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Chapter 5

Common Gene Polymorphisms Associated with Thrombophilia

Christos Yapijakis, Zoe Serefoglou and Constantinos Voumvourakis

Additional information is available at the end of the chapter

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Abstract

Genetic association studies have revealed a correlation between DNA variations in genes encoding factors of the hemostatic system and thrombosis-related disease. Certain variant alleles of these genes that affect either gene expression or function of encoded protein are known to be genetic risk factors for thrombophilia. The chapter presents the current genetics and molecular biology knowledge of the most important DNA polymorphisms in thrombosis-related genes encoding coagulation factor V (FV), coagulation factor II (FII), coagulation factor XII (FXII), coagulation factor XIII A1 subunit (FXIIIA1), 5,10-methylene tetrahydrofolate reductase (MTHFR), serpine1 (SERPINE1), angiotensin I-converting enzyme (ACE), angiotensinogen (AGT), integrin A2 (ITGA2), plasma carboxypeptidase B2 (CPB2), platelet glycoprotein Ib α polypeptide (GP1BA), thrombomodulin (THBD) and protein Z (PROZ). The molecular detection methods of each DNA polymorphism is presented, in addition to the current knowledge regarding its influence on thrombophilia and related thrombotic events, including stroke, myocardial infarction, deep vein thrombosis, spontaneous abortion, etc. In addition, best thrombosis prevention strategies with a combination of genetic counseling and molecular testing are discussed.

Keywords: Thrombophilia, coagulation factors, genetic association, DNA polymorphisms, molecular analysis

1. Introduction

Thrombosis is a common underlying pathological event of venous thromboembolism, ischemic stroke and ischemic heart disorder. It is well established that all three diseases are associated with a major global thrombotic events, including stroke, myocardial infarction, deep vein thrombosis, spontaneous abortion, etc. In addition, best thrombosis prevention strategies with a combination of genetic counseling and molecular testing are discussed.

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is known to be influenced by several hereditary and lifestyle-related factors [3–5]. Genetic association studies have revealed a correlation between DNA variations in genes encoding factors of the hemostatic system and thrombosis-related disease [6–10].

Certain variant alleles of these thrombosis-associated genes that affect either gene expression or function of encoded protein are known to be genetic risk factors for thrombophilia. The genetic variants are found in genes involved in coagulation, fibrinolysis, platelet activity and other functions related to thrombosis [4, 9, 11]. Most of these are single-nucleotide polymorphisms (SNPs) and affect hemostatic mechanisms in a quantitative or in a qualitative manner [9, 11]. Primary and secondary prevention of death and disability incidence due to thrombotic events may be effected through optimized anticoagulant treatment at individual patient level. This chapter will discuss the current molecular biology and genetics knowledge of the most important DNA polymorphisms in thrombosis-related genes. In addition, best thrombosis prevention strategies with a combination of genetic counseling and molecular testing will be discussed.

2. Coagulation and thrombosis-related factors

Coagulation is a complex multienzymatic cascade by which fibrin is produced forming the basis of a clot. Thrombosis involves an imbalance of coagulation and fibrinolysis, which also includes formation and degradation of extracellular matrix (ECM).

The most essential components of the coagulation and fibrinolysis systems are associated with the endothelial cell membrane, including tissue factor (TF), thrombin and urokinase receptors. They become exposed when vessels are injured and when platelets are bound to them at early stages of either coagulation or inflammation. Consequently, platelets release vascular endothelial growth factor (VEGF) and other growth factors into the circulation, thus promoting angiogenesis and attracting inflammatory cells to the site of injury [12]. In addition, platelets also contain hemostatic factors that regulate the extent of tissue repair and inhibit vascularization, such as serpine1, previously known as plasminogen activator inhibitor-1 (PAI-1) [12].

The coagulatory cascade is artificially divided based on in vitro testing in the intrinsic or contact pathway and the extrinsic or tissue factor (TF) pathway. The two pathways interfere since both thrombin and TF may activate coagulation factor IX (FIX) [12].

