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Causal Relationship between Gut Microbiota and Pulmonary Embolism: An Analysis Using Mendelian Randomization

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Abstract

Previous research has demonstrated a connection between an unbalanced gut microbiome (GM) and lung diseases, suggesting that gut bacteria may affect lung health through the "gut-lung" axis. However, the direct connection between GM and pulmonary embolism (PE) is unclear. Mendelian randomization studies were used to investigate GM's genetic relationship with PE. A total of 18,340 independent genewide association studies (GWAS) yielded single nucleotide polymorphisms (SNPs) linked to the GM, which were then used as instrumental variables in a multiple regression analysis (MR) to examine the effect of GM on the risk of PE within the IEU Open GWAS project, which included 2,118 PE cases and 359,076 controls. The principal analytical methodology utilized in this research was inverse variance weighting (IVW), complemented by assessments for pleiotropy and heterogeneity to confirm the results' resilience. The findings of this study are predominantly derived from the IVW method, providing evidence for causal associations between four distinct genera of GM and the risk of PE. Specifically, our analysis suggests that *Slackia* ($p = 0.031$), *Oscillospira* ($p = 0.038$), *Bacteroides* ($p = 0.032$), and *Clostridium sensu stricto 1* ($p = 0.049$) may be linked to a decreased likelihood of developing PE. Importantly, our analysis yielded no evidence of heterogeneity or pleiotropy. In this MR study, we have established through genetic analysis that specific GM are significantly involved in the development of PE, underscoring the connection between the gut-lung axis and suggesting avenues for future research into the impact of GM on PE.

Keywords: gut microbiota, pulmonary embolism, Mendelian randomization, gut-lung axis, causality

Introduction

Venous thromboembolism (VTE) encompasses pulmonary embolism (PE) and deep vein thrombosis. A sudden onset characterizes PE and carries the potential for fatality, with limited options for early detection and management of changeable risk factors. The pathogenesis of PE is multifaceted, involving a mix of environmental and genetic risk factors, with inflammation having a significant impact on the advancement of this condition (Lutsey and Zakai 2023). Inflammation serves to activate endothelial cells, platelets, and white blood cells, thereby initiating clotting processes and contributing to the development of coagulation disorders as well as increased pro-inflammatory cytokine levels, chemokines, and diverse white cell subtypes (Saghazadeh and Rezaei 2016). The gut microbiome (GM) is connected to the etiology of numerous in-

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flammatory disorders, with intestinal dysbiosis being linked to an increased prevalence of venous thromboembolism (Kappelman et al. 2011; Freuer et al. 2022). The dysregulation of GM can induce the involvement of innate immune cells, platelets, and vascular endothelial cells in inflammatory pathways, which release multiple clotting proteins and promote a pro-thrombotic state (Lutsey and Zakai 2023). Concurrently, recent research has extensively investigated the "gut-lung axis" relationship, revealing additional connections between intestinal flora metabolites and alterations in pulmonary function. Data indicates that the composition of the gut microbiome influences the pathogenesis of long-term pulmonary obstruction (Hasan et al. 2020) and asthma (Barcik et al. 2020) through its regulatory impact on inflammation. While there is a known correlation between intestinal dysbiosis and a heightened risk of VTE, the specific molecular pathways through which gut microbiota contribute to the development of VTE, particularly PE, have not yet been thoroughly investigated.

After acute myocardial infarction and stroke, VTE, which includes PE and deep vein thrombosis, is the third most common vascular disease, with an estimated 10 million cases annually, posing a significant global health challenge (Raskob et al. 2014). PE, a frequently encountered and potentially life-threatening condition in the emergency department, typically results in mortality within a short timeframe post-diagnosis. PE can lead to a range of pathophysiological disorders, with clinical thrombosis resulting from of vascular wall damage, alterations in blood flow, and abnormalities in blood components (Ballard et al. 2012). The development of PE is intricate and is impacted by a confluence of environmental and genetic variables. Increasing data indicates that inflammation contributes significantly to the risk of developing PE (Zhang et al. 2023). Inflammation not only triggers the coagulation process, but also contributes to coagulation dysfunction and an elevation in pro-inflammatory cytokines, chemokines, and different types of white blood cells. Activated white blood cells are the primary origin of tissue factor positive procoagulant microparticles, which have the potential to induce thrombus formation and growth (Zhang et al. 2023). Despite significant progress in identifying and treating lung embolism, further research is warranted to identify modifiable risk factors in a timely manner for effective prevention and treatment. The GM is known to perform a significant role in the pathogenesis of obesity, sepsis/infection, inflammatory bowel disease, and intestinal failure and has been associated with a higher risk of VTE (Miehsler et al. 2004; Ley et al. 2006; Kaplan et al. 2015; Gonzalez-Hernandez et al. 2016). Disruption of the GM by environmental or genetic influences can trigger inflammatory pathways in innate immune cells, platelets, and vascular endothelial cells, which causes coagulation protein to be released and promotes an inflammatory response, leading to a prothrombotic state (Zhu et al. 2016; Jäckel et al. 2017). Research has demonstrated that dysregulation of the GM can result in cellular dysfunction and tissue damage, which are collectively referred to as lesions in multiple organs, including the lungs, a phenomenon known as the gastrointestinal-lung axis (Budden et al. 2017). The gastrointestinal-lung axis is a bidirectional communication system between the gut and the lungs, which is mainly mediated by the immune, nervous, and circulatory systems. In the gut, the normal gut microbiota plays a crucial role in maintaining intestinal homeostasis. When the gut microbiota is dysregulated, such as antibiotic use, diet changes, or infections, it can lead to increased intestinal permeability. This allows endotoxins, such as lipopolysaccharides, and microbial metabolites to translocate from the gut into the bloodstream. Once in the circulation, these substances can reach the lungs via the systemic circulation. In the lungs, they may trigger an inflammatory response, affecting pulmonary function and potentially contributing to the development of lung diseases. Additionally, gut microbiota changes can activate the immune cells in the gut-associated lymphoid tissue. These activated immune cells can then circulate to the lungs through the bloodstream and lymphatic system, modulating the local immune response. Conversely, lung inflammation can also impact the gut microbiota. For instance, cytokines and other inflammatory mediators produced in the lungs during a respiratory infection can reach the gut through circulation, altering the composition and function of the gut microbiota. As such, this axis helps hormones, cytokines, endotoxins, and microbial metabolites enter the bloodstream (Dang and Marsland 2019). These results offer insight into the association between gut microbiota and thromboembolism, suggesting a potential link to PE. Nevertheless, it is unclear how gut microbiota contributes to PE pathogenesis, and this mechanism remains incompletely understood.

