Review

Common genetic risk factors of venous thromboembolism in Western and Asian populations

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ABSTRACT. Venous thromboembolism (VTE) is a multifactorial disorder involving both acquired and genetic risk factors. The common genetic factors in Western populations have been studied and reported for several decades, while studies on Asian populations are relatively scarce. Evidence suggests that the prevalence and genetic risk factors of VTE vary significantly among ethnic populations. In this review, we summarize the common genetic risk factors of VTE in both Western and Asian populations. In addition to the development of DNA sequencing technology, genome-wide association studies have many advantages and are becoming more

important in identifying new genetic risk factors and susceptible loci. They can therefore help in the prediction and prevention of VTE.

Key words: Venous thromboembolism; Genetics; DNA; Asian population; Genome-wide association study;

INTRODUCTION

Venous thromboembolism (VTE) is a disease with high morbidity and mortality. Both acquired and genetic risk factors are involved in VTE. A variety of acquired risk factors, such as age, pregnancy, lactation, long-term confinement in bed or immobilization, surgery, fracture, trauma, oral contraceptives, hormone-replacement therapy, malignancy, and antiphospholipid syndrome, are closely related to VTE (Shaheen et al., 2012). Evidence also indicates that genetic defects play an important role in the occurrence of VTE. To date, over 88 genes have been associated with VTE, and approximately 30% of idiopathic VTE is due to genetic defects (Morange and Tregouet, 2010); the major landmark factors are given in Figure 1 and Table 1. Interestingly, genetic predisposition to VTE has been observed in different ethnic populations (Zakai and McClure, 2011). In North America, VTE appears to be most common in individuals of African descent, with an incidence of 138-141 cases per 100,000 individuals per year, followed by 103-149 cases per 100,000 among Europeans in North America and Europe, and half the incidence in individuals of Hispanic descent compared with Europeans in the US. In contrast, the incidence of VTE in Asian populations is as low as 21-29 cases per 100,000 individuals per year (Stein et al., 2004; Zakai and McClure, 2011). The ethnic variation in VTE is attributed to the genetic disparity among ethnicities. This review focuses on the genetic risk factors for VTE among Western and Asian populations.

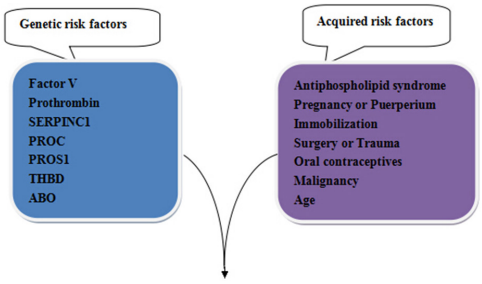

Figure 1. Risk factors of venous thromboembolism (VTE). Genetic risk factors and acquired risk factors synergistically induce VTE.

COMMON GENETIC RISK FACTORS IN WESTERN POPULATIONS

Factor V

Factor V (FV) is a single-chain glycoprotein encoded by the *F5* gene; it is synthesized in the liver and circulates in the plasma as an inactive precursor. After limited proteolysis by thrombin or FXa, FV is converted to its active form FVa, an essential non-enzymatic cofactor of FXa in prothrombin activation. Activated protein C (APC) inactivates FVa by proteolytic cleavage at Arg306, Arg506, and Arg679 (Kalafatis et al., 1994). A polymorphism of FV, R506Q (FV Leiden mutation),

is associated with APC resistance (APCR). FVa Q506 is hard to hydrolyze, and the cleavage activity of APC is reduced by 90% for FVa Q506 compared with the wild type. As a result, the protein retains its role as a procoagulant.

Table 1. Landmarks in genetic research on venous thromboembolism (VTE).

