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Abstract

Introduction
Previous observational studies have suggested a potential association between gut microbiota (GM) and venous thromboembolism (VTE), including pulmonary embolism (PE) and deep vein thrombosis (DVT). However, the causal nature of this association remains uncertain due to potential confounding factors.

Material and methods
The summary statistics for VTE, PE, and DVT were obtained from the GWAS conducted by the FinnGen consortium R9. The genetic data for relevant GM SNPs were extracted from the meta-analysis of GWAS performed by the global MiBioGen consortium. Using SNPs as instrumental variables, the IVW method was primarily employed to assess the bidirectional causal relationship between GM and VTE, PE, and DVT.

Results
For the risk of VTE onset, Candidatus Solea ferrea, Ruminococcaceae UCG002, and Ruminococcaceae UCG004 were negatively correlated, while Eubacterium hallii group, Butyricimonas, and Dorea were positively correlated. For PE, Intestinimonas, Unknown genus, and Firmicutes were negatively correlated, while Veillonella, Erysipelatoclostridium, and Lentisphaerae were positively correlated. For DVT, Mollicutes, Actinobacteria, and Bifidobacteriaceae were negatively correlated, while Adlercreutzia, Collinsella, and Desulfovibrio were positively correlated. After multiple corrections using the Bonferroni method, a significant causal relationship was identified between Ruminococcaceae and VTE. Cochran's Q test was performed to evaluate instrumental variable heterogeneity ($P > 0.05$), MR-Egger regression analyses were performed to examine pleiotropy ($P > 0.05$), and Leave-one-out analysis was conducted to assess the impact of each SNP on the outcome.

Conclusions
Specific GM may have causal effects on VTE, PE, and DVT, potentially contributing to the development of microbiota-centered therapeutic approaches and the identification of novel biomarkers for targeted preventive strategies.

Exploring the association between gut microbiota and venous thromboembolism using a Mendelian randomization analysis

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Abstract

Introduction: Previous observational studies have suggested a potential association between gut microbiota (GM) and venous thromboembolism (VTE), including pulmonary embolism (PE) and deep vein thrombosis (DVT). However, the causal nature of this association remains uncertain due to potential confounding factors.

Methods：The summary statistics for VTE, PE, and DVT were obtained from the meta-analysis of genome-wide association studies (GWAS) conducted by the FinnGen consortium R9. The genetic data for relevant GM single nucleotide polymorphisms (SNPs) were extracted from the meta-analysis of GWAS performed by the global MiBioGen consortium. Using SNPs as instrumental variables, the inverse variance weighting (IVW) method was primarily employed to assess the bidirectional causal relationship between GM and VTE, PE, and DVT.

Results: For the risk of VTE onset, *Candidatus Solea ferrea*, *Ruminococcaceae UCG002*, and *Ruminococcaceae UCG004* were negatively correlated, while *Eubacterium hallii group*, *Butyricimonas*, and *Dorea* were positively correlated. For PE, *Intestinimonas*, *Unknown genus*, and *Firmicutes* were negatively correlated, while *Veillonella*, *Erysipelatoclostridium*, and *Lentisphaerae* were positively correlated. For DVT, *Mollicutes*, *Actinobacteria*, and *Bifidobacteriaceae* were negatively correlated, while *Adlercreutzia*, *Collinsella*, and *Desulfovibrio* were positively correlated. After multiple corrections using the Bonferroni method, a significant causal relationship was identified between *Ruminococcaceae* and VTE. Cochran's Q test was performed to evaluate instrumental variable heterogeneity ($P > 0.05$), MR-Egger regression analyses were performed to examine pleiotropy ($P > 0.05$), and Leave-one-out analysis was conducted to assess the impact of

each SNP on the outcome.

Conclusion: Specific GM may have causal effects on VTE, PE, and DVT, potentially contributing to the development of microbiota-centered therapeutic approaches and the identification of novel biomarkers for targeted preventive strategies.

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Introduction

Venous thromboembolism (VTE), encompassing pulmonary embolism (PE) and deep vein thrombosis (DVT), is a serious thromboembolic disorder that remains a substantial challenge for global healthcare systems [1]. VTE is a multifactorial disease arising from the interplay between genetic and acquired risk factors, with its heritability estimated at 40% to 60% based on studies involving families, twins, and siblings [2]. A recent study indicated that the annual incidence of VTE in the USA is 123 cases per 100,000 people, with a higher rate among the elderly [3]. PE is more severe, often causing acute obstruction of the pulmonary vasculature, leading to hemodynamic instability and even death [4]. Among cardiovascular complications worldwide, DVT is the third leading cause of death and disability [5]. Therefore, ideveloping novel strategies for the prevention and treatment of VTE is essential to mitigate the socioeconomic consequences associated with its incidence and progression.

Anticoagulation therapy is the primary treatment for VTE, and delays in initiating or maintaining therapeutic levels may lead to poorer outcomes [6]. Rivaroxaban, a non-vitamin K oral anticoagulant (NOAC), is extensively employed for patients at heightened risk of thrombosis, especially VTE [7]. Preventing VTE is a crucial goal for medical professionals, requiring a thorough understanding of the risk factors to effectively mitigate the risk. A multicenter cohort study has identified the neutrophil-to-lymphocyte ratio (NLR), lactate dehydrogenase (LDH), C-reactive protein (CRP), and procalcitonin (PCT) as independent predictive factors for VTE [8]. Another Mendelian randomization (MR) analysis found that, among the 41 inflammatory cytokines included, only platelet-derived growth factor-BB (PDGF-BB) levels showed a causal relationship with an

increased risk of VTE, PE, and DVT [9]. Further research is needed to identify precise risk factors for VTE.

The human gut harbors trillions of microorganisms, which play a crucial role in maintaining digestive health and immune homeostasis [10]. Research showed that alterations in the composition of these GM are associated with various diseases, including gastrointestinal disorders, metabolic issues, and cardiovascular conditions [11-13]. Environmental or genetic disturbances in GM can trigger inflammatory reactions in blood vessels, platelets, and immune cells, potentially increasing the risk of thrombosis [14]. Disruption of the intestinal epithelial barrier, caused by factors such as inflammation, nutrition, and antibiotics, allowed microbial products and metabolites to enter the systemic circulation via the portal vein, potentially leading to thrombosis [15]. Disturbances in GM can activate pathways involving endothelial cells, platelets, and innate immune cells, leading to the release of coagulation proteins and the development of a prethrombotic state [16]. However, the precise role of GM in the development of thromboembolism is not yet fully understood.

MR uses natural variations in genetic variants across generations to determine causal relationships [17]. By employing single nucleotide polymorphisms (SNPs) associated with specific health conditions as proxies, MR helps identify causal relationships while avoiding the external biases often present in traditional epidemiological studies [18-20]. This approach provides a clearer understanding of the genetic influences on disease.

Our research investigates the influence of GM on venous thromboembolic conditions, including VTE, PE, and DVT, using two-sample summary MR with genetic variants associated with GM as instruments to explore these relationships.

Material and methods

Study design

This study was designed in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology Using Mendelian Randomization (STROBE-MR）. SNPs associated with the human GM were used as instrumental variables (IVs), with VTE, including PE and DVT, as the outcome variable. The study satisfies the three core assumptions of MR analysis: (1) the relevance assumption, indicating that the IVs are significantly associated with the exposure (GM); (2) the independence assumption, ensuring that the IVs are not associated with any confounding variables; and (3) the exclusion restriction assumption, which states that the selected genetic variants influence the outcome exclusively through the "IVs-exposure-outcome" pathway, without affecting the outcome via alternative pathways (Figure 1). The data for this study were aggregated from previously published research, for which participant consent and ethical clearance had been obtained.

