J Med Biochem 2014; 33 (1) DOI: 10.2478/jomb-2013-0037

**UDK 577.1 : 61** **ISSN 1452-8258**

*J Med Biochem 33: 22–27, 2014*

*Review article*
*Pregledni članak*

# MOLECULAR BASIS OF THROMBOPHILIA

## MOLEKULARNE OSNOVE TROMBOFILIJE

*Valentina Đorđević, Iva Pruner, Dragica Radojković*

*Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia*

## 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

Trombofilija nastaje kao rezultat kompleksne interakcije između negenetičkih i genetičkih faktora rizika koji hemostaznu ravnotežu pomeraju u smeru hiperkoagulacije i dovode do pojave tromboze. Veoma značajan faktor rizika za nastanak trombofilije je deficijencija inhibitora koagulacije: antitrombina, proteina C ili proteina S. Veliki korak u razumevanju genetičke osnove i molekularne dijagnostike trombofilije napravljen je otkrićem rezistencije na aktivirani protein C i faktor V Leiden mutacije. Ubrzo je otkrivena i varijanta u 3'-nekodirajućem regionu gena za faktor II (FII G20210A), za koju je pokazano da dovodi do povišene koncentracije protrombina u plazmi. Ove dve genske varijante su najučestaliji genetički faktori rizika za nastanak trombofilije. Nedavno je opisana nova mutacija u genu za protrombin (c.1787G >T) za koju je pokazano da dovodi do rezistencije na antitrombin, odnosno do smanjene mogućnosti inaktivacije mutiranog trombina od strane antitrombina, što predstavlja novi mehanizam za nastanak trombofilije. U toku poslednjih decenija, opisan je veliki broj genetičkih faktora rizika za nastanak trombofilije, uključujući one koji dovode do: nedostatka inhibitora koagulacije, povećanog nivoa ili smanjene inaktivacije koagulacionih faktora ili defekata sistema za fibrinolizu. Međutim, većina njih nije od dijagnostičke važnosti zbog njihovog malog ili još uvek nepoznatog uticaja na etiologiju trombofilije. Primena novih tehnologija koje omogućavaju analizu velikog broja gena kod jednog pacijenta otvoriće mogućnost individualnog utvrđivanja genetičkih faktora rizika, samim tim i adekvatan terapeutski pristup.

**Ključne reči:** trombofilija, genetički faktori rizika, genske varijante

*Address for correspondence:*

Valentina Đorđević
Institute of Molecular Genetics and Genetic Engineering
Vojvode Stepe 444A, P.O. Box 23, 11010 Belgrade, Serbia
Tel: +381 11 3976658
Fax: +381 11 3975808
e-mail: pg20210a@gmail.com

*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; NGS, 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 hemostasis 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 $\alpha$-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 Nangorff 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,

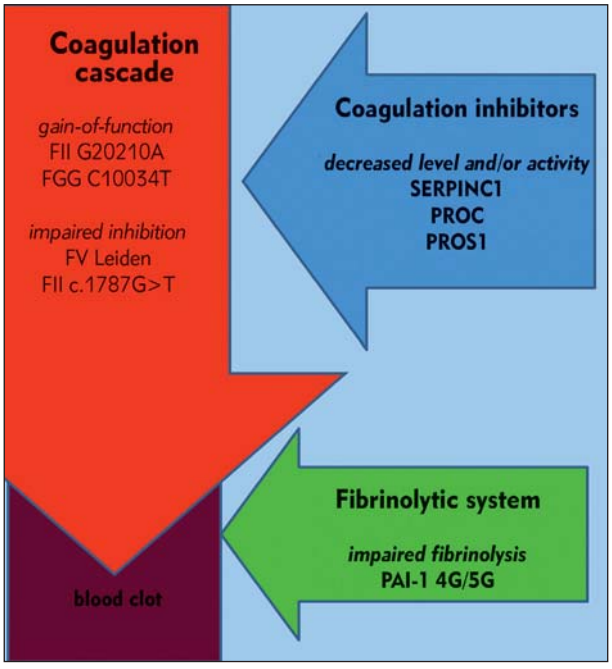


**Figure 1** Genetic risk factors related to thrombophilia.

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* that codes AT is a highly polymorphic gene, with more than 130 different mutations reported. In several families, AT deficiency develops due to »private mutations« (present in <0.001 of the population) (17). AT deficiency is rare in the general population (0.02%) but is associated with a high relative risk of DVT. Relative risk for heterozygous carriers is around 10. Homozygous AT deficiency is not compatible with life (18).

