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Circulating Cytokines and Venous Thromboembolism: A Bidirectional Two-Sample Mendelian Randomization Study

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Abstract

Background Epidemiological evidence has linked circulating cytokines to venous thromboembolism (VTE). However, it remains uncertain whether these associations are causal due to confounding factors or reverse causality. We aim to explore the causality between circulating cytokines and VTE, encompassing deep vein thrombosis (DVT) and pulmonary embolism (PE).

Methods In the current bidirectional Mendelian randomization (MR) study, instrumental variables of 41 circulating cytokines were obtained from the genome-wide association study meta-analyses (8,293 individuals). Summary statistics for the association of VTE (17,048 cases and 325,451 controls), DVT (8,077 cases and 295,014 controls), and PE (8,170 cases and 333,487 controls) were extracted from the FinnGen Study. A multivariable MR study was conducted to adjust for potential confounders. The inverse-variance weighted method was employed as the main analysis, and comprehensive sensitivity analyses were conducted in the supplementary analyses.

Results The MR analysis indicated stromal cell-derived factor-1α was suggestively associated with a reduced risk of VTE (odds ratio [OR]: 0.90; 95% confidence interval [CI]: 0.81–0.99; $p = 0.033$) and DVT (OR: 0.85; 95% CI: 0.75–0.97; $p = 0.015$). In addition, suggestive association of granulocyte colony-stimulating factor with PE (OR: 1.20; 95% CI: 1.06-1.37; $p = 0.005$) was observed. Multivariable MR analysis showed that the effect of cytokines on VTE was partly mediated through hemoglobin A1c and systolic blood pressure. Reverse MR analysis revealed that VTE was linked to decreased levels of several cytokines.

Conclusion We provide suggestive genetic evidence supporting the bidirectional causal effect between circulating cytokines and VTE, highlighting the importance of targeting circulating cytokines to reduce the incidence of VTE.

Keywords
- cytokines
- venous thromboembolism
- deep vein thrombosis
- pulmonary embolism
- Mendelian randomization

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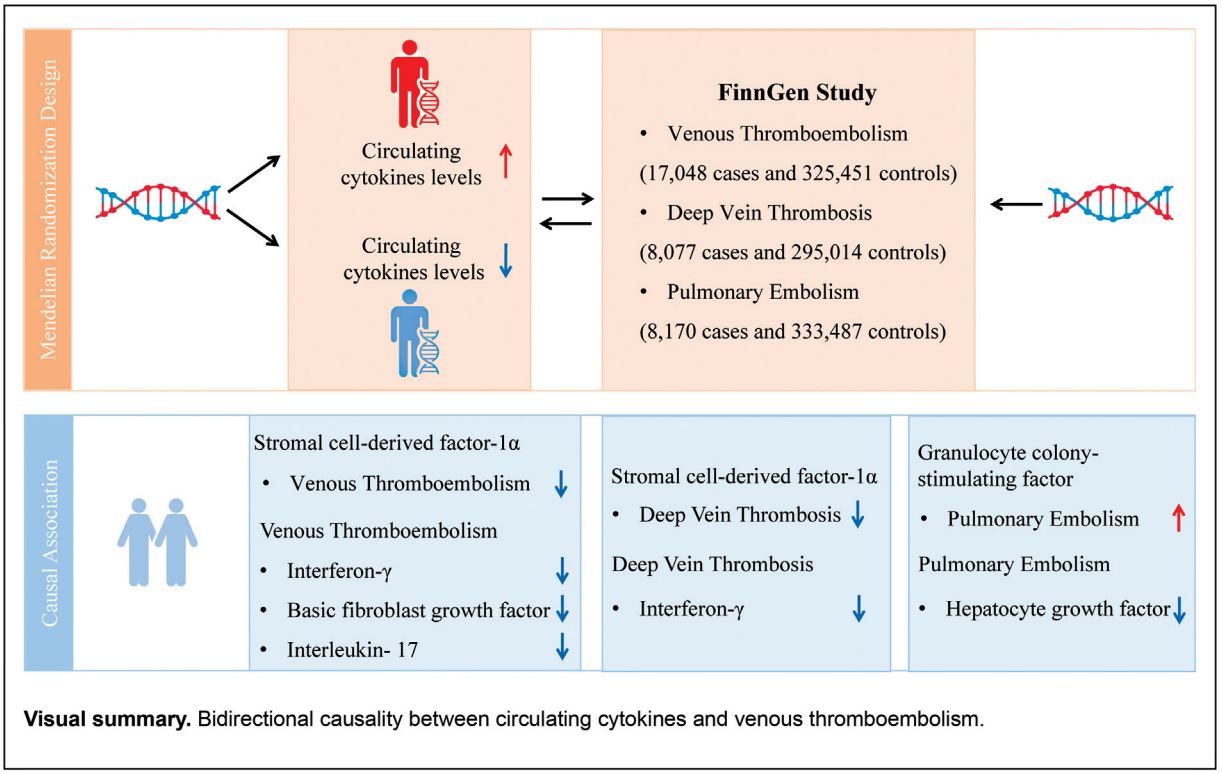

Visual summary. Bidirectional causality between circulating cytokines and venous thromboembolism.

Introduction

Venous thromboembolism (VTE), a common global health concern associated with substantial morbidity and mortality, encompasses the deep vein thrombosis (DVT) and pulmonary embolism (PE).[1,2] Among cardiovascular causes of death, VTE ranks as the third most prevalent worldwide, following coronary heart disease and ischemic stroke, with an annual incidence rate ranging from 1 to 4 per 1,000 adults.[3,4] A growing body of evidence reveals chronic inflammation plays a pivotal role in the progression of thrombosis.[5,6]

Circulating cytokines play a crucial role in the regulation of the inflammatory response, contributing to hypercoagulability, endothelial dysfunction, and hemodynamic alterations (stasis and turbulence) during the pathogenesis of venous thrombosis.[7] Extensive epidemiological studies have reported associations between cytokines and VTE, including DVT and PE.[8] For example, several studies have shown an association between plasma levels of interleukin-6 (IL-6) and VTE.[9,10] However, a large population-based study did not find any association between the levels of IL-1β, IL-6, IL-8 and tumor necrosis factor-alpha and the risk of VTE.[11] Therefore, the causal relationship between circulating cytokine levels and venous thrombosis remains under debate, due to confounding factors or potential reverse causation in traditional observational studies.