The Mendelian randomization (MR) approach utilizes integrated genome-wide association studies (GWAS) data to identify suitable single nucleotide polymorphisms (SNPs) serving as useful variables (IV) in evaluating the causal association among an exposure

combined with a result (Birney 2022). MR employs random allocation in allelic inheritance, mitigates the impact of confounding variables, and does not alter the genetic sequence of individuals, facilitating the investigation of the cause-and-effect link between GM and PE via MR research. MR methods have been utilized to evaluate potential causal associations between the gut microbiota (GMS) and lung conditions such as chronic obstructive pulmonary disease, asthma, and thromboembolism (Hasan et al. 2020; Freuer et al. 2022; Wei et al. 2023). However, while several recent studies have explored the relationship between gut microbiota and venous thromboembolism (Wang et al. 2023; Meng et al. 2024; Xi et al. 2024), the specific causal relationship between gut microbiota and PE using MR analysis warrants further investigation.

This research chose GM as the independent variable and PE as the dependent variable in a Mendelian randomization analysis. The study assessed the possible reason for the association between GM and PE through the lens of host genetics, offering a foundational framework for future investigations into the intricate mechanisms underlying PE. Furthermore, the identification of specific GM profiles in PE patients may lead to the finding of new biomarkers as well as the growth of improved diagnostic and therapeutic approaches.

Experimental

Materials and Methods

Study design. Two-sample Mendelian randomization examined the causal relationship between GM and PE. To mitigate the influence of confounding variables on the findings, the Mendelian randomization analysis followed three primary presumptions: i) IVs were exclusively linked to the exposure variables; ii) the selected IVs were not correlated with potential confounders (GM taxa and PE); iii) IVs could solely impact PE through GM taxa. Fig. 1 illustrates the fundamental principles underlying the relationship between GM and PE.

Sources of Exposure Data from the GWAS dataset on human gut microbiome made available by the MiBioGen collaboration, available at https://mibiogen.gcc.rug.nl. This dataset comprises genotyping and sequencing of 16S rRNA genes information from 18,340 samples, enabling investigation into the correlation between genetic diversity and GM. Through examination of GM taxa variation across diverse populations, the GWAS study identified 122,110 variation loci within 211 taxa, spanning 36 families, 131 genera, 20 orders, 9 phyla, and 16 classes (Kurilshikov et al. 2021).

We conducted a filtering process for SNPs, which were determined by earlier screening for SNP threshold. To guarantee more thorough results, SNPs with a threshold of $p < 1.0 \times 10^{-5}$ were chosen as possible IVs. The MR hypothesis was supported by the results of the linkage disequilibrium (LD) analysis ($R^2 < 0.001$, clumping distance = 10,000 kb), resulting in the removal of SNPs that did not meet the criteria. Each SNP was assessed on the F statistic, and those with $F < 10$ were excluded to prevent bias from weak instruments.

Outcome data source. The PE outcome data was taken from the Open GWAS database at IEU (https://gwas.mrcieu.ac.uk). This study utilized the UK Biobank dataset (ukb-d-I26) (Bycroft et al. 2018) in 2018, comprising 2,118 PE cases and 359,076 controls. Following the acquisition of SNP information related to exposure and outcomes, efforts were made to harmonize the data to ensure that an allele was the same for each SNP's effects on exposure and outcome variables.

MR Analysis. This study primarily utilizes the inverse variance weighting (IVW) technique as the main analytical method to evaluate the association between GM and PE, with the additional use of weighted Mode, Simple Mode, Weighted Median (WM), and MR-Egger as the four additional methods. Cochran's Q statistic was used to evaluate the heterogeneity of IVs; a $p > 0.05$ resulted in no heterogeneity. in IVs. The MR-Egger interject method was employed to identify pleiotropy within the data, with $p > 0.05$ indicating not having pleiotropy, confirming that each SNP adheres to the Mendelian randomization assumption, thus ensuring the dependability of the outcomes obtained through IVW analysis. Furthermore, Mendelian randomization-pleiotropy residual sum and outlier (MR-PRESSO) were analyzed to identify and address SNP outliers indicative of potential pleiotropic bias. An omission method sensitivity analysis was conducted to assess the effects of specific SNPs on the overarching causal estimate (Hemani et al. 2017). In summary, both MR analysis and sensitive assessment were employed to ensure the accuracy of GWAS data and the credibility of the findings.

Statistical analysis. R software v4.3.1 (R Core Team 2023) was used for data visualization and statistical analysis. "TwoSampleMR" packages for R and "MR-PRESSO" were utilized for GM and PE's causal relationship examined using Mendelian randomization analysis. Evidence of a possible causal relationship was assessed at a significance level of $p < 0.05$ relationship.