Year	Gene	Main discovery	Reference
1965	*SERPINC1*	Antithrombin III deficiency is the genetic risk factor for VTE.	(Egeberg, 1965)
1969	*ABO* locus	Non-O blood group was associated with increased risk of VTE.	(Jick et al., 1969)
1981	*PROC*	A heterozygous protein C (PC) deficiency in a family is associated with a history of recurrent VTE.	(Griffin et al., 1981)
1984	*PROS1*	The first VTE patients with PS deficiency were described.	(Comp and Esmon, 1984)
1993	*F5*	APCR co-segregated with the F5 gene, in particular with a single mutation (FV Leiden, Arg506Gln, rs6025) affecting one of the APC cleavage sites.	(Griffin et al., 1981)
1996	*F2*	Discovering a novel mutation (a nucleotide G to A transition at position 20210 in the 3' -UTR) from 28 families with unexplained venous thrombosis.	(Poort et al., 1996)
2005	*FGG*	Genetic variation in the fibrinogen gamma gene increases the risk for deep venous thrombosis by reducing plasma fibrinogen gamma' levels.	(Uitte de Willige et al., 2005)
2007	*F11*	F11 was associated with both DVT and coagulation factor XI level.	(Bezemer et al., 2008)
2008	*GP6*	Rs1613662 in GP6 was suspected to be associated with VTE.	(Trégouët et al., 2009)
2010	*HIVEP1*	The HIVEP1 locus was identified as a novel susceptibility locus for VTE.	(Trégouët et al., 2009)
2011	*STXBP5*	STXBP5 was a novel candidate gene for VTE.	(Smith et al., 2011)
2011	*VWF*	VWF rs1063856 was associated with VWF level	(Smith et al., 2011)
2011	*KNG1*	KNG1 Ile581Thr was associated with an increased risk of VTE.	(Morange et al., 2010)
2012	*PROCR*	PROCR rs867186 polymorphism may act in concert with some known genetic risk factors to increase VTE risk.	(Dennis et al., 2012)
2013	*THBD*	THBD rs16984852 was one of the common variants in Chinese population.	(Tang et al., 2013b)

The heterozygous FV Leiden mutation has been found in 90-95% of APCR patients, while only a small group of patients have the homozygous mutation. Compared with heterozygous FV Leiden mutation carriers, the homozygous carriers are much more likely to have VTE. A previous study showed that the relative risk of VTE increased seven times in heterozygous carriers compared with wild-type carriers, while the relative risk was 80 times greater in homozygous carriers (Bertina et al., 1994). The homozygous carriers had a higher risk of thrombosis due to the lack of normal FVa, but both FV Leiden mutation and normal FV existed in the blood of heterozygous carriers (Shaheen et al., 2012). The FV Leiden mutation rate is as high as 2% in Dutch people, while it is rare among Asians.

Prothrombin

Prothrombin, encoded by the *F2* gene, is a single-chain glycoprotein and one of the vitamin K-dependent factors synthesized in the liver. Prothrombin plays an essential role in the process of clotting. After activation to thrombin by the prothrombinase complex, it has procoagulant, anticoagulant, and antifibrinolytic activities (Poort et al., 1996).

A genetic variation of the *F2* gene, G20210A, was found to cause venous thrombosis, following sequencing studies on 28 families with VTE. G20210A is a missense mutation of a nucleotide located in the 3'-untranslated region (Poort et al., 1996). The *F2* G20210A allele is associated with a 3-4-fold increase in the risk of VTE, and slightly increased plasma levels of prothrombin,

resulting in an elevated thrombin level and a hypercoagulable state (Bucciarelli et al., 2013). In the general population, the mutation rate is 2%, and it does not cause VTE.

Extensive gene studies have shown that the G20210A mutation in the prothrombin gene can help to predict the occurrence and recurrence of VTE in Caucasian populations in Europe and America. However, it is still difficult to draw conclusions from the current data (Lindmarker et al., 1999; Christiansen et al., 2005; Lijfering et al., 2010). A case-control study, including 6079 individuals from five large retrospective cohorts, explored the relationship between the FV Leiden genotype and FII G20210A mutations and the risk for recurrent VTE. The results indicate that individuals with homozygous factor V Leiden and/or homozygous prothrombin G20210A or double-heterozygous carriers do not have a higher risk of recurrent VTE. However, single-heterozygous factor V Leiden carriers have a mildly increased risk of recurrent VTE compared with non-carriers.

Antithrombin (AT)

Serpin peptidase inhibitor, clade C [antithrombin (AT)], member 1 (SERPINC1) is a major physiological coagulant that is primarily synthesized in the liver. It comprises a single glycoprotein and belongs to the serpin superfamily. AT regulates coagulation by inhibiting procoagulant serine proteases such as thrombin, Factor X, and IXa.

Egeberg (1965) first discovered a defect of AT in a Norwegian family. AT deficiency is a rare autosomal dominant disorder with 0.09-6.4% prevalence in Asian populations, and it has a prevalence of 1.7-9.64% in Asian patients with VTE (Yin and Miyata, 2014). Perry et al. (1991) discovered a Cambridge II mutation, A384S, due to a G1246T gene substitution in exon 6. The mutation does not affect the protein expression level; however, it does lead to borderline or mildly reduced anti-Xa and anti-IIa activity in the presence of heparin. Later, Corral et al. (2007) confirmed this mutation in a British population, and realized its importance for VTE. The mutation rate of antithrombin Cambridge II is 0.2% in the general population and 1.7% in British VTE patients (Corral et al., 2007). Studies show that this mutation is associated with increased expression of thrombin. Until now, this mutation has not been identified in a Chinese population (Zhang et al., 2010).