Mendelian randomization (MR) analysis is an epidemiological analytical approach to infer the causal association of an exposure on an outcome using genetic variations.[12] MR analysis effectively reduces the impact of environmental confounders and reverse causation, as genetic variants are randomly assigned during conception.[13] Here, we employed a two-sample bidirectional MR analysis to systematically investigate the causal relationships between genetic liability to 41 circulating cytokines and the risk of venous VTE, encompassing DVT and PE.

Methods

Study Design

A two-sample bidirectional MR analysis was performed to explore the causal association between genetic liability to circulating cytokines and the risk of VTE (including DVT and PE). Furthermore, a multivariate MR analysis was conducted to adjust for well-established risk factors associated with VTE. The conceptual diagram depicting the current MR study is illustrated in ►**Fig. 1**. The single nucleotide polymorphisms (SNPs) chosen as instrumental variables (IVs) are based on three core assumptions of MR analysis: (1) IVs should be strongly associated with the exposure, (2) IVs should be independent of any potential confounders, and (3) IVs should only be linked to outcome through the exposure.[13]

Genetic Instrument Selection

The summary statistics and IVs for circulating levels of circulating cytokines were derived from a meta-analysis of genome-wide association study (GWAS), encompassing

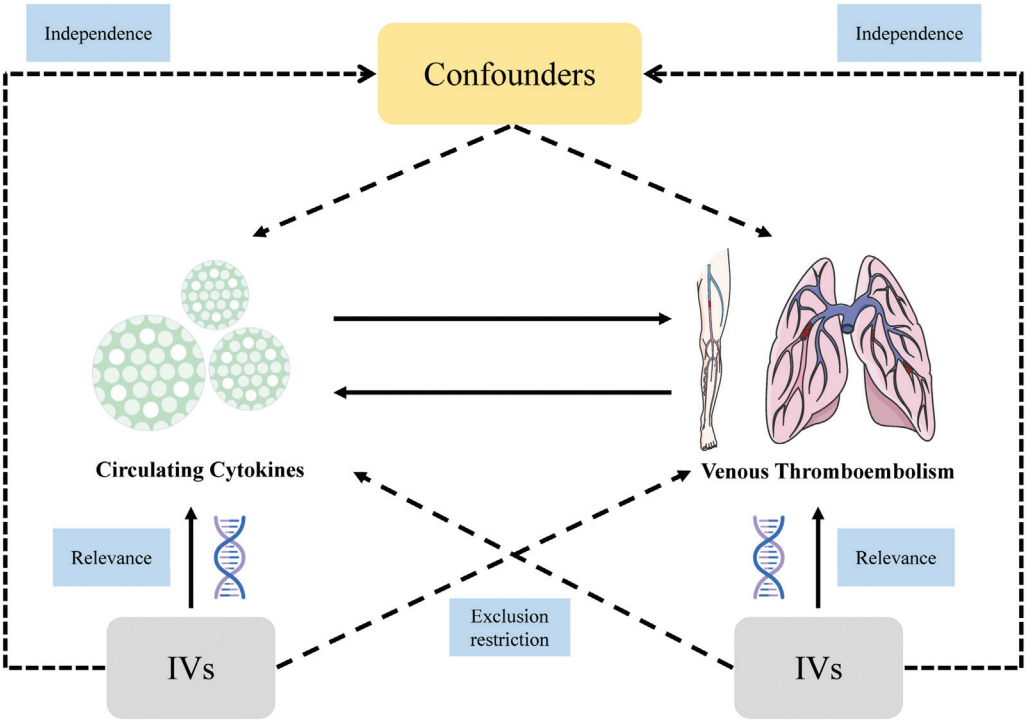

Fig. 1 Design of the current two-sample bidirectional Mendelian randomization study. Three core assumptions were as follows: (α) Relevance assumption; (β) independence assumption; (γ) exclusion restriction. IVs, instrumental variables.

8,293 individuals with European ancestry (►**Table 1**).[14,15] Similarly, the IVs for VTE, DVT, and PE were obtained from FinnGen study FinnGen consortium (Release 8, https://finngen.gitbook.io/documentation/v/r8/data-download). To select robust IVs, we initially identified SNPs that strongly predicted cytokines levels at a genome-wide significance ($p < 5 \times 10^{-8}$). However, due to the majority of cytokines having no or limited (<3) SNPs at this threshold, a more lenient significance threshold ($p < 5 \times 10^{-6}$) was employed to select IVs. Then, we conducted linkage disequilibrium tests among SNPs utilizing the European 1000 Genomes Project reference panel ($r^2 < 0.001$ and clump distance $> 10{,}000$ kb). If SNPs were in linkage disequilibrium, the SNP with higher *p*-value would be excluded from further analysis. Palindromic SNPs with a minor allele frequency greater than 0.42 were considered uninferrable and removed. Furthermore, to assess the robustness of the genetic instruments, we calculated both the variance explained (r^2) and *F*-statistic for each genetic variant in relation to levels of circulating cytokines. The F-statistic exceeding 10 indicated relatively strong estimated effects of the IVs in the MR analyses, while IVs with an F-statistic below 10 were excluded. Finally, the SNPs that were significantly associated with the outcomes or confounding factors were manually excluded using PhenoScanner (http://www.phenoscanner.medschl.cam.ac.uk/).

Data Sources

The summary-level statistics for the associations of venous thromboembolic diseases were obtained from a publicly available GWAS conducted by the FinnGen consortium (Release 8, https://finngen.gitbook.io/documentation/v/r8/data-download, ►**Table 1**).[16] A total of 17,048 cases and 325,451 controls for VTE (Phenocode: I9_VTE), 8,077 cases and 295,014 controls for DVT (Phenocode: I9_PHLETHROMBDVTLOW), and 8,170 cases and 333,487 controls for PE (Phenocode: I9_PULMEMB) were included in this GWAS. The FinnGen consortium comprised 342,499 individuals and a total of 16,962,023 variants were analyzed using a mixed-model logistic regression model that was adjusted for age, sex, 10 principal components, and genotyping batch.[16] The definitions of VTE, DVT, and PE were based on the International Classification of Diseases revision 9.

