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

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Submitted: 23 November 2024; **Accepted:** 1 April 2025
Online publication: 18 May 2025

Arch Med Sci
DOI: https://doi.org/10.5114/aoms/203603

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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 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*, an 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.

Key words: gut microbiota, venous thromboembolism, pulmonary embolism, deep vein thrombosis, Mendelian randomization.

Introduction

Venous thromboembolism (VTE), encompassing pulmonary embolism (PE) and deep vein thrombosis (DVT), is a serious thromboembolic disor-

der 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, developing 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 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 investigated 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 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

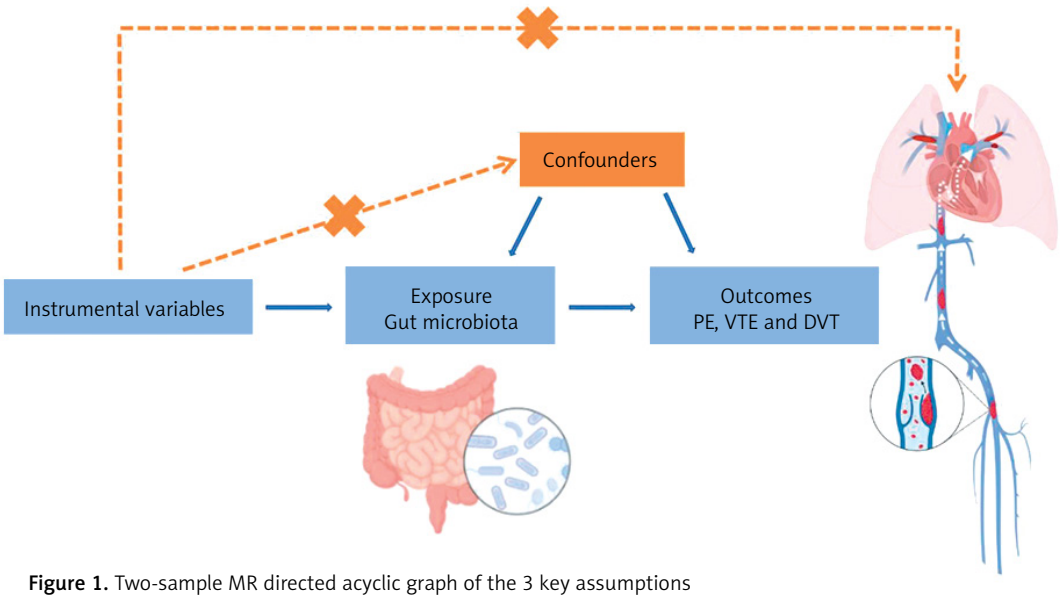


Figure 1. Two-sample MR directed acyclic graph of the 3 key assumptions

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

Reverse MR data

For reverse MR, we used data similar to those of the forward MR approach. In this context, VTE, PE, and DVT are considered variables of interest, and SNPs strongly associated with these conditions ($p < 5 \times 10^{-8}$) 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.

IV 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 ($p < 5 \times 10^{-8}$) 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 $p \geq 0.05$, 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

Table I. GWAS summary data sources of outcomes

Phenotype (Trait)	Data source (Consortium)	Sample size (Case/Control)	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.

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 aimed to enhance the accuracy and robustness of causal effect estimates.

Statistical analysis

In this study, sensitivity analyses included Cochran's Q test, the MR-Egger intercept test, the MR-PRESSO method, and leave-one-out analysis. Cochran's Q test was performed 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

IV 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 SI), 14 SNPs associated with PE (Supplementary Table SII), and 17 SNPs associated with DVT (Supplementary Table SIII).

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, $p = 0.036$), an 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).

This MR analysis revealed a positive causal relationship between certain GM and the risk levels of VTE, PE, and DVT. After multiple testing

