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Causal role of gut microbiota in intracranial aneurysm: evidence from a Mendelian randomization study

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Abstract. – OBJECTIVE: An increasing number of studies suggest that the alteration of gut microbiota may affect the pathogenesis of intracranial aneurysm (IA). However, the exact causal relationship between gut microbiota and IA has not been confirmed.

MATERIALS AND METHODS: The instrumental variables (IVs) for gut microbiota were obtained from a meta-analysis of a genome-wide association study (GWAS) conducted by the MiBioGen consortium (n = 13,266). The summary of GWAS data for IA was obtained from a large genome-wide meta-analysis involving 23 cohorts. Five Mendelian randomization (MR) methods were used to investigate the causal relationship between gut microbiota and IA (ruptured and unruptured), unruptured intracranial aneurysm only (uIA), and aneurysmal subarachnoid hemorrhage (aSAH) respectively, with inverse variance weighted (IVW) as the main MR method. All MR results were verified through sensitive analyses.

RESULTS: Based on the results of the IVW analyses, it was found that five gut microbiota taxa were causally associated with IA (ruptured and unruptured), seven gut microbiota taxa were causally associated with uIA, and six gut microbiota taxa were causally associated with aSAH. Among these taxa, the *genus Bilophila* was the only one identified to have significant protective effects against IA (ruptured and unruptured), uIA, and aSAH. The sensitivity analysis did not reveal any significant heterogeneity or horizontal pleiotropy among the included IVs.

CONCLUSIONS: MR analyses identified several gut microbiota taxa that have a causal relationship with IA. Future research should prioritize understanding the mechanisms underlying this causal relationship, as it is expected to contribute to the development of new methods for predicting and treating IA.

Key Words:
Gut microbiota, Intracranial aneurysm, Mendelian randomization, Causation.

Introduction

Intracranial aneurysm (IA) is an abnormal dilatation that occurs in the walls of intracranial arteries and is the first cause of subarachnoid hemorrhage (SAH)[1]. With an overall prevalence of 3.2%, IA has become a significant healthcare burden worldwide[2]. The formation of IA is a gradual process that is not yet fully understood. Hemodynamic factors and vascular risk factors, including hypertension, atherosclerosis, disorders of lipid metabolism, and smoking, have been shown in numerous studies[3-6] to be involved in the pathogenesis and development of IA. A Nordic Twin Study[7] showed that genetic susceptibility plays an important role in the development of IA, with genetic factors playing a 41% role in the development of aneurysmal subarachnoid hemorrhage (aSAH). This implies that environmental factors also play a key role in the pathogenesis of IA.

Several studies[8-10] have shown that one of the major risk factors for cerebrovascular disease is diet. Gut microbiota is a mediator in dietary influences on the pathogenesis of cerebrovascular disease[11-13]. The gut microbiota may influence the course of cerebrovascular disease by regulating metabolism and immunity[14,15]. Whether the presence of gut microbiota affects the formation of IA was explored in an animal study conducted by Shikata et al[16]. This study found that depleting the

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gut microbiota in mice with antibiotics reduced the incidence of IA. In a Metagenome-wide association study (MWAS), Li et al[17] found that the abundance of 47 gut microbiota species differed significantly between the unruptured intracranial aneurysm (uIA) and controls in a cohort. This study[17] also found that changes in the abundance of *Hungatella hathewayi* affected the occurrence of uIA by fecal transplantation into mice.

Understanding the role of gut microbiota in the occurrence and development of IA may inspire new ways in the prevention and treatment of IA. However, there is still a lack of research on the relationship between gut microbiota and IA in humans. Meanwhile, it is extremely costly and difficult to fully specify which gut microbiota taxa are protective or risk factors for IA in human or animal investigations. Mendelian randomization (MR) is considered a promising approach to identifying the causal relationship between gut microbiota and IA. MR analysis relies on three assumptions, and genetic variations are used as instrumental variables (IVs) to explore the causal relationship between exposure and outcome[18]. The key advantage of MR is that it utilizes the principle that genotypes are randomly assigned from parent to offspring, which means that confounding factors are excluded from influencing the relationship between genetic variation and outcome[19]. This makes it possible to rationally determine causal sequences. The largest cross-ancestry genome-wide relationship studies (GWAS) meta-analysis of the IA to date was performed by Bakker et al[20] in 2020. This GWAS study also included cases with both uIA and aSAHs (ruptured intracranial aneurysms). MiBioGen consortium[21] released gut microbiota GWAS data in 2021 based on 18,340 individuals (24 cohorts). Based on publicly available GWAS data on gut microbiota and IA, we conducted a two-sample MR study to determine the causal association between gut microbiota and IA.

Materials and Methods

Study Design

A two-sample MR framework was used to determine the causal relationship between gut microbiota and IA. Based on the data characteristics of gut microbiota GWAS, MR analysis was performed at five gut microbiota taxa levels (phylum, class, order, family, and genus). Figure 1 shows the MR assumptions and the full flow chart of our MR study.