ABO-related proteins

The *ABO* gene encodes proteins related to the blood group system, which determines the blood group. The “O” blood group arises from a deletion of guanine-258 in the gene, leading to a frameshift and the translation of an almost completely different protein.

Studies indicate that non-O blood groups elevate the risk of VTE by 1.8- to 2.5-fold. B and A1 blood groups can increase the risk of VTE by 2-fold more than O and A2 blood groups (Jick et al., 1969; Wu et al., 2008; Trégouët et al., 2009; Heit et al., 2012). Both Factor VIII and VWF undergo extensive glycosylation by glycosyltransferases, encoded by the ABO blood group genes (McGrath et al., 2010). Therefore, ABO phenotype correlates with plasma levels of FVIII and VWF. Compared with non-O blood groups, individuals with type O blood group have approximately 25% lower plasma FVIII and VWF levels. High plasma levels of FVIII and VWF are well-known risk factors of VTE. Interestingly, several studies have also shown that ABO blood groups remain significantly associated with VTE even after adjustment for FVIII or VWF levels (Ohira et al., 2007; Cohen et al., 2012). This suggests a VWF-independent pathway linking ABO to VTE.

Genetic variation in *VWF* and *STXBP5* genes can also influence the risk of VTE. Two genetic variations, *VWF* rs1063856 and *STXBP5* rs1039084, lead to elevated plasma levels of VWF

antigen and a higher risk for VTE. The *VWF* rs1063856 single nucleotide polymorphism, resulting in a T789A substitution in exon 18 (Smith et al., 2011), may increase the ability of VWF to transport or release FVIII into the circulation, thereby increasing the risk of VTE. The rs1039084 mutation with an N436S substitution is also associated with VWF expression and VTE risk levels. Therefore, *STXBP5* may be a candidate gene for VTE (Smith et al., 2011), although the relationship between *STXBP5* and the coagulation system is not yet clear.

COMMON GENETIC RISK FACTORS IN ASIANS

Researchers have also tried to define the genetic risk factors causing VTE in Asians. However, the common genetic mutations discussed above are rare in Asian populations. Until now, there has been no report on FII G20210A and antithrombin Cambridge II mutations, and only one case of the factor V Leiden mutation and APC resistance in a Chinese population (Kalafatis et al., 1994). Moreover, the rates of the factor V Leiden mutation and the prothrombin gene G20210A mutation were both approximately 0.2% in a Thai population (Angchaisuksiri et al., 2000). However, other genetic risk factors are thought to contribute to the occurrence of VTE in Asians (Table 2).

Table 2. Common genetic risk factors of venous thromboembolism (VTE).

Gene	Nucleotide change	Amino acid change	rs number	Prevalence in general population		Prevalence in VTE		Odds ratio (95%CI)	Reference
				Western	Asian	Western	Asian		
F5	c.1691G>A	p.Arg506Gln	rs6025	3-15%	0-1%	10%	0	Heterozygote: 7.0 homozygote: 80.0	(Bertina et al., 1994, Ko et al., 1996)
F2	c.20210G>A	-	rs1799963	2%	0-0.2%	1%-6%	0	20.0 (11.1-36.1)	(Poort et al., 1996; Angchaisuksiri et al., 2000; Ho, 2000)
*PROC**	c.565C>T	p.Arg189Trp	rs146922325	-	0.90%	-	5.88%	7.10 (3.50-4.39)	(Tang et al., 2013b)
*PROC**	c.574_576del	p.Lys193del	rs199469469	-	2.36%	-	6.78%	2.84 (1.88-4.29)	(Tang et al., 2013b)
*THBD**	c.151G>T	p.Lys196Glu	rs16984852	-	0.98%	-	2.68%	2.8 (1.88-4.26)	(Tang et al., 2013b)
PROS1[&]	c.586A>G	p.Lys196Glu	rs121918474	-	1.81%	-	9.32%	5.58 (3.11-10.01)	(Dahlbäck and Villoutreix, 2005)
SERPINC1[#]	c.1246G>T	p.Ala384Pro	-	0.2%	0	1.7%	0	9.75 (2.2-42.5)	(Corral et al., 2007; _Zhang et al., 2010)

*Common in Chinese population; [&]common in Japanese population; [#]common in British population.

Protein C

Protein C, encoded by the *PROC* gene, is a vitamin K-dependent plasma glycoprotein that is synthesized in the liver. It is stored as a serine protease precursor in the circulation. Protein C is activated by thrombomodulin (TM)-bound thrombin on the endothelial surface. The activated form of protein C (APC) inhibits the coagulation pathway by proteolysis of coagulation factors Va and VIIIa in the presence of protein S (Dahlbäck and Villoutreix, 2005).