3. Tissue factor pathway

TF is expressed constitutively on subendothelial fibroblasts and smooth muscle cells which are exposed to blood only upon vascular damage. Therefore, TF acts as the physiological initiator of coagulation [13]. In addition, TF is expressed on peripheral blood monocytes and on vascular endothelia after exposure to inflammatory or activating stimuli, such as endotoxin.

TF is a transmembrane glycoprotein with an extracellular ligand-binding domain that interacts with coagulation factor VII in both its inactive and active forms (FVII/FVIIa) [14]. When TF-
expressing cells in extravascular sites come in contact with plasma, a complex of TF with the circulating FVII is formed on the cell surface, which is further activated to FVIIa by limited proteolysis, producing activated coagulation factor X (FXa) and thrombin and leading to coagulation. The function of TF is kept at appropriate levels through TF pathway inhibitor (TFPI), a protease inhibitor constitutively released from endothelia. TFPI interacts with circulating FXa and the TFPI/FXa complex binds to the TF/FVIIa complex down-regulating TF-induced quaternary complex of Ca\(^{2+}\) and coagulation factors VIIIa, IXa and X [15].

In addition to its hemostatic role, TF is also involved in normal embryonic angiogenesis through the induction of vascular endothelial growth factor (VEGF) and the secretion up-regulation of urokinase plasminogen activator receptor [16–18]. Both TF and VEGF are stimulated by hypoxia through different pathways [19, 20].

4. Thrombin pathway

Thrombin plays a central role in coagulation, since it is the most effective agonist for platelet activation and leads to the formation of the fibrin clot by activating various zymogens and cofactors, including fibrinogen, coagulation factors XIII, V and VIII, platelet membrane GPV, protein S and protein C [21]. Much like TF, thrombin may promote angiogenesis through different pathways and independently of fibrin generation.

Thrombin also enhances the migratory potential of endothelia through basement membranes by activating gelatinase A (matrix metalloprotenase-2, MMP-2), which degrades collagen IV and releases tissue plasminogen (t-PA) and PAI-1 [22–25]. In addition, thrombin activates platelets, leading to their aggregation and release of numerous pro- and anti-angiogenic factors. Furthermore, angiogenesis is inhibited by the prothrombin kringle 2 domain as well as two prothrombin fragments (F1 and F2) generated during activation of thrombin [26, 27].

Thrombin inhibits dissolution of clots and modulates fibrinolysis through activation of thrombin activatable fibrinolysis inhibitor (TAFI). TAFI cleaves certain C-terminal lysine residues from fibrin, thereby preventing plasminogen, plasmin and t-PA from binding to fibrin [28].

Thrombin forms a complex with thrombomodulin, an endothelial cell membrane protein, leading to the activation of protein C. Consequently, activated protein C cleaves non-platelet-associated activated coagulation factors V and VII (FVa and FVIIa), thus down-regulating thrombin formation by inactivating the prothrombinase complex as well as the quaternary complex of Ca\(^{2+}\) and coagulation factors VIIIa, IXa and X. In addition to its anticoagulant role, thrombomodulin reduces fibrinolysis by activating TAFI in plasma [29]).

5. Fibrinogen, cross-linked fibrin and fibrinolysis

Fibrinogen plays a critical role in hemostasis. Following activation by thrombin, fibrinogen is subsequently cleaved to monomers of fibrin that are rapidly combined to form a fibrin matrix.
In addition, thrombin activates coagulation factor XIII (FXIII) that converts soluble fibrin into an insoluble polymer of fibrin. Activated FXIII also prevents fibrinolysis by linking α2-plasmin inhibitor to fibrin [30].

The fibrin gel promotes angiogenesis and cell growth, by providing a provisional matrix, enriched in growth factors which are protected from degradation, such as VEGF and insulin-like growth factor-1 [31, 32]. Furthermore, the fibrin gel provides a surface suitable for the prothrombinase assembly, a function platelets perform in intravascular clotting.

