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Common Gene Polymorphisms Associated with Thrombophilia

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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 (FXIII A1), 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 burden [1, 2]. Thrombophilia (a Greek composite word including “thrombos” meaning clot and “philia” meaning friendship) is a condition where individual susceptibility to thrombotic disease due to deregulation of the hemostatic system

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 metalloprotease-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 α 2-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 α 2-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- α (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 [11, 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 *ITGA2* gene which encodes integrin A2, also known as platelet glycoprotein Ia and variable number of tandem repeats (VNTR) in the *GP1BA* gene which encodes platelet glycoprotein Ib α 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 *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 *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 *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.

Factor (gene polymorphism)	Annealing temperature (°C)	Primers	Method
Coagulation factors			
Coagulation factor II (F2 G20210A)	54	F: 5'-AACAACCGCTGGTATCAAATGG-3'	RFLP
		R: 5'-GAGCTGCCCATGAATAGCACTG-3'	
Coagulation factor V (F5 Leiden)	54	F: 5'-GCA GAT CCC TGG ACA GTC-3'	RFLP
		R: 5'-TGT TAT CAC ACT GGT GCT AA-3'	
Coagulation factor (FXII C46T)	57	F: 5'-ACTTCCAGGACCGCCTTTGGAGGC-3'	RFLP

Factor (gene polymorphism)	Annealing temperature (°C)	Primers	Method
		R: 5'-GTTGACGCCCCGGGGCACCG-3'	
Coagulation factor FXIII A1subunit (F13A1 V34L)	55	F: 5'-ACTTCCAGGACCGCCTTTGGAGGC-3'	RFLP
		R: 5'-GTTGACGCCCCGGGGCACCG-3'	
Fibrinolysis-related factors			
Serpine1 (PAI-1 4G/5G)	57	F: 5'- CACAGAGAGAGTCTGGCCACGT-3'	RFLP
		R: 5'-CCAACAGAGGACTCTTGGTCT-3'	
Plasma carboxypeptidase (CPB2, TAFI C1040T)	57	F: 5'-CACAAAGAAAAACAGATCACACAG-3'	RFLP
		R: 5'-AAAGCCACCCAATTGTGATT-3'	
Thrombin-related factors			
Thrombomodulin (THBD -13G/A)	58	F: 5'-ACCAAGAGATGAAAGAGGGC-3'	RFLP
		R: 5'-CGGATCGGCCAGGGCTCGAGTTTATAAAGGCC-3'	
Protein Z (PROZ -33A/G)	64	F: 5'-GGGTCCTCTGAGCCTTCACCGTTCATTT-3'	RFLP
		R: 5'-CAGGCACAACAGACAGGTAAGCCAGATG-3'	
Platelet-adhesion factors			
Intergin A2 (ITGA2, GPIA C807T)	56	COMMON: 5'-GACAGCCCATTAATAAATGTCTCCTCTG-3'	ASO
		C: 5'-CCTTGCATATTGAATTGCTACG-3'	
		T: 5'-CCTTGCATATTGAATTGCTACA-3'	
Platelet glycoprotein Iba (GPIBA VNTR)	63	F: 5'-ACACTTCACATGGACTCCAT--3'	VNTR
		R: 5'-GGGTCATTTCTGGAGCTCTC--3'	
Other factors			
Methylenetetrahydrofolate reductase(MTHFR C677T)	57	F: 5'-TGAAGGAGAAGGTGTCTGCGGGA-3'	RFLP
		R: 5'-AGGACGGTGCGGTGAGAGTG-3'	
Angiotensin I-converting enzyme (ACE I/D)	60	F: 5'-CTGGAGACCACTCCCATCCTTTCT-3'	PCR (I or D is amplified)
		R: 5'-GATGTGGCCATCACATTGTCAGAT-3'	
		F1: 5'-TGGGACCACAGCGCCCGCCACTAC-3'	ASO (only I is amplified)
		R1: 5'-TCGCCAGCCCTCCCATGCCCATAA-3'	
Angiotensinogen (AGT M235T)	59	F: 5'-CAGGGTGCTGTCCACACTGGACCCC-3'	RFLP
		R: 5'-CCGTTTGTGCAGGGCCTGGCTCTCT-3'	

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.

