Causal effect of gut microbiota on venous thromboembolism: a two-sample mendelian randomization study

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Abstract

Background The gut microbiota of venous thromboembolism (VTE) patients exhibited significant alterations. However, the causal relationship between gut microbiota and VTE has not been fully understood. This study aimed to assess the causal relationship between gut microbiota and the risk of VTE using a two-sample Mendelian Randomization (MR) study.

Methods The gut microbiota and VTE genetic data were collected from the MiBioGen consortium and the UK biobank, respectively. The potential causal relationship between gut microbiota and VTE was investigated using a two-sample MR analysis, including inverse variance weighted (IVW), weighted median, MR-Egger, simple mode, and weighted mode methods. Cochran's Q-test, MR-PRESSO, and MR-Egger regression intercept analysis were utilized to perform sensitivity analysis.

Results At the genus level, the results of MR analysis found that *Coprococcus1* (OR: 1.0029, 95% CI: 1.0005–1.0054, $p = 0.0202$) was suggestively linked with an increased risk of VTE, while *Slackia* (odds ratio (OR): 0.9977, 95% confidence interval (CI): 0.9957–0.9998, $p = 0.0298$), *Butyricicoccus* (OR: 0.9971, 95% CI: 0.9945–0.9997, $p = 0.0309$), *Eubacterium coprostanoligenes group* (OR: 0.9972, 95% CI: 0.9946–0.9999, $p = 0.0445$), and *Bacteroides* (OR: 0.9964, 95% CI: 0.9932–0.9995, $p = 0.0234$) were suggestively associated with a reduced risk of VTE. No heterogeneity and horizontal pleiotropy was detected.

Conclusion This study found that there were potential causal relationships between five gut microbiota and VTE. Our findings may provide new insights into the mechanisms of VTE.

Keywords Gut microbiota, Venous thromboembolism, Causal relationship, Mendelian randomization analysis

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Introduction

Venous thromboembolism (VTE), encompassing deep vein thrombosis (DVT) and pulmonary embolism (PE), is a major global health concern with significant morbidity and mortality [1]. Annually, approximately 10 million individuals worldwide are affected by VTE, making it the third most common vascular disease after acute myocardial infarction and stroke [2]. Oral anticoagulation therapy is the primary treatment for most patients with VTE [3]. Traditional risk factors for VTE include surgery, trauma, cancer, pregnancy, advanced age, obesity, prolonged immobility, and genetic predisposition [3]. However, these factors can not fully explain the incidence of VTE, indicating the need to investigate additional risk factors [4].

Recent research has begun to explore the potential role of the gut microbiota in cardiovascular diseases, including VTE [5, 6]. The gut microbiota, a complex ecosystem of microorganisms residing in the human gastrointestinal tract, plays a crucial role in maintaining host health [7]. Advances in high-throughput sequencing technologies have expanded our understanding of the gut microbiome's composition and functions [8]. Environmental or genetic perturbations of the gut microbiome can trigger inflammatory cascades in vascular endothelium, platelets, and innate immune cells, leading to the release of procoagulant factors and the development of a prothrombotic state [9]. Emerging evidences suggest that alterations in the gut microbiota may be associated with VTE [9, 10]. For example, 16 S rRNA gene sequencing on feces samples revealed altered gut microbiota in VTE patients, with abnormal proliferation of *Blautia*, *Roseburia*, *Coprococcus*, and *Ruminococcus* compared to healthy controls [6]. However, the potential causal relationship of the gut microbiome on VTE remains unclear.

Mendelian randomization (MR) is a method that leverages genetic variants as instrumental variables (IVs) to infer causal relationship between exposure and outcome [11]. By exploiting the random assortment of alleles during meiosis, MR analysis reduces confounding factors, enhancing causal inference reliability [12]. This approach has been widely adopted to explore gut microbiome-disease associations, including metabolic, autoimmune, and cardiovascular disorders [13–15]. Our study employs MR analysis to investigate the potential causal link between the gut microbiome and VTE, aiming to unveil novel insights into VTE pathogenesis.

Materials and methods

Study design

In this study, the causal effect between gut microbiota and VTE was investigated using the MR analysis. The study schematic diagram is presented in Fig. 1. The exposure was gut microbiota, and the outcome was VTE. The MR analysis needs to satisfy the following assumptions: (1) The IVs must be closely related to exposure; (2) The IVs are not associated with confounders; (3) The IVs affect outcome only via exposure. This study was a secondary analysis of data from the original study and did not require additional ethical approval.

