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REVIEW



Genetic risk factors for venous thromboembolism

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ABSTRACT

Introduction: Venous thromboembolism (VTE) is a complex disease that aggregates in families. Both acquired and genetic risk factors are important. Proper recognition and management of high-risk individuals are important.

Areas covered: The genetic risk factors for VTE, the clinical consequences, and future perspectives are summarized. Classical thrombophilia i.e., factor V Leiden (rs6025), the prothrombin G20210A mutation (rs1799963), deficiencies of antithrombin, protein C, and protein S and the recent findings from genome wide association studies (GWAS), transcriptome-wide association studies (TWAS), genetic risk score (GRS), VTE candidate genes, expression studies, animal studies, studies using next generation sequencing, pathway analysis, and clinical implications are discussed.

Expert opinion: Screening of inherited thrombophilia should be performed in special cases. Identification of strong risk variants might affect the management. The increasing number of genetic risk variants is likely to change management of VTE.

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Venous thromboembolism; deep vein thrombosis; pulmonary embolism; genetics; molecular epidemiology; genome-wide association study; thrombophilia; protein S deficiency; protein C deficiency; antithrombin deficiency

1. Introduction

Venous thromboembolism (VTE) is next to coronary heart disease (CHD) and stroke, the third most common cardiovascular disorder [1–7]. The most frequent manifestations of VTE, includes superficial thrombophlebitis, deep vein thrombosis (DVT) of the lower extremities and pulmonary embolism (PE), though unusual manifestations also have been described: Budd–Chiari syndrome (hepatic vein occlusions) and thromboses of the vena cava and cerebral, portal, mesenteric, renal, and retinal veins [1–7]. Lifetime risk of VTE is rather high and has been estimated to be 5–10%. VTE recurrence risk is also high [1–8]. The risk of recurrent VTE is around 25% in 5 years.

VTE is a complex disorder with a multicausal origin [6]. Acquired VTE risk factors include immobilization, surgery, trauma, pregnancy, puerperium, malignancy, and oral contraceptives. Twenty-five to 50% of patients with VTE have a spontaneous or unprovoked (idiopathic) VTE event with no identifiable transient risk factors depending on exact definition [7,8]. Patients with unprovoked VTE have especially high risk of recurrence after discontinuation of anticoagulant (AC) treatment [7,8].

VTE has also a strong genetic component with an estimated heritability of 40–60% in studies of families, twins, siblings, and half-siblings [9–12]. That VTE clusters in families has been known for more than a century, reviewed by Zöller et al [13]. Although rare deficiencies of natural anticoagulants antithrombin, protein C, and protein S was discovered among VTE patients early on it was not clear until Dahlbäck et al discovered resistance to activated protein C (APC-resistance) in 1993 that genetic factors were demonstrated

to be common in VTE [14,15]. Soon APC-resistance was shown to be linked to a common *F5* gene mutation named Factor V Leiden (rs6025) [16–19]. Only two years later another common genetic variant associated with thrombosis was reported (prothrombin G20210A or rs1799963) [20]. Thrombophilic defects at these five gene loci are called classic thrombophilia [21–23]. The present article will not only review classic thrombophilia but will also go beyond and focus on latest discoveries and future prospects of the genetic risk factors for VTE.

2. Classical thrombophilia

Thrombophilia is defined as an acquired or inherited condition with hypercoagulability that leads to an increased risk for VTE [22]. Such hypercoagulable states involve either alterations in the vessel wall, the blood flow, and/or the composition of the blood (Virchow's triad). Rudolf Virchow postulated this already in 1856. So far there are five classical inherited thrombophilias: antithrombin deficiency, protein C deficiency, protein S deficiency, resistance to activated protein C due to Factor V Leiden (rs6025), and the prothrombin mutation (prothrombin G20210A, rs1799963) [21–25]. Thus, all inherited thrombophilias are either genetic defect in a natural anticoagulant (antithrombin, protein C, and protein S) or a coagulation factor (factor V and factor II) (Figure 1). In Figure 1, the more recent and weaker discovered VTE gene loci are marked with orange and the classical thrombophilia loci with red.

- Venous thromboembolism is a complex disease that clusters in families with moderate to high heritability.
- Five classical thrombophilia exist: rs6025, rs1799963, and deficiencies of the natural anticoagulants antithrombin, protein C, and protein S
- Recent GWAS have established a number of weak VTE variants and made GRS of large effect size possible.
- Factor V Leiden is associated not only with VTE risk but also peripheral artery disease.
- The agnostic methods WES and WGS will be important in future research to detect multiple rare variants in new gene loci.
- Development of clinical risk scores including the total burden of acquired risk factors (even transient) and genetic risk burden might lead to tailored treatment and prophylaxis.
- ESC 2019 guidelines recommend thrombophilia screening for young VTE patients below 50 years of age and unprovoked VTE, especially in the presence of family history of VTE.