Data Sources

The genetic information of gut microbiota was obtained from the GWAS performed by the MiBioGen consortium. This GWAS study involved 24 cohorts and 18,340 participants, with the majority (n = 13,266) having European ancestry. The taxonomic classification in this analysis was conducted by analyzing the microbial composition and through direct taxonomic binning. A microbiota quantitative trait loci (mbQTL) mapping analysis was conducted to identify host genetic variants associated with the abundance levels of bacterial taxa in the gut microbiota. The GWAS data included a total of 211 taxa from 9 phyla, 16 classes, 20 orders, 35 families, and 131 genera. Therefore, our MR study was performed on these 211 gut microbiota taxa. Additional details about the GWAS data of gut microbiota can be found in the cited literature[21], and the comprehensive GWAS data is available at https://mibiogen.gcc.rug.nl/.

The summary of GWAS data for IA was obtained from a large genome-wide meta-analysis[22], including individual-level genotypes from 23 different cohorts, which were merged into 9 European-ancestry strata based on genotyping platform and country. Each stratum was then analyzed using a logistic mixed model, and the results were meta-analyzed. A total of 7,495 cases and 71,934 controls were included in this meta-analysis, with 4,471,083 single nucleotide polymorphisms (SNPs) passing quality control (QC) thresholds. Then, stratified GWAS analysis was performed to assess whether genetic risk factors differed between ruptured intracranial aneurysms and unruptured intracranial aneurysms (aSAH *vs.* uIA). In our study, we conducted MR analyses between gut microbiota and IA (ruptured and unruptured), gut microbiota and uIA, and gut microbiota and aSAH, based on three IA-associated GWAS data. For more detailed information on GWAS data of IA, please refer to the original literature[20].

Selection of IVs

The selection of IVs was guided by the following criteria: (1) Due to the limited number of SNPs meeting the genome-wide significance threshold of $p < 5\times10^{-8}$, a threshold of $p < 1\times10^{-5}$ was used to screen potential IVs in our study[23-25]; (2) Linkage disequilibrium (LD) analysis was performed based on the European-based 1,000 Genome Projects using a clumping distance of 10,000 kb and a threshold of $r^2 < 0.001$, and SNPs failing to meet these

requirements were excluded; (3) Palindromic SNPs were eliminated to avoid the influence of alleles.

Statistical Analysis

Our MR study employed several high-efficiency methods, including inverse variance weighted (IVW), MR-Egger, weighted median, weighted mode, and simple mode, to establish the causality between gut microbiota and IA, and the IVW method was considered the main method of MR analysis. The IVW method involves converting the outcome effects of instrumental variables into a weighted regression on the exposure effects. In IVW analysis, the intercept is constrained to zero[26]. By mitigating the influence of specific confounders, IVW can provide unbiased estimates even in the absence of horizontal pleiotropy. The MR-Egger is influenced by outlying IVs and may result in inaccurate estimates[27]. However, even if all the selected IVs are invalid, the MR-Egger method can still provide unbiased estimates for causality. The weighted median method outperforms MR-Egger in terms of the accuracy of outcomes. It can reliably predict causal effects even if up to 50% of the information in the study is derived from erroneous IVs. The validity of the weighted mode approach remains intact even when other IVs do not meet the requirements of the MR method[28]. The simple mode, serving as an unweighted alternative, utilizes the empirical density function to estimate the causal relationship[29]. The statistical tests to estimate causal relationships were regarded as statistically significant at a $p < 0.05$.

The heterogeneity of the chosen SNPs was examined through Cochran's Q statistic. Directional horizontal pleiotropy was assessed by intercept

