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Article

Gut microbiota affects aneurysms through biomechanical mechanisms: A mendelian randomization study

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Abstract: Purpose: To assess any potential associations between gut microbiota (GM) and aortic aneurysm and cerebral aneurysm in biomechanics (AA and CA). **Methods:** We performed a two-sample Mendelian randomization (MR) to assess the causal association between GM and AA, CA. The inverse-variance weighted (IVW) model was used as the main analytical method and was followed by sensitivity analysis, including heterogeneity test, horizontal pleiotropy test, and leave-one-out analysis, to appraise the robustness of the MR results. **Results:** Our IVW results showed that RuminococcaceaeUCG005 [OR = 1.43, 95% CI (1.17,1.76), *P* = 0.000] and Roseburia [OR = 1.16, 95% CI (1.00,1.34), *P* = 0.049] were positively associated with AA, while Prevotella9 [OR = 0.81, 95% CI (0.68,0.96), *P* = 0.022] and RuminococcaceaeNK4A214 [OR = 0.72, 95% CI (0.57,0.89), *P* = 0.003] were negative. Meanwhile, we also found that Betaproteobacteria [OR = 1.53, 95% CI (1.08,2.17), *P* = 0.017], cCoriobacteriaceae [OR = 1.39, 95% CI (1.07,1.80), *P* = 0.012], Eggerthella [OR = 1.34, 95% CI (1.12,1.61), *P* = 0.002], Burkholderiales [OR = 1.59, 95% CI (1.11,2.28), *P* = 0.011], Dorei [OR = 1.19, 95% CI (1.01,1.40), *P* = 0.038] and Dorea [OR = 1.09, 95% CI (1.00,1.18), *P* = 0.044] were positively correlated with CA, and there was a negative association between Bifidobacteriales [OR = 0.73, 95% CI (0.57,0.95), *P* = 0.018] and CA. Sensitivity analysis showed no facts of reverse causality, pleiotropy, and heterogeneity. **Conclusions:** Our study demonstrates that RuminococcaceaeUCG005 and Roseburia are related to an increased risk of AA, Prevotella9, and RuminococcaceaeNK4A214 can reduce the risk of AA. On the other hand, Coriobacteriaceae, Eggerthella, Burkholderiales, Dorei and Dorea are related to an increased risk of CA, Bifidobacteriales can reduce the risk of CA. In addition, gut microbiota may affect the occurrence of aneurysms through biomechanical mechanisms such as the elasticity and strength of blood vessel walls.

Keywords: biomechanical mechanisms; gut microbiota; aneurysms; mendelian randomization

1. Introduction

An artery that has permanently enlarged abnormally due to a weak arterial wall is called an aneurysm. Aneurysms can develop anywhere in the body, but the aortic and cerebral aneurysms (AA and CA) are the most common and dangerous types. Congenital abnormalities in the middle layer of the artery wall's muscle, inflammatory responses in the arteries, and atherosclerosis are the main reasons of aneurysm formation [1,2]. Aneurysm symptoms are relatively insidious, but once ruptured, the rate of death and disability is high. More than 50% of patients with ruptured aortic aneurysms die of circulatory failure before reaching the hospital [2]. The prevalence of cerebral aneurysms was found to be 3.2% in a global clinical

study involving 50-year-old participants. The prognosis for rupture of cerebral aneurysms resulting in spontaneous subarachnoid hemorrhage is poor, with a mortality rate of approximately 30% and serious complications left in half of the survivors [3,4]. The genesis of aneurysms is still unclear, despite growing advancements in vascular surgery and vascular intervention procedures that have decreased the lethality of aneurysms. Targeting the etiology to lower the occurrence of aneurysms would significantly lessen patient suffering and have more clinical impact.

Gut microbiota (GM), a group of microorganisms that are colonized in the human intestinal tract and interdependent with the human body for a long period, are widely distributed, complex, and diverse, and maintain the normal physiological and immune functions of the host's intestinal tract [5]. In recent years, research on gut microbiota has revealed its significant impact on human physiology and pathology. Studies have shown that gut microbiota plays a crucial role in the development of various diseases, particularly those involving inflammatory responses like atherosclerosis, diabetes mellitus, and ischemic stroke. This influence is attributed to the regulation of the metabolic-immune axis by gut microbiota. As our understanding of the intricate relationship between gut microbiota and disease continues to evolve, it becomes increasingly clear that targeting the gut microbiota could offer promising therapeutic strategies for managing and preventing these diseases [6–10]. Pathological studies of human aneurysms and animal models have demonstrated that inflammation also plays an important role in the pathophysiology of aneurysms [11,12] and that immune cells induce chronic inflammation in the arterial wall to promote aneurysm progression [13,14]. Shikata et al. [15] demonstrated that gut microbiota are involved in aneurysmal pathophysiological processes by regulating inflammation in an animal study. The current study suggests that gut microbiota and aneurysms are related, but the causal relationship between the two remains uncertain.

Mendelian randomization (MR) is a statistical method that applies genetics to estimate the causal effect of an exposure on an outcome by using genetic variation as an instrumental variable for testing the exposure, aiming to reduce the bias caused by confounders or reverse causality in epidemiological studies [16,17]. Therefore, in this study, two-sample MR analysis was used to explore the potential causal relationship between gut microbiota and cerebral aneurysms and aortic aneurysms to identify specific pathogenic gut microbiota.

1.1. Data and methods

1.1.1. Data sources

The data of aortic aneurysms and cerebral aneurysms were obtained from the GWAS database, aortic aneurysms included 3230 cases and 475,964 controls covering 24,191,825 single nucleotide polymorphisms (SNPs); similarly, cerebral aneurysms included 945 cases and 472,738 controls, including 24,191,825 SNPs; these data are from European populations. The GWAS ID of aortic aneurysms and cerebral aneurysms are ebi-a-GCST90018783 and ebi-a-GCST90018815. Summary statistics for gut microbiota were obtained from the most recent GWAS website and

included 411 gut microbiota exposures. Two-sample MR analysis was used to investigate the potential causal relationship between aortic aneurysms and cerebral aneurysms and gut microbiota. All data used in this study were obtained from public data, which are publicly available on the database website (https://gwas.mrcieu.ac.uk/).

1.1.2. Screening of instrumental variables

There are 3 core hypotheses for Mendelian randomization studies.

(1) Hypothesis 1: Correlation hypothesis. Instrumental variables (IVs) are strongly correlated with exposure factors (gut microbiota). Under the conventional threshold $P < 5 \times 10^{-8}$, there were only a few SNPs, which did not meet the needs of Mendelian analysis, and the threshold was adjusted to $P < 1 \times 10^{-5}$ to obtain enough SNPs after referring to the related literature. The chain disequilibrium r^2 was set to 0.001, and the width of the chain disequilibrium region was 10,000 kb to ensure that the individual SNPs were independent of each other, and to rule out the influence of gene pleiotropy on the results and bias [18,19]; (2) Hypothesis 2: Independence hypothesis. Genetic variants are independent of confounders, SNPs selected as instrumental variables cannot be associated with confounders (smoking, hypertension, atherosclerosis, etc. in this study); (3) Hypothesis 3: Exclusivity hypothesis. Genetic variation is not associated with the outcome variable, SNPs can only affect aneurysms through gut microbiota and are not directly associated with aneurysms, and SNPs directly associated with cerebral aneurysms or aortic aneurysms were excluded ($P < 5 \times 10^{-8}$).

