Original Research Article

The Prevalence of the Thrombotic SNPs rs6025, rs1799963, rs2066865, rs2289252 and rs8176719 in Patients with Venous Thromboembolism in the Czech Population

Clinical and Applied Thrombosis/Hemostasis
Volume 31: 1-7

DOI: 10.1177/10760296251324202
journals.sagepub.com/home/cat
Sage

Tomas Kvasnicka, MD, PhD[1], Renata Cifkova, MD, PhD[2], Zuzana Zenahlikova, MD, PhD[1], Petra Bobcikova, MSc[1], Alena Syruckova, MSc[1], Martin Sevcik, MSc, PhD[1], Daniela Dusková, MD, PhD[3,4], and Jan Kvasnicka, MD, PhD[3]

Abstract

Introduction: Study aimed to determine the occurrence of 5 thrombosis-related single-nucleotide polymorphisms (SNPs) in patients with venous thromboembolism (VTE) (n = 2630) and a control group (n = 2637) in the Czech population.

Methods: The following gene SNPs were detected in both groups: *F5* Leiden (rs6025), *F2* (rs1799963), *FGG*, fibrinogen gamma' (rs2066865), *F11* (rs2289252) and *ABO* (rs8176719). Statistical analysis was performed using SAS statistical software with population genetics tools.

Results: Heterozygotes for *F5* Leiden were associated with a 5.58-fold and homozygotes *F5* Leiden with a 33.46-fold increased risk of VTE. At SNP rs1799963 (*F2*, prothrombin), only heterozygotes had a significant 3.9-fold increased risk of VTE. The findings at SNP rs2066865 (fibrinogen gamma', *FGG*) showed a 1.37-fold increased risk of VTE for *FGG* heterozygotes and a 1.77-fold increased risk of VTE for *FGG* homozygotes. There is also a significant 1.42-fold increase risk of VTE in the heterozygotes and a 1.80-fold increase risk of VTE in the homozygotes of the SNP rs 2289252 (*F11*). Further higher increases in the risk of VTE in both variants were found in patients with VTE at rs8176719 (*ABO*, non-O). It corresponds to a 2.2-fold increase in the risk of VTE in heterozygotes and a 3.5-fold increase in the risk of VTE in homozygotes.

Conclusion: Besides *F5* Leiden and prothrombin mutation, the study suggests that the gene polymorphisms of *FGG* (rs2066865), *F11* (rs2289252) and *ABO* (rs8176719) play a role as an independent heritable risk factor for VTE in the Czech population.

Keywords

venous thromboembolism, rs6025, rs1799963, rs2066865, rs2289252, rs8176719

Date received: 22 November 2024; revised: 4 February 2025; accepted: 10 February 2025

[1] Thrombotic Centre, Institute of Medical Biochemistry and Laboratory Diagnostics, first Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic
[2] Center for Cardiovascular Prevention, first Faculty of Medicine, Charles University and Thomayer University Hospital, Prague, Czech Republic
[3] 1st Department of Medicine, Division of Hematology, first Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic
[4] Department of Blood Transfusion, first Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic

Corresponding Author:
Jan Kvasnička, M.D., Professor, DrSc., Thrombotic Centre, Institute of Medical Biochemistry and Laboratory Diagnostics, first Faculty of Medicine, Charles University and General University Hospital in Prague, Karlovo nám. 32, 121 11 Prague 2, Czech Republic.
Email: jbkvasnicka@seznam.cz

Table 1. An Overview of Five Single Nucleotide Polymorphisms (SNPs) Associated with Venous Thromboembolism as Reported in Studies Conducted Within the European Population.