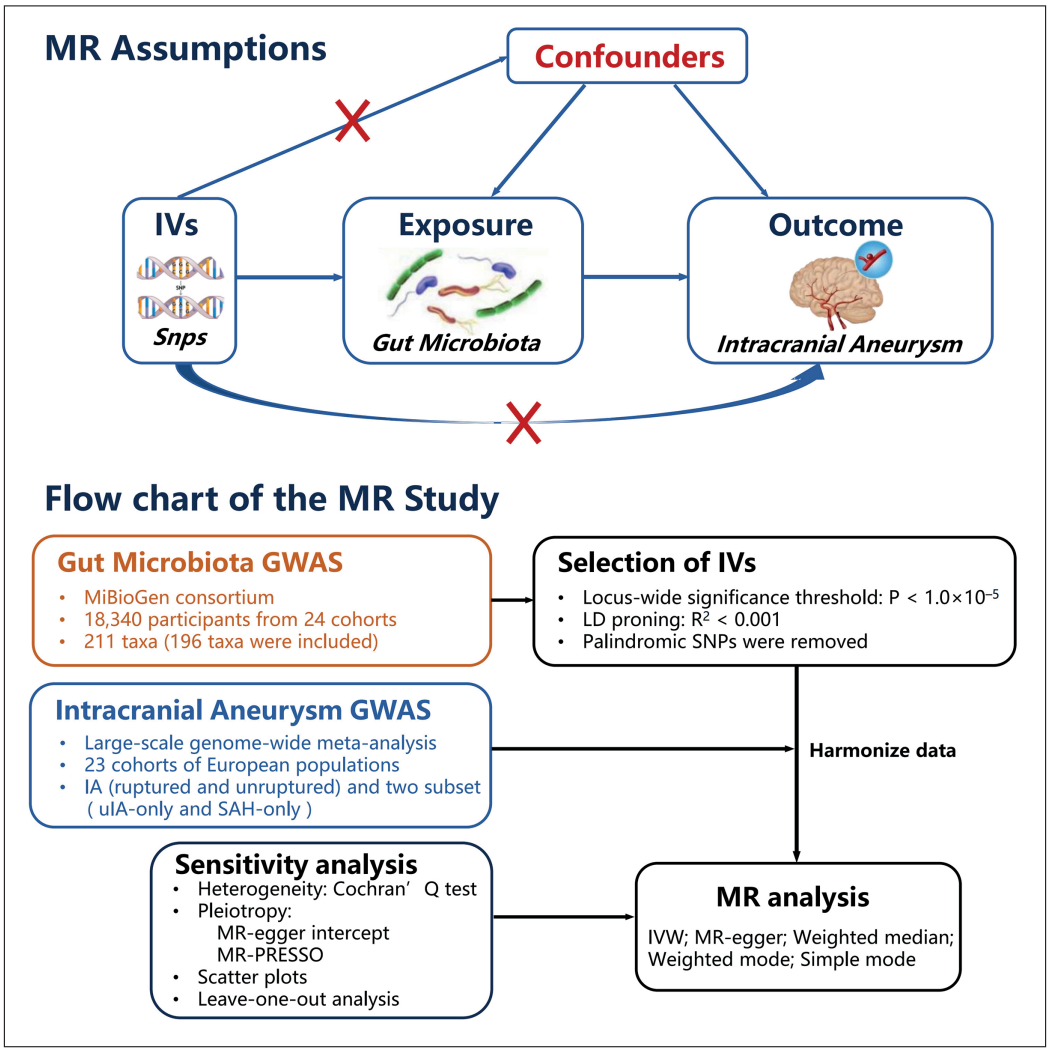

Figure 1. Overview of MR basic assumptions and flow chart of the present MR study.

term in MR-egger of the association between gut microbiota and outcomes. MR-Pleiotropy RESidual Sum and Outlier (MR-PRESSO) test was used to identify the outliers that may indicate pleiotropic biases. We also conducted a leave-one-out sensitivity analysis to confirm the reliability and stability of the IVs for causal effect estimates. F-statistic was calculated to assess the strength of IVs using the formula $F=Beta^2/SE^2$. The threshold for the absence of significant weak instrumental bias is an F-statistic for the corresponding IV greater than 10^{30}. *p*-value was set at 0.05 for statistical significance for both pleiotropy and heterogeneity tests.

R program (version 4.3.1, Bristol, UK) was used for statistical analysis.

Results

A total of 196 gut microbiota taxa were included in the MR study investigating causality with IA (ruptured and unruptured), uIA, and aSAH. Fifteen gut microbiota taxa were excluded due to insufficient validated SNPs in the IVW analyses or MR-PRESSO test. All SNPs used in the study were not identified as weak IVs. The F-statistic of the included SNPs ranged from 14.59 to 36.57.

MR Analysis Between Gut Microbiota and IA (Ruptured and Unruptured)

In the MR analysis of the 196 gut microbiota taxa with IA (ruptured and unruptured), a total of 1,530 SNPs met the selection criteria for IVs. All SNPs were not identified as weak IVs.

A total of five gut microbiota taxa were identified as causally related to IA (ruptured and unruptured). Among these taxa, three bacteria were found to be protective factors, while two were identified as risk factors ($p < 0.05$). The IVW analysis showed that family *Porphyromonadaceae* (OR, 0.60; 95% CI, 0.44 - 0.83; $p = 1.67\times10^{-3}$), genus *Bilophila* (OR, 0.66; 95% CI, 0.50 - 0.86; $p = 2.10 \times 10^{-3}$) and genus *Ruminococcus1* (OR, 0.62; 95% CI, 0.41 - 0.94; $p = 2.49 \times 10^{-2}$) had protective effects on IA (ruptured and unruptured). Meanwhile, the results of IVW showed that the risk gut microbiota taxa were family *Streptococcaceae* (OR, 1.30; 95% CI, 1.04 - 1.62; $p = 2.13 \times 10^{-2}$) and genus *Prevotella7* (OR, 1.18; 95% CI, 1.02 - 1.37; $p = 2.94 \times 10^{-2}$). Figure 2 presents the outcomes of the MR analysis examining the causal effects of the five gut microbiota taxa on IA (ruptured and unruptured).

MR Analysis Between Gut Microbiota and uIA

In the MR analysis of the 196 gut microbiota taxa with uIA, a total of 1,523 SNPs met the selection criteria for IVs.

