

INVITED REVIEW

Current knowledge on the genetics of incident venous thrombosis

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Summary. The genetic burden underlying venous thrombosis (VT) is characterized by a sibling relative risk of 2.5 and a strong heritability whose estimates varied from 35% to 60% according to different studies. However, the genetic factors identified so far only explain about 5% of VT heritability and just 16 genes have been robustly associated with the susceptibility to VT, most of them affecting the coagulation cascade. Eight of these have been identified during the last 5 years, thanks to the development of high-throughput micro-array genotyping technologies, which have radically changed the research landscape in human genetics. The present work is aimed at providing a historical review of the known genetic factors contributing to VT risk, as well as discussing future research strategies to follow to disentangle the whole spectrum of genetic variants associated with VT.

Keywords: coagulation, genetics, heredity, pulmonary embolism, venous thrombosis.

Introduction

As early as 1856, Virchow discussed three broad categories of factors contributing to venous thrombosis (VT), including alterations in the blood flow, changes in the constitution of the blood, and changes in the vessel wall [1]. Each year, approximately one in 1000 individuals in industrialized countries develop deep vein thrombosis (DVT) of the lower extremities [2,3]. Between 1% and 2% of these patients die from its complication, pulmonary embolism (PE), and about 25% suffer the chronic

effects of the post-thrombotic syndrome. VT (including DVT and PE) is a multifactorial disease with both established environmental and genetic risk factors [4]. The genetic burden underlying VT is characterized by a sibling relative risk of 2.5 and a strong heritability whose estimates varied from 35% to 60% according to various studies [5–7]. However, the genetic factors identified so far explain only about 5% of VT heritability [8]. From 1965 to 2013, 16 genes/loci have been robustly associated with the susceptibility to VT (Fig. 1), most of them affecting the coagulation cascade. The recent availability of high-throughput genotyping technologies and their application in the framework of genome-wide association studies (GWAS) have enabled the identification of eight of them. In the present review, we adopted a historical perspective to describe all genetic risk factors known so far for VT.

1965 – *SERPINC1* gene

Egeberg was the first to identify a genetic risk factor for VT, antithrombin (AT) deficiency, by studying a family with many affected relatives with VT [9]. AT belongs to the serpin family and regulates coagulation by inhibiting procoagulant serine proteases such as thrombin, activated (a) factor X and IXa [10]. AT deficiency is due to private mutations (i.e. present in < 0.001 of the population) in the *SERPINC1* gene encoding AT, with more than 130 different mutations reported and described in the gene database (<http://www1.imperial.ac.uk/departmentsofmedicine/divisions/experimentalmedicine/hematology/coag/antithrombin/>). AT deficiency is relatively rare in the general population (0.02%) and is associated with a relative risk of VT around 10 [11]. In addition to AT deficiencies, decreased AT plasma levels have been associated with VT risk in a dose–response manner [12], suggesting that common single-nucleotide polymorphisms (SNPs) modulating AT levels variability could also be good candidates for VT risk. Consistent with this hypothesis is the reported association of the *SERPINC1* rs2227589 polymorphism with both AT levels [13] and DVT risk [14]. With this in

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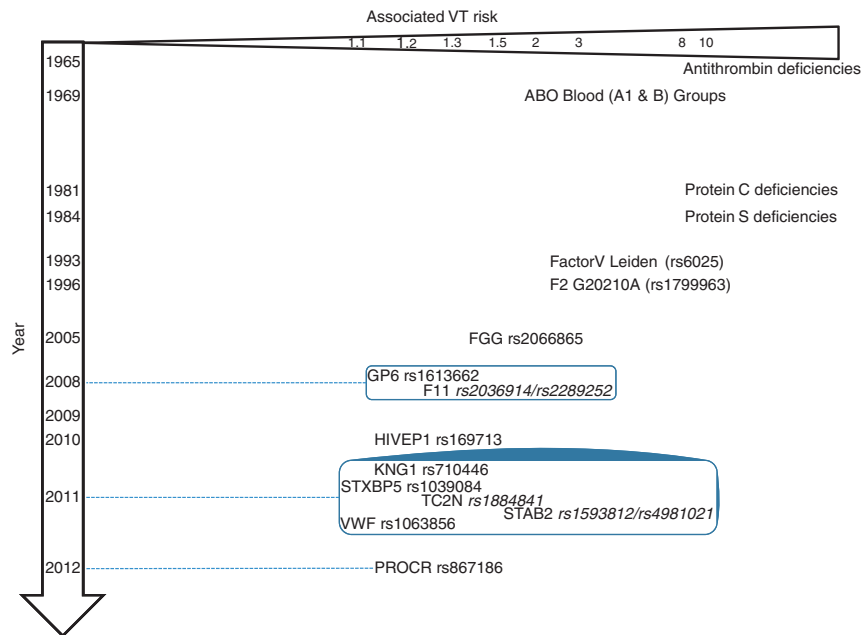