Chromosome	Gene	SNP	Reference						
			(16)	(17)	(18)	(19)	(20)	(21)	(22)
1	*F5*	rs6025	X	X	X	X	X	X	X
11	*F2*	rs1799963	X	X	X	/	X	X	X
4	*FGG*	rs2066865	X	X	X	X	X	/	X
4	*F11*	rs2289252	X	*	*	X	*	X	X
9	*ABO*	rs8176719	X	X	X	X	*	/	X
Studies			MEGA LETS	MEGA GWAS	UK Biobank GWAS metaanalyse	INVENT 18 GWAS metaanalyse	13 GWAS metaanalyse	6 GWAS metaanalyse	ARIC GWAS

X indicates that the SNP was tested; */*, the SNP was not tested; *, the next SNP in the gene was tested; GWAS, genome-wide association study; ARIC, Atherosclerosis Risk in Communities Study; INVENT, International Network Against Venous Thrombosis; LETS; Leiden Thrombophilia Study; MEGA, Multiple Environmental and Genetic Assessment Study

Introduction and Study aim

In Europe, approximately 500,000 people die each year from complications of VTE, even though this disorder can be prevented with appropriate prophylaxis.[1] The incidence of VTE is still relatively high, with 148 cases of venous thrombosis and 95 cases of acute pulmonary embolism per 100,000 individuals occurring each year.[2] It is the third most common disease and the third leading cause of death from cardiovascular disease.[3] The lifetime risk of VTE is also relatively high,[4] likely due to the increasing prevalence of obesity, cancer, and ageing populations.[5] However, the course of VTE is not stationary, and anticoagulation therapy may not influence its dynamics. After acute venous thrombosis, many patients develop post-thrombotic syndrome, which clinically manifests as varying degrees of chronic venous insufficiency.[6] The most severe complication of acute venous thrombosis, however, is acute pulmonary embolism (PE). Its incidence is approximately 100 per 100,000 population per year.[7] Another serious complication of embolization into the pulmonary circulation is chronic thromboembolic pulmonary hypertension (CTEPH), which occurs with a certain time lag in 2%–4% of patients with PE. Without treatment, the prognosis for CTEPH is poor, with historical data showing mortality rates of 70%–90% after 5 years.[8] Another complication of PE is the so-called post-pulmonary syndrome, manifested as new or progressive dyspnea and exercise intolerance after the end of anticoagulation therapy.[9] Up to 31% of cases of VTE recur after the first episode, too.[10] A higher number of recurrences is observed in so-called unprovoked VTE,[11] which leads to a tendency to prolong anticoagulation therapy.[12] The reason for this is also the increased mortality (up to 34%) observed in patients with recurrent VTE.[13] The goal of effective treatment to reduce the incidence and complications of VTE, including its recurrence, may therefore be individualized prevention in the future. The disease itself is multifactorial,[14] with many acquired, but also hereditary risk factors and varying prevalence among different ethnic groups.[15] Since 2008, newly published results of genome-wide association studies (GWAS) have included the examination of hundreds of thousands to millions of known SNPs in cohorts of tens of thousands to hundreds of thousands of individuals who have had VTE and controls that have not had VTE.[16–22] With the gradual increase in the number of identified SNPs and participants in new GWAS, dozens of the latest genetic associations with VTE have been discovered, mainly with a small impact on their risk. Therefore, the selection criterion for their use was set to require alleles associated with VTE to reach statistical significance in GWAS.[23] In addition to the F5 Leiden and prothrombin gene mutation, which Manuzzi et al[24] already consider "classic polymorphisms" associated with the risk of VTE, this requirement was met only by newly discovered SNPs in the fibrinogen gamma' gene /FGG/ (rs2066895), F11 gene (rs2289252), and ABO gene /non-O/ (rs8176719), which were repeatedly identified in the GWAS above and their recent meta-analyses in European population. Their overview is presented in Table 1.

However, this is no longer true for the Asian population[25,26] or American blacks.[22]

Our study aimed to determine the prevalence of these 5 SNPs in patients with VTE compared to their findings in individuals who did not have VTE in the Czech population, which as an ethnic group is referred to as the European Western Slavic population.