The IVW method identified seven gut microbiota taxa having causal relationships with uIA ($p < 0.05$). Six gut microbiota taxa were risk factors, and one was a protective factor for uIA. Gut microbiota taxa with protective effects include genus *Adlercreutzia* (OR, 1.82; 95% CI, 1.15 - 2.88; $p = 1.09 \times 10^{-2}$), class *Clostridia* (OR, 2.26; 95% CI, 1.13 - 4.51; $p = 2.05 \times 10^{-2}$), genus *Intestinimonas* (OR, 1.47; 95% CI, 1.04 - 2.07; $p = 2.78 \times 10^{-2}$), genus *Intestinimonas* (OR, 1.99; 95% CI, 1.05 - 3.78; $p = 3.58 \times 10^{-2}$), family *Oxalobacteraceae* (OR, 1.34; 95% CI, 1.01 - 1.76; $p = 4.04 \times 10^{-2}$) and genus *Victivallis* (OR, 1.38; 95% CI, 1.01 - 1.88; $p = 4.37 \times 10^{-2}$). Genus *Bilophila* (OR, 0.54; 95% CI, 0.31 - 0.94; $p = 2.85 \times 10^{-2}$) was identified as a risk factor for uIA. Figure 3 presents the outcomes of the MR analysis examining the causal effects of the seven gut microbiota taxa on uIA.

MR Analysis Between Gut Microbiota and aSAH

In the MR analysis of the 196 gut microbiota taxa with aSAH, a total of 1,530 SNPs met the selection criteria for IVs.

The IVW analysis identified six gut microbiota taxa that were protective against aSAH ($p < 0.05$), but no gut microbiota taxa were found to be risk factors. The six gut microbiota taxa include genus *Ruminococcus1* (OR, 0.51; 95% CI, 0.3 - 0.84; $p = 7.33 \times 10^{-3}$), genus *Bilophila* (OR, 0.68; 95% CI, 0.50 - 0.94; $p = 1.67 \times 10^{-2}$), family *Porphyromonadaceae* (OR, 0.64; 95% CI, 0.43 - 0.95; $p = 2.51 \times 10^{-2}$), class *Lentisphaeria* (OR, 0.79; 95% CI, 0.62 - 0.99; $p = 4.73 \times 10^{-2}$) and order *Victivallales* (OR, 0.79; 95% CI, 0.62 - 0.99; $p = 4.73 \times 10^{-2}$). Figure 4 presents the outcomes of the MR analysis examining the causal effects of the five gut microbiota taxa on aSAH.

To gain further insights into the role of gut microbiota in various outcomes, we have performed a summary of all the gut microbiota taxa that have been causally associated with IA (ruptured and unruptured), uIA, and aSAH. Notably, the genus *Bilophila* demonstrated significant protective effects against IA (ruptured and unruptured), uIA, and aSAH. Additionally, the family *Porphyromonadaceae* and the genus *Ruminococcus1* exhibited significant protective effects on IA

