MOLECULAR BASIS OF THROMBOPHILIA
MOLEKULARNE OSNOVE TROMBOFILIJE

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Summary

Thrombophilia is a multifactorial disorder, involving both genetic and acquired risk factors that affect the balance between procoagulant and anticoagulant factors and lead to increased thrombotic tendency. The severe forms of thrombophilia are caused by a deficiency of natural anticoagulants: antithrombin, protein C and protein S. The advances in DNA technology played an important role in the identification of the exact nature of these deficiencies and opened up new possibilities in genetic research and molecular diagnostics of thrombophilia. The major breakthrough came with the discovery of activated protein C resistance and the Factor V Leiden gene mutation. Shortly afterwards, a variant in the 3' untranslated region of the Factor II gene (FII G20210A) associated with an increased concentration of Factor II in plasma was described. These two gene variants represent the most common thrombophilic genetic risk factors. Recently, a novel prothrombin mutation (c.1787G>T) was identified in a Japanese family with juvenile thrombosis. This mutation leads to impaired inhibition of mutant thrombin by antithrombin, proposing a new mechanism of thrombophilia named resistance to antithrombin. In the last decade, several prothrombotic genetic risk factors have been described, including gene variants associated with defects of natural coagulation inhibitors, increased levels of coagulation factors or their impaired inhibition and defects of the fibrinolytic system. However, most of them are not of diagnostic value, due to their minor or unknown impact on the thrombotic risk. Large-scale DNA analysis systems are now becoming available, opening a new era in the genetic studies of the molecular basis of thrombophilia.

Keywords: thrombophilia, genetic risk factors, gene variants

Kratak sadržaj


Ključne reči: trombofilija, genetički faktori rizika, genske varijante

List of non-standard abbreviations: AT, antithrombin; PC, protein C; PS, protein S; tPA, tissue plasminogen activator; PAI-1, plasminogen activator inhibitor; DVT, deep venous thrombosis; PE, pulmonary embolism; APC, activated protein C; FV, factor V; FII, factor II; FGG, fibrinogen gamma; VWF, Von Willebrand factor; FVIII, factor VIII; NG5, next-generation sequencing; GWAS, Genome-Wide Association Studies.
**Introduction**

Hemostasis is the physiological response that prevents blood loss after vascular injury. It is a very complex balance that involves several factors: the blood vessel cells, platelets, coagulation factors, coagulation inhibitors and the fibrinolytic system (1). The endothelium of blood vessels is a natural barrier for blood loss, and disruption of a vessel wall causes vasoconstriction, collagen exposure and platelet activation (2, 3). Platelet activation further results in the transport of negatively charged phospholipids to the platelet membrane, which provide a catalytic surface for the complexes of coagulation factors (1, 3). Coagulation factors play a central role in the generation of fibrin, which allows the formation of a blood clot and prevents blood loss. This process is usually presented as a series of enzymatic reactions that involves activation of coagulation factors (the cascade principle) that ultimately results in cross-linked fibrin. The coagulation cascade has two pathways leading to fibrin formation: contact activation pathway–intrinsic pathway, and the tissue factor pathway–extrinsic pathway (4, 5). Thrombin is a central regulatory molecule, which affects the whole process of coagulation through the mechanism of positive and negative feedbacks (6, 7). Coagulation inhibition is very important for the maintenance of hemostatic balance. Natural inhibitors of coagulation factors include: antithrombin (AT), protein C (PC), protein S (PS), thrombomodulin (in interaction with thrombin) and others (1). These proteins inactivate specific coagulation factors and provide a regulatory mechanism that controls the coagulation response and limits the unnecessary extension of the clot. The fibrinolytic system has a role to remove the product of a coagulation–fibrin clot, preventing the pathological extension of the blood clots. The central enzyme of this system is plasmin, generated by the activation of his zymogen–plasminogen. Plasmin activity is regulated by several activators and inhibitors: tissue plasminogen activator (tPA), plasminogen activator inhibitor (PAI-1 and PAI-2) and α-2-antiplasmin (8).

