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Lack of association between potential prothrombotic genetic risk factors and arterial and venous thrombosis

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ABSTRACT. Recent studies have shown an association between thrombosis and factor VII (FVII), tissue factor (TF), and angiotensin-converting enzyme (ACE). This suggests that individuals with FVII-402 G/A, FVII-401 G/T, TF+5466 A/G, and ACE-287 insertion/deletion (I/D) polymorphisms present an increased risk of venous thrombosis, heart disease, and ischemic stroke compared with controls. In this study, we investigated the frequencies of these polymorphisms and their association with arterial and venous thrombosis. For the FVII-402 G/A polymorphism, there were 57.3% heterozygote (HT) genotypes and 8.3% homozygote (HM) genotypes in the patients, and 45.2% HT genotypes and 15.4% HM genotypes in the controls. For

the FVII-401 G/T polymorphism, there were 37.5% HT genotypes and 3.1% HM genotypes in the patients, and 32.7% HT genotypes and 4.8% HM genotypes in the controls. The polymorphism TF+5466 A/G was not found in any of the samples analyzed. For the ACE-287 I/D polymorphism, there were 43 (40.6%) HT genotypes and 63 (59.4%) HM genotypes in the controls and 28 (45.2%) HT genotypes and 34 (54.8%) HM genotypes in the patients. No significant difference was observed by comparing patients and controls. In this study, no association was found between the presence of the evaluated polymorphisms and the occurrence of thrombotic events.

Key words: Polymorphisms; Tissue factor; Factor VII; Thrombosis; Angiotensin-converting enzyme

INTRODUCTION

The development of thrombosis is the result of activation of the clotting mechanism and/or hypoactivation of natural anticoagulation or fibrinolytic mechanisms, with the subsequent formation of a thrombus, which results in arterial or venous thrombosis (Spronk et al., 2004; Hemmeryckx et al., 2012).

Arterial thrombosis, a cerebrovascular disease, is one of the most common causes of death in the USA (Zakai et al., 2011). Specifically, the stroke occurs by blockage of the arteries (ischemic form) or by extravasation of blood (hemorrhagic form). Generally, the main factor associated with arterial thrombosis is the instability or rupture of an atherosclerotic plaque. This rupture triggers the formation of a localized blood clot, rich in activated platelets that, together with macrophages and monocytes, causes the occlusion of the vessel, preventing the normal liberation of antithrombotic agents such as nitric oxide and promoting prothrombotic substances such as tissue factor (TF). The human atherosclerotic plaque contains large amounts of tissue factor and it has been suggested that, after plaque rupture, tissue factor initiates the activation of the coagulation system that contributes to the formation of an occlusive clot (Boles and Mackman, 2010; Lopaciuk et al., 2010).

Venous thromboembolism (VTE) is a vascular disease characterized by acute formation of a thrombus within a vein. Venous thrombi are composed mainly of fibrin and red blood cells. Venous thrombosis is represented by two major clinical events: deep venous thrombosis (DVT), in which there is an occurrence of deep vein thrombi; and pulmonary embolism (PE), which is a complication of DVT in which there is a detachment of the entire thrombus or a fragment thereof, which can migrate to the pulmonary artery tree (Previtali et al., 2011).

Although VTE is a common disease, its pathogenic mechanism is only partially understood compared with that of atherothrombosis. Over recent decades, progress has been made in identifying and characterizing the cellular and molecular mechanisms that interdependently influence Virchow's triad (de Meis and Levy, 2007). It is accepted that the combination of stasis and hypercoagulability (a homeostatic mechanism that alters the formation of clots under conditions that increase the concentration or the activation of coagulation factors), much more than endothelial damage, is necessary for the occurrence of VTE (Litzendorf and Satiani, 2011).

Genetic abnormalities that compromise the production, activity, bioavailability, or metabolism of specific factors can alter the physiological balance and predispose to premature

atherothrombotic and thromboembolic events (Litzendorf and Satiani, 2011).

Several genetic markers have been associated with thrombotic events, such as mutations or polymorphisms in factor V (factor V Leiden), prothrombin (FII mutant), methylenetetrahydrofolate reductase, apolipoprotein E, plasminogen activator inhibitor-1, TF, factor VII (FVII), and angiotensin-converting enzyme (ACE).

