Understanding hypercoagulability: determinants and impact on cardiometabolic disease

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Chapter 3

Association between venous thromboembolism-associated genetic variants, coagulation factor levels, and thrombin generation potential

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EJHaem. 2024 Feb 18;5(1):47-54.

Abstract

Recently three large meta-analyses of genome-wide association studies for venous thromboembolism (VTE) identified over 130 genetic variants. However, mechanisms by which newly identified and therefore underexplored VTE-associated genetic variants influence VTE remain unclear. To elucidate the mechanism, we investigated the association between 61 newly identified VTE-associated genetic variants and the levels of coagulation factor (F) VIII, FIX, FXI, and fibrinogen as well as thrombin generation parameters (lag time, peak, endogenous thrombin potential, time-to-peak, and velocity), which are well-known biological traits associated with VTE. This study was conducted on 5341 participants of the Netherlands Epidemiology of Obesity study. The associations between VTE-associated genetic variants and coagulation factor levels and thrombin generation parameters were examined using linear regression analyses, adjusted for age, sex, body mass index, oral contraceptive use, hormone replacement therapy, and menopausal status. Of 61 genetic variants, 33 were associated with one or more of coagulation factor levels and thrombin generation parameters. Following multiple testing corrections, five genetic variants remained significant, of which MAP1A rs55707100 exhibited the most robust association with thrombin generation parameters and FXI levels (β=-5.33%, 95%CI: -8.44, -2.22). Our findings shed light on the underlying mechanisms by which these genetic variants influence the risk of VTE.

***Keywords*:** Venous thromboembolism, Genetic variant, Procoagulant factor, Thrombin generation potential, the Netherlands Epidemiology of Obesity study

Introduction

Venous thromboembolism (VTE) is a multicausal disease influenced by acquired and genetic risk factors. Genome-wide association studies (GWAS) have identified genetic variants associated with VTE (1-6). Recently, three large GWAS meta-analyses identified over 130 genetic loci associated with VTE. In 2019, a GWAS meta-analysis was performed in 30,234 VTE cases and 172,122 controls, which identified 37 novel genetic variants associated with VTE (7). This included 6 loci that were newly identified and replicated in an independent cohort. Klarin and colleagues reported 39 genetic loci from GWAS meta-analysis, of which 22 replicated loci had not previously been reported in VTE (8). In 2022, a cross-ancestry GWAS meta-analysis identified 135 independent genetic loci, of which 34 novel genetic loci were replicated in an independent cohort (9).

Knowledge of associations between novel genetic loci and the coagulation system may lead to a better understanding of biological mechanism by which genetic loci influence VTE risks. However, although in individual GWAS, associations between newly identified genetic variants and intermediate hemostasis phenotypes (fibrinogen, fibrin D-dimer, coagulation factors (F) VII, FVIII, FXI, von Willebrand factor (VWF), tissue plasminogen activator, plasminogen activator inhibitor-1, activated partial thromboplastin time, and prothrombin time) was investigated, functional role of these genetic variants remains to be fully understood (7, 9). To better understand the mechanism, more extensive intermediate hemostasis phenotypes of VTE need to be investigated, e.g., by assessing the association with FIX, which is associated with an increased risk of VTE (10-12).

In addition to individual coagulation factors, to capture dynamics of coagulation cascade, global hemostatic coagulation tests are developed, and thrombin generation assay is one example. The thrombin generation assay measures potential to generate thrombin, which plays a crucial role in response to vascular injury as the major enzyme involved in the hemostatic system. In the thrombin generation assay, coagulation is activated with a small amount of tissue factor after which five parameters of thrombin generation can be assessed: lag time, peak height, endogenous thrombin potential (ETP), time-to-peak, and velocity. An increased ETP and a high peak of thrombin generation have consistently been associated with an increased risk of VTE (13-16).