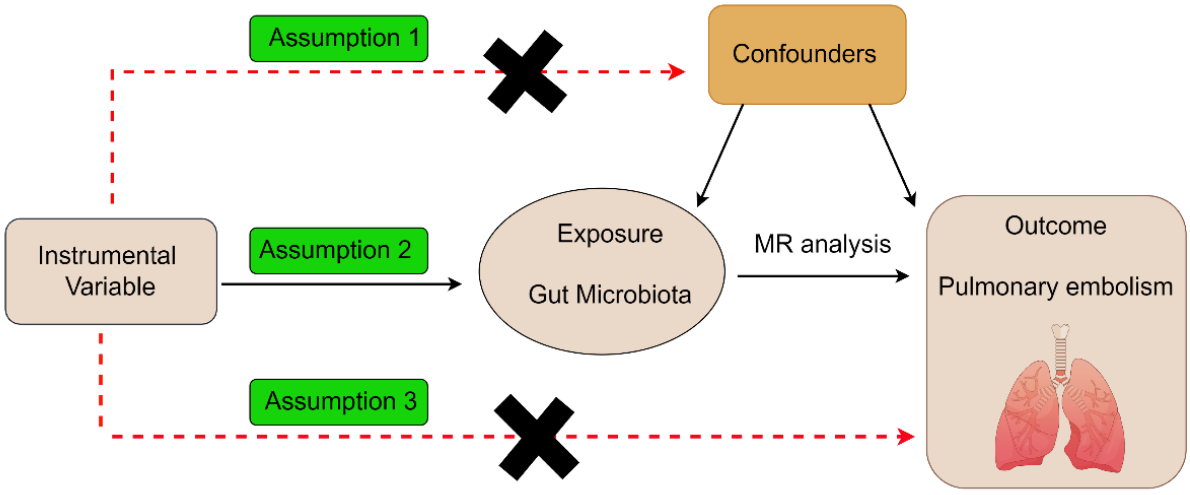

Fig. 1. Illustrates the principles of Mendelian randomization (MR) analysis and its three fundamental assumptions. The diagram was generated using Figdraw2.0 software, with the unique identifier RRSAAba96a.

Results

Screening IVs. 1,531 SNPs in all that are linked to PE were found across 211 bacterial groups using an LD test, MR-PRESSO test, *F*, and genome-wide significance threshold ($p < 1 \times 10^{-5}$) statistic verification ($F > 10$).

GM and PE: causal relationships. The IVW method was utilized to ascertain four GMs that exhibited robust causal associations with PE, as evidenced in Table I.

We found that four gut microbiota (GMS) were linked to a lower chance of PE (Fig. 2): *Slackia* (OR = 0.998, 95%CI: 0.997–1.000, $p = 0.031$), *Oscillospira* (OR = 0.998, 95%CI: 0.996–1.000, $p = 0.038$), *Bacteroides* (OR = 0.997, 95%CI: 0.995–1.000, $p = 0.032$), and *Clostridium sensu stricto 1* (OR = 0.996, 95%CI: 0.996–1.000, $p = 0.049$). Scatter plots indicate that causal approximations derived based on MBE, MR-Egger, and WM analyses are consistent but lack statistical significance (Fig. 3). This correlation implies a possible safeguarding consequence of certain bacteria

Table I
MR results of causal links between gut microbiota and pulmonary embolism risk.

Classification	SNP	SE	*p*-value	OR (95%Cl)	Pleiotropy			Heterogeneity			MR-PRESSO
					Egger intercept	SE	*p*-value	Method	Q	*p*-value	
Slackia	9	0.0007	0.031	0.998 (0.997–1.000)	0.0003	0.0003	0.424	MR-Egger	5.228	0.632	0.630
								IVW	5.948	0.653	
Oscillospira	9	0.0006	0.038	0.998 (0.996–1.000)	-7.75×10^{-5}	0.0004	0.851	MR-Egger	7.609	0.368	0.494
								IVW	7.650	0.468	
Bacteroides	11	0.0012	0.032	0.997 (0.995–1.000)	−0.0003	0.0004	0.537	MR-Egger	10.079	0.344	0.403
								IVW	10.541	0.394	
Clostridium sensu stricto 1	9	0.0009	0.049	0.998 (0.996–1.000)	−0.0001	0.0002	0.634	MR-Egger	4.684	0.698	0.794
								IVW	4.931	0.765	

SNP – single nucleotide polymorphism; SE – standard error; OR – odds ratio; Q – Cochran's Q; MR-PRESSO – Mendelian randomization pleiotropy residual sum and outlier