Data resources

Forward MR data

The genetic data related to GM were obtained from the global MiBioGen consortium database (https://mibiogen.gcc.rug.nl/), which integrates data from 25 cohorts across multiple countries, including the USA and Italy, involving a total of 18,340 individuals. The primary objective of this study was to identify the relationship between autosomal human genetic variants and GM by analyzing the participants' 16S rRNA sequencing profiles [21]. The genome-wide association study (GWAS) data for VTE, PE, and DVT were derived from the FinnGen consortium R9 release dataset. This genetic dataset includes 19,372 VTE cases and 357,905 controls, 9,109 9,243 PE cases and

367,108 controls, as well as DVT cases and 324,121 controls (Table I).

Table I. GWAS summary data sources of outcomes.

Phenotype (Trait)	Data source (Consortium)	Sample size (Case/Contro)	Ancestry	Covariates	Link
VTE	FinnGen	19,372/357,905	European	sex, age, 10 PCs, genotyping batch.	FinnGen_ Access_Results
PE		9,243/367,108			
DVT		9,109/324,121			

VTE-venous thromboembolism, PE-pulmonary embolism, DVT-deep vein thrombosis, PCs-principal components.

Reverse MR data

For reverse MR, we used data similar to that of the forward MR approach. In this context, VTE, PE, and DVT are considered variables of interest, and SNPs strongly associated with these conditions ($P < 5\times10^{-8}$) are identified as exposure variables. This process involves removing instances of linkage disequilibrium, palindromic sequences, and weakly correlated variables, as well as SNPs influenced by confounding factors, just as in forward MR.

IVs selection

Based on the three core assumptions of MR analysis, we first selected SNPs significantly associated with GM as IVs. SNPs with *P*-values less than the genome-wide significance threshold ($P < 5\times10^{-8}$) were chosen as the initial IVs. Secondly, to eliminate linkage disequilibrium (LD), SNPs within an LD region defined by a distance of 10,000 kb and an LD $r^2 < 0.001$ were excluded. Subsequently, SNPs associated with known confounding factors, such as cancer, prolonged bed rest, and fractures [22], were removed (http://www.phenoscanner.medschl.cam.ac.uk/). Finally, to ensure

allele alignment accuracy, harmonization of SNPs was performed [23]. The F-statistic was calculated using the formula: $F = R^2 (N - K - 1) / [K(1 - R^2)]$, where R^2 represents the proportion of variance explained by the SNPs, N is the number of participants in the exposure group, and K is the total number of SNPs included in the final analysis. An F-statistic ranging from 26.604 to 26.992 indicates a low risk of weak instrument bias. These screening steps ensure the robustness and reliability of the study results.

MR Analysis

In this study, the inverse variance weighted (IVW) method was employed as the primary analytical approach. In the forward MR analysis, GM served as the exposure variable, with SNPs associated with GM used as IVs to evaluate the causal relationships between VTE, PE, and DVT. Cochran's Q test was conducted to assess heterogeneity across genetic instruments. When $P < 0.05$, the IVW random-effects model was applied to estimate the causal effect; when the $P \geqslant 0.05$, a fixed-effects model was used [24]. To further validate and complement the causal inference results, additional methods were applied, including MR-Egger regression, weighted median estimator (WME), simple mode (SM), and weighted mode (WM) approaches. The MR-Egger method incorporates an intercept into the regression model to detect and adjust for horizontal pleiotropy in IVs, thereby enhancing the robustness of causal effect estimates [25]. The WME method calculates the weighted median of IV effects, thereby reducing the influence of outliers or biased IVs on the overall results [26]. The SM method estimates the mode of the causal effect distribution, providing reliable estimates when most IVs exhibit similar effects [27]. The WM method calculates the weighted mode of effect results, offering more robust estimates in the presence of effect

heterogeneity, particularly when data points have different weights or when outliers are present [28]. By integrating these methods, this study aims to enhance the accuracy and robustness of causal effect estimates.

Statistical analysis

In this study, sensitivity analyses included Cochran's Q test, MR-Egger intercept test, MR-PRESSO method, and Leave-one-out analysis. Cochran's Q test was conducted to assess the heterogeneity of the effects of the included IVs or SNPs, aiming to determine whether significant differences exist between data and estimates from different sources. $P < 0.05$ indicates significant heterogeneity, in which case the IVW random-effects model should be applied; if no significant heterogeneity is detected, the IVW fixed-effects model is used. The MR-Egger intercept test was employed to evaluate and quantify the horizontal pleiotropy of the instrumental variables, aiming to detect and correct potential bias in causal effect estimates. The MR-PRESSO method improves the accuracy of causal inference by identifying and removing outlier SNPs, thereby detecting and adjusting for horizontal pleiotropy. The Leave-one-out analysis assesses the robustness and reliability of the results by sequentially removing each SNP and re-evaluating the causal estimates using the remaining SNPs. MR analyses were reported using *P* values, odds ratios (*ORs*), and 95% confidence intervals (*CIs*). A significance level of $\alpha = 0.05$ was applied for causal inference. $\beta > 0$ indicates a positive association between the microbiota and the disease, whereas $\beta < 0$ suggests a negative association. Similarly, an $OR > 1$ indicates a positive association, while an $OR < 1$ suggests a negative association between the microbiota and the disease. All MR analyses were performed using R software (version 4.3.1) with the "Two Sample MR" package (version 0.5.6).

Results

IVs selection

According to the selection criteria for IVs, we identified 2,779 eligible SNPs. This dataset includes 211 GM taxa, comprising 131 genera, 35 families, 20 orders, 16 classes, and 9 phyla. F-statistics were calculated for each of the 2,779 SNPs, and no SNPs with $F < 10$ were identified, suggesting a low likelihood of weak instrument bias affecting the causal associations. Based on the IVW analysis, we found that most of the 211 GM taxa were not associated with VTE, whereas only a few taxa showed significant associations. Moreover, different types of thrombotic diseases were associated with specific bacterial taxa (Figure 2). In total, we identified 17 SNPs causally related to VTE (Supplementary Table 1), 14 SNPs associated with PE (Supplementary Table 2), and 17 SNPs associated with DVT (Supplementary Table 3).

Forward-direction MR analyses

A total of 14 gut bacterial taxa were found to have significant associations with VTE. Among them, *Candidatus Soleaferrea* (id.11350, *OR* = 0.92, *95% CI*: 0.85-1.00, *P* = 0.047), *Ruminococcaceae UCG002* (id.11360, *OR* = 0.92, *95% CI*: 0.85-1.00, *P* = 0.046), and *Ruminococcaceae UCG004* (id.11362, *OR* = 0.92, *95% CI*: 0.85-1.00, *P* = 0.046) were negatively associated with VTE, while the *Eubacterium hallii group* (id.11338, *OR* = 1.12, *95% CI*: 1.01-1.23, *P* = 0.025), *Butyricimonas* (id.945, *OR* = 1.11, 95% CI: 1.01-1.22, *P* = 0.027), and *Dorea* (id.1997, *OR* = 1.14, *95% CI*: 1.00-1.31, *P* = 0.047) were positively associated with VTE.

A total of 9 gut bacterial taxa were found to have significant associations with PE. Among them, *Intestinimonas* (id.2062, *OR* = 0.88, *95% CI*: 0.78-0.99, *P* = 0.036), *Unknown genus* (id.2041, *OR* =

0.87, *95% CI*: 0.77-0.97, *P* = 0.015), and *Firmicutes* (id.1672, *OR* = 0.86, *95% CI*: 0.74-0.99, *P* = 0.036) were negatively associated with PE, while *Veillonella* (id.2198, *OR* = 1.22, *95% CI*: 1.01-1.48, *P* = 0.043), *Erysipelatoclostridium* (id.11381, *OR* = 1.16, *95% CI*: 1.04-1.30, *P* = 0.007), and *Lentisphaerae* (id.2250, *OR* = 1.14, *95% CI*: 1.03-1.26, *P* = 0.023) were positively associated with PE.