Methods

The sample consisted of 2630 patients with VTE, including 2143 patients registered at the Thrombotic Center of the Institute of Clinical Biochemistry and Laboratory Diagnostics of the General University Hospital and the First Faculty of Medicine of Charles University in Prague. This registry included adult individuals (≥ 18 years) from the capital city of Prague and the Central Bohemian Region of the Czech Republic who were newly diagnosed with VTE between the years 2011–2017. Diagnostic criteria for VTE were a positive finding of venous thrombosis with duplex sonography and confirmation of acute embolism with multidetector computed

Table 2. Baseline and Clinical Data of Patients with Venous Thromboembolism (VTE) and Control Subjects.

	Controls	VTE patients
total–number (%)	2637 (100)	2630 (100)
male – number (%)	1516 (57.5)	926 (35.2)
female–number (%)	1121 (42.5)	1704 (64.8)
age–mean (SD), years	43.6 (12.8)	40.8 (14.8)
only deep venous thrombosis (%)	–	1816 (69)
only pulmonary embolism (%)	–	342 (13)
deep venous thrombosis and pulmonary embolism (%)	–	472 (18)
VTE recurrence (%)	–	613 (23)

tomography or perfusion scintigraphy of the lung ventilation. All these patients received anticoagulation treatment in the acute phase of VTE. This sample was supplemented by 487 individuals from the nationwide Czech post-Monica study register[27] who reported in the questionnaire that they had experienced VTE and were being treated with anticoagulants at that time. According to the completed questionnaire, the control group consisted of 2637 healthy individuals who had not experienced VTE. This group consisted of 1512 blood donors, healthy volunteers, and 1125 participants in the Czech post-MONICA study.[27] Baseline and clinical data for the control group and individuals who had experienced VTE are presented in Table 2. All patients with VTE and the control group provided written informed consent for DNA testing in accordance with the Helsinki Declaration. The genetic study focusing on VTE risk factors was approved by the Ethics Committee of the General University Hospital and the First Faculty of Medicine of Charles University in Prague (No. NT 11176-5). The Czech post-MONICA population study was approved by the Institute for Clinical and Experimental Medicine Ethics Committee and the Faculty of Thomayer University Hospital in Prague. All study participants were Caucasians living in the Czech Republic. Ethnically, the Czechs are considered a Western Slavic nation.

In the laboratory analyses, the SNPs of the following genes were investigated: F5 Leiden (rs6025), F2 prothrombin (rs1799963), FGG (rs8176719), F11 (rs2289252) and ABO /non-O/ (rs8176719) in uncoagulated peripheral blood samples. Genomic DNA was extracted from their leukocytes and isolated using the MagNA Pure LC Nucleic Acid Extraction System™ with the MagNA Pure DNA Isolation Kit I™ (all products from Roche Diagnostics, Mannheim, Germany). Mutations were determined using real-time polymerase reaction in a process called FRET (Fluorescence Resonance Energy Transfer). The analyses were performed using the LightCycler® 480 System with LC® 480 Genotyping Master Kits (all products supplied by Roche Diagnostics, Mannheim, Germany). Specific primers and fluorescence-labelled probes were designed and custom-made in collaboration with TIB MOLBIOL (Berlin, Germany). Categorical variables were expressed as numbers and percentages. Comparisons between groups were made using Fisher's exact test. Logistic analysis tests were performed to assess the clinical impact of alleles and genotypes. Results were presented as odds ratios (ORs) for risk versus non-risk homozygotes and corresponding 95% confidence intervals (CIs). Statistical analysis was performed using SAS statistical software (SAS version 9.4, SAS/Genetics™ 13.1, SAS Institute Inc., NC, USA) with population genetics tools. We consider findings with $p < 0.05$ as significant differences in the representation of risk alleles and genotypes.