In 1981, Griffin et al. (19) described a heterozygous PC deficiency in a family with a history of recurrent thrombosis. After that, more than 160 mutations were described in the *PROC* 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 population of Caucasian origin, but with significant regional differences in prevalence (2–16%) (13, 14, 23, 24). FV Leiden affects 15%–25% of patients with DVT and the risk of DVT in heterozygous carriers is approximately fivefold higher than in a control population (18). FV Leiden mutation was so prevalent in the general population that the focus shifted from single families, which was the case in the defects of AT, PC and PS, to population-based case-control studies (14).

*FII G20210A gene variant*

Shortly after the FV Leiden variant, substitution G to A at position 20210 in the 3'-untranslated region of the Factor II (FII) gene was described (26). FII G20210A variant was associated with increased concentrations of FII in plasma, leading to increased thrombin generation and hypercoagulability. This gene variant was also found to be common in healthy Caucasian populations (1–6%). FII G20210A was detected in 6–18% of thrombophilic patients and the presence of FII 20210A allele is associated with an approximately 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 hypercoagulation, 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 90$^{th}$ 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.

## References


1. Colman RW, Clowes AW, George JN, Goldhaber SZ, Marder VJ. Overview of hemostasis. In: Colman RW, Marder VJ, Clowes AW, George JN, Goldhaber SZ. Hemostasis and Thrombosis: basic principles and clinical practice. 5th ed. Philadelphia: Lippencott Wiliams & Wilkins, 2006: 2–15.
2. Hoffman M, Monroe DM. A cell-based model of hemostasis. Thromb Haemost 2001; 85: 958–65.
3. Ruggeri ZM. Mechanisms Initiating Platelet Thrombus Formation. Thromb Haemost 1997; 78: 611–16.
4. Saito H, Takamatsu J. Disorders of Prothrombin Conversion. In: Gross S, Roarh S, editors. Hematology: A problem-oriented approach. Baltimor: Williams & Wilkins, 2000: 570–1.
5. Roberts HR, Monroe DM, Oliver JA, Chang JY, Hoffman M. Newer concepts of blood coagulation. Haemophilia 1998; 4: 331–4.
6. Dang QD, Vindigni A, Dicera E. An allosteric switch controls the procoagulant and anticoagulant activites of thrombin. Proc Natl Acad Sci USA 1995; 92: 5977–81.
7. Fuentes-Prior P, Iwanaga Y, Huber R, Pagila R, Rumennik G, Seto M, et al. Structural basis for the anticoagulant activity of the thrombin-thrombomodulin complex. Nature 2000; 404: 518–25.
8. Degen JL. Genetic interaction between the coagulation and fibrinolytic system. Thromb Haemost 2001; 86: 30–7.
9. Elezović I. Urođene i stečene trombofilije. Bilten za transfuziologiju 2000; Suppl 1: 16–25.
10. Heit JA. The epidemiology of venous thromboembolism in the community. Arterioscler Thromb Vasc Biol 2008; 28: 370–2.
11. Lowe GD. Common risk factors for both arterial and venous thrombosis. Br J Haematol 2008; 140: 488–95.
12. Jordan FL, Nandorff A. The familial tendency in thromboembolic disease. Acta Med Scand 1956; 156: 267–75.