In this study, we aim to better understand potential biological mechanisms by which yet functionally underexplored genetic variants identified from three recent large GWAS meta-analyses affect VTE risks. We investigated associations between functionally underexplored genetic variants reported in three recent VTE GWAS meta-analyses (7-9) and the levels of FVIII, FIX, FXI, and fibrinogen as well as the five thrombin generation parameters including lag time, peak, time-to-peak, ETP, and velocity measured in the Netherlands Epidemiology of Obesity (NEO) study.

Methods

Study population

The NEO study is a population-based cohort study and included 6671 men and women aged between 45 and 65 years at baseline (2008-2012), with an oversampling of individuals with a self-reported body mass index (BMI) of 27kg/m^2 or higher. The study was approved by the Medical Ethical Committee of the Leiden University Medical Center (LUMC), Leiden, the Netherlands. All participants gave their written informed consent. The study design has been described in detail previously (17). Briefly, people with a BMI of 27kg/m^2 or higher living in

the greater area of Leiden in the west of the Netherlands were invited to participate in the NEO study. Additionally, people living in the neighboring municipality Leiderdorp were invited regardless of BMI, representing a reference distribution of BMI similar to the general Dutch population.

The present study consists of cross-sectional analyses using baseline measurements of the NEO study. Participants were excluded from analyses if 1) they used vitamin K antagonist or heparin, 2) had active cancer, and 3) had non-white ethnicity in self-report (Figure 1). Active cancer patients were defined as those who have been diagnosed within 5 years and have not been medically cured based on self-report. We also excluded participants who did not pass genotype quality control as described earlier (18). We additionally excluded participants with missing data on any needed variables and with outlier values (z-score >5) in outcomes. Finally, this study was performed on 5341 participants.

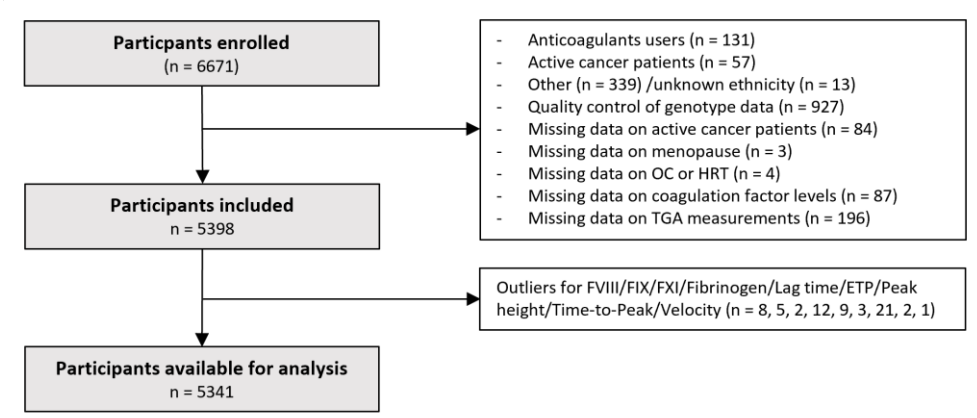

Figure 1. Flow chart of the study. Abbreviations: ETP, endogenous thrombin potential; F, coagulation factor; HRT, hormone replacement therapy; OC, oral contraceptive; TGA, thrombin generation assay.

Outcome measurements

In 5341 participants at baseline, fasting blood samples to measure the levels of coagulation factors and thrombin generation parameters were drawn into tubes containing 0.106M trisodium citrate (Sarstedt, Etten Leur, the Netherlands) and processed within 4 hours without the measurement of residual platelets (17). Tubes were centrifuged for 10 minutes at 2500g at 18°C and aliquoted plasma was stored at -80°C until further use, only after the first time thawing for 3 min at 37°C of a water bath. FVIII, FIX, and FXI activity were measured using a factor-specific clotting assay with ACL TOP 700 analyzer (Werfen, Barcelona, Spain). Fibrinogen levels were measured by the method of Clauss (19). The reproducibility of measurements was evaluated by using the same protocol with fasting blood samples collected from a random subset of 100 participants after three to five months from the first visit. The reproducibility of measurements was presented in a previous publication (20). Thrombin generation was measured using protocols described previously by Hemker *et al* (21): calibrated automated thrombogram (Thrombinoscope BV) (22). Briefly, 20µL of PPP-Reagent LOW (86194, TS31.00; Stago) and thrombin calibrator (86192, TS30.00; Stago) were

