVD subtypes. Furthermore, our findings suggest that elevated levels of Macrophage colony-stimulating factor 1 may be associated with an increased risk of coronary artery disease, heart failure, and angina pectoris. Conversely, decreased levels of C-C motif chemokine 25 may be linked to a reduced risk of atrial fibrillation, calcific aortic valve stenosis, and aortic aneurysm. Prior research has demonstrated that a deficiency in M-CSF can significantly decrease the progression of atherosclerosis in mice with hyperlipidemia. Additionally, a study has demonstrated that macrophage colony-stimulating factor plays a crucial role in neointimal thickening formation following arterial injury [46]. Chemokines, in turn, contribute to disease pathogenesis by directing leukocyte migration to inflammatory sites [47-48]. Studies utilizing serum proteomic profiling in heart failure cohorts have demonstrated an age-related increase in circulating C-C motif chemokine ligand 17 (CCL17) levels, which correlate with cardiac insufficiency [49]. Nevertheless, the impact of C-C motif chemokine 25 levels on the development of cardiovascular diseases remains poorly understood.

Furthermore, our study investigated the reverse causal effects of 8 CVD subtypes on gut microbiota and cytokine levels

Table 2. Mediation analysis of the causal pathways from gut microbiota to cardiovascular diseases via inflammatory cytokines.

Exposure	Mediator	Outcome	Total effect	Direct effect	Mediation effect (95% CI)	P value	Mediation Proportion
family Veillonellaceae	Macrophage colony-stimulating factor 1 levels	CAD	0.063	0.057	0.006 (0, 0.016)	0.134	9.55%
genus Parabacteroides	C-X-C motif chemokine 10 levels		-0.144	-0.123	-0.021 (-0.058, 0.002)	0.189	14.56%
order Lactobacillales	Interleukin-5 levels		-0.084	-0.076	-0.008 (-0.024, 0.004)	0.253	9.51%
phylum Actinobacteria	Leukemia inhibitory factor receptor levels	CAS	-0.092	-0.079	-0.013 (-0.033, 0)	0.104	14.06%
genus Bifidobacterium	C-C motif chemokine 25 levels	AF	-0.132	-0.125	-0.007 (-0.017, 0)	0.162	5.29%
phylum Actinobacteria	C-C motif chemokine 25 levels		-0.699	-0.690	-0.009 (-0.023, 0)	0.134	1.29%
phylum Actinobacteria	Leukemia inhibitory factor receptor levels		-0.699	-0.681	-0.018 (-0.043, 0)	0.102	2.57%
phylum Actinobacteria	Leukemia inhibitory factor receptor levels	AP	-0.110	-0.099	-0.011 (-0.028, 0)	0.116	9.98%
genus Subdoligranulum	C-C motif chemokine 25 levels	CAVS	-0.178	-0.161	**-0.017 (-0.035, -0.002)**	**0.034**	9.55%
phylum Actinobacteria	C-C motif chemokine 25 levels		-0.173	-0.155	**-0.018 (-0.035, -0.005)**	**0.024**	10.41%
genus Ruminococcaceae NK4A214 group	Fractalkine levels	Aortic Aneurysm	-0.390	-0.361	-0.029 (-0.071, -0.001)	0.107	7.44%

through bidirectional MR analysis. The findings indicated that 4 CVD subtypes exhibited bidirectional causal effects on the abundance of class Gammaproteobacteria, family Pasteurellaceae, and order Pasteurellales, while 3 CVD subtypes had bidirectional causal effects on levels of Interleukin-12 receptor subunit beta and T-cell surface glycoprotein CD5. These reverse causal findings suggest that the occurrence and progression of CVDs may further exacerbate gut microbiota dysbiosis and systemic inflammatory dysregulation, forming a vicious cycle of "microbiota dysregulation - inflammatory activation - CVD progression", which provides new insights for the long-term management of CVD patients. Additionally, we conducted further analysis to identify potential mediators of inflammatory cytokines in the pathway linking gut microbiota to cardiovascular diseases. Our research revealed that C-C motif chemokine 25 levels act as a mediator in the pathways linking the genera Subdoligranulum and phylum Actinobacteria to CAVS, with mediation effects of 9.55% and 10.41% respectively.

This study represents the initial exploration of the causal connections between gut microbiota, inflammatory cytokines, and eight CVDs utilizing a thorough and integrated MR Framework. Moreover, we established a pathway from gut microbiota to CVDs through the mediation analysis of inflammatory factors. In order to assess the validity of our findings in light of potential violations of Mendelian randomization assumptions, we conducted various sensitivity analyses using multiple Mendelian randomization approaches. Nonetheless, this study is subject to certain limitations. Firstly, the sample size for cardiovascular disease subtypes was inadequate. Secondly, the study only included populations of European descent due to limited availability of genome-wide association study data from other populations. Lastly, additional experiments and in-depth analysis of clinical patient data will be necessary to confirm the results.