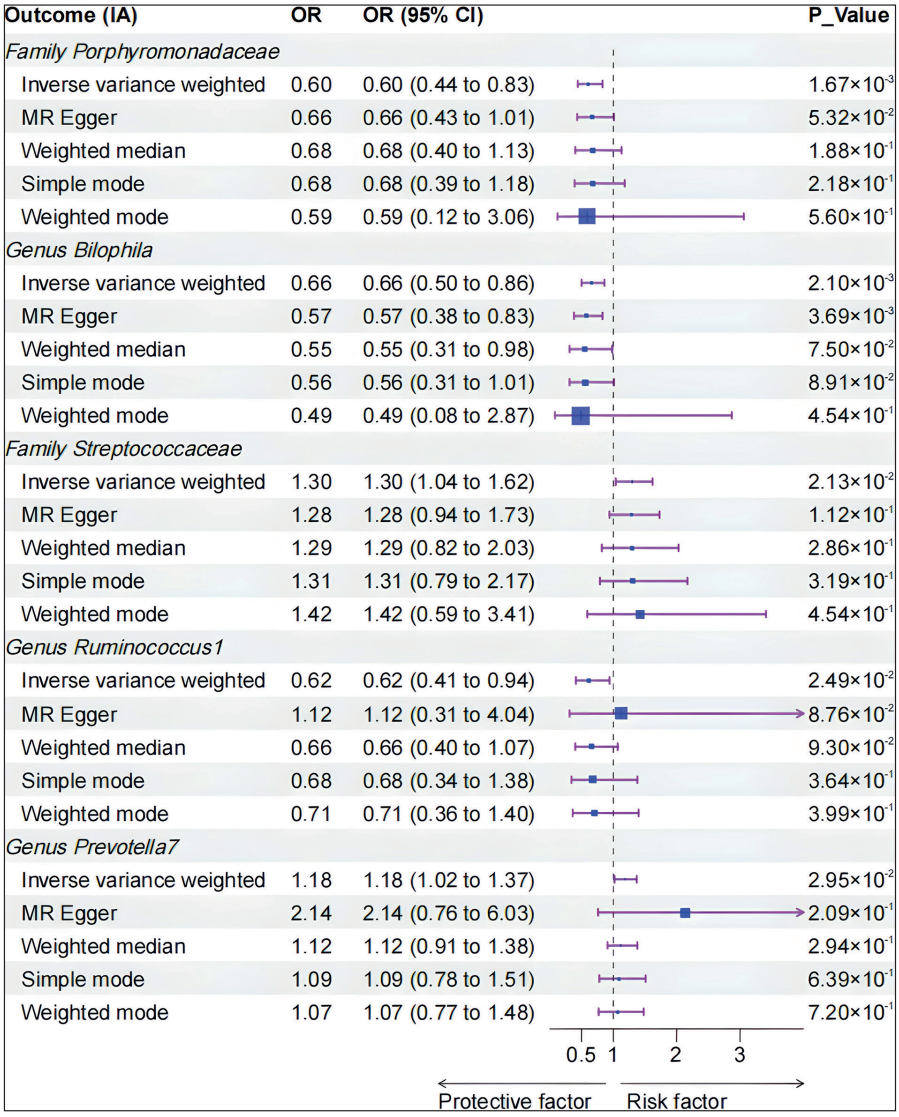

Figure 2. The results of MR analysis on the causal effect of the gut microbiota on IA (ruptured and unruptured) ($p < 0.05$ in IVM method).

(ruptured and unruptured) and aSAH. Figure 5 provides an overview of these findings.

Sensitivity Analyses

In the MR analyses of gut microbiota associated with IA (ruptured and unruptured), uIA, and aSAH, Cochran's Q test did not find significant heterogeneity in the IVs associated with the positive gut microbiota taxa ($p > 0.05$). MR-Egger regression intercept analysis indicated no significant directional horizontal pleiotropy and no significant outliers were found in the MR-PRESSO analysis ($p > 0.05$). The visual inspection in scatter plots and leave-one-out plots of the IVs did not show any potential outliers for all positive gut microbiota taxa (**Supplementary Figures 1-6**). Overall, the sensitivity analysis demonstrated that the MR results were unbiased, robust, and reliable.

Discussion

Over the past decade, gut microbiota in gut-brain communication has attracted more and more attention in scientific research. A large number of studies[31-33] have suggested that there is a strong connection between gut microbiota and the brain. For example, specific gut microbiota taxa