dispensed into the wells of a round-bottom, 96-well plate (#3655; Thermo Scientific). A thermostable inhibitor of contact activation (PS-0177-oxoxox; Synapse Research Institute) was added to plasma samples from the participants in the NEO study and to normal pooled plasma (as an internal control for each plate). Then, 80µL of mixed plasma was added to the plate, and the plate was placed in a fluorometer for incubation at 37°C for 10 minutes. Thrombin formation was initiated by adding 20µL of a fluorogenic substrate with calcium (FluCa-kit, 86197, TS 50.00; Stago). The final reaction volume was 120µL. Thrombin formation was determined every 10 seconds for 50 minutes and corrected for the calibrator using the Thrombinoscope software. The inter-assay coefficient variation determined in normal pooled plasma ranged between 5.3% and 17.2% for thrombin generation parameters. Lag time measured in minutes represents the time from induction to the initial thrombin generation. ETP, the area under the thrombin generation curve is a measure of the total amount of thrombin formed during the assay. Peak height measured in nM indicates the highest point of thrombin level. Time-to-peak measured in minutes represents the time from induction to peak. Velocity indicates how fast thrombin levels rise from the initial point of thrombin generation to the peak amount formed.

Selection of VTE-associated genetic variants

As this study aims to better understand biological functions of recently identified and therefore less explored VTE-associated genetic variants, we focused on the single nucleotide polymorphisms (SNPs), which were defined as "novel" findings in three original GWASs published between 2019 and 2022 (7-9). These GWAS meta-analyses reported 58 "novel" genetic variants, i.e., which were newly identified to be associated with VTE risk and were successfully replicated in a different study population in the original publication. Additionally, we included 6 novel variants in articles by Lindström et al (7) and Klarin et al (8), which were not replicated within the original publication but were identified as VTE-associated SNPs in GWAS by Thibord *et al* (9). Therefore, we aimed to assess the phenotype association of 64 VTE-associated SNPs from imputed genotype data in the NEO study.

Detailed information on genotyping and imputation procedure was described elsewhere (18). Briefly, genotyping was conducted using the Illumina HumanCoreExome-24 BeadChip (Illumina Inc., San Diego, California, USA). Because not all genetic variants were directly genotyped, we performed genotype imputation to infer the missing genotypes based on a reference panel from the 1000 Genome project (v3 2011) with IMPUTE (v2.2) software after the quality control. This procedure increased genome coverage and power for the investigation of the association with coagulation factor levels and thrombin generation parameters. After genotype imputation, a total of 29,556,346 genetic variants were included in the imputed genotype data, including 61 out of the 64 VTE-associated SNPs that we aimed to study. Among the studied genetic variants in the current study, 12 were directly genotyped and 49 were imputed genetic variants. Three of the 64 genetic variants (rs2074492, rs35208412, rs142140545) were not covered in the imputed genotype data in the NEO study therefore we analyzed associations between 61 VTE-associated SNPs and the levels of coagulation factors and thrombin generation parameters. An imputation quality score below 0.3 indicates a low accuracy of imputation. For these 61 genetic variants, imputation quality was higher than 0.55 with a median of 0.97 (Supplemental table 1).

Imputed SNPs have uncertainties in inferring their genotypes, i.e., the lower the imputation quality, the higher the chance to deviate from real genotypes. To evaluate potential measurement error from imputed genotype data, we performed TaqMan Assay (Applied Biosystems, Foster City, CA, USA) using a polymerase chain reaction mix (TaqPath™

ProAmp™ Master Mix, Applied Biosystems) for nine SNPs identified by Lindström et al (7). Five of nine genetic variants succeeded to be genotyped. We assessed the correspondence of these five genotypes between TaqMan measures and imputed genotypes and developed a prediction model to infer the misclassification rate of the other imputed variants without TaqMan measures.