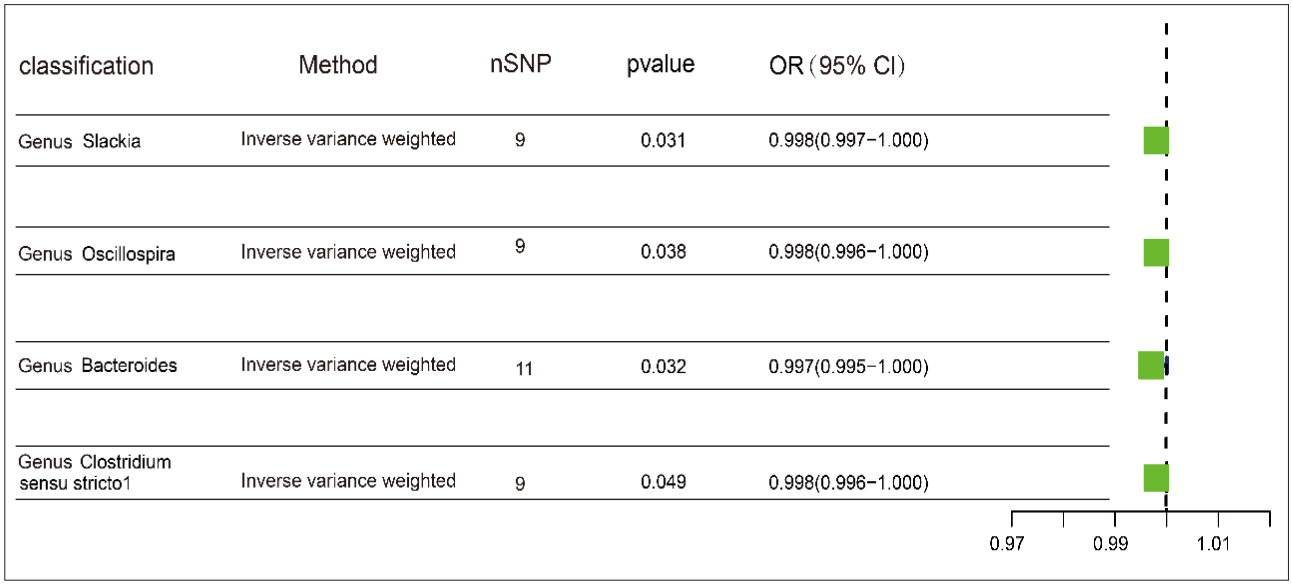

Fig. 2. The forest plot illustrates the causal relationships between four GM (*Slackia*, *Oscillospira*, *Bacteroides*, and *Clostridium sensu stricto 1*) and the risk of PE.

against PE, although no specific gut flora connected to a higher chance of developing PE was investigated. Collectively, these findings support a stable causal relationship between GM and PE, as determined by genetic factors.

Sensitivity analysis and potential pleiotropy detection. In order to mitigate potential bias, various actions were implemented to assess the MR's sensitivity analyses and identify any possible pleiotropy of instrumental variables in each phenotype in turn. According to Cochrane's Q test ($p > 0.05$), the selected SNPs showed no significant heterogeneity. The pleiotropic MR Egger test results revealed no evidence of pleiotropic effects in our Mendelian randomization study ($p > 0.05$, as shown in Table I). As a result of the MR-PRESSO analysis, no significant outliers were found ($p > 0.05$). The omission method sensitivity testing showed no fundamental difference between the results when any SNP was removed (Fig. 4). A symmetric funnel plot (Fig. 5) indicates no heterogeneity in the results.

Discussion

Recently, several MR studies (Wang et al. 2023; Meng et al. 2024; Xi et al. 2024) have indeed investigated the relationship between gut microbiota and VTE. Similar to these studies, our research also found that *Slackia* and *Bacteroides* were associated with a reduced risk of VTE. However, our study offers unique contributions. Unlike previous studies limited to specific population subsets or particular gut microbiota, we explored the associations between multiple gut microbiota genera and pulmonary embolism (PE), a specific type of VTE, in a broader context. This broader scope provides a more comprehensive view of the role of gut microbiota in VTE pathogenesis. Regarding research methods, there are differences in the selection of instrumental variables and the emphasis on analysis methods These previous studies may have used different SNP screening criteria or relied more on specific MR methods. Our study, however, comprehensively applied multiple MR methods and conducted a more thorough sensitivity analysis to ensure the reliability of the results.

This research investigated the potential causal link between GM and PE through a genetic perspective utilizing MR techniques. The study employed independent GM-related SNPs identified in GWAS as instrumental variables and evaluated their impact on PE risk using MR methodologies. Following the implementation of the IVW technique as the principal causal analysis, the results indicated that four specific GM taxa - *Slackia*, *Oscillospira*, *Bacteroides*, and *Clostridium sensu stricto 1* – correlated with a reduced likelihood of developing PE. A test of pleiotropy and heterogeneity confirmed that these findings were reliable, as no significant pleiotropy or heterogeneity was detected.

Our findings align with some recent studies that have explored the relationship between gut microbiota and pulmonary diseases. For example, similar to our results on *Oscillospira*, previous research has shown that an increase in SCFA-producing bacteria, such as *Oscillospira*, is often associated with a reduced inflammatory state in the body, which may contribute to a

