# Association between gut microbiota and venous thromboembolism: a two-sample Mendelian randomization study

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**Research Article**

**Keywords:** Gut microbiota, Venous thromboembolism, Mendelian randomization, Causal effect, Single nucleotide polymorphisms

**Posted Date:** August 28th, 2023

**DOI:** https://doi.org/10.21203/rs.3.rs-3284945/v1



**Additional Declarations:** No competing interests reported.

# Abstract

**Background:** Recent studies have suggested an association between gut microbiota (GM) and venous thromboembolism (VTE). However, observational studies cannot indicate causality and population-level studies with a higher evidence level for causality are lacking. Therefore, our study aimed to explore the causal association of GM with VTE.

**Methods:** This study utilized the summary-level data of respective genome-wide association study for 196 gut microbial taxa and VTE. Two-sample Mendelian randomization (MR) design was deployed and comprehensively sensitive analyses were followed to validate the robustness of results. We used the inverse-variance weighted (IVW) method, the weighted median method, weighted mode method, simple mode method, MR-Egger regression, MR-Egger intercept test, Cochrane's Q-test, outlier test, and leave-one-out analysis as the primary analysis.

**Results:** We identified suggestive associations between 17 bacterial traits and the risk of VTE. Porphyromonadaceae (IVW odds ratio (OR): 1.3729, p=0.0035) and Cyanobacteria (IVW OR: 1.2151, p=0.0048) were associated with increased risk of VTE. Three gut microbiota taxa (Eubacteriumrectalegroup (IVW OR: 1.0038, p=0.0278), Coprococcus2 (IVW OR: 1.0041, p = 0.0063), and LachnospiraceaeUCG001 (IVW OR: 1.0041, p=0.0009) were predicted to play a causal role in enhancing the risk of encompassing deep vein thrombosis. And three gut microbiota taxa (Christensenellaceae (IVW OR: 1.0023, p=0.0497), Streptococcaceae (IVW OR: 1.0031, p=0.0279), Victivallaceae (IVW OR: 1.0014, p=0.0493) were positively associated with pulmonary embolism.

**Conclusions:** This study suggested the role of the specific GM on the risk for VTE, which may provide new ideas and a theoretical basis for the prevention and treatment of VTE in the future.


# 1. Introduction

Venous thromboembolism (VTE), encompassing deep vein thrombosis (DVT) and pulmonary embolism (PE), affects approximately 10 million individuals across diverse racial backgrounds annually on a global scale, and incidence rises exponentially in correlation with advancing age(1). Of particular significance, severe PE poses a grave threat to the lives of afflicted patients(2). VTE is instigated by the interplay of various predisposing factors, including major surgical procedures and the presence of active cancer, contributing to nearly 50% of such occurrences(3). The Virchow triad encompasses the primary factors contributing to the pathogenesis of VTE, which comprise disturbances in blood flow, a hypercoagulable state of the blood, and a procoagulant condition of the blood vessel wall(4). The comprehensive comprehension of the modifiable risk factors associated with VTE and its extensive range of consequences is consistently regarded as a crucial and formidable inquiry, particularly at the microscale dimensions. Consequently, there exists a pressing necessity to ascertain modifiable risk factors from a microscopic standpoint in order to avert thrombotic occurrences and subsequently mitigate unfavorable long-term ramifications.

Microbiotas in the human gut have been recognized as host partners in recent years. Through the microbiota and their metabolites, gut microbiota (GM), which are thought to constitute a new organ, influence other organs and systems in the body like the brain, lungs, liver, and cardiovascular system(5). Research conducted on both animals and humans has demonstrated that probiotics can play a crucial role in modulating immune and anti-inflammatory mechanisms(6). Dysbiosis, which refers to an imbalance in microbial composition, is commonly characterized by a reduction in microbial diversity. Conversely, microbial dysbiosis has the potential to induce an inflammatory response by influencing the differentiation of T helper (TH) cell lineages(7). Recent evidence suggests that the GM actively participates in immunometabolism by influencing the production of metabolites, including short-chain fatty acids, bile acids, and tryptophan metabolites(8). GM can produce Trimethylamine N-oxide (TMAO) by metabolizing dietary (Western diets) phosphatidylcholine, L-carnitine, and choline, which contain trimethylamine (TMA)(9). Consequently, Western diets have been found to be associated with elevated levels of TMAO in the bloodstream, which in turn have been correlated with unfavorable outcomes pertaining to thrombotic events(10).There is an increasing body of evidence indicating an intricate interplay between coagulation and inflammation, whereby the initiation of

the coagulation cascade stimulates the immune system, subsequently leading to the promotion of thrombosis by innate immune cells through a phenomenon referred to as immunothrombosis(11).

The human gut microbiome is a vast assemblage of microorganisms that assumes an indispensable function in human existence. Owing to its intricate and mutually beneficial symbiotic association with the host, the GM exhibits a close association with human well-being, extending beyond intestinal disorders. It is noteworthy to highlight recent investigations that have established a connection between microbial dysbiosis and VTE. The GM potentially serves as a reservoir for systemic inflammation and coagulation activation by facilitating the translocation of lipopolysaccharide from Gram-negative bacteria into the systemic circulation(12). The impact of GM on human diseases has been increasingly investigated through research, revealing that dysbiosis of GM can have detrimental effects on human health, potentially leading to the development of chronic diseases(13). However, the specific connections between the GM and VTE remain largely unexplored. Therefore, it is crucial to establish a causal relationship for this correlation and identify the most relevant gut microbial taxa in order to inform clinical practices for managing VTE.

Mendelian randomization (MR) serves as an alternative approach for mitigating observational bias by utilizing genetic variation, typically single nucleotide polymorphisms (SNPs), as instrumental variables (IVs) to ascertain causal associations between exposures and disease outcomes(14). The primary advantage of MR in establishing causality stems from its utilization of genetic variants as IVs. MR capitalizes on the principles that (1) genetic variants are randomly inherited, with one allele originating from each parent (i.e., the law of segregation assortment), and (2) the transmission of alleles to offspring occurs independently(15). Hence, it is improbable for the results of MR to be susceptible to environmental factors that could potentially distort the estimated association. Numerous prior MR studies have successfully elucidated modest risk factors for diverse ailments, encompassing cancers, cardiovascular diseases (CVD), and others, thereby offering effective solutions in the field of epidemiology(16, 17). The MiBioGen consortium has recently published a significant number of microbiome abundance-associated loci(18), presenting an exceptional opportunity to investigate the causal connection between the GM and VTE. Leveraging the available genetic database, gene variants that regulate VTE can be considered IVs for further examination of the causal association between GM and the risk of VTE.

In this study, we conducted a two-sample MR analysis using publicly available large-scale genome-wide association study (GWAS) data on GM and VTE. Our aim was to investigate the potential causal effects of 196 gut microbial taxa on VTE. These results confirm the involvement of specific GM in either increasing or reducing the risk of VTE, many of which have not previously been implicated in VTE research. Therefore, our findings not only expand the current understanding of GM associated with VTE, but also establish GM's specific causal relationship with VTE, offering a novel approach for the clinical management of VTE.

## 2. Materials and Methods

## 2.1 Data resources

The data utilized in this study, pertaining to the human gut microbiome, was obtained from publicly available sources, specifically existing and published GWAS. Consequently, all original studies involved in the acquisition of this data have obtained ethical approval and informed consent. The GWAS data for the human gut microbiome was derived from the genome-wide genotypes and 16S fecal microbiome data of a total of 18,340 individuals across 24 cohorts. This data was meticulously curated and analyzed by the MiBioGen consortium. The primary sources of this data were conveniently accessed via the MiBioGen website (https://mibiogen.gcc.rug.nl/). The summary data for VTE, DVT, and PE were all obtained from the GWAS summary data repository (https://gwas.mrcieu.ac.uk/). This dataset includes 9,176 cases of VTE, 9,241 cases of DVT, and 1,846 cases of PE, with all participants being of European ancestry. Further details of information regarding the data in this MR study can be found in Table 1.

Table 1
Details of the genome-wide association studies (GWASs) used in the Mendelian randomization.

| Phenotype | Sample size | Ancestry | Data source |
|---|---|---|---|
| **Exposure** | | | |
| Gut microbiota | 18,340 | Mixed | https://mibiogen.gcc.rug.nl/ |
| **Outcomes** | **Cases/Controls** | | |
| VTE | 9,176/ 209,616 | European | finn-b-I9_VTE; https://gwas.mrcieu.ac.uk/ |
| DVT | 9,241/ 453,692 | European | ukb-b-12040; https://gwas.mrcieu.ac.uk/ |
| PE | 1,846/ 461,164 | European | ukb-b-18366; https://gwas.mrcieu.ac.uk/ |
| Abbreviations: | | | |
| VTE, venous thromboembolism; DVT, deep vein thrombosis; PE, pulmonary embolism. | | | |

# 2.2 Selection of IVs

The accurate and robust establishment of causal inference in MR heavily relies on the careful selection of IVs, which must adhere to three fundamental assumptions: Firstly, the genetic IV(s) should exhibit a strong association with the risk factor under investigation; Secondly, the variation in the IV(s) should remain unaffected by any confounding factors that may influence the relationship between the exposure and outcome variables; Lastly, the exposure variable should solely impact the outcome variable, thereby eliminating any potential pleiotropic effects and satisfying the exclusion restriction assumption (Fig. 1).