Factor (gene polymorphism)	Method	Enzyme (Temperature, °C)	DNA fragments of normal allele (bp)	DNA fragments of variant allele (bp)
<i>Coagulation factors</i>				
Coagulation factor II (F2 G20210A))	RFLP	TaqI (65)	98 + 10	108
Coagulation factor V (F5 Leiden)	RFLP	TaqI (65)	157 + 18	175
Coagulation factor (FXII C46T))	RFLP	HgaI (37)	369	247 + 122
Coagulation factor FXIII A1subunit (F13A1 V34L)	RFLP	HhaI (37)	94 + 20	114
<i>Fibrinolysis-related factors</i>				
Fibrinolysis-related factors Serpine 1 (PAI-1 4G/5G)	RFLP	BsII (37)	77 + 22	99
Plasma carboxypeptidase (CPB2, TAFI C1040T)	RFLP	SpeI (37)	245 + 118	363
<i>Thrombin-related factors</i>				
Thrombomodulin (THBD -13G/A)	RFLP	StuI (37)	259	235 + 24
Protein Z (PROZ -33A/G)	RFLP	HhaI (37)	272	157 + 115
<i>Platelet-adhesion factors</i>				
Intergin A2 (ITGA2, GPIA C807T))	ASO		148	148
Platelet glycoprotein Iba (GPIBA VNTR)	VNTR		315, 237, 198	276
<i>Other factors</i>				
Methylenetetrahydrofolate reductase (MTHFR C677T)	RFLP	HinfI (37)	198	176 + 22
Angiotensin-converting enzyme (ACE I/D)	A ASO		190	490
Angiotensinogen(AGT M235T)	RFLP	TthIII (37)	165	141 + 24
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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References

- [1] Lozano R, Naghavi M, Foreman K et al. Global and regional mortality from 235 cases of death for 20 age groups in 1990 and 2010. *Lancet*. 2012;380:2095–2128.
- [2] Raskob GE, Angchaisuksiri P, Blanco AN et al. Thrombosis: A major contributor to the global disease burden. *J Thromb Haemost*. 2014;12:1580–1590.
- [3] Albisetti M, Moeller A, Waldvogel K, Bernet-Buettiker V, Cannizzaro V, Anagnostopoulos A, Balmer C and Schmugge M. Congenital prothrombotic disorders in children with peripheral venous and arterial thromboses. *Acta Haematol*. 2007;117:149–155.
- [4] Bick RL. Hereditary and acquired thrombophilic disorders. *Clin Appl Thromb Hemost*. 2006;12:125–135.
- [5] Gary T, Hafner F, Froehlich H, Stojakovic T, Scharnagl H, Pilger E and Brodmann M. High factor VIII activity, high plasminogen activator inhibitor 1 antigen levels and low factor XII activity contribute to a thrombophilic tendency in elderly venous thromboembolism patients. *Acta Haematol*. 2010;124:214–217.
- [6] Banerjee I, Gupta V and Ganesh S. Association of gene polymorphism with genetic susceptibility to stroke in Asian populations: A meta-analysis. *J Hum Genet*. 2007;52:205–219.
- [7] Kim RJ and Becker RC. Association between factor V Leiden, prothrombin G20210A, and methylene tetrahydrofolate reductase C677T mutations and events of the arterial circulatory system: A meta-analysis of published studies. *Am Heart J*. 2003;146:948–957.