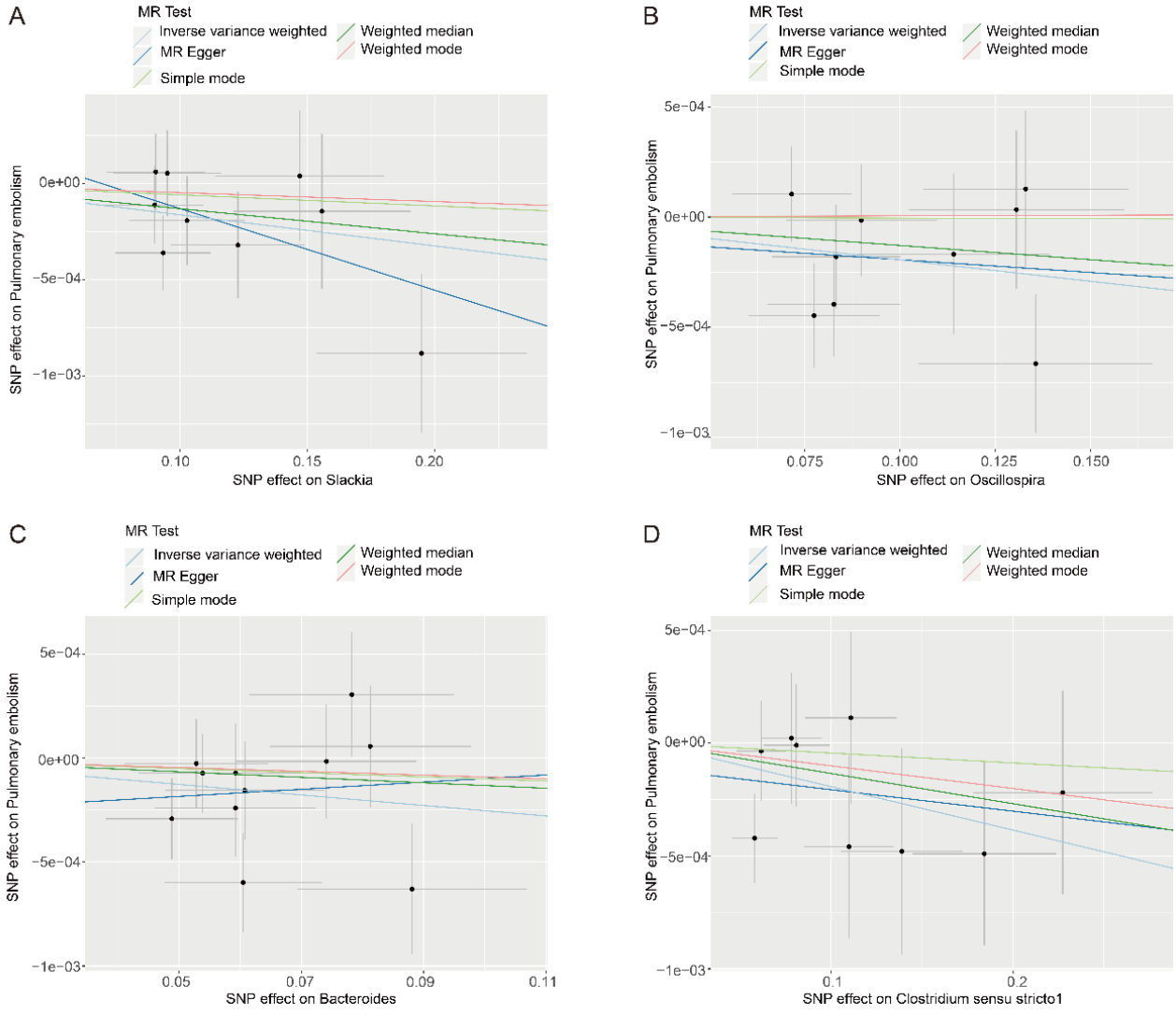

Fig. 3. The scatter plots were used to examine the correlation between four GM and the likelihood of developing PE disease: A) *Slackia*'s potential causal association with PE; B) potential causal relationship between *Oscillospira* and PE; C) the potential causal relationship between *Bacteroides* and PE; D) potential causal relationship between *Clostridium sensu stricto1* and PE.

lower risk of PE. However, it should be noted that in some other studies on lung dysfunction, the role of bacteria can be complex. Some bacteria may have a dual-edged effect, being beneficial in specific contexts but harmful in others. In our study, the inverse relationship between *Bacteroides* abundance and PE formation contradicts the general pattern in some literature where *Bacteroides* is sometimes considered a pathogen in the development of various diseases. This discrepancy might be due to differences in study populations, research methods, or the specific subsets of *Bacteroides* species studied. For instance, the mouse model in Zhang et al.'s study (2023) may not fully represent the human situation, and the diversity of *Bacteroides* species and their functions in different hosts could lead to such conflicting results.

Regarding new clinical findings, while previous studies mainly focused on the overall relationship between gut microbiota and VTE, our study specifically identified four gut microbiota genera with potential protective effects against PE. This provides more targeted information for future clinical research. For example, it may guide the development of personalized probiotic therapies. Supplementing specific bacteria such as *Slackia*, *Oscillospira*, *Bacteroides*, or *Clostridium sensu stricto 1* can modulate the gut microbiota and potentially reduce the risk of PE. This finding also enriches our understanding of the role of gut microbiota in developing PE and offers new ideas for preventing and treating this disease.

This study represents the initial Mendelian randomized investigation into the causal involvement of