We further obtained genetic summary statistics for body mass index (BMI), hemoglobin A1c (HbA1c), moderate to vigorous physical activity (MVPA), systolic blood pressure (SBP), and sedentary behavior (time spent watching television) from the corresponding consortium (►**Table 1**).[17–21]

All studies included in our analysis have obtained relevant ethical review approvals from the respective ethical review committees, and all participants involved in the original studies have provided written informed consent.

Statistical Analysis

The inverse-variance weighted (IVW) method was utilized as the main statistical model to evaluate the associations of genetic liability to circulating cytokines with VTE, DVT, and PE. This method could yield the most reliable causal estimates, but it would be relatively susceptible to pleiotropy and outliers.[22] Therefore, the Weighted Median method, MR–Egger method, and MR Pleiotropy Residual Sum and Outlier (MR-PRESSO) method were employed in the

Table 1 Characteristics of data for MR analyses

Phenotype	Source	Sample size	Ancestry	Adjustments in the GWAS
Exposure/outcome				
Inflammatory cytokines	The Cardiovascular Risk in Young Finns Study	8,293 individuals	European ancestry	Age, sex, and the first 10 genetic principal components
	FINRISK 1997			
	FINRISK 2002			
Exposure/outcome				
Venous thromboembolism	FinnGen	17,048 cases and 325,451 controls	European ancestry	Age, sex, 10 principal components, and genotyping batch
Deep vein thrombosis	FinnGen	8,077 cases and 295,014 controls	European ancestry	Age, sex, 10 principal components, and genotyping batch
Pulmonary embolism	FinnGen	8,170 cases and 333,487 controls	European ancestry	Age, sex, 10 principal components, and genotyping batch
Adjustment				
Body mass index	Genetic Investigation of ANthropometric Traits consortium	~700,000 individuals	European ancestry	Age, sex, recruitment center, genotyping batches, and 10 principal components
Hemoglobin A1c	Meta-Analysis of Glucose and Insulin-related Traits Consortium	281,416 individuals	70% European ancestry	Not mentioned
Moderate to vigorous physical activity	UK Biobank	377,234 individuals	European ancestry	Age, sex, genotyping chip, first 10 genomic principal components
Systolic blood pressure	International Consortium of Blood Pressure-Genome Wide Association Studies and UK biobank	757,601 individuals	European ancestry	Sex, age, body mass index, and a binary indicator variable for UKB
Sedentary behavior	UK biobank	437,887 individuals	European ancestry	Age, sex, genotyping chip, first 10 genomic principal components

Abbreviation: GWAS, genome-wide association study.

following sensitivity analyses to evaluate the robustness of associations and potential pleiotropy. Specifically, the Weighted Median method can yield consistent causal estimates even when half of the weight is derived from invalid instruments.[23] The MR–Egger regression is capable of detecting potential pleiotropy between associations through intercept tests, while also providing more accurate estimates with correction for pleiotropy.[24] However, this approach would result in a reduction of statistical power for analyses. The MR-PRESSO method could provide relatively unbiased causal estimates by detecting and removing potential outliers.[25]

Leave-one-out analyses and scatter plots depicting the associations of genetically determined levels of circulating cytokines with VTE, DVT, and PE were also conducted. The multivariable IVW method was employed as the primary approach to assess whether potential confounders, such as BMI, HbA1c, MVPA, SBP, and sedentary behavior, could influence the association between inflammatory cytokines and VTE, DVT, and PE. Cochran Q-derived p-value and I^2 statistic were employed to evaluate the heterogeneity in IVW analysis ($p < 0.05$ or $I^2 > 50\%$ as significant heterogeneity), while the p-value for the MR–Egger intercept was utilized to assess potential horizontal pleiotropy ($p < 0.05$).[26,27] If pleiotropy was detected by the MR–Egger intercept test, MR–Egger regression would be employed as the primary analysis.[28] The Bonferroni correction was applied to account for multiple testing in examining the association between exposures and outcomes. Associations with a p-value $<1.2 \times 10^{-3}$ (0.05/41 exposures) were deemed statistically significant after adjusting for multiple testing, while associations with p-values ranging from 1.2×10^{-3} to 0.05 were considered suggestive. We considered our results as causal associations only if they exhibited consistent directionality and achieved statistical significance in at least two sensitivity analyses. In the present study, we conducted statistical analyses utilizing R software (version 4.3.0; R Foundation for Statistical Computing, Vienna, Austria) and the TwoSampleMR R package (https://github.com/MRCIEU/TwoSampleMR), in conjunction with MR-PRESSO (https://github.com/rondolab/MR-PRESSO).

Results

A total of 430 SNPs for 41 systemic circulating cytokines were identified as IVs to investigate the causal relationship among circulating cytokines in VTE, including DVT and PE. The variance explained by SNPs for each circulating cytokines ranged from 2 to 14%, and with all F-statistic values exceeding 10, indicating a low likelihood of significant weak instrument bias (►**Supplementary Tables S1**, available in the online version). Additionally, 17 SNPs were employed as IVs for VTE, 10 SNPs for DVT, and 5 SNPs for PE in reverse MR analysis (►**Supplementary Tables S2**, available in the online version).

The causal associations between genetically predicted levels of circulating cytokines and the risk of VTE, DVT, and PE are depicted in ►**Figs 2** to **4**, while detailed information can be found in ►**Supplementary Tables S3** to **S5** (available in the online version). The IVW analysis indicated that an increase of 1 standard deviation (SD) in stromal cell-derived factor-1 alpha (SDF1α) concentrations was suggestively associated with a lower risk of VTE (odds ratio [OR]: 0.90; 95% confidence interval [CI]: 0.81–0.99; $p = 0.033$) and DVT (OR: 0.85; 95% CI: 0.75–0.97; $p = 0.015$; ►**Figs. 2** and **3** and ►**Supplementary Tables S3** and **S4,** available in the online version). Similarly, suggestive evidence was found for the causal associations between IL-6 with the lower risk of VTE (OR: 0.88; 95% CI: 0.77–1.00; $p = 0.049$) and DVT (OR: 0.83; 95% CI: 0.70–0.98; $p = 0.031$; ►**Figs. 2** and **3** and ►**Supplementary Tables S3** and **S4**, available in the online version). Conversely, 1 SD increased in the concentrations of beta nerve growth factor (βNGF) was suggestively linked to an increased risk of VTE (OR: 1.13; 95% CI: 1.00–1.28; $p = 0.043$) and PE (OR: 1.16; 95% CI: 1.00–1.34; $p = 0.044$; ►**Fig. 4** and ►**Supplementary Table S5**, available in the online version). In addition, we also observed a suggestive causal relationship between an increase of 1 SD in genetic liability to IL-18 with a decreased risk of DVT (OR: 0.91; 95% CI: 0.83–0.99; $p = 0.030$) and granulocyte colony-stimulating factor (G-CSF; OR: 1.20; 95% CI: 1.06–1.37; $p = 0.005$) with an elevated risk of PE (►**Figs. 3** and **4** and ►**Supplementary Tables S4** and **S5**, available in the online version). No significant relationships were found with genetic liability to other circulating cytokines with VTE, DVT, and PE (►**Figs. 2**–**34** and ►**Supplementary Tables S3**–**S5**, available in the online version).