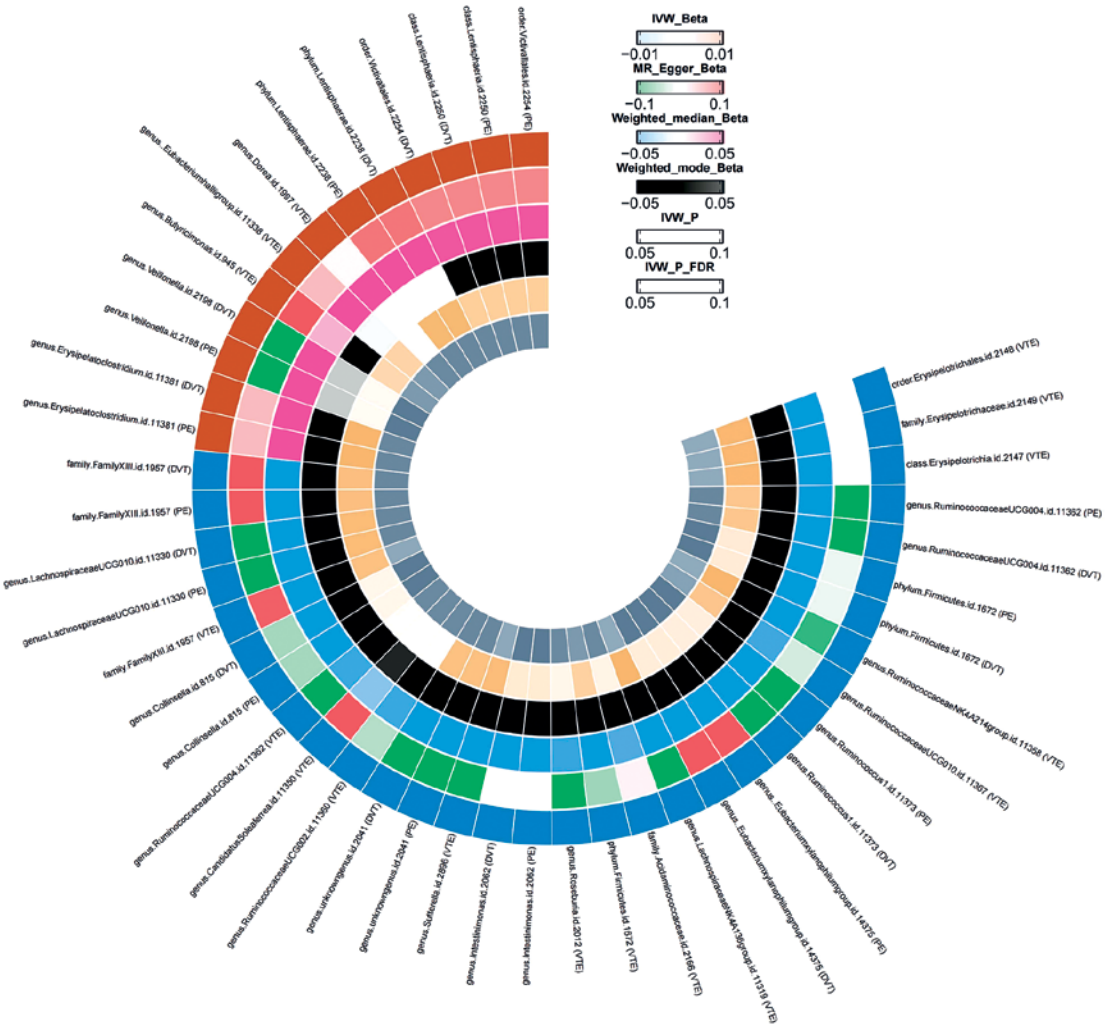


Figure 2. The results of MR analysis reveal the association between GM and VTE, PE, and DVT

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)
	an 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 (Lentisphaerae????)	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 – pulmonary embolism, DVT – deep vein thrombosis, nSNP – number of single nucleotide polymorphisms.

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 (Figures 3 and 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 Tables SIV–SVI).

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

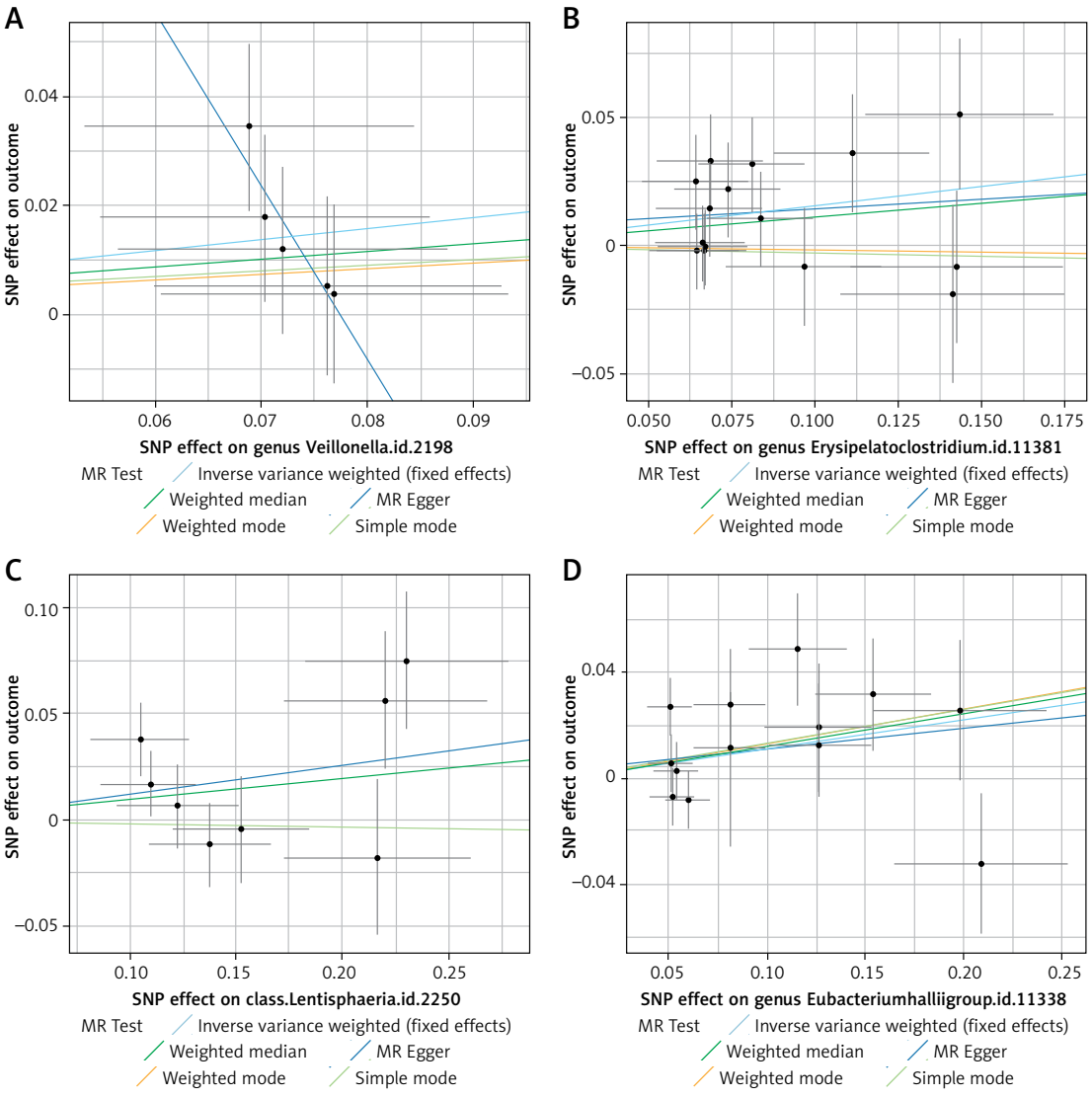
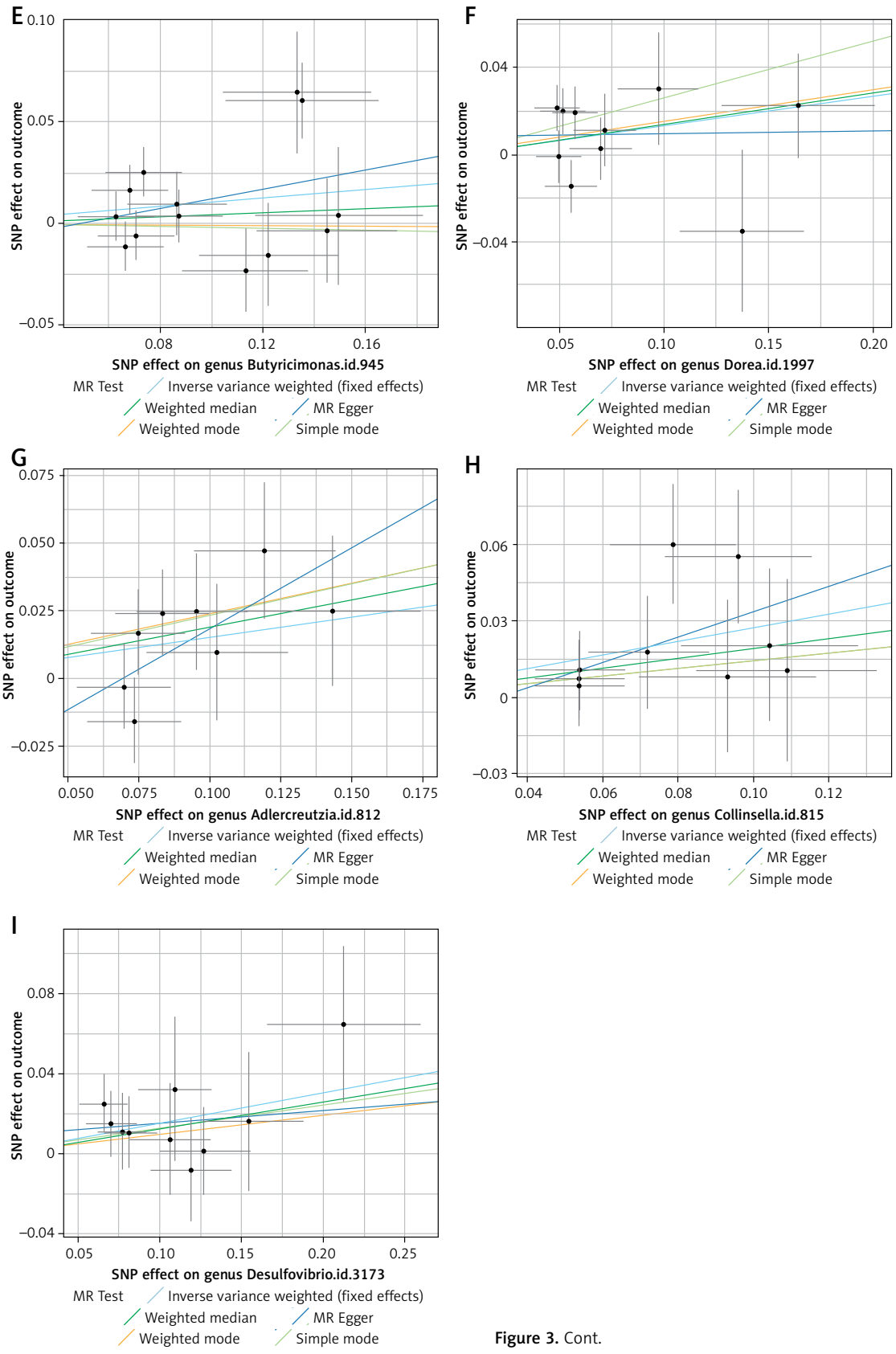