Initially, we excluded 15 bacterial traits lacking specific names and subsequently identified a total of 196 bacterial traits as the focus of our investigation. These traits encompassed 9 phyla, 16 classes, 20 orders, 32 families, and 119 genera. Subsequently, we employed the “TwoSampleMR” packages within the R statistical programming software (version 4.2.1) to select independent IVs at a genomewide significance level of $< 1.0\times10^{-5}$. This selection was based on criteria of linkage disequilibrium (LD) $r^2 < 0.001$ and a clumping distance of 10000 kb. A comprehensive analysis yielded a total of 6404 (2548 SNPs for VTE, 2587 SNPs for DVT, 1269 SNPs for PE) autonomous SNPs that exhibited associations with 196 bacterial traits. Subsequently, pertinent data such as the effect allele, effect size (including β-value), standard error, and p-value were extracted for each SNP, detailed information of the SNPs can be found in Table S1. Utilizing this information, we determined the proportion of variation explained ($R^2$) and employed F-statistics to quantify the instrument strength. The $R^2$ value represents the proportion of variance in a risk factor that can be explained by the IVs. The F statistic is frequently used as a measure of the strength of the association between the instruments and the exposure of interest, with values exceeding 10 being considered robust instruments(19). The $R^2$ and F-statistics were computed using the following formula (Table S1):

$$R^2 = 2 \times (1 - MAF) \times MAF \times \beta^2$$

$$F=(R^2/(1-R^2)) \times ((N-k-1)/k)$$

**Note**

N represents sample size; β represents the genetic estimation of each SNP on the exposure; k represents the number of SNPs. MAF minor allele frequency of SNPs used as IVs.

# 2.3 Statistical analysis

Various high-efficiency methodologies, such as the inverse-variance weighted (IVW) method, weighted median method, weighted mode method, simple mode method, MR-Egger regression, MR-Egger intercept test, Cochrane’s Q-test, outlier (MR-PRESSO) test,

and leave-one-out analysis were employed to estimate the causal effects between exposures (GM) and outcomes (VTE, DVT, and PE). The primary method utilized for assessing the causal relationship between exposure and outcome was the IVW method(20, 21). The Cochran's Q and $I^2$ test ($I^2 = (Q - df)/Q$; df, degree of freedom; Q, Cochran's Q test) was employed to assess the heterogeneity among SNPs, as the presence of heterogeneity may indicate pleiotropy of the IVs(22). Furthermore, the leave-one-out analysis was conducted wherein each instrumental SNP was systematically excluded to ascertain any potential presence of heterogeneous SNPs. The MR-Egger regression method was employed to mitigate potential concerns regarding the causal effect and directional pleiotropy, while also accounting for the presence of invalid instruments(23). In cases where over 50% of the weight originated from valid instrumental variables, the weighted median estimator was utilized to obtain a reliable estimate(24). If the hypothesis of instrument strength independent of direct effect is violated, it has been observed that the weighted model estimate exhibits enhanced statistical power in detecting a causal effect, reduced bias, and decreased rates of type I error compared to MR-Egger regression(25). The p-value obtained from the MR-Egger intercept assessment was employed to examine the potential presence of horizontal pleiotropy across the instrument SNPs. If the resulting p-value was below 0.05, it suggested the existence of horizontal pleiotropy among the SNPs. The MR-PRESSO test was conducted to identify potential outliers among the SNPs and subsequently obtain adjusted association results by excluding these outliers. The associations between the human GM and the risk of VTE were reported as ORs along with their corresponding 95% CI. All MR analyses were carried out using the “TwoSampleMR” package in R version 4.2.1 (https://www.rproject.org/).

## 3. Results

# 3.1 Main results of the 196 bacterial traits with the risk of VTE.

The results of the MR analysis examining the associations between all 196 bacterial traits and the risk of VTE can be found in Table S1. In summary, our findings suggest that 17 bacterial traits exhibit a potential association with the risk of VTE, as determined by the IVW method (Fig. 2). The specific IVs utilized for these 17 bacterial traits are listed in Table S2.

Family Porphyromonadaceae.id.943 has been found to have a positive association with the risk of VTE (IVW OR: 1.3090; 95% CI: 1.0764–1.5919, p = 0.007) (Table 2). Additionally, the MRPRESSO test did not identify any outliers, and the results were consistent with the primary method (OR: 1.3090; 95% CI: 1.0976–1.5611; p = 0.0134) (Table 3). The MR-Egger regression analysis also did not provide evidence of pleiotropic effects (Egger_intercept: 0.0150; p = 0.6071) (Table 4). Furthermore, our findings indicate a significant association between an increase in genetically predicted VTE and a higher abundance of phylum Cyanobacteria.id.1500 (IVW OR: 1.1412; 95% CI: 1.0041–1.2970, p = 0.0430). Additionally, the intercept of the MR-Egger regression analysis revealed no evidence of potential horizontal pleiotropy (Egger_intercept: 0.0012; p = 0.9653). In view of heterogeneous results, the Cochran's Q tests provide no indication of heterogeneity among the 17 taxa (Table 5). The leave-one-out analysis demonstrated stability except for genus Actinomyces.id.423 (Fig. 3).

Table 2
Effect estimates of the associations between 17 bacterial traits and the risk of VTE, DVT and PE in MR analysis.

| Gut microbiota (Exposure) | Outcome | Method | N.SNP | P-value | OR | Low95% CI | High95% CI |
|---|---|---|---|---|---|---|---|
| family.Porphyromonadaceae.id.943 | VTE | MR Egger | 11 | 0.95905 | 1.02510 | 0.40839 | 2.57308 |
| | | Weighted median | 11 | 0.04197 | 1.30379 | 1.00968 | 1.68357 |
| | | IVW | 11 | 0.00699 | 1.30899 | 1.07638 | 1.59187 |
| | | Simple mode | 11 | 0.13265 | 1.45514 | 0.92869 | 2.28002 |
| | | Weighted mode | 11 | 0.14671 | 1.41785 | 0.91778 | 2.19040 |
| phylum.Cyanobacteria.id.1500 | VTE | MR Egger | 10 | 0.59928 | 1.13035 | 0.72870 | 1.75340 |
| | | Weighted median | 10 | 0.15293 | 1.13353 | 0.95453 | 1.34610 |
| | | IVW | 10 | 0.04304 | 1.14120 | 1.00414 | 1.29697 |
| | | Simple mode | 10 | 0.43905 | 1.12444 | 0.84649 | 1.49367 |
| | | Weighted mode | 10 | 0.41896 | 1.11283 | 0.86894 | 1.42517 |
| family.Defluviitaleaceae.id.1924 | DVT | MR Egger | 12 | 0.39941 | 0.99668 | 0.98933 | 1.00409 |
| | | Weighted median | 12 | 0.02609 | 0.99662 | 0.99365 | 0.99960 |
| | | IVW | 12 | 0.02658 | 0.99752 | 0.99533 | 0.99971 |
| | | Simple mode | 12 | 0.07295 | 0.99532 | 0.99072 | 0.99995 |
| | | Weighted mode | 12 | 0.08948 | 0.99540 | 0.99058 | 1.00024 |
| family.Oxalobacteraceae.id.2966 | DVT | MR Egger | 15 | 0.83472 | 0.99915 | 0.99137 | 1.00700 |
| | | Weighted median | 15 | 0.06126 | 0.99776 | 0.99542 | 1.00011 |
| | | IVW | 15 | 0.04254 | 0.99805 | 0.99617 | 0.99993 |
| | | Simple mode | 15 | 0.21443 | 0.99665 | 0.99163 | 1.00170 |
| | | Weighted mode | 15 | 0.40021 | 0.99802 | 0.99356 | 1.00250 |
| family.Ruminococcaceae.id.2050 | DVT | MR Egger | 12 | 0.28783 | 0.99574 | 0.98834 | 1.00319 |
| | | Weighted median | 12 | 0.00040 | 0.99265 | 0.98861 | 0.99671 |
| | | IVW | 12 | 0.01971 | 0.99611 | 0.99286 | 0.99938 |

Abbreviations: IVW, inverse-variance weighted.