Statistical analysis

In the NEO study, participants with a BMI ≥ 27kg/m^2 were oversampled. We first invited individuals aged between 45 and 65 years with self-reported BMI ≥ 27kg/m^2 who lived in Leiden and its surrounding area. In addition, individuals aged between 45 and 65 years living in the neighboring municipality, Leiderdorp, were invited regardless of BMI. A total of 1671 individuals participated in the NEO study from the latter invitation, representing a reference BMI distribution similar to the BMI distribution of the general Dutch population (23). Because of the skewed BMI distribution in the NEO study due to the oversampling of overweight and obese individuals, inferences made on the general population might be biased. To correctly represent associations in the general population, adjustments for the oversampling of individuals with a BMI ≥ 27kg/m^2 have been made (24, 25). This was done by calculating weight factors for the NEO population based on the 1671 participants with a reference BMI distribution. By using these weight factors, we assigned a higher weight to the participants with lower BMI when we estimated the association between genetic variants and coagulation factor levels as well as thrombin generation parameters (26). Consequently, the results of the present study applied to a population-based study without oversampling of individuals with a BMI ≥ 27kg/m^2.

We assessed distributions for the levels of coagulation factors and thrombin generation parameters. All were normally distributed except thrombin generation velocity. We applied natural logarithm transformation to obtain normal distribution for thrombin generation velocity. Linear regression analysis was used to estimate the regression coefficient (β) with 95% confidence intervals (CI) for the association between genetic variants and the levels of coagulation factors and thrombin generation parameters under an additive genetic model and adjusted for age, sex, body mass index, oral contraceptive use, hormone replacement therapy, and menopausal status. We considered a minor allele as an effect allele in the analysis. As lag time and time-to-peak are negatively associated with VTE risks, a reversed linear regression analysis was performed for these two outcomes. Due to natural logarithm transformations applied to velocity, we exponentiated the obtained coefficients and 95% CI, subtracted one from the values, and multiplied by 100 to report the results in Table 2, Table 3, and Supplemental Tables 2 and 3. The results are interpreted as the percent increase or decrease of velocity per allele. We employed a false discovery rate (FDR) for multiple testing correction with q-value of 0.1 as significant threshold. To assess the impact of potential misclassification on the estimated effect sizes, we replicated the analyses with the genotype data obtained from the TaqMan assay after adjustment for the same confounders. All statistical analyses were conducted in R version 4.2.1.

Results

Participant characteristics

Table 1 describes the baseline characteristics of 5341 study participants as proportion for categorical variables and mean with standard deviation (SD) or median with interquartile range for continuous variables. The mean (SD) age of participants was 56 (6) years. The study

population consisted of 56% women, of whom 83% were peri- or postmenopausal and 6.2% and 3.2% were taking oral contraceptives or hormone replacement therapy, respectively.

Association between VTE-associated genetic variants and coagulation factor levels and thrombin generation parameters

Supplemental Table 1 presents the characteristics of genetic variants included. Associations between 61 genetic variants and the levels of coagulation factors and thrombin generation parameters are shown in Supplemental Table 2 with the crude model and Supplemental Table 3 with the adjusted model. Of 61 genetic variants, 33 were associated with one or more coagulation factor levels and thrombin generation parameters, of which five genetic variants remained after FDR corrections (Table 2 and Table 3).