Data sources

The gut microbiota genetic data were obtained from the largest genome-wide meta-analysis published to date for gut microbiota composition conducted by the MiBioGen consortium, which included genome-wide association studies (GWAS) data and 16 S fecal microbiome data for 18,340 individuals from 24 cohorts, approximately 78% of whom were European (14,306 individuals from 18 cohorts). Additionally, sex, age, and other covariates were adjusted in all cohorts. The GWAS summary data included a total of 211 gut microbiota taxa (131 genera, 35 families, 20 orders, 16 classes, and 9 phyla). After excluding 12 unknown genera, 119 taxa at the genera level as the exposure were included in the follow-up study. For those interested in further details, the complete information of the dataset can be accessed through the link (https://gwas.mrcieu.ac.uk/datasets/ebi-a-GCST90016908/) and literature [16].

The GWAS summary data of VTE was collected from the UK biobank. The phenotypic code for VTE was "ukb-d-I9_VTE", including 4,620 VTE cases, 356,574 controls, and 11,901,177 single nucleotide polymorphisms (SNPs) (https://gwas.mrcieu.ac.uk/datasets/ukb-d-I9_VTE/).

IVs selection

The IVs were selected according to the following criteria: (1) SNPs that significantly (p-value$<1\times10^{-5}$) associated with gut microbiota were selected as potential IVs; (2) Independent SNPs were further acquired to minimize the effect of linkage disequilibrium (LD), with parameters set to $r^2<0.001$ and clumping distance$=10,000$ kb; (3) Palindromic SNPs (SNPs with A/T or G/C alleles) and SNPs that were not present in the outcome were eliminated; (4) The strength of SNPs with F-statistic>10 were retained to avoid weak instruments bias [17].