The FVII gene is located on the long arm of chromosome 13. Two of its most common polymorphisms are -401 G/T and -402 G/A. These polymorphisms of the promoter region of the FVII gene are associated with changes in FVII blood levels, and represent important risk factors for quantitative cardiovascular diseases, including ischemic heart disease and arterial or venous thrombosis (Jackson et al., 2000; Eroğlu et al., 2010, 2011).

The TF gene is located on chromosome 1 and one of its most studied polymorphisms is +5466 A/G. Studies have associated this polymorphism with high levels of TF and increased risk of thrombotic events, and have been used to predict future events (Opstad et al., 2010b; Eroğlu et al., 2010; Silveira et al., 2012). Thus, polymorphisms linked to these coagulation factors cause an inappropriate expression of their genes and can predispose to thrombotic events (Litzendorf and Satiani, 2011).

Blood coagulation is based on the high affinity of TF for both activated FVII (FVIIa) and FVII, forming a 1:1 complex on the cell surface. After binding to TF, FVII is rapidly converted to FVIIa. However, it is important to note that factor VII bound to TF undergoes self-activation. Only the TF/FVIIa complex is active (Mitrophanov and Reifman, 2011; Silveira et al., 2012; Mercer and Chambers, 2013). This complex can activate blood clotting in two ways: activation of factor IX by proteolysis, and direct activation of factor X, which seems to be the predominant mechanism in *in vitro* studies. These two pathways initiate reactions leading to the formation of fibrin at the site of endothelial injury (Hoffman, 2003; Mercer and Chambers, 2013). Several experimental and clinical studies have clearly demonstrated that the TF/FVIIa complex is the initiator of the coagulation cascade in cardiovascular disease (Petrillo et al., 2010; Silveira et al., 2012).

In addition to genetic factors, acquired factors have an important impact on the risk of vascular obstruction (Singh et al., 2012). Age, obesity, smoking, use of hormones, surgery, pregnancy, hypertension, and immobilization are common risk factors associated with venous and arterial thrombosis contributing to 90 and 60% of cardiac diseases and stroke cases, respectively.

However, ACE is a cellular membrane peptidase, and functions as an ectoenzyme with its catalyst site exposed on the extracellular surface of the cell. ACE is a zinc-dependent metallopeptidase with broad substrate specificity, but it was first described for its catalytic properties in two vasoactive peptides: angiotensin I and bradykinin (Kalyesubula et al., 2014).

The human ACE gene is located on the short arm of chromosome 17 (Fatini et al., 2009). The plasma levels of this enzyme in humans are related to the insertion/deletion (I/D) polymorphism in the ACE gene. The deletion of a 287-bp sequence in intron 16 of the ACE gene is associated with increased levels of transcription of messenger RNA and hence with increased ACE expression (Jackson et al., 2000; Fatini et al., 2009). Thus, carriers of the DD genotype are associated with higher plasma levels of the enzyme than those with the ID (intermediate levels) or II (low levels) genotypes (Jackson et al., 2000; Zhang et al., 2012).

The association between the ACE gene polymorphism and risk of occlusive vessel processes such as stroke has attracted a great deal of attention and has been a focus of research. Thus, the identification and characterization of variants of genes that are related to diseases

of importance, such as those mentioned above, may result in better prognoses, therapy, and prevention (Fatini et al., 2009; Khan et al., 2014). The aim of this study was to investigate TF+5466 A/G, FVII-402 G/A, FVII-401 G/T, and ACE-287 I/D polymorphisms in patients with arterial and venous thrombosis in an attempt to identify additional insights into the underlying thrombotic processes.

MATERIAL AND METHODS

Patients and control subjects

A total of 210 individuals participated in the study. Patients who had survived an arterial thrombotic event (N = 27) with the diagnosis of ischemic stroke (IS), and patients who had suffered a venous thrombotic event (N = 77) were consecutively selected by physicians at the Hematology Unit of the University Hospital (Universidade Federal de Minas Gerais, Belo Horizonte, MG) to participate in the present study between July 2007 and December 2010. Diagnosis of arterial thrombosis was confirmed by magnetic resonance, brain computed tomography, and/or arteriography. Diagnosis of DVT was confirmed by duplex scan while PE was diagnosed by scintilography and angiotomography.