Results

The genetic findings in patients with VTE (n 2630) and controls (n 2637) are shown in Table 3. All findings in controls met the Hardy-Weinberg equation (HWE). Compared to the control group, an increased representation of SNP rs6025 (*F5* Leiden), rs1799963 (*F2*, prothrombin), rs2066865 (fibrinogen gamma', FGG), rs2289252 (*F11*) and rs8176719 (ABO, non-O) was found in patients with VTE (all with $p < 0.001$). Heterozygotes for *F5* Leiden were associated with a 5.58-fold increased in the risk of VTE (OR 5.58; 95% CI 4.74-6.56), while homozygotes had up to a 33.46-fold increased risk of VTE (OR 33.46; 95% CI 10.52-106.40). At SNP rs1799963 (*F2*, prothrombin), only heterozygotes had a significant 3.9-fold increased risk of VTE (OR 3.90; 95% CI: 2.90-5.25). Compared to the control and VTE patient groups, the OR for the homozygous variant of *F2* was not significant. The findings at SNP rs2066865 (fibrinogen gamma', *FGG*) showed a 1.37-fold increased risk of VTE for *FGG* heterozygotes (OR 1.37; 95% CI: 1.22-1.54) and a 1.77-fold increased risk of VTE for *FGG* homozygotes (OR 1.77; 95% CI: 1.43-2.18). There is also a significant 1.42-fold increase risk of VTE in the heterozygotes (OR 1.42; 95% CI: 1.25-1.6) and a 1.80-fold increase risk of VTE in the homozygotes (OR 1.80; 95% CI: 1.55-2.13) of the SNP rs 2289252 (*F11*). Further higher increases in the risk of VTE in both variants were found in patients with VTE at rs8176719 (*ABO*, non-O). It corresponds to a 2.2-fold increase in the risk of VTE in heterozygotes (OR 2.19; 95% CI: 1.91-2.51) and a 3.5-fold increase in the risk of VTE in homozygotes (OR 3.52; 95% CI: 2.99-4.13).

Discussion

Significant associations of the tested 5 SNPs, rs6025 (*F5* Leiden), rs1799963 (*F2*, prothrombin), rs2066865 (*FGG*, fibrinogen gamma'), rs2289252 (*F11*) and rs8176719 (*ABO*, non-O) with VTE were demonstrated alongside findings of the GWAS above in patients with ischemic stroke and VTE,[28] in patients with cancer and VTE,[29] in patients with CTEPH[30] and in pregnant women with VTE.[31] Examination of these 5 SNPs was also used to determine the likelihood of first VTE[16] or the likelihood of VTE recurrence after the completion of anticoagulation therapy.[17,32] In both studies, the

Table 3. Results of Alleles and Genotypes Frequencies, Logistic Regression (Odds Ratio with 95% Wald Confidence Limits) and Fisherś Exat Test p-Value Between Controls (n 2637) and VTE Patients (n 2630) fof the Five VTE Risk SNPs.

			Risk Allele Frequencies		Genotypes Frekvencies			
Gene and dbID	SNP	Risk Allele	Controls	VTE Patients	Controls	VTE Patients	Odds Ratio (95% CI)	Fisher's Exat Test: P-Value
F5 (Leiden) rs6025	691 G>A	A	4.19%	18.82%	A/A 0.11% G/A 8.15% G/G 91.73%	A/A 2.7% G/A 32.24% G/G 65.06%	A/A versus GG:33.46(10.52-106.40) G/A versus G/G:5.58 (4.74-6.56)	<0.0001
F2 (protrombin) rs1799963	20210 G>A	A	1.16%	4.26%	A/A 0.04% G/A 2.24% G/G 97.72%	A/A 0.15% G/A 8.21% G/G 91.63%	A/A versus GG:4.28(0.48-8.29) G/A versus G/G:3.9(2.9-5.25)	<0.0001
FGG rs2066865	10043 C>T	T	24.49%	30.45%	C/C 57.25% C/T 36.53% T/T 6.22%	C/C 48.39% C/T 42.33% T/T 9.29%	T/T versus C/C:1.77(1.43-2.18) C/T versus C/C:1.37(1.22-1.54)	<0.0001
F11 rs2289252	22771 C>T	T	39.59%	47.19%	C/C 37.18% C/T46.47% T/T 16.35%	C/C 28.00% C/T 49.61% T/T 22.38%	T/T versus C/C:1.80(1.55-2.13) C/T versus C/C:1.42(1.25-1.60)	<0.0001
ABO rs8176719	c.261 del G	G	41.17%	56.51%	-/- 35.06% -/G 47.55% G/G 17.39%	-/- 17.48% -/G 52.02% G/G 30.50%	G/G versus -/-:3.52(2.99-4.13) -/G versus -/-:2.19(1.91-2.51)	<0.0001