Statistical methods

The inverse variance weighted (IVW), weighted median, MR-Egger, simple mode, and weighted mode methods were utilized to perform a two-sample MR analysis to investigate the causal effect of gut microbiota on VTE. Among these, the IVW method served as the primary approach, integrating Wald ratio estimates from multiple SNPs and providing a consistent assessment of the causal effect between exposure and outcome when the IV assumptions are met [18]. The IVW method yields the most reliable results when the IVs are not subject to
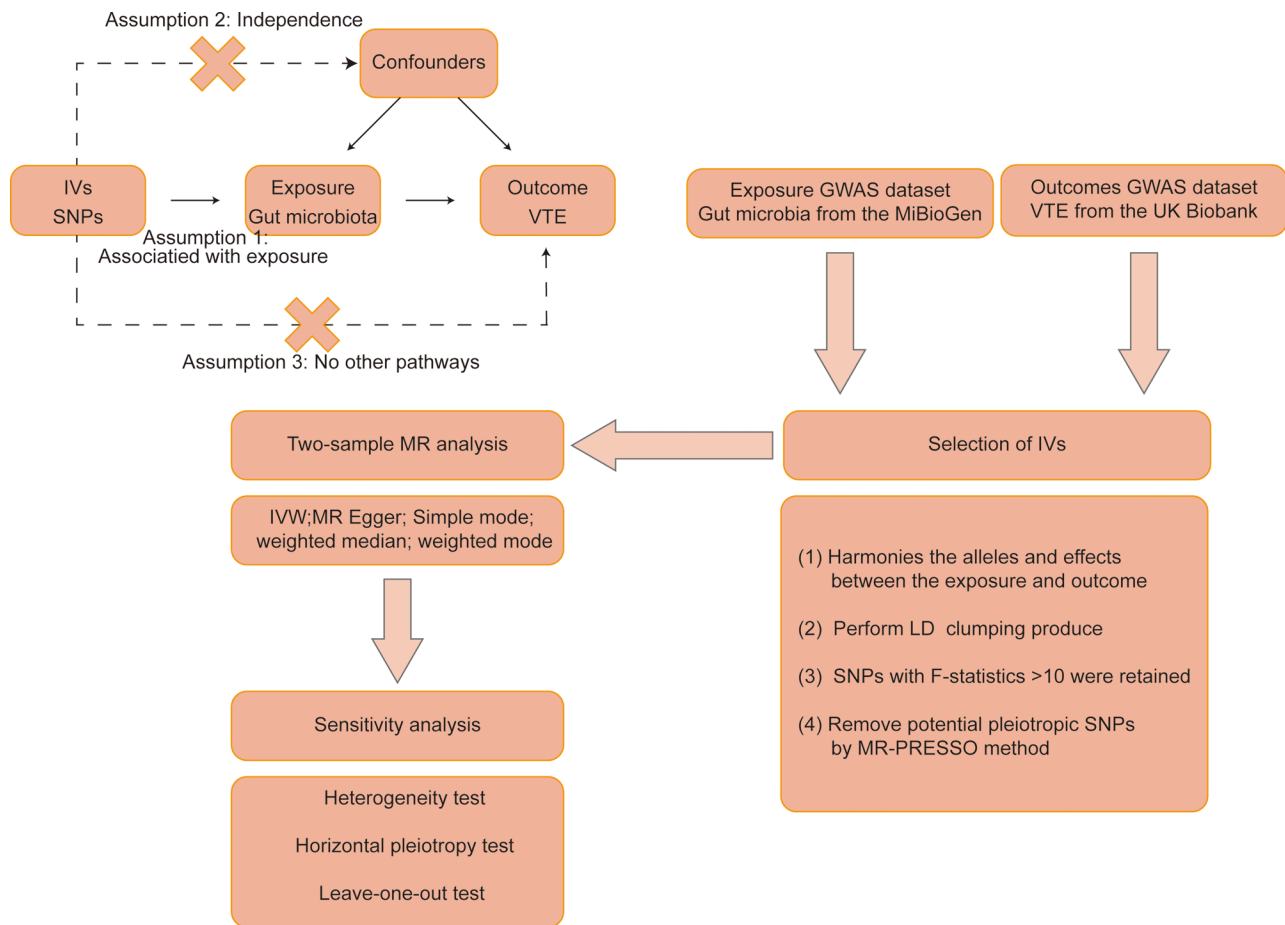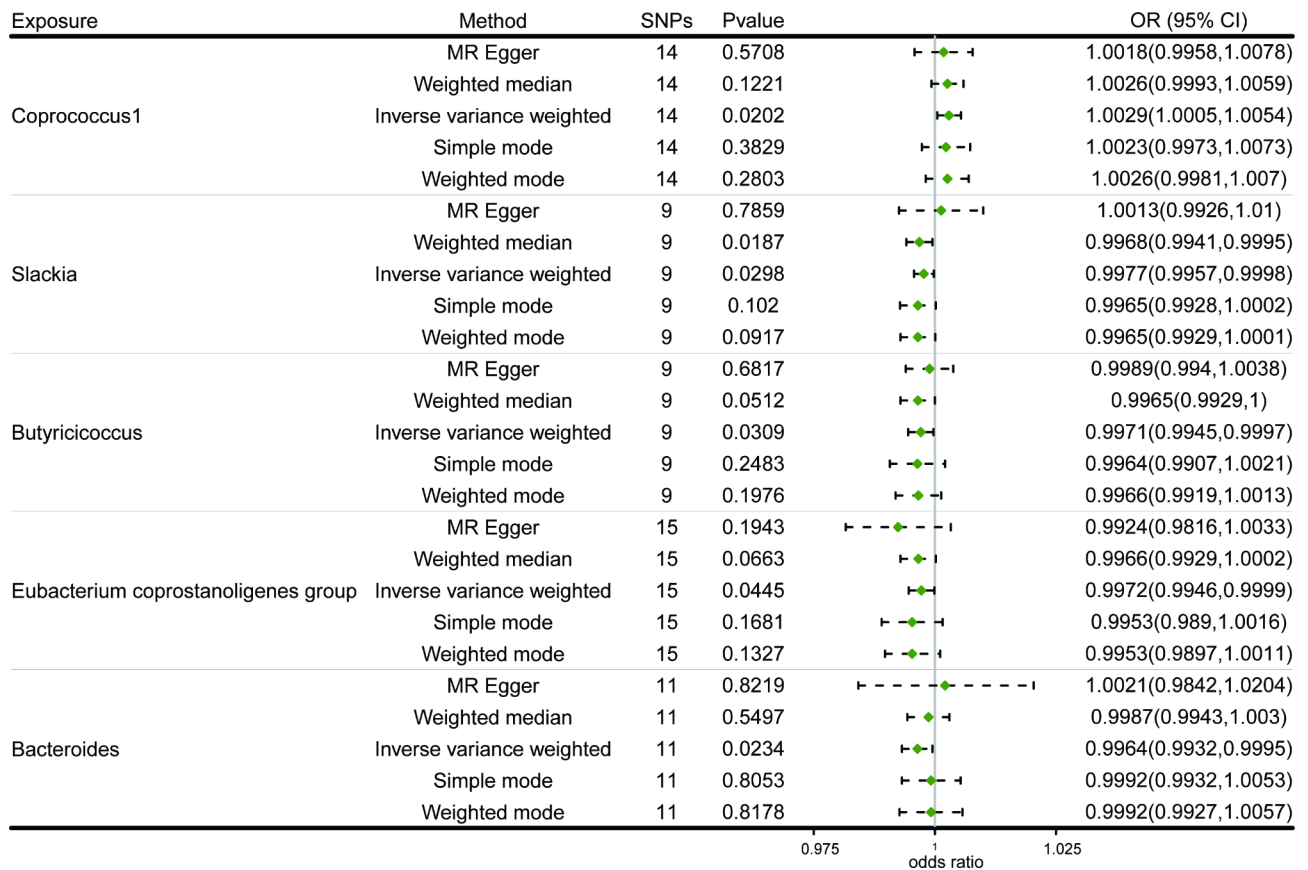
Fig. 1 The schematic diagram of this study. IVs: instrumental variables, SNPs: single-nucleotide polymorphisms, VTE: venous thromboembolism, MR: Mendelian randomization, GWAS: genome-wide association study, IVW: inverse-variance weighted, LD: linkage disequilibrium, MR-PRESSO: MR pleiotropy residual sum and outlier