Fig. 1. Historical perspectives of known genetic risk factors for venous thrombosis. SNPs in italic are markers for yet unidentified functional variants.

mind, we conducted a GWAS in ~900 individuals in the MARTHA project to identify common SNPs (i.e. with minor allele frequency MAF > 5%) associated with altered AT plasma levels [15]. However, we were unable to robustly identify any common SNP that could explain even 5% of plasma AT variability.

1969 – *ABO* locus

Jick *et al.* [16] were the first to report that non-O blood group was associated with increased risk of VT. Recent studies [17–19] have clarified this association and demonstrated that B and A1 blood groups were at higher risk, with a relative risk around 2, than O and A2 blood groups. The latter two groups can be genetically characterized by the rs8176719 and rs8176750 whose minor alleles, respectively, tag for O and A2 and have been consistently found at lower frequency in VT patients than in controls in the three GWAS reported so far on VT [8,18,19]. The *ABO* rs8176719, a deletion of one nucleotide in exon 6, leads to a reading frameshift and premature termination of the polypeptide before the N-terminal catalytic domain, producing the functionless O allele. The *ABO* rs8176750 that is also a deletion of one nucleotide results in a frameshift that abrogates the wild-type stop codon within exon 7 and likely leads to an elongated transcript and an absent or dysfunctional protein similar to blood type O [20]. Interestingly, in-depth analysis of the *ABO* locus has recently identified several intronic and 3'UTR SNPs with evidence for association with VT that could be independent of the

rs8176750 and rs8176719 [19,21]. These results emphasize the known complexity of the link between *ABO* locus and VT risk.

ABO blood group is believed to contribute to VT risk through modifications of von Willebrand Factor (VWF) and factor VIII (FVIII) levels in plasma, likely by affecting VWF glycosylation and thus its proteolysis and clearance [22,23]. However, several studies have shown that *ABO* blood groups remain significantly associated with VT even after adjustment for FVIII or VWF levels [24,25]. This suggests that *ABO* may affect cardiovascular risk by other means than solely modifications of FVIII and VWF molecules. Several biological arguments support the existence of a VWF-independent pathway linking *ABO* to human diseases. First, although traditionally regarded as red blood cell antigen, *ABO* antigens are also expressed on various other tissues including platelets, the vascular endothelium, and epithelial surfaces [22]. Second, the *ABO* locus was found associated with plasma levels of soluble intercellular adhesion molecule-1, P-selectin, and E-selectin [26], three intermediate phenotypes of cardiovascular risk. Consistent with these results is the association of *ABO* locus with coronary artery disease (CAD) risk observed in the recent GWAS on CAD [27]. Of note, the *ABO* locus has also been identified through GWAS as a susceptibility locus for different type of cancer [28], a situation prone to VT occurrence.