likelihood of first VTE and VTE recurrence increased with the increased finding of risk alleles. Mutation in the gene for coagulation Factor V (*F5* Leiden, rs6025) is associated with resistance to activated protein C (APC). APC limits clot formation during normal hemostasis by proteolytic inactivation of Factors Va.[33] Therefore, the mutated coagulation Factor FV Leiden is less effectively degraded by APC than standard coagulation Factor V after activation, leading to increased thrombin formation and hypercoagulable state. In the control group, a similar frequency of the *F5* Leiden heterozygotes (8.15%) was found as in the previously published prevalence of the *F5* Leiden heterozygotes (8.91%) in the Czech population.[34] However, the *F5* Leiden heterozygotes in patients with VTE were found to be 32.2%. This means that *F5* Leiden plays a significant role in the etiopathogenesis of VTE, which is consistent with the preventive measures currently being carried out in the Czech Republic when genetic testing is indicated.[35] Mutation of the *F2* gene G20210A (rs1799963) is associated with increased prothrombin levels and the risk of venous thrombosis.[36] Variant 20210 A has a more effective poly (A) site, leading to increased mRNA and protein expression of prothrombin regardless of promoter and gene.[37] In our cohort of patients with VTE, a higher frequency of heterozygous variant (8.21%) of *F2* was recorded compared to 2.24% in the control group ($p < 0.001$). The homozygous variant of *F2* is rare, with a frequency of 0.04% in the control group and 0.15% in the group of individuals with a history of VTE. However, the OR finding for the homozygous variant of *F2* was insignificant compared to the control and VTE patient groups. SNP rs2066865 (fibrinogen gamma', FGG, 10034 C/T) is associated with reduced levels of fibrinogen gamma' and a reduced ratio of fibrinogen gamma' to total fibrinogen. This reduced level of fibrinogen gamma' and increased level of total fibrinogen are associated with an increased risk of venous thrombosis.[38,39] According to the results of our study, the association between VTE and *FGG* mutation is also demonstrated in the Czech Republic. Another SNP, rs2289252, in the *F11* gene, is independently associated with VTE.[40] In our study, after statistical analysis, we confirmed this finding. Higher levels of the coagulation Factor XI antigen and its activity have been reported in VTE patients with homozygous and heterozygous rs2289252 variants.[41] Therefore, higher levels of coagulation Factor XI will also be found in our VTE patients if they are carriers of rs2289252.An important outcome of our study is that up to 16.35% of homozygous *F11* variant rs2289252 was observed even in the control group. The importance of increased coagulation Factor XI levels as an etiological cause of VTE and arterial thrombosis is now widely discussed, particularly after the discovery of new anticoa-gulants, Factor XI inhibitors. These inhibitors have been reported to have a more favourable effect (less bleeding) than treatment with other oral anticoagulation therapy.[42]

The *ABO* genotype ("non-O") allows for better differentiation of heterozygous and homozygous variants than serological determination of ABO blood groups. Rs8176719 represents a site in the *ABO* gene, designated as c.261delG, and is a key SNP in determining blood type O.[43] An allele that encodes blood type A or B will have the G allele at this site. If a deletion has occurred at this site, which completely removed this nucleotide, the corresponding allele is considered rs8176719 (-) and encodes the most common blood type O allele. The ABO blood group ("non-O") is now commonly considered a risk factor for VTE.[44] We also found a significant increase in "non-O" in our group of patients with VTE. Rs8176719 is one of the top polymorphisms associated with increased plasma levels of coagulation Factor VIII and its carrier von Willebrand Factor.[45] These elevated plasma levels of Factor VIII[46] and von Willebrand Factor[47] have previously been described as a risk factor for VTE.