A total of 13 gut bacterial taxa were found to have significant associations with DVT. Among them, *Mollicutes* (id.3920, *OR* = 0.96, *95% CI*: 0.84-1.09, *P* = 0.004), *Actinobacteria* (id.419, *OR* = 0.85, *95% CI*: 0.74-0.97, *P* = 0.017), and *Bifidobacteriaceae* (id.433, *OR* = 0.85, *95% CI*: 0.74-0.99, *P* = 0.031) were negatively associated with the disease, while *Adlercreutzia* (id.812, *OR* = 1.80, *95% CI*: 0.93-3.50, *P* = 0.046), *Collinsella* (id.815, *OR* = 1.31, *95% CI*: 1.08-1.59, *P* = 0.006），and *Desulfovibrio*（id.3173, *OR* = 1.16, *95% CI*: 1.01-1.33, *P* = 0.006) were positively associated with the disease (Table II).

Table II. MR analysis of the causal relationship between GM and VTE, PE, and DVT

Outcome	**Exposure**	**nSNP**	***P* value**	***OR* (95% CI)**
VTE	*Candidatus Soleaferrea*	9	0.047	0.92 (0.85-1.00)
	Ruminococcaceae UCG002	20	0.046	0.92 (0.85-1.00)
	Ruminococcaceae UCG004	11	0.045	0.91 (0.83-1.00)
	Eubacterium hallii group	13	0.025	1.12 (1.01-1.23)
	Butyricimonas	13	0.027	1.11 (1.01-1.22)
	Dorea	10	0.047	1.14 (1.00-1.31)
PE	*Intestinimonas*	16	0.036	0.88 (0.78-0.99)
	Unknown genus	12	0.015	0.87 (0.77-0.97)
	Firmicutes	14	0.036	0.86 (0.74-0.99)
	Veillonella	5	0.043	1.22 (1.01-1.48)
	Erysipelatoclostridium	15	0.007	1.16 (1.04-1.30)
	Lentisphaeria	8	0.023	1.13 (1.02-1.26)
DVT	*Mollicutes*	11	0.004	0.82 (0.71-0.94)
	Actinobacteria	14	0.017	0.85 (0.74-0.97)
	Bifidobacteriaceae	11	0.031	0.85 (0.74-0.99)
	Adlercreutzia	8	0.046	1.16 (1.00-1.35)

Collinsella	9	0.006	1.31 (1.08-1.59)
Desulfovibrio	10	0.034	1.16 (1.01-1.33)

MR-Mendelian randomization, GM-gut microbiome, VTE-venous thromboembolism, PE-encompassing pulmonary embolism, DVT-deep vein thrombosis, nSNP-number of single nucleotide polymorphisms.

This MR analysis revealed a positive causal relationship between certain GM and the risk levels of VTE, PE, and DVT. After multiple testing correction using the Bonferroni method, a significant causal association was identified between *Ruminococcaceae* and VTE. This suggests that changes in GM may have corresponding effects on thrombotic diseases such as VTE, PE, and DVT. Additionally, the results of supplementary analyses, including MR-Egger, WME, and ML methods, were consistent with the direction of the IVW method. The statistically significant associations are illustrated by scatter plots and forest plots (Figure 3 and Figure 4).

Reverse-direction MR analyses

In the reverse MR analysis, we applied the same analytical procedures, setting GM as the outcome and VTE, PE, and DVT as the exposure factors. The results showed no causal relationships between GM and VTE ($P > 0.05$), PE ($P > 0.05$), or DVT ($P > 0.05$) (Supplementary Table 4-6).

Sensitivity analysis

The funnel plot generated from the MR-Egger regression (Figure 5) indicated no evidence of heterogeneity or horizontal pleiotropy in the forward MR results. Additionally, no outliers were detected in the MR-PRESSO analysis or Cochran's Q test. A Leave-one-out analysis was subsequently performed, and the corresponding forest plot (Figure 6) further confirmed the stability of these results.

DISCUSSION

To gain a more comprehensive understanding of the impact of GM on VTE development, this study conducted bidirectional two-sample MR analyses using summary statistics for VTE, PE, and DVT from the FinnGen Consortium R9 data, along with GM meta-analysis data from the global MiBioGen Consortium. A total of 211 GM taxa were analyzed for causal associations with VTE, PE, and DVT. The analysis identified causal associations between 18 GM taxa and the incidence of VTE, PE, and DVT. Specifically, *Candidatus Soleaferrea*, *Ruminococcaceae UCG002*, and *Ruminococcaceae UCG004* were negatively associated with VTE, while *Eubacterium hallii group*, *Butyricimonas*, and *Dorea* showed positive associations with VTE ($P < 0.05$ and $OR > 1$). For PE risk, *Intestinimonas*, *Unknown genus*, and *Firmicutes* were negatively associated, whereas *Veillonella*, *Erysipelatoclostridium*, and *Lentisphaerae* showed positive associations ($P < 0.05$ and $OR > 1$). Regarding DVT risk, *Mollicutes*, *Actinobacteria*, and *Bifidobacteriaceae* were negatively associated, while *Adlercreutzia*, *Collinsella*, and *Desulfovibrio* were positively associated ($P < 0.05$ and $OR > 1$). These findings enhance the understanding of GM's role in VTE pathogenesis and highlight specific taxa that may contribute to or protect against VTE progression.

The pathogenesis of VTE is highly complex. Thrombosis is a pathological process characterized by the abnormal aggregation and solidification of blood components within blood vessels, leading to vascular obstruction, driven by endothelial injury, altered hemodynamics, and a hypercoagulable state [29]. While thrombosis is essential for hemostasis in damaged vessels, it can also result in adverse events such as vascular occlusion, embolism, and pathological clot formation. Conversely, impaired thrombosis may lead to excessive bleeding [30]. The regulatory role of GM in VTE

development has increasingly become a research focus, with different bacterial taxa potentially influencing VTE risk through metabolic products, inflammation modulation, and gut barrier function. *Ruminococcaceae* is a key GM family closely associated with various metabolic processes and diseases. Although direct studies on the association between *Ruminococcaceae* and VTE are limited, metabolites produced by GM, particularly metabolite trimethylamine-N-oxide (TMAO), have been shown to be associated with VTE [31]. One study reported that, compared to low TMAO levels, patients with moderate and high TMAO had a 38% and 44% increased risk of VTE recurrence, respectively. However, the results were not statistically significant [32]. Papa et al. reported that TMAO is a risk factor for inflammatory bowel disease, with *Ruminococcaceae* playing a crucial role in its production and potentially influencing the thrombotic process [33]. *Ruminococcaceae* metabolize dietary lipids, including choline, phosphatidylcholine, and L-alpha glyceryl phosphorylcholine, into trimethylamine (TMA), which is subsequently oxidized to TMAO in the liver. Elevated TMAO levels have been linked to endothelial dysfunction, platelet hyperreactivity, and increased thrombosis risk [34]. Notably, *Ruminococcaceae* abundance is negatively correlated with thrombosis formation, suggesting that a higher abundance of *Ruminococcaceae* may be associated with a lower risk of VTE.