| Gut microbiota (Exposure) | Outcome | Method | N.SNP | P-value | OR | Low95% CI | High95% CI |
|---|---|---|---|---|---|---|---|
| | | Simple mode | 12 | 0.08342 | 0.99203 | 0.98388 | 1.00024 |
| | | Weighted mode | 12 | 0.04091 | 0.99191 | 0.98512 | 0.99875 |
| genus.Eubacteriumrectalegroup.id.14374 | DVT | MR Egger | 12 | 0.46204 | 1.00358 | 0.99443 | 1.01283 |
| | | Weighted median | 12 | 0.06875 | 1.00378 | 0.99971 | 1.00788 |
| | | IVW | 12 | 0.02532 | 1.00358 | 1.00044 | 1.00673 |
| | | Simple mode | 12 | 0.12336 | 1.00563 | 0.99902 | 1.01228 |
| | | Weighted mode | 12 | 0.57312 | 1.00196 | 0.99536 | 1.00860 |
| genus.Coprococcus2.id.11302 | DVT | MR Egger | 10 | 0.42474 | 1.00721 | 0.99049 | 1.02420 |
| | | Weighted median | 10 | 0.03000 | 1.00413 | 1.00040 | 1.00788 |
| | | IVW | 10 | 0.00629 | 1.00408 | 1.00115 | 1.00701 |
| | | Simple mode | 10 | 0.08907 | 1.00529 | 0.99985 | 1.01077 |
| | | Weighted mode | 10 | 0.14574 | 1.00513 | 0.99882 | 1.01148 |
| genus.Erysipelatoclostridium.id.11381 | DVT | MR Egger | 16 | 0.44727 | 1.00344 | 0.99484 | 1.01211 |
| | | Weighted median | 16 | 0.06614 | 0.99730 | 0.99442 | 1.00018 |
| | | IVW | 16 | 0.00419 | 0.99669 | 0.99444 | 0.99896 |
| | | Simple mode | 16 | 0.33073 | 0.99746 | 0.99253 | 1.00242 |
| | | Weighted mode | 16 | 0.32379 | 0.99746 | 0.99261 | 1.00234 |
| genus.LachnospiraceaeUCG001.id.11321 | DVT | MR Egger | 16 | 0.47318 | 1.00434 | 0.99284 | 1.01598 |
| | | Weighted median | 16 | 0.01383 | 1.00381 | 1.00077 | 1.00685 |
| | | IVW | 16 | 0.00285 | 1.00364 | 1.00125 | 1.00603 |
| | | Simple mode | 16 | 0.12872 | 1.00429 | 0.99906 | 1.00956 |
| | | Weighted mode | 16 | 0.15933 | 1.00396 | 0.99872 | 1.00922 |
| genus.Slackia.id.825 | DVT | MR Egger | 9 | 0.99146 | 1.00005 | 0.99052 | 1.00968 |

Abbreviations: IVW, inverse-variance weighted.

| Gut microbiota (Exposure) | Outcome | Method | N.SNP | P-value | OR | Low95% CI | High95% CI |
|---|---|---|---|---|---|---|---|
| | | Weighted median | 9 | 0.11593 | 0.99753 | 0.99446 | 1.00061 |
| | | IVW | 9 | 0.01987 | 0.99731 | 0.99506 | 0.99957 |
| | | Simple mode | 9 | 0.44120 | 0.99806 | 0.99339 | 1.00276 |
| | | Weighted mode | 9 | 0.42009 | 0.99803 | 0.99351 | 1.00258 |
| family.Alcaligenaceae.id.2875 | PE | MR Egger | 11 | 0.06773 | 1.01406 | 1.00078 | 1.02753 |
| | | Weighted median | 11 | 0.27287 | 1.00122 | 0.99904 | 1.00341 |
| | | IVW | 11 | 0.04954 | 1.00164 | 1.00000 | 1.00329 |
| | | Simple mode | 11 | 0.87615 | 1.00029 | 0.99670 | 1.00390 |
| | | Weighted mode | 11 | 0.82056 | 1.00044 | 0.99677 | 1.00412 |
| family.Christensenellaceae.id.1866 | PE | MR Egger | 6 | 0.09733 | 1.02377 | 1.00214 | 1.04586 |
| | | Weighted median | 6 | 0.51835 | 1.00093 | 0.99810 | 1.00377 |
| | | IVW | 6 | 0.04382 | 1.00236 | 1.00007 | 1.00467 |
| | | Simple mode | 6 | 0.85831 | 1.00039 | 0.99629 | 1.00451 |
| | | Weighted mode | 6 | 0.86687 | 1.00039 | 0.99602 | 1.00478 |
| family.Streptococcaceae.id.1850 | PE | MR Egger | 4 | 0.80341 | 1.00551 | 0.96803 | 1.04444 |
| | | Weighted median | 4 | 0.04907 | 1.00288 | 1.00001 | 1.00575 |
| | | IVW | 4 | 0.03261 | 1.00263 | 1.00022 | 1.00504 |
| | | Simple mode | 4 | 0.20485 | 1.00325 | 0.99931 | 1.00721 |
| | | Weighted mode | 4 | 0.20408 | 1.00326 | 0.99931 | 1.00723 |
| family.Victivallaceae.id.2255 | PE | MR Egger | 5 | 0.41187 | 1.00796 | 0.99162 | 1.02457 |
| | | Weighted median | 5 | 0.58866 | 1.00045 | 0.99881 | 1.00209 |
| | | IVW | 5 | 0.04646 | 1.00138 | 1.00002 | 1.00273 |
| | | Simple mode | 5 | 0.84960 | 1.00023 | 0.99797 | 1.00251 |

Abbreviations: IVW, inverse-variance weighted.

| Gut microbiota (Exposure) | Outcome | Method | N.SNP | P-value | OR | Low95% CI | High95% CI |
|---|---|---|---|---|---|---|---|
| | | Weighted mode | 5 | 0.85569 | 1.00023 | 0.99787 | 1.00260 |
| genus.Eubacteriumhalliigroup.id.11338 | PE | MR Egger | 7 | 0.28556 | 1.02082 | 0.98691 | 1.05589 |
| | | Weighted median | 7 | 0.06019 | 0.99766 | 0.99523 | 1.00010 |
| | | IVW | 7 | 0.00418 | 0.99719 | 0.99527 | 0.99911 |
| | | Simple mode | 7 | 0.47345 | 0.99844 | 0.99447 | 1.00244 |
| | | Weighted mode | 7 | 0.48257 | 0.99853 | 0.99469 | 1.00238 |
| genus.Actinomyces.id.423 | PE | MR Egger | / | / | / | / | / |
| | | Weighted median | / 2 | / 0.03283 | / 0.99732 | / 0.99486 | / 0.99978 |
| | | IVW | / | / | / | / | / |
| | | Simple mode | / | / | / | / | / |
| | | Weighted mode | | | | | |
| genus.Anaerotruncus.id.2054 | PE | MR Egger | 9 | 0.12853 | 0.98346 | 0.96498 | 1.00230 |
| | | Weighted median | 9 | 0.07373 | 0.99786 | 0.99553 | 1.00021 |
| | | IVW | 9 | 0.01521 | 0.99790 | 0.99621 | 0.99960 |
| | | Simple mode | 9 | 0.22454 | 0.99740 | 0.99355 | 1.00127 |
| | | Weighted mode | 9 | 0.21357 | 0.99743 | 0.99372 | 1.00116 |

Abbreviations: IVW, inverse-variance weighted.

Table 3
Effect estimates of the associations of VTE, DVT, and PE with 17 bacterial traits in the MR PRESSO analysis.

| Gut microbiota(exposure) | Outcome | Outlier test | P-value | OR | Low95% CI | High95% CI |
|---|---|---|---|---|---|---|
| family.Porphyromonadaceae.id.943 | VTE | MR-PRESSO | 0.01343 | 1.30899 | 1.09759 | 1.56110 |
| phylum.Cyanobacteria.id.1500 | VTE | MR-PRESSO | 0.07373 | 1.14120 | 1.00414 | 1.29697 |
| family.Defluviitaleaceae.id.1924 | DVT | MR-PRESSO | 0.01811 | 0.99752 | 0.99577 | 0.99927 |
| family.Oxalobacteraceae.id.2966 | DVT | MR-PRESSO | 0.06201 | 0.99805 | 0.99617 | 0.99993 |
| family.Ruminococcaceae.id.2050 | DVT | MR-PRESSO | 0.03974 | 0.99611 | 0.99286 | 0.99938 |
| genus.Eubacteriumrectalegroup.id.14374 | DVT | MR-PRESSO | 0.00407 | 1.00358 | 1.00164 | 1.00553 |
| genus.Coprococcus2.id.11302 | DVT | MR-PRESSO | 0.00055 | 1.00408 | 1.00254 | 1.00561 |
| genus.Erysipelatoclostridium.id.11381 | DVT | MR-PRESSO | 0.01184 | 0.99669 | 0.99444 | 0.99896 |
| genus.LachnospiraceaeUCG001.id.11321 | DVT | MR-PRESSO | 0.00928 | 1.00364 | 1.00125 | 1.00603 |
| genus.Slackia.id.825 | DVT | MR-PRESSO | 0.01919 | 0.99731 | 0.99552 | 0.99911 |
| family.Alcaligenaceae.id.2875 | PE | MR-PRESSO | 0.07793 | 1.00164 | 1.00000 | 1.00329 |
| family.Christensenellaceae.id.1866 | PE | MR-PRESSO | 0.09990 | 1.00236 | 1.00007 | 1.00467 |
| family.Streptococcaceae.id.1850 | PE | MR-PRESSO | 0.02610 | 1.00263 | 1.00137 | 1.00388 |
| family.Victivallaceae.id.2255 | PE | MR-PRESSO | 0.11729 | 1.00138 | 1.00002 | 1.00273 |
| genus.Eubacteriumhalliigroup.id.11338 | PE | MR-PRESSO | 0.01852 | 0.99719 | 0.99547 | 0.99891 |
| genus.Actinomyces.id.423 | PE | MR-PRESSO | / | / | / | / |
| genus.Anaerotruncus.id.2054 | PE | MR-PRESSO | 0.02680 | 0.99790 | 0.99639 | 0.99942 |

Table 4
Effect estimates of the associations of VTE, DVT, and PE with 17 bacterial traits in the pleiotropy analysis.