The provisional fibrin matrix undergoes remodeling and is transformed into mature connective tissue stroma, which in its constituent elements resembles the stroma of healing wounds [33, 34]. There is a balance between deposition of fibrin gel from clotting activation and its dissolution through activation of fibrinolysis [34]. Fibrinolytic activity depends on the balance between several factors including coagulation XIIa (FXIIa), which converts plasminogen to plasmin, plasminogen activators (PA) and their inhibitors, such as PAI-1 and α2-antiplasmin.

6. Angiogenesis

Angiogenesis (vascularization) involves a sequence of key events which include focal detachment of endothelial cells from the basement membrane, localized proteolytic degradation of the basement membrane and ECM invasion, leading to capillary tube formation and vascular remodeling [35]. Hemostatic mechanisms may influence angiogenesis directly or indirectly through a number of different pathways, involving the release of pro-angiogenic and anti-angiogenic factors from activated platelets. These angiogenesis-related factors, such as tumor necrosis factor-alpha (TNF-α), promote both formation of fibrin-rich ECM and thrombin signaling through activation of G-protein-coupled protease-activated receptors on the endothelial cell surface [21, 36].

7. Thrombosis and cancer

Cancer-related thrombosis represents a complex imbalance of coagulation and fibrinolysis, also involving the formation and degradation of ECM through interaction of angiogenesis-related factors [37–40]. Within a tumor, new vessels leak fibrin into the extravascular space and therefore persistently activate the coagulatory system, both locally and systemically, producing the clinical appearance of an unhealed wound [12, 41].

The disruption of hemostatic mechanisms is not merely a secondary effect of cancer but it appears to be a frequent event [41–43]. Sometimes, the activation of coagulation, such as venous thromboembolism, may even precede the clinical manifestations of cancer [42, 43]. It is well established that bleeding diathesis and systemic activation of the coagulation cascade contribute significantly to morbidity and mortality of patients with malignancies [44, 45].
8. Genetic association studies

Susceptibility in thrombophilia, like in all multifactorial diseases, implicates the interaction between environmental factors, such as diet or smoking, as well as genetic factors [46]. In the last couple of decades, population-based genetic association studies have been extensively investigating DNA polymorphisms in genes putatively involved in multifactorial diseases [39, 40, 46]. Such studies estimate the risk of developing a certain disease by comparing the frequency of polymorphic genotypes and allele frequencies between patients and matched healthy controls of the same population. A variant allele or a genotype is associated with increased risk for a disease when its detected frequency is significantly higher in cases than controls [47]. A significant association result indicates that the studied DNA polymorphism tested either directly affects the risk of a certain disease or acts as a genetic marker for a linked genetic variant that influences risk for that disease [47].

Genetic association studies usually investigate common SNPs in genes of cohorts of individuals. Over 10 million known SNPs are included in public databases and more of them are constantly identified. The frequency of the less common variant allele of SNPs in the general population is at least 1%, while mutations are usually much rarer. Therefore, due to their high incidence in the population, SNPs are usually very informative [39, 40, 46]. However, genetic association studies have to be conducted based on certain basic criteria, such as the adequate number of studied individuals, the gender ratio, age and ethnic compatibility of patients and healthy controls in order to avoid false positive and false negative results [46, 48]. The observed genotypic frequencies are considered representative of the population either of patients or healthy people if they are compatible with the Hardy–Weinberg equilibrium [49]. Highly significant associations replicated by a number of studies may be a useful tool for the prognosis and prevention of disease [40, 50].

The spectrum of coagulation and fibrinolysis factors and their respective encoding genes suggests that a great number of functional DNA polymorphisms might confer a major or minor risk of thrombosis [4, 7–11, 51, 52]. The most important DNA polymorphisms in thrombosis-related genes will be discussed, including those encoding (a) coagulation factors, (b) thrombin-related factors, (c) fibrinolysis-related factors, (d) platelet-adhesion factors and (e) other factors. A handful of variants in genes encoding for certain factors of the hemostatic system are positively known to confer a highly significant thrombotic phenotype, such as coagulation factor V (FV) Leiden, while for other variants there is only some emerging evidence implicating them in thrombotic disease, such as serpine1 (PAI-1) 4G/5G.