Among five variants, *STXBP5* rs7739314, *VWF* rs216296, and *MAP1A* rs55707100 were associated with phenotypes with a q-value of FDR <0.05. *STXBP5* rs7739314 (β = -3.45%, 95% CI: -5.13, -1.77) and *VWF* rs216296 (β = -6.05%, 95% CI: -8.79, -3.31) were associated with FVIII levels. *MAP1A* rs55707100 was associated with ETP (β = -120.31nM*min, 95% CI: -180.38, -60.24), peak height (β = -10.73nM, 95% CI: -15.77, -5.70), and velocity (β = -15.00%, 95% CI: -22.14, -7.21). Additionally, we observed that *MAP1A* rs55707100 (β = -5.33%, 95% CI: -8.44, -2.22) and *ABCA6* rs77542162 (β = 5.18%, 95% CI: 2.05, 8.31) were associated with FXI levels with a q-value of FDR <0.1. *SBNO1* rs12824685 was associated with FIX levels (β = -1.97%, 95% CI: -3.13, -0.80) with a q-value of FDR <0.1. Direction of these associations aligned with the effect direction observed in associations between these genetic variants and VTE risks.

For 56 genetic variants without TaqMan measures, the predicted misclassification rate ranged between 1.54 to 17.86% with a median of 2.69% (Supplemental Table 1). Supplemental Table 4 describes associations between genotyped variants obtained by TaqMan assay and the levels of coagulation factors and thrombin generation parameters showing only minor changes from the main observations.

Table 1. Characteristics of the study population

	Study population (n = 5341)
Age (y)	56 (6)
Sex (% women)	56
Menopause status (% peri or post)	82.50
Oral contraceptive user (%)	6.20
Hormone replacement therapy (%)	3.19
Body mass index	29.4 (27.3 – 32.1)
FVIII activity (%)	120.87 (30.87)
FIX activity (%)	116.08 (19.07)
FXI activity (%)	116.46 (19.44)
Fibrinogen (mg/dL)	291.18 (55.50)
Lag time (min)	7.08 (1.55)
ETP (nM*min)	1077.16 (354.72)
Peak height (nM)	79.49 (32.95)
Time-to-Peak (min)	14.91 (2.05)
Velocity (nM/min)	9.42 (6.70 - 13.27)

Results are based on analyses weighted toward the BMI distribution of the general population. Values are mean with standard deviation, median with interquartile ranges or percentages. The values of menopause, oral contraceptive user, and hormone replacement therapy were calculated in women. Abbreviations: ETP, endogenous thrombin potential.

Table 2. VTE-associated genetic variants associated with thrombin generation parameters

Chr	rsID	Gene	Lag time	ETP	Peak	Time-to-peak	Velocity
6	rs7739314	*STXBP5*	-0.01 (-0.09, 0.08)	-14.5 (-31.6, 2.6)	-1.73 (-3.34, -0.13)	-0.08 (-0.18, 0.03)	-3.63 (-6.13, -1.06)
12	rs216296	*VWF*	-0.04 (-0.18, 0.10)	-15.2 (-47.6, 17.2)	-3.32 (-6.06, -0.58)	-0.23 (-0.41, -0.04)	-6.44 (-10.66, -2.01)
12	rs12824685	*SBNO1*	0.03 (-0.08, 0.14)	-5.5 (-28.1, 17.0)	-1.68 (-3.72, 0.36)	-0.02 (-0.16, 0.12)	-2.71 (-5.93, 0.62)
15	rs55707100	*MAP1A*	-0.50 (-0.84, -0.17)	-120.3 (-180.4, -60.2)	-10.73 (-15.77, -5.70)	-0.63 (-1.05, -0.21)	-15.00 (-22.14, -7.21)
17	rs77542162	*ABCA6*	-0.16 (-0.42, 0.10)	5.2 (-53.8, 64.1)	1.98 (-3.41, 7.38)	-0.03 (-0.36, 0.31)	2.51 (-6.29, 12.14)

Table 3. VTE-associated genetic variants associated with coagulation factor levels