Additionally, a study by Huang et al. involving 33 patients with liver cirrhosis found a positive correlation between *Eubacterium hallii group* and the occurrence of VTE. This bacterial group was significantly enriched in patients with both liver cirrhosis and VTE, and its abundance was positively associated with coagulation factor parameters [35]. These findings align with the results of our study. *Butyricimonas* is a GM primarily known for producing butyrate, a key short-chain fatty acid with

anti-inflammatory properties that plays a crucial role in maintaining intestinal barrier function. Disruption of inflammation and intestinal barrier integrity has been identified as a potential trigger for VTE [36]. Therefore, it has been hypothesized that *Butyricimonas* may indirectly influence VTE risk by modulating inflammation or preserving gut health. A reduction in *Butyricimonas* abundance could theoretically promote thrombosis through inflammatory pathways. However, our findings contradict this assumption, suggesting that the association between *Butyricimonas* and VTE may involve more complex underlying mechanisms. *Dorea* belongs to the *Firmicutesphylum* and is frequently associated with metabolic diseases such as obesity and diabetes, as well as inflammatory conditions, all of which are important risk factors for VTE [37]. In cardiovascular disease-related studies, changes in *Dorea* abundance have been linked to inflammation and metabolic dysregulation, potentially influencing VTE risk by activating coagulation pathways or increasing blood viscosity [38]. If an increase in *Dorea* abundance is associated with exacerbated inflammation or metabolic disturbances, it may indirectly elevate the risk of VTE through these mechanisms. However, *Dorea* may also contribute to reducing VTE risk by maintaining GM stability or supporting anti-inflammatory effects. Therefore, the precise association between *Dorea* and VTE (whether positive or negative) requires further investigation and validation. Currently, no clear research has explored the association between *Candidatus Soleaferrea* and VTE risk, highlighting a potential direction for future studies.

The GM may play a crucial role in the occurrence and progression of PE through the regulation of the "gut-lung axis". Wu et al. demonstrated that certain drugs could alleviate pulmonary inflammation in mice by modulating *Intestinimonas*, and this inflammatory response is closely

associated with thrombosis risk. It has been hypothesized that an increase in *Intestinimonas* abundance may exert a protective effect against PE [39]. Further research has revealed that *Intestinimonas* influences pulmonary inflammation and immune responses through the "gut-lung axis", contributing to immune homeostasis in the lungs [40]. Conversely, a decrease in *Intestinimonas* abundance may exacerbate systemic inflammation, thereby increasing the risk of PE. Our findings are consistent with this evidence. *Veillonella* is an anaerobic bacterium that may play a crucial role in PE formation. A case report described a 38-year-old female who developed Lemierre's syndrome following a throat infection, with imaging revealing thrombosis in the jugular and subclavian veins, accompanied by systemic complications. Blood culture identified *Veillonella parvula*, suggesting that this bacterium may contribute to the formation of infectious thrombosis [41]. This finding also supports our study conclusions. Additionally, recent studies have found that the GM composition in patients with idiopathic pulmonary arterial hypertension differs from that of healthy controls, with *Firmicutes* exhibiting the highest abundance at 53.16% and 57.08%, respectively [42].Another study demonstrated that *cryptotanshinone* alleviates pulmonary fibrosis by modulating GM and bile acid metabolism, significantly reducing the proportion of *Erysipelatoclostridium*, suggesting its potential involvement in pulmonary diseases [43]. However, the specific association between *Erysipelatoclostridium* and PE requires further investigation. Regarding *Unknown genus* and *Lentisphaerae*, there is a lack of research on their association with PE in the existing literature, and no direct evidence currently supports their association with PE.

Research on the role of GM in DVT remains in its early stages. A metabolomics study identified altered metabolic profiles in DVT patients, suggesting that GM may contribute to DVT pathogenesis

[44]. Studies indicated that *Collinsella* is enriched in DVT patients with myelofibrosis, producing short-chain fatty acids and other metabolites that influence host metabolism and immune function [45]. This underscores the significance of GM in DVT progression and its potential as a diagnostic and therapeutic target. However, no studies have directly reported associations between *Mollicutes*, *Actinobacteria*, *Bifidobacteriaceae*, *Adlercreutzia*, *Desulfovibrio*, and DVT.

With aging, GM diversity increases, and its composition and function tend to stabilize. The dominant bacterial phyla in the adult gut include *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, and *Proteobacteria*. These active microbial communities play a crucial role in carbohydrate metabolism, energy production, cell component synthesis, nutrient processing, and immune system development [46]. However, the vast diversity of GM poses significant challenges for comprehensive measurement and quality control. Additionally, the effects of the same GM taxa may vary across different diseases. Therefore, this study employs MR analysis to investigate the association between GM and the risk of VTE, PE, and DVT. The strategy of targeting the GM as a host regulatory factor has gradually attracted attention in emerging therapeutic approaches for chronic diseases. These approaches include fecal microbiota transplantation, probiotic supplementation, dietary interventions, targeted use of antibiotics, and inhibition of specific microbial enzymes [47-51]. GM is also closely linked to intestinal inflammation. However, most supporting evidence for these associations is indirect, necessitating further direct experiments, modeling studies, and comprehensive investigations for validation.

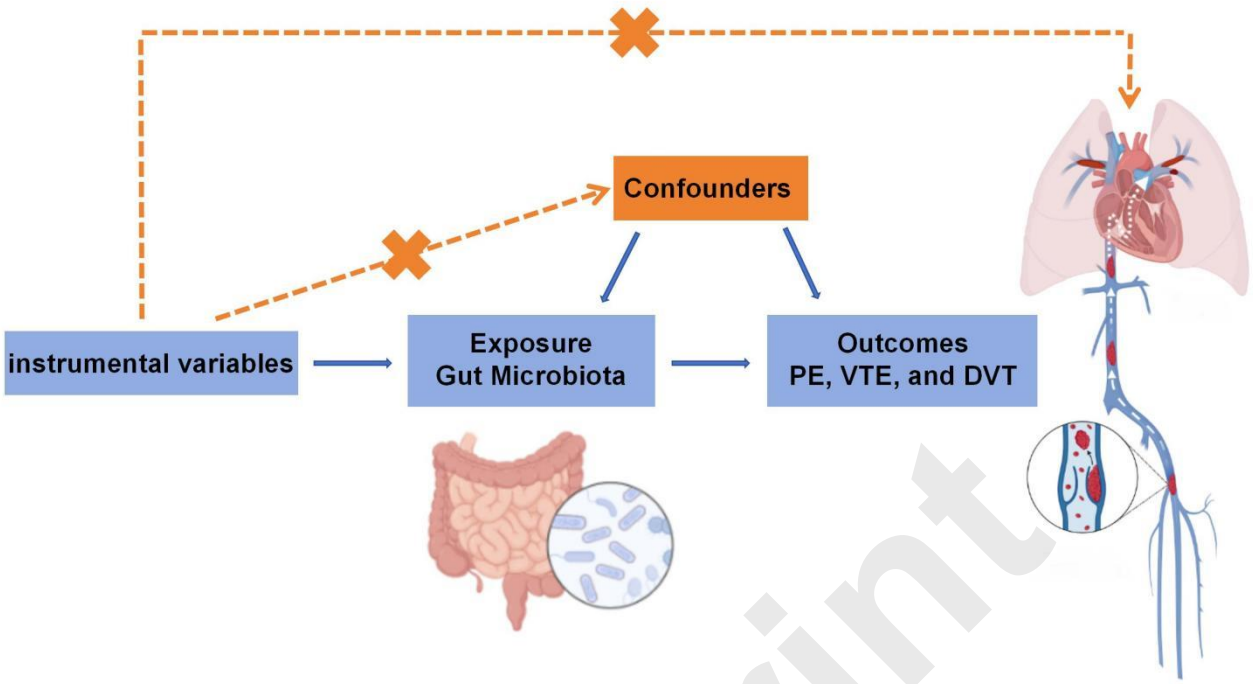
Our study offers several advantages. It is among the few MR analyses that investigate the causal association between GM and VTE. We utilized extensive GWAS data from multiple databases,

ensuring high-quality instrumental variables with $F > 10$, thereby reducing the risk of weak instrumental bias and enhancing explanatory power. However, certain limitations should be acknowledged. The GWAS data primarily involve individuals of European descent, which may restrict the generalizability of our findings to other ethnic groups. Additionally, most GWAS studies employ 16S rRNA gene sequencing at the genus level, preventing precise association of specific strains or species with our findings. Moreover, due to limitations in the outcomes database, the phenotypes discussed do not completely encompass all types of VTE, PE, and DVT, thereby somewhat reducing the clinical relevance and interpretative depth of our conclusions. Future large-scale clinical trials and cohort studies are necessary to further validate our findings.