Some of the thrombosis-associated variants are very common in different populations, such as serpine1 (PAI-1) 4G/5G, which ranges between 42% and 54% worldwide [11]. Other polymorphic variants differ widely among populations, such as thrombomodulin (THBD) -G13A, which is extremely rare in Caucasians (<1%) and ranges between 8% and 19% in East Asians [53–59].

9. Coagulation factors

FV Leiden is probably the most important hereditary thrombosis-associated factor in Caucasians, with heterozygotes exhibiting up to 10-fold greater relative risk and Leiden homozy-
gotes 50–100-fold greater relative risk of venous thrombosis [60, 61]. *FV* Leiden is responsible for 20–25% of isolated thrombotic events and for 40–45% of cases of familial thrombophilia and fetal loss [3, 62–64]. The *FV* gene encodes a plasma glycoprotein which is activated by thrombin/coagulation factor Xa (FXa) and subsequently converted into factor Va (FVa). The Leiden variant (mutation G1691A) destroys a cleavage site of the anticoagulant-activated protein C in FVa. This variant has a wide allelic frequency range (1–9%) in European populations, while it is virtually absent from non-Caucasian populations [3, 60, 65]. *FV* Leiden is thought to have arisen approximately 21,000 to 34,000 years ago in Caucasians [60].

The gene encoding coagulation factor II (*FII*) or prothrombin contains another common defect in its 3′ untranslated region (G20210A). *FII* is a plasma glycoprotein which is activated to thrombin by coagulation factors FXa and FVa. The G20210A mutation is related to elevated plasma prothrombin levels, and heterozygotes have up to fourfold increased risk of venous thrombosis [66, 67]. The variant allele has a frequency of 1.3–4.5% in Caucasian populations [11, 65–67].

A common DNA polymorphism in the gene encoding coagulation factor XII (*F12*) is C46T. The prevalence of the thrombosis-related 46T allele ranges between 18% and 37% in various populations [11, 68]. In the gene *F13A1* coding for coagulation factor XIII A1 subunit, a SNP results in amino acid substitution Val34Leu [69]. There is emerging evidence that the *F13* 34Leu allele confers protection against thrombosis [69]. The prevalence of this particular allele varies considerably between populations ranging between 13% and 28% [11, 69].

### 9.1. Thrombin-related factors

The prevalence of the thrombin-related SNPs thrombomodulin (*THBD*) -G13A and protein Z (*PROZ*)-A33G is low in Caucasian populations (<1% and 6%, respectively) [11, 54, 70]. Both SNPs are located in the promoter region of the respective genes and the variant alleles result in lower levels of gene expression and lower protein production of thrombomodulin and protein Z. The role of both DNA polymorphisms in susceptibility for thrombosis appears to be minor.

### 9.2. Fibrinolysis-related factors

Variants in genes of fibrinolysis-related factors such as serpine1 (also known as plasminogen activator inhibitor-1, PAI-1) and thrombin-activatable fibrinolysis inhibitor (TAFI, also known as plasma carboxypeptidase B2, CPB2) have been shown to have an association with increased risk of venous thrombosis and myocardial infarction to a diverse extent [56, 71, 72]. A deletion/insertion polymorphism (4G/5G) in the promoter of the *serpine1* gene interferes with regulation of its transcription [72]. The 4G allele binds only an activator, while the 5G allele binds both a repressor and an activator. Therefore, the presence of the 4G variant results in higher gene expression, increased inhibition of the plasminogen activators and decreased plasminogen conversion to plasmin. Several studies have indicated that 4G/4G homozygosity is a risk factor for deep vein thrombosis, myocardial infarction and miscarriage during pregnancy [71, 72]. The prevalence of 4G variant in various populations ranges from 31% to 63% [11, 71, 72].
The effect of the C1040T polymorphism on TAFI (CPB2) function and its modest role in thrombosis is well established [56, 73]. The C to T substitution at codon 325 leads to a Thr to Ile change, which has an impact on the thermal stability of the enzyme, resulting in longer half-life and increased overall antifibrinolytic activity [74]. The prevalence of the 1040T variant in Caucasians and East Asians (31–40%) is much higher than African populations (11%) [11].