Patients with major systemic diseases that are known to predispose to thrombosis, such as cancer, infections, hepatic diseases, or autoimmune disorders, as well as those with coagulation disorders, were excluded. Baseline information on smoking habits, medication use, and personal history of disease were gathered by trained medical staff during a standardized interview. In addition, all participants underwent an extensive standardized medical examination. Body mass index was calculated as weight in kilograms divided by height in square meters. Systolic and diastolic blood pressures were measured in the right arm with the patient in a sitting position, according to the recommendations of the American Heart Association. Patients aware of having hypertension, taking antihypertensive medication, and/or having blood pressure values of 160/90 mmHg at baseline were defined as actual hypertension cases. Individuals were classified as having diabetes mellitus if their plasma glucose level was equal to or greater than 126 mg/dL in the fasting state, or if individuals were receiving oral anti-diabetes medications or insulin. Hypercholesterolemia was considered if total cholesterol was greater than 239 mg/dL. None of the patients was using lipid-lowering therapy. A regular smoker was defined as a subject who currently smoked at least five cigarettes per day. No renal disease was observed or detected by the trained medical staff. Patients had normal levels of urea and creatinine.

The control group comprised 106 subjects with no previous history of arterial or venous thrombosis from the same demographic area as the patients, but with no familial relationship to them. The institutional Ethics Committee of Universidade Federal de Minas Gerais approved this study, and informed consent was obtained from all participants.

Sample collection

Venous blood was extracted from all subjects into ethylenediaminetetraacetic (5 mL) tubes using the Vacutainer® System (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), and was subjected to genomic DNA extraction using the Wizard purification system

(Promega Inc., Madison, WI, USA), following the manufacturer instructions. The DNA bank was prepared and stored at -80°C for future analysis and was approved by the Institutional Ethics Committee of Universidade Federal de Minas Gerais.

Genotyping the FVII (-401 G/T and -402 G/A), TF (+5466 A/G), and ACE (-287 I/D) polymorphisms

The FVII and TF polymorphisms were investigated by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and the ACE polymorphism was investigated by single PCR. Briefly, amplification of the FVII and TF genes was achieved through two separate reactions and only one was used for ACE amplification using oligonucleotides that have been described previously. The PCRs were performed in a PT100 PCR thermocycler (MJ Research, Waltham, Massachusetts, USA) using 10 mM of each primer (Invitrogen, São Paulo, SP, Brazil), 2 mM deoxynucleoside triphosphate (GIBCO BRL, São Paulo, SP, Brazil), and 0.5 units Taq polymerase (Phoneutria, Belo Horizonte, MG, Brazil). PCRs were submitted to 40 cycles consisting of 2 min at 94°C for initial denaturing, 10 s at 94°C for denaturing, 30 s at 67.5°, 59°, and 60°C for primer annealing of the FVII, TF, and ACE genes, respectively, and 30 s for primer extension. The PCR products of FVII-401 G/T, FVII-402 G/A, and TF were subjected to endonuclease digestion for 3 h at 65°C with enzyme Tail (Promega Inc.), for 4 h at 55°C with enzyme *Bsa*Il (Promega Inc.), and for 4 h at 37°C with enzyme *Hin*fl (Promega Inc.), respectively, in a single reaction. Digested fragments were analyzed by polyacrylamide gel electrophoresis. These methods allowed the differentiation of wild type, heterozygous, and homozygous FVII and TF genes, and I/D in the ACE gene. DNAs from previously typed individuals were included in each set of analyzed samples to control enzyme activity.

Statistical analysis

Statistical comparisons were performed using version 7.0 of the Epi Info (Dean et al., 1996). The patient group was tested against the control group for each polymorphism frequency. Odds ratios (ORs) were used as a measure of the association between each polymorphism of the TF, FVII, and ACE genes, and to identify predictors of arterial and venous thrombosis. Confidence intervals were determined for ORs. Significance levels were estimated by applying the chi-square test. Differences were considered significant when $P < 0.05$.