Despite these advancements, our study has several limitations that warrant consideration. First, the sample sizes for CVD subtypes, particularly rare conditions such as CAVS and aortic aneurysm, were relatively small, which may reduce the statistical power to detect causal associations and increase the risk of type II errors. Second, the exclusive use of GWAS data from European populations limits the generalizability of our findings to other ethnic groups. Given the known heterogeneity in gut microbiota composition and genetic susceptibility to CVDs across populations, future studies should prioritize multi-ethnic cohorts to validate these associations. Third, while MR analysis minimizes confounding and reverse causation, it cannot fully replace experimental validation. The mechanisms linking specific bacterial taxa (e.g., Actinobacteria) to CVDs protection remain speculative, necessitating functional studies using animal models or in vitro systems to elucidate microbial metabolite production, host immune interactions, and vascular remodeling pathways.

To address these gaps, we propose the following research priorities: (1) Large-scale longitudinal studies integrating multi-omics data (metagenomics, metabolomics, and proteomics) to track dynamic microbiota-cytokine-CVD interactions; (2) Randomized controlled trials testing dietary, probiotic, or anti-cytokine interventions targeting identified mediators (e.g., CCL25 or FGF5); (3) Development of ethnic-specific microbiota biomarkers for CVD risk stratification; and (4) Mechanistic investigations into how bidirectional CVD-microbiota effects influence disease progression (e.g., through gut-barrier integrity or systemic inflammation).

Additionally, we emphasize the need for standardization in microbiota nomenclature and cytokine measurement across studies to enhance comparability. Future MR analyses should also explore sex-specific and age-stratified causal effects, given the demographic variability in CVD presentations.

Conclusion

In this study, a comprehensive Mendelian randomization analysis was conducted to investigate the relationships between gut microbiota, inflammatory cytokines, and eight types of CVDs. The analysis revealed 34 positive causal effects and 24 negative causal effects between genetic predisposition in gut microbiota and CVDs, as well as 16 protective factors and 30 risk factors between cytokines and CVDs. We further identified two significant mediating pathways, in which CCL25 mediated the protective effect of Subdoligranulum and Actinobacteria against CAVS. The gut microbiota taxa and inflammatory cytokines identified in this investigation may serve as novel biomarkers for CVD risk stratification and management, as well as enhance our understanding of the pathophysiological mechanisms underlying CVDs. In conclusion, our findings provide novel genetic evidence for the gut-heart axis, while the outlined limitations highlight the necessity for translational research to bridge these genetic associations with clinical applications.

Abbreviations

Angina Pectoris: AP; Atrial Fibrillation: AF; Calcific Aortic Valve Stenosis: CAVS; Cardiovascular Diseases: CVDs; C-C Motif Chemokine 25: CCL25; C-C Motif Chemokine Ligand 17: CCL17; Confidence Intervals: CI; Coronary Atherosclerosis: CAS; Coronary Artery Disease: CAD; Endothelial Cells: EC; Fibroblast Growth Factor 5: FGF5; Genome-Wide Association Studies: GWAS; Heart Failure: HF; Instrumental Variables: IVs; Inverse Variance Weighted: IVW; Linkage Disequilibrium: LD; Macrophage Colony-Stimulating Factor 1: M-CSF; Mendelian Randomization: MR; Multivariable Mendelian Randomization: MVMR; Odds Ratios: OR; Randomized Controlled Trials: RCTs; Single-Nucleotide Polymorphisms: SNPs; Smooth Muscle Cells: SMC; Trimethylamine N-Oxide: TMAO.

Author Contributions

Xuekun Hou: Conceptualization, Methodology, Formal Analysis, Writing – Original Draft Preparation. Yunxiao Meng: Methodology, Formal Analysis, Writing – Original Draft Preparation. Yuxuan Zhang: Formal Analysis, Data Curation. Xianghan Zheng: Formal Analysis, Data Curation. Hongqi Guo: Validation, Visualization. Yichen Zhang: Validation, Visualization. Hongmei Yuan: Project Administration, Writing – Review & Editing. Zefei Chu: Conceptualization, Writing – Review & Editing, Supervision, Funding Acquisition. All authors read and approved the final manuscript.

Acknowledgements

Not Applicable.

Funding Information

The project was financially supported by Medical Science Research Project of Hebei Provincial Health Commission (20251061/20251060); Scientific Research Project for Institutions of Higher Education, Department of Education of Hebei Province (QN2025430/QN2025264); HBUCM College Students' Innovation Training Program(S202514432008/S202514432025/S202514432080).

Ethics Approval and Consent to Participate

Not Applicable.

Competing Interests

The authors declare that they have no existing or potential commercial or financial relationships that could create a conflict of interest at the time of conducting this study.

Data Availability

All data needed to evaluate the conclusions in the paper are present in the paper or the Supplementary Materials. Additional data related to this paper may be requested from the authors.

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