The main findings remained consistent for SDF1α in relation to VTE and DVT, as well as G-CSF in relation to PE, based on the results of subsequent sensitivity analyses (►**Supplementary Tables S3**–**S5**, available in the online version). The scatterplots illustrating the aforementioned associations are presented in ►**Supplementary Figs. S1** to **S3**. However, the suggestive associations of βNGF with VTE and PE, as well as IL-6 and IL-18 with DVT did not persist in sensitivity analyses (►**Supplementary Tables S3**–**S5**, available in the online version). Therefore, we considered these associations to be statistically unstable. Meanwhile, several outliers of IL-6 were identified in the MR-PRESSO analyses of VTE, and the significance of results did not remain after removing outliers (►**Supplementary Table S3**, available in the online version). Multivariable MR analyses indicated that the association pattern of SDF1α in VTE remained consistent after adjusting for BMI, MVPA, and sedentary behavior (►**Fig. 5** and ►**Supplementary Table S6**, available in the online version). Similarly, the association pattern of SDF1α in DVT remained unchanged following adjustment for MVPA and sedentary behavior (►**Fig. 5** and ►**Supplementary Table S6**, available in the online version). However, only after adjusting for MVPA, did the association pattern of SCGF in PE remain unaltered (►**Fig. 5** and ►**Supplementary Table S6**, available in the online version).

Furthermore, to explore the potential reverse causality, a reverse-direction MR analysis was conducted. After considering the results of the sensitivity analysis, the reverse MR analysis results suggested that genetic liability to VTE was associated with decreased levels of multiple cytokines,

