Understanding hypercoagulability: determinants and impact on cardiometabolic disease

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Part I.

In-depth genetic exploration of coagulation parameters and venous thromboembolism

Chapter 2

Genomic science of risk prediction for venous thromboembolic disease: convenient clarification or compounding complexity

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Abstract

Venous thromboembolism (VTE) refers to abnormal blood clots in veins occurring 1-2 in 1000 individuals every year. While anticoagulant treatment can prevent VTE, it increases the risk of bleeding. This emphasizes the importance of identifying individuals with a high risk of VTE and providing prophylactic interventions to these individuals to reduce both VTE and bleeding risks. Current risk assessment of VTE is based on the combination of mainly clinical risk factors. With the identification of an increasing number of genetic variants associated with the risk of VTE, the addition of genetic findings to clinical prediction models can improve risk prediction for VTE. Especially, for individuals in high-risk situations, the added value of genetic findings to clinical prediction models may have benefits such as better prophylaxis of VTE and the reduced side effect of bleeding from unnecessary treatment. Nevertheless, the question of whether these models eventually will have clinical utility remains to be proven. Here, we review the current state of knowledge on genetic risk factors for VTE, explore genetic prediction models for VTE, and discuss their clinical implications and challenges.

Keywords: Venous thromboembolism, Genetics, Review, Prediction, Genetic risk prediction

Introduction

Venous thromboembolism (VTE) is a disease characterized by abnormal formation of blood clots in veins. Manifestations of VTE include deep vein thrombosis (DVT) and pulmonary embolism (PE). VTE occurs in 1-2 per 1000 individuals per year (1-4). Approximately 30% of VTE patients develop recurrent VTE within 10 years after the first VTE (3-5). Treatment with anticoagulants may prevent first and recurrent VTE but comes at an increased risk of bleeding. This highlights the clinical importance of identifying individuals at particularly high risk of VTE and focusing (prophylactic) treatment on these individuals to reduce the risk of both VTE and bleeding.

Current risk assessment of VTE is based on the combination of mainly clinical risk factors. Well-established risk factors for VTE include advanced age, sex, family history, obesity, cancer, oral contraceptive use, hormone replacement therapy, pregnancy, puerperium, immobilization, plaster cast, major surgery, and biochemical risk factors such as elevated coagulation factors VIII, IX, and XI (6). As VTE is a multicausal disease (7), an increasing number of genetic variants have also been identified to be associated with the risk of VTE. Several studies have indicated that genetic risk factors are potentially useful in risk prediction among which the well-known genetic risk factors, i.e., the factor V Leiden (FVL) and the prothrombin 20210A mutation are included (8-11). However, it is unclear whether these models are clinically relevant and under what conditions this holds.

In this review, we summarize the current state of knowledge on genetic risk factors for VTE. In addition, we review genetic prediction models for VTE and discuss clinical implications and current challenges in applying genetics to improve risk prediction of VTE.

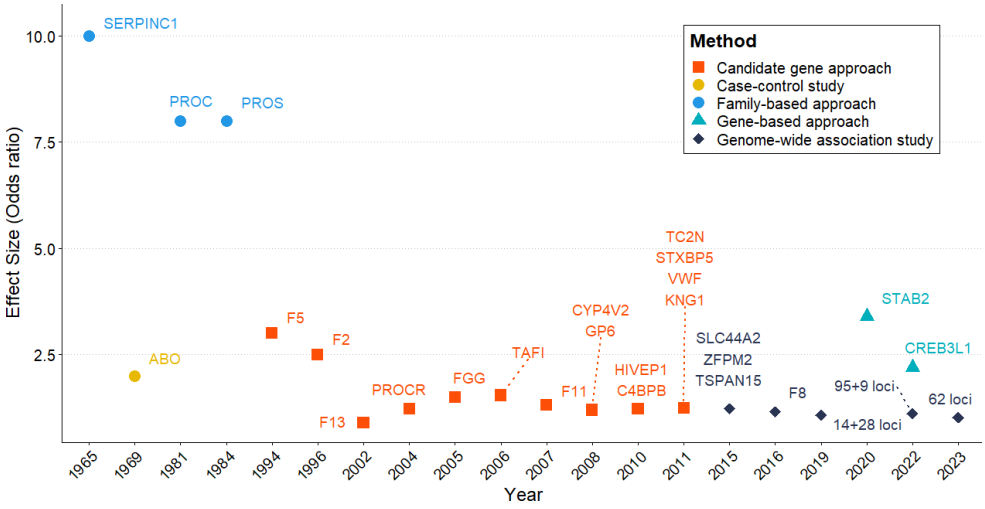

Figure. History of genetic study for the first venous thromboembolism. Only novel loci are reported. The average effect size of genetic variants is provided if multiple variants are identified in a year [17–25,33,36,37,39–42,45,50–56,59,60]

Genetic and genomic studies for VTE

Genetics account for a substantial part of VTE risk. When the heritability was estimated based on common variants with over 5% of minor allele frequency only, it was reported that nearly one-third of VTE is driven by genetics (12). However, familial studies showed that 40 to 60% of VTE risk can be attributed to genetic factors, which shows that also rare variants contribute to the heritability of the disease (13-16). To identify genetic factors associated with VTE, many studies explored the genetic architecture of VTE, with various approaches from family-based analysis to next-generation sequencing (Figure).