Conclusion

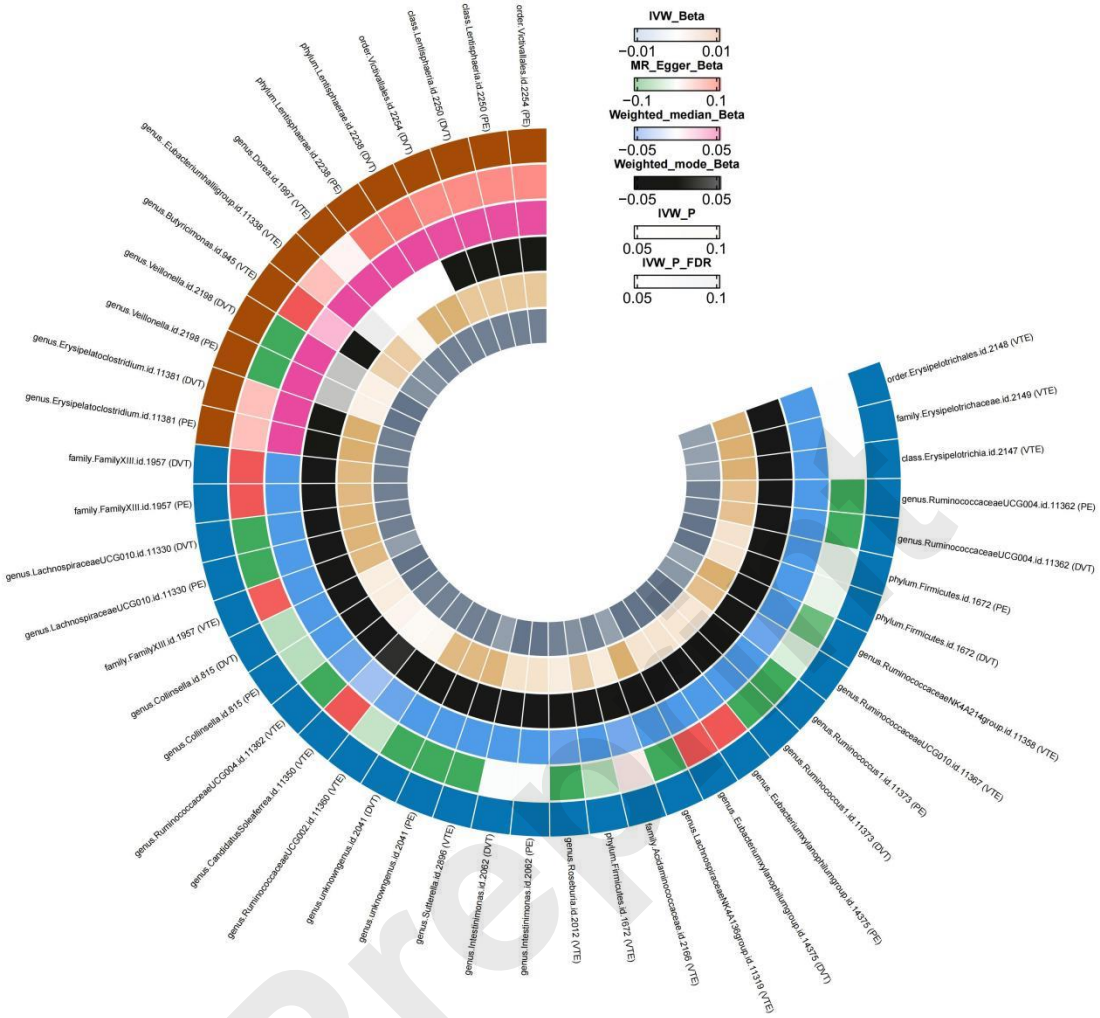
In summary, specific GM exhibit a clear causal relationship with the development of VTE, PE, and DVT. Ruminococcaceae was found to significantly reduce the risk of VTE. This study enhances the understanding of the role of GM in VTE pathogenesis, particularly in the potential mechanisms of the microbiota-immune-coagulation network. Moreover, it provides important theoretical support for the development of innovative therapeutic approaches based on probiotics or microbiota transplantation.

384 # Figure Legends



385

386 **Figure1.** Two-sample MR directed acyclic graph of the 3 key assumptions



387

388 **Figure2.** The results of MR analysis reveal the association between GM and VTE, PE, and DVT

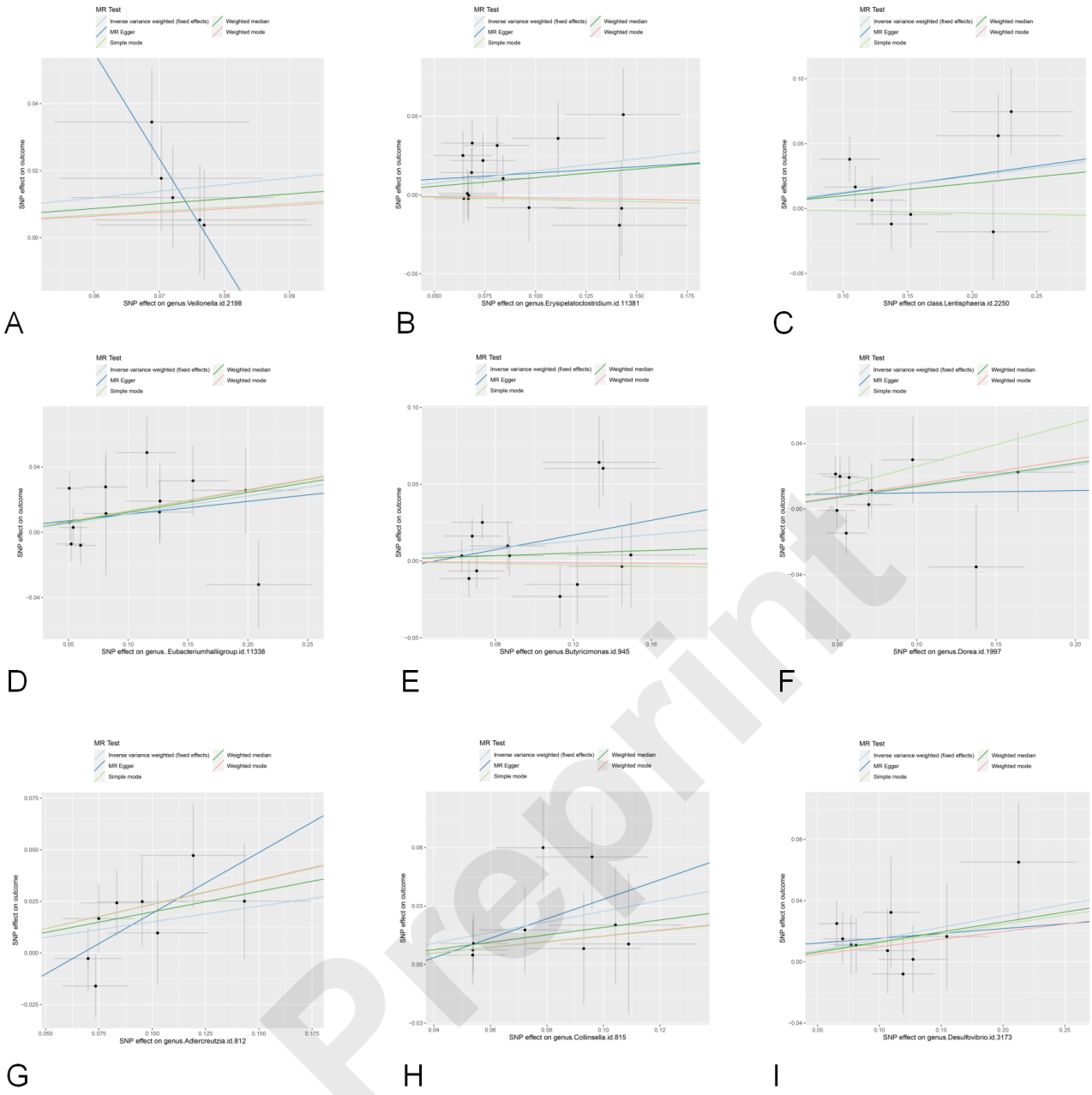


Figure3. Scatter Plots of MR Effect size for causal association between gut microbiota and VTE, PE, and DVT