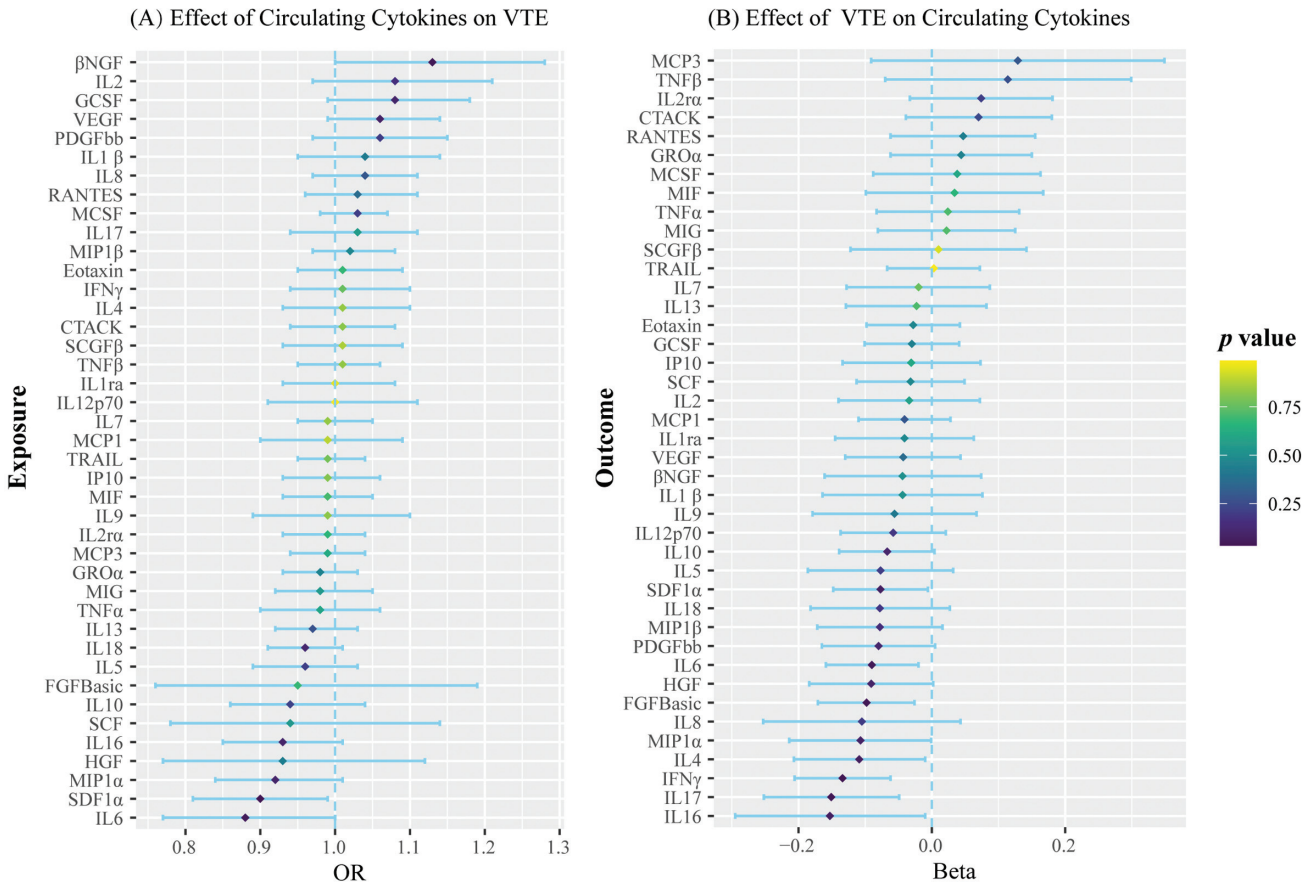

Fig. 2 Bidirectional associations between genetic liability to circulating cytokines and venous thromboembolism. (**A**) Effect of circulating cytokines on VTE; (**B**) effect of VTE on circulating cytokines. βNGF, beta nerve growth factor; CTACK, cutaneous T cell-attracting chemokine; FGFBasic, basic fibroblast growth factor; G-CSF, granulocyte colony-stimulating factor; GROα, growth-regulated oncogene-a; HGF, hepatocyte growth factor; IFNγ, interferon gamma; IL, interleukin; IP10, interferon gamma-induced protein 10; MCP1, monocyte chemotactic protein 1; MCP3, monocyte-specific chemokine 3; MCSF, macrophage colony-stimulating factor; MIF, macrophage migration inhibitory factor; MIG, monokine induced by interferon gamma; MIP1α, macrophage inflammatory protein-1a; MIP1β, macrophage inflammatory protein-1b; OR, odds ratio; PDGFbb, platelet-derived growth factor BB; RANTES, regulated upon activation normal T cell expressed and secreted factor; SCF, stem cell factor; SCGFβ, stem cell growth factor beta; SDF1α, stromal cell-derived factor-1 alpha; SNPs, single-nucleotide polymorphisms; TNFα, tumor necrosis factor alpha; TNFβ, tumor necrosis factor beta; TRAIL, TNF-related apoptosis-inducing ligand; VEGF, vascular endothelial growth factor.

including basic fibroblast growth factor (FGFBasic), interferon-γ (IFNγ), and IL-17 (beta, −0.098; 95% CI: −0.171 to −0.026; $p = 0.008$; beta, −0.134; 95% CI: −0.206 to −0.062; $p = 2.61E-04$; beta, −0.151; 95% CI: −0.252 to −0.049; $p = 0.004$, ►**Fig. 2** and ►**Supplementary Table S7**, available in the online version). Genetically predicted DVT was found to be suggestively associated with reduced levels of IFNγ (beta, −0.086; 95% CI: −0.155 to −0.016; $p = 0.016$, ►**Fig. 3** and ►**Supplementary Table S8**, available in the online version). Additionally, genetic predisposition to PE was observed to be suggestively linked with reduced levels of hepatocyte growth factor (HGF; beta, −0.091; 95% CI: −0.163 to −0.018; $p = 0.014$, ►**Fig. 4** and ►**Supplementary Table S9**, available in the online version).

The I^2 statistic and corresponding p-value revealed a modest level of heterogeneity in our principal analyses (►**Supplementary Tables S10** and **S11**, available in the online versio). However, the MR–Egger intercept analyses provided limited evidence of heterogeneity for the associations between circulating cytokines and VTE, DVT, as well as PE (►**Supplementary Tables S10** and **S11**, available in the online version). The leave-one-out sensitivity analyses revealed that no individual SNP had a significant impact on the causal association in our primary analysis (►**Supplementary Figs. S4–S6**, available in the online version).

Discussion

In the present comprehensive MR analysis, genetic data obtained from GWAS were utilized to assess potential bidirectional causal associations between genetic predisposition to levels of 41 circulating cytokines and the risk of VTE, DVT, and PE. Our findings provided suggestive evidence supporting a causal relationship between genetically predicted elevated levels of SDF1α and a reduced risk of VTE and DVT. Besides, a potential association of genetic liability to G-CSF with PE was found. Furthermore, suggestive evidence was found supporting the reverse causal effect of VTE on the