From family-based approach to candidate gene approach

The first studies reporting on genetic risk factors for VTE were performed in families with a high incidence of VTE at young age. In these studies, researchers identified genetic loci causing a deficiency of natural anticoagulants i.e., antithrombin, protein C, and protein S in 1965, 1981, and 1984, respectively, which were strongly associated with the risk of VTE with relative risks ranging between 8 and 12 (17-19). These mutations are rare in the general population, with allele frequencies less than 1%. Furthermore, the relative risks associated with these mutations were much lower outside of these thrombophilic families (20-23), which suggested that these deficiencies in thrombophilic families interacted with other genetic risk factors (24). For instance, individuals with both protein C deficiency and FVL had an increased risk of VTE compared with individuals with only one of these risk factors (25). This was also shown for protein S deficiency, which effect was substantially attenuated when FVL was taken into account (26).

In addition to these rare variants with strong effects on VTE, relatively common variants with moderate effects on VTE were also identified early. In 1969, ABO blood type was identified to be associated with VTE risk in a case-control study (27). Individuals with non-O blood type have an increased risk of VTE compared with individuals with O blood type. Two additional genetic variants were discovered via candidate gene approach in the 1990s. In 1993, activated protein C resistance was identified as a risk factor for VTE (28, 29). One year later, researchers showed that a mutation in the gene of coagulation *F5* (rs6025, FVL) was the underlying genetic cause of resistance to activated protein C (30). In 1996, prothrombin 20210A (rs1799963) was identified, resulting in increased levels of prothrombin (31). All these genetic variants are relatively common in the general population and are defined as moderate risk factors for VTE (30-32).

Similar to FVL and prothrombin 20210A, by targeting the genes involved in hemostasis, researchers further identified genetic variants associated with VTE. *F13* rs5985 (33), *PROCR* rs867186 (34), *FGG* rs2066865 (35), *F5* rs4524, *F11* rs2289252, *PROC* rs5937 (36), and *ABO* rs2519093 (37) were associated with VTE risk with relative risk estimates between 1 and 2. However, the findings from a candidate gene approach were often hard to replicate (38). Also, the method only applied to genes that were known or likely to play a role in disease etiology. The intricate nature of VTE pathogenesis, coupled with our incomplete understanding, catalyzed researchers to explore genes across the entire genome, rather than solely focusing on genes directly implicated in VTE pathogenesis.

Genome-wide association study

Genome-wide association studies (GWAS) enable researchers to examine millions of variants throughout the genome to search for novel genetic risk factors for VTE (Table 1). On the road to GWAS for VTE, the first study resembling a GWAS was conducted in 2008. Bezemer and

colleagues investigated the associations between 19,682 single nucleotide polymorphisms (SNP) in 10,887 genes and the risk of DVT (39). They identified 3 variants (*CYP4V2* rs13146272, *SERPINC1* rs2227589, and *GP6* rs1613662). The first GWAS for VTE without a prior hypothesis was performed in 2009 (40). In 419 cases and 1228 controls, researchers found three genetic variants associated with VTE (*F5* rs2420371, *ABO* rs505922, and *ABO* rs657152) across 291,872 SNPs tested. However, none of the associations remained after adjustment for FVL and SNPs tagging ABO blood group (i.e., A2 rs8176750, B rs8176746, and O rs8176719). In subsequent years, additional GWASs identified several genetic variants, but no novel variants were identified after adjustment for known variants (41-43).

Table 1. Overview of genetic variants associated with first venous thromboembolism and reported in at least three studies.

