

An Overview of Genetic Risk Factors in Thrombophilia

Valentina Djordjević, Ljiljana Rakićević, Dragica Radojković

Institute of Molecular Genetics and Genetic Engineering, 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 tendency to thrombosis. The concept that thrombophilia could be associated with genetic defects was first proposed in 1965 after the discovery of familiar antithrombin III deficiency. Further family studies showed that deficiency of protein C or protein S also increased thrombotic risk. In the coming years the advent in DNA technology, especially the invention of PCR reaction, played an important role in the identification of the exact nature of these deficiencies and opened new possibilities in the genetic research of thrombophilia. The breakthrough came with the discovery of activated protein C resistance and Factor V Leiden mutation. Shortly afterwards a mutation in the 3' untranslated region of Factor II gene (FII G20210A) associated with increased concentration of factor II in plasma, was described. Large epidemiologic studies have conformed that these two common mutations represent significant risk factors for thrombophilia. In the last decade several prothrombotic genetic risk factors have been described, including genes variants associated with increased levels of coagulation factors, defects of natural coagulation inhibitors, defects of the fibrinolytic system and hyperhomocysteinemia. These genetic defects or their combination have been extensively studied in an attempt to elucidate the possible association with increased thrombotic tendency. The large-scale DNA analysis systems are now becoming available, opening a new era in the genetic studies of thrombophilia. New technology will enable many genes to be studied in a single patient bringing us closer to the "personalized" medicine.

Keywords: thrombophilia; genetic risk factors; mutation

INTRODUCTION

The term thrombophilia was coined by Jordan and Nangorff in 1956; it refers to increased tendency to develop clots in blood vessels. It is a multicausal disease in which both acquired and genetic risk factors may play important roles affecting the natural haemostatic balance between procoagulant and anticoagulant factors [1, 2]. The concept that thrombophilia could be associated with genetic defects was first proposed in 1965 after the discovery of familiar antithrombin III (ATIII) deficiency [3]. The ATIII deficiency is a very rare disorder with the incidence of 0.02% in general population and it is associated with a high risk of venous thromboembolism (VTE) [4]. It took almost 20 years before the deficiency of protein C (PC) and protein S (PS) was recognized as very strong, but rare thrombotic risk factors [5, 6]. Deficiencies of ATIII, PC, PS are found in less than 1% of the population [4]. These first studies were based on the analysis of plasma levels of natural anticoagulants in family members with deficiency status, and were carried out in order to document inheritance patterns. In the coming years the advent in DNA technology, especially the invention of PCR reaction, opened up new possibilities in genetic research of thrombophilia.

FACTOR V LEIDEN AND FACTOR II G20210A MUTATIONS

The breakthrough came with the discovery of activated protein C (APC) resistance and Factor V G1691A (FV Leiden) mutation. Dahlback et al [7] found that the plasma of patients with familiar thrombosis showed a

reduced response to the addition of APC. APC resistance is the consequence of impaired ability of APC to cleave Factor V (FV) due to amino-acid substitutions within the FV at the cleavage sites. APC inactivates FV by proteolysis at Arg³⁰⁶, Arg⁵⁰⁶ and Arg⁶⁷⁹. FV is first cleaved at Arg⁵⁰⁶, and this peptide bond cleavage is essential for the optimal exposure of the cleavage sites of Arg³⁰⁶ and Arg⁶⁷⁹ [8]. Shortly after Dahlback's finding, Bertina and coworkers reported that single point mutation G1691A in the FV gene, which results in the substitution of arginine at position 506 by glutamine, was responsible for the observed APC resistance phenotype [9]. FV Leiden mutation is common in the healthy population of Caucasian origin, but with significant regional differences in prevalence (2-16%) [10, 11, 12]. The frequency is increased up to 15-50% in patients with VTE [13, 14]. Heterozygous carriers have approximately a 5-fold increased risk for thrombosis and in homozygous carriers the risk is 50-fold increased [15]. Studies showed that the most common clinical manifestation of FV Leiden mutation was a deep vein thrombosis (DVT) with or without pulmonary embolism (PE) [16, 17, 18]. Although originally identified within the context of family study, FV Leiden mutation was so prevalent in the general population that the focus shifted from family to population-based case-control studies. Shortly after FV Leiden mutation, G to A substitution at position 20210 of the 3'-untranslated region of the Factor II (FII) gene was described. The G20210A gene variant was associated with increased concentration of FII in plasma, and also found to be common in Caucasian populations (1-6%) [19, 20]. The risk of thrombosis is 2- to 3-fold increased in carriers. Large epidemiologic studies have conformed that these two common mutations