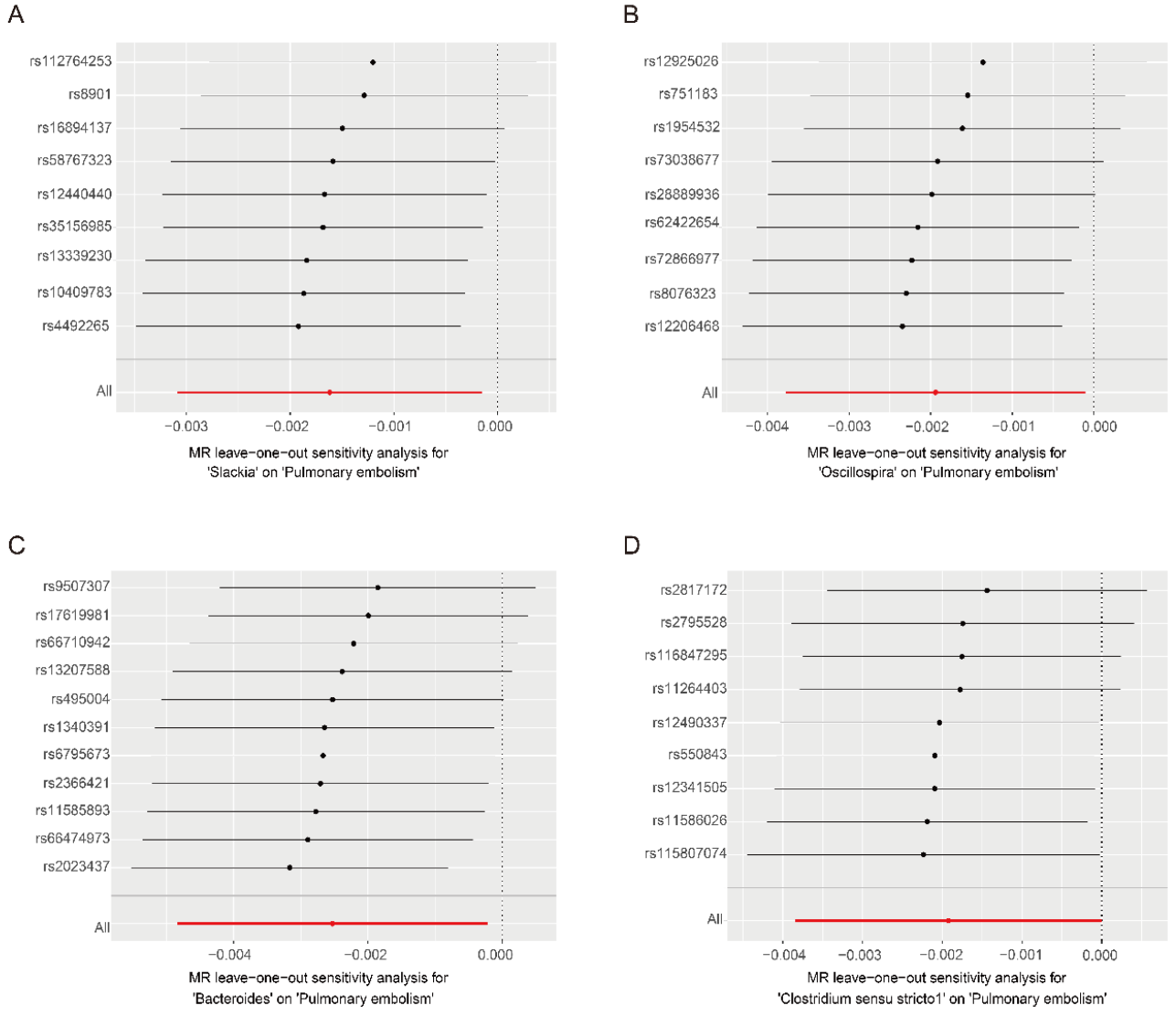

Fig. 4. The leave-one-out sensitivity analysis demonstrated that the exclusion of any single SNP did not significantly impact the outcomes.

GM in PE. Employing MR Analysis, we examined the GM taxa and possible causal relationships PE through the lens of host genetics, confirming its impact on altering susceptibility to PE. The composition of the gut microbiome is known to change during disease progression, and in the case of pulmonary embolism, it likely undergoes multiple changes as the disease develops through its earlier stages. The protective bacteria identified in our study, such as *Slackia*, *Oscillospira*, *Bacteroides*, and *Clostridium sensu stricto 1*, represent a long-term attempt by the microbiome to mitigate adverse responses within the host. This could be achieved through various mechanisms. For example, these bacteria might modulate the immune response by reducing inflammation or enhancing the host's defense mechanisms. They could also influence the production of metabolites that benefit the host's physiological state, potentially counteracting the processes that lead to PE. However, further research is needed to confirm this hypothesis. Understanding these potential regulatory mechanisms of the gut microbiome could provide new insights into the pathogenesis of PE and open up new avenues for therapeutic interventions. The protective effects of four genus levels of intestinal bacteria, namely *Slackia*, *Oscillospira*, *Bacteroides*, and *Clostridium sensu stricto 1*, on PE may offer potential biomarkers for future research. Additionally, our findings suggest the possibility of targeted dysregulation of specific gut microbiota taxa as a method for treating and preventing PE. The development of pulmonary embolism is a complex process that likely involves multiple circulatory and tissue disorders and is not solely due to the direct action of the microbiome or its metabolites. Numerous potential confounding factors could influence