RESULTS

Considering the baseline characteristics of patients, the majority of patients was young adults and 92% developed the first episode of thrombosis before the age of 45. Men comprised 40.4% of the patients and the median age was similar in patients and controls. Smoking (14.8%) and hypertension (7.4%) were the most frequently acquired risk factors for arterial thrombosis among patients. Familial history of thrombosis was noted in 24.7% of patients and there was recurrence in 22.7% (Table 1).

The early occurrence of a thrombotic event was noted among patients, including those aged 8-14 years (N = 17). According to the anatomical site of the event, IS (26%) and DVT of the lower limbs (74%) were the most frequent events.

Table 1. Baseline characteristics of patients and controls.

	Patients (N = 104)	Controls (N =106)	P value
Age (median)	36.0	32.5	>0.05
Male (%)	42 (40.4)	45 (42.5)	>0.05
BMI > 30 kg/m^2	2	0	-
Diabetes (%)	1 (1.2)	1 (0.4)	>0.05
Hypertension (%)	6 (7.4)	1 (0.4)	<0.01
Regular smoker (%)	12 (14.8)	9 (4.4)	<0.01

BMI = body mass index.

No significant differences were observed for FVII-402 G/A, FVII-401 G/T, and ACE I/D polymorphisms by comparing controls and patients considering the genotypes found. For TF genotyping, no differences in heterozygous or homozygous genotypes were found between patients and controls. No significant differences were observed for FVII-402 G/A, FVII-401 G/T, and ACE I/D allelic frequencies (Tables 2 and 3, respectively).

Table 2. Frequency of factor VII (FVII)-402 G/A and FVII-401 G/T polymorphisms in patients and controls.

Polymorphisms	Patients [N = 96 (%)]	Controls [N = 104 (%)]	OR	95%CI	P value
FVII-402 G/G	33 (34.4)	41 (39.4)			
FVII-402 G/A	55 (57.3)	47 (45.2)	1.41	0.78-2.57	0.33
FVII-402 A/A	8 (8.3)	16 (15.4)	0.53	0.19-1.43	0.31
FVII-401 G/G	57 (59.4)	65 (62.5)			
FVII-401 G/T	36 (37.5)	34 (32.7)	1.2	0.67-2.17	0.63
FVII-401 T/T	3 (3.1)	5 (4.8)	0.68	0.16-2.99	0.88

Table 3. Frequency of angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphisms in patients and controls.

Polymorphisms	Patients [N = 104 (%)]	Controls [N = 106 (%)]	OR	95%CI	P value
ACE D/D	40 (38.5)	50 (47.2)	0.70	0.40-1.21	0.26
ACE I/D	47 (45.2)	43 (40.6)	1.21	0.69-2.09	0.59
ACE I/I	17 (16.3)	13 (12.2)	1.39	0.64-3.05	0.52

The frequencies of the polymorphisms studied were also analyzed according to the anatomical site, such as arterial (N = 26) and venous (N = 78) thrombosis, but no significant differences were observed (tables not shown).

DISCUSSION

It is known that the frequencies of genetic factors and their association with risk of development of venous or arterial thrombosis vary widely according to the ethnic origin and other epidemiological aspects of individuals and populations.

In this study, no significant differences were found among the polymorphisms investigated. Several studies have examined these polymorphisms in patients with venous and arterial thrombosis, but the results are very variable and sometimes contradictory (Athanasiadis et al., 2010; Lopaciuk et al., 2010; Eroğlu et al., 2010, 2011; Previtali et al., 2011; Friso et al., 2012).

Studies have shown that increases in the levels of prothrombotic factors and decreases in the levels of inhibitors can induce the production of thrombin and the subsequent formation of a thrombus, and the proportion of these factors arising from genetic variations may contribute to a hypercoagulable state (Opstad et al., 2010a; Campo et al., 2013; Turfan et al., 2014).