Inherited deficiencies of the natural coagulation inhibitors antithrombin, protein C, and protein S are all strong risk factors for VTE [23–25]. However, these deficiencies are rare (<1%) in the population. Typically, these disorders are due to different loss of function (LOF), i.e. gene inactivating, mutations in the *SERPINC1*, *PROC*, and *PROS1* genes, respectively. Several hundreds of causative mutations have been described responsible for these defects. The most common mutations in

Mutations causing inherited deficiencies of antithrombin, protein C or protein S may be associated with decreased plasma immunological and functional levels (Type I deficiency), whereas type II deficiency is associated with functionally abnormal protein molecules [23–25]. Type II antithrombin deficiency is subdivided into three type: HBS (heparin binding site variants), RS (reactive site variants), and PE (variants giving pleiotropic effects). For protein C there exist just two types without subtypes: type I and type II deficiency. Protein S, the cofactor to protein C is a special case. Protein S exists in plasma as free or bound to C4b-binding protein (C4BP). Type I protein S deficiency is characterized by low levels of both free and total protein S. Type II is

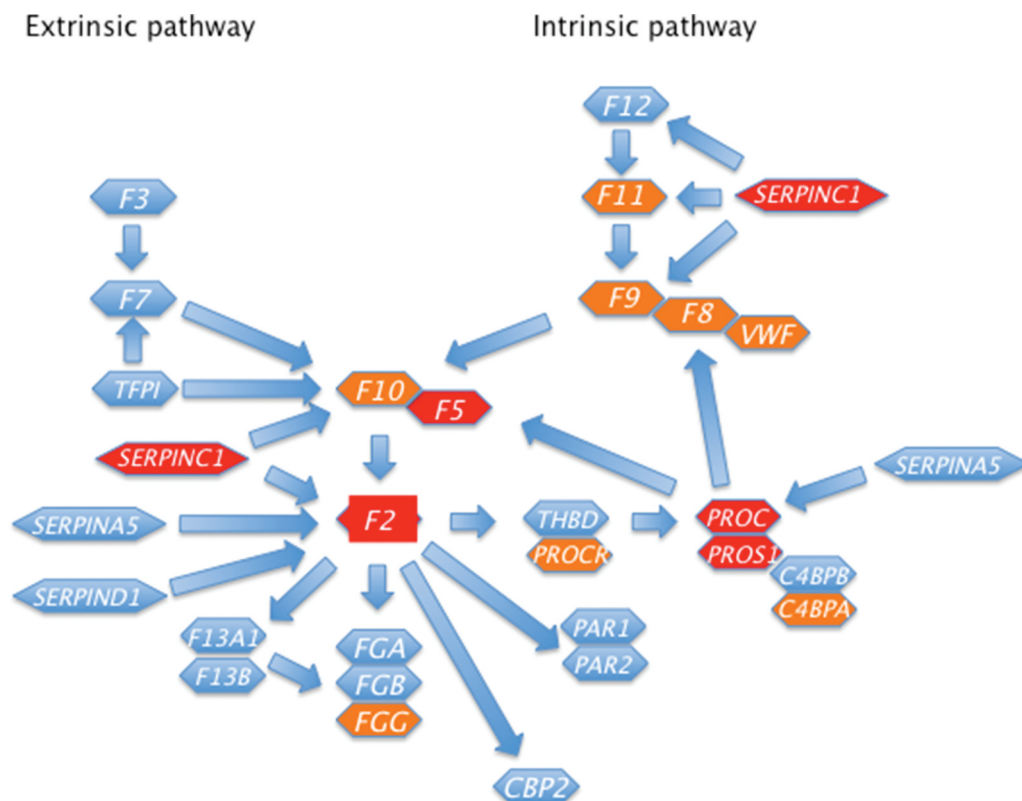


Figure 1. The Coagulations system and its regulating anticoagulant genes are depicted. The official HGNC (HUGO Gene Nomenclature Committee) abbreviations are used. Classical thrombophilia genes are marked with red and minor VTE risk genes are marked with orange.

reserved for qualitative protein S deficiency, while type III deficiency is characterized by low free protein S and normal total protein S. It has been shown that the level of free protein S in plasma discriminates better between those with and without protein S deficiency than the level of total protein S.

2.2. *F5 gene and resistance to activated protein C (APC-resistance)*

Dahlbäck et al discovered resistance to activated protein C (APC-resistance) in 1993 as a common and strong risk factor for VTE [14,15]. Soon it became clear that in a majority of cases a single mutation (factor V Leiden, rs6025) in the factor V gene (*F5*) was the major cause of inherited APC-resistance [16–19]. Factor V Leiden is a common gain-of-function (GOF) *F5* gene mutation, i.e. gene activating, with a prevalence of carriers among Caucasians of 5–10% [16–19,23–25]. Among patients with VTE it is found in 20–30%, and in around 50% of patients with familial thrombophilia [23–25]. The variant leads to resistance to activated protein C (APC-resistance) [23–25]. The Arg506 to Gln mutation is located in the part of the gene encoding for one of the three cleavage sites in factor V (Arg306, Arg506, and Arg679), where APC inactivates factor Va [23–25,28]. The risk of thrombosis is 5-fold increased in heterozygotes, and fiftyfold in homozygotes [23–25].

More recent studies have identified a number of other GOF variants in the *F5* gene among VTE patients, reviewed by Segers et al [28]. However, except the R2 haplotype (rs6033, rs1800595, rs6030, rs6027) and A/G allele variants (rs6021, rs4524, rs4525, rs6032) these APC-resistance associated *F5* gene variants are all rare [28]. The rare variants Factor V Cambridge (rs118203906) and Factor V Hong Kong (rs118203905) both affect the APC cleavage site at Arg306 (or Arg534 when numbered from the starting Met including the signal peptide). More recently, some other rare *F5* gene mutations such as FV Nara (Trp1920Arg) and FV Bonn (Ala512Val) that also cause APC-resistance and thrombosis have been identified [29–32]. No *F8* gene variants have been associated with APC-resistance.

2.3. *Prothrombin G20210A (rs1799963)*

A G-to-A transition at nucleotide 20210 (prothrombin G20210A, rs1799963) in the 3'-untranslated part of the prothrombin gene (*F2*) causes increased prothrombin levels and an increased risk of VTE [20]. This GOF mutation is also prevalent (around 2%) and almost only found in Caucasians. Carriers have a 2- to 3-fold increased risk of venous thrombosis, and the variant is found in approximately 6% of patients with VTE [23–25]. Homozygosity for the rs1799963 variant occurs though rarer than homozygosity for the rs6025 variant. However, the risk for VTE is high and has been reported to be 30 times increased for homozygotes for the rs1799963 variant [23].