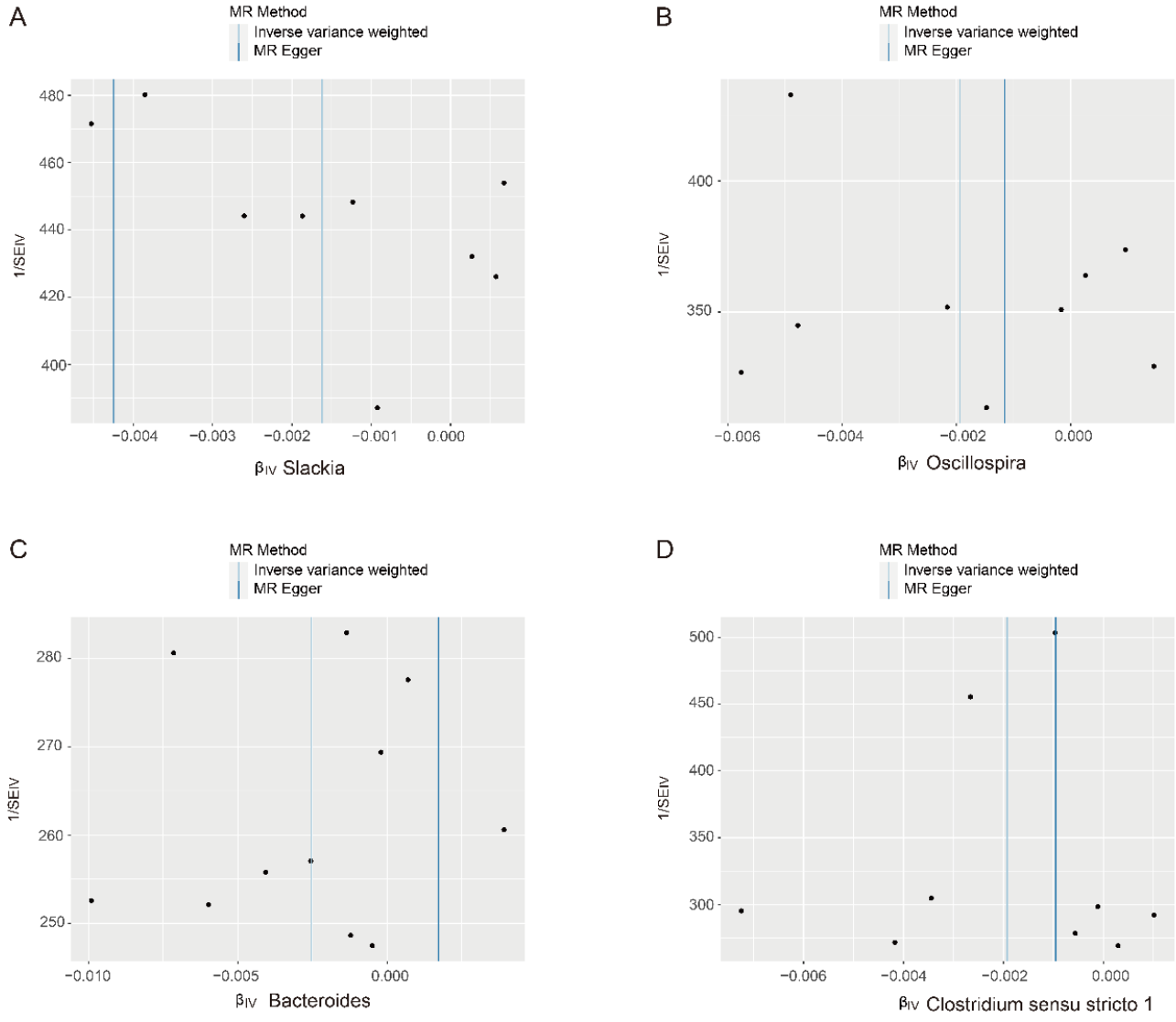

Fig. 5. The funnel plot analysis indicated the absence of heterogeneity in the experiments as confirmed by the IVW and MR-Egger tests.

the relationship between gut microbiota and PE. General health status plays a crucial role. For example, individuals with pre-existing chronic diseases may have a disrupted gut microbiota composition. At the same time, these diseases can also increase the risk of PE through various pathways, such as inflammation and coagulation disorders. Nutritional status also cannot be ignored. Malnutrition or an imbalanced diet may lead to changes in the gut microbiota, and specific nutrients (or lack thereof) can affect the body's inflammatory response and blood coagulation function, both related to PE development. Dietary habits, like a diet high in fat or low in fiber, can alter the community structure and function of the gut microbiota. A high-fat diet may promote the growth of certain bacteria associated with an increased risk of inflammation. In contrast, a low-fiber diet may reduce the production of beneficial microbial metabolites such as short-chain fatty acids, which are important for maintaining gut and overall health. These factors interact with the gut microbiota in complex ways and can significantly impact the susceptibility to PE.

Oscillospira is a significant contributor to the synthesis of fatty acids with short chains (SCFA), which serve as a crucial criterion for the evaluation of potential "Probiotics of the next generation" (Yang et al. 2021). SCFA is primarily generated via the fermentation of carbohydrates by intestinal microorganisms and is essential for controlling metabolic and immune equilibrium. Butyric acid, a product of SCFA, serves as a key component in the preservation of intestinal health and has been demonstrated to bolster the function of the intestinal barrier through the upregulation of proteins in tight junctions such as Claudin-1 and

Zonula occludens 1 (Wang et al. 2012). Numerous investigations have demonstrated a noteworthy correlation between inflammation and *Oscillospira*, with most findings indicating a negative correlation (Konikoff and Gophna 2016), aligning with our own research. Xu et al. (2021) observed a negative correlation between the profusion of *Oscillospira* and disease severity in ulcerative colitis patients, while Lima and Longman (2021) reported a decreased presence of *Oscillospira* in children's inflammatory bowel disease intestines. Nevertheless, the precise mechanism by which *Oscillospira* influences inflammation, including PE, remains uncertain.

Bacteroides, a commensal microorganism in the gut, can become opportunistic when extraintestinal translocation occurs (Zafar and Saier 2021), leading to the development of various disease conditions upon its migration into sterile tissues (Koropatkin et al. 2012). Factors contributing to this translocation include impaired immune function, intestinal barrier disruption, surgical trauma, excessive antibiotic use, and aging (Vaishnavi 2013; Archambaud et al. 2019). Zhang et al. (2023) conducted a study in which a PE mouse model was established using vancomycin to modulate the gut microbiome. The findings indicated that PE mice with reduced levels of *Bacteroides* exhibited a higher survival rate (Zhang et al. 2023). Conversely, our research revealed an inverse relationship between *Bacteroides* abundance and PE formation. This paradoxical outcome may be attributed to interspecies variations in our respective studies. Recent research has also shown that *Bacteroides* can have different effects depending on the stage of development and the overall gut microbiota composition. For example, in the context of infancy, a *Bacteroides*-dominant gut microbiota may enhance neurodevelopment, while in other situations, it could be involved in disease processes. This further emphasizes the need for a more in-depth understanding of *Bacteroides* species and their functions in different host-microbe interactions. Nevertheless, it is worth noting that not all instances of extraneous translocations result in adverse health conditions. Previous studies have indicated the significance of *Bacteroides* as a primary producer of vitamin K, a crucial nutrient synthesized by human gut bacteria (Walther et al. 2013). It can boost bone density to prevent or treat osteoporosis (Fujita et al. 2012). Tamana and colleagues (2021) discovered that in the later stages of infancy development, a gut microbiota dominated by *Bacteroides* can potentially enhance neurodevelopment. These findings indicate that some *Bacteroides* species could exhibit both pathogenic and helpful effects, contingent upon their host location. Therefore, a comprehensive understanding of the species and functions of *Bacteroides* is essential in elucidating their effects on illness and host health within the context of gut microbiome research.

The bacterial genera *Slackia* and *Clostridium sensu stricto 1* have been underrepresented in scientific literature, resulting in a limited understanding of their physiological functions and impact on host health. The study of these lesser-known genera remains a prominent focus in microbiology and medical research, with the potential for significant advancements in our knowledge of their ecological roles and health implications. Further research holds promise for a more comprehensive understanding of *Slackia* and *Clostridium sensu stricto 1* bacteria and their relevance to human health.

