Review Article

Progress in research into the genes associated with venous thromboembolism

Lian-xing Zhao, Bo Liu, Chun-sheng Li

Department of Emergency Medicine, Beijing Chaoyang Hospital, Capital Medical University, Beijing 100020, China

Corresponding Author: Chun-sheng Li, Email: lcsyyy@163.com

BACKGROUND: Venous thromboembolism (VTE), including both deep vein thrombosis (DVT) and pulmonary embolism (PE), is a common, lethal disorder that affects hospitalized and non-hospitalized patients. This study aimed to review the progress in the research into VTE.

DATA SOURCES: We reviewed the studies about VTE and verified different genetic polymorphisms of VTE.

RESULTS: The pathogenesis of VTE involves hereditary and acquired factors. Many studies indicated that the disorder of coagulation and fibrinolytic system is of utmost importance to this disease. Genetic polymorphism-related VTE demonstrated significant differences among geographies and ethnicities.

CONCLUSION: VTE has many risk factors, but genetic factors play an important role.

KEY WORDS: Venous thrombosis; Gene; Hereditary; Polymorphism

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INTRODUCTION

Venous thromboembolism (VTE), including both deep vein thrombosis (DVT) and pulmonary embolism (PE), is a common, lethal disorder that affects hospitalized and nonhospitalized patients.^[1] VTE results from both hereditary and acquired risk factors. In addition, vessel wall damage, venous stasis, and increased activation of clotting factors remain the fundamental basis for our understanding of thrombosis. In Asia, VTE increases in frequency in older patients.^[2] Mutations and gene polymorphisms play an important role in the development of VTE, and genetic factors are differentiated in geographies and races.^[3] Thrombophilia refers to congenital hereditary to thrombosis. Though significant advances in western countries have been made in understanding congenital thrombophilia, there may be many more heritable forms of thrombophilia as yet undiscovered. This study aimed to review the progress in the study of VTE.

POLYMORPHISMS IN EUROPEANS AND AMERICANS

Biologically, polymorphism refers to two or more clearly different phenotypes in the same population or to two or more discontinuous genotype variations generated from mutation including DNA fragment length polymorphism, DNA repeat sequence polymorphism and signal nucleotide polymorphism. Polymorphism does not change all the lifetime, and it follows Mendel's laws.

Coagulation factor V (FV)

FV Leiden is the most common mutation. Dahlbäck et al^[4] described a previously unrecognized mechanism for familial thromboembolic disease that is characterized by poor anticoagulant response to activated protein C (APC). Bertina et al^[5] reported that the phenotype of APC resistance was associated with a single point mutation in the FV gene, which at nucleotide position 1691 G→A predicts the synthesis of a FV molecule 506 R→Q. APC

plays a role in anticoagulation by cutting activated FV in three positions (R306, R506, R679).^[6,7] After mutation, FV has normal clotting activity, but is resistant to APC. This can be detected in 5% of the general population, 20%–25% of patients with VTE, and 50% of patients with familial thrombophilia.^[8,9] Brugge et al^[10] reported the thrombotic risk of FV Leiden homozygotes was higher than that of heterozygotes. The mutation was different between different ethnic groups and regions.^[11]

FV also has other polymorphisms. HR2 (4070A>G, His1299Arg), cooperated with FV Leiden, increases the impact of APC-R and risk of venous thromboembolism.^[12] FV Cambridge (1091G>C, Arg306Thr) and FV Liverpool (1250T>C, Ile359Thr) may be related to APC-R.^[12] FV Hong Kong (1090A>G, Arg306Gly) can be found in 4.7% and 4.5% of patients with thrombosis and controls, respectively in Hong Kong.^[7]

Prothrombin

Prothrombin (FII) G202100A is common in Caucasians. FII consists of 14 exons, 13 introns and 5' and 3' untranslated regions. The mutation was located in the 3' untranslated region, which increases the concentrations and activities of prothrombin,^[13] and increases the risk of venous thrombosis by 3 times.^[14] Some DVT patients, especially recurrent and familial patients, showed resistance to warfarin.^[15] FII can be detected in 2%–6% of Caucasians, 1.7% of healthy Northern Europeans, 3% of Southern Europeans, but none of American Indians and Asians.