Figure4. Leave-one-out assessing the impact of individual SNPs on the overall causal estimate

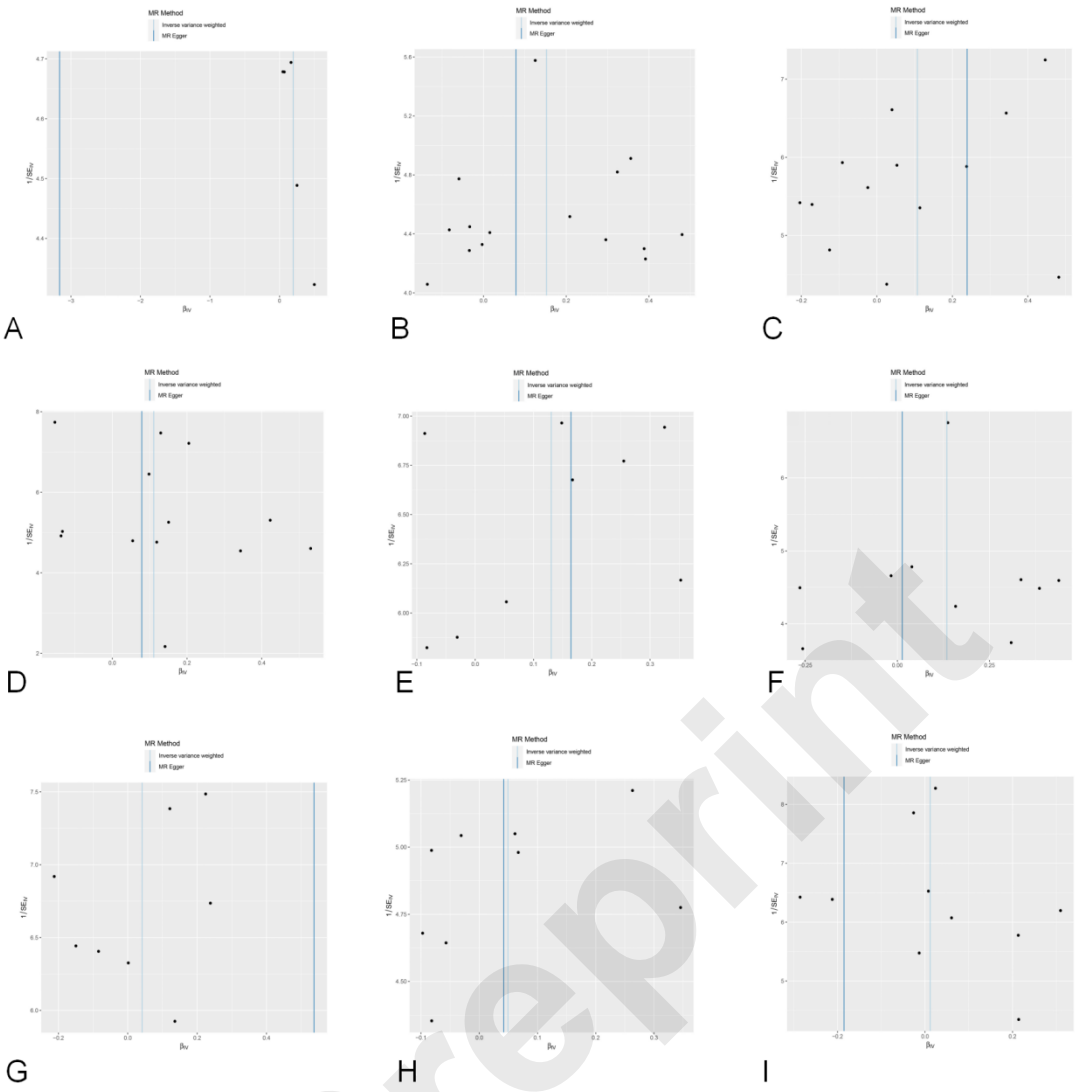


Figure5. Funnel Plots of MR Analysis among VTE, PE, and DVT

Figure6. Forest Plots of MR Analysis between GM and VTE, PE, and DVT

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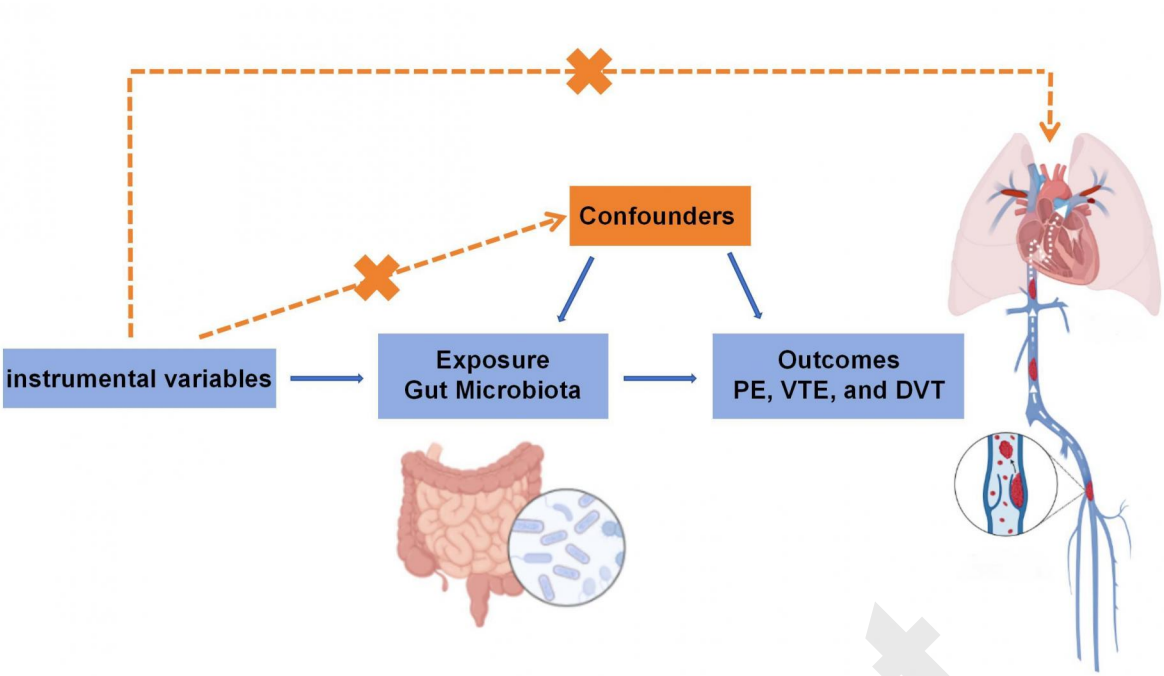

Confounders
instrumental variables
Exposure
Gut Microbiota
Outcomes
PE, VTE, and DVT