- [8] Klerk M, Verhoef P, Clarke R, Blom HJ, Kok FJ and Schouten EG: MTHFR 677C>T polymorphism and risk of coronary heart disease: A meta-analysis. *JAMA*. 2002;288:2023–2031.
- [9] Lane DA and Grant PJ: Role of hemostatic gene polymorphisms in venous and arterial thrombotic disease. *Blood*. 2000;95:1517–1532.
- [10] Reitsma PH. Genetics in thrombophilia. An update. *Hämostaseologie*. 2015;35:47–51.
- [11] Yapijakis C, Serefoglou Z, Nixon AM, Vylliotis A, Ragos V, Vairaktaris E. Prevalence of thrombosis- related DNA polymorphisms in a healthy Greek population. *In Vivo*. 2012;26:1095–1102.
- [12] Nash GF, Walsh DC and Kakkar AK: The role of the coagulation system in tumour angiogenesis. *Lancet Oncol*. 2001;2:608–613.
- [13] Gomez K and McVey JH: Tissue factor initiated blood coagulation. *Front Biosci*. 2006;11:1349–1359.
- [14] Peppelenbosch MP and Versteeg HH: Cell biology of tissue factor, an unusual member of the cytokine receptor family. *Trends Cardiovasc Med*. 2001;11:335–339.
- [15] Crawley JT and Lane DA: The haemostatic role of tissue factor pathway inhibitor. *Arterioscler Thromb Vasc Biol*. 2008;28(2):233–242.
- [16] Carmeliet P, Ferreira V, Breier G, Pollefeyt S, Kieckens L, Gertsenstein M, Fahrig M, Vandenhoek A, Harpal K, Eberhardt C, Declercq C, Pawling J, Moons L, Collen D, Risau W and Nagy A: Abnormal blood vessel development and lethality in embryos lacking a single *VEGF* allele. *Nature*. 1996;380:435–439.
- [17] Olivier V, Bentolila S, Chabbat J, Hakim J and de Prost D: Tissue factor-dependent vascular endothelial growth factor production by human fibroblasts in response to activated factor VII. *Blood*. 1998;91:2698–2703.
- [18] Taniguchi T, Kakkar AK, Tuddenham EG, Williamson RC and Lemoine NR: Enhanced expression of urokinase receptor induced through the tissue factor-factor VIIa pathway in human pancreatic cancer. *Cancer Res*. 1998;58:4461–4467.
- [19] Mechtcheriakova D, Wlachos A, Holzmüller, Binder BR and Hofer E: Vascular endothelial cell growth factor-induced tissue factor expression in endothelial cells is mediated by EGR-1. *Blood*. 1999;93:3811–3823.
- [20] Yapijakis C, Vairaktaris E, Vassiliou S, Vylliotis A, Nkenke E, Nixon AM, Derka S, Spyridonidou S, Vorris E, Neukam F and Patsouris E: The low VEGF production allele of the +936C/T polymorphism is strongly associated with increased risk for oral cancer. *J Cancer Res Clin Oncol*. 2007;133:787–791.
- [21] Coughlin SR: Thrombin signalling and protease-activated receptors. *Nature*. 2000;407:258–264.

- [22] Maragoudakis ME, Kraniti N, Giannopoulou E, Alexopoulos K and Matsoukas J: Modulation of angiogenesis and progelatinase a by thrombin receptor mimetics and antagonists. *Endothelium*. 2001;8:195–205.
- [23] Tsopanoglou NE and Maragoudakis ME: On the mechanism of thrombin-induced angiogenesis. Potentiation of vascular endothelial growth factor activity on endothelial cells by upregulation of its receptors. *J Biol Chem*. 1999;274:23969–23976.
- [24] Tsopanoglou NE, Andriopoulou P and Maragoudakis ME: On the mechanism of thrombin-induced angiogenesis: Involvement of $\alpha v\beta 3$ -integrin. *Am J Physiol Cell Physiol*. 2002;283:1501–1510.
- [25] Tsopanoglou NE and Maragoudakis ME: Role of thrombin in angiogenesis and tumor progression. *Semin Thromb Hemost*. 2004;30:63–69.
- [26] Lee TH, Rhim T and Kim SS: Prothrombin kringle-2 domain has a growth inhibitory activity against basic fibroblast growth factor-stimulated capillary endothelial cells. *J Biol Chem*. 1998;273:28805–28812.
- [27] Rhim TY, Park CS, Kim E and Kim SS: Human prothrombin fragment 1 and 2 inhibit bFGF-induced BCE cell growth. *Biochem Biophys Res Commun*. 1998;252:513–516.
- [28] Bouma BN and Mosnier LO: Thrombin activatable fibrinolysis inhibitor (TAFI) – How does thrombin regulate fibrinolysis? *Ann Med*. 2006;38:378–388.
- [29] Koutsi A, Papapanagiotou A and Papavassiliou AG: Thrombomodulin: From haemostasis to inflammation and tumorigenesis. *Int J Biochem Cell Biol*. 2008;40:1669–1673.
- [30] Mosesson MW: Fibrinogen and fibrin structure and functions. *J Thromb Haemost*. 2005;3:1894–1904.
- [31] Fernandez PM, Patierno SR and Rickles FR: Tissue factor and fibrin in tumor angiogenesis. *Semin Thromb Hemost*. 2004;30:31–44.
- [32] Sahni A, Simpson-Haidaris PJ, Sahni SK, Vaday GG and Francis CW: Fibrinogen synthesized by cancer cells augments the proliferative effect of FGF-2. *J Thromb Haemost*. 2008;6:176–183.
- [33] Dvorak HF: Tumors: Wounds that do not heal. Similarities between tumor stroma generation and wound healing. *N Engl J Med*. 1986;315:1650–1659.
- [34] Dvorak HF: Rous-Whipple Award Lecture. How tumors make bad blood vessels and stroma. *Am J Pathol*. 2003;162:1747–1757.
- [35] Engelse MA, Hanemaaijer R, Koolwijk P and van Hinsbergh VW: The fibrinolytic system and matrix metalloproteinases in angiogenesis and tumor progression. *Semin Thromb Hemost*. 2004;30:71–82.