Deficiencies of protein C (PC), protein S (PS) and antithrombin (AT)

Deficiencies of PC, PS and AT were genetic factors identified earlier for venous thrombosis.

PC, a vitamin K-dependent glycoprotein, is a serine protease precursor with anticoagulation inhibiting action of FVa and FVIIIa by proteolytic action. There are two types of PC deficiency: type I, patients with decreased levels of antigen and activity, and type II, patients with normal antigen but decreased level of activity. There are 200 different mutations that have been reported.^[16] Bu-Amro et al^[17] reported that individuals with low levels of PC were generally born with fatal thrombotic complications. PC deficiency is rarely seen, with an incidence of 0.2%–0.4% in the general population and 3.7% in patients with venous thrombosis. This does not show the advantage of mutation.

PS is an important cofactor of PC anticoagulant

pathway and tissue factor pathway inhibitors synthesized in the liver,^[18] inhibiting the activation of FX in combination with Zn²⁺.^[19] Hereditary PS deficiency is rarely seen in Western countries, with an incidence of 0.03%–0.13% in the healthy population and 2.3% in patients with venous thrombosis. Mutations could be found in about 50% of patients with PS deficiency, and there is no advantage of mutation.

AT is the natural inhibitor of thrombin and FX_a. Congenital AT deficiency is an autosomal dominant genetic disease, which is a strong risk factor of venous thrombosis. More than 180 gene mutations attributing to hereditary AT deficiency showed significant heterogeneity since 1993 when Olds et al^[20] reported AT gene sequence. In Western countries, the proportion of hereditary AT deficiency is 0.02%–0.20% in the general population, 1%–2% in patients with VTE, and 4%–7% in patients with familial VTE.

GENE POLYMORPHISM AND MUTATIONS IN CHINA

There are some differences in gene polymorphisms between Chinese and Western populations. For example, FV Leiden and FII G20210A are rare in China; inherited deficiency of AT, PC and PS might play an important role in the occurrence of venous thromboembolism in Chinese.^[21] Shen et al^[22] demonstrated that PC and PS deficiencies are the most important risk factors associated with thrombosis in patients with venous thrombophilia in Taiwan province.

AT gene mutation

The AT gene consists of 7 exons, located in 1q23-25.1. AT deficiency is classified into two phenotypes: type I, patients have a concordant decrease in AT antigen and functional levels; type II, patients have a normal antigen level but a decreased functional level. Clinically, type II are common, but patients of type I account for 80% in patients with symptomatic thrombosis.^[23] It has been found that more than 180 gene mutations were related to AT deficiency. The tendency to form venous thrombosis is different among different heterozygous mutations.^[24]

Zhou et al^[25] investigated a patient with recurrent mesenteric venous thrombosis for 3 times and his 6 relatives of 3 generations. They found a heterozygous mutation of G13328A, which contributes to the AT mutation of Ala404Thr. In the first-degree relatives, 3

who had a mutation were of type II deficiency caused by the hereditary of AT deficiency. Wang et al^[26] also found the same mutation in their study. The proportion in the general population with AT deficiency was 0.08%, but it was not estimated in a large cohort of patients with thrombosis. We did not find any advantage of mutation because of the high heterogeneity of gene mutation.

Protein C

The PC gene, about 10 802 bp and located in 2q13-q14, consists of 9 exons and introns. More than 300 mutations occurred in this gene, and most of them were reported in Western countries.

Tasy et al^[27] reported that C6152T could be found in 43% of patients with VTE and in 0.85% of the general population (95%CI 0.35–1.35). PC gene C6152T was located in the seventh exon, which contributes arginine to tryptophan in the 147th position. Ding et al^[28] confirmed that Arg147Trp was the hot spot mutation in VTE patients with PC deficiency. The mutation rate was about 43.5%. Ye et al^[29] also found the same mutation in a PE patient and his family members. This finding showed the correlation between the mutation and the type II of PC deficiency.

Hu et al^[30,31] found two gene polymorphisms. One was PROC p.Arg189Trp (rs 146922325: C>T) detected in 17 of 34 patients with protein deficiency, and the morbidity of their relatives was 8.8 times higher than that of those without gene mutation. A large case-control study detected 5.88% of patients with PROC p.Arg189Trp in the patient group and 0.87% in the control group, and the morbidity of patients was 6 to 7 times higher than normal controls. The other was PROC p.Lys192del (rs 199469469: AAG/-), found in 6.77% of the patients and 2.4% in the normal controls, with a morbidity of the patients was 2.9 times higher than the normal controls.