Phenotypes represented by all eligible SNPs were searched by Pheno Scanner (http://www.phenoscanner.medschl.cam.ac.uk/) [20] to exclude confounders associated with aneurysms (e.g., smoking, hypertension, atherosclerosis, infection, trauma, etc.) [21] and the aneurysm-related SNPs.

The statistical strength of the correlation between SNPs and exposure was assessed in Mendelian randomization using the F statistic, which is calculated by the formula $F = R^2(\mathrm{N} - \mathrm{K} - 1)/(1 - R^2)K$, where N is the sample size of the gut microbiota database, K is the number of SNPs, and R^2 represents the proportion of variance in gut microbiota explained by SNPs, $R^2 = 2 \times (1 - \mathrm{MAF})(\mathrm{MAF}) \times (\beta/\mathrm{SD})^2$, $F < 10$ is considered as a weak correlation between SNPs and exposure that needs to be excluded, and $F > 10$ is considered as an instrumental variable that meets the requirements. EAF (Effect Allele Frequency) is the effect allele frequency of each SNP, the frequency of allele occurrence and β is the effect value of the allele [20,22].

1.1.3. Mendelian randomization analysis

The inverse variance weighted (IVW) analysis method [23–25] was used as the main analysis method in this study and combined with Wald estimation to analyze the causal relationship of gut microbiota on aneurysms [26]. To guard against false positives in multiple testing, we applied a Bonferroni correction to establish a statistically adjusted significance threshold [$P = 1.21 \times 10^{-4}$ (0.05/411)] [27]. The inverse variance weighted analysis method, inspired by meta-analysis, was proposed to reduce the variance by weighted averaging. IVW was subdivided into m ixed-effects model and random-effects model. If heterogeneity was shown in the sensitivity test, the random-effects model should be chosen to reduce the error; if

there was no heterogeneity, the results of the random-effects model and the fixed-effects model were consistent. Other analytical methods such as weighted median (WM) [28] and MR-Egger regression are also used as an aid. WM assumes that at least half of the IVs in the analysis are valid, and then obtains a consistent estimate of causality. MR-Egger regression adal intercept term, which is mainly used to determine the existence of horizontal pleiotropy, considering the presence of pleiotropy that does not provide evidence of a causal effect.

1.1.4. Sensitivity analysis

In this study, horizontal pleiotropy was tested by the MR-Egger method, if the intercept term in the MR-Egger analysis is significant, it indicates that the study has horizontal polyvalence. The heterogeneity of SNPs was determined by Cochran's Q test if Cochran's Q statistic test was statistically significant ($P < 0.05$), it proved that the analysis results had significant heterogeneity, and heterogeneity was considered to exist when $P < 0.05$, and then causal inference was made by using the random effect model of IVW. The leave-one-out analysis was used to assess whether the significant results of the combined IVW were determined by a single SNP.

1.1.5. Statistical software version and name

MR analyses in the study were realized with *R* (version 4.3.1) and the Two Sample MR package and MR-PRESSO package.

2. Results

Four gut microbiota associated with aortic aneurysms and seven gut microbiota associated with cerebral aneurysms were obtained from 411 human gut microbiota by IVW method in MR analysis. Some positive exposures in the presence of pleiotropy are filtered out. The influence of each SNP locus on aortic aneurysms or cerebral aneurysms was obtained by MR analysis.

2.1. Two-sample mendelian randomization analysis

The relationship between the gut microbiota and the aneurysm as a whole is shown in the diagram as a whole (**Tables 1** and **2**, **Figures 1** and **2**).

The consistent direction of causality demonstrated by the three methods underscores a significant finding: the groups of Prevotella_9 and RuminococcaceaeNK4A214 exhibit a negative association with the risk of aortic aneurysm [OR = 0.81, 95% CI (0.68–0.96), $P = 0.014$; OR = 0.72, 95% CI (0.57–0.89), $P = 0.003$]. This suggests that they may serve as protective factors against aortic aneurysm. On the contrary, the RuminococcaceaeUCG005 group and Roseburia are positively linked to the risk of aortic aneurysm [OR = 1.43, 95% CI (1.17–1.76), $P < 0.001$; OR = 1.16, 95% CI (1.00–1.34), $P = 0.049$] (**Table 1**, **Figure 1**, **Figure 3B**, Supplementary **Figures S1B–S3B**). Moreover, an examination of relevant SNPs through Phenoscanner unveiled that one SNP in Prevotella_9 was connected to confounding factors. Upon the exclusion of these SNPs and subsequent reapplication of MR analysis, Prevotella_9. "rs1304512" was found to be associated with the phenotype "self-reported hypertension," a statistically significant result.

Table 1. MR analysis results of gut microbiota and aortic aneurysms.

GWAS ID	Exposure	snps	Methods	OR (95% CI)	*P*	Cochran's Q (*P*)	Egger intercept (*P*)
ebi-a-GCST90017045	Prevotella_9	14	MR-Egger	0.85 (0.50–1.43)	0.546	13 (0.752)	−0.002 (0.939)
			IVW	0.81 (0.68–0.96)	0.014	14 (0.815)	
			Weight median	0.77 (0.61–0.96)	0.022		
ebi-a-GCST90017052	RuminococcaceaeNK4A214	13	MR-Egger	0.68 (0.31–1.47)	0.345	11 (0.892)	0.004 (0.887)
			IVW	0.72 (0.57–0.89)	0.003	12 (0.929)	
			Weight median	0.72 (0.54–0.97)	0.03		
ebi-a-GCST90017056	RuminococcaceaeUCG005	14	MR-Egger	1.38 (0.76–2.49)	0.315	12 (0.438)	0.004 (0.885)
			IVW	1.43 (1.17–1.76)	< 0.001	13 (0.517)	
			Weight median	1.36 (1.02–1.82)	0.034		
ebi-a-GCST90027713	Roseburia	14	MR-Egger	1.55 (0.85–2.83)	0.181	12 (0.935)	−0.031 (0.349)
			IVW	1.16 (1.00–1.34)	0.049	13 (0.924)	
			Weight median	1.21 (0.99–1.47)	0.055		