Figure 3. Scatter plots of MR effect size for causal association between gut microbiota and VTE, PE, and DVT



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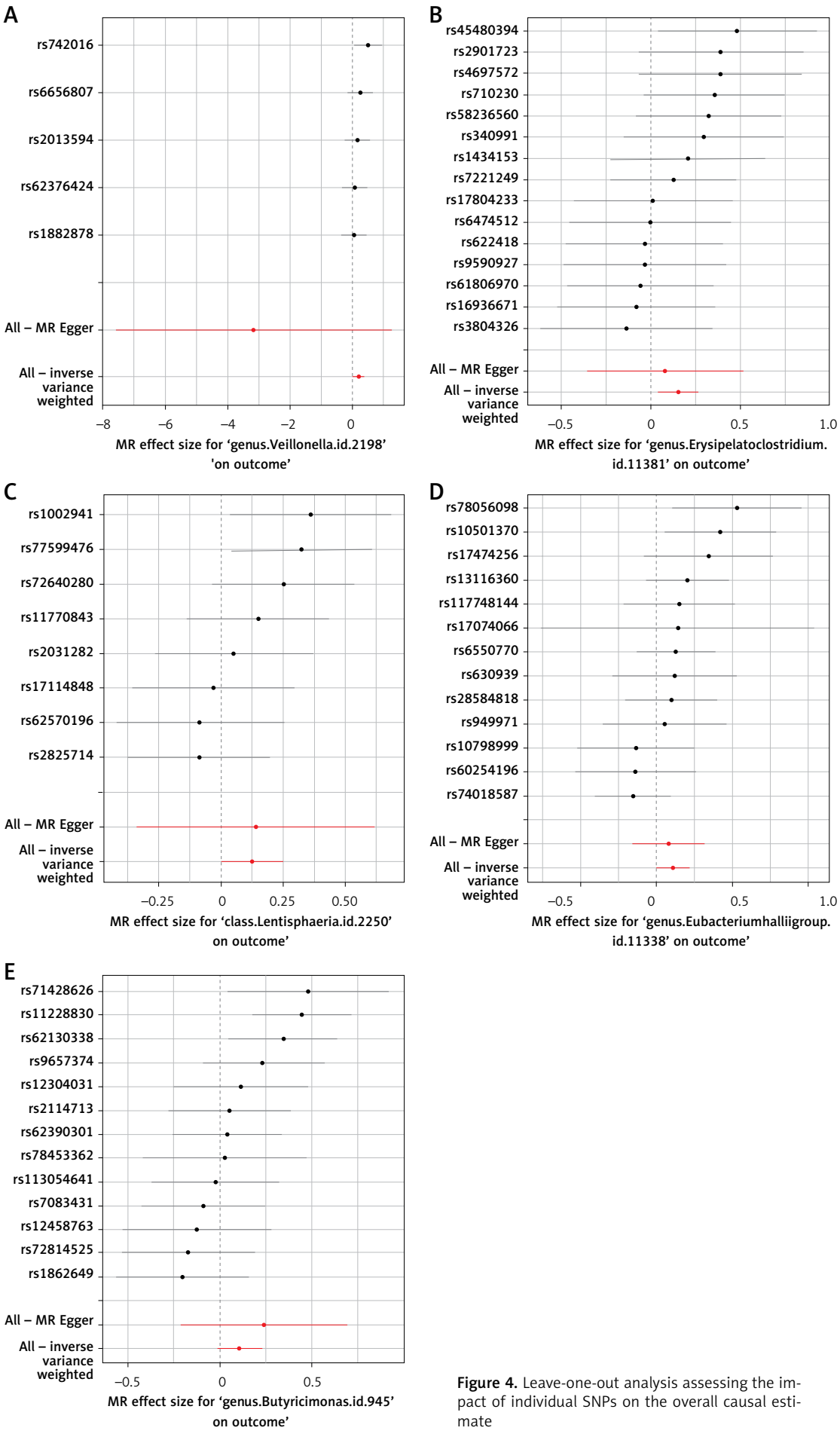
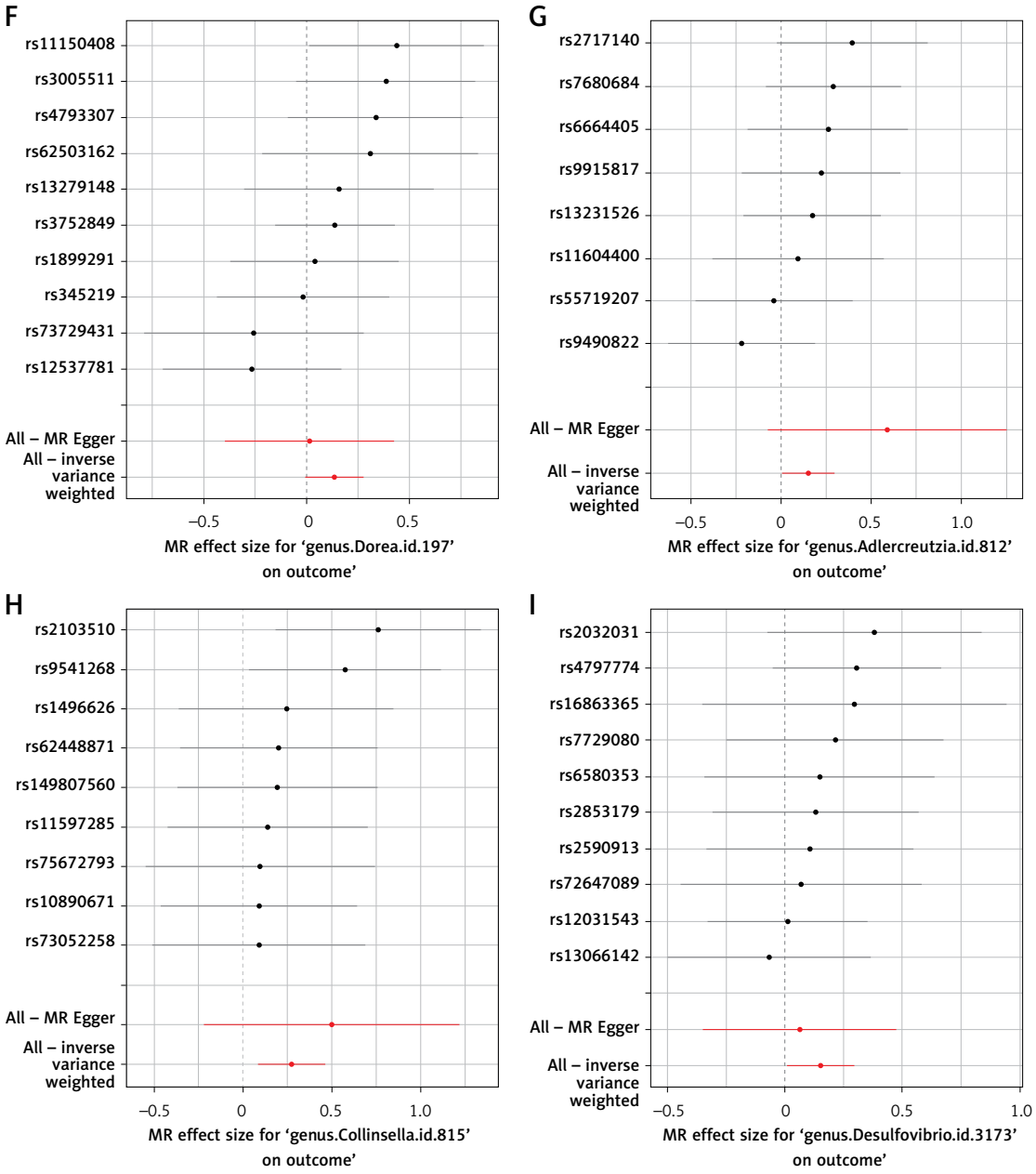


Figure 4. Leave-one-out analysis assessing the impact of individual SNPs on the overall causal estimate



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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*, an 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, em-