9.3. Platelet-adhesion factors

The role of DNA polymorphisms in genes encoding for platelet-adhesion factors, regarding the risk for certain thrombotic diseases, remains still uncertain although a minor contribution may not yet be ruled out. Among the most promising polymorphisms, possibly associated with risk for cerebrovascular diseases, appear to be C807T in the \textit{ITGA2} gene which encodes integrin A2, also known as platelet glycoprotein Ia and variable number of tandem repeats (VNTR) in the \textit{GP1BA} gene which encodes platelet glycoprotein Ibα polypeptide [11, 53, 75].

9.4. Other factors

Methylenetetrahydrofolate reductase (MTHFR) is an enzyme that plays an important role in folate metabolism [76]. A C677T mutation in the \textit{MTHFR} gene involves an alanine/valine change that renders the protein temperature sensitive and diminishes its activity, resulting in the pro-thrombotic condition of hyperhomocysteinemia, particularly when dietary intake of folate is inadequate [8, 77–79]. Homozygotes for the 677T allele account for 8.5% of the general population in Caucasians, and they are known to have a higher risk for thrombotic events [11, 80].

Two other factors that are related directly to blood pressure and indirectly to thrombotic events are angiotensinogen (AGT) and angiotensin-converting enzyme (ACE), two major players in the rennin–angiotensin system, a circulatory cascade primarily involved in the regulation of blood pressure and serum electrolytes. AGT is hydrolyzed into angiotensin I by rennin, which is subsequently converted to potent vasoconstrictor angiotensin II by ACE [81].

Among several DNA polymorphisms in the \textit{AGT} gene, the only well-studied one is a SNP at codon 235 because it influences gene expression and therefore has been associated with hypertension and thrombosis [82–85]. It involves an amino acid substitution (methionine to threonine, M235T). The high gene expression T allele has been associated with increased hypertension and risk for thrombosis [11, 82–84, 86]. The prevalence of the T variant is higher in Africans and Asians (78%), while it is lower in Caucasians ranging between 34% and 55%. [11, 86].

ACE activity is mainly determined by the insertion/deletion (I/D) polymorphism of a 287 bp Alu repeat sequence inside intron 16 of the \textit{ACE} gene [87]. Homozygotes for the I allele may display as low as half of the plasma ACE level compared to the homozygotes for the D allele, whereas the ID heterozygotes display an intermediate level [88]. The presence of the D allele causes elevation in angiotensin II levels and results in higher blood pressure. The D allele frequency is more abundant in Europeans (49–58%) than Asians (32–34%) [11].
10. Molecular detection methodology

Molecular analysis of DNA polymorphisms is usually performed in samples of genomic DNA extracted from blood, saliva, tissue, hair, semen or any other biological material. DNA testing of blood or saliva samples is a common practice. As in all occasions of genetic testing, a signed informed consent has to be obtained from any individual whose DNA would be examined.

The molecular detection methods of each DNA polymorphism depends on several factors including the nature of the nucleotide variation, the local sequence of the gene, the possible availability of a restriction enzyme recognizing the sequence of one allele, cost of the detection method, etc. There are several in-house methods that have been published, while a number of kits made by private companies are available. In order to study a DNA polymorphism, molecular geneticists either sequence the gene region of interest or use a method involving polymerase chain reaction (PCR), followed by analysis of resulting DNA fragments by agarose gel electrophoresis.

The PCR method usually involves the following: (a) simple use of a pair of primers, if the two alleles differ in size, as in the case of the ACE I/D polymorphism; (b) PCR followed by restriction enzyme, if a restriction fragment length polymorphism (RFLP) is present, as in the case of FV Leiden (G1691A) in which endonuclease TaqI is used; (c) an allele-specific primers, when a common primer is coupled with each allele-specific oligonucleotide (ASO) primer in a separate PCR, as in the case of the ITGA2 C807T polymorphism.