			Reference											
Ch	**Gene**	**SNP**	**45**	**46**	**47**	**48**	**49**	**12**	**50**	**51**	**52**	**53**	**54**	**Ct**
1	*F5*	rs6025			3.6	3.3	2.9	3.5	2.4	3.0	3.0	3.0	2.3	9
11	*F2*	rs1799963				2.3	0.5	2.6		2.3	2.1	2.2	1.7	7
4	*FGG*	rs2066865		N		1.2		1.2		1.3		1.2		5
10	*GRK5*	rs10886430							1.1	1.2	0.9	1.1	0.9	5
10	*TSPAN15*	rs78707713				1.3			0.8	1.3		0.8	1.2	5
17	*SMG6*	rs1048483							1.1	1.1		1.1	1.1	4
1	*C4BPA*	rs2842700							1.1	1.1	1.1			3
2	*PLEK*	rs1867312							1.1	1.1			1.0	3
4	*FGG*	rs2066864							1.2		1.2		1.2	3
7	*JAZF1-AS1*	rs1513275									1.1	0.9	1.0	3
8	*SCARA5*	rs10087301								1.1		0.9	1.1	3
8	*ZFPM2*	rs6993770									1.1	0.9	1.1	3
9	*ABO*	rs505922	1.9	N							0.7			3
9	*ABO*	rs2519093			1.7			1.4	1.4					3
13	*F10*	rs3211752								1.1	1.0		0.9	3
16	*PLCG2*	rs12445050							1.1	1.2			1.1	3
23	*F9*	rs6048								1.1	1.1	1.0		3

Genetic variants are ranked high to low according to the count reported. Values represent effect estimates mentioned in the original literature. Abbreviations: Chr, chromosome; SNP, single nucleotide polymorphism; Ct, count

With the increased genomic coverage from 1000 genome reference panel and collaborative initiatives, additional VTE-associated genetic variants were identified via GWAS. In 2015, a GWAS meta-analysis identified 9 loci from 12 cohorts consisting of 7,507 cases and 52,632 controls with 6.7 million SNPs tested (44). Of the 9 loci identified, *TSPAN15* rs78707713, *SLC44A2* rs2288904, and *ZFPM2* rs4602861 were novel loci, of which the first two variants were subsequently replicated. With the same reference panel, two additional GWASs were conducted in 2016 and 2017, respectively, from which *F8* rs114209171 was a novel finding (12, 45). Through an updated version of the genotype imputation reference panel (i.e., 1000 Genomes Project phase 3 v.5) with even more genetic variants covered and increased collaborative efforts, two GWASs in 2019 identified 14 and 28 novel genetic variants associated with VTE, respectively (46, 47).

More recently, cross-ancestry genomic data provided a new opportunity to discover novel genetic risk factors for VTE. In 2022, two cross-ancestry GWAS meta-analyses were performed (48, 49). Thibord *et al.* conducted a discovery analysis in three ethnicities, including 55,330 cases and 1,081,973 controls, followed by replication analysis, combined meta-analysis, and ethnicity-specific analysis. In total, they found 135 loci including 95 novel

loci associated with VTE. The other cross-ancestry GWAS meta-analysis was conducted in 9 studies including six ethnicities in 27,987 cases and 135,290 controls. The study found 29 known and 9 novel loci (49). The most recent GWAS identified 93 genetic loci associated with VTE based on 81,190 cases and 1,419,671 controls from six studies, of which 62 loci were novel findings (50). Future collaborations and advanced genome coverage may allow for the potential discovery of more genetic risk factors for VTE.

Gene-based analysis

Through GWAS we identified many common genetic variants. While these findings add to a better understanding of the role of genetics in VTE, limited statistical power makes investigating rare variants in standard GWAS difficult. As an alternative to GWAS, gene-based analysis has been used to assess the joint effect of multiple rare variants aggregated in a gene on disease risk.

Several gene-based analyses were conducted to detect genetic contributions to the risk of VTE. Lindström and colleagues incorporating 8,332 VTE cases and 16,087 controls of European ancestry, performed a gene-based analysis using exome array genotype data (51). However, no novel gene was identified, which could be due to a lower genetic variant density in the array. Tang *et al.* adopted advanced whole-exome sequencing (WES) for the gene-based analysis of VTE, however, the sample size was limited and only *PROC* was identified (52). Another gene-based analysis was performed on WES data from 393 individuals with VTE and 6114 controls, which pinpointed 4 genes including a novel VTE gene *STAB2* (53). In 2022, three genes associated with DVT were reported through gene-based analysis with UK Biobank WES data from approximately 200,000 participants, namely known genes *F5* and *F2* as well as a novel gene *CREB3L1* (54). In 2023, the first whole genome sequencing analysis was performed in 3793 cases and 7834 controls, in which the authors replicated the association between *PROC* and VTE (55). These findings showed the possibility of detecting novel genetic contributions to VTE through gene-based analysis and the importance of conducting gene-based analysis with data generated from advanced sequencing techniques in a large sample size.