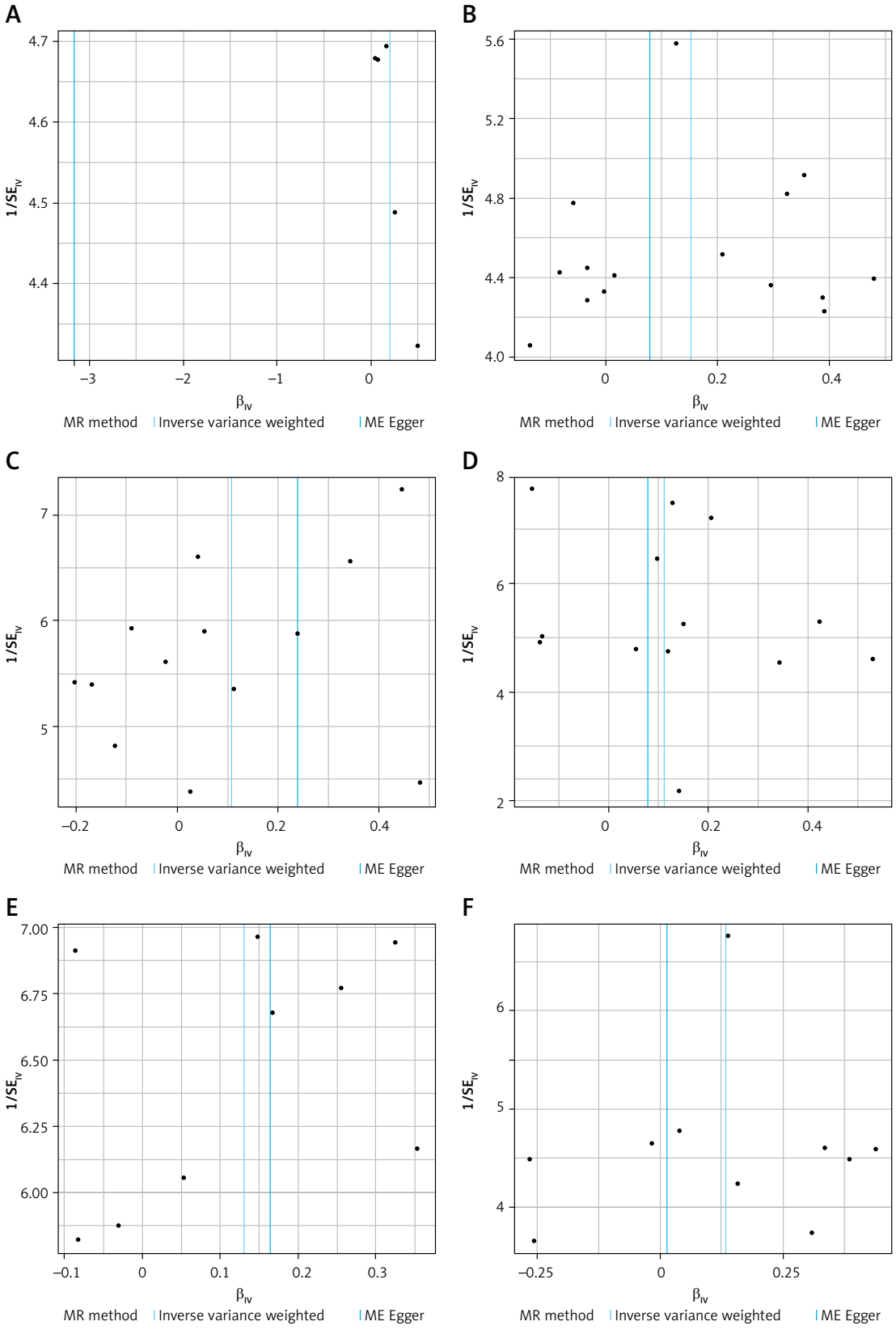
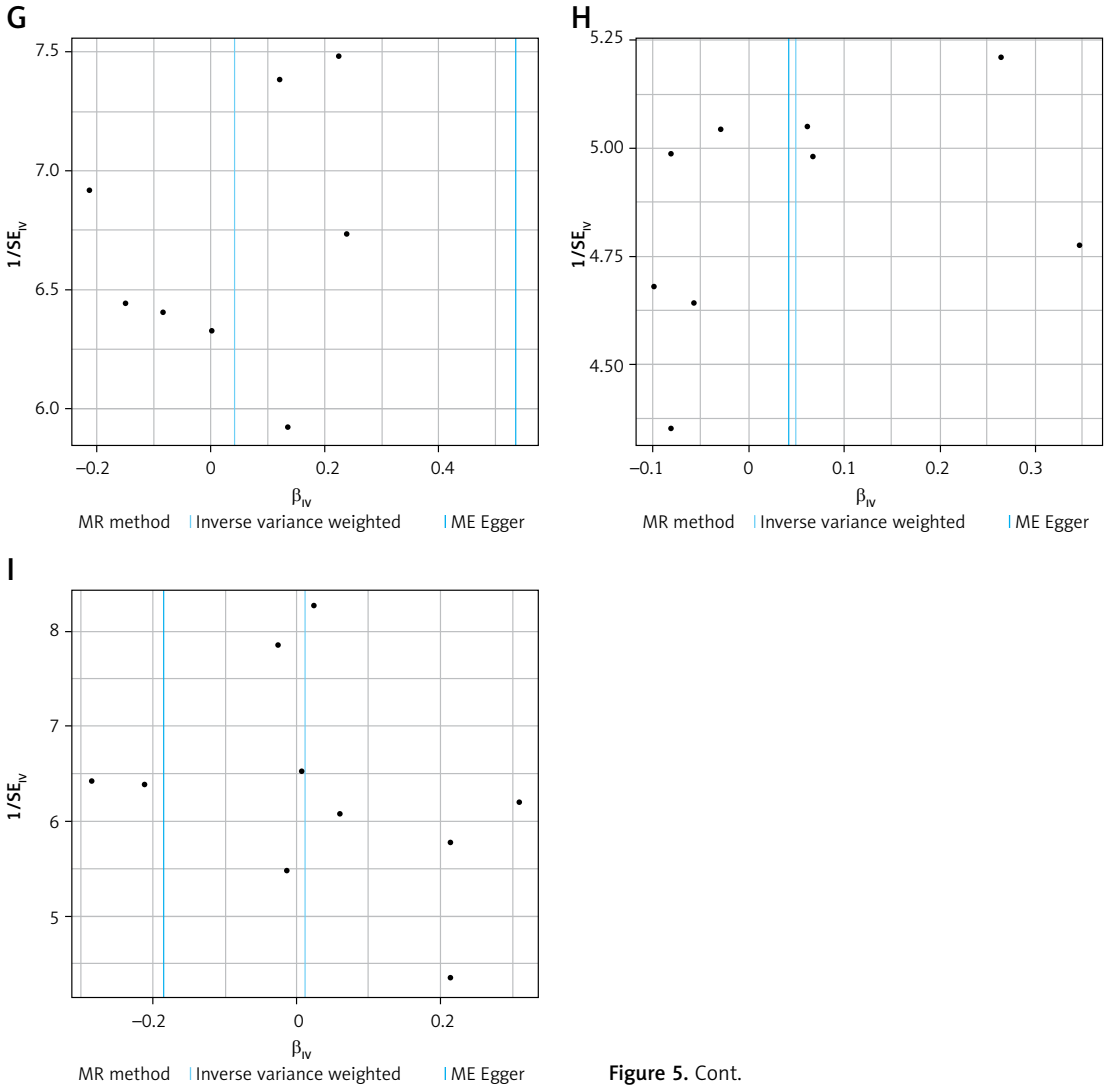