horizontal pleiotropy [19]. MR-Egger regression is based on the assumption of instrument strength independent of direct effect (InSIDE), which makes it possible to evaluate the existence of pleiotropy with the intercept term. MR-Egger regression was used to detect the presence of horizontal pleiotropy in the IVs and to provide unbiased estimates of causal effect even in the presence of horizontal pleiotropy [20]. The MR-Egger method is able to generate reliable causal estimates even in situations where all IVs are invalid. The weighted median method provides consistent causal effect estimates when at least 50% of the SNPs are valid IVs [21]. Compared to the MR-Egger method, the weighted median method improves the accuracy of the results [22]. The simple mode and weighted mode approaches were employed as supplementary methods to further enhance the robustness of the analysis [23]. The simple mode is a model-based assessment approach that offers pleiotropy robustness. For mode assessment, the weighted mode is sensitive to the hard throughput collection [24].

For sensitivity analysis, Cochran's Q-test for random effect IVW was used to assess heterogeneity. MR-PRESSO and MR-Egger regression intercept analysis were applied to identify horizontal pleiotropy [25]. Additionally, MR-PRESSO analysis was used to eliminate outliers and correct for horizontal pleiotropy [26]. Moreover, leave-one-out analysis was conducted to evaluate whether the causal effect was driven by single SNPs, and the results were visualized by forest plot [27]. The Bonferroni correction method was used to identify false-positive results caused by multiple tests. Associations with $p < 4.2 \times 10^{-4}$ (0.05 divided by 119) were considered statistically significant, whereas associations with $p > 4.2 \times 10^{-4}$ and $p < 0.05$ were defined as suggestive associations. The "TwoSampleMR" and "MR-PRESSO" packages in R software (version 4.4.0) were used to carry out statistical analysis [28, 29].

Results

IVs selection

A total of 1,529 SNPs with p-value $< 1 \times 10^{-5}$ were selected as IVs from 119 taxa at the genus level after eliminating LD ($r^2 < 0.001$ and clumping distance = 10,000 kb), and removing palindromic SNPs and SNPs that were not present in the outcome (Supplementary Table 1). The F-statistic values of all IVs were more than 14 in this study, indicating no evidence of weak instrument bias.

The causal effect of gut microbiota on VTE

The causal effect of gut microbiota on VTE was investigated using a two-sample MR analysis (Fig. 2). We found that five microbial taxa were suggestively associated with the risk of VTE. According to the IVW method, four microbial taxa showed a potential protective effect on VTE, while one was associated with an increased risk of VTE (Figs. 3 and 4). The microbial taxa that play a protective role in VTE are as follows: *Slackia* (odds ratio (OR): 0.9977, 95% confidence interval (CI): 0.9957–0.9998, $p = 0.0298$), *Butyricicoccus* (OR: 0.9971, 95% CI: 0.9945–0.9997, $p = 0.0309$), *Eubacterium coprostanoligenes group*

Fig. 2 A circus plot showing the causal effects of gut microbiota on VTE. From the outer circle to the inner circle, five methods are represented: weighted mode, simple mode, inverse variance weighted, weighted median, and MR Egger. The colour shades indicated the size of the p-value