| Gut microbiota(exposure) | Outcome | Egger_intercept | se | P-value |
|---|---|---|---|---|
| family.Porphyromonadaceae.id.943 | VTE | 0.0149564 | 0.02807 | 0.60706 |
| phylum.Cyanobacteria.id.1500 | VTE | 0.001205467 | 0.02689 | 0.96534 |
| family.Defluviitaleaceae.id.1924 | DVT | 9.30E-05 | 0.00040 | 0.82080 |
| family.Oxalobacteraceae.id.2966 | DVT | -0.000152408 | 0.00054 | 0.78025 |
| family.Ruminococcaceae.id.2050 | DVT | 3.47E-05 | 0.00031 | 0.91353 |
| genus.Eubacteriumrectalegroup.id.14374 | DVT | -1.41E-08 | 0.00032 | 0.99997 |
| genus.Coprococcus2.id.11302 | DVT | -0.000232996 | 0.00063 | 0.72070 |
| genus.Erysipelatoclostridium.id.11381 | DVT | -0.000561972 | 0.00035 | 0.13479 |
| genus.LachnospiraceaeUCG001.id.11321 | DVT | -6.26E-05 | 0.00051 | 0.90422 |
| genus.Slackia.id.825 | DVT | -0.000308425 | 0.00053 | 0.58163 |
| family.Alcaligenaceae.id.2875 | PE | -0.000732879 | 0.00040 | 0.09835 |
| family.Christensenellaceae.id.1866 | PE | -0.001095885 | 0.00056 | 0.12311 |
| family.Streptococcaceae.id.1850 | PE | -0.000172677 | 0.00116 | 0.89554 |
| family.Victivallaceae.id.2255 | PE | -0.000790018 | 0.00100 | 0.48776 |
| genus.Eubacteriumhalliigroup.id.11338 | PE | -0.001228841 | 0.00090 | 0.23167 |
| genus.Actinomyces.id.423 | PE | / | / | / |
| genus.Anaerotruncus.id.2054 | PE | 0.000808834 | 0.00053 | 0.17420 |

Table 5
Effect estimates of the associations of VTE, DVT, and PE with 17 bacterial traits in the heterogeneity analyses.

| Gut microbiota(exposure) | Outcome | Method | Q | Q_df | Q_pval | $I^2$ |
|---|---|---|---|---|---|---|
| family.Porphyromonadaceae.id.943 | VTE | MR Egger | 7.820375 | 9 | 0.55234 | 0.15084 |
| | | IVW | 8.104274 | 10 | 0.61865 | 0.23392 |
| phylum.Cyanobacteria.id.1500 | VTE | MR Egger | 9.812704 | 8 | 0.278419 | 0.18473 |
| | | IVW | 9.81517 | 9 | 0.365655 | 0.08305 |
| family.Defluviitaleaceae.id.1924 | DVT | MR Egger | 6.976908 | 10 | 0.72762 | 0.43330 |
| | | IVW | 7.030987 | 11 | 0.796569 | 0.56450 |
| family.Oxalobacteraceae.id.2966 | DVT | MR Egger | 19.96524 | 13 | 0.09608 | 0.34887 |
| | | IVW | 20.08984 | 14 | 0.127334 | 0.30313 |
| family.Ruminococcaceae.id.2050 | DVT | MR Egger | 13.69062 | 10 | 0.187577 | 0.26957 |
| | | IVW | 13.7076 | 11 | 0.249598 | 0.19753 |
| genus.Eubacteriumrectalegroup.id.14374 | DVT | MR Egger | 4.213974 | 10 | 0.937178 | 1.37306 |
| | | IVW | 4.213974 | 11 | 0.963234 | 1.61036 |
| genus.Coprococcus2.id.11302 | DVT | MR Egger | 2.3283 | 8 | 0.969257 | 2.43598 |
| | | IVW | 2.465496 | 9 | 0.981797 | 2.65038 |
| genus.Erysipelatoclostridium.id.11381 | DVT | MR Egger | 14.82443 | 14 | 0.390261 | 0.05561 |
| | | IVW | 17.49204 | 15 | 0.29031 | 0.14247 |
| genus.LachnospiraceaeUCG001.id.11321 | DVT | MR Egger | 18.54459 | 14 | 0.183091 | 0.24506 |
| | | IVW | 18.56448 | 15 | 0.234152 | 0.19201 |
| genus.Slackia.id.825 | DVT | MR Egger | 4.742767 | 7 | 0.691321 | 0.47593 |
| | | IVW | 5.076388 | 8 | 0.749381 | 0.57592 |
| family.Alcaligenaceae.id.2875 | PE | MR Egger | 8.633527 | 9 | 0.471767 | 0.04245 |
| | | IVW | 12.03221 | 10 | 0.282907 | 0.16890 |
| family.Christensenellaceae.id.1866 | PE | MR Egger | 2.094235 | 4 | 0.718432 | 0.91001 |
| | | IVW | 5.892568 | 5 | 0.316813 | 0.15147 |
| family.Streptococcaceae.id.1850 | PE | MR Egger | 0.789298 | 2 | 0.673917 | 1.53390 |
| | | Weighted median | 0.811364 | 3 | 0.846747 | 2.69748 |
| family.Victivallaceae.id.2255 | PE | MR Egger | 5.559926 | 3 | 0.135098 | 0.46042 |
| | | Weighted median | 6.713435 | 4 | 0.151829 | 0.40418 |
| genus.Eubacteriumhalliigroup.id.11338 | PE | MR Egger | 2.942885 | 5 | 0.708791 | 0.69901 |
| | | IVW | 4.794926 | 6 | 0.570372 | 0.25132 |
| genus.Actinomyces.id.423 | PE | MR Egger | / | / | / | / |
| | | IVW | 0.548136 | 1 | 0.45908 | 0.82437 |
| genus.Anaerotruncus.id.2054 | PE | MR Egger | 4.146845 | 7 | 0.762717 | 0.68803 |

| Gut microbiota(exposure) | Outcome | Method | Q | Q_df | Q_pval | $I^2$ |
|---|---|---|---|---|---|---|
| | | IVW | 6.434065 | 8 | 0.59873 | 0.24338 |

Regarding the association with DVT, we have identified potential causal relationships between the remaining eight taxa and DVT. This is supported by the significant differences observed in the IVW analyses for all eight phenotypes ($p < 0.05$, Table 2). Among these taxa, three have been found to potentially exert a positive causal effect on DVT, thereby increasing the risk of developing this condition. Specifically, these taxa include genus Eubacteriumrectalegroup.id.14374 (IVW OR: 1.0036, 95% CI: 1.0004–1.0067, p = 0.0253), genus Coprococcus2.id.11302 (IVW OR: 1.0041, 95% CI: 1.0012–1.0070, p = 0.0063), and genus LachnospiraceaeUCG001.id.11321 (IVW OR: 1.0036, 95% CI: 1.0012–1.0060, p = 0.0028). Contrarily, five taxa, namely family Defluviitaleaceae.id.1924 (IVW OR: 0.9975, 95% CI: 0.9953–0.9997, p = 0.0266), family Oxalobacteraceae.id.2966 (IVW OR: 0.9981, 95% CI: 0.9962–0.9999, p = 0.0425), family Ruminococcaceae.id.2050 (IVW OR: 0.9961, 95% CI: 0.9929–0.9994, p = 0.0197), genus Erysipelatoclostridium.id.11381 (IVW OR: 0.9967, 95% CI: 0.9944–0.9990, p = 0.0042), and genus Slackia.id.825 (IVW OR:0.9973, 95% CI: 0.9951–0.9996, p = 0.0199) have been identified as exhibiting a negative causal effect on DVT and demonstrating a tendency to causally reduce the risk of DVT(Fig. 2).

In the analysis of GM and the occurrence of PE, the use of GM as an exposure variable revealed that 7 specific gut microbial taxa were causally associated with PE, as indicated by the IVV results (Fig. 2). Notably, the IVW method suggested that 4 bacterial traits, namely family Alcaligenaceae.id.2875 (IVW OR: 1.0016, 95% CI: 1.0000-1.0033, p = 0.0495), family Christensenellaceae.id.1866 (IVW OR: 1.0024, 95% CI: 1.0001–1.0047, p = 0.0438), family Streptococcaceae.id.1850 (IVW OR: 1.0026, 95% CI: 1.0002–1.0050, p = 0.0326), and family Victivallaceae.id.2255(IVW OR: 1.0014, 95% CI: 1.000-1.0027, p = 0.0465) were suggestively associated with a higher risk of PE. In contrast, genus Eubacteriumhalliigroup.id.11338 (IVW OR: 0.9972, 95% CI: 0.9953–0.9991, p = 0.0042), genus Actinomyces.id.423 (IVW OR: 0.9973, 95% CI:0.9949–0.9998, p = 0.0328), and genus Anaerotruncus.id.2054 (IVW OR: 0.9979, 95% CI: 0.9962–0.9996, p = 0.0152) exhibited a negative association with the risk of PE when employing the IVW method (Fig. 2, Table 2).