- [36] Wojtukiewicz MZ, Sierko E and Rak J: Contribution of the hemostatic system to angiogenesis in cancer. *Semin Thromb Hemost.* 2004;30:5–20.
- [37] Nakasaki T, Wada H, Shigemori C, Miki C, Gabazza EC, Nobori T, Nakamura S and Shiku H: Expression of tissue factor and vascular endothelial growth factor is associated with angiogenesis in colorectal cancer. *Am J Hematol.* 2002;69:247–254.
- [38] Shoji M, Hancock WW, Abe K, Micko C, Casper KA, Baine RM, Wilcox JN, Danave I, Dillehay DL, Matthews E, Contrino J, Morrissey JH, Gordon S, Edgington TS, Kudryk B, Kreutzer DL and Rickles FR: Activation of coagulation and angiogenesis in cancer: Immunohistochemical localization *in situ* of clotting proteins and vascular endothelial growth factor in human cancer. *Am J Pathol.* 1998;152:399–411.
- [39] Tsigris C, Chatzitheofylaktou A, Xiromeritis C, Nikiteas N and Yannopoulos A: Genetic association studies in digestive system malignancies. *Anticancer Res.* 2007;27(5B):3577–3587.
- [40] Vairaktaris E, Serefoglou Z, Avgoustidis D, Yapijakis C, Critselis E, Vylliotis A, Spyridonidou S, Derka S, Vassiliou S, Nkenke E and Patsouris E: Gene polymorphisms related to angiogenesis, inflammation and thrombosis that influence risk for oral cancer. *Oral Oncol.* 2009;45:247–253.
- [41] Franchini M, Montagnana M, Targher G, Manzato F and Lippi G: Pathogenesis, clinical and laboratory aspects of thrombosis in cancer. *J Thromb Thrombolysis.* 2007;24:29–38.
- [42] De Cicco M: The prothrombotic state in cancer: Pathogenic mechanisms. *Crit Rev Oncol Hematol.* 2004;50:187–196.
- [43] Falanga A, Panova-Noeva M and Russo L: Procoagulant mechanisms in tumour cells. *Best Pract Res Clin Haematol.* 2009;22:49–60.
- [44] Elice F, Jacoub J, Rickles FR, Falanga and Liebman HA: Hemostatic complications of angiogenesis inhibitors in cancer patients. *Am J Hematol.* 2008;83:862–870.
- [45] Sutherland DE, Weitz IC and Liebman HA: Thromboembolic complications of cancer: Epidemiology, pathogenesis, diagnosis, and treatment. *Am J Hematol.* 2003;72:43–52.
- [46] Wünsch Filho V and Zago MA: Modern cancer epidemiological research: Genetic polymorphisms and environment. *Rev Saude Publica.* 2005;39(3):490–497.
- [47] Hirschhorn JN, Lohmueller Kirk, Byrne E and Hirschhorn K: A comprehensive review of genetic association studies. *Genet Med.* 2002;4(2):45–61.
- [48] Cooper DN, Nussbaum RL and Krawczak M: Proposed guidelines for papers describing DNA polymorphism-disease associations. *Hum Genet.* 2002;110(3):207–208.
- [49] Guo SW and Thompson EA: Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics.* 1992;48:361–372.