Exposure	Method	SNPs	Pvalue		OR (95% CI)
Coprococcus1	MR Egger	14	0.5708		1.0018(0.9958,1.0078)
	Weighted median	14	0.1221		1.0026(0.9993,1.0059)
	Inverse variance weighted	14	0.0202		1.0029(1.0005,1.0054)
	Simple mode	14	0.3829		1.0023(0.9973,1.0073)
	Weighted mode	14	0.2803		1.0026(0.9981,1.007)
Slackia	MR Egger	9	0.7859		1.0013(0.9926,1.01)
	Weighted median	9	0.0187		0.9968(0.9941,0.9995)
	Inverse variance weighted	9	0.0298		0.9977(0.9957,0.9998)
	Simple mode	9	0.102		0.9965(0.9928,1.0002)
	Weighted mode	9	0.0917		0.9965(0.9929,1.0001)
Butyricicoccus	MR Egger	9	0.6817		0.9989(0.994,1.0038)
	Weighted median	9	0.0512		0.9965(0.9929,1)
	Inverse variance weighted	9	0.0309		0.9971(0.9945,0.9997)
	Simple mode	9	0.2483		0.9964(0.9907,1.0021)
	Weighted mode	9	0.1976		0.9966(0.9919,1.0013)
Eubacterium coprostanoligenes group	MR Egger	15	0.1943		0.9924(0.9816,1.0033)
	Weighted median	15	0.0663		0.9966(0.9929,1.0002)
	Inverse variance weighted	15	0.0445		0.9972(0.9946,0.9999)
	Simple mode	15	0.1681		0.9953(0.989,1.0016)
	Weighted mode	15	0.1327		0.9953(0.9897,1.0011)
Bacteroides	MR Egger	11	0.8219		1.0021(0.9842,1.0204)
	Weighted median	11	0.5497		0.9987(0.9943,1.003)
	Inverse variance weighted	11	0.0234		0.9964(0.9932,0.9995)
	Simple mode	11	0.8053		0.9992(0.9932,1.0053)
	Weighted mode	11	0.8178		0.9992(0.9927,1.0057)

0.975   1   1.025
odds ratio

Fig. 3 Forest plot of the causal effects of *Coprococcus1*, *Slackia*, *Butyricicoccus*, *Eubacterium coprostanoligenes group*, and *Bacteroides* on VTE. SNPs: single nucleotide polymorphisms, OR: odds ratio, CI: confidence interval

(OR: 0.9972, 95% CI: 0.9946–0.9999, $p = 0.0445$), and *Bacteroides* (OR: 0.9964. 95% CI: 0.9932–0.9995, $p = 0.0234$). In contrast, *Coprococcus1* (OR: 1.0029, 95% CI: 1.0005–1.0054, $p = 0.0202$) was associated with an increased risk of VTE.

For *Slackia* and *Bacteroides*, the causal effects evaluated by ME-Egger are opposite to those estimated by the other four methods, although not significant (Fig. 4B, E). These potential causal effects warrant cautious interpretation. However, given the absence of horizontal pleiotropy and heterogeneity (explained later), the IVW method has the strongest statistical power compared to the MR-Egger method. Consequently, *Slackia* and *Bacteroides* may reduce the risk of VTE.

Sensitivity analyses
The results of the sensitivity analyses are presented in Table 1. Cochran's Q tests revealed no significant heterogeneity (p-value > 0.05, Table 1). Furthermore, the MR-Egger intercept and MR-PRESSO analyses showed no horizontal pleiotropy (p-value > 0.05, Table 1). Leave-one-out analyses indicated that the results were not influenced by any single SNP (Fig. 5). These sensitivity analyses support the robustness of our main findings.

Discussion
The potential causal relationship of gut microbiota on VTE was explored in this study using GWAS data through two-sample MR analysis. We found that *Slackia*, *Butyricicoccus*, *Eubacterium coprostanoligenes group*, and *Bacteroides* were suggestively associated with a reduced risk of VTE, whereas *Coprococcus1* was suggestively associated with an increased risk of VTE. These findings contribute to the current understanding of the potential role of gut microbiota in VTE pathogenesis and elucidate specific taxa that may either promote or inhibit VTE development.