In order to further evaluate the potential impact of directional pleiotropy on the estimates of causal effects, we conducted a scan of the SNPs associated with the 17 bacterial traits using the GWAS Catalog. Our analysis revealed that 34 SNPs were found to be accompanied by other traits (Table S3). After excluding these pleiotropic SNPs, we observed that the associations between family Porphyromonadaceae.id.943 (IVW OR: 1.3729, 95% CI: 1.1102–1.6978, p = 0.0035), phylum Cyanobacteria.id.1500 (IVW OR: 1.2151, 95% CI: 1.0612–1.3914, p = 0.0048), family Oxalobacteraceae.id.2966 (IVW OR: 0.9978, 95% CI: 0.9958–0.9997, p = 0.0233), family Ruminococcaceae.id.2050 (IVW OR: 0.9956, 95%CI: 0.9923–0.9990, p = 0.0106), genus Eubacteriumrectalegroup.id.14374 (IVW OR: 1.0038, 95% CI : 1.0004–1.0072, p = 0.0278), genus Erysipelatoclostridium.id.11381 (IVW OR: 0.9970, 95% CI: 0.9948–0.9993, p = 0.0110), genus LachnospiraceaeUCG001.id.11321 (IVW OR: 1.0041, 95% CI: 1.0017–1.0065, p = 0.0009), genus Slackia.id.825 (IVW OR: 0.9974, 95% CI:0.9951–0.9996, p = 0.0221), family Christensenellaceae.id.1866 (IVW OR:1.0023, 95% CI:1.0000-1.0047, p = 0.0497), family Streptococcaceae.id.1850 (IVW OR: 1.0031, 95% CI: 1.0003–1.0059, p = 0.0279), family Victivallaceae.id.2255(IVW OR: 1.0014, 95% CI: 1.0000-1.0028, p = 0.0493), genus Actinomyces.id.423(IVW OR: 0.9973, 95% CI: 0.9949–0.9998,p = 0.0328), and genus Anaerotruncus.id.2054(IVW OR: 0.9979, 95% CI: 0.9962–0.9996, p = 0.0149) with the risk of VTE remained consistent when using the IVW method. However, the associations between the family Defluviitaleaceae.id.1924(IVW OR: 0.9978, 95% CI: 0.9955–1.0001, p = 0.0616), family Alcaligenaceae.id.2875 (IVW OR: 1.0017, 95% CI: 0.9998–1.0036, p = 0.0737), genus Eubacteriumhalliigroup.id.11338(IVW OR: 0.9981, 95% CI: 0.9958-1.000362957, p = 0.0988) and VTE were found to be unstable (Fig. 4).

# 3.2 Sensitivity analyses and detection of pleiotropy

In order to mitigate the impact of undue bias, pleiotropic analyses are performed. After excluding pleiotropic SNPs, the MRPRESSO test did not identify any outliers, and the results were consistent with the IVW method except for family Streptococcaceae.id.1850, family Christensenellaceae.id.1866, and family Victivallaceae.id.2255 (Table S4). Furthermore, the conclusions drawn from these analyses are substantiated by the leave-one-out sensitivity analysis (Fig. 5). Additionally, the

presence of symmetrically distributed SNPs in the funnel plots suggests a reduced likelihood of causal associations being influenced by potential biases (Fig. S1). In view of heterogeneous results, the Cochran's Q tests indicate a lack of heterogeneity among the 15 taxa ($p > 0.05$) (Table S5). Furthermore, the MR-Egger intercept tests do not provide any indication of horizontal pleiotropy within the same 14 taxa except for family.Streptococcaceae.id.1850 (Table S6). Scatter plot and forest plot of the causal effect of GM on VTE, DVT, and PE were shown in Figure S2 and Figure S3, respectively. In conclusion, our MR analyses demonstrate reliability and robustness. These findings collectively suggest that the identified causal associations between GM and VTE are likely mediated by the aforementioned gut bacterial taxa.

# 4. Discussion

To the best of our understanding, this study represents one of the initial attempts to comprehensively assess the causal associations between the GM and VTE, focusing on a genetic standpoint. In addition, employing the two-sample MR design, our investigation provides compelling evidence that the predicted abundance of certain gut microbial taxa, as determined by genetic factors, significantly contributes to the development and progression of VTE. By utilizing molecular genetic markers as IVs, the MR approaches effectively mitigate the influence of confounding factors such as socioeconomic status and cultural influences, as well as address issues of reverse causality(26). Family Porphyromonadaceae.id.943, phylum Cyanobacteria.id.1500, genus Eubacteriumrectalegroup.id.14374, genus LachnospiraceaeUCG001.id.11321, genus Coprococcus2.id.11302, family Christensenellaceae.id.1866, family Streptococcaceae.id.1850, and family Victivallaceae.id.2255 have been identified as playing causal roles in promoting the initiation of VTE. Conversely, family Oxalobacteraceae.id.2966, family Ruminococcaceae.id.2050, genus Erysipelatoclostridium.id.11381, genus Slackia.id.825, genus Actinomyces.id.423, and genus Anaerotruncus.id.2054 have been found to causally reduce the risk of VTE. The complete understanding of the involvement of the GM in the development of thromboembolism remains incomplete. However, our MR study has addressed this knowledge gap by investigating the potential contribution of the GM to VTE. Moreover, our study has explored the specific taxa that may either promote or hinder the initiation of VTE, providing a fresh perspective on this matter.

The recognition of the gut microbial involvement in diverse facets of human health is steadily growing. It assumes a pivotal function in multiple inflammatory conditions, and its manipulation presents a potential therapeutic avenue for such ailments. Several observational studies have documented the correlation between GM and VTE(27, 28). The activation of inflammatory pathways in vascular endothelial cells, platelets, and innate immune cells can be induced by perturbations of the gut microbiome caused by environmental or genetic factors(29). This activation subsequently leads to the release of various coagulation proteins, ultimately resulting in a prothrombotic state. The development of thrombosis is a multifaceted process involving intricate interactions among the coagulation system, innate immune system, and inflammation(30–32). Furthermore, inflammation is closely associated with dysbiosis, an increase in intestinal permeability, and the production of specific metabolites(33).

Porphyromonadaceae have been associated with cognitive dysfunction and the advancement of diabetes, as well as negative cardiac phenotype and obesity, as evidenced by previous studies conducted on both humans and animals(34–37). While primarily originating in the oral cavity, Porphyromonadaceae have also been linked to gut-related implications that extend beyond the local oral environment(38). Due to the metabolic activities of gut bacteria, particularly in relation to aromatic amino acids like tyrosine and phenylalanine, Porphyromonadaceae have the potential to produce phenolic compounds, with p-Cresyl sulfate (pCS) being one of its constituents(39). pCS is a prototype protein-bound uremic toxin that has been associated with numerous biological and biochemical (toxic) effects, including uremic cardiovascular disease (CVD)(40). Previous studies have shown a higher prevalence of Cyanobacteria, a phylum of bacteria, in individuals with CVD (41), as well as in mouse models of progeria(42). In the present study, an increased prevalence of family Porphyromonadaceae.id.943 and phylum Cyanobacteria.id.1500 is indicative of an elevated susceptibility to VTE. These findings propose that the identification of these specific bacterial strains in fecal samples may serve as a prognostic biomarker and a potential focus for effective intervention strategies in VTE.

Furthermore, we have identified three gut microbial taxa (Eubacteriumrectalegroup.id.14374, LachnospiraceaeUCG001.id.11321, Coprococcus2.id.11302) that have been found to have a positive causal relationship with DVT, in addition to the

aforementioned two taxa that promote VTE. It is worth noting that Eubacteriumrectale is among the most prevalent bacterial species detected in human fecal samples(43). Previous MR analyses have provided support for a potential causal effect of Eubacteriumrectale in reducing plasma levels of hydrogen sulfite, a toxin known to impact cardiovascular function(44). Additionally, the reduction in Eubacteriumrectale has been found to be associated with cerebrovascular events in patients with refractory hypertension(45). LachnospiraceaeUCG001 has been identified as being involved in the synthesis of glutamate, butyrate, serotonin, and gamma amino butyric acid (GABA), which are crucial neurotransmitters associated with depression(46). Additionally, this microbial species may potentially contribute to the health of adults at risk for cardiovascular conditions(47). Notably, studies have suggested that Coprococcus2, as part of the intestinal microbiome, may influence age-related phenotypes, such as weight loss(48). This study has provided initial evidence of a potential causal association between the three taxa and the risk of DVT. However, further research is warranted to elucidate the underlying biological mechanisms linking these taxa to DVT. Additionally, our investigation has identified Oxalobacteraceae.id.2966, Ruminococcaceae.id.2050, Erysipelatoclostridium.id.11381, and Slackia.id.825 as being associated with the suppression of DVT. A previous study has indicated the presence of distinct members of the Oxalobacteraceae family in human atherosclerotic plaques derived from common carotid arteries of individuals with atherosclerosis, albeit at relatively low abundances within the total population of the 16S rRNA gene(49). Furthermore Ruminococcaceae, Ruminococcaceae UCG-002 and Ruminococcaceae UCG-003 have been identified as the primary contributors to the observed positive correlation between chronic insomnia and CVD(50). Atherosclerosis serves as the primary etiology of CVD, with hypercholesterolemia and hyperlipidemia acting as the principal risk factors for atherosclerosis development. The mitigation of hypercholesterolemia can be achieved through the reduction of Erysipelatoclostridium abundance and the activation of butanoate and vitamin B6 metabolism(51). In heart failure patients without sarcopenia, Slackia was found to be significantly enriched. Consequently, targeting this taxon may present a novel approach for preventing and treating sarcopenia in heart failure patients(52). The present study provides evidence supporting a reasonable correlation between specific gut microbiota taxa, as identified in our MR study, and the occurrence of DVT. The present study provides evidence supporting a reasonable correlation between specific gut microbiota taxa, as identified in our MR study, and the occurrence of DVT.