The research presents several strengths alongside certain constraints. These strengths include the novel utilization of two samples for MR analysis, allowing for an analysis of potential resonal relationships between GM and PE at the genus level. Additionally, as compared to previous randomized controlled studies, the gut microbiota data we used is the GWAS to date, giving the study a larger sample size. Lastly, the significant epidemiological implications of MR analysis suggest its ongoing relevance and potential expansion later on. It is important to recognize this study's limitations. Besides the issues we have already mentioned, other potential confounding factors could influence the results. For example, diet is closely related to the composition of gut microbiota. Different dietary patterns, such as high-fiber or high-fat diets, may affect the abundance and function of the gut microbiota, and at the same time, diet can also impact the body's inflammatory state and coagulation function, which are relevant to the development of PE. Lifestyle factors like exercise and smoking also cannot be ignored. Regular exercise may modulate the gut-lung axis through various mechanisms, and smoking can disrupt the balance of the gut microbiota and increase the risk of inflammation and thrombosis. However, due to the nature of our data source, these factors were not accounted for in the current analysis. In addition, it is important to rec-

ognize this study's limitations, including the following: i) While our study supports the MR hypothesis, it does not definitively eliminate the potential for weak instrument bias. ii) Incorporating individuals of European descent in the GWAS data may limit the study's findings' applicability to other ethnic populations. iii) The exposure data only classified genus, restricting the ability to analyze relationships at the species level. Insufficient IV availability for reverse MR analysis precludes the determination of the possible link of causation between GM and PE. To address the concern about the generalizability of our findings, we acquired GWAS data of PE from https://ngdc.cncb.ac.cn/omix/release/OMIX001381 and GWAS data of gut microbiota from https://db.cngb.org/search/project/CNP0000794 for Asian populations. Repeating the Mendelian randomization analysis with these Asian datasets, we found that some of the gut microbiota genera, such as *Slackia*, *Oscillospira*, *Bacteroides*, and *Clostridium sensu stricto 1* , still showed significant associations with the risk of PE in the Asian population. However, the strength of these associations differed from those observed in the European population used in our original study. These differences may be attributed to genetic, environmental, and lifestyle variations among ethnic groups. For example, genetic background can influence the composition and function of the gut microbiota, and dietary habits, which vary across ethnicities, can also play a role in modulating the gut-lung axis. This finding emphasizes the importance of researching diverse ethnic populations to understand the relationship between gut microbiota and PE fully.

While bacteria are the major component of the gut microbiome, other components such as the mycobiome, viruses, archaea, and protists can also significantly modulate circulatory and tissue dysfunction. For example, certain fungi in the gut mycobiome may interact with the immune system and influence inflammation, which is closely related to the development of PE. Viruses in the gut virome can affect the gut epithelial barrier function, potentially leading to changes in the translocation of microbial products and subsequent effects on the host's health. Archaea may contribute to the metabolism of the gut microbiota, and protists can interact with bacteria and the host immune system in complex ways. However, in the present study, we mainly focused on bacteria and did not fully consider the impact of these other microbial components. This is a limitation of our study. Future research should aim to comprehensively investigate the relationships between all components of the gut microbiome and PE to gain a more complete understanding of the underlying mechanisms.

Hence, this finding represents a preliminary investigation at the species level. Subsequent research endeavors intend to broaden the sample size and investigate the correlation between GM taxa and PE at the species level to offer enhanced theoretical backing for investigating the "gut-lung" axis mechanism.

Conclusions

Our study employs a Mendelian randomization analysis with two samples using publicly available GWAS aggregate research to establish the causal relationship among GM (specifically *Slackia*, *Oscillospira*, *Bacteroides*, and *Clostridium sensu stricto 1*) and PE. The outcomes of our investigation suggest that gut flora could significantly influence the onset and progress of PE, offering a novel understanding of the processes through which gut microbiota impacts the susceptibility to lung diseases, specifically PE.

These findings have important implications for future research and clinical practice. Regarding potential therapeutic interventions, we could consider developing probiotic-based therapies. For example, supplementing with *Slackia*, *Oscillospira*, *Bacteroides*, or *Clostridium sensu stricto 1* strains may help modulate the gut microbiota and potentially reduce the risk of PE. Additionally, dietary interventions that promote the growth of these beneficial bacteria could be explored. This might involve increasing the intake of prebiotic foods rich in fiber, which can serve as substrates for the growth of these bacteria.

On the clinical side, the identified associations could be used to develop new risk prediction models for PE. By incorporating information about an individual's gut microbiota composition, we can more accurately assess their risk of developing PE, especially in high-risk populations. This could enable earlier preventive measures, such as targeted anticoagulant therapy or lifestyle modifications.

Furthermore, these discoveries could offer a theoretical framework for advancing innovative interventions and therapies for PE targeted at enhancing or altering GM.

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Abbreviations
GM – Gut microbiome
GMS – Gut microbiota
GWAS – Genome-wide association studies
IV – instrumental variables
IVW – Inverse variance weighting
MR – Mendelian randomization
PE – Pulmonary embolism
SCFA – short-chain fatty acids
SNPs – Single nucleotide polymorphisms
VTE – Venous thromboembolism
WM – Weighted Median

Availability of data and material
The datasets generated during and/or analyzed during the current study are available in the IEU openGWAS project (https://gwas.mrcieu.ac.uk/), and the GWAS ID is ukb-d-I26. The human gut microbiome GWAS dataset provided by the MiBioGen consortium, available at https://mibiogen.gcc.rug.nl.

Ethical statement
The data utilized in this investigation were discovered exclusively from the publicly accessible GWAS-Summary Data. The present research does not necessitate ethical approval since every GWAS data used in the research is openly accessible and has been sanctioned by the relevant ethical review board. No further ethical approval is necessary for this study, as it involves the re-analysis of data that has already been made publicly accessible.

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Author contributions
Lilan Cen and Ling Qin made significant contributions to data analysis and manuscript writing; Wanling Chen was responsible for data collection; Lihua Wei, CaixiaTang and Xiang Teng conducted the visualization of correlation analysis; Zhe Tian designed the experiments and refined the manuscript.

Conflict of interest
The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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