Genetic polymorphisms are reported to account for about one-third of increased FVII plasma levels in individuals. The allele -402 G/A has been independently associated with the increased transcriptional activity of FVII (Lopaciuk et al., 2010). The allele -401 G/T is significantly associated with lower plasma levels of FVII (Eroğlu et al., 2010). There is controversy regarding the genetic variations that interfere with blood levels of FVII. Some studies indicate that high levels of FVII may be related to a hypercoagulable state, and certain polymorphisms in the FVII gene locus contribute to variability in the activity of FVII in the plasma (Folsom et al., 2007; Eroğlu et al., 2010). However, other studies have failed to find any association between the FVII gene polymorphism and arterial and venous thrombosis (Folsom et al., 2007; Serve et al., 2007). Some studies also suggest that the alleles associated with low levels of FVII can play a protective role against, for example, myocardial infarction. Previous studies have shown that the rare alleles of polymorphisms at positions -401 and -402 are related to marked changes in the rate of FVII gene transcription. However, other studies have investigated the role of common polymorphisms affecting the plasma levels of FVII with thrombotic disorders and found quite conflicting results. Recently, Eroğlu et al. (2010) have shown that the polymorphism -402 G/A of FVII is not associated with venous thromboembolism.

Some studies have described the role of the polymorphism +5466 A/G of the TF gene, showing that it is associated with the risk of thrombosis (Reny et al., 2004; Mälarstig et al., 2005; Morange et al., 2007; Davis and Erlich, 2008; Boles and Mackman, 2010) and a threefold increase in the risk of thrombosis in individuals that possess this polymorphism (Opstad et al., 2010a). Thus, several studies have reported the relationship between this polymorphism and regulation of TF expression and high levels of TF in cardiovascular disease (Campo et al., 2006; Undas et al., 2009; Opstad et al., 2010b). Other studies have also shown that the TF +5466 A/G polymorphism modulates thrombin generation initiated by vascular injury. However, other studies show no relationship between the expression of TF and increased risk of thrombus formation (Davis and Erlich, 2008; Steppich et al., 2009; Zhou et al., 2011).

Studies have shown that ACE plays an important role in blood pressure and the remodeling of the heart and blood vessels (Poorgholi et al., 2013). The deletion of a 287-bp sequence in intron 16 of the ACE gene is associated with increased levels of transcription of messenger RNA, and hence with increased ACE expression. Thus, in carriers of the DD genotype, ACE levels are higher than in those with genotype ID or II (Zhang et al., 2012). Prandoni's study (2007) of patients with venous thrombosis showed a moderate increase in risk for male patients with the DD genotype, but no increase in risk for women patients with the same genotype.

Jackson et al. (2000) in another case-control study of 500 unselected patients showed that the ID polymorphism in the ACE gene was not a risk factor for venous thromboembolism. Lopaciuk et al. (2010) analyzed the genetic profiles of 38 patients who had a postoperative symptomatic pulmonary embolism or proximal deep venous thrombosis after arthroplasty, and 241 control subjects without thrombosis. No difference between the groups was observed. These results suggest that there is no association between the presence of the D allele and increased risk of thromboembolic events. Poorgholi et al. (2013) addressed that issue in a large prospective study that included 348 patients, and reported a lack of association between ACE polymorphism and IS in a US population.

However, Khan et al. (2014) reported a positive correlation between the ACE gene polymorphism and carotid artery stenosis and ischemic cerebrovascular disease. Zhang et al. (2012) also reported that the deletion polymorphism in the ACE gene is an important risk factor for venous thrombosis after orthopedic surgery; the homozygous DD genotype increased thrombotic risk by more than 11 times, and the heterozygous ID genotype increased the risk by about five times.

Studies have shown that venous and arterial disorders share common risk factors, including age, obesity, smoking, diabetes mellitus, hypertension, hyperlipidemia, and metabolic syndrome, but the nature of these associations is not yet fully understood (Prandoni, 2007). It should be noted that most of the patients in the present study were not under the influence of such classic risk factors for vascular obstruction, and no associations were observed for the polymorphisms studied. Most probably, others genetic risk factors, not investigated in this study, are related to these manifestations.