13. März W, Nauck M, Wieland H. The molecular mechanisms of inherited thrombophilia. Z Kardiol 2000; 89: 575–86.
14. Rosendaal FR, Reitisma PH. Genetic of venous thrombosis. J Thromb Haemost 2009; 7: 301–4.
15. Egeberg O. Inherited antithrombin III deficiency causing thrombophilia. Thromb Diath Haemorrh 1965; 13: 516–30.
16. Cooper PC, Coath F, Daly ME, Makris M. The phenotypic and genetic assessment of antithrombin deficiency. Int J Lab Hematol 2011; 33: 227–37.
17. Bucciarelli P, Passamonti SM, Biguzzi E, Gianniello F, Franchi F, Mannucci PM, et al. Low borderline plasma levels of antithrombin, protein C and protein S are risk factors for venous thromboembolism. J Thromb Haemost 2012; 10: 1783–91.
18. Seligsohn U, Lubetsky A. Genetic susceptibility to venous thrombosis. N Engl J Med 2001; 344: 1222–31.
19. Griffin JH, Evatt B, Zimmerman TS, Kleiss AJ, Wideman C. Deficiency of protein C in congenital thrombotic disease. J Clin Investig 1981; 68: 1370–3.
20. Van Cott EM, Laposata M. Laboratory evaluation of hypercoagulable states. Hematol Oncol Clin North Am 1998; 12: 1141–66.
21. Comp PC, Esmon CT. Recurrent venous thromboembolism in patients with a partial deficiency of protein S. N Engl J Med 1984; 311: 1525–8.
22. Beauchamp NJ, Dykes AC, Parikh N, Campbell Tait R, Daly ME. The prevalence of, and molecular defects underlying, inherited protein S deficiency in the general population. Br J Haematol 2004; 125: 647–54.
23. Dahlback B, Carlsson M, Svensson PJ. Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C. Proc Natl Acad Sci USA 1993; 90: 1004–8.
24. Bertina RM, Koeleman BPC, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994; 369: 64–6.
25. Rees DC, Cox M, Clegg JB. World distribution of factor V Leiden. Lancet 1995; 346: 1133–4.
26. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood 1996; 88: 3698–703.
27. Bertina RM. Genetic approach to thrombophilia. Thromb Heamost 2001; 86: 92–103.
28. Danckwardt S, Hartmann K, Gehring NH, Hentze MW, Kulozik AE. 3' end processing of the prothrombin mRNA in thrombophilia. Acta Haematol 2006; 115: 192–7.
29. Đorđević V, Pruner I, Kovač M, Miljić P, Antonijević N, Kojić S, Radojković D. Three novel 3'end prothrombin gene polymorphisms and their association with thrombophilia (abstract). J Thromb Haemost 2012; 9: Suppl 2: 873.
30. Danckwardt S, Gehring NH, Neu-Yilik G, Hundsdoerfer P, Pforsich M, Frede U, Hentze MW, Kulozik AE. The prothrombin 3'end formation signal reveals a unique architecture that is sensitive to thrombophilic gain-of-function mutations. Blood 2004; 104: 428–35.
31. Miyawaki Y, Suzuki A, Fujita J, Maki A, Okuyama E, Murata M, et al. Thrombosis from a prothrombin mutation conveying antithrombin resistance. N Engl J Med 2012; 366: 2390–6.
32. Đorđević V, Kovač M, Pruner I, Francuski D, Radojković D. A novel prothrombin c.1787G>A mutation in a Serbian family with recurrent thromboembolism – another case of antithrombin resistance (abstract). J Thromb Haemost 2013; 11: 375.
33. Uitte de Willige S, de Visser MC, Houwing-Duistermaat JJ, Rosendaal FR, Vos HL, Bertina RM. Genetic variation in the fibrinogen gamma gene increases the risk for deep

venous thrombosis by reducing plasma fibrinogen gamma' levels. Blood 2005; 106: 4176–83.