- [50] Vairaktaris E, Yapijakis C, Serefoglou Z, Avgoustidis D, Critselis E, Spyridonidou S, Vylliotis A, Derka S, Vassiliou S, Nkenke E and Patsouris E: Gene expression polymorphisms of interleukins-1beta, -4, -6, -8, -10, and tumor necrosis factors alpha, -beta: Regression analysis of their effect upon oral squamous cell carcinoma. *J Cancer Res Clin Oncol*. 2008;134:821–832.
- [51] Ageno W, Squizzato A, Garcia D and Imberti D: Epidemiology and risk factors of venous thromboembolism. *Semin Thromb Hemost*. 2006;32:651–658.
- [52] Musunuru K and Kathiresan S: HapMap and mapping genes for cardiovascular disease. *Circ Cardiovasc Genet*. 2008;1:66–71.
- [53] Hoppe B, Tolou F, Dorner T, Kieseewetter H and Salama A: Gene polymorphisms implicated in influencing susceptibility to venous and arterial thromboembolism: Frequency distribution in a healthy German population. *Thromb Haemost*. 2006;96:465–470.
- [54] Ireland H, Kunz G, Kyriakoulis K, Stubbs PJ and Lane DA: Thrombomodulin gene mutations associated with myocardial infarction. *Circulation*. 1997;96:15–18.
- [55] Koumas L, Costeas PA, Papaloizou A and Giantsiou-Kyriakou A: Genetic assessment of cardiovascular risk factors in the Greek Cypriot population. *Thromb Res*. 2003;112:143–146.
- [56] Martini CH, Doggen CJ, Cavallini C, Rosendaal FR and Mannucci PM: No effect of polymorphisms in prothrombotic genes on the risk of myocardial infarction in young adults without cardiovascular risk factors. *J Thromb Haemost*. 2005;3:177–179.
- [57] Vairaktaris E, Yapijakis C, Serefoglou Z, Vylliotis A, Ries J, Nkenke E, Wiltfang J, Derka S, Vassiliou S, Springer I, Kessler P and Neukam FW: Plasminogen activator inhibitor-1 polymorphism is associated with increased risk for oral cancer. *Oral Oncol*. 2006;42:888–892.
- [58] Zama T, Murata M, Ono F, Watanabe K, Watanabe R, Moriki T, Yokoyama K, Tokuhira M and Ikeda Y: Low prevalence of activated protein C resistance and coagulation factor V Arg506 to Gln mutation among Japanese patients with various forms of thrombosis, and normal individuals. *Int J Hematol*. 1996;65:71–78.
- [59] Zhao J, Zhou X, Huang J, Chen J and Gu D: Association study of the thrombomodulin -33G>A polymorphism with coronary artery disease and myocardial infarction in Chinese Han population. *Int J Cardiol*. 2005;100:383–388.
- [60] Bauduer F and Lacombe D: Factor V Leiden, prothrombin 20210A, methylene tetrahydrofolate reductase 677T, and population genetics. *Mol Genet Metab*. 2005;86:91–99.
- [61] Bertina RM: Factor V Leiden and other coagulation factor mutations affecting thrombotic risk. *Clin Chem*. 1997;43:1678–1684.