As described above, there are controversies regarding the importance of FVII and ACE polymorphisms in arterial and venous thrombosis. In this study, the presence of polymorphisms in FVII, TF, and ACE genes was not related to arterial thrombosis, particularly stroke and venous thrombosis. The difference between the results of this study and those in which significant differences were detected in the frequency of FVII, TF, and ACE polymorphisms can possibly be explained by differences in the experimental design and the demographic characteristics of the populations studied. One thing to be noted is that the patients in our study were not selected with regard to the anatomical site of thrombosis.

Another important aspect to be considered is that the patients evaluated in this study were young; 17 patients aged 8-14 years had suffered a stroke. We know that people aged over 45 have a higher risk of developing vaso-occlusive processes than younger people because age is an important risk factor for the occurrence of these events, as the body goes through many changes, including changes in the hemostatic system. These changes are often associated with lifestyle habits that are acquired and vary from individual to individual. In this study, one aspect of note is that more than 92% of the patients were below 40 years of age and had suffered an early thrombotic event.

The limitations of this study should be considered, such as the small sample size, the population studied, and the lack of determination of FVII, TF, and ACE levels.

Our data indicate that even in homozygous genotypes, these polymorphisms do not contribute to the development of thrombotic events in the population studied. Thus, considering the multifactorial and multigenic occurrence of thrombotic events, apart from the classic factors for thrombosis discussed above, there may be a relationship between polymorphisms in thrombotic events, although more studies are needed to confirm this.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES

Athanasiadis G, Esteban E, Gayà-Vidal M, Dugoujon JM, et al. (2010). Different evolutionary histories of the coagulation factor VII gene in human populations? *Ann. Hum. Genet.* 74: 34-45.

Boles J and Mackman N (2010). Role of tissue factor in thrombosis in antiphospholipid antibody syndrome. *Lupus* 19: 370-378.

Campo G, Valgimigli M, Ferraresi P, Malagutti P, et al. (2006). Tissue factor and coagulation factor VII levels during acute myocardial infarction: association with genotype and adverse events. *Arterioscler. Thromb. Vasc. Biol.* 26: 2800-2806.

Campo G, Pavasini R, Pollina A, Tebaldi M, et al. (2013). Coagulation factors and recurrence of ischemic and bleeding adverse events in patients with acute coronary syndromes. *Thromb. Res.* 132: 151-157.

Davis DR and Erlich JH (2008). Cardiac tissue factor: roles in physiology and fibrosis. *Clin. Exp. Pharmacol. Physiol.* 35: 342-348.

de Meis E and Levy RA (2007). Câncer e trombose : uma revisão da literatura Cancer and thrombosis : a literature review. *Rev. Bras. Cancerol.* 53: 183-193.

Dean AG, Dean JA, Coulombier D, Brendel KA, et al. (1996). Epi Info, Version 6: a word processing, database, and statistics program for public health on IBM-compatible microcomputers. Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

Eroğlu A, Oztürk A, Cam R and Akar N (2010). No significant association between the promoter region polymorphisms of factor VII gene and risk of venous thrombosis in cancer patients. *Exp. Oncol.* 32: 15-18.

Eroğlu A, Oztürk A and Akar N (2011). Association between the -402GA, -401GT, and -323ins10-bp polymorphisms of factor VII gene and breast cancer. *Breast Cancer* 18: 282-285.

Fatini C, Sticchi E, Sofi F, Said AA, et al. (2009). Multilocus analysis in candidate genes ACE, AGT, and AGTR1 and predisposition to peripheral arterial disease: role of ACE D/-240T haplotype. *J. Vasc. Surg.* 50: 1399-1404.

Folsom AR, Cushman M, Heckbert SR, Ohira T, et al. (2007). Factor VII coagulant activity, factor VII -670A/C and -402G/A polymorphisms, and risk of venous thromboembolism. *J. Thromb. Haemost*. 5: 1674-1678.

Friso S, Lotto V, Choi SW, Girelli D, et al. (2012). Promoter methylation in coagulation F7 gene influences plasma FVII concentrations and relates to coronary artery disease. *J. Med. Genet.* 49: 192-199.

Hemmeryckx B, Emmerechts J, Bovill EG, Hoylaerts MF, et al. (2012). Effect of ageing on the murine venous circulation. *Histochem. Cell Biol.* 137: 537-546.