A typical PCR in total volume of 25–50 μl includes the following steps: (a) an initial denaturation step at 94°C for 4–5 min; (b) 30–35 cycles of a denaturation step at 94°C for 0.5–1 min, followed by an annealing step at primer-specific temperature for 0.5–1 min, followed by an elongation step at 72°C for 0.5–1 min; and (c) a final elongation step at 72°C for 5–7 min. Table 1 presents PCR annealing conditions and primers which may be used for the detection of functional DNA polymorphisms in thrombosis-related genes, using in-house protocols [11]. Table 2 mentions the expected sizes of DNA fragments viewed by agarose gel electrophoresis analysis.

<table>
<thead>
<tr>
<th>Factor (gene polymorphism)</th>
<th>Annealing temperature (°C)</th>
<th>Primers</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulation factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coagulation factor II (F2 G20210A)</td>
<td>54</td>
<td>F: 5’-AAACAACGCTGGTATCAAATGG-3’</td>
<td>RFLP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: 5’-GAGCTGCCCATGAATAGCACTG-3’</td>
<td></td>
</tr>
<tr>
<td>Coagulation factor V (F5 Leiden)</td>
<td>54</td>
<td>F: 5’-GCA GAT CCC TGG ACA GTC-3’</td>
<td>RFLP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: 5’-TGATATCACACTGACTAA-3’</td>
<td></td>
</tr>
<tr>
<td>Coagulation factor (FXII C46T)</td>
<td>57</td>
<td>F: 5’-ACTTCCAGGACCCTTCTGGAGGC-3’</td>
<td>RFLP</td>
</tr>
<tr>
<td>Factor (gene polymorphism)</td>
<td>Annealing temperature (°C)</td>
<td>Primers</td>
<td>Method</td>
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<tr>
<td>Coagulation factor FXIII A1 subunit (F13A1 V34L)</td>
<td>55</td>
<td>R: 5’-GTTGACGCCCGGCAACGC-3’</td>
<td>RFLP</td>
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<tr>
<td>Fibrinolysis-related factors</td>
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<tr>
<td>Serpinel (PAI-1 4G/5G)</td>
<td>57</td>
<td>F: 5’- CACAGAGAGATGGCCACGT-3’</td>
<td>RFLP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: 5’-GTTGACCGCCCGGCAACGC-3’</td>
<td></td>
</tr>
<tr>
<td>Plasma carboxypeptidase (CPB2, TAFI C1040T)</td>
<td>57</td>
<td>F: 5’-CACAAGAAAACAGATCACACAG-3’</td>
<td>RFLP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: 5’-AAAGCCACCCAATTGTGAT-3’</td>
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<tr>
<td>Thrombin-related factors</td>
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<tr>
<td>Thrombomodulin (THBD -13G/A)</td>
<td>58</td>
<td>F: 5’-ACCAAGAGATGAAAGAGGGC-3’</td>
<td>RFLP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: 5’-CGGATCGGCCAGGGCTCGAGTTTATAAGGCC-3’</td>
<td></td>
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<tr>
<td>Protein Z (PROZ -33A/G)</td>
<td>64</td>
<td>F: 5’-GGGTCACTCTGAGCTCCTACGT-3’</td>
<td>RFLP</td>
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<tr>
<td></td>
<td></td>
<td>R: 5’-CAGGCACAACAGACAGTGTAAGCCAGAT-3’</td>
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<td>Platelet-adhesion factors</td>
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<td>Intergin A2 (ITGA2, GPA I C807T)</td>
<td>56</td>
<td>COMMON: 5’- GACAGCCCCATTAATATAATGTCTCCTCTG-3’</td>
<td>ASO</td>
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<tr>
<td></td>
<td></td>
<td>C: 5’-CCTGATATGAAATATGCATCTG-3’</td>
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<td></td>
<td></td>
<td>T: 5’-CCTGATATGAAATATGCATCTG-3’</td>
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<tr>
<td>Platelet glycoprotein Iba (GPBA VNTR)</td>
<td>63</td>
<td>F: 5’-ACACTTCACATGGACTCCAT-3’</td>
<td>VNTR</td>
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<tr>
<td></td>
<td></td>
<td>R: 5’-GGGTCAATTTCTGGAGCTCTC-3’</td>
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<td>Other factors</td>
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<td>Methylene tetrahydrofolate reductase (MTHFR C677T)</td>
<td>57</td>
<td>F: 5’-TGAAGGAGAAGGTGTCTGCGGGA-3’</td>
<td>RFLP</td>
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<tr>
<td></td>
<td></td>
<td>R: 5’-AGGACGGTGCGGTGAGAGTG-3’</td>
<td></td>
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<tr>
<td>Angiotensin I-converting enzyme (ACE I/D)</td>
<td>60</td>
<td>F: 5’-CTGGAGACCCACTCCCACCTCTTCTCTG-3’</td>
<td>PCR (I or D is amplified)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: 5’-GATGTGGCCATCACATTCGTCAGAT-3’</td>
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<tr>
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<td></td>
<td>F1: 5’-TGGGACCACAGCGCCCGCCAGCTAC-3’</td>
<td>ASO (only I is amplified)</td>
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<td>R1: 5’-TCGCCAGCCCTCCCCATGCCCAATA-3’</td>
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<td>Angiotensinogen (AGT M235T)</td>
<td>59</td>
<td>F: 5’-CAGGTTGCTGTCCACACTGACC-3’</td>
<td>RFLP</td>
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<tr>
<td></td>
<td></td>
<td>R: 5’-CCGTTCGACAGGCGCGCCCTGCTCT-3’</td>
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</table>