2.4. *Antithrombin resistance due to prothrombin mutations*

In analogy with the APC-resistance phenomena resistance to antithrombin has been reported in young patients with VTE

[33–36]. Mutations in the last exon of *F2* with substitution of Arginine at residue 596 (Arg596Leu, Arg596Gln, Arg596Trp) have been identified as the cause of antithrombin resistance [33–36]. However, these variants are rare and are not identified in gnomAD (<https://gnomad.broadinstitute.org/>, accessed 21 July 2020). A formal statistical association with VTE therefore remains to be proven.

2.5. *Abnormal factor IX (Padua)*

Simioni et al reported in 2009 a 25-year-old man with VTE and around eight times increased factor IX coagulation activity with normal factor IX level [37]. This was due to a factor IX mutation with substitution of Arginine 338 for a Lysine (factor IX Padua). Recombinant factor IX–Arg338Lys had a specific activity that was 5- to 10- times increased compared with recombinant wild-type factor IX activity. However, factor IX Padua is just as the prothrombin variants causing antithrombin resistance rare and is not identified in gnomAD (<https://gnomad.broadinstitute.org/>, accessed 21 July 2020). A formal statistical association with VTE therefore remains to be proven also for FIX Padua.

2.6. *Gene-gene interaction*

As factor V Leiden and prothrombin 20210A both are common, compound heterozygotes are not extremely rare among individuals with deficiencies of antithrombin, protein C, and protein S. Individuals may have up to 30- to 100- times increased risk for VTE [25]. However, these risks are estimated from family studies and might constitute an overestimation of the actual VTE risk. Moreover, the confidence intervals are wide. Whether newly created genetic risk scores (GRS) scores will interact in similar ways with classical thrombophilia remains to be determined.

3. *New findings from genome wide association study*

Genome wide association studies (GWAS) enable hypothesis-free methods for testing associations between loci (genetic regions) and diseases [38]. Typically, hundreds of thousands to millions of genetic variants across the genomes of thousands of individuals are tested to identify genotype–phenotype associations. These associations have led to insights into the architecture of disease susceptibility for a number of diseases including VTE [39–41]. Several novel VTE disease loci have been discovered and previously discovered VTE loci have been confirmed by GWAS studies, reviewed by Trégouët and Morange [42].

In 2019, Lindstrom et al and Klarin et al. published two large GWAS studies, respectively [43,44] (Table 1). Lindstrom et al meta-analyzed GWAS data for 30,234 VTE cases and 172,122 controls and tested the association between 12,923,718 SNPs and VTE. They identified 34 independent genetic signals for VTE risk from GWAS meta-analysis, of which 14 were novel associations. In Table 1 the replicated loci together with loci identified by the other large GWAS by

Table 1. Significant and replicated loci and variants in two large Genome wide association studies by Lindstrom et al [43] and by Klarin et al [44].