(FV Leiden and FII G20210A) represent moderate but significant risk factors for thrombophilia [13, 16, 21].

OTHER GENETIC RISK FACTORS

In the last decade, several prothrombotic genetic risk factors have been described; genes variants associated with levels of coagulation factors (factor XIII Val34Leu, fibrinogen γ C10034T, fibrinogen β -455G/A), defects of natural coagulation inhibitors (protein C -1641G/A, protein C -1654G/A), defects of the fibrinolytic system (plasminogen activator inhibitor (PAI-1) -675 4G/5G) and hyperhomocysteinemia (MTHFR C677T) [22-26]. Homozygous carriers of FXIII gene variant have a reduced risk of venous thrombosis (approximately 30%) with the prevalence of 10% in general population [22]. Single nucleotide polymorphisms (SNPs) in fibrinogen genes have been associated with small thrombotic risk increases (1.2-1.3-fold), except for fibrinogen γ C10034T, which increases thrombotic risk approximately 2-fold [23]. Variants in the promoter region of protein C gene are associated with 1.3-fold increased risk of thrombosis [24]. A common single guanine (4G/5G) polymorphism located 675 bp upstream from the transcription site of the PAI-1 gene was associated with elevated PAI-1 level, which appears to be a thrombotic risk factor [25]. The variant C677T of the MTHFR gene is also very common. This polymorphism renders the enzyme thermolabile and tends to a slightly elevated homocysteine level. Published studies have shown conflicting results regarding the effect of carriership on the risk of thrombosis [2, 4, 12, 26, 27].

Although numerous, due to their minor or unknown impact on the thrombotic risk, most of described gene variants are not of diagnostic value. The combinations of candidate genes variants have been and still are extensively studied in an attempt to elucidate their possible association with increased thrombotic tendency [20, 28, 29].

In the past years there has been increasing evidence that genetic factors may play important roles in the patient's

anticoagulant response. Polymorphisms for the CYP2C9 gene, which encodes the main cytochrome P450 enzyme that metabolizes warfarin, and VKORC1, the gene encoding the warfarin target vitamin K epoxide reductase, are associated with variability in the dose requirement of warfarin [30, 31]. The current knowledge of genetic factors affecting other anticoagulants is more limited and this area requires futures studies [31].

A large-scale DNA analysis systems (sequencing, microarray) are now becoming available opening a new era in the genetic studies of thrombophilia. In the recent study, nearly 20,000 SNPs in over 11,000 genes were tested in 3,000 patients with thrombosis and 5,000 control subjects. Several polymorphisms located in CYP4V2, SERPINC1, Factor IX, Glycoprotein 6 were found to be associated with thrombosis [32]. Among them, a sequence variant in CYP4V2 was located close to the Factor XI gene, and probably related to the Factor XI level, which was found to be associated with thrombosis in previous studies [32].

CONCLUSION

In order to determine new prothrombotic genetic risk factors several genome-wide associated studies are foreseen, as well as their potential clinical utility. The comprehensive knowledge of all genetic risk factors will substantially improve our diagnostics and the prevention of thrombophilia. New technology will enable many genes to be studied in a single patient and the determination of "personalized" genetic risk factors for thrombophilia, and finally, therapeutic approach tailored for the individual patient.

ACKNOWLEDGEMENT

This work was supported by a grant 143051 from the Ministry for Science, Serbia.