High plasma levels of FVIII and VWF are well-established risk factor for VT, even after adjusting for *ABO* blood group [29] which explains about 25% of the vari-

ability of these two traits. Genetic variation influencing the variability of these phenotypes could also influence the risk of VT. This is the case for the rs1063856 SNP in the structural *VWF* gene, resulting in a Thr789Ala substitution in exon 18 that is associated with both VWF levels and VT risk. The rs1063856-C allele (Ala789) is associated with higher VWF levels and a higher risk (odds ratio (OR) ~ 1.15) for VT [30]. The meta-analysis of several GWAS datasets conducted under the CHARGE consortium [31] identified six novel loci modulating VWF plasma levels, *CLEC4M*, *STX2*, *TC2N*, *STXBP5*, *SCARA5*, and *STAB 2* among which the latter three also influenced FVIII levels. These loci were then natural candidates for VT risk. None of the *VWF*-associated SNPs at the *CLEC4M*, *STX2*, and *SCARA5* have been found so far associated with VT risk. Conversely, *STAB 2* [8,32], *TC2N* [33], and *STXBP5* [31] SNPs have been observed associated with VT risk. While there is yet no evidence for a functional role of the *STAB 2* and *TC2N* VT-associated SNPs, the identified *STXBP5* SNP, rs1039084, involves an Asn436-Ser substitution suggesting it could be functional. Evidence for a role of *STXBP5* in the regulation of VWF has been recently reinforced by the finding that *STXBP5* SNPs were associated with bleeding phenotype in women with type 1 von Willebrand disease [34]. Worthy of note is the association between *STX2* SNPs with the risk of arterial thrombosis [35]. Syntaxin 2 (*STX2*) is a binding substrate for the syntaxin-binding protein 5 (*SXBP5*) and is a member of the soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein receptor (SNARE) protein family. These proteins drive vesicle exocytosis by fusion of granules and target membranes, a process involved in the regulation of numerous secretory events [36]. *STXBP5* and *STX2* interact specifically with SNARE complex proteins, such as SNAP23 and syntaxin-4. These proteins have been shown to be involved in Weibel-Palade Body exocytosis, the well-known mechanism for the secretion of VWF molecules by endothelial cells [37].

1981 – *PROC* gene

Griffin *et al.* [38] described a heterozygous protein C (PC) deficiency in a family with a history of recurrent VT. PC circulates in plasma as an inactive zymogen. It is activated at the endothelial surface by the membrane-bound thrombin–thrombomodulin complex [39]. When activated PC (APC) is bound to its cofactor protein S (PS), it inactivates the procoagulant FVa and FVIIIa, limiting the coagulation cascade and fibrin formation. PC deficiency is due to private mutations in the *PROC* gene encoding PC and occurs in one in 2000–4000 of the adult population [11]. The thrombotic risk associated with heterozygous PC deficiency is approximately 8 [11].

The database published on behalf of the ISTH coagulation inhibitor subcommittee lists more than 160 different mutations (<http://www.hgmd.cf.ac.uk>).

In the general population, a low level of circulating PC is associated with increased risk of VT [40]. Several SNPs (including rs1799810, rs2069910, and rs2069915) located in the promoter of the *PROC* gene have been shown to mildly influence PC plasma levels in candidate gene approaches [41–43]. Their association with the risk of VT deserves in-depth investigation. A GWAS performed within the ARIC Study confirmed the association of *PROC* SNPs with PC levels but additionally identified the *GCKR* and *PROCR* loci as influencing plasma PC levels [44]. Very interestingly, the strongest association was observed at the *PROCR* locus encoding the endothelium protein C receptor (EPCR). PC activation is enhanced approximately 20-fold when PC binds to EPCR [45]. The *PROCR* SNP that influences the most PC levels is the rs866186 resulting in a serine-to-glycine substitution at codon 219 in the membrane-spanning domain of EPCR. This variant is known to explain between 56% and 87% of the variations in soluble EPCR levels [43,46–49]. The G allele tags the A3 haplotype (4 *PROCR* haplotypes have been identified in whites) and is associated with increased shedding of EPCR from the endothelial membrane, both by rendering the receptor more sensitive to cleavage and by leading to a truncated mRNA through alternative splicing [50,51]. In addition to its effect on PC activation, EPCR also works to limit thrombus formation by binding FVII/FVIIa, facilitating the clearance of FVIIa, and limiting downstream activation of factor X [52,53]. Accordingly, the rs867186 was found to be associated also with FVII plasma levels and prothrombin time (PT), which measures the clotting time from the activation of FVII, and reflects the integrity of the extrinsic and common coagulation pathways [31,54]. Despite evidence supporting a functional role for the rs867186 SNP, its direct association with VT risk has been questionable. However, a recent meta-analysis relying on a systematic review of all available case–control studies that had investigated the association of rs867186 and VT demonstrated a mild but robust association [55]. The increased risk associated with the rs867186-G allele (Gly219) was 1.22.