Genetic studies for recurrent VTE

The genetic risk for recurrent VTE is not fully explained by the genetic risk factors for the first VTE. Researchers studied whether the genetic risk factors for a first VTE were also associated with the risk of recurrent VTE (56). Genetic risk factors for a first VTE had relatively smaller effects on the risk of recurrent VTE than on a first VTE. Similarly, two meta-analyses showed that FVL and prothrombin 20210A were associated with recurrent VTE. However, relative risk ratios of 1.46 for FVL and 1.72 for prothrombin 20210A were lower than those for a first VTE (57, 58). Possible explanations for the lower relative risks of recurrence than of a first event are the different absolute risk of a first VTE in the general population and among individuals who had a first VTE (i.e., a scaling effect), and selection of individuals with a first VTE who have risk factors including unknown risk factors. Because of the presence of other (sometimes unknown) risk factors, the relative risk of individual genetic variants among individuals who had a first VTE is lower for the risk of recurrence.

Few studies specifically identified genetic risk factors for recurrent VTE (Table 2). A long GT-repeat in *HMOX1* (rs3074372), *APOM* rs805297 and *SELE* rs5361 were associated with an increased risk of recurrent VTE (59-61). Additionally, the association between 86 genetic variants in 56 genes and recurrent VTE were investigated, in which four genetic variants, i.e.,

CCR2 rs1799864, *MMP3* rs3025058, *PON1* rs662, and *CETP* rs1800775 were associated with the risk of recurrent VTE (62). In 2018, the first GWAS for recurrent VTE was conducted in 447 recurrent VTE cases and 832 first VTE cases, where around 8.6 million variants were tested to be associated with recurrent VTE. The study found associations between FVL and *RP11-638L3.1* rs9946608 at 18q22.1 and recurrent VTE (63). In addition, the study replicated their findings in combined data from three cohorts with 350 first VTE cases, showing that *RP11-638L3.1* rs9946608 was associated with recurrent VTE (63). To our knowledge, there has been no gene-based analysis for recurrent VTE. Since with increased sample sizes, expanded genome coverage, and cross-ancestry studies the likelihood rose to discover novel variants from GWAS for a first VTE, further studies with an improved study setting may also provide opportunities to identify additional recurrence-specific genetic risk factors.

Overall, in the past decades, a large number of studies in the field of VTE have identified a very large number of genetic variants, all contributing to a better understanding of VTE. For most of the genetic variants recently identified, the mechanism by which these variants lead to an increased risk of VTE remains to be elucidated. It should be noted that good predictors are not necessarily causally related to disease, but potentially all these all these genetic findings contribute to a better prediction of VTE risk. We will discuss the use of genetic findings in risk prediction for VTE in different target populations in the next section.

Table 2. Overview of genetic variants associated with recurrent venous thromboembolism.

			Reference				
Chr	**Gene**	**SNP**	**65**	**66**	**67**	**68**	**69**
1	*SELE*	rs5361	4.10				
1	*F5*	rs6025					2.35
3	*CCR2*	rs1799864				2.00	
6	*APOM*	rs805297			1.72		
7	*PON1*	rs662				1.79	
11	*MMP3*	rs3025058				1.66	
16	*CETP*	rs1800775				0.63	
18	*RP11-638L3.1*	rs9946608					1.73
22	*HMOX1*	rs3074372		2.20			

Values represent effect estimates mentioned in the original literature. Abbreviations: Chr, chromosome; SNP, single nucleotide polymorphism.

Genetics in risk prediction for VTE

Prediction models for VTE aim to assess an individual's risk of developing VTE and thereby discriminate between those with a high risk and those with a low risk of disease. For those who are at a high risk of VTE, anticoagulant treatment can be prescribed to prevent VTE. Since anticoagulant treatment comes with an increased risk of bleeding, the goal of a prediction model is to, as accurately as possible, identify individuals whose risk of VTE is higher than their risk of bleeding or vice versa, to optimally target prophylactic treatment to those who will benefit the most. Numerous studies developed prediction models comprising clinical risk factors, genetic risk factors, or a combination of both for prediction of a first or recurrent VTE.

Risk prediction for first VTE

Four clinical prediction models were developed to estimate the risk of a first VTE in general populations. Table 3 describes the clinical prediction models and the clinical risk factors included in each prediction model (64-67). The number of included risk factors ranged from 5 to 14 including socio-demographic factors, medical history, medication use, and biomarkers. The discriminatory ability of the models was assessed by calculating the area under the receiver operating characteristic curve (AUC), which ranged between 0.65 and 0.77 indicating a moderate discriminatory ability of these models. Of the models, the models of Hippisley-Cox *et al.* (64), and Lin *et al.* (66) provided calibration values which showed the models were well calibrated with close similarities between the predicted risks and the observed risks.