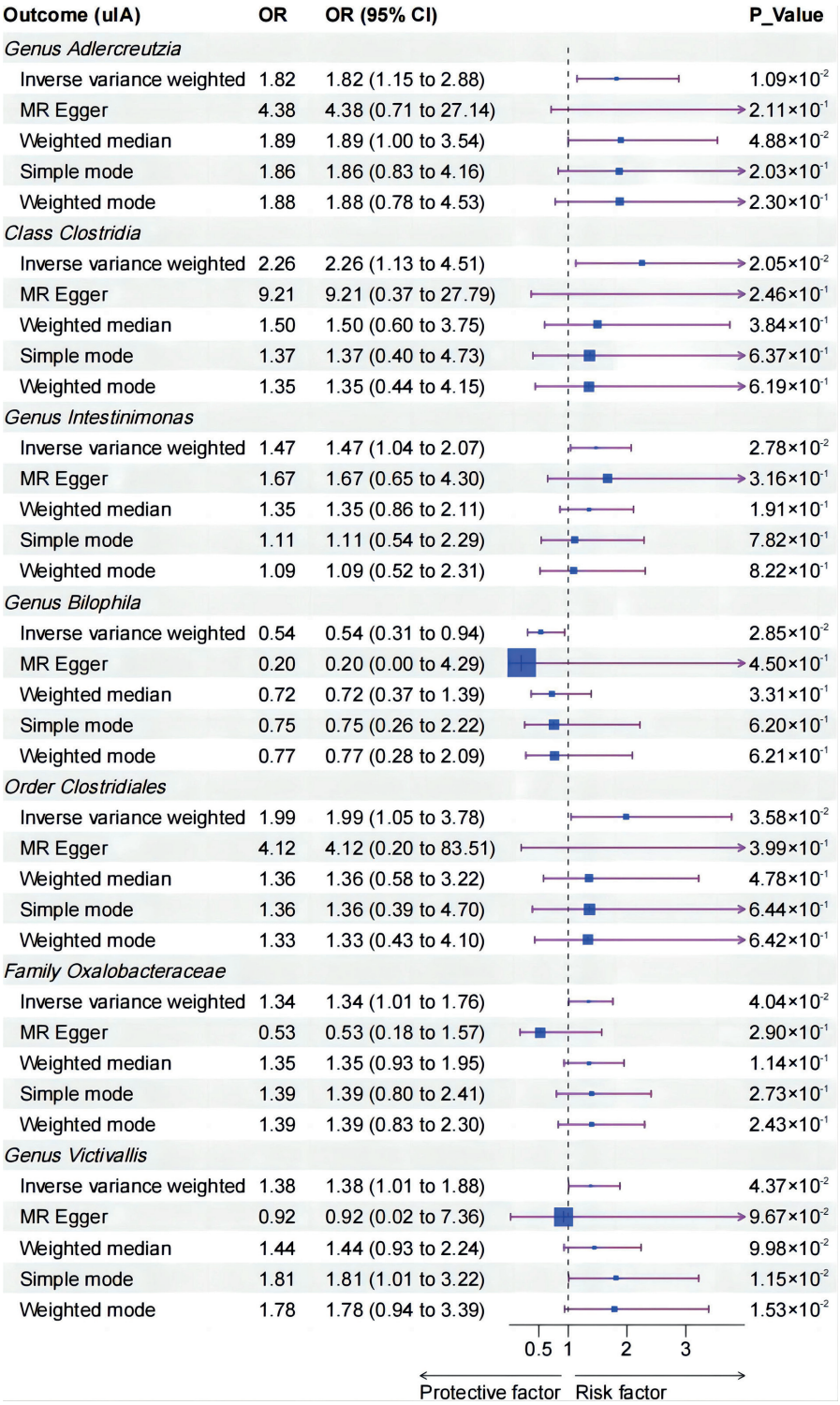

Figure 3. The results of MR analysis on the causal effect of the gut microbiota on uIA ($p < 0.05$ in IVM method).

are associated with cognition, mood, and even neurodegenerative disorders in humans[34,35]. For cerebrovascular diseases, increasing evidence[8,9] has shown that the gut microbiota is a risk factor in cardiovascular diseases by influencing immune homeostasis and host metabolism. IA is the primary cause of cerebrovascular diseases; however, there is a lack of studies on the relationship

between gut microbiota and IA. Li et al[17] performed a case-control metagenome-wide association study in humans and mice and identified *Hungatella hathewayi* protecting mice against the formation and rupture of IA through regulating circulating taurine levels. The data presented by Frösen et al[36] also suggest a potential association between antibiotic use and aneurysm formation in humans. The potential role of the gut microbiota in the pathophysiology of intracranial aneurysm is further supported by clinical studies by Pyysalo et al[37] and Hallikainen et al[38].

However, there is currently no direct evidence that establishes a causal relationship between gut microbiota and IA. In our study, we conducted a Mendelian randomization (MR) analysis and