1984 – *PROS1* gene

The first VT patients with PS deficiency were described by Comp *et al.* [56]. PS functions as a non-enzymatic cofactor to APC in the inactivation of factors Va and VIIIa. In the circulation, PS forms inactive complexes with C4b-binding protein (C4b-BP). Free (f-) PS represents approximately 40% of the total circulating level, and only this fraction has APC cofactor activity. As for AT and PC deficiencies, PS deficiency is due to rare and private mutations within *PROS1* gene (database available on line:

<http://www.hgmd.cf.ac.uk>). The relative risk associated with heterozygous PS deficiency is approximately 8 [57]. As for AT and PC levels, the risk of VT increases with decrease levels of PS [12]. Two GWAS have been conducted to identify common SNPs associated with PS levels, in the MARTHA [15] and GAIT [58] studies. These two studies failed to detect robust associations despite some suggestive elements in favor of an association of *DNAJC6* SNPs with f-PS levels [58].

The GAIT study has also been subject to a GWAS focusing on the C4b-BP levels [59]. C4b-BP is a heterodimeric molecule existing in three different isoforms $\alpha7\beta1$, $\alpha6\beta1$, and $\alpha7\beta0$; only isoforms with a β chain, which normally represent 80% of circulating C4b-BP, being able to bind PS. This work showed that the rs3813948 at the *C4BPB/C4BPA* locus *C4BPA* and *C4BPB* genes coding for the α - and β -chains, respectively, explained about 10% of plasma levels of $\alpha7\beta0$ and 10% of *C4BPA* monocyte mRNA expression. Interestingly, the rs3813948-C allele associated with increased levels of both $\alpha7\beta0$ and *C4BPA* expression was further found associated with increased risk (OR ~1.20) of VT in two French case-control studies [59]. This association with VT has, however, not yet been replicated in other studies. As the rs3813948 was not associated with PS levels but only with $\alpha7\beta0$ levels, which do not carry PS, this emphasizes the previously raised possibility that C4b-BP can be independently active in the coagulation pathway [60]. C4b-BP also belongs to complement inactivator proteins which are thought to be involved in immune response and inflammation, and this could favor a role of inflammation in VT-related mechanisms [61].

1993 – F5 gene

Dahlbäck *et al.* [62] described three families in whom APC did not yield the expected prolongation of the clotting time in an activated partial thromboplastin time (aPTT) assay; this defined a new phenotype, called APC resistance (APCR). The study by Bertina *et al.* [63] later showed that APCR co-segregated with the *F5* gene, in particular with a single mutation (FV Leiden, Arg506Gln, rs6025) affecting one of the APC cleavage sites. In Caucasians, the frequency of the so-called FV Leiden mutation is around 5%. FV Leiden affects 15%–25% of patients with VT, and the risk of VT in heterozygous carriers is approximately fivefold higher than in a control population [11]. More recently, by in-depth analysis of *F5* gene, Smith *et al.* [64] identified the rs4524 variant, a non-synonymous substitution of lysine (K) for Arginine (R) at amino acid position 858, as a new VT-associated SNP. The rs4524 SNP in *F5* was consistently associated with DVT in three large Dutch case-control studies [65]. This variant is not in linkage disequilibrium with the FV Leiden mutation and is associated with an increased risk of VT of 1.21. In the two French GWAS conducted on VT,

the allelic OR associated with rs4524 was 1.17 ($P = 0.013$; PE Morange, DA Tregouet, unpublished data). The functionality of the rs4524 remained to be addressed.