Griffin et al. (1981) identified a heterozygous protein C deficiency in a family with a history of VTE. Hereditary protein C deficiency is usually inherited as an autosomal dominant trait, with 1.6-8.6% prevalence in European VTE patients. However, the rates for the general population and Asian VTE patients are as high as 0.29-4 and 4-31.76%, respectively (Yin and Miyata, 2014).

Recently, the p.Arg189Trp mutation in protein C has been investigated by two independent groups. The studies show that this mutation is the most frequent variant in protein C deficiency and confirm that protein C is a significant risk factor for VTE in Chinese populations. Although this mutation is rare in western populations, it is present in approximately 0.9% of the general Chinese

population (Tsay and Shen, 2004; Tang et al., 2012a,b). Among 34 unrelated probands with hereditary protein C deficiency, 17 carried this mutation, and the first-degree relatives of these 17 probands had an 8.8-fold increased risk of VTE (Tang et al., 2012a). Further studies have shown that the mutation is located at the C-terminal region of the light chain near to the EGF-2-like domain, and may affect its interaction with other molecules.

In 2012, another common genetic mutation of VTE, the *PROC* p.Lys193del mutation, was investigated by Tang et al. The mutation reduced the anticoagulant activity of protein C and elevated the risk of VTE in a Chinese population with an odds ratio of 2.71. The prevalence was 2.36% in the general Chinese population and 6.78% in Chinese VTE patients (Tang et al., 2012b). Although they exhibit lower protein C anticoagulant activity, VTE patients with this mutation show relatively normal amidolytic activity compared with the wild-type carriers (Miyata et al., 1998, 2009; Tang et al., 2012b). Interestingly, this mutation is rare in Japanese populations.

Protein S

Protein S, encoded by the *PROS1* gene, is a vitamin K-dependent single glycoprotein that is synthesized in the liver. As a cofactor of APC, protein S plays an essential role in the regulation of physiological anticoagulants.

The prevalence of hereditary protein S deficiency is 1.4-7.5% in Caucasian VTE patients and 8-47.06% in Asian patients (Yin and Miyata, 2014). Protein S deficiency is one of the most common causes of VTE among Asians, especially in Japanese populations. To date, more than 200 mutations have been reported in the *PROS1* gene. Among these, large deletions/duplications are the major causes of protein S deficiency in Asians (Gandrille et al., 2000; Yin et al., 2007; Tang et al., 2013a). In addition, the *PROS1* p.Lys196Glu mutation, also known as the protein S Tokushima mutation, accounts for 9-30% of abnormalities in protein S among patients of Japanese descent (Yin et al., 2007; Ikejiri et al., 2010; Miyata et al., 2012; Hamasaki et al., 2013; Tang et al., 2013a). Three independent studies have concluded that this mutation is a risk factor for VTE with an odds ratio of 3.74-8.56 in Japanese patients (Kinoshita et al., 2005; Kimura et al., 2006; Ikejiri et al., 2010). Interestingly, this mutation is race-specific and is only identified in Japanese populations.

Thrombomodulin

TM, encoded by the *THBD* gene, is a transmembrane glycoprotein mainly expressed in the endothelial cells. It is a critical component in the protein C anticoagulant system, and interacts with thrombin and protein C (Esmon and Owen, 1981). The TM-thrombin complex increases the rate of thrombin-dependent protein C activation by more than 1000-fold (Weiler and Isermann, 2003).

Recently, sequencing and genotyping of the *THBD* gene in a large case-control study of the Chinese population comprising 1304 VTE patients and 1334 controls discovered that the *THBD* c.151G>T mutation is associated with soluble TM levels and the risk of VTE. The *THBD* c.151G>T mutation is a genetic risk factor for VTE among Chinese people. Carriers with this mutation have a 2.8-fold increased risk of developing VTE, and their first-degree relatives have a 3.42-fold increased risk of VTE (Tang et al., 2013b). The occurrence rate of this mutation is 0.98% in the Chinese population and the population-attributable risk is approximately 1.48% (Tang et al., 2013b).

This mutation, causing VTE, may contribute to the significant reduction of the protein expression in cells

The three common genetic risk factors, *PROC* p.Arg189Trp, *PROC* p.Lys193del, and *THBD* c.151G>T account for at least 10% of the total VTE events in the Chinese population. However, the prevalence of these variants is much lower than the most common genetic risk factor in the Western population: the factor V Leiden mutation (5-10%) (Dahlbäck, 2008).