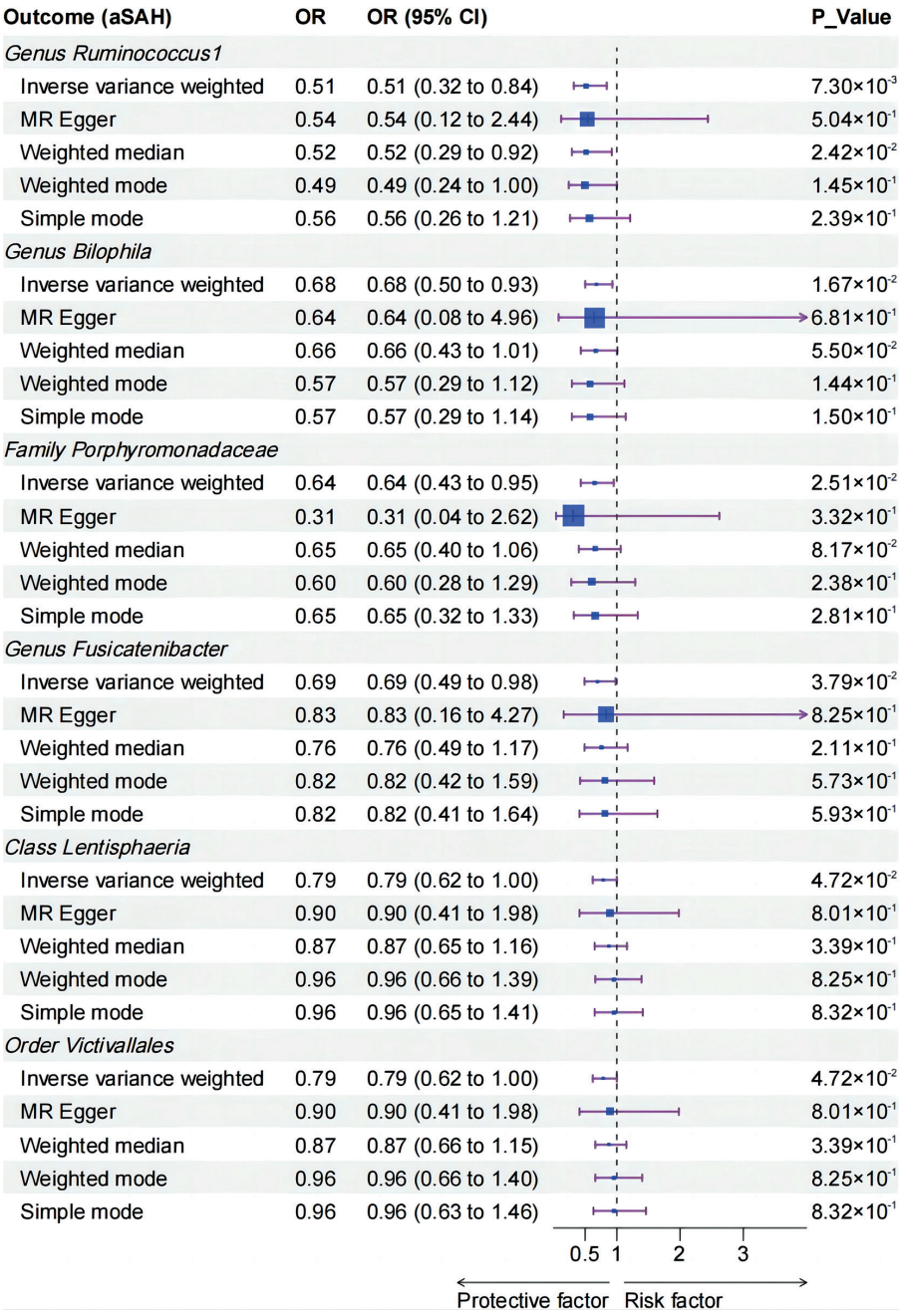

Figure 4. The results of MR analysis on the causal effect of the gut microbiota on aSAH ($p < 0.05$ in IVM method).

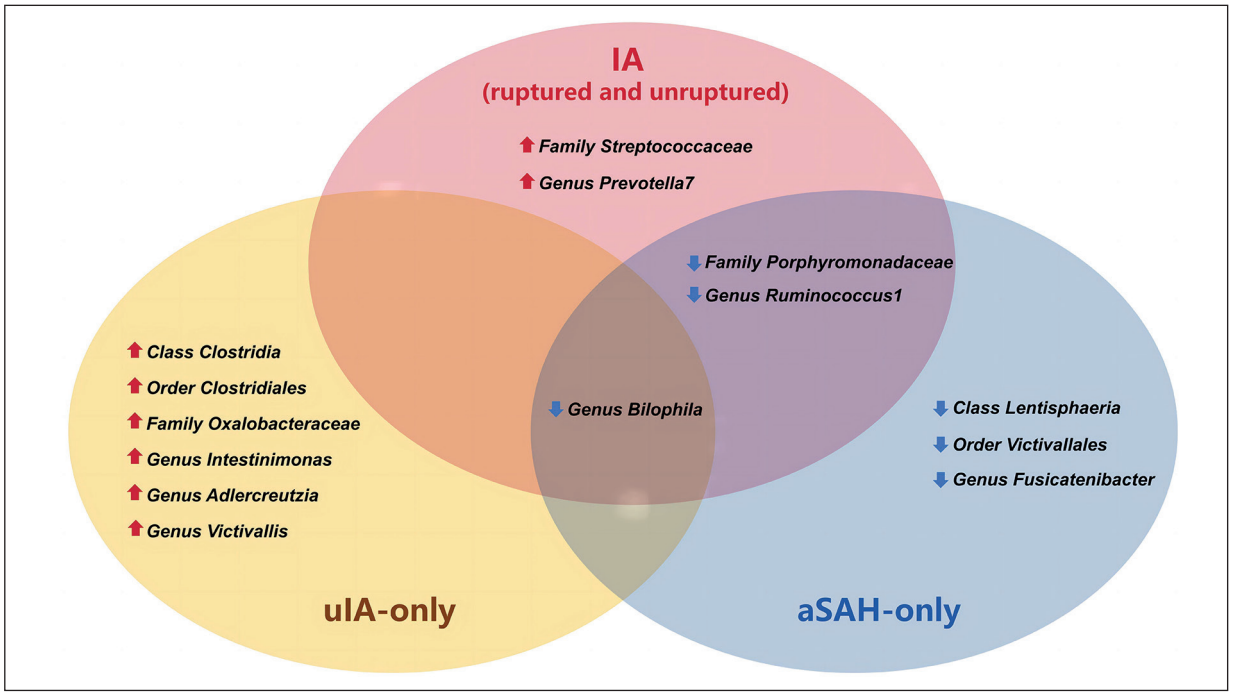

Figure 5. Summary plot of all gut microbiota taxa causally related to IA (ruptured and unruptured), uIA, and aSAH. An upward arrow indicates that the bacterium is a risk factor for the outcome, and a downward arrow indicates that the bacterium is a protective factor for the outcome.