A similar epidemiological study of the occurrence of mutations in *FGG, F11* and *ABO*, "non- O" in patients with VTE compared to their occurrence in a control group has not yet been published in the Czech Republic. However, the impact of our study is limited by the fact that the occurrence of VTE was determined in the nationwide Czech post-Monica study based only on self-reported data from a completed questionnaire, without objective verification.

Therefore, it would be helpful to expand the study on the significance of 5 SNP in the *F5, F2*, *FGG, F11*, and *ABO* genes in a prospective study. It would also be helpful to evaluate the significance of their combined occurrence of risk alleles and the determination of the so-called weighted risk score. Using this score, the risk of VTE in Caucasians increased 1.41 times (95% CI 1.27-1.56) with each additional risk allele.[48]

Conclusion

In addition to *F5* Leiden and prothrombin mutation, the study indicates that the gene polymorphisms of *FGG* (rs2066865), *F11* (rs2289252), and *ABO* (rs8176719) are independent heritable risk factors for VTE in the Czech population.

Addendum: All authors contributed to the critical revision of the manuscript and approved the final version. In addition, Tomas Kvasnicka contributed to concept and design study and drafted the manuscript; Kovarova-Kudrnova Zuzana and Brzezkova Radka participated in the study design and performed the classification of VTE cases; Renata Cifkova classified control cases; Daniela Duskova classified control cases; Petra Bobcikova and Alena Syruckova analysed the SNPs; Martin Sevcik and Zuzana Zenahlikova provided statistical advice and Jan Kvasnicka contributed to concept and design study.

Acknowledgements

The authors thank the staff and participants of the study for their important contributions. This study was supported by grant MH CZ DRO VFN 64 165 (given by the Czech Ministry of Health).

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Grant MH CZ DRO VFN 64 165 (given by the Czech Ministry of Health,