Gene	rs ID	Alt/ ref	Consequence	MAF ¹ [43]	MAF ² [44]	OR ¹ [43]	OR ² [44]
<i>F5</i>	rs6025	T/C	Arg534Gln	0,03	0,0247	2,39	2,53
<i>F5</i>	rs4524	C/T	Lys858Arg	0,26	-	0,88	-
<i>C4BPA</i>	rs2842700	A/C	intron	0,06	0,1045	1,14	1,11
<i>CYP27C1</i>	rs7585314	T/C	intron	-	0,3362	-	1,07
<i>PLEK</i>	rs1867312	C/A	intron	0,43	0,4398	1,06	1,06
<i>CSRP1</i>	rs13084580	T/C	5'UTR	-	0,1148	-	1,08
<i>PROS1</i>	rs6795524	G/A	intron	-	0,0733	-	1,15
<i>FGG</i>	rs2066865	A/G	downstream	-	0,2465	-	1,22
<i>FGG</i>	rs2066864	A/G	intron	0,24	NS	1,20	-
<i>F11</i>	rs4253417	C/T	intron	-	0,3874	-	1,22
<i>F11</i>	rs2289252	T/C	intron	0,40	-	1,19	-
<i>F11</i>	rs2036914	T/C	intron	0,47	-	0,86	-
<i>F11</i>	rs4253421	A/G	intron	0,12	-	0,80	-
<i>OSMR-AS1</i>	rs4869589	T/G	intron	0,26	-	1,07	-
<i>RP11-122C5.1</i>	rs16867574	C/T	downstream	-	0,3175	-	1,06
<i>STXBP5</i>	rs9373523	T/G	intron	0,42	-	0,94	-
<i>STXBP5</i>	rs7739314	C/A	downstream	-	0,5345	-	1,06
<i>HLA-C</i>	rs2074492	T/C	upstream	-	0,6191	-	1,05
<i>ZFPM2</i>	rs4541868	A/C	intron	0,25	-	0,92	-
<i>ZFPM2</i>	rs4734879	A/G	intron	-	0,7114	-	1,09
<i>SCARA5</i>	rs10087301	A/G	intron	-	0,8135	-	1,07
<i>ABO</i>	rs8176749	A/T	synonymous	0,08	-	1,23	-
<i>ABO</i>	rs687289	G/C	intron	0,35	-	1,34	-
<i>ABO</i>	rs2519093	T/C	intron	0,21	-	1,40	-
<i>ABO</i>	rs9411377	A/C	intron	-	0,3129	-	1,36
<i>ABO</i>	rs579459	C/T	intron	0,23	-	1,36	-
<i>GRK5</i>	rs10886430	G/A	intron	0,13	0,1202	1,12	1,14
<i>TSPAN15</i>	rs78707713	T/C	intron	-	0,875	-	1,27
<i>TSPAN15</i>	rs78707713	C/T	intron	0,12	-	0,83	-
<i>F2</i>	rs191945075	A/G	Downstream (intron)	0,01	-	1,86	-
<i>(LRP4)</i>							
<i>F2</i>	rs1799963	A/G	3'UTR	-	0,0111	-	1,88
<i>F2</i>	rs3136516	G/A	intron	0,47	-	1,06	-
<i>MYRF</i>	rs174536	A/C	intron	-	0,6649	-	1,08
<i>MPHOSPH9</i>	rs2851436	G/T	intron	0,21	-	0,94	-
<i>SBNO1</i>	rs12824685	G/T	intron	-	0,8033	-	1,07
<i>VWF</i>	rs1558519	G/A	intron	0,35	-	1,08	-
<i>VWF</i>	rs216296	G/A	intron	-	0,9095	-	1,14
<i>VWF</i>	rs216311	T/C	Thr1381Ala	0,39	-	0,98	-
<i>F10</i>	rs3211752	G/A	intron	-	0,4771	-	1,07
<i>CATSPERB</i>	rs57328376	G/A	intron	-	0,2993	-	1,06
<i>PLCG2</i>	rs12445050	T/C	intron	0,14	0,1366	1,11	1,13
<i>SMG6</i>	rs1048483	T/C	intron	0,49	0,4717	1,06	1,06
<i>SLC44A2</i>	rs4548995	G/C	intron	0,22	-	0,90	-
<i>SLC44A2</i>	rs2288904	G/A	Gln154Arg	-	0,7849	-	1,12
<i>PEPD</i>	rs731839	A/G	intron	-	0,6599	-	1,05
<i>GP6</i>	rs1671135	G/C	Downstream (intron)	0,21	-	0,93	-
<i>(NLRP2)</i>							
<i>GP6</i>	rs1654425	C/T	synonymous	-	0,8426	-	1,09
<i>(CD93)</i>	rs6083037	A/T	intergenic	-	0,8253	-	1,09
<i>PROCR</i>	rs6088735	T/C	intron	0,23	-	1,08	-
<i>PROCR</i>	rs867186	G/A	Ser219Gly	0,10	-	1,11	-
<i>EDEM2</i>	rs10747514	A/G	intron	-	0,3183	-	1,12
<i>A4GALT</i>	rs9607928	A/C	intron	-	0,1339	-	1,09
<i>F9</i>	rs6048	A/G	Thr194Ala	-	0,716	-	1,07
<i>BRCC3</i>	rs7051718	T/C	intron	-	0,7507	-	1,07
<i>F8</i>	rs143478537	G/C	upstream	0,14	-	1,08	-
<i>(BCOR)</i>	rs3002417	T/C	intergenic	-	0,5281	-	1,06

MAF = minor allele frequency, OR = odds ratio, ¹ = Lindstrom et al [43].
² = Klarin et al [44].

Klarin et al [44] are presented. Klarin et al performed a discovery GWAS with testing of around 13 million DNA sequence variants for association with VTE (26,066 VTE cases and 624,053 controls) and replicated findings among 17,672 VTE cases and 167,295 controls. They identified 22 novel replicated loci shown in Table 1. The genome wide significant and replicated genes from the Lindstrom et al and Klarin et al GWAS are presented in Figure 2 according to potential linkage

with VTE. For instance, the choline transporter *SLC44A2* has been identified in red blood cell membrane [45] and *A4GALT* is related to P1 blood group status [46]. A gene variant (rs395967) of *OSMR-AS1* has been associated with monocyte count [47] and *PEDP* has been suggested to be involved in rheumatoid arthritis and immune response in tuberculosis [48,49]. Some loci involve different systems such as *ABO* gene. *ABO* gene is linked both to VWF (platelets) and factor VIII (coagulation) levels but also determine ABO blood group (erythrocytes). Whether all the *ABO*-associated risk is related to VWF and factor VIII levels remains to be determined. In Figure 1 are minor coagulation and anticoagulation GWAS loci marked with orange and classical thrombophilia loci marked with red. Several loci that reached genome wide significance but could not be replicated are shown in Table 2 [43,44]. Whether these are spurious association or not remains to be determined.

Worth to mention is another recent GWAS of peripheral artery disease (PAD) including 31,307 PAD cases and 211,753 controls, by Klarin et al [50]. For the first time they formally linked Factor V Leiden to an arterial disorder. Factor V Leiden was the second strongest variant associated with PAD with an odds ratio of 1.2 (95%CI 1.14–1.26, p value = 1.63E-12). Most interestingly, the Factor V Leiden variant's effect estimate increased as PAD severity increased, with factor V Leiden carriers having a 62% increased risk of major amputation (OR = 1.62; 95%CI: 1.16–2.26). Moreover, no associations between Factor V Leiden and ischemic stroke or coronary heart disease were observed. This is in line with recent reports showing a protective effect of rivaroxaban against limb event in PAD [51,52].

4. Genetic risk score (GRS)

A drawback of GWAS studies is that most identified genetic variants are weak risk factors, i.e. with relative risks between 1.0 and 1.5. However, the identification of a large number of weak variants allows for creation of GRS [43,44]. Lindstrom et al created a GRS including 37 variants. Individuals belonging to the bottom fifth percentile had a 50% lower risk for VTE (OR = 0.51) and those among the top fifth percentile had a 3.2-fold greater odds ratio for VTE compared to the population in the middle range [43]. Klarin et al created a 297 variants GRS for VTE that identified 5% of the population at a risk estimate similar to carriers of the factor V Leiden (rs6025) and prothrombin G20210A mutations [rs1799963] [44].