Hemostasis disorders occur as a consequence of impaired or altered function of one or more participants in this complex process. Hypercoagulability is a state in which the hemostatic balance shifts toward excessive platelet activation and fibrin generation, leading to the formation of a clot in a blood vessel and obstruction of the blood flow. Obstruction of the blood flow can have deleterious consequences in the form of venous and arterial thrombosis (1). Arterial thrombosis can manifest as myocardial infarction, ischemic stroke or arterial embolism. Deep venous thrombosis (DVT) and pulmonary embolism (PE) are the most frequent clinical manifestations of venous thrombosis (9). Thrombosis is a multifactorial disorder with both established environmental and genetic risk factors (10). Conventional environmental thrombosis risk factors are: aging, smoking, immobilization, blood pressure, cholesterol, obesity, metabolic syndrome and diabetes, pregnancy, cancer, surgery, trauma and infection (1, 11).

**Thrombophilia**

The term thrombophilia was introduced by Jordan and Nangorf in 1956 in order to describe the »familial tendency in thromboembolic disease« (12). In recent years, this term has been used with a variety of different similar meanings, referring broadly to an increased tendency to develop clots in blood vessels. In the last five decades, several genetic risk factors related to thrombophilia have been described. They can roughly be divided into three groups: 1. affecting coagulation inhibitors’ genes leading to reduced inhibition of coagulation; 2. affecting procoagulant factor genes resulting in their impaired inhibition or gain-of-function; 3. affecting fibrinolytic system genes leading to impaired fibrinolysis (1, 13, 14) (Figure 1).

**Defects of AT, PC and PS**

The concept that thrombophilia could be associated with genetic defects was first proposed in 1965, after the discovery of familiar AT deficiency (15). These initial studies were based on the analysis of plasma levels of AT in family members with a deficiency status, in order to document inheritance patterns. Further research has shown that AT deficiency occurs due to mutations in the SERPINC1 gene. There are two primary types of AT deficiency: type I,
which is characterized by an inadequate amount of normal AT level, and type II, in which the amount of AT present is normal, but the mutant protein is unable to carry out its functions (16). The SERPINC1 gene encoding PC. Deficiency of PC can manifest as Type I deficiency, in which the PC level is decreased, and Type II deficiency, in which low PC activity contrasts with normal protein levels. Type I defects are more common than Type II defects (20). PC deficiency occurs in one in 2000–4000 of the adult population and thrombotic risk associated with heterozygous PC deficiency is approximately 8 (18).

The first patients with PS deficiency were described by Comp et al. (21) in 1984. Protein S deficiency presents an autosomal dominant trait with complete penetrance. PS deficiency is caused by mutations within the PROS1 gene. The relative risk associated with heterozygous PS deficiency is approximately 8 (22).

**FV Leiden gene variant**

The breakthrough in genetic research of thrombophilia came with the discovery of activated protein C (APC) resistance and the Factor V G1691A (FV Leiden) mutation. In 1993, Dahlback et al. (23) found that the plasma of patients with familiar thrombosis showed a reduced response to the addition of APC. Later, it was discovered that the point mutation G1691A in the FV gene, which results in substitution of arginine at position 506 by glutamine, was responsible for the observed APC resistance phenotype (24, 25). FV Leiden mutation leads to impaired ability of APC to cleave mutant FV at position 506, resulting in an increase in thrombin generation (25). It was reported that FV Leiden was common in a healthy Caucasian population (1–6%). FV Leiden was detected in 6–18% of thrombophilic patients and the presence of FII 20210A allele was associated with a fourfold increased risk of DVT (14, 18, 24, 27). Apart from FII G20210A, many gene variants have been detected in the 3’ end of the prothrombin gene, such as: A19911G, C20068T and C20211T (28, 29). The propensity to the new gene variants, which might lead to hypercoagulability, has been explained by the unusual architecture of non-canonical sequence elements in the 3’ end of the prothrombin gene (28, 30).

**Antithrombin resistance**

Recently, a novel thrombophilia mechanism named AT resistance was proposed, based on the FII c.1787G>T mutation identified in a Japanese family with juvenile thrombosis. The mutation leads to impaired inhibition of mutant thrombin (p.Arg596Leu) by AT, resulting in AT resistance and an increased risk of thrombophilia (31).

A novel FII c.1787G>A mutation, affecting the same prothrombin Arg596 residue, has also been reported recently in two unrelated Serbian families with severe recurrent thrombosis (32). These studies have shown that the reported mutations are rare, but future investigations are needed to determine their frequency and clinical relevance.