34. Chung DW, Davie EW. Gamma and gamma' chains of human fibrinogen are produced by alternative mRNA processing. Biochemistry 1984; 23: 4232–6.
35. Eriksson P, Kallin B, Van't Hofft FM, Båvenholm P, Hamsten A. Allele-specific increase in basal transcription of the plasminogen activator inhibitor-1 gene is associated with myocardial infarction. Proc Natl Acad Sci USA 1995; 92: 1851–5.
36. Tsantes AE, Nikolopoulos GK, Bagos PG, Bonovas S, Kopterides P, Vaiopoulos G. The effect of the plasminogen activator inhibitor-1 4G/5G polymorphism on the thrombotic risk. Thromb Res 2008; 122: 736–42.
37. Jick H, Slone D, Westerholm B, Inman WH, Vessey MP, Shapiro S, et al. Venous thromboembolic disease and ABO blood type. A cooperative study. Lancet 1969; 1: 539–42.
38. Wu O, Bayoumi N, Vickers MA, Clark P. ABO(H) blood groups and vascular disease: a systematic review and meta-analysis. J Thromb Haemost 2008; 6: 62–9.
39. Tregouet DA, Heath S, Saut N, Biron-Andreani C, Schved JF, Pernod G, et al. Common susceptibility alleles are unlikely to contribute as strongly as the FV and ABO loci to VTE risk: results from a GWAS approach. Blood 2009; 113: 5298–303.
40. McGrath RT, McKinnon TA, Byrne B, O'Kennedy R, Terraube V, McRae E, et al. Expression of terminal alpha2-6-linked sialic acid on von Willebrand factor specifically enhances proteolysis by ADAMTS13. Blood 2010; 115: 2666–73.
41. Ohira T, Cushman M, Tsai MY, Zhang Y, Heckbert SR, Zakai NA, et al. ABO blood group, other risk factors and incidence of venous thromboembolism: the Longitudinal Investigation of Thromboembolism Etiology (LITE). J Thromb Haemost 2007; 5: 1455–61.
42. Cohen W, Castelli C, Alessi MC, Aillaud MF, Bouvet S, Saut N, et al. ABO blood group and von Willebrand factor levels partially explained the incomplete penetrance of congenital thrombophilia. Arterioscler Thromb Vasc Biol 2012; 32: 2021–8.
43. Koster T, Blann AD, Briet E, Vandenbroucke JP, Rosendaal FR. Role of clotting factor VIII in effect of von Willebrand factor on occurrence of deep vein thrombosis. Lancet 1995; 345: 152–5.
44. Kyrle PA, Minar E, Hirschl M, Bialonczyk C, Stain M, Schneider B, et al. High plasma levels of factor VIII and the risk of recurrent venous thromboembolism. N Engl J Med 2000; 343: 457–62.
45. Li C, Schwarz UI, Ritchie MD, Roden DM, Stein CM, Kurnik D. Relative contribution of CYP2C9 and VKORC1 genotypes and early INR response to the prediction of warfarin sensitivity during initiation of therapy. Blood 2009; 113: 3925–30.
46. Daly A. Pharmacogenomics of anticoagulants: steps toward personal dosage. Genom Med 2009; 1: 10.
47. Babić N. Clinical pharmacogenomics and concept of personalized medicine. J Med Biochem 2012; 31: 281–6.
48. Kovač MK, Rakićević LB, Radojković DP. Extreme sensitivity to acenocoumarol therapy in patient with both VKORC.-1639 A/A and CYP2C9*1/*3 genotypes. Thromb Thrombolysis 2011; 32: 368–71
49. Antonijević N, Stanojević M, Milošević R, Đorđević V, Jauković M, Vukčević V, et al. Combined thrombophilic risk factors and essential thrombocythemia in patient with recurrent venous thromboembolic episodes–thirty-three-year follow-up. J Thromb Thrombolysis 2005; 19: 93–5.
50. De Haan HG, Bezemer ID, Doggen CJ, Le Cessie S, Reitsma PH, Arellano AR, et al. Multiple SNP testing improves risk prediction of first venous thrombosis. Blood 2012; 120: 656–63.
51. Ocak G, Vossen CY, Verduijn M, Dekker FW, Rosendaal FR, Cannegieter SC, et al. Risk of venous thrombosis in patients with major illnesses: results from the MEGA study. J Thromb Haemost 2013; 11: 116–23.
52. Bezemer ID, Bare LA, Doggen CJ, Arellano AR, Tong C, Rowland CM, et al. Gene variants associated with deep vein thrombosis. JAMA 2008; 299: 1306–14.

*Received: July 12, 2013*

*Accepted: August 6, 2013*