Chr	rsID	Gene	FVIII	FIX	FXI	Fibrinogen
6	rs7739314	*STXBP5*	-3.45 (-5.13, -1.77)	-0.52 (-1.45, 0.42)	-0.26 (-1.30, 0.79)	0.86 (-2.06, 3.79)
12	rs216296	*VWF*	-6.05 (-8.79, -3.31)	-0.05 (-1.67, 1.56)	-1.04 (-2.86, 0.78)	4.04 (-1.49, 9.57)
12	rs12824685	*SBNO1*	-0.47 (-2.53, 1.59)	-1.97 (-3.13, -0.80)	-1.08 (-2.28, 0.12)	-0.17 (-3.95, 3.61)
15	rs55707100	*MAP1A*	-4.11 (-9.23, 1.02)	1.35 (-1.73, 4.44)	-5.33 (-8.44, -2.22)	-4.63 (-13.59, 4.32)
17	rs77542162	*ABCA6*	4.45 (-1.00, 9.90)	4.34 (1.54, 7.13)	5.18 (2.05, 8.31)	5.98 (-4.31, 16.27)

Results are based on analyses weighted toward the BMI distribution of the general population (n = 5341). Values are reported as regression coefficients with 95% confidence intervals. Analyses are adjusted for age, sex, body mass index, menopausal status, oral contraceptive use, and hormone replacement therapy. Abbreviations: VTE, venous thromboembolism; Chr, chromosome.

Discussion

We studied the association between 61 recently described VTE-associated genetic variants, which were functionally not yet well characterized, and the levels of coagulation factors and thrombin generation parameters. We observed that 33 genetic variants were associated with one or more of coagulation factor levels and thrombin generation parameters. Following multiple testing corrections, we identified five genetic variants with robust associations with one or more of these outcomes.

Intermediate phenotypes for clinical endpoints serve as indicators for understanding how disease-associated genetic variants influence the risk of disease. The coagulation system represents intermediate phenotypes for VTE risk with evidence that individuals with elevated levels of coagulation factors have an increased risk of VTE. Individual coagulation factors (i.e., FVIII, FXI and fibrinogen), therefore, have been used to study the mechanism through which genetic variants identified by GWAS for VTE mediate their effects (7, 9). We replicated all associations between genetic variants and FVIII and FXI identified in previous studies while we observed an association between *ARID4A* rs11158204 and fibrinogen levels among associations between genetic variants and fibrinogen. However, fibrinogen-associated genetic variants except for *PLCE1* rs2274224 in previous studies were associated with other coagulation factor levels and thrombin generation parameters in the current study. In addition to three coagulation factors, we further examined the association between functionally underexplored VTE-associated genetic variants and FIX levels and thrombin generation potential which represents global coagulation levels. Following multiple testing corrections, we identified five robust associations between genetic variants (i.e., *STXBP5* rs7739314, *VWF* rs216296, *SBNO1* rs12824685, *ABCA6* rs77542162, and *MAP1A* rs55707100) and coagulation factor levels and thrombin generation parameters. Our findings provide insight into the mechanisms by which these genetic variants influence VTE risks, underscoring the role of the coagulation system in VTE pathogenesis.

In the present study *MAP1A* rs55707100 was associated with thrombin generation ETP, peak, and velocity as well as FXI levels. *MAP1A* encodes microtubule-binding associated protein which plays a role in microtubule assembly. Microtubule is essential for various cellular processes such as cell division and maintenance. The genetic variant was associated with platelet count, triglycerides levels, high density lipoprotein cholesterol levels, and C-reactive protein levels (27-29). In addition, our results showed that these genetic variants is associated with coagulation system which likely explain the genetic association with VTE risks. However, the mechanism underlying the association between *MAP1A* rs55707100, thrombin generation parameters and FXI levels remains to be clarified yet.

We also identified that *STXBP5* rs7739314 and *VWF* rs216296 were associated with FVIII levels. These variants are correlated with *STXBP5* rs9390460 and *VWF* rs11064010, respectively, which were previously identified in a GWAS for FVIII and VWF plasma levels (30). In the coagulation system, VWF serves as a carrier for FVIII, protecting FVIII from proteolysis. *STXBP5* encodes a syntaxin 1 binding protein which plays a role in inhibiting endothelial release of VWF (31). Experimental evidence showed that decreased expression of STXBP5 by siRNA against *STXBP5* in vitro led to increased VWF release (31). Furthermore, mice with *Stxbp5* deficiency showed increased plasma VWF levels (31). Overall, our findings indicate that *STXBP5* rs7739314 and *VWF* rs216296 influence FVIII levels by regulating VWF, which in turn contributes to VTE risks. Further studies are needed to establish the validity of these assumptions.