ORCID iD

Tomas Kvasnicka https://orcid.org/0000-0001-9546-3750

References

1. Cohen AT, Agnelli G, Anderson FA, et al. Venous thromboembolism (VTE) in Europe. The number of VTE events and associated morbidity and mortality. *Thromb Haemost*. 2007;98:756–764.
2. Heit JA, Spencer FA, White RH. The epidemiology of venous thromboembolism. *J Thromb Thrombolysis*. 2016;41:3–14.
3. ISTH Steering Committee for World Thrombosis Day. Thrombosis: A major contributor to the global disease burden. *J Thromb Haemost*. 2014;12:1580–1590.
4. Bell EJ, Lutsey PL, Basu S, et al. Lifetime risk of venous thromboembolism in two cohort studies. *Am J Med*. 2016;129(339): e19–e26.
5. Heit JA, Ashrani A, Crusan DJ, McBane RD, Petterson TM, Bailey KR. Reasons for the persistent incidence of venous thromboembolism. *Thromb Haemost*. 2017;117:390–400.
6. Farrell JJ, Sutter C, Tavri S, Patel I. Incidence, and interventions for post-thrombotic syndrome. *Cardiovasc Diagn Ther*. 2016;6: 623–631.
7. Konstantinides SV, Meyer G, Becattini C, et al. ESC Guidelines for the diagnosis and management of acute pulmonary embolism developed in collaboration with the European respiratory society (ERS). *Eur Heart J*. 2019;2020(41):543–603.
8. Jansa P, Ambrož D, Kuhn M, et al. Epidemiology of chronic thromboembolic pulmonary hypertension (CTEPH) in the Czech Republic. *Pulm Circ*. 2022;12(1):e12038.
9. Klok FA, Ageno W, Ay C, et al. Optimal follow-up after acute pulmonary embolism: A position paper of the European Society of Cardiology working group on pulmonary circulation and right ventricular function, in collaboration with the European Society of Cardiology working group on atherosclerosis and vascular biology, endorsed by the European respiratory society. *Eur Heart J*. 2022;43:183–189.
10. Kyrle PA, Kammer M, Eischer L, et al. The long-term recurrence risk of patients with unprovoked venous thromboembolism: An observational cohort study. *J Thromb Haemost*. 2016;14: 2402–2409.
11. Jiménez D, Díaz G, Marín E, Vidal R, Sueiro A, Yusen RD. The risk of recurrent venous thromboembolism in patients with unprovoked symptomatic deep vein thrombosis and asymptomatic pulmonary embolism. *Thromb Haemost*. 2006;95:562–566.
12. Kearon C. Extended anticoagulation for unprovoked venous thromboembolism: A majority of patients should be treated. *J Thromb Thrombolysis*. 2011;31:295–300.
13. Barco S, Corti M, Trinchero A, et al. Survival and recurrent venous thromboembolism in patients with first proximal or isolated distal deep vein thrombosis and no pulmonary embolism. *J Thromb Haemost*. 2017;15:1436–1442.
14. Rosendaal F. Venous thrombosis: A multicausal disease. *Lancet* 1999;353(9159):1167–1173.
15. Zöller B, Svensson PJ, Dahlbäck B, Lind-Hallden C, Hallden C, Elf J. Genetic risk factors for venous thromboembolism. *Expert Rev Hematol*. 2020;13:971–981.
16. de Haan HG, Bezemer ID, Doggen CJ, et al. Multiple SNP testing improves risk prediction of first venous thrombosis. *Blood*. 2012;120:656–663.
17. van Hylckama Vlieg A, Flinterman LE, Bare LA, et al. Genetic variations associated with recurrent venous thrombosis. *Circ Cardiovasc Genet*. 2014;7:806–813.
18. Klarin D, Busenkell E, Judy R, et al. Genome- wide association analysis of venous thromboembolism identifies new risk loci and genetic overlap with arterial vascular disease. *Nat Genet*. 2019;51:1574–1579.
19. Lindström S, Wang L, Smith EN, et al. Genomic and transcriptomic association studies identify 16 novel susceptibility loci for venous thromboembolism. *Blood*. 2019;134:1645–1657.
20. Thibord F, Klarin D, Brody JA, et al. Cross-ancestry investigation of venous thromboembolism genomic predictors. *Circulation*. 2022;146:1225–1242.
21. Ghouse J, Tragante V, Ahlberg G, et al. Genome-wide meta-analysis identifies 93 risk loci and enables risk prediction equivalent to monogenic forms of venous thromboembolism. *Nat Genet*. 2023;55(3):399–409.
22. Folsom AR, Tang W, Hong CP, et al. Prediction of venous thromboembolism incidence in the general adult population using two published genetic risk scores. *PLoS One*. 2023;18(1):e0280657.
23. Tang W, Teichert M, Chasman DI, et al. A genome-wide association study for venous thromboembolism: The extended cohorts for heart and aging research in genomic epidemiology (CHARGE) consortium. *Genet Epidemiol*. 2013;37:512–521.
24. Mannucci PM, Franchini M. Classic thrombophilic gene variants. *Thromb Haemost*. 2015;114:885–889.
25. Zhang Z, Li H, Weng H, et al. China Pulmonary thromboembolism REgistry study (CURES) investigators. Genome-wide association analyses identified novel susceptibility loci for pulmonary embolism among han Chinese population. *BMC Med*. 2023; 21(1):153.
26. Liu C, Hou J, Li W, et al. Construction and optimization of a polygenic risk model for venous thromboembolism in the Chinese population. *J Vasc Surg Venous Lymphat Disord*. 2024;12(1): 101666.
27. Cífková R, Bruthans J, Wohlfahrt P, et al. 30 - year trends in major cardiovascular risk factors in the Czech population, Czech MONICA and Czech post-MONICA, 1985-2016/17. *PLoS One*. 2020;15:e0232845.
28. Rinde LB, Morelli VM, Småbrekke B, et al. Effect of prothrombotic genotypes on the risk of venous thromboembolism in patients with and without ischemic stroke. The tromsø study. *J Thromb Haemost*. 2019;17:749–758.
29. Skille H, Paulsen B, Hveem K, et al. Combined effects of five prothrombotic genotypes and cancer on the risk of a first venous thromboembolic event. *J Thromb Haemost*. 2020;18:2861–2869.