Additional to the models incorporating clinical parameters alone, several prediction models also included only well-established VTE-associated genetic variants to predict the risk of a first VTE. The simplest models only included genetic variants with strong to moderate effect (i.e., FVL and prothrombin 20210A) to identify individuals at a high risk of VTE. Since most genetic variants identified from GWAS have weak to moderate effects on VTE risk, genetic risk scores (GRS) aggregating the effect of each genetic variant or polygenic risk score (PRS) including all correlated genetic variants as an extension of GRS have been constructed to improve the predictive ability of genetic prediction models (Table 4). The number of genetic variants included in each model varied from only two to an extensive, and probably not very practical, model consisting of 1,092,045 genetic variants. Each model showed discriminative abilities with AUCs between 0.58 and 0.71. The first prediction model using only genetic risk factors was developed by de Haan and colleagues in 2012 (8). A 31-SNP GRS was developed by combining genetic variants which had previously been reported to be associated with VTE. Additionally, a 5-SNP GRS including *F5* rs6025, *F2* rs1799963, *ABO* rs8176719, *FGG* rs2066865, and *F11* rs2036914, which had the strongest associations with VTE, was tested as the added value of additional genetic variants was limited. Both models performed similarly with an AUC of 0.71 (95% CI: 0.69-0.72) and 0.69 (95% CI: 0.67-0.70), respectively. Soria *et al.* developed a GRS, i.e., the Thrombo inCode (TiC), and assessed the predictive ability (9). The TiC included 9 genetic variants associated with VTE and showed better discrimination with an AUC of 0.68 (95% CI: 0.63-0.72) than a GRS including only FVL and prothrombin 20210A (AUC: 0.58, 95% CI: 0.55-0.60). Bruzelius *et al.* constructed a GRS with 7 genetic variants and 4 SNP-SNP interactions with an AUC of 0.66 (95% CI: 0.64-0.68) in women (10). In 2021, Kolin *et al.* published a 36-SNP risk score of which the AUC was 0.62 (95% CI: 0.61-0.63) (11). Two recent GWAS studies proposed a GRS and a PRS from their GWAS findings (48, 50). However, these studies did not provide the discriminative ability of the scores themselves but showed the ability of the scores to identify individuals with a high risk of VTE. Similarly, several other studies also constructed GRSs (46-48, 50, 68), but again did not provide the discriminative ability of the GRSs, and only showed that individuals with a higher GRS had a higher risk of VTE than those with a lower GRS.

When looking at the combined clinical and genetic prediction models, these combined prediction models have a better predictive ability for a first VTE than models with only genetic factors. Combined prediction models are described in Table 4. Although the predictive ability of GRSs only is slightly lower than that of models consisting of clinical predictors only, it is obvious that the addition of genetic variants to the clinical prediction model improves clinical prediction models for a first VTE.

Table 3. Overview of variables that are used in clinical prediction models for first venous thromboembolism.

Reference	70	71	72	73
Socio-demographic factors				
Age	o	o	o	o
Sex	-	o	o	-
Body mass index	o	o	-	o
Smoking	o	o	-	o
Medical history				
Cardiovascular disease	-	o	-	-
Varicose veins	o	-	-	-
Congestive cardiac failure	o	-	-	-
Peripheral vascular disease	-	-	o	-
Hypertension	-	-	o	-
Chronic kidney disease	o	-	o	-
Any cancer	o	o	o	-
Chronic obstructive airways disease	o	-	-	-
Chronic obstructive pulmonary disease	-	-	o	-
Inflammatory bowel disease	o	-	-	-
Hospital admission	o	-	o	-
Medication use				
Antipsychotic drugs	o	-	-	-
Hypertension drugs	-	-	o	-
Cardiovascular drugs	-	-	o	-
Non-steroidal anti-inflammatory drugs	-	-	o	-
Antidiabetic drugs	-	-	o	-
Tamoxifen	o	-	-	-
Oral contraceptives	o	-	-	-
Hormone replacement therapy	o	o	-	-
Biomarker				
D-Dimer	-	o	-	o
Growth/Differentiation Factor-15	-	o	-	-
Activated Partial Thromboplastin Clotting Time	-	-	-	o

Table 4. Overview of genetic, clinical, and combined risk prediction models for first venous thromboembolism.