The etiology of VTE involves complex interactions among the coagulation cascade, innate immune system, and inflammatory processes [30]. Research in vivo has demonstrated that targeting inflammatory pathways can effectively reduce the incidence of venous thrombosis [31]. Isoflavones, phenolic compounds with estrogenic and anti-inflammatory properties, have been reported to inhibit thrombus formation and platelet aggregation in animal models [32, 33]. However, isoflavones require microbial transformation into bioactive metabolites for human absorption and utilization [34]. These active compounds include dihydrodaidzein, equol, and
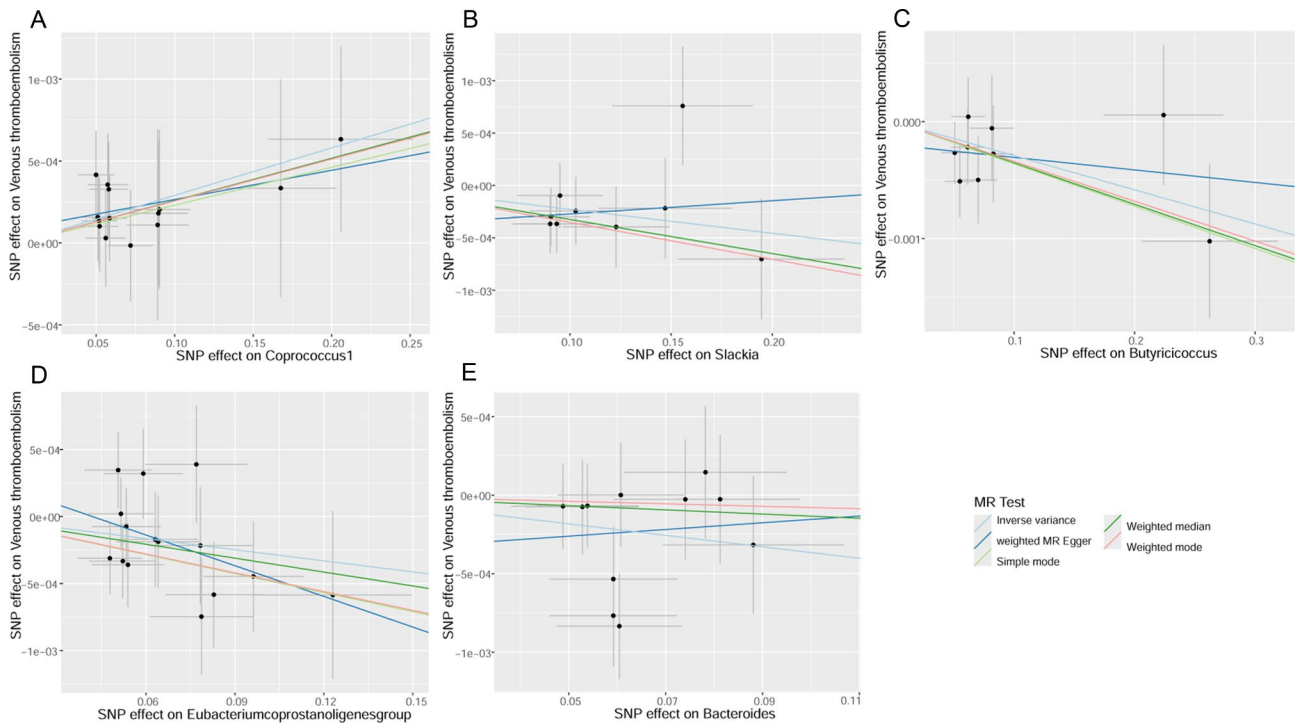
Fig. 4 Scatterplots of the causal effects of *Coprococcus1* (**A**), *Slackia* (**B**), *Butyricicoccus* (**C**), *Eubacterium coprostanoligenes group* (**D**), and *Bacteroides* (**E**) on VTE

Table 1 Heterogeneity and pleiotropy test

Exposure	Heterogeneity test						Pleiotropy test		
	MR-Egger			Inverse variance weighted			MR-Egger		
	Q	Q_df	Q_pval	Q	Q_df	Q_pval	Egger intercept	se	pval
Coprococcus1	2.376	12	0.999	2.536	13	0.999	8.64E-05	0.0002	0.696
Slackia	4.513	7	0.719	5.182	8	0.738	-0.0004	0.0005	0.441
Butyricicoccus	3.543	7	0.831	4.312	8	0.828	-0.0002	0.0002	0.409
Eubacterium coprostanoligenes group	10.539	13	0.649	11.352	14	0.658	0.0003	0.0004	0.384
Bacteroides	9.633	9	0.381	10.071	10	0.434	-0.0004	0.0006	0.538

Q: Heterogeneity static Q; df: Degree of freedom; se: Standard error

5-hydroxy-equol [34]. *Slackia isoflaviconvertens*, a human gut bacterium, converts isoflavones into equol and 5-hydroxy-equol [35, 36]. Additionally, the activation and aggregation of platelets contributed to the venous thrombus formation [37]. It is notable that thromboxane A2 (TxA2), a primary platelet metabolite, promotes these processes [38]. Muñoz et al. reported that equol, which has the highest affinity for TxA2 receptors compared to daidzein and genistein, inhibits platelet activation and aggregation through competitive receptor antagonism [39].