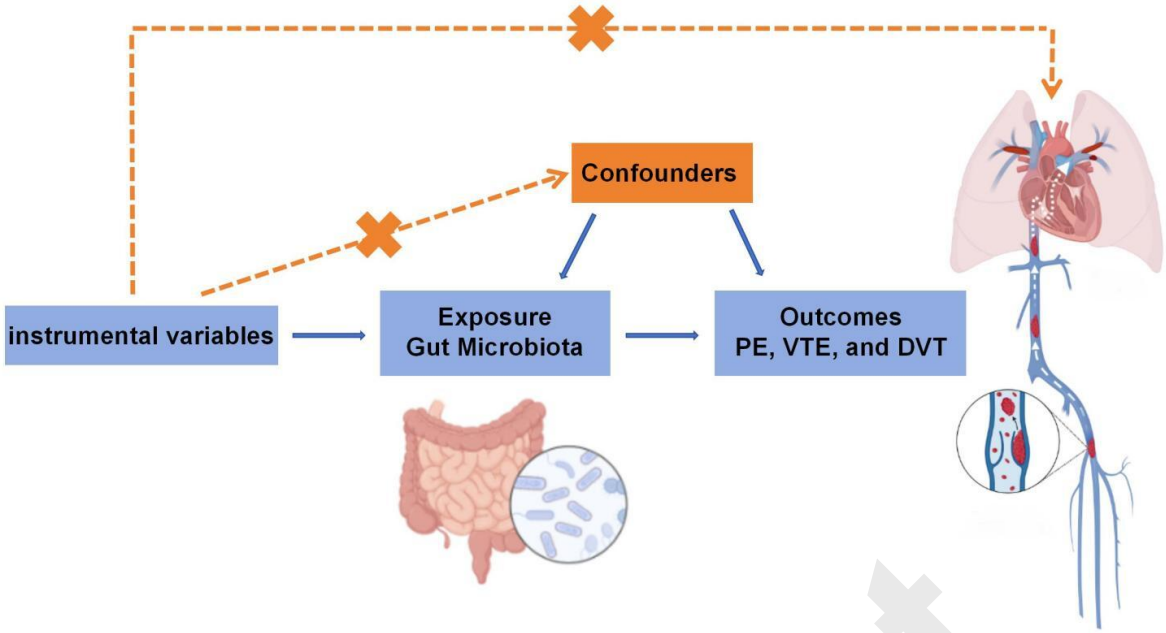


FIGURE 1: Two-sample MR directed acyclic graph of the three key assumption.

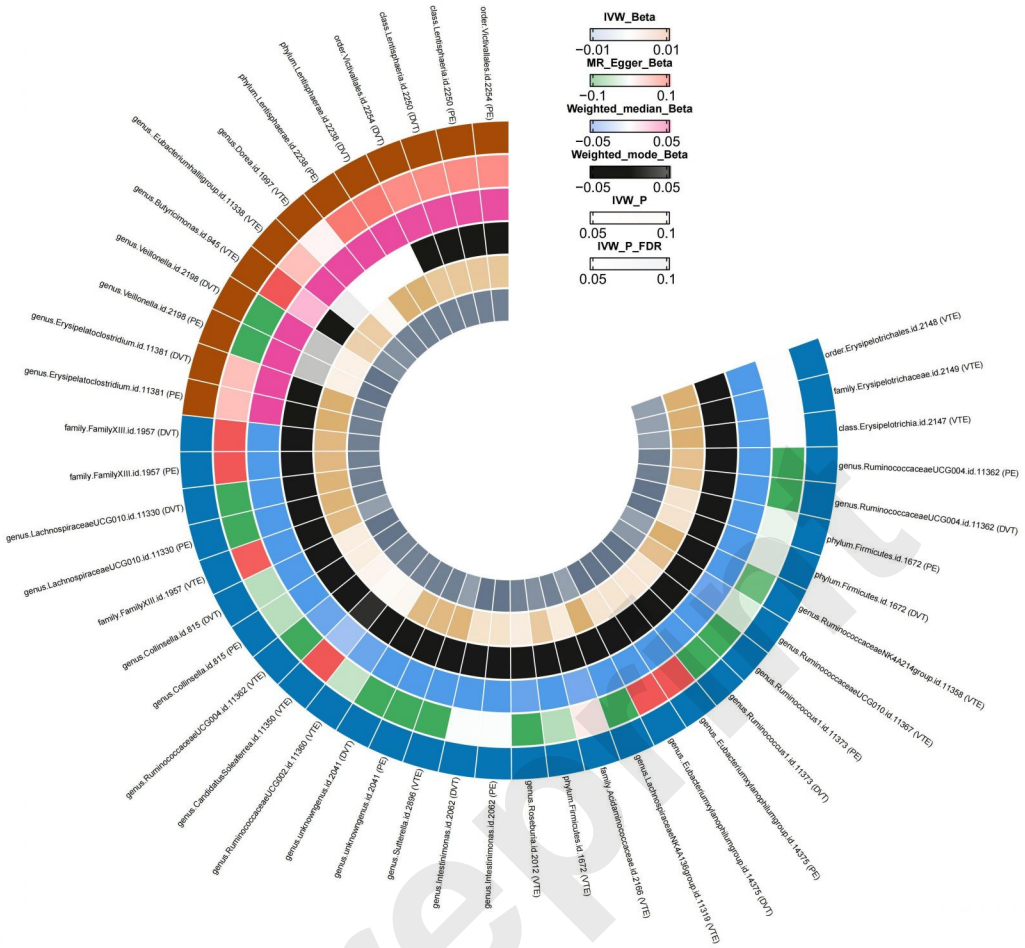
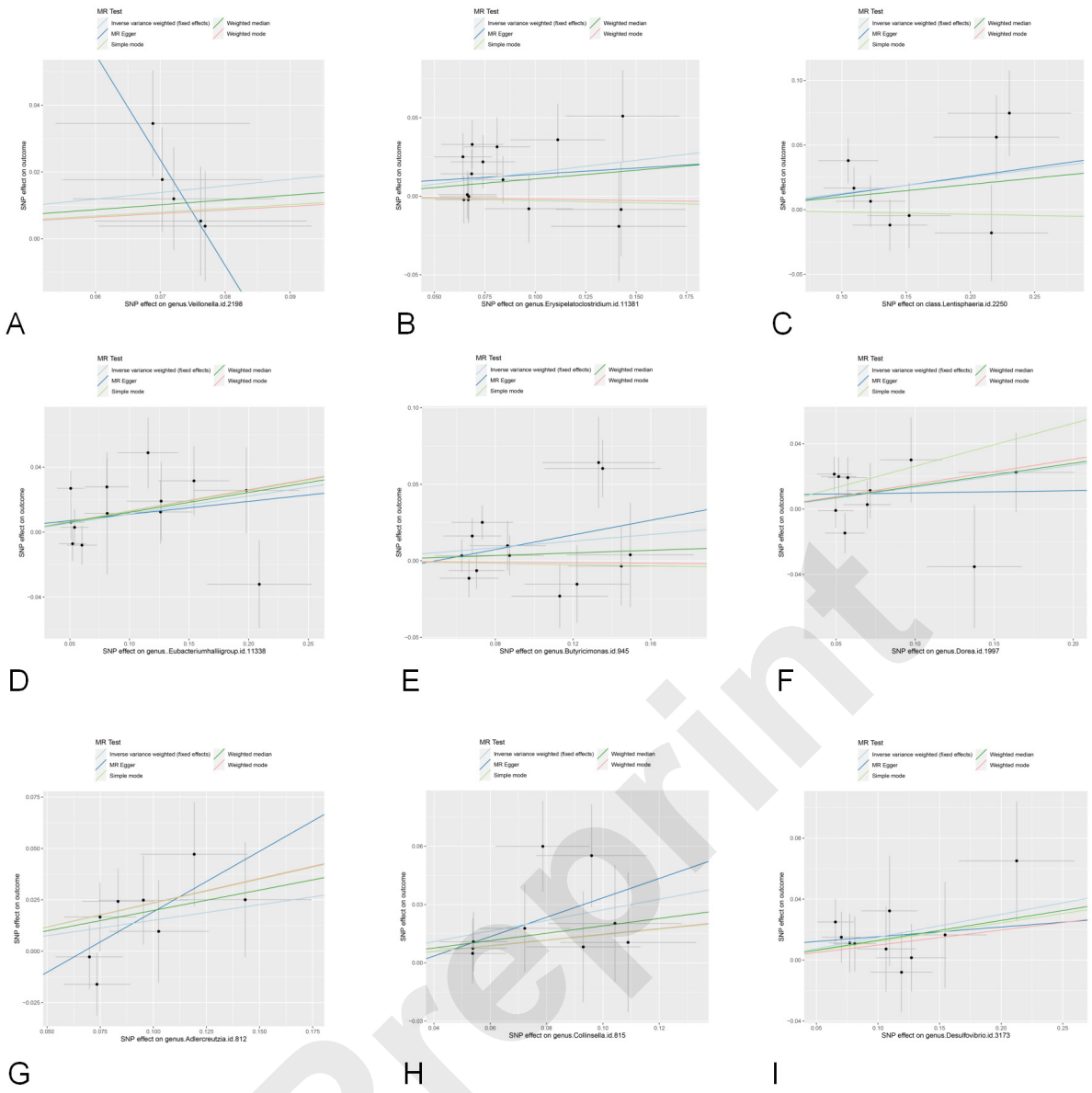