5. Transcriptome-wide association studies (TWAS)

Transcriptome-wide association studies (TWAS) integrate genome-wide association studies (GWAS) and gene expression datasets to identify gene-trait associations [53]. TWAS is thus an agnostic approach to prioritize causal genes at GWAS loci. Some loci will be non-causal, owing to sharing of expression quantitative trait loci (eQTL). TWAS is especially prone to spurious prioritization if expression data from non-trait-related tissues or cell types are used. Lindstrom et al [43] used TWAS to identify 5 genetic loci with imputed gene expression levels differing between cases (n = 29 435) and controls (n = 157 769) of european ancestry in whole

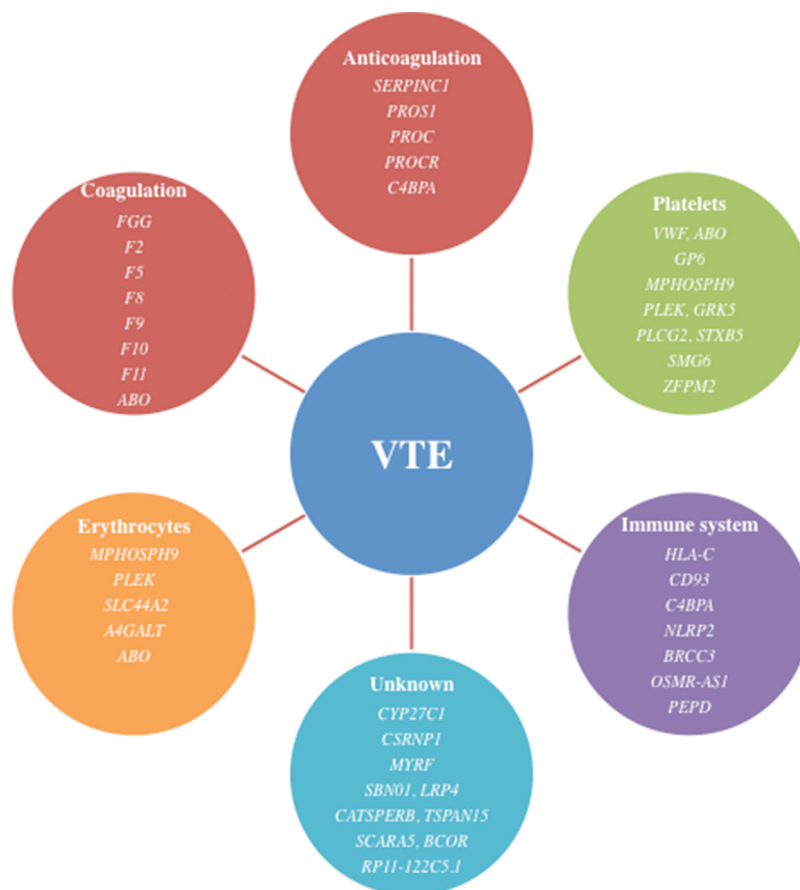


Figure 2. Confirmed genes linked to venous thromboembolism (VTE) in family studies [23–25] or genome wide association studies [43,44] grouped according to potential relation to VTE, i.e. anticoagulation, coagulation, platelets, erythrocytes, immune system, and unknown.

Table 2. Significant but not replicated loci and variants in two large Genome wide association studies by Lindstrom et al [43] and by Klarin et al [44].

Gene	rs ID	Alt/ ref	Consequence	MAF ¹ [43]	MAF ² [44]	OR ¹ [43]	OR ² [44]
<i>LMOD1</i>	rs2644120	C/G	intron	-	0,5461	-	1,06
<i>C1orf198</i>	rs145470028	T/G	intron	0,02	-	0,78	-
<i>(EPHA3)</i>	rs141912156	T/C	intergenic	-	0,0274	-	1,18
<i>NUGGC</i> (<i>SCARA5</i>)	rs12675621	G/A	Intron (upstream)	0,29	-	1,07	-
<i>QSER1</i>	rs563259534	T/C	intron	-	0,0844	-	1,11
<i>RP11-347H15.5</i>	rs117653193	T/C	downstream	-	0,9154	-	1,11
<i>ARID4A</i>	rs11158204	T/C	downstream (intergenic)	0,25	-	0,94	-
<i>EIF5A</i>	rs12450494	A/T	upstream (Intergenic)	0,47	-	1,06	-
<i>ABCA6</i>	rs77542162	G/A	Cys1359Arg	-	0,0214	-	1,21
<i>CHD3</i>	rs74252295	G/A	upstream	-	0,0726	-	1,10
<i>STX10</i>	rs7508633	G/A	intron	0,45	-	1,06	-

MAF = minor allele frequency, OR = odds ratio, ¹ = Lindstrom et al [43]. ² = Klarin et al [44].

blood (*SH2B3*, *SPSB1*, *RP11-747H7.3*, *RP4-737E23.2*) and in liver (*ERAP1*). However, these loci remain to be confirmed.

6. High-throughput nucleotide sequencing

Another drawback of GWAS studies are that rare variants will not be detected. For instance, the variants causing deficiencies of antithrombin, protein C, and protein S are not detected by

even very large GWAS studies [43,44]. The development of several so-called next generation sequencing (NGS) or High-Throughput Nucleotide Sequencing platforms is offering the possibility of generating fast, inexpensive and accurate genomic information, reviewed by Cunha et al [54]. A few such studies have been published [55–64]. These studies could be either agnostic [61–64], i.e. whole exome sequencing (WES) or whole genome sequencing (WGS), or using NGS for re-sequencing of targeted candidate genes [55–60]. A major

problem is that the studied mutations are rare and even if studying mutation enrichment in genes a large study size will be necessary.