1996 – F2 gene

By extensively screening the prothrombin gene (*F2*), an obvious candidate for VT in 28 families with unexplained venous thrombosis, Poort *et al.* [66] identified a nucleotide G to A transition at position 20210 in the 3'UTR region (rs1799963). In 474 unselected patients with a first episode of VT and 474 controls of the LETS study, the frequency of the rs1799963-G rare allele was 6.2% and 2.3%, respectively [66]. Prothrombin (FII) has procoagulant, anticoagulant, and antifibrinolytic activities after its activation into thrombin by the prothrombinase complex. Thrombin acts by activating factors XIII, XI, and VIII, V, PC, and the thrombin-activatable fibrinolysis inhibitor and by cleaving fibrinogen to form fibrin. Carrying the *F2* 20210A allele is associated with a 3- to 4-fold increased risk of VT [11]. The *F2* 20210A allele is associated also with slightly increased plasma levels of FII leading to increased thrombin generation, which results in a hypercoagulable state. The mutation contributes also to the regulation of the APC anticoagulant pathway [67]. Another SNP *F2* rs3136516 (A19911G) has been reported to be associated with elevated FII levels [50,68]. An *in vitro* study [69] demonstrated that the rs3136516 (located within the 13th intron) is also functional through its effect on an intronic splicing enhancer motif. Its association with the risk of VT deserves in-depth investigation.

2005 – FGG gene

Fibrinogen is a key component of the hemostatic system, playing a role in both primary and secondary response [70]. Thrombin-catalyzed cleavage of fibrinopeptides (Fp) A and B converts fibrinogen into fibrin, which spontaneously polymerizes, forming the fibrin clot. The fibrinogen molecule is a disulfide-bonded dimer each consisting of three polypeptides termed $A\alpha$, $B\beta$, and γ . The three polypeptides are encoded by separate genes, *FGA*, *FGB*, and *FGG*, clustered in a region of approximately 50 kb on chromosome 4q31.3 [71]. The fibrinogen γ chain mRNA transcript is subject to alternative processing and polyadenylation [72]. The main form, the γA chain, consists of 411 amino acids, comprising 10 exons and 9 introns. For this chain to arise, polyadenylation occurs at the polyadenylation signal downstream of exon 10. The alternative γ' chain arises when polyadenylation occurs at an alternative polyadenylation signal in intron 9. Surprisingly, it appears that the relationship between $\gamma A/\gamma'$ fibrinogen level and thrombosis may depend on the type of vascular disease, whereby the γ' chain associates with a prothrombotic risk in arterial disease, but an antithrombotic effect in VT [73,74]. Patients with VT have reduced

γ A/ γ ' fibrinogen levels and reduced γ ' fibrinogen/total fibrinogen ratio compared with healthy controls [74]. Individuals with a γ ' fibrinogen/total fibrinogen ratio below the 10th percentile, as measured in the control group, had a 2.4-fold increased risk of VT in the Leiden Thrombophilia Study. By scanning the genes of the fibrinogen cluster, the rs2066865 SNP (10034C>T) was observed associated with reduced γ A/ γ ' fibrinogen levels [74]. The T allele associated with reduced γ A/ γ ' fibrinogen levels is robustly associated with a 1.47 increased risk of VT [74]. A GWAS recently performed on γ ' fibrinogen plasma levels demonstrated that the genetic loci associated with γ ' fibrinogen levels are all located in or near the fibrinogen gene locus, the strongest association being observed with the *FGG* rs2066865 [75].

2007 – *F11* gene

Meijers *et al.* [76] initially demonstrated in 2000 that high FXI levels were associated with elevated risk of VT. Plasma FXI circulates as a zymogen in human plasma as a complex with high molecular weight kininogen (HK) [77]. FXI contributes to hemostasis by activating FIX. FXI can be activated by four biologically relevant proteases: FXIIa, FXIa, thrombin, and meizothrombin. It has been suggested that the more relevant *in vivo* pathway for activation of plasma FXI might be the feedback activation by thrombin [78]. Later, in 2007, several SNPs at the *F11* locus were reported to be associated with risk of VT (with OR ~1.35) through modulation of FXI plasma levels [14,28,64]. The *F11* functional SNP(s) responsible for this association remain to be clearly identified, but an haplotype analysis of the *F11* locus revealed the existence of a ying/yang haplotype structure containing the culprit variant(s) [8].