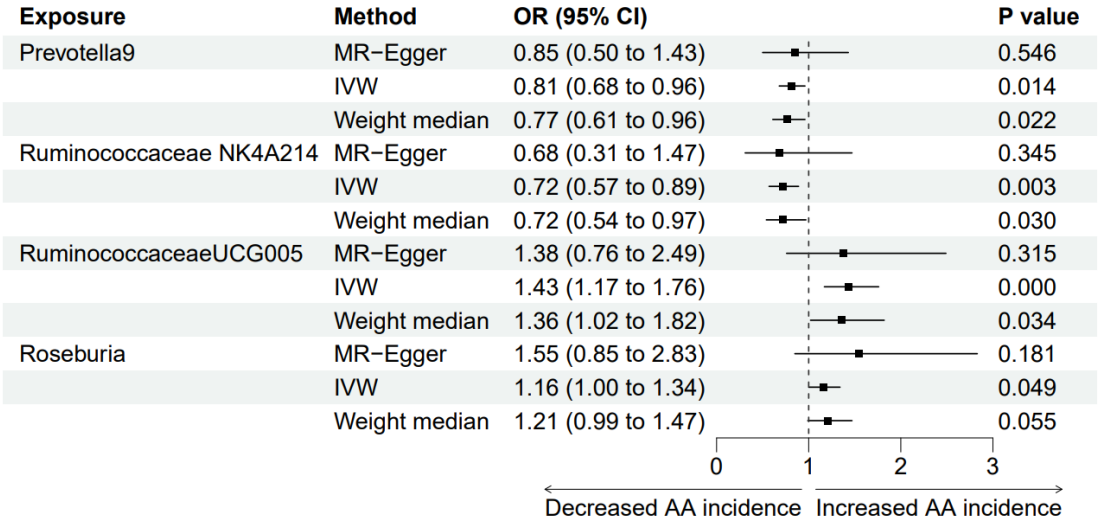

Figure 1. Forest plot of MR analysis of gut microbiota and aortic aneurysm.

In a study focusing on cerebral aneurysms, it was observed that Bifidobacteriales exhibited a negative association with the risk of cerebral aneurysms, with an odds ratio of 0.73 and a 95% confidence interval ranging from 0.57 to 0.95, yielding a statistically significant *P*-value of 0.018. Conversely, other bacterial taxa such as BetaProteobacteria, Coriobacteriaecae, Eggerthella, Burkholderiales, Dorei, and Dorea were found to be positively associated with the risk of cerebral aneurysm. Their respective odds ratios and confidence intervals were 1.53 (1.08–2.17), 1.39 (1.07–1.80), 1.34 (1.12–1.61), 1.59 (1.11–2.28), 1.19 (1.01–1.40), and 1.09 (1.00–1.18), with corresponding *P*-values of 0.017, 0.012, 0.002, 0.011, 0.038, and 0.044 (**Table 2**, **Figure 2**, **Figures 4B–5B**, Supplementary **Figures S4B–S8B**).

Table 2. MR analysis results of gut microbiota and cerebral aneurysms.

GWAS ID	Exposure	snps	Methods	OR (95%CI)	*P*	Cochran's Q (*P*)	Egger intercept (*P*)
ebi-a-GCST90016912	BetaProteobacteria	12	MR-Egger	2.16 (0.55–8.49)	0.295	10 (0.133)	−0.023 (0.619)
			IVW	1.53 (1.08–2.17)	0.017	11 (0.166)	
			Weight median	1.42 (0.96–2.11)	0.081		
ebi-a-GCST90016914	Coriobacteriaecae	14	MR-Egger	1.92 (0.69–5.32)	0.233	12 (0.817)	−0.023 (0.532)
			IVW	1.39 (1.07–1.80)	0.012	13 (0.844)	
			Weight median	1.44 (1.03–2.02)	0.031		
ebi-a-GCST90017093	Bifidobacteriales	12	MR-Egger	0.64 (0.27–1.50)	0.328	10 (0.491)	0.011 (0.750)
			IVW	0.73 (0.57–0.95)	0.018	11 (0.571)	
			Weight median	0.73 (0.51–1.06)	0.095		
ebi-a-GCST90016990	Eggerthella	11	MR-Egger	2.71 (0.82–8.96)	0.137	9 (0.362)	−0.074 (0.275)
			IVW	1.34 (1.12–1.61)	0.002	10 (0.332)	
			Weight median	1.24 (0.97–1.58)	0.074		
ebi-a-GCST90017094	Burkholderiales	11	MR-Egger	1.69 (0.42–6.82)	0.477	9 (0.117)	−0.004 (0.931)
			IVW	1.59 (1.11–2.28)	0.011	10 (0.165)	
			Weight median	1.53 (1.01–2.30)	0.043		
ebi-a-GCST90027824	Dorei	15	MR-Egger	1.36 (0.49–3.77)	0.563	13 (0.293)	−0.013 (0.794)
			IVW	1.19 (1.01–1.40)	0.038	14 (0.357)	
			Weight median	1.12 (0.89–1.40)	0.328		
ebi-a-GCST90027847	Dorea	11	MR-Egger	1.10 (0.87–1.41)	0.444	9 (0.590)	−0.003 (0.911)
			IVW	1.09 (1.00–1.18)	0.044	10 (0.681)	
			Weight median	1.11 (1.00–1.25)	0.059		

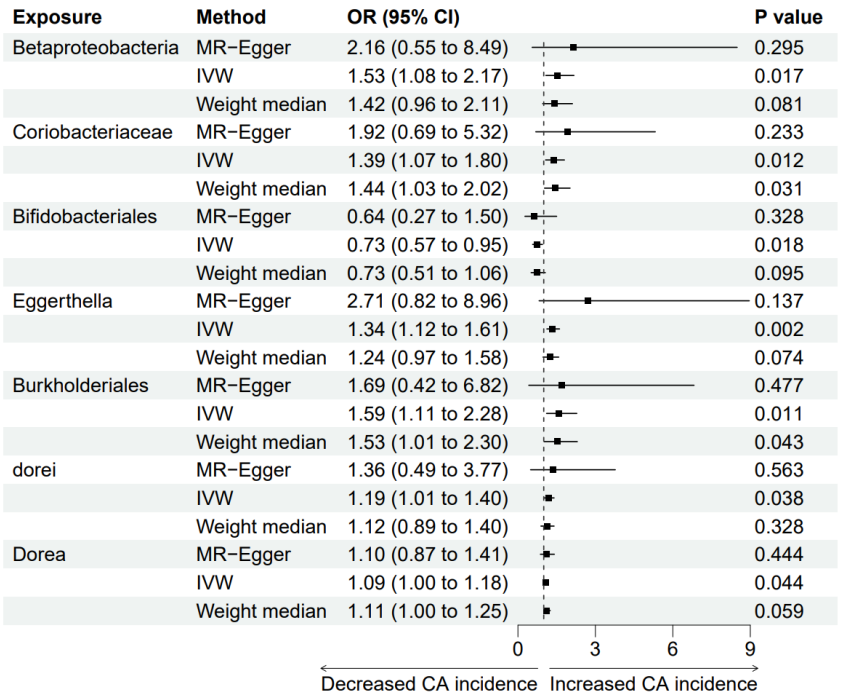

Figure 2. Forest plot of MR analysis of gut microbiota and cerebral aneurysm.