		Genetic model		Clinical model		Combined model	
Year	**Ref**	**No.SNPs**	**AUC (95% CI)**	**Included clinical markers**	**AUC (95% CI)**	**AUC (95% CI)**	**Imp**
2012	8	5	0.69 (0.67-0.70)	Leg injury, surgery, pregnancy, plaster cast, bedridden at home, travel, hospitalization, OC use, HRT, obesity, malignancy, and family history	0.77 (0.76-0.78)	0.82 (0.81-0.83)	6.5%
2012	8	31	0.71 (0.69-0.72)	Leg injury, surgery, pregnancy, plaster cast, bedridden at home, travel, hospitalization, OC use, HRT, obesity, malignancy, and family history	0.77 (0.76-0.78)	0.82 (0.81-0.83)	6.5%
2014	9	2	0.58 (0.55-0.60)	Family history	0.59 (0.55-0.63)	0.65 (0.60-0.69)	10.2%
2014	9	9	0.68 (0.63-0.72)	Family history	0.59 (0.55-0.63)	0.70 (0.65-0.75)	18.6%
2014	9	13	0.67 (0.62-0.72)	Family history	0.59 (0.55-0.63)	0.70 (0.65-0.75)	18.6%
2015	10	11[a]	0.66 (0.64-0.68)	Height, BMI, surgery, plaster cast, travel, OC use, HRT, and family history	0.80 (0.79-0.82)	0.84 (0.82-0.85)	5.0%
2021	11	36	0.62 (0.61-0.63)	Age, sex, BMI, smoking status, fracture in the last 5 years, and previous cancer diagnosis	0.65 (0.64-0.65)	0.69 (0.68-0.70)	6.2%
2022	52	100	-	Age, sex, and the first 10 principal components	0.52 (0.51-0.52)	0.62 (0.62-0.63)	19.2%
2023	54	1092045	-	Age, sex, four principal components, BMI, FII/FV, CVD, hypertension, type 2 diabetes, chronic kidney disease, COPD/pulmonary fibrosis, varicose veins, cancer, and autoimmune disease	0.67 (0.67-0.68)	0.70 (0.69-0.70)	4.5%

Abbreviations: No.SNP, Number of single nucleotide polymorphism; AUC, area under the curve; OC, oral contraceptive; HRT, hormone replacement therapy; BMI, body mass index; CVD, cardiovascular disease; COPD, chronic obstructive pulmonary disease

Risk prediction for recurrent VTE

More clinical prediction models have been developed for recurrent VTE than for a first VTE. This is probably because recurrent VTE has a much higher frequency than first VTE, and thereby is perceived as a larger medical problem. Also, for such prediction models information from patients with VTE suffices, and no control group of healthy individuals needs to be included. A recent review summarized the clinical prediction models for recurrent VTE (69). There are now 17 published clinical prediction models for recurrent VTE. The models vary in terms of included population, outcome measurements, predictive ability, and included predictors. The number of included predictors ranged between 3 and 16. Predictors that were most frequently used were sex, age, VTE type (i.e., DVT or PE), location of the index event, and D-dimer test results.

Several studies assessed the risk of recurrent VTE with only genetic risk factors. In 2008, researchers provided proof of principle on whether genetic risk factors associated with VTE can be used to identify individuals at a high risk of recurrent VTE (70). Adding genetic variants one by one in a rank order of risk based on the effect estimation, researchers found patients with the first two, three, and four variants (*F7* rs6046, *F5* rs6025, *MTHFR* rs1801133, *FGB* rs1800790 in rank order) had a 1.7, 2.7, and 5.1-fold increased risk of recurrent VTE among 817 patients after a first VTE. However, the number of participants with multiple risk alleles was low, and therefore the authors concluded that the clinical utility of this approach was limited. In addition, two studies developed GRSs by counting the number of risk-increasing alleles in patients and stratified patients into low-risk and high-risk groups based on the GRSs. In 4100 VTE patients with a mean follow-up of 5.4 years, van Hylckama Vlieg *et al.* developed 31 and 5-SNP GRS with genetic variants that de Haan *et al.* used for the first VTE (8, 71). Both GRSs showed similar discriminative ability for recurrent VTE. Using 5-SNP GRS, they found that high-risk patients (≥5 risk alleles) had a 6-year cumulative incidence of 20.3% (95% CI: 16.5-24.1) compared with low-risk patients with a 6-year cumulative incidence of 9.4% (95% CI: 6.7-12.1). In 131 VTE patients with a mean follow-up of 3.9 years, Ahmad *et al.* developed 12 and 8-SNP GRSs which also showed similar discriminative ability (72). Using the 8-SNP GRS, high-risk patients (≥5 risk alleles) showed a VTE recurrence probability of 15.4% (95% CI: 12.4-18.5), compared with low-risk patients with a probability of 8.3% (95% CI: 5.9-10.7).