identified several gut microbiota taxa that are causally associated with the development of IA. Ma et al[39] employed the MR method to investigate the causal relationship between gut microbiota and intracranial aneurysms. However, the GWAS data used in that study only involved uIA patients and controls. It is important to consider that intracranial aneurysms encompass both ruptured and non-ruptured states. To comprehensively explore the causal relationship between gut microbiota and intracranial aneurysms, including their specific disease states, our study utilized the largest GWAS data of intracranial aneurysms available to date. This GWAS dataset includes not only uIA-only cases but also aSAH and the summary data of both. This allows us to further identify those gut microbiota taxa that have a significant causal relationship with intracranial aneurysms (ruptured and unruptured) and aSAH, and further compare the differences between these bacteria, as shown in Figure 5. Genus *Bilophila* is a unique gram-negative anaerobic rod that was recovered from appendicitis specimens and human feces in 1989[40]. In the normal human gut microbiota, the genus *Bilophila* usually makes up less than 0.1% of the total population, including species that are resistant to bile, do not ferment sugars, and are capable of reducing sulfate[41]. In our present research, this bacterium is the only gut microbiota taxon that has been identified to have a significant causal relationship with the occurrence of IA (ruptured and unruptured), uIA, and aSAH. However, no relevant human or animal studies have found that the genus *Bilophila* is associated with the development of IA, and the specific mechanism of their causal relationship is still unclear. It is worth noting that the genus *Bilophila* was found[42-44] to be significantly altered in the development of Alzheimer's disease, Parkinson's disease and some psychiatric disorders, implicating an important role of genus *Bilophila* in the gut-brain axis.

The findings of our research indicate that various gut microbiota have distinct impacts on aneurysm rupture, although the mechanisms behind this phenomenon remain unknown. Noce et al[45]'s study suggested that the disturbance of the gut microbiota can potentially impact arterial blood vessels by influencing systemic inflammatory responses. This can lead to changes in the strength of the arterial wall, which may be a contributing factor in the occurrence and progression of intracranial aneurysms. In a study conducted by Chen et al[46], it was observed that there are differentially expressed genes in ruptured intracranial aneu-

rysms compared to non-ruptured intracranial aneurysms. These genetic variations may potentially contribute to the disparities in gut microbiota that are causally associated with the development of these two diseases. Inflammation may be another pathway for the formation and development of various aneurysms. For example, the gut microbiota has been found[47-49] to play a critical role in abdominal aortic aneurysm formation. The change of certain gut microbiota taxons markedly alleviates abdominal aortic aneurysm development by reducing neutrophil infiltration and NOX^2-dependent neutrophil extracellular trap formation[50]. Chronic inflammation has been identified[51] as a significant factor in the development of IA. Metabolic profiling revealed that changes in certain circulating metabolites showed[17] varying degrees of correlation with gut microbial species that differed in abundance between uIA patients and controls and may have a key impact on the pathophysiology of uIA development. These metabolic changes include the accumulation of stearic acid in the arterial wall and the reduction of taurine in the serum[52]. Taurine has been proven[53-55] to play a protective role in many diseases, including aortic aneurysm formation, subarachnoid hemorrhage and acute ischemic stroke, by reducing inflammatory response. Additionally, other possible mechanisms involved in the pathophysiological processes of IA include reduced extracellular matrix remodeling and maintenance of the structural integrity of cerebral arteries[51,56,57].

The identification of the causal relationship between gut microbiota and intracranial aneurysms may hold potential for therapeutic and preventive interventions for intracranial aneurysms. Gut microbiota-based treatments play an important role in many diseases, and our study may provide new insights into future intracranial aneurysm treatments. Currently, fecal transplantation and microbial agents have been instrumental in treating diseases like polycystic ovary syndrome and non-alcoholic fatty liver disease[58,59]. Specifically, the discovery of microorganisms through MR methods is anticipated to play a significant role in the treatment and prevention of intracranial aneurysms in the future.

Further investigation is required through basic, translational, and clinical studies to gain a comprehensive understanding of the potential mechanism of gut microbiota in the pathophysiology of IA. A deeper understanding of the role of gut microbiota in IA may lead to the identification of new biomarkers for predicting the formation and rupture of IA. Moreover, establishing a causal relationship between gut microbiota and IA could indicate that regulating the gut microbiota holds promise as a therapeutic approach for treating IA.

Conclusions

In the present study, we conducted an MR analysis to identify the gut microbiota taxa that are causally related to IA, including both uIA and aSAH. The findings from this MR study provide evidence for the significant role of gut microbiota in the occurrence and progression of IA, thereby expanding our understanding of the 'gut-brain axis'. Future research should focus on investigating the potential mechanisms underlying this causal relationship, which could offer valuable insights for the development of effective prevention and treatment strategies for IA.

Conflict of Interest
The authors declare that they have no conflict of interest.

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We express our gratitude to the MiBioGen consortium for providing the gut microbiota GWAS summary statistics on their online platform. Additionally, we would like to acknowledge Bakker et al[20] for their valuable contribution by sharing the GWAS data about intracranial aneurysms.

Ethics Approval
Not applicable.

Informed Consent
Not applicable.

Authors' Contribution
The study was designed by Junwei Sun and Yanbing Yu, who also analyzed the data and drafted the manuscript. Junwei Sun was responsible for analyzing and interpreting the data. Yanbing Yu revised the manuscript. The final manuscript was approved by all the authors.

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Data Availability
The supplementary materials generated during the current study are available upon request.

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