Figure 5. Funnel plots of MR analysis among VTE, PE, and DVT



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bolism, 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 the 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

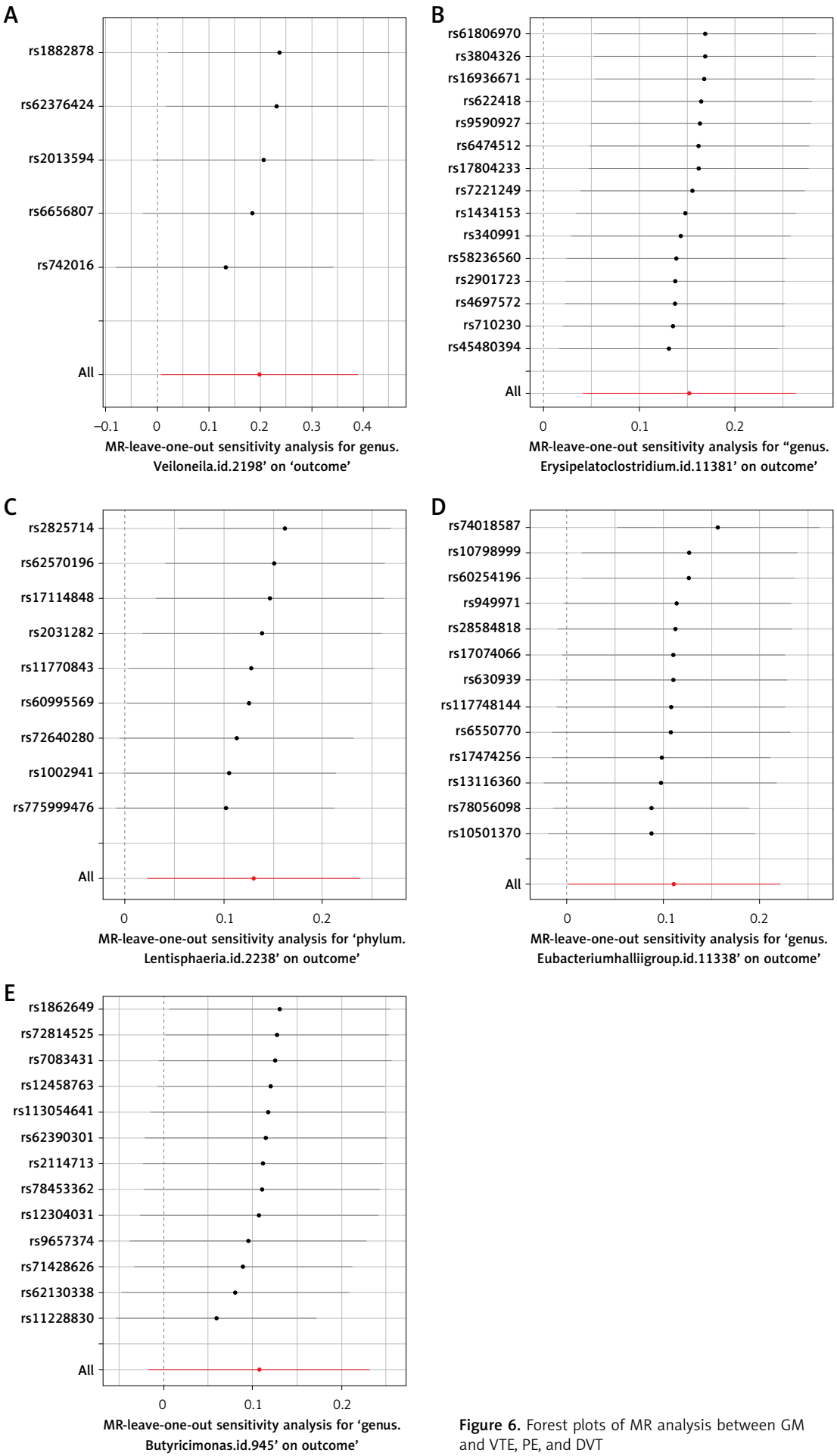

Figure 6. Forest plots of MR analysis between GM and VTE, PE, and DVT

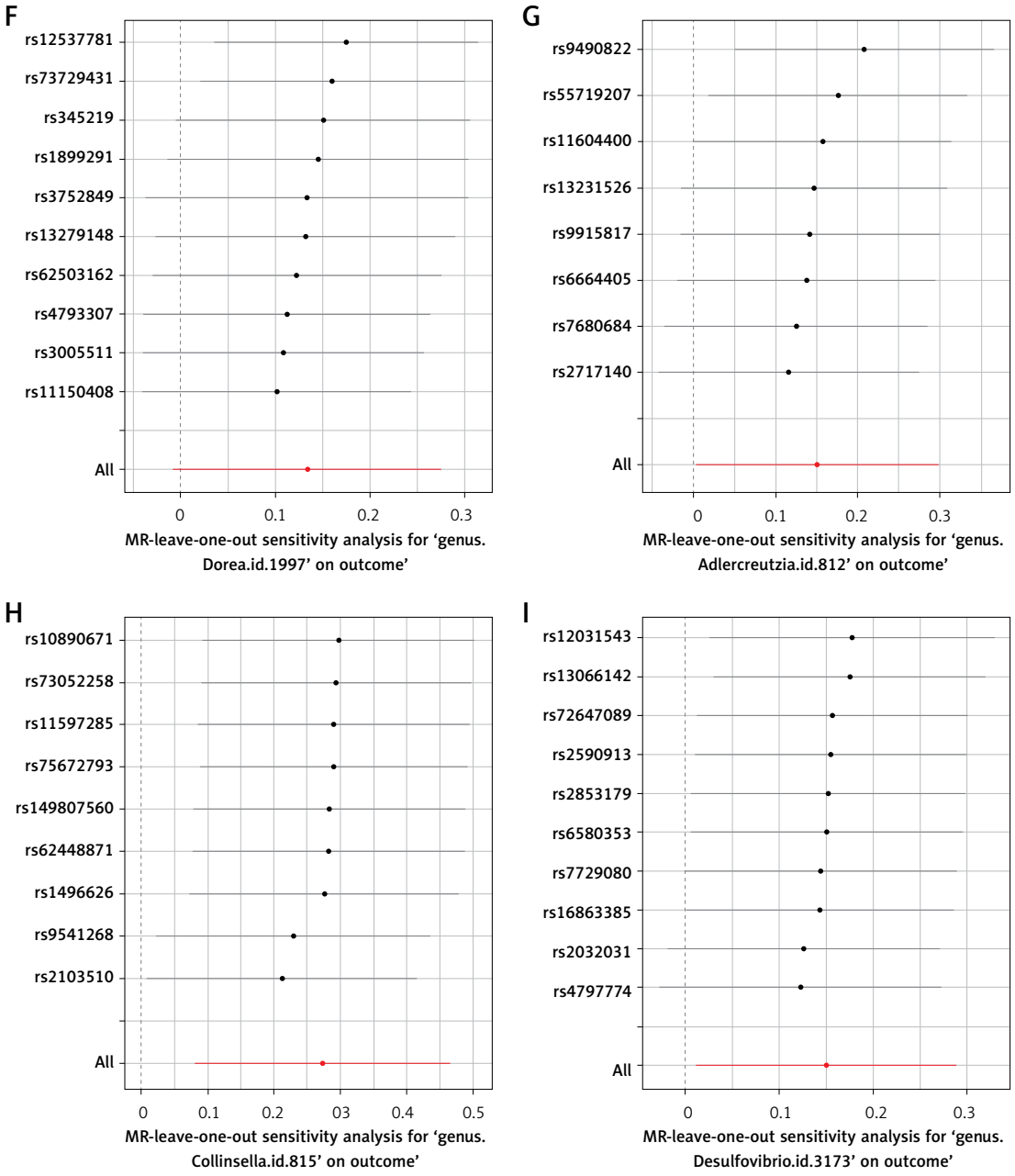

Figure 6. Cont.

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 *Firmicutes* phylum 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 patient 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 the 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 have investigated the causal association between GM and VTE. We incorporated 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.

In conclusion, 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.

Funding

National Natural Science Foundation of China (82274528); Shanghai Municipal Health Commission Scientific Research rogramme Mission Statement (202240228); Training Program for High-caliber Talents of Clinical Research at Affiliated Hospitals of SHUTCM (2023LCRC06).

Ethical approval

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

References

1. Al Raizah A, Alrizah M. Artificial intelligence in thrombosis: transformative potential and emerging challenges. Thromb J 2025; 23: 2.
2. Xia YQ, Tang L, Hu Y. [Advances in the genetics of venous thromboembolic disease]. Zhonghua Xue Ye Xue Za Zhi 2024; 45: 1144-7.
3. Saad M, Batool RM, Waqas SA, et al. Unveiling the trends: growing cancer and venous thromboembolism mortality in older adults in the United States, 1999-2020. Thromb Res 2025; 247: 109259.
4. Opitz CF, Meyer FJ. Pulmonary embolism: an update based on the revised AWMF-S2k guideline. Hamostaseologie 2024; 44: 111-8.
5. Navarrete S, Solar C, Tapia R, et al. Pathophysiology of deep vein thrombosis. Clin Exp Med 2023; 23: 645-54.
6. Szymanski K, Weber C, Daugherty K, et al. A review of venous thromboembolism for the hospitalist. Postgrad Med 2025; 137: 131-8.
7. Wang X, Zhang C, Pan M, et al. Design and rationale of the multicenter randomized clinical trial (REVERSE): efficacy and safety of rivaroxaban in the early postoperative period for patients with bioprosthetic valve replacement or valve repair. Int J Cardiol 2025; 425: 133023.
8. Zeng J, Feng J, Luo Y, et al. Inflammatory biomarkers as predictors of symptomatic venous thromboembolism in hospitalized patients with AECOPD: a multicenter cohort study. J Atheroscler Thromb 2025; 32: 439-57.
9. Liu Q, Yang F, Kong K, et al. Potential causal relationships between blood metabolites, inflammatory cytokines, and venous thromboembolism. Front Immunol 2024; 15: 1445790.
10. Dong Y, Zhang K, Wei J, et al. Gut microbiota-derived short-chain fatty acids regulate gastrointestinal tumor immunity: a novel therapeutic strategy? Front Immunol 2023; 14: 1158200.
11. Larsson SC, Butterworth AS, Burgess S. Mendelian randomization for cardiovascular diseases: principles and applications. Eur Heart J 2023; 44: 4913-24.