Worth to mention is the finding from a pilot study by Manderstedt et al using targeted sequencing of 17 candidate genes associated with VTE [60]. Ninety-six VTE patients from the population-based Malmö Thrombophilia Study (MATS) were analyzed using an AmpliSeq strategy and Ion Torrent sequencing compared with data from public databases (gnomAD and SweGen). Determination of the nsyn/syn ratio (number of non-synonymous variants per non-synonymous site, nsyn, to the number of synonymous variants per synonymous site, syn) showed, as expected in VTE patients, an increase of non-synonymous relative to synonymous anticoagulant variants (*SERPINC1*, *PROC*, *PROS1*, *PROCR*, *THBD*) compared with controls (nsyn/syn, 0.95 vs. 0.68). In contrast, non-synonymous procoagulant (*FGG*, *F2*, *F5*, *F9*, *F11*, *KNG1*, *GP6*, *VWF*) variants (nsyn/syn, 0.31 vs. 0.63) showed a decrease in VTE patients compared to controls. This suggests that a deficit of non-synonymous variants in procoagulant genes is a potential novel mechanism contributing to VTE.

7. Candidate VTE genes, expression studies, and animal studies

Anticoagulation and coagulation genes are natural candidate genes for VTE. For instance, several genetic variants have been found among VTE patients in the *THBD* gene [65]. Some of these *THBD* genetic variants also exhibit reduced functional activity [65]. Still, the *THBD* gene remains to be formally linked to VTE [43,44]. A Ser219Gly missense variant in the endothelial protein C receptor (EPCR), i.e. *PROCR* gene, has been linked to VTE [66]. This has been confirmed in the GWAS study by Lindstrom et al [43]. Many other genes involved in the regulation of the coagulation system has not been genetically linked to VTE such as tissue factor pathway inhibitor (*TFPI*), Heparin cofactor II (*SERPIND1*), protein Z (*PROZ*), and genes involved in the fibrinolytic system.

Mouse models have turned out to be useful for understanding the biology of VTE and testing new therapeutics [67,68]. Mouse models have been used to study the hypercoagulability caused by Factor V Leiden [69]. Two recent mouse studies indicated *GAS6/TAM* pathway signaling might be involved in VTE and also that *PCSK9* inhibitors may protect against VTE [70,71]. Thus, these genes and pathways might be worthwhile for further studies. Still, it is important to remember that there are differences between animal models and human studies. For instance, Erickson showed in a transgenic mice study that elevated levels of plasminogen activator inhibitor-1 (PAI-1) contribute to the development of venous but not arterial occlusions [72]. However, so far PAI-1 (*SERPINE1* gene) and other fibrinolytic genes like *PLG*, *PLAT*, *PLAU*, *SERPINF2*, *SERPINB2*, and *CPB2* remains to be genetically linked to VTE risk in humans [43,44].

8. Pathways involved in the genetic pathogenesis of venous thromboembolism

All classical thrombophilias either involve the coagulation cascade (*F2* and *F5*) or its regulation by anticoagulants, i.e. antithrombin and the protein C system [*SERPINC1*, *PROC*, *PROS1*, *F5*] [73]. However, recent GWAS have expanded and revised the genetic architecture of VTE [39–44,74]. Though weaker associations GWAS shows that besides coagulation and the natural anticoagulants also platelets, erythrocytes, and inflammation are involved in the genetic pathogenesis of VTE (Figures 1 and 2). For some VTE loci identified in GWAS studies no evident link to VTE have been identified.

Pathway analysis is a way to better understand the findings from genetic studies. Pathway analysis may also identify novel VTE candidate genes for further study. Figures 3(a,b) present the result of GeneMANIA analysis of all confirmed genes linked to VTE (<https://genemania.org/>) [75]. The GeneMANIA analysis is based on available genomics and proteomics data. Totally 587 network connections were identified. Twenty genes (filled circles) were identified and they were involved in coagulation (*F3*, *F7*, *F2R*), anticoagulation (*TFPI*, *THBD*, *SERPINA5*), platelets (*GP5*, *GP9*, *GP1 BA*, *GP1BB*, *ADAMTS13*), and immune system (*ITGAD*, *KIR2DL1*, *KIR2DS2*, *KIR2DS1*, *PYCARD*). Some genes had unknown potential relations to VTE (*HDAC5*, *WHRN*, *USP7*, *FAM175A*).

9. Clinical implications

Thrombophilia screening is performed in many institutions. According to European Society of Cardiology (ESC) 2019 guidelines testing for thrombophilia (including the acquired risk factors antiphospholipid antibodies and lupus anticoagulant) could be considered in patients with VTE at a young age (e.g. aged <50 years) and in VTE patients without any identifiable risk factor, i.e. unprovoked or spontaneous VTE [76]. With unprovoked means VTE occurring in the absence of any major reversible risk factor. A strong family history of VTE further increase the indication for thrombophilia testing. VTE patients who are carriers of some forms of major hereditary thrombophilia, notably those with confirmed deficiency of antithrombin, protein C, or protein S, and patients with homozygous factor V Leiden or homozygous prothrombin G20210A mutation, are potential candidates for indefinite anticoagulant treatment after a first episode of unprovoked VTE. Thrombophilia testing in these cases may help to tailor the regimen of the anticoagulant treatment over the long-term [76]. ESC 2019 guidelines on the other hand point out that, no evidence of a clinical benefit of extended anticoagulant treatment is currently available for carriers of heterozygous factor V Leiden or prothrombin 20210A mutation. There exist clinical risk prediction models but the clinical value and the possible therapeutic implications of these models in the NOAC era are unclear [76]. In addition to estimate the risk of recurrence, physicians also have to take into consideration the risk of bleeding. Incidence estimates from cohort studies conducted more than 15 years ago reported a 3% annual incidence of major bleeding in patients treated with VKAs (Vitamin