REFERENCES

- Jordan FL, Nandorff A. The familial tendency in thrombo-embolic disease. *Acta Med Scan.* 1956; 156:267-75.
- Lane DA, Mannucci PM, Bauer KA, Berina RM, Bochkov NP, Boulyjenkov V, et al. Inherited thrombophilia: Part 1. *Thromb Haemost.* 1996; 76(5):651-62.
- Egeberg O. Inherited antithrombin III deficiency causing thrombophilia. *Thromb Diath Haemorrh.* 1965; 13:516-30.
- Seligsohn U, Lubetsky A. genetic susceptibility to venous thrombosis. *N Engl J Med.* 20010; 344:1222-31.
- Griffin JH, Evatt B, Zimmerman TS, Kleiss AJ, Wideman C. Deficiency of prothrombin C in congenital thrombotic disease. *J Clin Invest.* 1981; 68:1370-3.
- Comp PC, Nixon RR, Cooper MR, Esmon CT. Familial prepeitin S deficiency is associated with recurrent thrombosis. *J Clin Invest.* 1984; 74:2082-8.
- 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.
- Kalafatis M, Rand MD, Mann KG. The mechanism of inactivation of human factor Va by activated protein C. *J Biol Chem.* 1994; 269:31869-80.
- 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.
- Rees DC, Cox M, Clegg JB. World distribution of factor v Leiden. *Lancet.* 1995; 346:1133-4.
- Rees DC. The population genetics of factor V Leiden (Arg506Gln). *Br J Haematol.* 1996; 95:579-86.
- Djordjevic V, Rakicevic LJ, Mikovic D, Kovac M, Miljic P, Radojkovic D, et al. Prevalence of factor V Leiden, factor V Cambridge, factor II G20210A and methylenetetrahydrofolate reductase C677T mutations in healthy and thrombophilic Serbian population. *Acta Haematol.* 2004; 112(4):227-9.
- De Stefano V, Chiusolo P, Paciaroni K, Leone G. Epidemiology of factor V Leiden: Clinical implications. *Semin Thromb Hemost.* 1998; 24:367-79.
- Koster T, Rosendaal FR, de Ronde, briet E, Vandenbroucke JP, Bertina RM. Venous thrombosis due to a poor anticoagulant response to activated protein C: Leiden Thrombophilia Study. *Lancet.* 1993; 342:1503-6.
- Dahlback B. Are we ready for factor V Leiden screening? *Lancet.* 1996; 347:1346-7.
- Svensson PJ, Dahlback B. Resistance to activated protein C as a