It is important to note that VTE, encompassing PE, is a prevalent condition associated with substantial morbidity and mortality. Our findings indicate that certain gut microbiota, such as Christensenellaceae.id.1866, Streptococcaceae.id.1850, and Victivallaceae.id.2255 are linked to the development of PE. Christensenellaceae, a producer of short-chain fatty acids, exhibited a noteworthy decrease in fecal samples obtained from Ldlr-/-( Casp1-/-) mice, potentially impacting the progression of atherosclerosis(53). In addition, Christensenellaceae_R-7 was observed to be significantly more abundant in the normotensive group compared to the hypertensive group(54). Numerous studies conducted on hypertensive individuals similarly revealed a loss and reduced abundance of bacteria capable of producing butyrate following the onset of obesity. In contrast, the augmentation in the abundance of certain streptococcus taxa may be attributed to variances in BMI and waist circumference(55). Furthermore, a prior MR analysis demonstrated an association between Victivallaceae and an increased risk of chronic obstructive pulmonary disease(56). Furthermore, we identify two gut microbiota taxa (Actinomyces.id.423, Anaerotruncus.id.2054) that exhibit a negative causal relationship with PE, which is a novel finding. Nitric oxide (NO), a small gaseous and multifunctional signaling molecule, plays a crucial role in maintaining metabolic and cardiovascular homeostasis. Actinomyces, being the most abundant among nitrate-reducing bacteria, has been linked to endothelial dysfunction and cardiovascular risk(57). The administration of chenpi extract resulted in an increase in both the abundance and diversity of fecal microbiota. Additionally, the abundance of Anaerotruncus was significantly elevated following chenpi extract treatment, and this increase showed a significant negative correlation with serum lipid parameters(58). In conjunction with the previously obtained findings, our MR study posits the potential for mitigating and managing PE through the augmentation of negatively influential gut microbiota taxa via diverse approaches. The collective evidence from our MR study substantiates a plausible association between the GM and PE.

Given the direct interconnection of gastrointestinal tracts and the established impact of GM on the progression of systemic diseases, a comprehensive understanding of the precise role played by distinct gut microbe taxa in VTE could offer novel prospects for enhanced preventive and management strategies. Despite previous endeavors to elucidate the correlation between VTE and GM, no conclusive evidence has been put forth to establish a causal relationship. Moreover, despite the identification of

dysbiosis of the GM as a phenotype in patients with VTE, it is important to note that this dysbiosis is a result of a complex combination of multiple factors, and the specific changes in diverse taxa of microbiota are not consistent. Additionally, the composition of the GM may vary due to inconsistencies in the staging of VTE. As previously mentioned, MR is an ideal study design for investigating the causal relationship between potential risk factors and diseases of interest. In recent times, numerous MR studies have been conducted to elucidate the impact of modest risk factors on VTE. By conducting studies on the factors that influence the risk of VTE, MR research aids in the development of public health policies and clinical interventions that efficiently decrease the occurrence and societal impact of VTE.

This study represents a pioneering effort in the field of MR research, as it utilizes extensive data on the GM and genetic factors related to VTE to investigate the potential causal relationship between GM and the risk of VTE. The current MR analysis offers several notable advantages. Firstly, our study effectively addresses the issue of causality and confounding variables through the robust implementation of the MR method. Secondly, our MR study encompasses a broad population sample at a relatively low cost, thereby enhancing the practicality and persuasiveness of our findings compared to traditional observational studies. Thirdly, this study presents the first evidence of a causal relationship between GM and VTE from a genetic standpoint. Additionally, the F-statistic of the instrumental variables (IVs) employed in our analysis all exceeded the threshold of > 10, indicating a reduced likelihood of weak instrument bias. Future research in the field will focus on investigating causal associations to better understand the role of GM in disease development. The findings of our MR analysis can serve as a valuable resource for selecting specific gut bacteria for further investigation into the pathogenesis of VTE.

Nevertheless, it is important to acknowledge the limitations of this study. Firstly, the analysis of bacterial taxa was limited to the genus level, without further exploration at more specific levels such as species or strain. Secondly, it is worth noting that most participants in this GWAS were of European descent, potentially impacting the generalizability of the findings. Therefore, caution should be exercised when applying the results of this study to populations of different ethnic backgrounds. Thirdly, in order to ensure an adequate number of instrumental variables (IVs), we employed a significance threshold of $p < 1.0\times10^{-5}$ for the selection of GM IVs, surpassing the conventional genome-wide significance level of $p < 5\times10^{-8}$. Moreover, it is important to note that the impact of the bacterial traits we observed was relatively modest, and there were no other independent GWAS investigating VTE with a sufficiently large sample size to corroborate our findings. Fourthly, our study specifically aimed to elucidate the risk factors for VTE in order to facilitate comprehensive clinical intervention and mitigate the incidence of this condition. Consequently, we focused on examining the unidirectional influence of 196 gut microbial taxa on VTE. Fifthly, this study has not fully explored the precise mechanisms through which the aforementioned gut microbial taxa impact the risk of VTE. Additionally, despite the identification of numerous VTE cases in the present GWAS analysis, stratification or adjustment for these cases was not feasible. Naturally, larger sample sizes in clinical studies and experiments are necessary to validate our findings. Lastly, given the unavailability of information regarding VTE subtypes, further investigations are warranted once this information becomes accessible.

# 5. Conclusion

In summary, by utilizing publicly accessible genetic databases, this study successfully established causal associations between specific intestinal microbes and VTE. Consequently, these findings hold promise for elucidating the pathogenesis of VTE and exploring innovative treatment approaches, thereby offering valuable insights into the potential role of these microorganisms.

# Declarations


### Acknowledgements

Not applicable


### Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Chao Wang, Jia Wang and Bojian Fei. The first draft of the manuscript was written by Chao Wang and all authors

commented on previous versions of the manuscript. All authors read and approved the final manuscript.


### Funding

This work was supported by grants from the Wuxi Health Commission Youth Project(Q202242).


### Availability of data and materials

The data used in this study is freely available for download in the MiBioGen Repository, https://mibiogen.gcc.rug.nl/, and GWAS repository, https://gwas.mrcieu.ac.uk/cs, respectively.

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors state that they have no competing interests.

### Competing Interests

The authors have no relevant financial or non-financial interests to disclose.