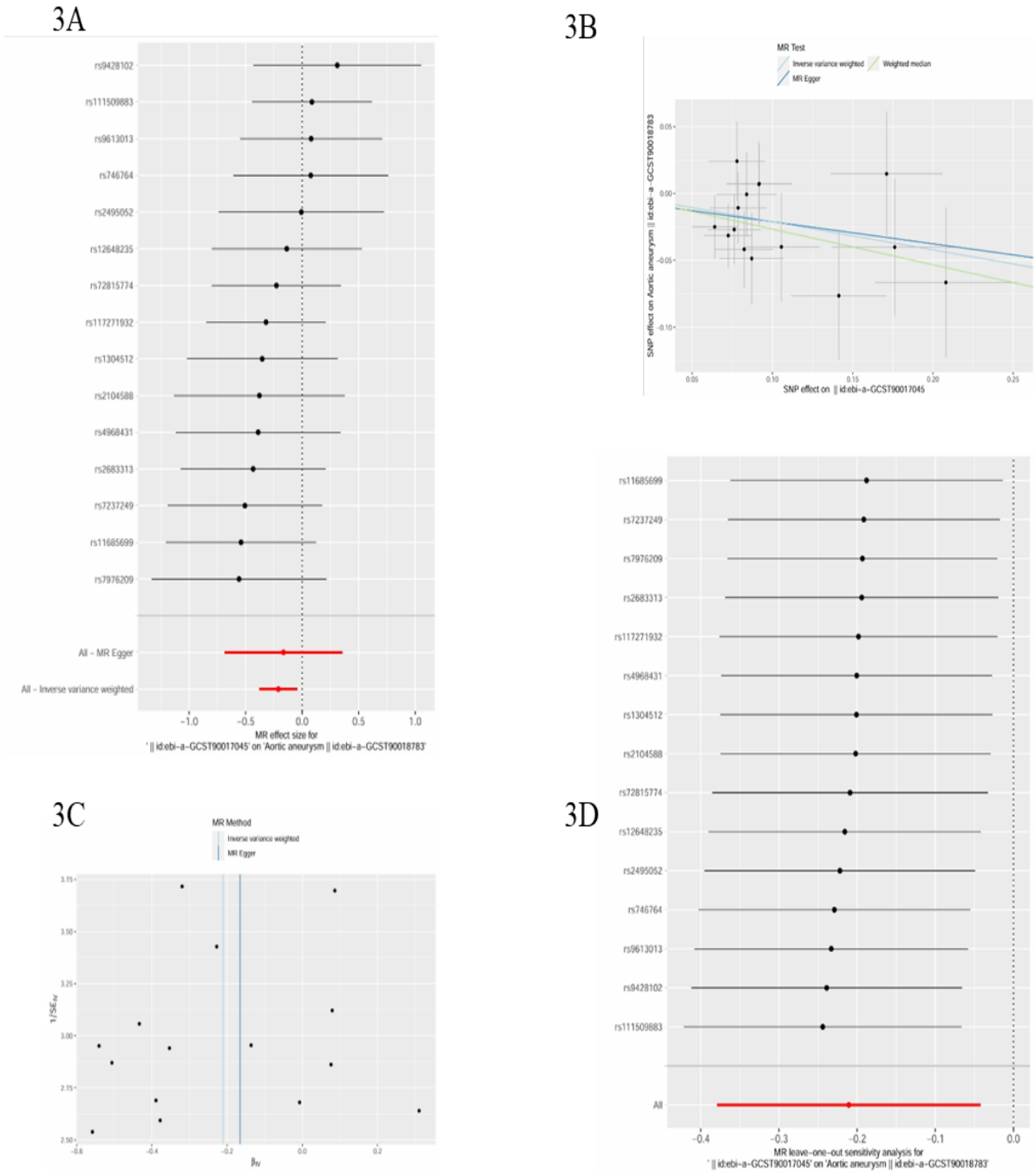

Figure 3. Causal relationship between Prevotella_9 and aortic aneurysms. **(A)** Forest plot of causality between Prevotella_9 and aortic aneurysms; **(B)** Scatter plot of causality between Prevotella_9 and aortic aneurysms; **(C)** Funnel plot of causality between Prevotella_9 and aortic aneurysms; **(D)** "leave-one-out" plot of causality between Prevotella_9 and aortic aneurysms.