Newly identified risk factors of VTE beyond the coagulation/fibrinolysis pathway

Most of the common genetic defects discussed above belong to the coagulation or fibrinolysis systems, and were identified by the candidate gene approach. However, to obtain a better understanding of the influence of genetics in VTE, more genetic risk factors and novel study methods need to be explored. During the past decade, genome-wide association studies (GWASs) have revolutionized the identification of susceptible loci associated with VTE. GWASs of VTE have not only confirmed the well-established genetic risk factors of VTE, such as *F5* rs6025, *ABO* rs8176719 and rs2519093, and *F2* rs1799963 (Heit et al., 2012), but have also identified new genetic risk factors, such as *GP6*, *HIVEP1*, *KNG1*, and *BAI3*. These newly identified factors are beyond the traditional coagulation/fibrinolysis pathway.

Glycoprotein VI

The first large-scale GWAS on VTE was performed in 2008 (Bezemer et al., 2008), and revealed three new loci: the *CYP4V2*/*KLKB1*/*F11* gene cluster, *GP6*, and *SERPINC1*. These three loci are associated with F11, platelet-activation, and antithrombin, respectively. The *GP6* gene is the first identified VTE-associated locus beyond the traditional coagulation pathway. *GP6* encodes platelet membrane glycoprotein VI, which belongs to the immunoglobulin superfamily. The rs1613662 mutation is an A/G substitution in the *GP6* gene, and results in an S219P mutation. Carriers of this mutation have a 15% increased risk of VTE (Trégouët et al., 2009).

Human immunodeficiency virus type I enhancer binding protein 1 (HIVEP1)

A novel susceptibility locus, *HIVEP1*, has been associated with VTE, but is outside the traditional coagulation/fibrinolysis pathway according to the multi-stage GWAS, MARTHA and FARIVE studies (Morange et al., 2010). The HIVEP1 transcription factor is encoded by the *HIVEP1* gene, and belongs to the ZAS family (Wu, 2002). The *HIVEP1* rs169713C allele is associated with an increased risk for VTE with an odds ratio of 1.20 (Morange et al., 2010). Since HIVEP1 is involved in inflammation, it implies that the inflammation process may affect VTE.

Kininogen (HK)

High-molecular weight HK, encoded by the *KNG1* gene, is another newly identified factor beyond the traditional coagulation/fibrinolysis pathway. The authors of a GWAS reported that the *KNG1* T581 allele is associated with decreased APTT levels and increased risk of VTE, with an odds ratio of 1.19 (Morange et al., 2011). *KNG1* c.I581T is associated with activated partial thromboplastin time, and HK may have a role in the regulation of plasma FXI concentration.

SUMMARY AND PROSPECTIVE

In this review, we have discussed the major genetic risk factors in Western populations: the FV Leiden, prothrombin G20210A, and antithrombin Cambridge II mutations, and ABO blood groups. However, these risk factors are rare in Asian populations. Instead, *PROC* (c.565C>T and c.574_576dup/del), *PROS1* (c.586A>G), and *THBD* (c.151G>T) are common among Asians. In addition, we summarized several newly identified factors discovered by GWAS, which are beyond the traditional coagulation/fibrinolysis pathway.

GWAS is an effective means of identifying risk loci for VTE. However, it has some limitations. For example, GWAS can only identify common mutations (>5%) with relatively low risk (odds ratio < 1.5). For complex diseases, including VTE, rare variants have a lower frequency but a potentially significant phenotypic impact (Manolio, 2013). As genomic technologies, such as deeper sequencing, fine mapping, copy-number variations, whole-exome sequencing, and even whole genome sequencing, improve, lower frequency variants become possible to detect.

Rapid genetic sequencing results in the discovery of novel risk factors for many complex diseases. Emerging evidence proves that genetic factors play a crucial role in the development of VTE. Currently, the genetic screening of VTE, also known as thrombophilia screening, established about two decades ago, includes the screening of AT, PC, and PS, as well as factor V Leiden and FII G20210A mutations. However, most of the emerging genetic variations are not in the panel, although several recommendations or guidelines have been published (Howard and Hughes, 2013). Identification of the risk factors of inherited thrombophilia can help prevent the occurrence and development of VTE, and even help develop personalized medicine or gene treatment (Sandset, 2013). Next-generation sequencing is a promising technology that may revolutionize the current approach; whole-genome sequencing will soon be available for the targeted panel at an acceptable cost. In addition, to better understand and prevent the disease, biochemical and molecular biology studies are required to determine the roles of each risk factor in causing VTE.

Conflicts of interest

The authors declare no conflict of interest.

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