**FGG C10034T gene variant**

A single nucleotide polymorphism C10034T in the fibrinogen gamma-γ (FGG) gene has been associated with increased thrombophilia tendency (33). The fibrinogen chain mRNA transcript is the subject of alternative processing and polyadenylation. The main form is the γA chain, while the alternative γ chain arises when polyadenylation occurs at an alternative polyadenylation signal in intron 9 (34). Fibrinogen γ contains a unique high-affinity non-substrate binding site for thrombin, which seems critical for the expression of the AT activity during fibrin formation. The FGG 10034T allele is associated with reduced γA/γ fibrinogen levels and with a 1.47 increased risk of DVT (33).

**PAI-1 4G/5G gene variant**

A deletion/insertion (4G/5G) polymorphism of the PAI-1 gene has been correlated with levels of plasma PAI-1. This gene variant is located at position
–675 in the promoter region of the PAI-1 gene and leads to increased expression of PAI-1. The 4G allele is associated with higher levels of PAI-1, and might increase the risk for thrombotic events through impaired fibrinolysis (35, 36).

Other genetic risk factors

In 1969, Jick et al. (37) reported an association between non-O blood group and increased risk of DVT. Recent studies have clarified this association and verified that B and A1 blood groups are at higher risk than O and A2 blood groups, with the relative risk of approximately 2 (38, 39). It is assumed that an ABO blood group could contribute to thrombosis risk through modifications of von Willebrand factor (VWF) and factor VIII (FVIII) levels in plasma (40, 41). On the other hand, several studies have revealed that ABO blood groups remain significantly associated with elevated prothrombotic risk, even after adjustment for FVIII or VWF levels (41, 42). Elevated plasma levels of FVIII and VWF are also established risk factors for DVT (43). It was found that the relative risk of recurrent thrombosis was 6.7-fold increased in patients with FVIII levels greater than the 90th percentile (44). The genetic variation influencing the variability of these phenotypes is not yet clearly defined.

In the past years, increasing evidence has shown that genetic factors may play important roles in patient response to anticoagulant therapy. Polymorphisms in genes CYP2C9 (encoding the main cytochrome P4590 enzyme) and VKORC1 (encoding the warfarin target vitamin K epoxide reductase) were associated with variability in warfarin dose requirement (45–48). Current knowledge about the genetic factors affecting other anticoagulants is more limited and this area requires future studies (46).

Interaction between genetic risk factors

It has been shown that two or more risk factors, rather than just one particular genetic risk factor, lead to thrombotic disorders (11, 27). Combinations of different candidate gene variants have been extensively studied in an attempt to elucidate their possible association with increased thrombotic tendency (11, 13, 27, 49). In a recent study, De Haan et al. (50) analyzed the interaction of 31 prothrombotic gene variants. Their results showed that even though some of the studied variants showed very weak association with disease, their combination could be useful for predicting patients’ thrombophilia susceptibility.

Future: from GWAS to personal medicine

Despite of many studies within this field, the pathogenesis of thrombophilia in a large number of patients still remains unexplained. The advances in DNA technology, from the PCR reaction to large-scale analysis systems such as sequencing and microarrays, opened up new possibilities in the genetic research and molecular diagnostics of thrombophilia. Nowadays, large population-based case-control studies involving thousands of patients are carried out in order to determine significant thrombophilia risk factors (51, 52). Also, using new technology approaches, especially next-generation sequencing (NGS) and Genome-Wide Association Studies (GWAS), a number of new genetic variants possibly involved in the pathogenesis of thrombophilia have been described. Although numerous, most of these gene variants are not of diagnostic value, due to their minor or unknown impact on the thrombotic risk.

In the future, large amounts of research data will allow to establish a prediction score for the thrombophilia risk. New technological developments will enable many genes to be studied in a single patient in a cost-effective manner. This genetic profiling and environmental risk factors data will allow determination of »personalized« thrombophilia risk factor scores and, finally, a therapeutic and prevention approach tailored for an individual patient.

Acknowledgements. This work was supported by grant No 173008 from the Ministry of Science and Technological Development, Republic of Serbia.

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.
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Received: July 12, 2013
Accepted: August 6, 2013