2008 – *gp6*

GP6 is the first VT-associated locus that lies outside the standard coagulation cascade. It was identified through a large-scale association study, focusing mainly on non-synonymous polymorphisms [14]. *GP6* encodes the receptor glycoprotein (GP) VI that has a major role in collagen-induced signaling. The rs1613662, which refers to an A/G substitution in amino acid 219 resulting in a serine to a proline substitution, was found to be associated with the risk of VT in several other studies [18,79]. A carriers having a 15% increased risk of VT. The potential mechanism in VT of this Ser219Pro change of the collagen receptor GPVI is unknown. However, it has been shown to reduce, in a dose-dependent manner, collagen-induced platelet activation [80]. The identification of *GP6*, as VT-associated locus, is striking as it adds to several lines of research indicating that platelets play a determining role in VT although they have historically been ignored in this pathological setting. Activated platelets are important catalysts of both intrinsic and extrinsic thrombin generation

and, therefore, fibrin deposition [81]. Moreover, the use of aspirin may decrease the risk of first and recurrent VT [82,83].

2010 – *HIVEP1* gene

The *HIVEP1* locus was identified as a novel susceptibility locus for VT through a multi-stage genetic association study where the most promising, but not genome-wide significant results of a first GWAS on VT [18] were assessed for replication in three additional studies. This work relying on 5862 VT patients and 7112 healthy individuals revealed that the *HIVEP1* rs169713-C allele was associated with an increased risk for VT of 1.20 [84]. *HIVEP1* encodes a protein that participates in the transcriptional regulation of inflammatory target genes. The human immunodeficiency virus type 1 enhancer-binding protein 1 (*HIVEP1*/*PRDIIBF1*/*MBP-1*) is part of the *ZAS* gene family, in which a characteristic pair of C2H2 zinc fingers is linked to an acidic-rich and serine/threonine-rich region. *HIVEP1* has been shown to bind nuclear factor kappa-light-chain-enhancer of activated B cells consensus sequences and related motifs within the enhancer element of human immunodeficiency virus type 1 and promoter regions of immunoglobulin kappa, major histocompatibility complex class I, interferon- β , and interleukin-2 receptor [85–88]. A recent haplotype analysis suggested that the effect on VT risk of the rs169713 was due to its linkage disequilibrium with other *HIVEP1* SNPs suggesting that the functional(s) SNP remain to be identified [8]. *HIVEP1* identification as a locus of susceptibility for VT underlines again the association between inflammation process and VT (see section on *PROS1*).

2011 – *KNG1* gene

Shortened aPTT levels have been shown to be a reliable predictor of VT [89]. In 2010, Houlihan *et al.* [90] identified the *KNG1* gene, encoding HK, as a major susceptibility locus influencing aPTT levels. The top SNP of the *KNG1* gene for the association with aPTT levels was the rs710446 (Ile581Thr) [54]. In 2011, we demonstrated that the Thr581 allele, originally found in the GWAS associated with decreased aPTT levels, was also associated with increased risk of VT with an OR of 1.19 [91]. HK plays an important role in blood coagulation by positioning prekallikrein and FXI near FXII [92]. In addition, *KNG1* knockout mice demonstrate prolonged aPTT and delayed arterial thrombosis [93]. Moreover, antibodies against mouse FXI that directly interfere with the FXI-HK interaction prevented arterial occlusion induced by FeCl₃ to a similar degree as complete FXI deficiency [94]. More recently, by adopting a GWAS strategy, Sabater-Leal *et al.* [95] have shown that the rs710446 SNP was also associated with plasma FXI concentration in the GAIT study.