- [62] Coppage KH, Hinton AC, Moldenhauer J, Kovilam O, Barton JR and Sibai BM: Maternal and perinatal outcome in women with a history of stroke. *Am J Obstet Gynecol.* 2004;190:1331–1334.
- [63] Foka ZJ, Lambropoulos AF, Saravelos H, Karas GB, Karavida A, Agorastos T, Zournatzi V, Makris PE, Bontis J and Kotsis A: Factor V leiden and prothrombin G20210A mutations, but not methylene tetrahydrofolate reductase C677T, are associated with recurrent miscarriages. *Hum Reprod.* 2000;15:458–462.
- [64] Ridker PM, Hennekens CH, Lindpainter K, Stampfer MJ, Eisenberg PR and Miletich JP: Mutation in the gene coding for coagulation factor V and the risk of myocardial infarction, stroke, and venous thrombosis in apparently healthy men. *N Engl J Med.* 1995;332:912–917.
- [65] Yapijakis C, Antoniadis T, Salavoura K, Voumvourakis C and Vairaktaris E: Potential prevention of thromboembolism by genetic counseling and testing for two common thrombophilia mutations. *In Vivo.* 2012;26:165–172.
- [66] De Stefano V, Martinelli I, Mannucci PM, Paciaroni K, Chiusolo P, Casorelli I, Rossi E, and Leone G: The risk of recurrent deep venous thrombosis among heterozygous carriers of both factor V Leiden and the G20210A prothrombin mutation. *N Engl J Med.* 1999;341:801–806.
- [67] Poort SR, Rosendaal FR, Reitsma PH and 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;88:3698–3703.
- [68] Santamaria A, Mateo J, Tirado I, Oliver A, Belvis R, Marti-Fabregas J, Felices R, Soria JM, Souto JC and Fontcuberta J: Homozygosity of the T allele of the 46 C/T polymorphism in the F12 gene is a risk factor for ischemic stroke in the Spanish population. *Stroke.* 2004;35:1795–1799.
- [69] Wells PS, Anderson JL, Scarvelis DK, Doucette SP and Gagnon F: Factor XIII Val34Leu variant is protective against venous thromboembolism: A HuGE review and meta-analysis. *Am J Epidemiol.* 2006;164:101–109.
- [70] Cesari F, Fatini C, Sticchi E, Fedi S, Abbate R, Gensini GF and Sofi F: Protein Z gene polymorphisms (intron F 79 G>A; –13A>G) are not associated with acute coronary syndromes. *Thromb Haemost.* 2006;96:98–99.
- [71] Eriksson P, Kallin B, van't Hooft FM, Bavenholm P and Hamsten A: Allele-specific increase in basal transcription of the plasminogen-activator inhibitor 1 gene is associated with myocardial infarction. *Proc Natl Acad Sci U S A.* 1995;92:1851–1855.
- [72] Grubic N, Stegnar M, Peternel P, Kaider A and Binder BR: A novel G/A and the 4G/5G polymorphism within the promoter of the plasminogen activator inhibitor-1 gene in patients with deep vein thrombosis. *Thromb Res.* 1996;84:431–443.

- [73] Morange PE, Tregouet DA, Frere C, Luc G, Arveiler D, Ferrieres J, Amouyel P, Evans A, Ducimetiere P, Cambien F, Tired L and Juhan-Vague I; Prime Study Group: TAFI gene haplotypes, TAFI plasma levels and future risk of coronary heart disease: The PRIME Study. *J Thromb Haemost.* 2005;3:1503–1510.
- [74] Schneider M, Boffa M, Stewart R et al. Two naturally occurring variants of TAFI (Thr-325 and Ile-325) differ substantially with respect to thermal stability and antifibrinolytic activity of the enzyme. *J Biol Chem.* 2002;277:1021–1030.
- [75] Ozelo MC, Origa AF, Aranha FJ, Mansur AP, Annichino-Bizzacchi JM, Costa FF, Pollak ES and Arruda VR: Platelet glycoprotein Ib alpha polymorphisms modulate the risk for myocardial infarction. *Thromb Haemost.* 2004;92:384–386.
- [76] Wagner C and Bailey LB (eds): Folate in health and disease. Marcel Dekker, New York, 1995.
- [77] Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA and van den Heuvel LP: A candidate genetic risk factor for vascular disease: A common mutation in methylenetetrahydrofolate reductase. *Nat Genet.* 1995;10:111–113.
- [78] Goracy I, Cyrylowski L, Kaczmarczyk M, Fabian A, Koziarska D, Goracy J and Ciechanowicz A: C677T polymorphism of the methylene tetrahydrofolate reductase gene and the risk of ischemic stroke in Polish subjects. *J Appl Genet.* 2009;50:63–67.
- [79] Jacques PF, Bostom AG, Williams RR, Ellison RC, Eckfeldt JH, Rosenberg IH, Selhub J and Rosen R: Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. *Circulation.* 1996;93:7–9.
- [80] Gudnason V, Stansbie D, Scott J, Browron A, Nicaud V and Humphries S. C677T (thermolabile alanine/valine) polymorphism in methylenetetrahydrofolate reductase (MTHFR): its frequency and impact on plasma homocysteine concentration in different European populations. *Atherosclerosis.* 1998;136:347–354.
- [81] Stroth U and Unger T. The renin angiotensin system and its receptors. *J Cardiovasc Pharmacol.* 1999;33:21–28.
- [82] Caulfield M, Lavender P, Farrall M, Munroe P, Lawson M, Turner P and Clark AJ: Linkage of the angiotensinogen gene to essential hypertension. *N Engl J Med.* 1994;330:1629–1633.
- [83] Hata A, Namikawa C, Sasaki M, Sato K, Nakamura T, Tamura K and Lalouel JM: Angiotensinogen as a risk factor for essential hypertension in Japan. *J Clin Invest.* 1994;93:1285–1287.
- [84] Hegele RA, Brunt JH and Connelly PW: A polymorphism of the angiotensinogen gene associated with variation in blood pressure in a genetic isolate. *Circulation.* 1994;90:2207–2212.