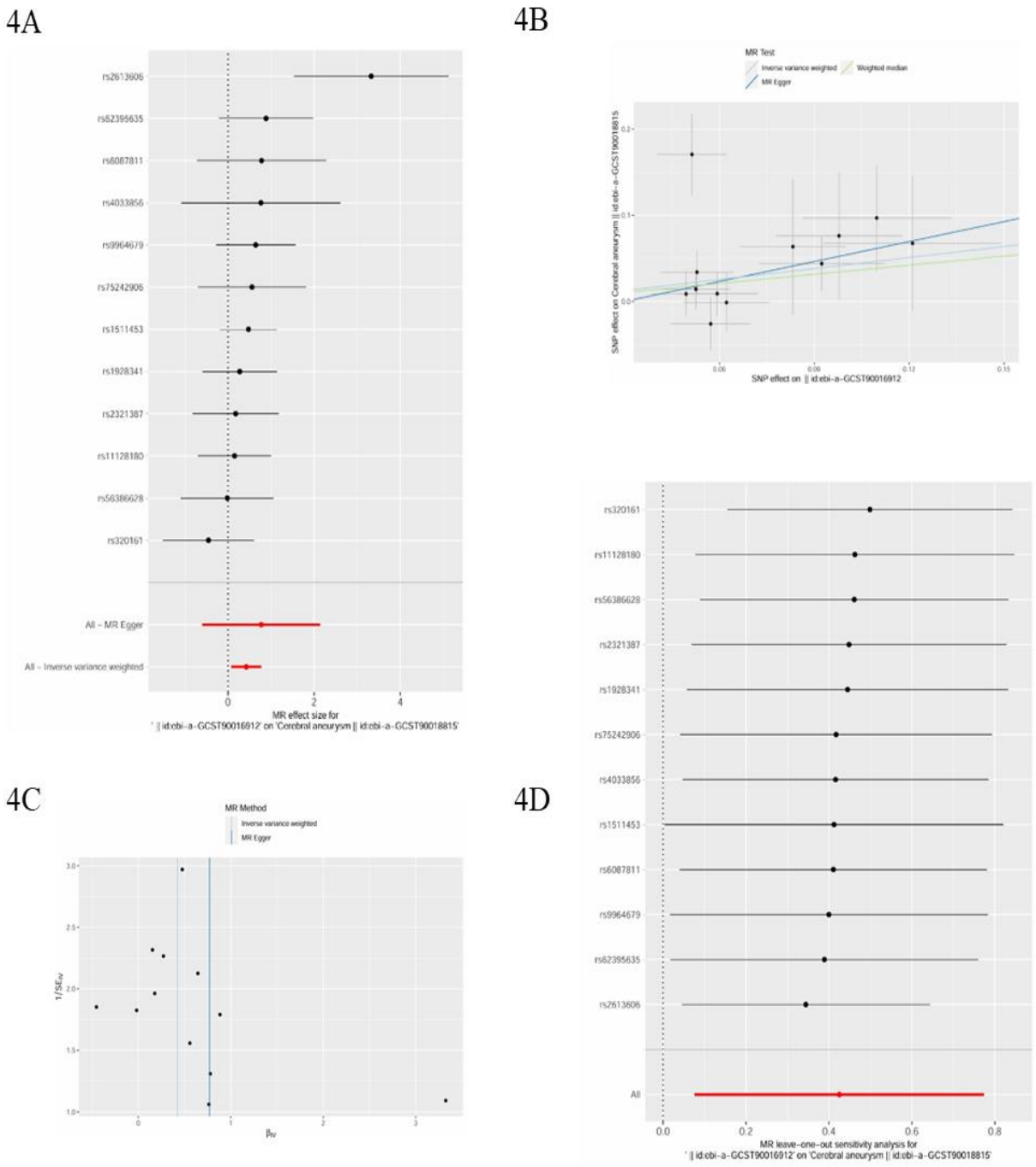

Figure 4. Causal relationship between BetaProteobacteria and cerebral aneurysms. **(A)** Forest plot of causality between BetaProteobacteria and cerebral aneurysms; **(B)** Scatter plot of causality between BetaProteobacteria and cerebral aneurysms; **(C)** Funnel plot of causality between BetaProteobacteria and cerebral aneurysms; **(D)** "leave-one-out" plot of causality between BetaProteobacteria and cerebral aneurysms.

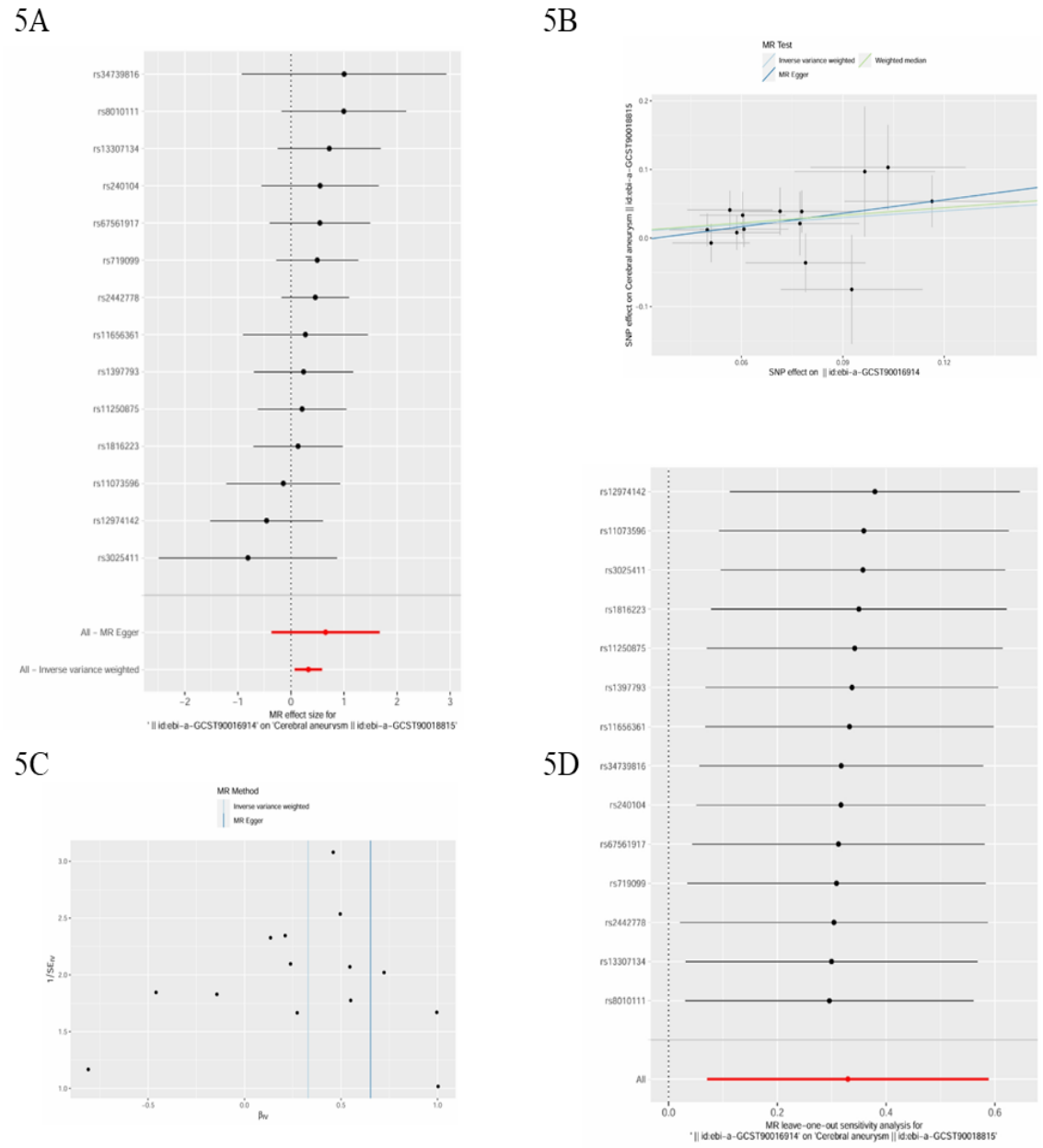

Figure 5. Causal relationship between Coriobacteriaecae and cerebral aneurysms. **(A)** Forest plot of causality between Coriobacteriaecae and cerebral aneurysms; **(B)** Scatter plot of causality between Coriobacteriaecae and cerebral aneurysms; **(C)** Funnel plot of causality between Coriobacteriaecae and cerebral aneurysms; **(D)** "leave-one-out" plot of causality between Coriobacteriaecae and cerebral aneurysms.

Furthermore, an investigation into the horizontal pleiotropy of single nucleotide polymorphisms (SNPs) associated with these bacterial taxa was conducted using the Phenoscanner website. The analysis revealed no SNPs that were linked to potential confounders, as indicated in **Table 2** and **Figure 2**. This research sheds light on the complex interplay between microbial composition and the development of cerebral aneurysms, underscoring the need for further exploration in this area.

2.2. Sensitivity analysis

The results of MR Egger regression showed that in aortic aneurysms, there was no horizontal pleiotropy in 4 bacterial groups including Prevotella_9 (intercept P = 0.939), (intercept P = 0.887), (intercept P = 0.885), and (intercept P = 0.349), which indicated that the results of MR analysis were reliable (**Table 1**, **Figure 3C**, Supplementary **Figures S1C–S3C**).

In cerebral aneurysms, BetaProteobacteria (Intercept P = 0.619), Coriobacteriaecae (Intercept P = 0.532) Bifidobacteriales (Intercept P = 0.750), Eggerthella (Intercept P = 0.275), Burkholderiales (Intercept P = 0.931), Dorei (Intercept P = 0.794), and Dorea (Intercept P = 0.911) likewise did not show horizontal pleiotropy (**Table 2**, **Figures 4C–5C**, Supplementary **Figures S4C–S8C**).