Besides, we showed the association between *SBNO1* rs12824685 and FIX levels. Protein encoded by *SBNO1* are suggested to play a role in regulation of transcription. Previous studies suggested that SBNO1 is associated with testicular development via Wnt signaling pathway in mice (32, 33). Moreover, Takano *et al.* showed that knockdown of *sbno1* resulted in abnormal brain development in zebrafish (34). *SBNO1* rs12824685 was also identified to be associated with brain morphology via GWAS (35, 36). Nevertheless, no evidence has been reported to explain the detailed role and function of *SBNO1* rs12824685 for FIX levels and coagulation cascades.

Additionally, we observed an association between *ABCA6* rs77542162 and FXI levels. *ABCA6* encodes membrane-associated protein that belongs to the ATP binding cassette A transporter superfamily. Notably, *ABCA6* rs77542162 was associated with total and low-density lipoprotein (LDL) cholesterol (37). Elevated total and LDL cholesterol levels were associated with increased FXI activity (38) whereas the use of lipid-lowering drugs resulted in decreased levels of FXI (39-41). Proteomics analysis indicated associations between proteins involved in lipid metabolism and FXI activity in patients with VTE at 12 months follow-up (42). Lowering FXI activity was associated with improved fibrin clot permeability, which indicated increased susceptibility to clot lysis in coronary artery disease (41). Decreased FXI levels reduced macrophage accumulation and improved the early-stage thrombus resolution in mice (43). Overall, the association between *ABCA6* rs77542162 and FXI levels may be mediated through lipid metabolism, which may influence VTE risks. The complex interplay between lipid metabolism, FXI levels, and thrombosis warrants further investigation to better understand the underlying mechanisms and potential clinical implications.

We did not observe a clear association between a large number of newly identified genetic variants associated with VTE and any coagulation factor levels or thrombin generation parameters after multiple testing corrections. The lack of the associations in our study might be explained by the difference in statistical power as most GWASs include more participants. Also, these variants may be associated with other intermediate phenotypes of VTE. For example, other genetic variants in *GRK5* and *SMG6,* but not the variants studied here, have been suggested to involve platelet traits, which are also considered intermediate phenotypes associated with VTE risk (44). Considering the complexity of VTE, further studies should focus on the association between genetic variants and other intermediate phenotypes including blood traits, as well as environmental factors, or epigenetic markers to characterize the biological mechanisms of genetic loci that have no identified functional role associated with coagulation factors and thrombin generation parameters in VTE risks.

This study investigated the association between newly identified VTE-associated genetic variants and individual coagulation factors and thrombin generation parameters in a large population-based cohort study. However, there are several limitations. First, we only focused on white ethnicity. Further studies are warranted to replicate the findings in other populations. Secondly, we used imputed genotype data, which may potentially be affected by measurement error. However, when we estimated the misclassification rate by comparing the genotypes obtained from imputation and genotyping by TaqMan assay, we confirmed that 82 to 98% of the classification was estimated correctly across variants. Also, the results only marginally changed when we replicated the analyses with the genotype data obtained from the TaqMan assay.

In conclusion, among functionally underexplored VTE-associated genetic variants, we identified that five VTE-associated genetic variants were associated with one or more coagulation factor levels or thrombin generation parameters, shedding light on the

underlying mechanisms by which these genetic variants influence the risk of VTE. Future studies should study the role of other VTE-associated variants, which may lay outside the coagulation system. Further research and integration with clinical data will contribute to elucidate the clinical relevance of these genetic variants and their impact on VTE risk.

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