PS gene mutation

The human genome of PS has active (Psa or PROS1) and false types without activity (PSβ or PROS2), which are located in 3q 11.1–11.2. The deficiency of PS is common in Japanese,^[32] in which PS Tokushima (p.Lys196Glu) has the advantage of mutation. Tang et al^[33] found 17 different mutations in 18 of 40 patients with venous thrombosis associated with PS deficiency, but they didn't find the advantage of mutation except for C.-168C>T in two probands. In 200 consecutive patients with venous thrombosis and 50 healthy controls, the same mutation was not found but the heterogeneity. As

mutations contributing to PS deficiency^[22] are common in Chinese patients with thrombophilia, we must pay more attention to the mutations.

Thrombomodulin (TM)

TM as a transmembrane glycoprotein expressed in vascular endothelial cells, plays an important role in anticoagulation. The gene of TM (TMBD) located in 20p11.21 is 4kbp with only one exon. Hu et al^[34] found that the mutation rates of THBD were 2.68% and 0.97% in 1 304 patients and 1 334 controls respectively. Polymorphism is in the untranslated region of gene 5'-end, in which expression level decreased by 50% confirmed *in vitro*. The risk of thrombosis in heterozygous patients was 2.8 times higher than in healthy controls. The risk of thrombosis in the first-degree relatives was 3.42 times higher than in healthy controls as shown by a further study of 176 first-degree relatives of 38 probands.

Endothelial NO synthase (eNOS)

NO, an important regulator for vascular homeostasis, can relax vascular smooth muscle cells, prevent the adhesion of platelet and monocyte to endothelial cells, reduce the migration and proliferation of vascular smooth muscle, and inhibit the development of atherosclerosis. eNOS located in 7q35-36 is 21kb consisting of 26 exons. Akhter et al^[35] demonstrated that eNOS 894G/T was associated with venous thrombosis in Northern Indians. Li et al^[36] found that the presence of GT and GT+TT was significantly higher in patients than in controls (18.1% vs. 12.3%, $P=0.014$; 20.3% vs. 13.4%, $P=0.005$), so was the T allele (12.5% vs. 7.1%, $P=0.006$).

FV Leiden

Cai et al^[37] investigated a Chinese family with a history of venous thrombosis. They found PC resistance (APC-R) in 4 of the 5 family members by experimental screening of coagulation. They found a new mutation G2172C in the 13th exon in all APC-R family members by sequencing the FV, but not in family members without APC-R. The mutation predicted the replacement of glutamate by aspartate at position 666, close to one of the APC cleavage sites. This hypothesis needs to be confirmed in other Chinese families with APC-R.

Copy number variations of the FVIII gene

FVIII plays a role in the final process of coagulation. Shen et al^[38] found that FVIII in plasma and copy number of the FVIII gene was significantly higher in patients

than in controls in a case-control study including 179 patients with VTE and 176 healthy controls. Copy number variations (CNV) were caused by the genome rearrangement, with an increase or decrease of DNA large fragments of more than 1kb. They believed that changing the gene copy number (eg, simple deletion, insertion and replication) could influence the individual susceptibility to disease. This was a dose-dependent risk factor for primary and recurrent venous thrombosis, in which activities increased by amplification was associated with the occurrence of VTE.

Other related gene polymorphism

Beckers et al^[39] found that gene polymorphism of inflammatory factors IL-1A, IL-4, IL-6 and IL-13 may be associated with venous thrombosis in Dutch. Methylenetetrahydrofolate reductase (MTHFR) C677T is common in Caucasians, resulting in a slight elevation of homocysteine. He et al^[40] reported that the plasma level of homocysteine was associated with the MTHFR genotype of TT, which may be a genetic risk factor in patients with mesenteric venous thrombosis. Ma et al^[41] found that plasminogen activator inhibitor-1 gene polymorphism was associated with acute pulmonary embolism, and that the genotype of 4G/4G significantly increased the risk of pulmonary embolism to individuals without environmental factors. In addition, more genetic factors such as ACE I/D and CYP11B2 (-344C/T) need to be further studied because of genetic differences, small sample size, single center, uncertain relations or weak risk.

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