Figure2. The results of MR analysis reveal the association between GM and VTE, PE, and DVT



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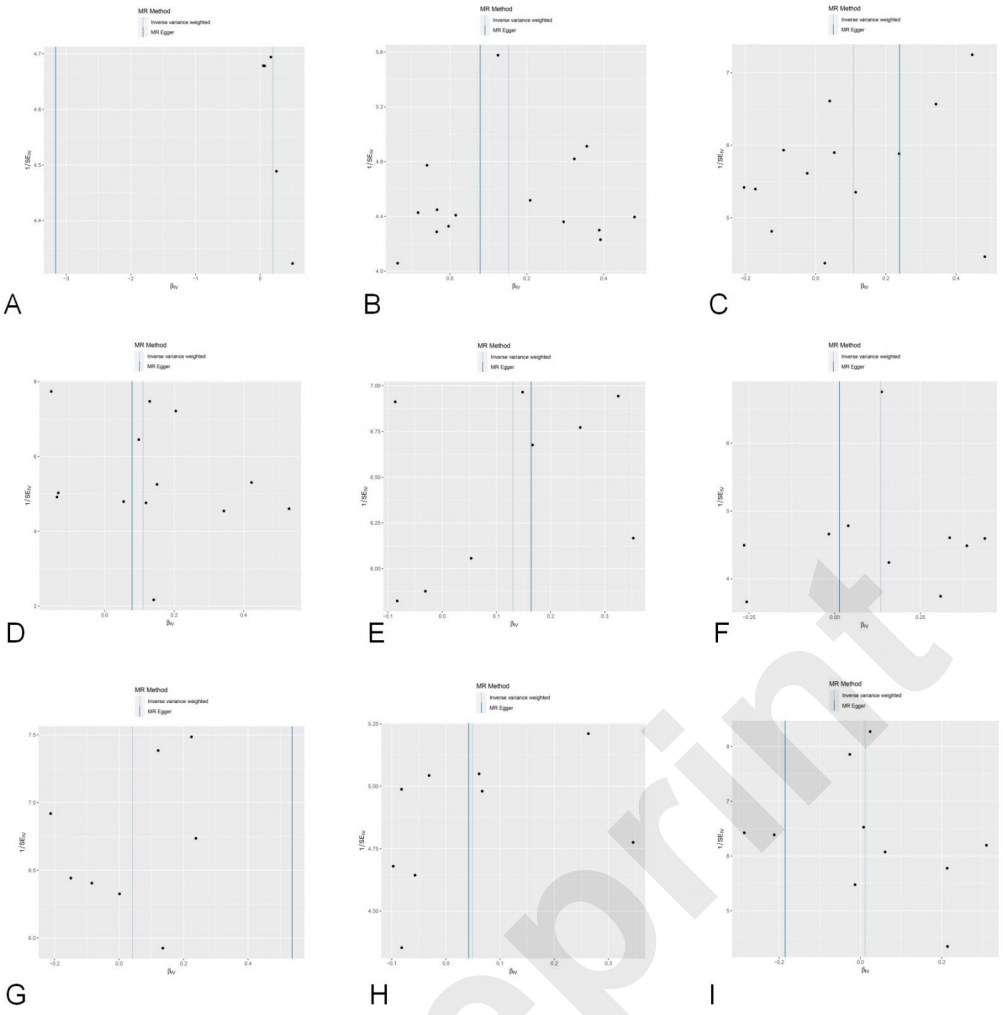

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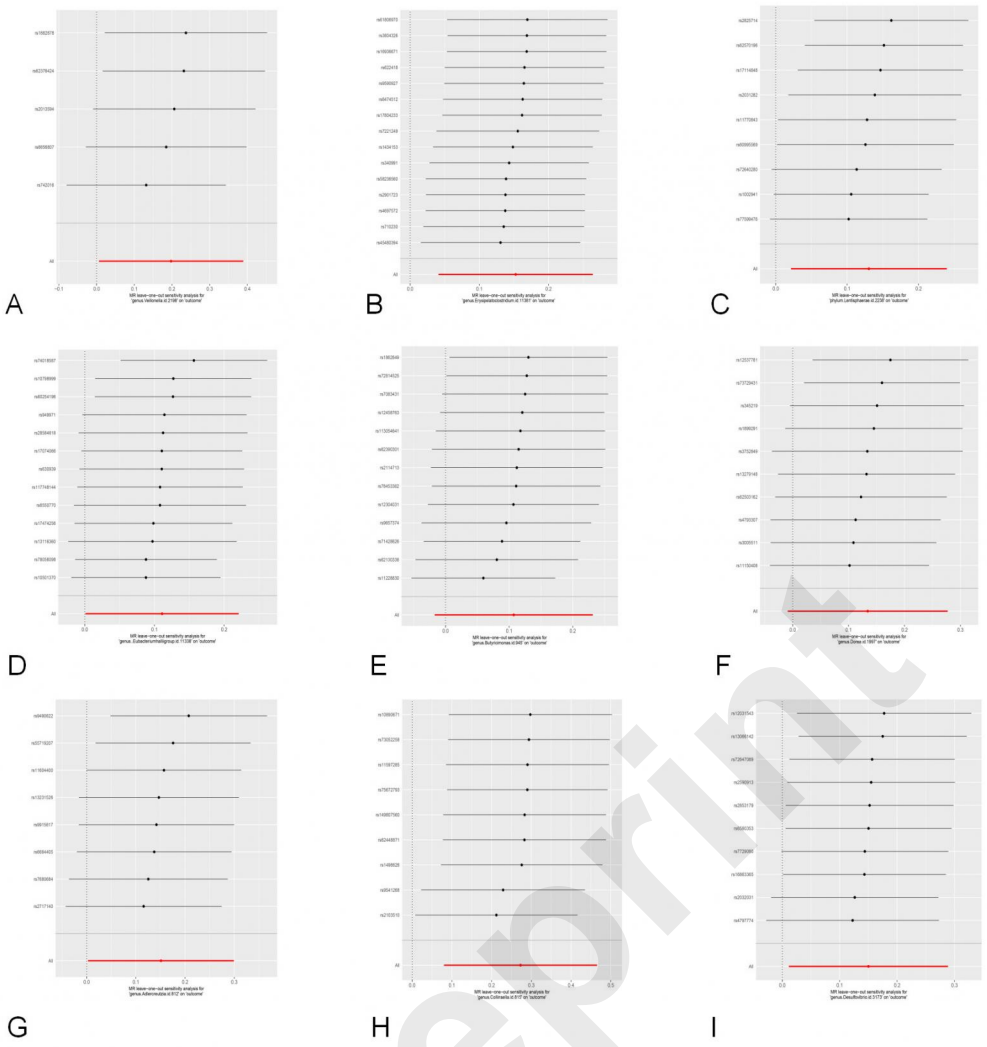

Figure6. Forest Plots of MR Analysis between GM and VTE, PE, and DVT