RFLP: restriction fragment length polymorphism; ASO: allele-specific oligonucleotide; VNTR: variable number tandem repeats.

Table 1. Conditions and primers which may be used for molecular detection of functional polymorphisms associated with thrombophilia.
<table>
<thead>
<tr>
<th>Factor (gene polymorphism)</th>
<th>Method</th>
<th>Enzyme (Temperature, °C)</th>
<th>DNA fragments of normal allele (bp)</th>
<th>DNA fragments of variant allele (bp)</th>
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<td>Coagulation factors</td>
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<tr>
<td>Coagulation factor II (F2 G20210A))</td>
<td>RFLP</td>
<td>TaqI (65)</td>
<td>98 + 10</td>
<td>108</td>
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<td>Coagulation factor V (F5 Leiden)</td>
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<td>TaqI (65)</td>
<td>157 + 18</td>
<td>175</td>
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<tr>
<td>Coagulation factor (FXII C46T))</td>
<td>RFLP</td>
<td>Hgal (37)</td>
<td>369</td>
<td>247 + 122</td>
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<td>Coagulation factor FXIII A1subunit (F13A1 V34L)</td>
<td>RFLP</td>
<td>Hhal (37)</td>
<td>94 + 20</td>
<td>114</td>
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<td>Fibrinolysis-related factors</td>
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<tr>
<td>Serpine 1 (PAI-1 4G/5G)</td>
<td>RFLP</td>
<td>BslI (37)</td>
<td>77 + 22</td>
<td>99</td>
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<td>Plasma carboxypeptidase (CPB2, TAFI C1040T)</td>
<td>RFLP</td>
<td>Spel (37)</td>
<td>245 + 118</td>
<td>363</td>
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<td>Thrombin-related factors</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombomodulin (THBD -13G/A)</td>
<td>RFLP</td>
<td>Stul (37)</td>
<td>259</td>
<td>235 + 24</td>
</tr>
<tr>
<td>Protein Z (PROZ -33A/G)</td>
<td>RFLP</td>
<td>Hhal (37)</td>
<td>272</td>
<td>157 + 115</td>
</tr>
<tr>
<td>Platelet-adhesion factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intergin A2 (ITGA2, GPIA C807T))</td>
<td>ASO</td>
<td></td>
<td>148</td>
<td>148</td>
</tr>
<tr>
<td>Platelet glycoprotein Ibα (GPIBA VNTR)</td>
<td>VNTR</td>
<td></td>
<td>315, 237, 198</td>
<td>276</td>
</tr>
<tr>
<td>Other factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylene tetrahydrofolate reductase (MTHFR C677T)</td>
<td>RFLP</td>
<td>HinfI (37)</td>
<td>198</td>
<td>176 + 22</td>
</tr>
<tr>
<td>Angiotensin-converting enzyme (ACE I/D)</td>
<td>A ASO</td>
<td></td>
<td>190</td>
<td>490</td>
</tr>
<tr>
<td>Angiotensinogen (AGT M235T) RFLP</td>
<td>TthIII (37)</td>
<td></td>
<td>165</td>
<td>141 + 24</td>
</tr>
</tbody>
</table>