Only a limited number of studies developed prediction models for recurrent VTE with combined clinical and genetic risk factors. Franco Moreno *et al.* developed a prediction model using age, sex, BMI, varicose veins, D-dimer, and FVIII levels as well as FVL and prothrombin 20210A in 398 first VTE patients with a median follow-up of 21.3 months (73). The model showed good discriminative ability with an AUC of 0.91. However, the authors did not compare the combined models with prediction models containing clinical or genetic risk factors only. Timp *et al.* assessed the added value of genetic variants to a clinical model in 3750 first VTE patients with a median follow-up of 5.7 years (74). The authors developed four different prediction models: Model A with clinical, laboratory, and genetic factors; Model B with clinical and fewer laboratory factors; Model C with clinical and genetic factors; and Model D with clinical factors only. The genetic factors included were FVL, ABO blood group, and the 5-SNP GRS that was used by de Haan *et al.* (8) and van Hylckama Vlieg *et al.* (71). After backward selection which systematically eliminates predictors from all candidate variables to create the most suitable prediction model, model A did not include any genetic markers. On the other hand, model B included FVL, and model C included FVL and ABO blood type. The discriminative ability of combined models B and C was similar to that of clinical

prediction models A and D. Currently, there is no study in which common genetic variants identified from GWAS are added to the clinical prediction model for recurrent VTE. Given that addition of common variants to the clinical model improved the predictive ability of a first VTE, further improvement may be possible by combining additional genetic variants to the clinical models for recurrent VTE.

Overall, a genetic prediction model can be used to identify individuals at high risk of first and recurrent VTE. Although the models comprising clinical risk factors only, outperformed genetic prediction models (i.e., GRSs), it is clear that the addition of genetic risk factors to clinical prediction models improves the accuracy of risk prediction.

Clinical implication of genetics in risk prediction for VTE: Clinically relevant or not?

Results from the literature clearly showed that the addition of genetic variants to clinical prediction models improved predictive ability of the models. Furthermore, the addition of a GRS to a clinical prediction model showed better discriminative ability than the addition of individual genetic variants (9, 50). However, before implementing GRSs into clinical practice, we need to think about the clinical value of using genetic variants in risk prediction. The four clinical prediction models for the first VTE in Table 3 showed AUCs over 0.70 on average, which was considered an acceptable discriminative ability in general. However, by adding genetic findings to the clinical prediction models, discriminative abilities are improved by 4 to 20% and the absolute difference of AUCs ranged between 0.01 and 0.11. For recurrent VTE the prediction ability was not improved with the addition of genetic findings to the clinical models although there was only one study in which only FVL and ABO blood group were included in the combined model. Therefore, current models are still unlikely to be able to guide clinical practice, although randomized trials to test these models in various circumstances are underway (e.g., Netherlands trial register: NL9003). Furthermore, an impact study is required to investigate the added value before implementation. However, to our knowledge, no study has been conducted to explore the clinical impact of using genetic factors for risk prediction in clinics. Therefore, whether the improvement is clinically meaningful remains to be determined.

Moreover, the cost-effectiveness of the use of genetic factors in risk prediction of VTE should be considered. Although the cost of genotyping has decreased, its use results in additional costs in risk prediction compared with the current system, which increases the entire healthcare cost. Eckman *et al.* estimated the cost-effectiveness of testing FVL in patients with a first VTE (75). The authors concluded that patients who have no obvious risk factors (i.e., idiopathic VTE) and a low risk of bleeding after anticoagulant treatment may benefit from FVL testing followed by prolonged anticoagulant treatment in carriers. In contrast, they concluded that in populations with a very low prevalence of FVL (i.e., African, and Asian), patients with a low risk of recurrent VTE (i.e., clear indication of first VTE), or patients with a high risk of bleeding from anticoagulant treatment are not of interest for cost-effective FVL testing followed by possible prolonged anticoagulant treatment. Similarly, Simpson *et al.* performed a systematic review to investigate the cost-effectiveness of thrombophilia screening in patients with first VTE (76). Thrombophilia testing was likely cost-effective in certain subgroups such as men below the age of 70 years with first VTE. These results suggest that screening the entire population is not clinically relevant.

Instead, it may be clinically useful and less costly to assess the risk of VTE among those who are in high-risk situations, e.g., cancer patients, surgery patients, or women initiating oral

contraceptive use, as their absolute risk of developing VTE is much higher than that in the general population. According to the current clinical guidelines, the use of anticoagulants as VTE prophylaxis is considered when individuals with persistent and temporary risk conditions have a moderate to high risk of VTE without a high risk of major bleeding (77). Similar to high-risk groups to develop a first VTE, in individuals who experienced a first VTE the incidence rate of recurrence is much higher than that of a first VTE in general populations, indicating that risk assessment for recurrence may be clinically useful and cost-effective. In general, patients with a first VTE are treated with anticoagulants at least for three months. After this treatment, prolonged anticoagulant treatment is recommended for only those in whom the risk of VTE outweighs the risk of bleeding. Therefore, better prediction tools improve the ability to identify those who are at high risk of VTE in whom treatment will outweigh the risk of bleeding. This will lead to the proper use of anticoagulants, i.e., reducing the risk of VTE in those who benefit and withholding anticoagulants in those who would have an unacceptable risk of subsequent bleeding.