Short-chain fatty acids (SCFAs), including acetate, propionate, and butyrate, are produced by the gut microbiota through dietary fermentation [40]. SCFAs play crucial roles in maintaining intestinal homeostasis and modulating immune and inflammatory responses [41]. These SCFAs are mainly produced by the phyla Firmicutes and Bacteroidetes [42]. Bacterial lipopolysaccharide (LPS) is a key mediator linking the microbiome to a hypercoagulable state [43]. By binding to Toll-like receptors on immune cells, LPS activates endothelial cells and platelets, triggering the coagulation cascade and promoting thrombosis [43]. Butyrate and propionate inhibit LPS-induced IL-6 and IL-12 expression in monocyte-derived mature dendritic cells [44]. Moreover, the NF-κB signaling pathway is closely linked to thrombosis [45]. Its activation elevates pro-inflammatory factors and fibrinolytic inhibitors while reducing anti-thrombotic factors, exacerbating inflammation and thrombosis [45]. Many studies have demonstrated that butyrate exerts anti-inflammatory effects by inhibiting NF-κB activation in macrophages [46–48].

Butyricicoccus and *Bacteroides* genera belong to the phylum Bacteroidetes [49]. *Butyricicoccus pullicaecorum*
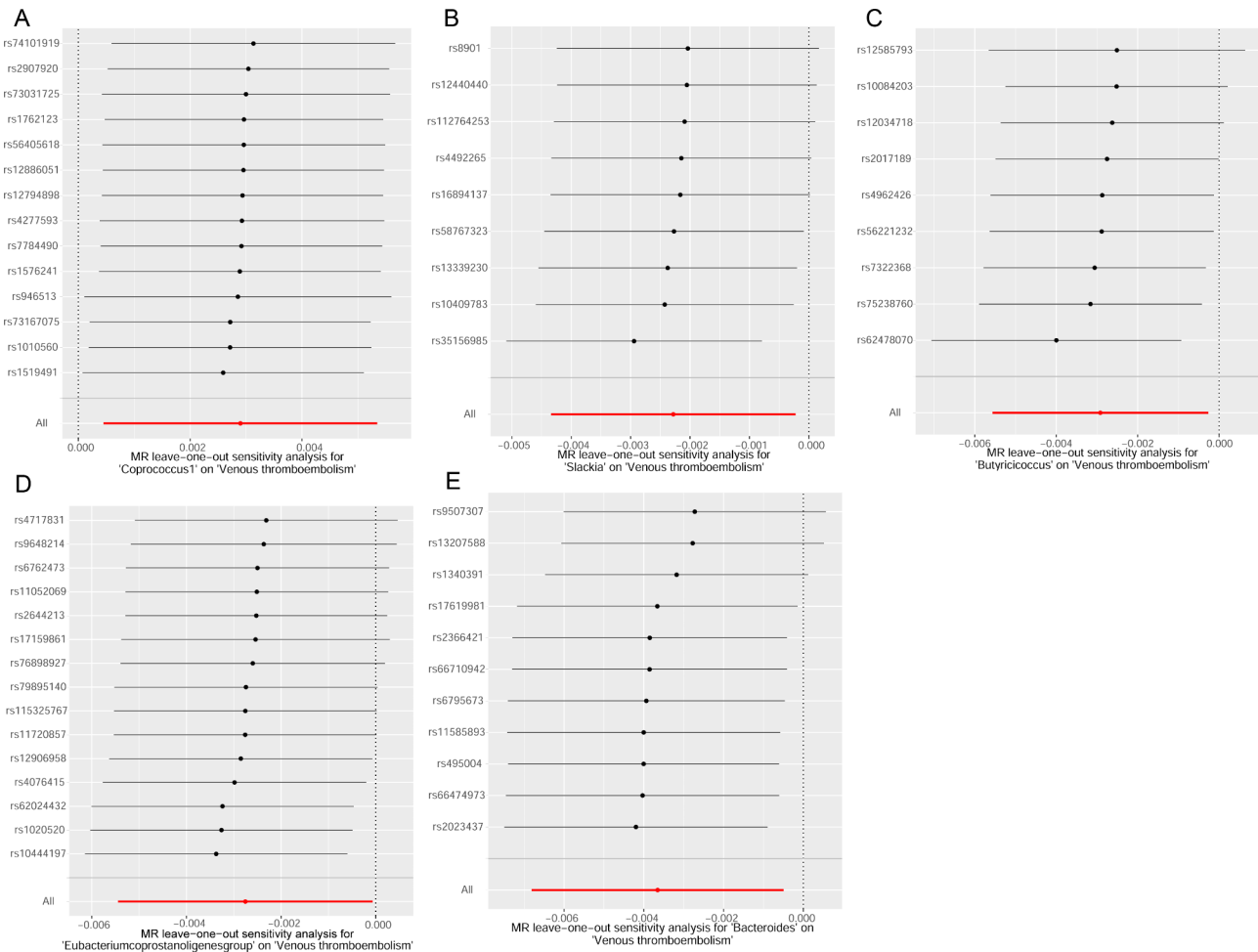
Fig. 5 Leave-one-out plots of the causal effects of *Coprococcus1* (**A**), *Slackia* (**B**), *Butyricicoccus* (**C**), *Eubacterium coprostanoligenes group* (**D**), and *Bacteroides* (**E**) on VTE