The one-by-one removal test showed that no single SNP had a significant impact on the robustness of the results, indicating that this study is stable (**Figures 3D–5D**, Supplementary **Figures S1D–S8D**).

The results of Cochran's test showed that the Q p-value of IVW and MR-Egger were > 0.05 for all the bacterial groups and there was no heterogeneity. (No outliers were detected by the MR-PRESSO method, P > 0.05).

3. Discussion

The gut microbiome is a complex and dynamic collection of ecological communities mainly consisting of four bacterial phyla, including Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidetes. From a structural point of view, arteries have been defined as a composite material consisting of fibres, elastin, and collagen, embedded in a compliant and viscoelastic matrix, made of ground substance and cells. Collagen and elastin are two major constituents of the arterial wall. The mechanical effects of blood flow and shear stress in the endothelium and vascular smooth muscles, inflammation, as well as the controls from the renin-angiotensin-aldosterone system, endothelins, adipokines from perivascular adipose tissue (PVAT), are the key factors in the pathophysiology of arterial remodelling and the progression of Hypertension [29].

The dysbiosis of gut microbiomes may cause metabolic, immune, and neurological diseases [29,30]. As an invisible organ, it could directly influence the vascular wall inflammatory cell infiltration manifested in an enhanced vascular wall, which is important in the progression of aneurysms [31]. Our study through MR analysis explored gut microbiomes that were associated with the formation and progressionof aneurysms, and shed light on the potential role of gut microbiomes in preventing the occurrence and progression of aneurysms [31].

The effect of gut microbiota on intracranial aneurysms has also been reported in the literature, where gut microbiota affects intracranial aneurysm formation through inflammation within the walls of intracranial aneurysms in mice, which was further confirmed by antibiotic depletion of gut microbiota experiments [15]. These gut microbiomes may play a role in modifiable risk factors of aneurysm or participate in the formation of aneurysms through other potential mechanisms instead of having a causal association with aneurysms. Moreover, the intricate symbiotic or antagonistic relationship among gut microbiomes should not be neglected [32]. Additionally,

intestinal barrier dysfunction could cause the spread of inflammation and allow gut microbiomes or their metabolites to enter into the systemic circulation, even colonizing in aneurysms [33,34]. Vasoconstrictors, such as noradrenaline, endothelin-1 (ET-1) orangiotensin II (Ang II), increase artery stiffness, whereas vasodilators such as glyceryl trinitrate elicit opposite effects. Systemic inflammation may also result in greater permeability of the blood–brain barrier, leading to the colonization of gut microbiomes in aneurysms [35]. Changes in endothelial function and smooth muscle tone can influence the stiffness of the elastic and muscular arteries. Studies have shown that the interaction of chemicals secreted by intestinal flora and blood vessel clotting factors can lead to increased permeability of the blood vessel wall and change the biomechanical properties of the blood vessel wall, leading to inflammation or exudation [14].

By controlling for confounding factors and reverse causality effects, a total of four gut microbiota were associated with aortic aneurysms in this study by Mendelian randomization, including Prevotella_9, RuminococcaceaeNK4A214, RuminococcaceaeUCG005, Roseburia and seven gut microbiota were associated with cerebral aneurysms, including Beta proteobacteria, Coriobacteriaecae, Bifidobacteriales, Eggerthella, Burkholderiales, Dorei and Dorea. The reason for the different gut microbiota analyzed is that we speculate that it may be related to the fact that the embryonic origin of the aorta and cerebral arteries is not identical; although the endothelium of all blood vessels originates from the mesoderm, and the embryonic source of the vast majority of arterial intima and epithelium is also mesodermal, the source of the smooth muscle cells of a portion of the blood vessels in the neural crest (NC), including the diaphragmatic layer of the heart, the aorta, vena cava, and some cerebral arteries [36].

The analysis of gut microbiota about aortic aneurysm reveals significant associations. Specifically, RuminococcaceaeUCG005 and Roseburia have been linked to an elevated risk of aortic aneurysm, while Prevotella_9 and RuminococcaceaeNK4A214 have shown potential in reducing this risk. In an animal study, it was demonstrated that Clostridium butyricum can suppress pathogenic bacteria in the gut and support the proliferation of beneficial bacteria such as Bifidobacterium and Lactobacillus [37]. This resulted in a notable increase in the relative abundance of these beneficial bacteria in the Clostridium butyricum and RuminococcaceaeNK4A214 groups compared to the control group, whereas the relative abundance in the RuminococcaceaeUCG005 group was notably lower. Additionally, another study highlighted the role of Prevotella in mitigating inflammatory responses and lowering the risk of developing psoriasis, with patients suffering from psoriasis exhibiting significantly lower levels of Prevotella abundance [38].

Additionally, analyzing the gut microbiota associated with cerebral aneurysms, BetaProteobacteria, Coriobacteriaecae, Bifidobacteriales, Eggerthella, Burkholderiales, Dorei were associated with an increased risk of cerebral aneurysms, most of these gut microbiota were shown to be associated with inflammatory responses in the body in different studies, and all of them increased localized or systemic inflammatory responses in the body [39], and the abundance of Coriobacteriaecae was significantly higher in HIV-1 seroconverted individuals than

in the general population, which is associated with an increase in blood biomarkers of inflammation [40]; increased abundance of Eggerthella was positively associated with the risk of systemic lupus erythematosus [41]; increased abundance of Burkholderiales was associated with the risk of cystic fibrosis in Russia [42]; and, in patients with irritable bowel syndrome, the gut microbiota of subjects with IBS, compared to healthy controls, was significantly increased in Dorei and Dorea species were significantly increased and Lactobacillus and Bifidobacterium species were decreased [43]. This point is consistent with our finding that Bifidobacteriales are protective factors for cerebral aneurysm development. Bifidobacteriales have the role of regulating the immune function, stimulating the intestinal mucosa, activating the immune system of the intestinal mucosa, causing it to produce antibodies and cytokines, and improving the immune and anti-inflammatory ability of the intestinal mucosa [44]. At the same time, in a recent study, the researchers analyzed the gut bacteria of 76 patients with Takayasu arteritis and 56 healthy individuals. They discovered that patients with Campylobacter in their gut were much more likely to need treatment for aortic dilatation compared to those without Campylobacter [45]. Meta-analysis research demonstrated that the relative abundance of Hungatella hathewayi is a protective factor against aneurysm growth and rupture. Meanwhile, the relative abundance of Campylobacter ureolyticus is associated with stroke-aneurysmal subarachnoid hemorrhage [46]. Another group applied 16S sequencing and found that the abundance of the genus Campylobacter and Campylobacter ureolyticus was significantly higher in the ruptured aneurysms group [47]. Gut microbiota has also been shown to exacerbate Ang II-induced arterial hypertension, vascular inflammation and dysfunction in conventional mice compared to germ-free mice [48]. In addition, interleukin-4 (IL-4) and IL-10 are increased in the Ang II-treated conventional mice but not in germ-free mice. The translocation of gut bacteria to the intraperitoneal space, due to epithelial layer damage, can induce transitory infection with systemic elevation of IL-12 [49].