- [85] Jeunemaitre X, Soubrier F, Kotelevtsev YV, Lifton RP, Williams CS, Charru A, Hunt SC, Hopkins PN, Williams RR, Lalouel JM et al: Molecular basis of human hypertension: role of angiotensinogen. *Cell*. 71:7–20.
- [86] Staessen J, Kuznetsova T, Wang J, Emelianov D, Vlietinck R and Fagard R: M235T angiotensinogen gene polymorphism and cardiovascular renal risk. *J Hypertens*. 1999;17:9–17.
- [87] Zhu X, McKenzie CA, Forrester T, Nickerson DA, Broeckel U and Schunkert H et al. Localization of a small genomic region associated with elevated ACE. *Am J Hum Genet*. 2000;67:1144–1153.
- [88] McKenzie CA, Julier C, Forrester T, McFarlane-Anderson N, Keavney B, Lathrop GM et al. Segregation and linkage analysis of serum angiotensin I-converting enzyme levels: Evidence for two quantitative-trait loci. *Am J Hum Genet*. 1995;57:1426–1435.
- [89] Bang SM, Jang MJ, Kim KH, Yhim HY, Kim YK, Nam SH, Hwang HG, Bae SH, Kim SH, Mun YC, Kim YK, Kim I, Choi WI, Jung CW, Park NH, Choi NK, Park BJ and Oh D: Prevention of Venous Thromboembolism, 2nd Edition: Korean Society of Thrombosis and Hemostasis Evidence-Based Clinical Practice Guidelines. *Korean Med Sci*. 2014;29:164–171.
- [90] Cardiovascular Disease Educational and Research Trust, Cyprus Cardiovascular Disease Educational and Research Trust, European Venous Forum, International Surgical Thrombosis Forum, International Union of Angiology, Union Internationale de Phlébologie: Prevention and treatment of venous thromboembolism: International Consensus Statement (guidelines according to scientific evidence). *Int Angiol*. 2006;25:101–161.
- [91] Guyatt GH, Akl EA, Crowther M, Gutterman DD, Schünemann HJ, American College of Chest Physicians Antithrombotic Therapy and Prevention of Thrombosis Panel: Executive summary: Antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest*. 1012;141:7S–47S.
- [92] Federici C, Gianetti J and Andreassi MG: Genomic medicine and thrombotic risk: Who, when, how and why? *Int J Cardiol*. 2006;106:3–9.
- [93] Reich LM, Bower M and Keys NS. Role of the geneticist in testing and counseling for inherited thrombophilia. *Genet Med*. 2003;5:133–143.
- [94] Coppens M, van de Poel MH, Bank I, Hamulyak K, van der Meer J, Veeger NJ, Prins MH, Buller HR and Middeldorp S: A prospective cohort study on the absolute incidence of venous thromboembolism and arterial cardiovascular disease in asymptomatic carriers of the prothrombin 20210A mutation. *Blood*. 2006;108:2604–2607.