12. Wu Q, Li J, Sun X, et al. Multi-stage metabolomics and genetic analyses identified metabolite biomarkers of metabolic syndrome and their genetic determinants. EBioMedicine 2021; 74: 103707.
13. Xu S, Li X, Zhang S, et al. Oxidative stress gene expression, DNA methylation, and gut microbiota interaction trigger Crohn's disease: a multi-omics Mendelian randomization study. BMC Med 2023; 21: 179.
14. Pasqualini J, Facchin S, Rinaldo A, et al. Emergent ecological patterns and modelling of gut microbiomes in health and in disease. PLoS Comput Biol 2024; 20: e1012482.
15. Gong F, Zheng X, Zhao S, et al. Disseminated intravascular coagulation: cause, molecular mechanism, diagnosis, and therapy. MedComm 2025; 6: e70058.
16. Johnson TA, Mukhopadhyay S, Buzza MS, et al. Regulation of macrophage fibrinolysis during venous thrombus resolution. Thromb Res 2024; 243: 109149.
17. Birney E. Mendelian randomization. Cold Spring Harb Perspect Med 2022; 12: a041302.
18. Song Q, Huang T, Song J, et al. Causal associations of body mass index and waist-to-hip ratio with cardiometabolic traits among Chinese children: a Mendelian randomization study. Nutr Metab Cardiovasc Dis 2020; 30: 1554-63.
19. Zhou J, Li Y, Lin Y, et al. The genetic causal association between hip or knee osteoarthritis and frailty: a two-sample Mendelian randomization analysis. Arch Med Sci 2024; 20: 938-46.
20. Jiang R, Qu Q, Wang Z, et al. Association between air pollution and bone mineral density: a Mendelian randomization study. Arch Med Sci 2024; 20: 1334-8.
21. Kurilshikov A, Medina-Gomez C, Bacigalupe R, et al. Large-scale association analyses identify host factors influencing human gut microbiome composition. Nat Genet 2021; 53: 156-65.
22. Kamat MA, Blackshaw JA, Young R, et al. PhenoScanner V2: an expanded tool for searching human genotype-phenotype associations. Bioinformatics 2019; 35: 4851-3.
23. Emdin CA, Khera AV, Kathiresan S. Mendelian randomization. JAMA 2017; 318: 1925-6.
24. Bowden J, Del Greco MF, Minelli C, et al. A framework for the investigation of pleiotropy in two-sample summary data Mendelian randomization. Stat Med 2017; 36: 1783-802.
25. Zhang Y, Li D, Zhu Z, et al. Evaluating the impact of metformin targets on the risk of osteoarthritis: a mendelian randomization study. Osteoarthritis Cartilage 2022; 30: 1506-14.
26. Zhao JV, Schooling CM. Using Mendelian randomization study to assess the renal effects of antihypertensive drugs. BMC Med 2021; 19: 79.
27. Liu Z, Zhang H, Sun X, et al. Causal association between metabolites and age-related macular degeneration: a bidirectional two-sample mendelian randomization study. Hereditas 2024; 161: 51.
28. Wu Y, Shen Z, Chen B, et al. Investigation of bidirectional causal association between temporomandibular disorders and five mental disorders. Arch Oral Biol 2024; 171: 106169.
29. Zhou C, Zhou Y, Ma W, et al. Revisiting Virchow's triad: exploring the cellular and molecular alterations in cerebral venous congestion. Cell Biosci 2024; 14: 131.
30. Lv K, Chen S, Xu X, et al. Protein disulfide isomerase cleaves allosteric disulfides in histidine-rich glycoprotein to regulate thrombosis. Nat Commun 2024; 15: 3129.