However, our study has some limitations. First, all GWAS data were from European populations, and the findings of this study cannot be fully applied to other populations; second, when we analyzed the gut microbiota, most of them were analyzed at the genus level, individually up to the level of the order, phylum, and family, and not at the level of the specific species or strains; and furthermore, to obtain a sufficient intestinal microbiota, we chose a higher than the traditional genome-wide significance level ($P < 5 \times 10^{-8}$) for instrumental variables in gut microbiota ($P < 1 \times 10^{-5}$); finally, our Mendelian Randomization (MR) analysis primarily relied on the significance ($p < 0.05$) of the Inverse Variance Weighted (IVW) method. It is prudent to interpret the significance derived from a single method cautiously. Therefore, future studies should aim to validate these findings with larger datasets and explore other robust MR methods to further strengthen causal inference. The MR analysis results did not meet the Bonferroni correction threshold [$p = 1.21 \times 10^{-4}$ (0.05/411)], meaning the associations in this study are not statistically significant. Hence, these findings are indicative of potential associations rather than definitive evidence. More research is needed to reveal the specific mechanisms involved.

Supplementary materials: Supplementary material shows the relationship between the remaining 8 kinds of bacteria and aneurysms.

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References

1. Sakalihasan N, Limet R, Defawe OD. Abdominal aortic aneurysm. Lancet. 2005 Apr 30-May 6;365(9470):1577-89. doi: 10.1016/S0140-6736(05)66459-8.
2. Anagnostakos J, Lal BK. Abdominal aortic aneurysms. Prog Cardiovasc Dis. 2021 Mar-Apr; 65: 34-43. doi: 10.1016/j.pcad.2021.03.009.
3. Chen Y, Wright N, Guo Y, et al.; China Kadoorie Biobank Collaborative Group. Mortality and recurrent vascular events after first incident stroke: a 9-year community-based study of 0.5 million Chinese adults. Lancet Glob Health. 2020 Apr;8(4):e580-e590. doi: 10.1016/S2214-109X (20)30069-3.
4. Nieuwkamp DJ, Setz LE, Algra A, et al. Changes in case fatality of aneurysmal subarachnoid haemorrhage over time, according to age, sex, and region: a meta-analysis. Lancet Neurol. 2009 Jul;8(7):635-42. doi: 10.1016/S1474-4422(09)70126-7.
5. Sampson TR, Mazmanian SK. Control of brain development, function, and behavior by the microbiome. Cell Host Microbe. 2015 May 13;17(5):565-76. doi: 10.1016/j.chom.2015.04.011.
6. Koeth RA, Wang Z, Levison BS, et al. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. Nat Med. 2013 May;19(5):576-85. doi: 10.1038/nm.3145
7. Holmes E, Li JV, Marchesi JR, et al. Gut microbiota composition and activity in relation to host metabolic phenotype and disease risk. Cell Metab. 2012 Nov 7;16(5):559-64. doi: 10.1016/j.cmet.2012.10.007.
8. Wang Z, Klipfell E, Bennett BJ, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature. 2011 Apr 7;472(7341):57-63. doi: 10.1038/nature09922.
9. Le Chatelier E, Nielsen T, Qin J, et al. Richness of human gut microbiome correlates with metabolic markers. Nature. 2013 Aug 29;500(7464):541-6. doi: 10.1038/nature12506.
10. Claesson MJ, Jeffery IB, Conde S,et al. Gut microbiota composition correlates with diet and health in the elderly. Nature. 2012 Aug 9;488(7410):178-84. doi: 10.1038/nature11319.
11. Hosaka K, Hoh BL. Inflammation and cerebral aneurysms. Transl Stroke Res. 2014 Apr;5(2):190-8. doi: 10.1007/s12975-013-0313-y.
12. Frösen J, Tulamo R, Paetau A,et al. Saccular intracranial aneurysm: pathology and mechanisms. Acta Neuropathol. 2012 Jun;123(6):773-86. doi: 10.1007/s00401-011-0939-3.
13. Raffort J, Lareyre F, Clément M,et al. Monocytes and macrophages in abdominal aortic aneurysm. Nat Rev Cardiol. 2017 Aug;14(8):457-471. doi: 10.1038/nrcardio.2017.52.
14. Dale MA, Ruhlman MK, Baxter BT. Inflammatory cell phenotypes in AAAs: their role and potential as targets for therapy. Arterioscler Thromb Vasc Biol. 2015 Aug;35(8):1746-55. doi: 10.1161/ATVBAHA.115.305269.
15. Shikata F, Shimada K, Sato H,et al. Potential Influences of Gut Microbiota on the Formation of Intracranial Aneurysm. Hypertension.2019Feb;73(2):491-496. doi: 10.1161/HYPERTENSIONAHA.118.11804.