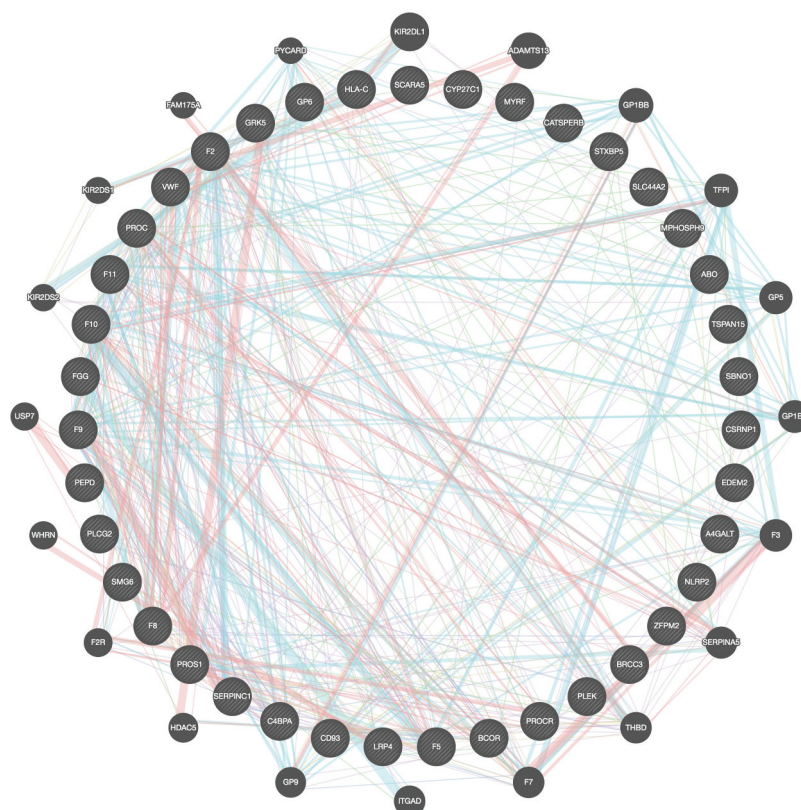


Figure 3A. GeneMANIA analysis [75] showing 39 genes (dashed circles) linked to venous thromboembolism (dashed circles) [23–25,43,44] and 20 related genes (filled circles) identified by GeneMANIA. Color of the 587 lines denotes different network connections: pathways (turquoise), co-expression (purple), physical interactions (red), co-localization (blue), shared protein domains (yellow), and genetic interaction (green). *OSMR-AS1* and *RP11-122C5.1* could not be found by GeneMANIA.

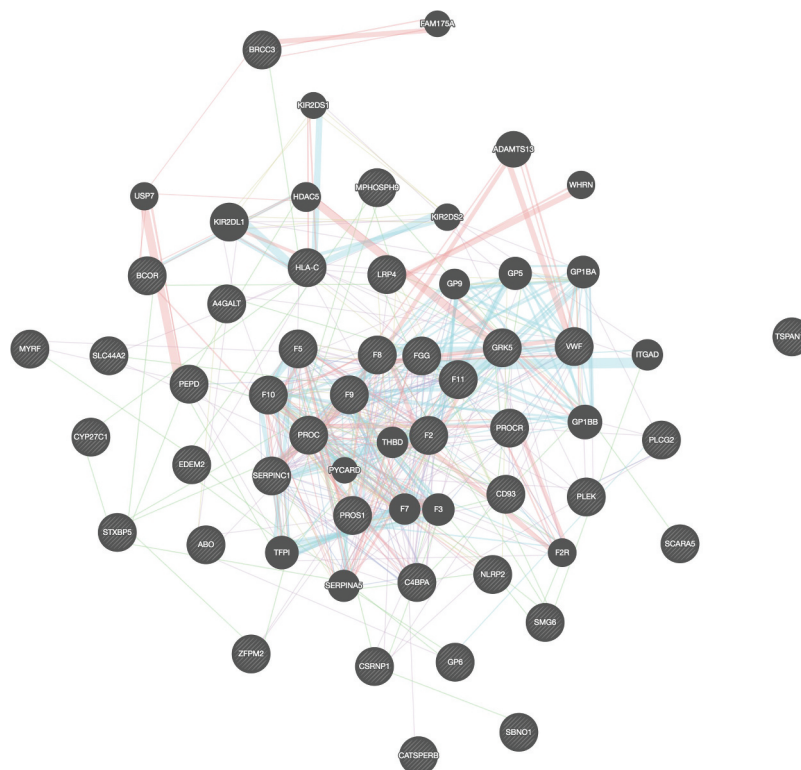


Figure 3B. GeneMANIA analysis [75] showing the 587 connections between 39 genes (dashed circles) linked to venous thromboembolism (dashed circles) [23–25,43,44] and 20 related genes (filled circles) identified by GeneMANIA. Only *TSPAN15* has no connections to any other gene. The twenty identified genes by GeneMANIA involve coagulation (*F3*, *F7*, *F2* R), anticoagulation (*TFPI*, *THBD*, *SERPINA5*), platelets (*GP5*, *GP9*, *GP1 BA*, *GP1BB*, *ADAMTS13*), immune system (*ITGAD*, *KIR2DL1*, *KIR2DS2*, *KIR2DS1*, *PYCARD*), and genes with unknown potential relation to VTE (*HDAC5*, *WHRN*, *USP7*, *FAM175A*).