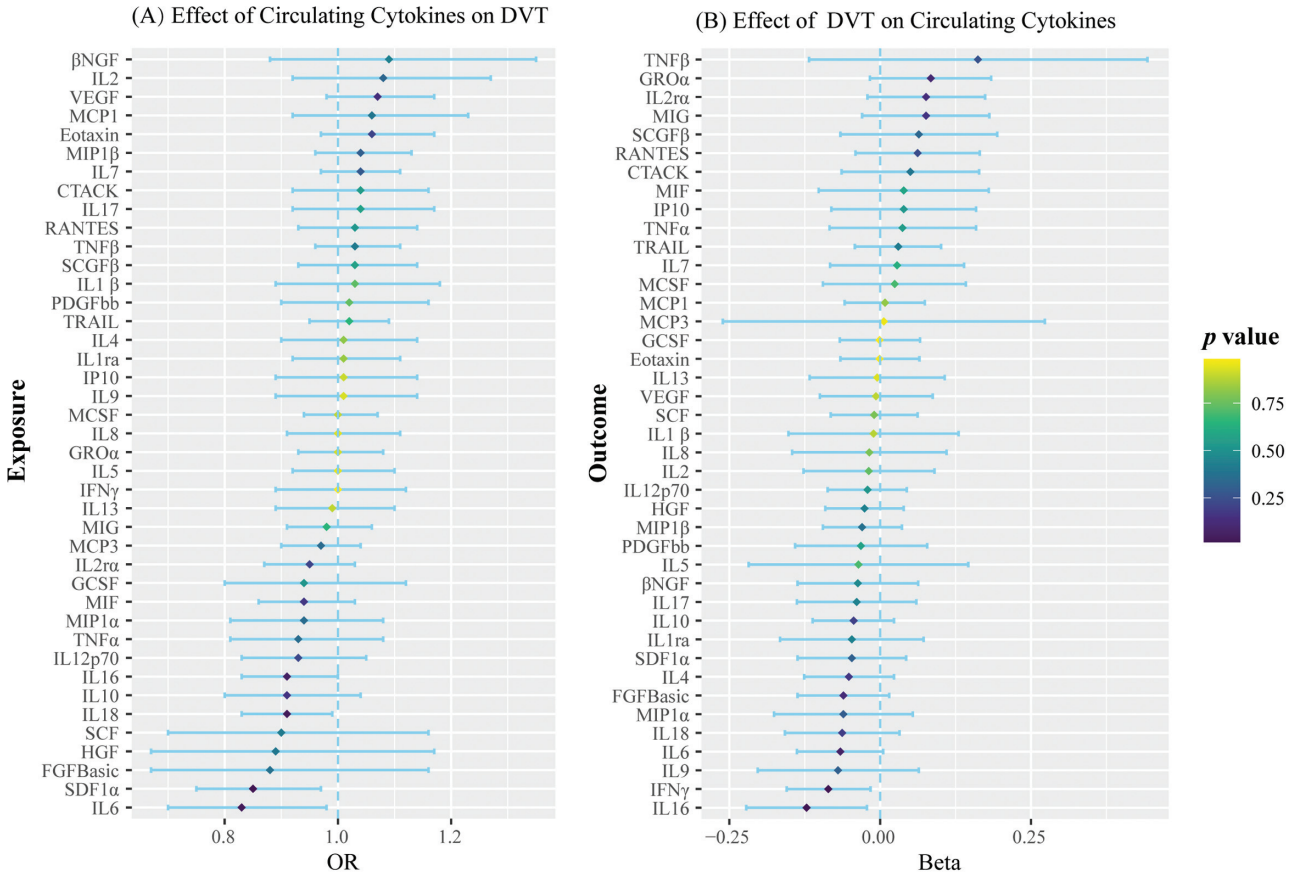

Fig. 3 Bidirectional associations between genetic liability to circulating cytokines and deep vein thrombosis. (**A**) Effect of circulating cytokines on DVT; (**B**) effect of DVT on circulating cytokines. βNGF, beta nerve growth factor; CTACK, cutaneous T cell-attracting chemokine; FGFBasic, basic fibroblast growth factor; G-CSF, granulocyte colony-stimulating factor; GROα, growth-regulated oncogene-a; HGF, hepatocyte growth factor; IFNγ, interferon gamma; IL, interleukin; IP10, interferon gamma-induced protein 10; MCP1, monocyte chemotactic protein 1; MCP3, monocyte-specific chemokine 3; MCSF, macrophage colony-stimulating factor; MIF, macrophage migration inhibitory factor; MIG, monokine induced by interferon gamma; MIP1α, macrophage inflammatory protein-1a; MIP1β, macrophage inflammatory protein-1b; OR, odds ratio; PDGFbb, platelet-derived growth factor BB; RANTES, regulated upon activation normal T cell expressed and secreted factor; SCF, stem cell factor; SCGFβ, stem cell growth factor beta; SDF1α, stromal cell-derived factor-1 alpha; SNPs, single-nucleotide polymorphisms; TNFα, tumor necrosis factor alpha; TNFβ, tumor necrosis factor beta; TRAIL, TNF-related apoptosis-inducing ligand; VEGF, vascular endothelial growth factor.

levels of several cytokines. Those association patterns persisted in the subsequent supplementary analyses.

An increasing body of evidence indicates the functional interdependence between inflammation and thrombosis, with circulating cytokines being suggested as significant contributors to VTE.[29,30] SDF-1α, a member of the CXC chemokine family, is upregulated in injured tissue and facilitates recruitment of innate immune cells that directly and indirectly contribute to microvascular network growth.[31] Several studies have found that biomaterials based on SDF-1α can expedite endothelialization, enhance anticoagulation, and modulate intimal regeneration, thereby mitigating thrombosis in vascular scaffold.[32–34] Additionally, numerous animal studies have demonstrated that upregulation of SDF-1α confers anti-inflammatory and anti-apoptotic effects under acute myocardial infarction setting, thereby ultimately contributing to the improvement of cardiac function.[35,36] Furthermore, a clinical trial based on the CASSINI study (a multicenter, randomized, double-blind, placebo-controlled, parallel-group, phase 3b trial) demonstrated a significant reduction of SDF-1 in cancer patients without VTE and identified it as a potential biomarker for cancer-associated VTE.[37] However, to the best of our knowledge, there has been limited investigation into the direct association between SDF1α and VTE in the general population. Our MR results provided novel genetic insights into the protective role of SDF1α against VTE and DVT.

The relationship between SDF1α and VTE is complex and multifaceted, with the primary mechanism potentially involving anti-inflammatory effects and endothelialization.[38] SDF1α has been demonstrated to exert an anti-inflammatory effect in biomaterials by suppressing the expression of inflammatory factors and recruiting anti-inflammatory monocytes.[39] The healthy endothelium exerts an inhibitory effect on thrombosis by the secretion of a cascade of anticoagulant molecules.[40] Several studies have reported that nanofibrous vascular scaffolds fabricated using a miscible polymer of heparin and SDF1α exhibit enhanced endothelialization and reduced thrombogenicity.[32,41] The statistical significance of the causal relationship between SDF1α and