Future research

The GWAS approach has been very powerful to identify novel susceptibility loci to human complex diseases. In most cases, though, identified risk alleles explain only a small percentage of the heritability of the disease. These findings support the hypothesis of a polygenic framework underlying complex diseases in which susceptibility is due to a large number of cumulative mainly weak effects brought by variants covering the whole spectrum of allele frequencies. VT is not an exception to the rule, as established susceptibility loci are hypothesized to explain <5% of the VT heritability. All VT susceptibility alleles identified in the framework of GWAS have frequencies higher than 0.10 and are characterized by allelic ORs for VT of 1.15 or more. In the field of arterial thrombosis, common SNPs associated with OR as low as 1.04 were detectable through the use of a collection of more than 60 000 patients [96]. Similar efforts have been initiated in the field of VT genetics to perform a meta-analysis of all available GWAS datasets. Even if it is unlikely that more than 10 000 VT patients will be assembled, such a project will undoubtedly identify additional common SNPs with lower risk of disease. Besides, coupled with the use of imputation techniques relying on the latest public databases reporting genetic information on less common (MAF between 1% and 5%) (e.g. <http://www.1000genomes.org/>), this project will also be able to detect rarer SNPs expected to be associated with higher risk of disease [8]. Another attractive strategy to identify less common variants associated with higher risk of disease is to perform target re-sequencing of the whole sequence of a known susceptibility gene [97,98]. All genes we discussed in this review are obviously good candidates for re-sequencing. Supporting this hypothesis are the identification of novel rare variants in *F2* and *F9* (encoding FIX) responsible for VT phenotype in families [99,100]. A preliminary step toward this direction has been made by the group of Lotta *et al.* [101] who have just set up a pilot study investigating the coding sequences of ~200 candidate genes using a next-generation sequencing approach. Would one be more interested in an agnostic search for rare variants, the application of the latest exome chip array enriched for rare coding SNPs discovered by whole-exome-sequencing projects would be a nice and promising approach [102]. While numerous whole-exome- and whole-genome-sequencing projects have demonstrated the power of the next-generation sequencing technology to identify very rare variants responsible for familial forms of human diseases [103–105], their application to complex diseases, including VT, is still questionable. Nevertheless, such strategy may be efficient if applied to idiopathic patients belonging to families with multiple affected relatives.

To date, the genetics of VT has been studied most frequently in patients with European ancestry. Studies of

hospital discharge data and large observational cohorts show that the incidence of VT varies by race, with blacks having the highest rates, followed by whites and then Hispanics and Asians. The overall incidence of VT is 30–60% higher in blacks than in whites [106–109]. Many genetics variants known to affect risk of VT have been investigated to determine whether they might confer a disproportionate risk of VT in blacks; however, none of these known defects have been clearly shown to contribute significantly to the increased risk [109]. Blacks have the highest levels of FVIII and VWF among all ethnic groups studied [110]. In addition, markers of activation of the coagulation system such as plasma D-dimer are higher in blacks [111]. Whether these findings represent an underlying genetic defect or are simply markers of increased coagulation due to another cause is unknown.

In previous work [32], we discussed the possibility that gene–gene and gene–environment interactions, copy-number variations, genome-wide transcriptomic analysis, epigenetics, and metabolomics could help in disentangling the genetic and genomic architecture underlying VT susceptibility. So far, such approaches failed to yield any promising findings [112].

Genetic testing in clinical setting

Screening tests are performed to identify individuals at risk of a preventable disease or complications of the disease. In individuals with a personal history of VT, genetic screening would be considered if the results of such testing provide information that would influence treatment decisions. In asymptomatic individuals (i.e. those without a personal history of VT), screening would only be considered if the following criteria are met:—genetic defect occurs at a high frequency in the general population;—the penetrance of the defect is high;—safe and effective methods for prevention of VT are available. In both cases, genetic testing is at present of limited value.

In the context of VT, the genetic screening also called thrombophilia screening nowadays includes the search for AT, PC, PS deficiency, Factor V Leiden, and F2 20210A. This list of tested risk factors has not changed since the discovery of the F2 G20210A in 1996. Despite the publications of several recommendations, there is no consensus yet of the subjects who qualify for this thrombophilia screening, the type of laboratory test to use, or the clinical treatment of patients with thrombophilia [11,113–120]. In individuals with a personal history of VT, the presence of an inherited thrombophilia has no effect on the intensity nor on the duration of anticoagulation treatment because those disorders have no or very limited influence on either the response to anticoagulant agents or the rate of recurrence [121,122]. However, for selected patients, knowledge of an inherited thrombophilia might affect management as, for example, in the setting of pregnancy [123,124]. Genetic counseling in families with VT based