K antagonists) [76]. However, meta-analysis indicates a 40% lower major bleeding risk for novel oral anticoagulants (NOAC) compared with VKAs [77]. In agreement with these meta-analysis Agnelli et al have shown that extended anticoagulation with apixaban at either a treatment dose (5 mg) or a thromboprophylactic dose (2.5 mg) twice daily reduced the risk of recurrent VTE without increasing the risk of major bleeding [78]. More detailed reviews of the management of classical thrombophilia have been published by Campello and coworkers and is recommended for further reading [79,80].

It is most likely that in the future weighted GRS using GWAS detected VTE risk variants may turn out to be useful for risk assessment instead of using individuals risk variants such as factor V Leiden or prothrombin 20210A mutation [43,44]. Moreover, high-throughput nucleotide sequencing is most useful for identifying individuals with mutations in the natural anticoagulant genes and should be considered to be included in thrombophilia assessment (i.e., *SERPINC1*, *PROC*, *PROS1*) [60].

9.1. Combined oral contraceptives and classical thrombophilia

The Agency for Healthcare Policy and Research and the World Health Organization (WHO) advise patients with thrombogenic mutations to avoid combined oral contraceptives (COCs) [81–83]. They recommend, for example, that ‘a personal history of VTE or a known thrombogenic mutation are conditions that represent an unacceptable health risk if combined hormonal contraception is used’ [81–83]. However, present guidelines including WHO recommend against universal screening due to the low prevalence of thrombophilia and the high cost of screening [83–86]. A systematic review by van Vlijmen et al indicates that the absolute VTE risk of heterozygosity for either the factor V Leiden or the prothrombin-G20210A mutation is modest, when no other risk factors are present (e.g., family history) [87]. By contrast, the study discourages COC-use in women with antithrombin deficiency, protein C deficiency, protein S deficiency, double heterozygosity or homozygosity of factor V Leiden and prothrombin-G20210A mutation [87]. It remains to be resolved whether a universal determination of GRS before COC-use could be useful for better risk assessment.

10. Conclusions

GWAS studies have added a number of weak loci to the strong classical five thrombophilia. Not only coagulation and anticoagulation but also platelets, erythrocytes, and the immune system and a number of genes with unknown linkage to VTE are involved in the genetic pathogenesis of VTE. GRS will be helpful instruments for risk assessment. Hopefully, even larger GWAS and especially WES and WGS in combination with bioinformatic tools will resolve the remaining heritability of VTE.

11. Expert opinion

The most important genetic risk factors for VTE (ie, classical thrombophilia) are all related to coagulation (*F2* and *F5* genes)

or the anticoagulation system (*SERPINC1*, *PROC*, and *PROS1* genes) (Figures 1 and 2). However, the recent two large GWAS studies added several novel gene loci all involved directly or indirectly in platelet biology, erythrocyte biology, or inflammation (Table 1 and Figure 2) [43,44,74]. The GWAS study by Lindstrom et al also used Mendelian randomization and showed that some blood traits are causally associated with VTE risk [43]. Thus, platelets that before having been implicated mostly in arterial thrombosis are also of importance for VTE [43,44,74]. As erythrocytes also are constituents of thrombi it is possible that certain erythrocyte traits could increase the VTE risk [88,89]. For instance, in a large, population-based cohort study a graded independent association between red cell distribution width (RDW) and risk of first event of VTE was observed [89]. This relationship was independent of potential confounders including hematological, multiple biological, socioeconomic, and lifestyle factors. Thus, evidence is accumulating that the formed elements in the blood need more attention from researchers in the field of VTE [43,44,74,88,89]. Inflammatory disorders, such as infection and immune-mediated diseases, have been linked to VTE [90]. However, the two recent GWAS studies are the first to link several genes involved in inflammation to VTE underlining the importance of inflammation in VTE pathogenesis [43,44,74]. Whether inflammation per se is a driver of thrombosis or whether inflammation induced VTE is due to its effects on coagulation, anticoagulation, and platelets remains to be determined.

With increasing number of cases and controls more risk SNP for VTE will probably be identified in future GWAS studies. The effect size of these risk variants will probably all be minor. A natural development of these GWAS detected variants will therefore be the development and validation of separate GRS both for primary VTE and recurrent VTE. The combination of GRS with acquired risk factors will be useful for better risk assessment and tailored prophylaxis of both primary VTE and recurrent VTE. Another avenue will be the detection of novel risk loci due to enrichment of rare risk variants using WES and WGS technology. Though each variant is rare it is possible that together these variants will make a considerable contribution to the missing heritability of VTE.

GWAS studies have identified many novel weak VTE variants [43,44]. With even larger study size even weaker VTE variants might be detected. The question is however, if there are more rare variants causing VTE in other genes than *SERPINC1*, *PROC*, and *PROS1*. An important tool for the success is the development of NGS enabling WES and WGS in combination with bioinformatic tools. Maybe in the near future knowledge of all genetic risk factors in an individual may lead to relevant genetic risk estimation. This will likely include GRS and also rare variants. However, it should be noted, that several acquired risk factors such as surgery and pregnancy confer even higher risks than most genetic variants associated with VTE. As acquired risk factors often are transient, they offer the best opportunity to reduce the burden of VTE by improved and individualized anticoagulant prophylaxis. In this context a better estimate of genetic risk may help for tailoring individualized anticoagulant prophylaxis. Risk scores including the total burden of acquired risk factors (even

transient) and genetic risk burden must be the goal in order to tailor adequate treatment, prevention and prophylaxis.

Of special interest will be the research studying the importance of VTE risk factors such as FV Leiden and peripheral artery disease [44,50]. We early on reported about arterial thrombosis in APC-resistant patients [91–93] and also among protein S deficient patients [94], and now it seems that at least FV Leiden is an important graded risk factor for peripheral artery disease [44,50]. This will also be an important research field.

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Declaration of interest

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