31. Gong D, Zhang L, Zhang Y, et al. Gut microbial metabolite trimethylamine N-oxide is related to thrombus formation in atrial fibrillation patients. Am J Med Sci 2019; 358: 422-8.
32. Reiner MF, Muller D, Gobbato S, et al. Gut microbiota-dependent trimethylamine-N-oxide (TMAO) shows a U-shaped association with mortality but not with recurrent venous thromboembolism. Thromb Res 2019; 174: 40-7.
33. Papa A, Santini P, De Lucia SS, et al. Gut dysbiosis-related thrombosis in inflammatory bowel disease: potential disease mechanisms and emerging therapeutic strategies. Thromb Res 2023; 232: 77-88.
34. Jonsson AL, Backhed F. Role of gut microbiota in atherosclerosis. Nat Rev Cardiol 2017; 14: 79-87.
35. Huang X, Zhang Y, Yi S, et al. Potential contribution of the gut microbiota to the development of portal vein thrombosis in liver cirrhosis. Front Microbiol 2023; 14: 1217338.
36. Lee H, An J, Kim J, et al. A novel Bacterium, Butyricimonas virosa, preventing HFD-induced diabetes and metabolic disorders in mice via GLP-1 receptor. Front Microbiol 2022; 13: 858192.
37. Tsai Y, Tai W, Liang C, et al. Alternations of the gut microbiota and the Firmicutes/Bacteroidetes ratio after biologic treatment in inflammatory bowel disease. J Microbiol Immunol Infect 2025; 58: 62-9.
38. Mi HTN, Chaiyasarn S, Kim H, et al. C-glycoside-metabolizing human gut Bacterium, Dorea sp. MRG-IFC3. J Microbiol Biotechnol 2023; 33: 1606-14.
39. Wu Y, Chen Y, Li Q, et al. Tetrahydrocurcumin alleviates allergic airway inflammation in asthmatic mice by modulating the gut microbiota. Food Funct 2021; 12: 6830-40.
40. Wang L, Cai Y, Garssen J, et al. The bidirectional gut-lung axis in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2023; 207: 1145-60.
41. Montatore M, Zagaria A, Masino F, et al. A rare case of Lemierre's syndrome due to Veillonella Parvula: a dangerous and forgotten complication of a septic condition. Indian J Otolaryngol Head Neck Surg 2024; 76: 3570-5.
42. Li J, Liu C, Xu Y, et al. Gut microbiota alterations in adolescent idiopathic scoliosis are associated with aberrant bone homeostasis. Orthop Surg 2024; 16: 965-75.
43. Li T, Chu Y, Yan K, et al. Simultaneous determination of tanshinol, protocatechuic aldehyde, protocatechuic acid, notoginsenoside R1, ginsenoside Rg1 and Rb1 in rat plasma by LC-MS/MS and its application. Biomed Chromatogr 2017; 31: doi: 10.1002/bmc.3889.
44. Cao J, An G, Li R, et al. Novel strategy for human deep vein thrombosis diagnosis based on metabolomics and stacking machine learning. Anal Chem 2024; 96: 14560-70.
45. Barone M, Barone M, Ricci F, et al. A specific host/microbial signature of plasma-derived extracellular vesicles is associated to thrombosis and marrow fibrosis in polycythemia vera. Cancers 2021; 13: 4968.
46. An J, Kwon H, Kim YJ. The Firmicutes/Bacteroidetes ratio as a risk factor of breast cancer. J Clin Med 2023; 12: 2216.
47. Rafie E, Zugman M, Pal SK, et al. What is the role of fecal microbiota transplantation in immunotherapy trials? Current perspectives and future directions. Eur Urol Focus 2024; 10: 882-5.
48. Tiwari S, Paramanik V. Role of probiotics in depression: connecting dots of gut-brain-axis through hypothalamic-pituitary adrenal axis and tryptophan/kynurenic pathway involving indoleamine-2,3-dioxygenase. Mol Neurobiol 2025; 62: 7230-41.
49. Yu J, Wu Y, Zhu Z, et al. The impact of dietary patterns on gut microbiota for the primary and secondary prevention of cardiovascular disease: a systematic review. Nutr J 2025; 24: 17.
50. Almeida-Santos AC, Duarte B, Tedim AP, et al. The healthy human gut can take it all: vancomycin-variable, linezolid-resistant strains and specific bacteriocin-species interplay in Enterococcus spp. Appl Environ Microbiol 2025; 91: e169924.
51. Abdullah, Ahmad N, Xiao J, et al. Gingerols: preparation, encapsulation, and bioactivities focusing gut microbiome modulation and attenuation of disease symptoms. Phytomedicine 2025; 136: 156352.