30. Kvasnička J, Jansa P, Cífková R, et al. The incidence of the thrombophilic SNPs rs6025, rs1799963, rs2066865, rs2289252, and rs8176719 in chronic thromboembolic pulmonary hypertension. *Clin Appl Thromb Hemost*. 2024;30:10760296241271369.
31. Bare LA, de Haan HG, Arellano AR, Tong C H, Devlin JJ, Rosendaal FR. A simple genetic thrombosis score of five single nucleotide polymorphisms is associated with risk of first venous thrombosis in pregnant women. *Blood*. 2013;122(21):3617.
32. Trégouët DA, Heath S, Saut N, et al. Common susceptibility alleles are unlikely to contribute as strongly as the FV and ABO loci to VTE risk: Results from a GWAS approach. *Blood*. 2009; 113:5298–5303.
33. Bertina RM, Koeleman BP, Koster T, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature*. 1994;369(6475):64–67.
34. Kvasnicka T, Hajkova J, Bobcikova P, et al. The frequencies of six important thrombophilic mutations in a population of the Czech Republic. *Physiol Res*. 2014;63:245–253.
35. Kvasnicka J. Hereditary thrombophilias-recommendations for genetic testing in the clinical praxis. *Cas Lek Cesk*. 2010;149: 468–471.
36. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'- untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood*. 1996;88:3698–3703.
37. Ceelie H, Spaargaren-van Riel CC, Bertina RM, Vos HL. G20210a is a functional mutation in the prothrombin gene; effect on protein levels and 3'-end formation. *J Thromb Haemost*. 2004;2:119–127.
38. Uitte de Willige S, de Visser MC, Houwing-Duistermaat JJ, Rosendaal FR, Vos HL, Bertina RM. Genetic variation in the fibrinogen gamma gene increases the risk for deep venous thrombosis by reducing plasma fibrinogen gamma' levels. *Blood*. 2005;106:4176–4183.
39. Uitte de Willige S, Rietveld IM, De Visser MC, Vos HL, Bertina RM. Polymorphism 10034 C> T is located in a region regulating polyadenylation of FGG transcripts and influences the fibrinogen gamma'/gammaA mRNA ratio. *J Thromb Haemost*. 2007; 5:1243–1249.
40. Li Y, Bezemer ID, Rowland CM, et al. Genetic variants associated with deep vein thrombosis: The F11 locus. *J Thromb Haemost*. 2009;7:1802–1808.
41. Lunghi B, Cini M, Legnani C, Bernardi F, Marchetti G. The F11 rs2289252 polymorphism is associated with FXI activity levels and APTT ratio in women with thrombosis. *Thromb Res*. 2012; 130:563–564.
42. Gailani D, Gruber A. Targeting factor XI and factor XIa to prevent thrombosis. *Blood*. 2024;143:1465–1475.
43. Yamamoto F, Clausen H, White T, Marken J, Hakomori S. Molecular genetic basis of the histo-blood group ABO system. *Nature*. 1990;345(6272):229–233.
44. Groot HE, Villegas Sierra LE, Said MA, Lipsic E, Karper JC, van der Harst P. Genetically determined ABO blood group and its associations with health and disease. *Arterioscler Thromb Vasc Biol*. 2020;40:830–838.
45. Sabater-Lleal M, Huffman JE, de Vries PS, et al. Genome-wide association transethnic meta-analyses identifies novel associations regulating coagulation factor VIII and von willebrand factor plasma levels. *Circulation*. 2019;139:620–635.
46. Michels A, Lillicrap D, Yacob M. Role of von willebrand factor in venous thromboembolic disease. *JVS Vasc Sci*. 2021;3:17–29.
47. Kyrle PA, Minar E, Hirschl M, et al. High plasma levels of factor VIII and the risk of recurrent venous thromboembolism. *N Engl J Med*. 2000;343:457–462.
48. Folsom AR, Tang W, Weng LC, et al. Replication of a genetic risk score for venous thromboembolism in whites but not in African Americans. *J Thromb Haemost*. 2016;14:83–88.