16. Emdin CA, Khera AV, Kathiresan S. Mendelian Randomization. JAMA. 2017 Nov 21;318(19):1925-1926. doi: 10.1001/jama.2017.17219.
17. Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. Hum Mol Genet. 2014 Sep 15;23(R1):R89-98. doi: 10.1093/hmg/ddu328.
18. Ma J, Li J, Jin C, et al. Association of gut microbiome and primary liver cancer: A two-sample mendelian randomization and case control study. Liver Int, 2023, 43(1): 221-233. doi: 10.1111/liv.15466
19. Liu B, Ye D, Yang H, et al. Assessing the relationship between gut microbiota and irritable bowel syndrome: A two-sample Mendelian randomization analysis. BMC Gastroenterology, 2023, 23(1): 150. doi: 10.1186/s12876-023-02791-7
20. Kamat MA, Blackshaw JA, Young R, et al. PhenoScanner V2: An expanded tool for searching human genotype-phenotype associations. Bioinformatics (Oxford, England), 2019, 35(22): 4851- 4853. doi: 10.1093/bioinformatics/btz469
21. Vardulaki KA, Walker NM, Day NE, et al. Quantifying the risks of hypertension, age, sex and smoking in patients with abdominal aortic aneurysm. Br J Surg. 2000 Feb;87(2):195-200. doi: 10.1046/j.1365-2168.2000.01353.x.
22. Palmer TM, Lawlor DA, Harbord RM, et al. Using multiple genetic variants as instrumental variables for modifiable risk factors. Statistic Meth Med Res, 2012, 21(3): 223-242. doi: 10.1177/0962280210394459
23. Chen L, Yang H, Li H, et al. Insights into modifiable risk factors of cholelithiasis: A Mendelian randomization study. Hepatology, 2022, 75(4): 785-796. doi: 10.1002/hep.32183
24. Jia Y, Hui L, Sun L, et al. Association between human blood metabolome and the risk of psychiatric disorders. Schizophrenia Bulletin, 2023, 49(2): 428-443. doi: 10.1093/schbul/sbac130
25. Yavorska OO, Burgess S. Mendelian randomization: An R package for performing Mendelian randomization analyses using summarized data. Inter J Epidemiol, 2017, 46(6): 1734-1739. doi: 10.1093/ije/dyx034
26. Burgess S, Bowden J, Fall T, et al. Sensitivity analyses for robust causal inference from mendelian randomization analyses with multiple genetic variants. Epidemiology, 2017, 28 (1): 30-42. doi: 10.1097/EDE.0000000000000559
27. Li B, HanY,FuZ,ChaiY,GuoX,DuS,etal.Thecausalrelationship between gut microbiota and lymphoma: a two-sample Mendelian randomization study. Front Immunol. (2024) 15:1397485. doi: 10.3389/fimmu.2024.1397485
28. Bowden J, Davey Snith G, Haycock P C, et al. Consistent estimation in mendelian randomization with some invalid instruments using a weighted median estimator. Genet Epidemiol, 2016, 40(4): 304-314. doi: 10.1002/gepi.21965
29. Strandwitz, P. (2018). Neurotransmitter modulation by the gut microbiota. Brain Res.1693, 128–133. doi: 10.1016/j.brainres.2018.03.015
30. Xu, Q., Ni, J. J., Han, B. X., Yan, S. S., Wei, X. T., Feng, G. J., et al. (2021). Causal relationship between gut microbiota and autoimmune diseases: a two-sample Mendelian randomization study. Front. Immunol. 12:746998. doi: 10.3389/fimmu.2021.746998
31. Ling, X., Jie, W., Qin, X., Zhang, S., Shi, K., Li, T., et al. (2022). Gut microbiome sheds light on the development and treatment of abdominal aortic aneurysm. Front. Cardiovasc. Med. 9:1063683. doi: 10.3389/fcvm.2022.1063683
32. Tian, Z., Zhang, Y., Zheng, Z., Zhang, M., Zhang, T., Jin, J., et al. (2022). Gut microbiome dysbiosis contributes to abdominal aortic aneurysm by promoting neutrophil extracellular trap formation. Cell Host Microbe 30, 1450–1463.e8. doi: 10.1016/j.chom.2022.09.004
33. Marques Da Silva, R., Caugant, D. A., Eribe, E. R., Aas, J. A., Lingaas, P. S., Geiran, O., et al. (2006). Bacterial diversity in aortic aneurysms determined by 16S ribosomal Rna gene analysis. J. Vasc. Surg. 44, 1055–1060. doi: 10.1016/j.jvs.2006.07.021
34. Clifford, A., and Hoffman, G. S. (2015). Evidence for a vascular microbiome and its role in vessel health and disease. Curr. Opin. Rheumatol. 27, 397–405. doi: 10.1097/BOR.0000000000000184
35. Nelson, J. W., Phillips, S. C., Ganesh, B. P., Petrosino, J. F., Durgan, D. J., and Bryan, R. M. (2021). The gut microbiome contributes to blood-brain barrier disruption in spontaneously hypertensive stroke prone rats. FASEB J. 35:e21201. doi: 10.1096/fj.202001117R
36. Vega-Lopez GA, Cerrizuela S, Tribulo C, et al. Neurocristopathies: New insights 150 years after the neural crest discovery. Dev Biol. 2018 Dec 1;444 Suppl 1:S110-S143. doi: 10.1016/j.ydbio.2018.05.013.
37. Liang J, Kou S, Chen C, et al. Effects of Clostridium butyricum on growth performance, metabonomics and intestinal microbial differences of weaned piglets. BMC Microbiol. 2021 Mar 22;21(1):85. doi: 10.1186/s12866-021-02143-z.
38. Zang C, Liu J, Mao M, et al. Causal Associations Between Gut Microbiota and Psoriasis: A Mendelian Randomization Study. Dermatol Ther (Heidelb). 2023 Oct;13(10):2331-2343. doi: 10.1007/s13555-023-01007-w.

39. Qin H, Yang F, Hao P, et al. Gut microbiota and cerebrovascular diseases: a Mendelian randomization study. Front Microbiol. 2023 Aug 10;14:1228815. doi: 10.3389/fmicb.2023.1228815.
40. Mohammad NS, Nazli R, Zafar H, et al. Effects of lipid based Multiple Micronutrients Supplement on the birth outcome of underweight pre-eclamptic women: A randomized clinical trial. Pak J Med Sci. 2022 Jan-Feb;38(1):219-226. doi: 10.12669/pjms.38.1.4396.
41. Xiang K, Wang P, Xu Z, et al. Causal Effects of Gut Microbiome on Systemic Lupus Erythematosus: A Two-Sample Mendelian Randomization Study. Front Immunol. 2021 Sep 7;12:667097. doi: 10.3389/fimmu.2021.667097.
42. Voronina OL, Kunda MS, Ryzhova NN, et al. On Burkholderiales order microorganisms and cystic fibrosis in Russia. BMC Genomics. 2018 Feb 9;19(Suppl 3):74. doi: 10.1186/s12864-018-4472-9.
43. Cristina M. SABO, Dan L. DUMITRASCU， Microbiota and the irritable bowel syndrome [J], Minerva Gastroenterology 2021 December;67(4):377-84DOI: 10.23736/S2724-5985.21.02923-5.
44. Uusitupa HM, Rasinkangas P, Lehtinen MJ, et al. Bifidobacterium animalis subsp. lactis 420 for Metabolic Health: Review of the Research. Nutrients. 2020 Mar 25;12(4):892. doi: 10.3390/nu12040892.
45. Manabe Y, Ishibashi T, Asano R, et al. Gut dysbiosis is associated with aortic aneurysm formation and progression in Takayasu arteritis. Arthritis Res Ther. 2023 Mar 24;25(1):46. doi: 10.1186/s13075-023-03031-9.
46. Klepinowski T, Skonieczna-Żydecka K, Pala B, et al. Gut microbiome in intracranial aneurysm growth, subarachnoid hemorrhage, and cerebral vasospasm: a systematic review with a narrative synthesis. Front Neurosci. 2023; 17: 1247151. doi: 10.3389/fnins.2023.1247151.
47. Kawabata S, Takagaki M, Nakamura H, et al. Dysbiosis of Gut Microbiome Is Associated With Rupture of Cerebral Aneurysms. Stroke. 2022 Mar;53(3):895-903. doi: 10.1161/STROKEAHA.121.034792.
48. Karbach SH, Schönfelder T, Brandão I, et al. Gut microbiota promote angiotensin II–induced arterial hypertension and vascular dysfunction. J. Am. Heart Assoc. 2016; 5: e003698. doi: 10.1161/JAHA.116.003698
49. Paulos CM, Wrzesinski C, Kaiser A, et al. Microbial translocation augments the function of adoptively transferredself/tumor-specific CD8 + T cells via TLR4 signaling. J. Clin. Investig. 2007; 117: 2197–2204. doi: 10.1172/JCI32205