RFLP: restriction fragment length polymorphism; ASO: allele-specific oligonucleotide; VNTR: variable number tandem repeats.

Table 2. DNA fragments observed after agarose gel electrophoresis.
11. Prevention of thrombosis

Individual susceptibility to thrombotic diseases, including venous thromboembolism, ischemic stroke and ischemic heart disorder, is known to be influenced by genetic factor in addition to lifestyle as reported in earlier studies [1–5, 10]. Prevention of idiopathic thrombosis is imperative, since it is very common and life-threatening [89–91]. Genetic counseling and presymptomatic DNA testing is especially important for people with a positive family history of thrombophilia. Several studies have indicated that pretest genetic counseling would be helpful in reducing anxiety and confusion about thrombophilia facts [10, 67, 92, 93]. Geneticists may play a significant role in the prevention of thrombophilia if, during their routine collection of family history data during counseling for other diseases, they recognize the individuals at risk for thrombosis and inform them about preventive measures, including the available molecular tests.

The ability to routinely detect the inherited genetic predisposition for thrombosis (either mutation- or disease-associated polymorphisms) may significantly contribute to early diagnosis and make possible early intervention and prevention of thrombotic incidents. Individuals with increased risk for thrombophilia should best be referred to hematologists who may advise some of them to receive preventive anticoagulant therapy. Particularly, women at risk for thrombosis should consult a hematologist before taking contraceptive pills, as well as in case of pregnancy. The optimal management of asymptomatic at risk individuals remains unclear, but it is generally agreed that thromboprophylaxis should be provided, at least in high-risk periods such as during surgery or pregnancy [10, 94–104].

Molecular diagnostics and prevention of thrombophilia will be greatly benefited when the relative contribution of each thrombosis-related DNA polymorphism to vascular disease is better understood, possibly with the aid of large-scale epidemiological studies. Only then, a population-wide screening might be warranted for preventative purposes. At this stage, initial information about the prevalence of major DNA polymorphisms is recommended for each population in order to shape local health policies for prevention of thrombosis by identification of individuals at risk.

As an example, in southern and eastern Europeans prevention of thrombophilia may be significantly effected by studying genetic variants FV Leiden and FII G20210A since they both appear to be found in relatively high frequencies and to be major susceptibility factors for thromboembolic incidents in these populations [55, 67, 105–110]. A study in Greeks indicated that the combination of genetic counseling and molecular testing for these two common thrombophilia may increase up to fivefold the identification of at risk individuals compared to population-wide screening and has a significant impact on the prevention of thromboembolic incidents [66].

As genetic testing becomes a routine approach, it is expected that it will be extensively used both in hospital and community preventive medicine. Accordingly, it may be envisaged that thrombophilia, which is currently such a major global burden, shall be routinely preventable in the future with the aid of genetic testing and proper anticoagulant treatment, thus safeguarding the life and health status of asymptomatic individuals at risk.
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Author details

Christos Yapijakis*, Zoe Serefoglou and Constantinos Voumvourakis

*Address all correspondence to: cyapijakis_ua_gr@yahoo.com

Department of Neurology, University of Athens Medical School, Athens, Greece

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