on the screening for these five defects is also still debated. Indeed, identifying the defect in asymptomatic family members from a proband with VT would identify those at high risk and therefore allow avoidance of high-risk situations or facilitate targeted prophylaxis at time of unavoidable high risk. However, despite harboring the same gene mutation, affected individuals even within families frequently exhibit marked clinical heterogeneity. As an example, the penetrance of the FV Leiden is low with only 10% of heterozygotes and 80% of homozygotes develop VT in their lifetime, with varying severity among affected individuals. Moreover, in these families, individuals without the defect also have a markedly increased risk of VT compared with the general population, reflecting a selection of families with a strong thrombotic tendency in which as-yet-unknown thrombophilic defects have co-segregated. Last, family history of VT remains significantly associated with the risk of VT even after adjustment for the thrombophilic defects [125]. We have recently shown that ABO blood group and VWF plasma levels partially explained the incomplete penetrance of congenital thrombophilia within these families [25].

As discussed above, the list of weak but common genetics risk factors have slightly increased. Even if each VT-associated SNP has little predictive value *per se*, their combination may improve the predictive ability and could be used to model susceptibility to VT. A first attempt has been published to examine the potential of multiple SNP analysis for the prediction of recurrent VT. Fifteen SNPs known at this time to be either established or putative risk factors for VT were studied [126]. While none of the individual SNPs were more than weakly associated with the risk of recurrence VT, a genetic score based on the number of at-risk alleles carried by each individual was derived and shown of promising added predictive value. The hazard ratios for recurrence increased according to the number of at-risk alleles, by a 1.7-fold increased risk for carriers of the two alleles with strongest effects, a 2.7-fold risk for carriers of the three strongest associated alleles, and 5.1-fold risk for carriers of four alleles. Thus, although there was an increased risk of recurrent VT for carriers of several genetic variants, the clinical utility of this approach was poor, as it did not accurately identify individuals who will have a recurrence. The predictive ability of multiple SNP analysis has been recently studied for first events of VT using a larger number of SNPs ($n = 31$) in two large population-based case-control studies [127]. Genetic risk scores based on all 31 SNPs or on the five most strongly associated SNPs performed similarly (areas under receiver-operating characteristic curves [AUCs] of 0.70 and 0.69, respectively). For the 5-SNP risk score (which includes rs6025 [*F5*, factor V Leiden], rs1799963 [*F2*, 20210G<A], rs8176719 [*ABO*], rs2066865 [*FGG*], and rs2036914 [*F11*]), the ORs for VT ranged from 0.37 (95% confidence interval [CI], 0.25–0.53) for persons with 0 risk alleles to 7.48 (95% CI, 4.49–12.46) for per-

sons with more than or equal to six risk alleles. The AUC of a risk model based on known non-genetic risk factors was 0.77 (95% CI, 0.76–0.78). Combining the non-genetic and genetic risk models improved the AUC to 0.82 (95% CI, 0.81–0.83), indicating good diagnostic accuracy. In the future, adding newly discovered predictive SNPs to the model may further improve discrimination. However, to become clinically useful, subgroups of high-risk persons must be identified in whom genetic profiling will also be cost-effective. Usefulness of this genetic score should also be evaluated on the risk of recurrence. It is tempting to speculate that the much higher number of SNPs used (35 vs. 17 in the first study) might improve the accuracy of the score particularly if environmental and clinical factors known to be related to the recurrence are added.

All the genetics elements we discussed in this review were addressed without distinguishing DVT from PE, the two main VT manifestations, as it is frequently carried out in current studies aiming at discovering genetic factors underlying VT susceptibility. This could be contradictory to what is already known about these two clinical entities as strong line of evidence suggests that risk factors for PE are different from those of DVT [128]. This emphasizes the need, when possible, to consider PE and DVT as two distinct phenotypes in future studies looking at the genetics of VT. Similarly, further studies specifically addressing the genetics of recurrence of VT are mandatory [129].

Disclosure of Conflicts of Interest

The authors state that they have no conflict of interest.

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