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## Figures

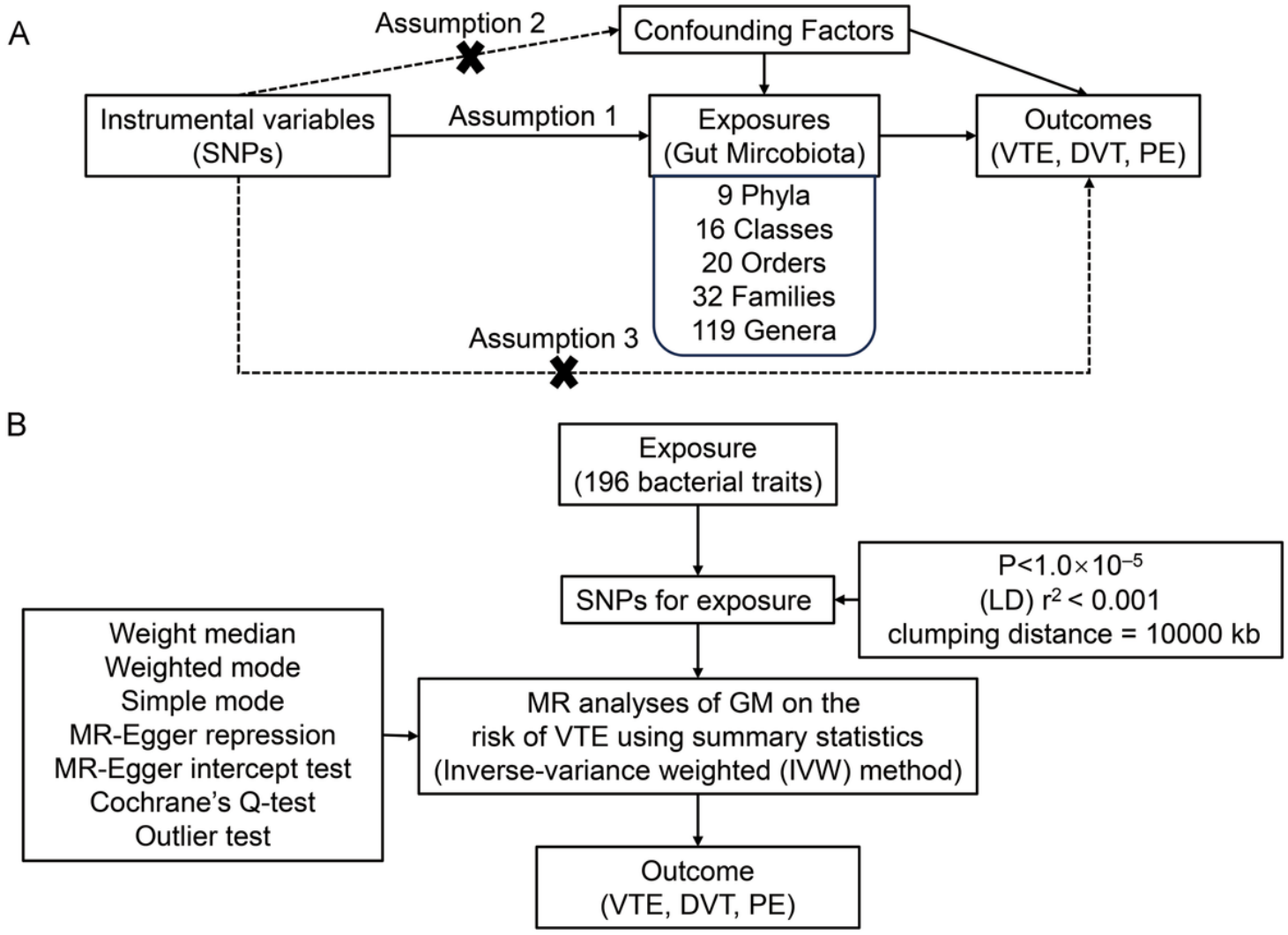



**Figure 1**

**Schematic depiction of the connections between gut microbiota (GM) and venous thromboembolism (VTE).**

The application of MR in this context relies on three fundamental assumptions: (1) Assumption 1, the genetic variants must exhibit associations with the exposures, (2) Assumption 2, the genetic variants must not demonstrate associations with confounding factors, and (3) Assumption 3, the genetic variants must solely influence outcomes through the exposures, excluding alternative pathways. Abbreviations: MR, Mendelian randomization; SNP, single nucleotide polymorphism; LD, linkage disequilibrium.

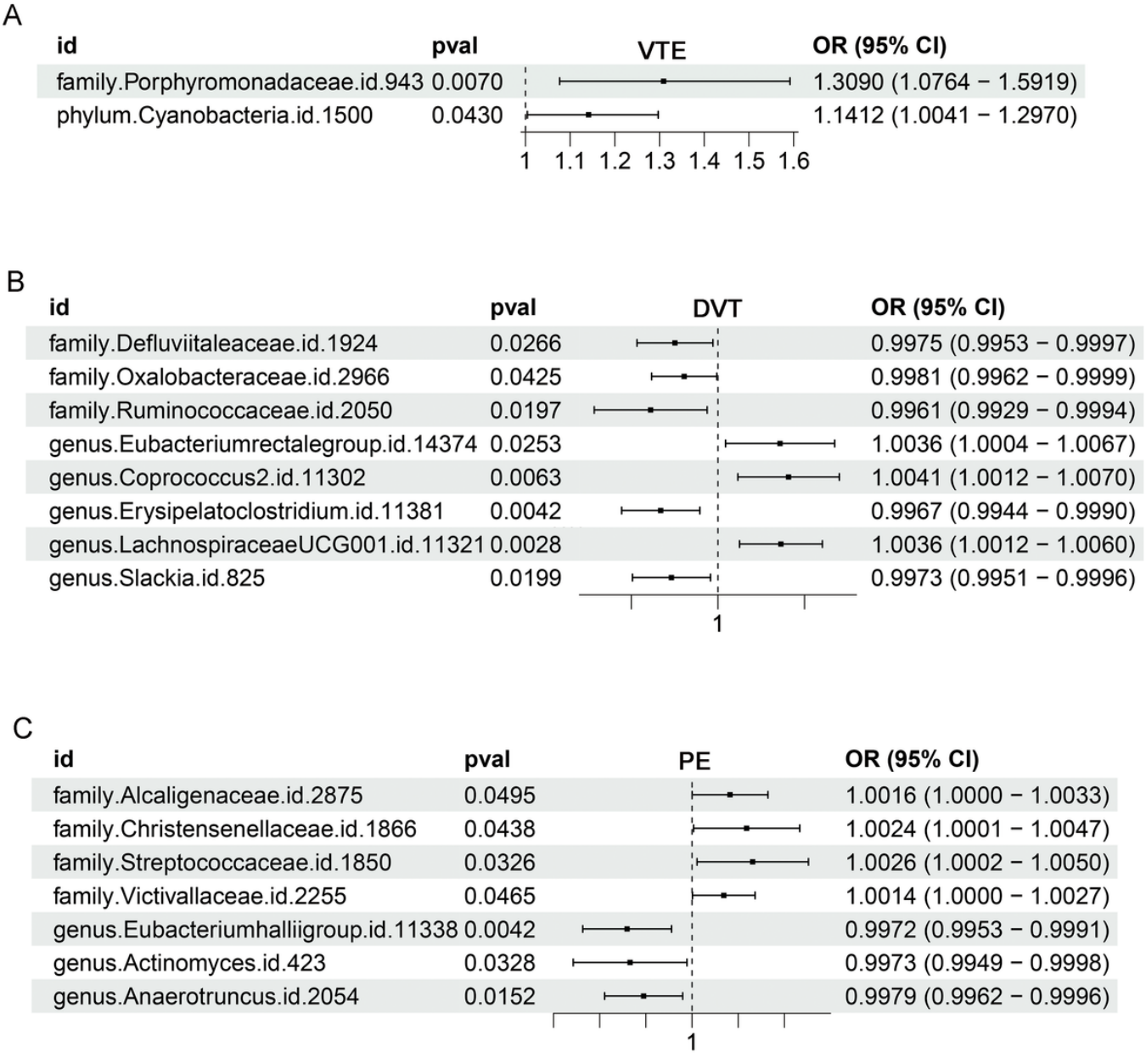
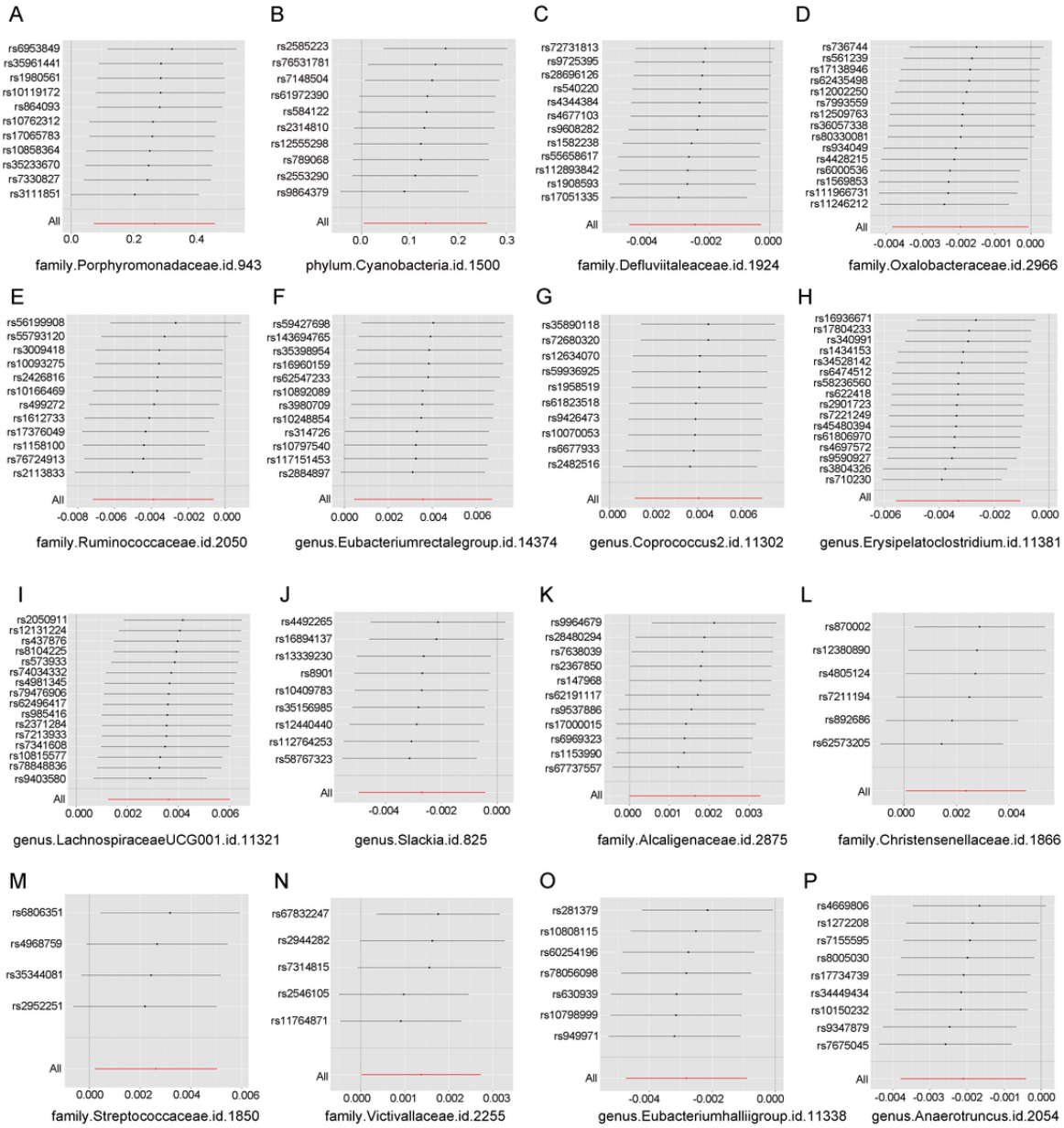



**Figure 2**

**Forest plot of causal effect of genetically determined 17 bacterial traits on VTE, DVT, and PE using IVW method.**

The figure showed the IVW estimates of significantly VTE-associated gut microbiota taxa. The black bars represent the IVW estimates. The OR > 1 indicates increased risk while < 1 indicates decreased risk. Abbreviations: VTE, venous thromboembolism; DVT, deep vein thrombosis; PE, pulmonary embolism; CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism.