- basis for venous thrombosis. *N Engl J Med.* 1994; 330:517-20.
17. Djordjevic V, Rakicevic LJ, Miljic P, Mikovic D, Kovac M, Radojkovic D, et al. Impact of acquired and genetic factors on thrombophilic phenotype in FV Leiden mutation carriers. *Yugoslav Med Biochem.* 2005; 24(2):141-6.
 18. Kovac M, Mikovic D, Antonijevic N, Rakicevic L, Djordjevic V, Radojkovic D, et al. FV Leiden mutation and risk of recurrent venous thromboembolism in Serbian population. *J Thromb Thrombolysis.* 2008; 25:284-7.
 19. 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.
 20. Bertina RM. Genetic approach to thrombophilia. *Thromb Haemost.* 2001; 86:92-103.
 21. Kovac M, Mitic G, Mikovic Z, Antonijevic N, Djordjevic V, Mikovic D, et al. Type and location of venous thromboembolism in carriers of factor V Leiden or prothrombin G20210A mutation versus patients with no mutation. *Clin Appl Thr Haemost.* in press.
 22. van Hylckama, Vlieg A, Komanasin N, Ariens RA, Poort SR, Grant PJ, et al. Factor XIII Val34Leu polymorphism, factor XIII antigen levels and activity and the risk of deep venous thrombosis. *Br J Haematol.* 2002; 119:169-75.
 23. Behague I, Poirier O, Nicaud V, Evans A, Arveiler D, Luc G, et al. Beta fibrinogen gene polymorphisms are associated with plasma fibrinogen and coronary artery disease in patients with myocardial infarction. The ECTIM Study. *Etude Cas-Temoins sur l'Infarctus du Myocarde.* *Circulation.* 1996; 93:440-9.
 24. Spek CA, Koster T, Rosendaal FR, Bertina RM, Reitsma PH. Genotypic variation in the promoter region of the protein C gene is associated with plasma protein C levels and thrombotic risk. *Arterioscler Thromb Vasc Biol.* 1995; 15(2):214-8.
 25. Burzotta F, Di Castelnuovo A, Amore C, D'Orazio A, Di Bitondo R, Donati MB, et al. 4G/5G promoter PAI-1 gene polymorphism is associated with plasmatic PAI-1 activity in Italians: a model of gene-environment interaction. *Thromb Haemost.* 1998; 79(2):354-8.
 26. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nature Genet.* 1995; 10:111-3.
 27. Beletic A, Mirkovic D, Antonijevic N, Djordjevic V, Sango V, Jakovljevic B, et al. Incidence of hyperhomocysteinemia and MTHFR C677T polymorphism among young patients with acute myocardial infarction. *J Med Biochem.* 2009; 28(1):41-5.
 28. Antonijevic N, Stanojevic M, Milosevic R, Djordjevic V, Jaukovic M, Vukcevic V, et al. Combined thrombophilic risk factors and essential thrombocytopenia in patient with recurrent venous thromboembolic episodes-thirty-three-year follow-up. *J Thromb Thrombolysis.* 2005; 19(2):93-5.
 29. Reitsma PH, Rosendaal FR. Past and future of genetic research in thrombosis. *J Thromb Haemost.* 2007; 5:264-9.
 30. Li C, Schwarz UJ, 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(17):3925-30.
 31. Daly A. Pharmacogenomics of anticoagulants: steps toward personal dosage. *Genom Med.* 2009; 1:10.
 32. Rosendaal FR, Reitsma PH. Genetic of venous thrombosis. *J Thromb Haemost.* 2009; 7:301-4.

Преглед генетичких фактора ризика код тромбофилије

Валентина Ђорђевић, Љиљана Ракићевић, Драгица Радојковић

Институт за молекуларну генетику и генетски инжењеринг, Београд, Србија

КРАТАК САДРЖАЈ

Тромбофилија настаје као резултат сложене интеракције не-генетичких и генетичких фактора ризика који хемостазну равнотежу померају у смеру хиперкоагулације и доводе до појаве тромбозе. Да генетички фактори могу имати значајну улогу у настанку тромбофилије први пут је указано 1965. године, када је недостатак антитромбина III описан у једној породици. Даља истраживања у оквиру „породичних“ студија показала су да недостатак протеина С и протеина С такође доводи до повећаног ризика за појаву тромбозе. Напредак на пољу ДНК технологије, а посебно откриће и примена *PCR*, отворио је нове могућности у истраживању генетичких фактора ризика. Велики корак напред направљен је открићем резистенције на активирани протеин С и мутације фактора V Лајден (*Leiden*), која до ње доводи. Убрзо је откривена и мутација у 3'-некодирани региону гена за фактор II (*FII G20210A*), за коју је пока-

зано да изазива повишену концентрацију протромбина у плазми. Даље епидемиолошке студије су потврдиле да су ове две честе мутације значајан фактор ризика за настанак тромбофилије. У последњој деценији описан је велики број генетичких фактора ризика, укључујући и оне који доводе до повећаног нивоа коагулационих фактора, недостатка природних инхибитора коагулације, оштећења система за фибринолизу и хиперхомоцистеинемije. Ове генетичке варијанте и њихове комбинације се интензивно проучавају, како би се утврдило колики је њихов значај у настанку тромбофилије. Примена нових технологија које омогућавају анализу великог броја гена код једног болесника отвориће могућност индивидуалног утврђивања генетичких фактора ризика и, самим тим, одговарајућег приступа у лечењу.

Кључне речи: тромбофилија; генетички фактори ризика; мутације