However, we still need careful use of genetic testing in even a high-risk group. For example, one might wonder whether all women should have genetic testing before oral contraceptive use (78). Assuming a death rate of PE of 5.7 per 100,000 a year and FVL prevalence of 0.05%, Vandenbroucke *et al.* estimated that 400,000 women should be screened for FVL and 20,000 women with FVL should avoid oral contraceptive use to prevent one death from VTE during one year (79). Also, to prevent one VTE, 666 women should be tested for FVL or prothrombin 20210A and 333 should not take oral contraceptives when assuming the risk difference of 0.3 between carriers and non-carriers (80). Because the prevalence of common variants is higher than that of FVL and prothrombin 20210A, the number of individuals who should be screened and denied treatment may be lower than these estimates, but likewise may also be the yield. Further investigation is required to study the impact of genetic testing in high-risk populations before the strategy can be recommended.

In summary, the question of whether these models eventually will have clinical utility, depends on the target population. Here, we discussed predicting the risk of a first or recurrent VTE where the absolute risk of an event is higher for recurrent VTE. This indicates that prediction models for recurrent VTE are more likely associated with a lower number needed to treat and therefore more likely to become cost-effective than prediction models for first VTE. Similarly, models for predicting the risk of a first VTE may be targeted to high-risk populations such as surgery patients or cancer patients to increase clinical utility, but this is beyond the scope of this review.

Future perspectives

We discussed current knowledge of genetic variants associated with the risk of VTE, the added value of genetic findings in clinical VTE risk prediction, and the considerations to implement genetic prediction models for VTE in clinics. Here, we will discuss additional research possibilities to apply genetic findings in risk prediction of VTE.

First, studies on genetics based on multiple ethnicities are required, if not in subpopulations. Most genetic studies are based on individuals with European ethnicity. Genetic variants have different allele frequencies and risks according to population background. For instance, FVL is hardly found in individuals with a non-European background (81), and, like prothrombin 20210A, even has a widely different prevalence within Europe (82, 83). Therefore, the application of findings from European individuals to others may have different consequences in risk prediction. Indeed, Folsom *et al.* tried to validate the 5-SNP risk score

developed by de Haan *et al.* in the Dutch population (8), in a population from the USA consisting of those with various European backgrounds and African Americans enrolled in the Atherosclerosis Risk in Communities study (84). The score was associated with VTE in the first subgroup, but not in African Americans. Therefore, it is necessary to find genetic variants associated with VTE in populations from all ethnic backgrounds and to apply the findings to risk prediction in each population.

Second, additional prevention strategies can be developed with genetic findings. The current prophylaxis regimens mostly depend on anticoagulants for high-risk populations or individuals in high-risk situations. If we identify individuals with genetically high risk at early life stages, however, we can recommend them to modify their lifestyle to prevent VTE. Ghouse *et al.* showed that although individuals have a genetically high risk of VTE, those who smoke, are obese, and do not exercise regularly have a two times higher risk of VTE than those who do not smoke, are not obese, and exercise regularly (50). In addition to prophylactical anticoagulant treatment, lifestyle changes can be a new strategy to prevent VTE.

Lastly, genetic findings may be used to predict VTE risks with other approaches. The current genetic studies only focus on the risk of a first or recurrent VTE. However, we may take this further and investigate whether the age of incident VTE is predicted genetically and whether genetic factors explain the time to recurrent VTE or bleeding without or with anticoagulants. Also, to decide on prophylactical treatment for individuals at high risk, bleeding risk should be considered in addition to the risk of VTE. Whereas many prediction models have been proposed to predict bleeding risk, as summarized in a recent review (69), none of these models include genetic findings, due to a lack of insights into the genetic contribution to bleeding during anticoagulant treatment.

Conclusion

Advancing technology has provided increasing opportunities to explore genetic contributions to VTE. This has improved our understanding of the hereditary susceptibility to VTE. Furthermore, the addition of genetic risk factors to clinical prediction models has improved risk prediction for VTE. For individuals in high-risk situations, the added value of genetic factors to risk prediction models may help better prophylaxis of VTE and reduce the side effect of bleeding from excessive or unnecessary treatment. However, the utility of genetic factors in risk prediction for VTE has not been proven.

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