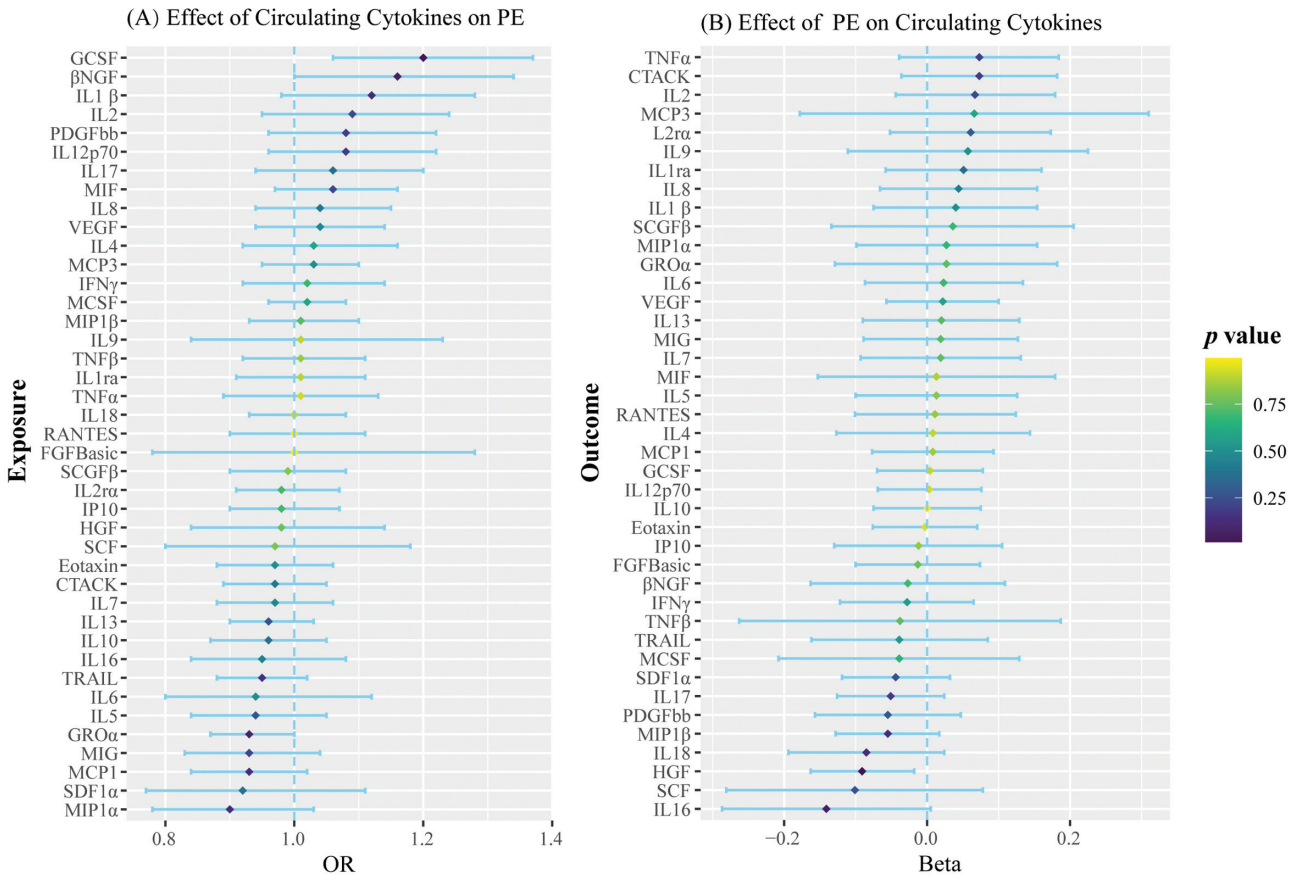

Fig. 4 Bidirectional associations between genetic liability to circulating cytokines and pulmonary embolism. (**A**) Effect of circulating cytokines on PE; (**B**) effect of PE on circulating cytokines. βNGF, beta nerve growth factor; CTACK, cutaneous T cell-attracting chemokine; FGFBasic, basic fibroblast growth factor; G-CSF, granulocyte colony-stimulating factor; GROα, growth-regulated oncogene-a; HGF, hepatocyte growth factor; IFNγ, interferon gamma; IL, interleukin; IP10, interferon gamma-induced protein 10; MCP1, monocyte chemotactic protein 1; MCP3, monocyte-specific chemokine 3; MCSF, macrophage colony-stimulating factor; MIF, macrophage migration inhibitory factor; MIG, monokine induced by interferon gamma; MIP1α, macrophage inflammatory protein-1a; MIP1β, macrophage inflammatory protein-1b; OR, odds ratio; PDGFbb, platelet-derived growth factor BB; RANTES, regulated upon activation normal T cell expressed and secreted factor; SCF, stem cell factor; SCGFβ, stem cell growth factor beta; SDF1α, stromal cell-derived factor-1 alpha; SNPs, single-nucleotide polymorphisms; TNFα, tumor necrosis factor alpha; TNFβ, tumor necrosis factor beta; TRAIL, TNF-related apoptosis-inducing ligand; VEGF, vascular endothelial growth factor.

VTE disappeared after adjusting for HbA1c and SBP in the present study, implying that a portion of the effect of SDF1α on VTE may be mediated through HbA1c and SBP. In a nested case–control study, it was observed that women with HbA1c levels >7.0% exhibited a 36 to 55% increased relative risk of VTE compared to those with HbA1c levels between 6.5 and 7.0%.[42] Another MR study demonstrated a significant association between genetically predicted SBP levels and a decreased risk of VTE.[43] Therefore, it is imperative to take into account the levels of HbA1c and SBP when interpreting our MR findings.

Our MR study also revealed that genetically determined elevated levels of circulating G-CSF concentrations were positively associated with an increased susceptibility to PE. G-CSF, a hematopoietic growth factor, possesses the capacity to mobilize cells from the bone marrow into the peripheral blood circulation and promote granulocyte maturation.[44] Currently, there is no direct evidence linking G-CSF to PE. However, a study has reported the presence of pulmonary micro-embolism in a healthy donor following the administration of G-CSF for mobilization of hemopoietic progenitor cells.[45] This may be attributed to the activation of endothelial cells and coagulation system induced by G-CSF. Similarly, in vitro studies have demonstrated that recombinant human G-CSF induces platelet aggregation,[46] corroborating our MR study findings that G-CSF serves as a risk factor for PE.

The reverse MR analysis revealed that VTE and DVT were negatively associated with the levels of IFNγ. The cytokine IFNγ, primarily produced by natural killer cells, natural killer T cells, and Th1 CD4 and CD8 cytotoxic T lymphocytes, plays a crucial role in enhancing immune surveillance during infection.[47] The presence of IFNγ has been documented to be linked with a decrease in neovascularization and collagen degradation, thereby leading to a delay in thrombus resolution.[48] The present study revealed a decrease in IFNγ levels following VTE and DVT, implying a potential depletion of this cytokine during thrombosis. The levels of IL17 and FGFBasic were observed to be decreased in patients with VTE in our study. IL-17A has been reported to promote thrombus formation by enhancing platelet activation, aggregation, and