and *Butyricicoccus desmolans comb. nov* were representative of butyrate-producing bacteria [50, 51]. *Bacteroides humanifaecis sp.* is a strain isolated from human feces that produces acetate and isobutyrate [52]. Furthermore, using certain oligosaccharides to cultivate healthy human fecal bacteria led to an enrichment of *Bacteroides* and increased SCFA production [53]. Additionally, *Bacteroides* has been reported to alleviate LPS-induced inflammation [54]. In mice with liver cirrhosis, treatment with *Bacteroides* alleviated the progression of portal vein thrombosis [55]. Besides, the *Eubacterium coprostanoligenes group* belongs to the phylum Firmicutes [56]. The *Eubacterium coprostanoligenes group* has been reported to enhance the intestinal mucus barrier and relieve chemotherapy-induced intestinal mucositis [57]. The correlation between *Slackia, Butyricicoccus, Eubacterium coprostanoligenes group,* and *Bacteroides* and VTE identified in our results are consistent with their known biological functions and previous research findings.

The genus *Coprococcus1* is the major butyrate producer and is often considered a probiotic [58, 59]. MR analyses have revealed a negative association between *Coprococcus1* and sepsis incidence and mortality [60, 61]. Conversely, *Coprococcus1* was identified as a risk factor for interstitial cystitis in MR studies [62, 63]. Our current investigation suggests that *Coprococcus1* may increase the risk of VTE. However, these findings warrant further investigation for validation.

In brief, this study has several strengths and limitations. For strengths, the causal relationship of gut microbiota on VTE was analyzed, avoiding the interference of reverse causal links and reducing confounder factors. Secondly, this study leveraged genetic data from a large population cohort, enhancing the reliability of our findings. Thirdly, compared to traditional observational studies, this approach proved more time-efficient and effectively mitigated the impact of confounding factors. For limitations, the data on gut microbiome and VTE primarily derive from European populations, potentially

limiting generalizability to other ethnicities. Secondly, although our findings suggest causal relationships between gut microbiota and VTE, these findings are based on predictions of genetic associations and should be considered preliminary, and the underlying mechanisms require further investigation. Moreover, our use of summary-level data precluded subgroup analyses, such as distinguishing between DVT and PE. Lastly, our study focused on genetic-level causality; applying these conclusions to VTE risk assessment necessitates careful consideration of the disease's multifactorial nature.

Conclusion
This study found five genera of gut microbiota were suggestively associated with the risk of VTE. *Slackia, Butyricicoccus, Eubacterium coprostanoligenes group*, and *Bacteroides* were associated with a reduced risk of VTE, while *Coprococcus1* was linked to an increased risk of VTE. These findings may offer new directions for clinical intervention in VTE management.

Abbreviations
VTE	Venous thromboembolism
MR	Mendelian randomization
IVW	Inverse variance weighted
OR	Odds ratio
CI	Confidence interval
DVT	Deep vein thrombosis
PE	Pulmonary embolism
GWAS	Genome-wide association studies
SNPs	Single nucleotide polymorphisms
LD	Linkage disequilibrium
TxA2	Thromboxane A2
SCFAs	Short-chain fatty acids
LPS	Lipopolysaccharide

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12959-024-00676-7.

Supplementary Material 1

Author contributions
M. L and C. W designed the study. L. X and H. W wrote the manuscript. L.X, H. W, J D, S. Z and M. L collected, analyzed, and interpreted the data. M.L, W.X and C. W critically reviewed, edited the manuscript. All authors reviewed and approved the final manuscript.

Data availability
The gut microbiota genetic data were obtained from the MiBioGen consortium. The GWAS summary data of VTE was collected from the UK biobank.

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