## Figure 3

**Leave-one-out analyses for the causal estimates of 16 gut microbiota taxa on the risk of VTE, DVT, and PE.**

Forest plot showing the causal effect of gut microbiota (exposure) on VTE, DVT, and PE (outcomes). A-B. Gut microbiota-VTE; C-J. Gut microbiota-DVT; K-P. Gut microbiota-PE.

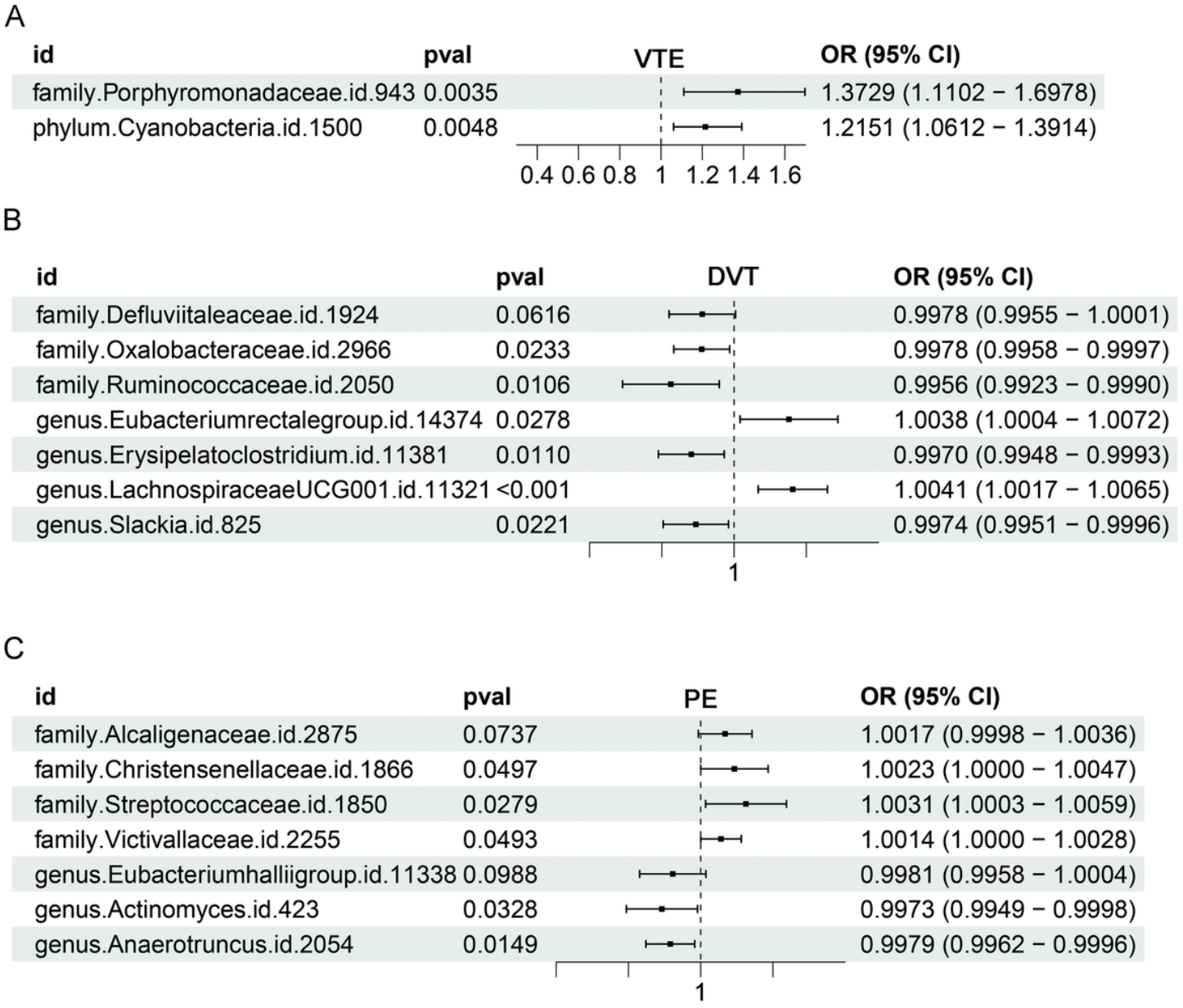



**Figure 4**

**Forest plot of causal effect of genetically determined 16 bacterial traits on VTE, DVT, and PE using IVW method after excluding these pleiotropic SNPs.**

The figure showed the IVW estimates of significantly VTE-associated gut microbiota taxa. The black bars represent the IVW estimates. The OR > 1 indicates increased risk while < 1 indicates decreased risk. Abbreviations: VTE, venous thromboembolism; DVT, deep vein thrombosis; PE, pulmonary embolism; CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism.

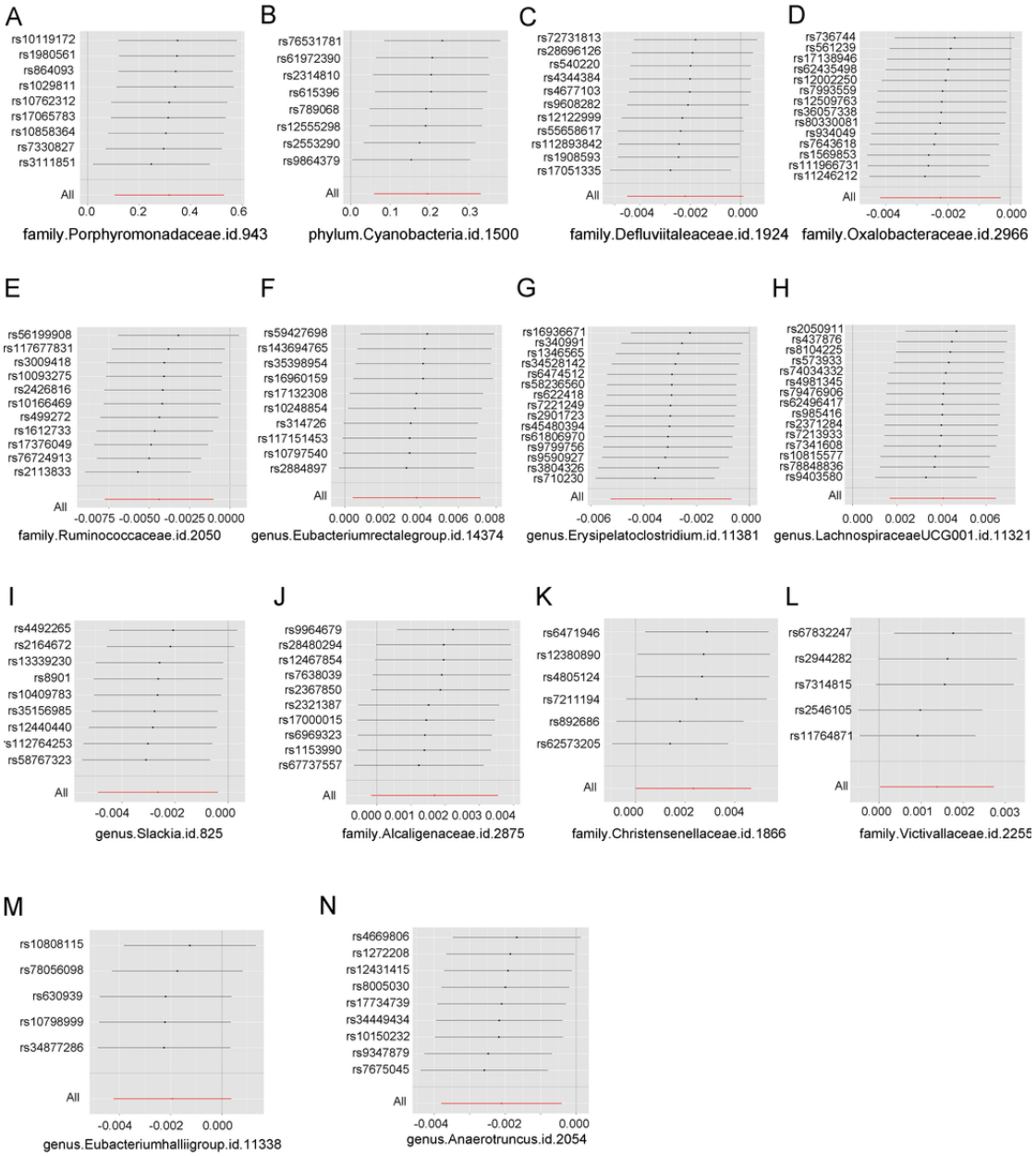



**Figure 5**

**Leave-one-out analyses for the causal estimates of 14 gut microbiota taxa on the risk of VTE, DVT, and PE after excluding these pleiotropic SNPs.**

Forest plot showing the causal effect of gut microbiota (exposure) on VTE, DVT, and PE (outcomes). A-B. Gut microbiota-VTE; C-I. Gut microbiota-DVT; J-N. Gut microbiota-PE.

# Supplementary Files

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