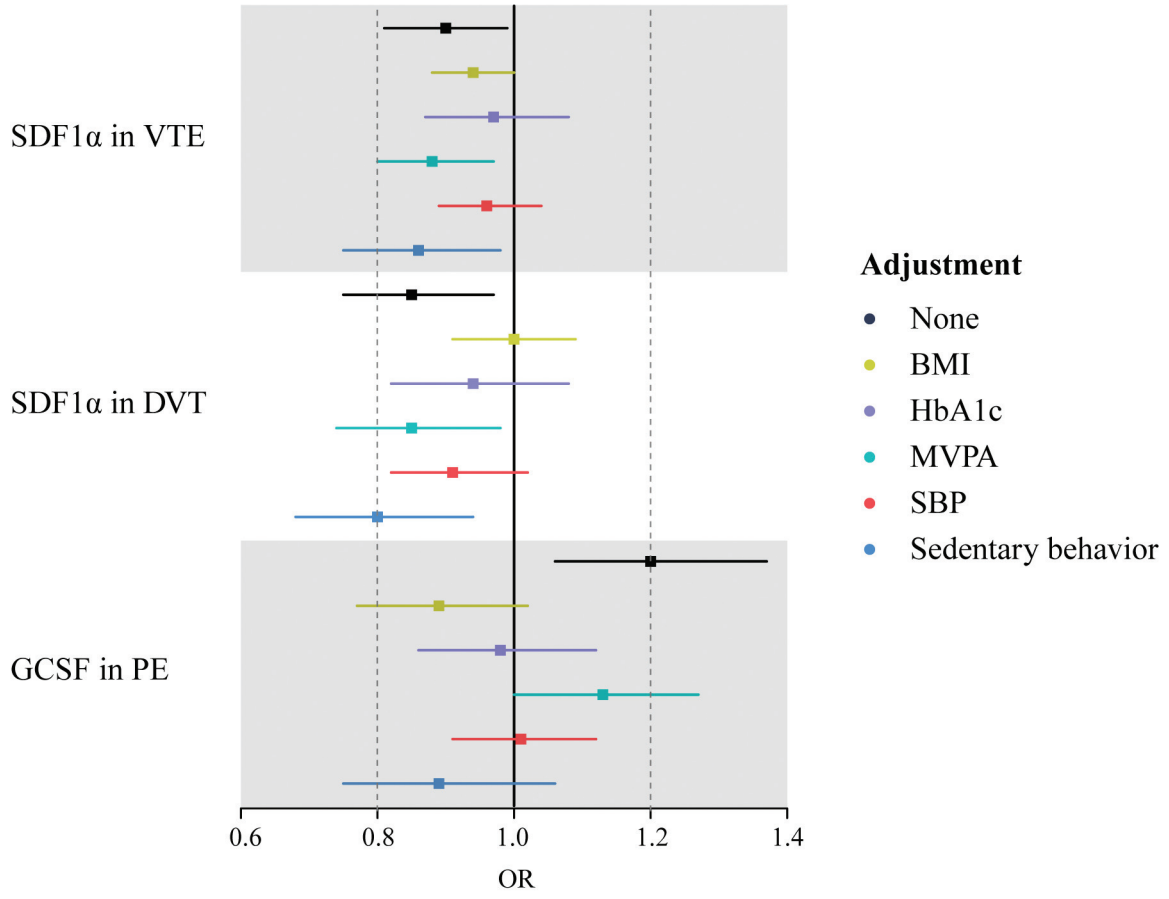

Fig. 5 Associations of circulating cytokines and venous thromboembolism after adjusting for potential confounders. BMI, body mass index; DVT, deep vein thrombosis; G-CSF, granulocyte colony-stimulating factor; HbA1c, hemoglobin A1c; IVW, inverse-variance weighted; MVPA, moderate to vigorous physical activity; OR, odds ratio; PE, pulmonary embolism; SBP, systolic blood pressure; SDF1α, stromal cell-derived factor-1 alpha; VTE, venous thromboembolism.

activating endothelial cells.[49] However, relevant studies investigating the potential association between VTE and FGFBasic remain scarce in the existing literature. The reverse MR analysis suggested an inverse correlation between PE and HGF. The observational study revealed a significant association between PE and an elevated level of HGF, which contrasts with our MR results.[50] This discrepancy suggests that potential confounding factors may have influenced the findings of the observational study.

The present MR study possessed multiple notable strengths. The major one was the MR design, which mitigated potential confounding and other biases that could have influenced the observed associations. Moreover, our study encompassed a comprehensive range of circulating cytokines to investigate the bidirectional causal relationship between cytokines and VTE, including DVT and PE. Additionally, the high *F*-statistics (all *F*-statistics >10) provided robust evidence of the strong predictive ability of the genetic instruments for the exposures, thereby ensuring sufficient statistical power in our study. Finally, a multivariable MR method was employed to adjust for established risk factors.

Several limitations should be acknowledged when interpreting our findings. First, a relatively lenient threshold was applied in our study to increase the number of instruments for circulating cytokines, which may result in reduced statistical power. Second, the influence of potential pleiotropy still could not be entirely ruled out, though weak evidence of horizontal pleiotropy was found in the MR–Egger intercept tests. Meanwhile, MR-PRESSO analyses were employed to obtain more reliable associations before and after removing identified outliers. Third, the absence of individual-level genotyping data precluded the evaluation of the causal association between circulating cytokines and VTE across different age groups and genders. Finally, the population included in the GWAS for this MR study was predominantly of European descent, which minimized potential population stratification bias, but at the same time limited the generalizability of findings to other populations.

Conclusion

In conclusion, this MR study provides consistent evidence supporting a bidirectional causal relationship between genetically predicted levels of specific circulating cytokines and the risk of VTE. Our findings suggest that strategies targeting circulating cytokines may act as prevention

approaches for VTE. Further research is necessary to validate these findings and explore underlying biological mechanisms.

What is known about this topic?

- Circulating cytokines play a pivotal role in the pathogenesis of venous thromboembolism (VTE), encompassing deep vein thrombosis (DVT) and pulmonary embolism (PE).
- Evidence from observational studies regarding the association between circulating cytokines and VTE remains controversial, due to confounding factors and reverse causation bias. Thus, it is unclear whether the association between specific circulating cytokines and VTE is causal or spurious.

What does this paper add?

- We identified a cytokine, stromal cell-derived factor-1α (SDF-1α), with potential clinical applications in the prevention of VTE and DVT.
- We observed a suggestive causal association between granulocyte colony-stimulating factor (GCSF) and the development of PE. In contrast to previous observational studies, our Mendelian randomization approach provides consistent and robust genetic evidence.

Data Availability Statement

All the data used in the present study had been publicly available. The original contributions presented in the study are included in the article/►**Supplementary Material**, and further inquiries can be directed to the corresponding author/s.

Authors' Contribution

T.H., F.Y., J.Y., and H.C. contributed to the conception or design of the work. P.S. and Y.C. contributed to the acquisition, analysis, or interpretation of data for the work. T.H. wrote the first draft of the manuscript with critical revisions from P.S., F.Y., and H.C. All gave final approval and agreed to be accountable for all aspects of work ensuring integrity and accuracy.

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Conflict of Interest

None declared.

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