- [95] de Maistre E, Terriat B, Lesne-Padieu AS, Abello N, Bouchot O and Steinmetz EF: High incidence of venous thrombosis after surgery for abdominal aortic aneurysm. *J Vasc Surg.* 2009;49:596–601.
- [96] Horne MK 3rd and McCloskey DJ: Factor V Leiden as a common genetic risk factor for venous thromboembolism. *J Nurs Scholarsh.* 2006;38:19–25.
- [97] Kakkos SK, Sabetai M, Tegos T, Stevens J, Thomas D, Griffin M, Geroulakos G and Nicolaidis AN: Asymptomatic Carotid Stenosis and Risk of Stroke (ACSRS) Study Group: Silent embolic infarcts on computed tomography brain scans and risk of ipsilateral hemispheric events in patients with asymptomatic internal carotid artery stenosis. *J Vasc Surg.* 2009;49:902–909.
- [98] King A and Markus HS: Doppler embolic signals in cerebrovascular disease and prediction of stroke risk: A systematic review and meta-analysis. *Stroke.* 2009;40:3711–3717.
- [99] Langlois NJ and Wells PS: Risk of venous thromboembolism in relatives of symptomatic probands with thrombophilia: A systematic review. *Thromb Haemost.* 2003;90:17–26.
- [100] Said JM, Higgins JR, Moses EK, Walker SP, Borg AJ, Monagle PT and Brennecke SP: Inherited thrombophilia polymorphisms and pregnancy outcomes in nulliparous women. *Obstet Gynecol.* 2010;115:5–13.
- [101] Selby R, Geerts W, Ofosu FA, Craven S, Dewar L, Phillips A and Szalai JP: Hypercoagulability after trauma: Hemostatic changes and relationship to venous thromboembolism. *Thromb Res.* 2009;124:281–287.
- [102] Simioni P, Tormene D, Prandoni P, Zerbinati P, Gavasso S, Cefalo P and Girolami A: Incidence of venous thromboembolism in asymptomatic family members who are carriers of factor V Leiden: A prospective cohort study. *Blood.* 2002;99:1938–1942.
- [103] Taniguchi S, Fukuda I, Daitoku K, Minakawa M, Odagiri S, Suzuki Y, Fukui K, Asano K and Ohkuma H: Prevalence of venous thromboembolism in neurosurgical patients. *Heart Vessels.* 2009;24:425–428.
- [104] Zubkov AY and Wijdicks EF: Deep venous thrombosis prophylaxis in cerebral hemorrhage. *Rev Neurol Dis.* 2009;6:21–25.
- [105] Antoniades C, Tousoulis D, Vasiliadou C, Stefanadi E, Marinou K and Stefanadis C: Genetic polymorphisms of platelet glycoprotein Ia and the risk for premature myocardial infarction: Effects on the release of sCD40L during the acute phase of premature myocardial infarction. *J Am Coll Cardiol.* 2006;47:1959–1966.
- [106] Antoniadi T, Hatzis T, Kroupis C, Economou-Petersen E and Petersen MB: Prevalence of factor V Leiden, prothrombin G20210A, and *MTHFR* C677T mutations in a Greek population of blood donors. *Am J Hematol.* 1999;61:265–267.

- [107] Chaida C, Gialeraki A, Tsoukala C and Mandalaki T: Prevalence of the FVQ506 mutation in the Hellenic population. *Thromb Haemost.* 1996;76:127.
- [108] Ioannou HV, Mitsis M, Eleftheriou A, Matsagas M, Nousias V, Rigopoulos C, Vartholomatos G and Kappas AM: The prevalence of factor V Leiden as a risk factor for venous thromboembolism in the population of North-Western Greece. *Int Angiol.* 2000;19:314–318.
- [109] Vassilikioti S, Doumas M, Douma S, Petidis K, Karagiannis A, Balaska K, Vyzantidis A and Zamboulis C: Angiotensin converting enzyme gene polymorphism is not related to essential hypertension in a Greek population. *Am J Hypertens.* 1996;9:700–702.
- [110] Xenophontos SL, Hadjivassiliou M, Ayrton N, Karagrigoriou A, Pantzaris M, Nicolaides AN and Cariolou MA: Spectrum and prevalence of prothrombotic single nucleotide polymorphism profiles in the Greek Cypriot population. *Int Angiol.* 2002;21:322–329.