Hoffman M (2003). Remodeling the blood coagulation cascade. *J. Thromb. Thrombolysis* 16: 17-20.

Jackson A, Brown K, Langdown J, Luddington R, et al. (2000). Effect of the angiotensin-converting enzyme gene deletion polymorphism on the risk of venous thromboembolism. *Br. J. Haematol.* 111: 562-564.

Kalyesubula R, Bagasha P and Perazella MA (2014). ACE-I/ARB therapy prior to contrast exposure: what should the clinician do? *Biomed. Res. Int.* 2014: 423848.

Khan IA, Jahan P, Hasan Q and Rao P (2014). Angiotensin-converting enzyme gene insertion/deletion polymorphism studies in Asian Indian pregnant women biochemically identifies gestational diabetes mellitus. *J. Renin Angiotensin Aldosterone Syst.* 15: 566-571.

Litzendorf ME and Satiani B (2011). Superficial venous thrombosis: disease progression and evolving treatment approaches. *Vasc. Health Risk Manag.* 7: 569-575.

Lopaciuk S, Windyga J, Watala CW, Bykowska K, et al. (2010). Polymorphisms in the factor VII gene and ischemic stroke in young adults. *Blood Coagul. Fibrinolysis* 21: 442-447.

Mälarstig A, Tenno T, Johnston N, Lagerqvist B, et al. (2005). Genetic variations in the tissue factor gene are associated with clinical outcome in acute coronary syndrome and expression levels in human monocytes. *Arterioscler. Thromb. Vasc. Biol.* 25: 2667-2672.

Mercer PF and Chambers RC (2013). Coagulation and coagulation signalling in fibrosis. *Biochim. Biophys. Acta* 1832: 1018-1027.

Mitrophanov AY and Reifman J (2011). Kinetic modeling sheds light on the mode of action of recombinant factor VIIa on thrombin generation. *Thromb. Res.* 128: 381-390.

Morange PE, Blankenberg S, Alessi MC, Bickel C, et al. (2007). Prognostic value of plasma tissue factor and tissue factor pathway inhibitor for cardiovascular death in patients with coronary artery disease: the AtheroGene study. *J. Thromb. Haemost.* 5: 475-482.

Opstad TB, Pettersen AA, Weiss T, Arnesen H, et al. (2010a). Gender differences of polymorphisms in the TF and TFPI genes, as related to phenotypes in patients with coronary heart disease and type-2 diabetes. *Thromb. J.* 8: 7.

Opstad TB, Pettersen AA, Bratseth V, Arnesen H, et al. (2010b). The influence of tissue factor and tissue factor pathway

inhibitor polymorphisms on thrombin generation in stable coronary artery disease. *Pathophysiol. Haemost. Thromb.* 37: 98-103.

Petrillo G, Cirillo P, D'Ascoli GL, Maresca F, et al. (2010). Tissue factor/factor FVII complex inhibitors in cardiovascular disease. Are things going well? *Curr. Ceardiol. Rev.* 6: 325-332.

Poorgholi L, Saffar H, Fathollahi MS, Davoodi G, et al. (2013). Angiotensin- converting enzyme insertion/deletion polymorphism and its association with coronary artery disease in an Iranian population. *J. Tehran Heart Cent.* 8: 89-94.

Prandoni P (2007). Venous thromboembolism and atherosclerosis: is there a link? *J. Thromb. Haemost.* 5: 270-275.

Previtali E, Bucciarelli P, Passamonti SM and Martinelli I (2011). Risk factors for venous and arterial thrombosis. *Blood Transfus.* 9: 120-138.

Reny JL, Laurendeau I, Fontana P, Bièche I, et al. (2004). The TF-603A/G gene promoter polymorphism and circulating monocyte tissue factor gene expression in healthy volunteers. *Thromb. Haemost.* 91: 248-254.

Serve E, Reny JL, Akhavan S, Emmerich J, et al. (2007). A two adenine insertion polymorphism in the 3' untranslated region of factor VII gene is associated with peripheral arterial disease but not with venous thrombosis. Results of case-control studies. *Thromb. Haemost.* 98: 733-737.

Silveira A, Scanavini D, Boquist S, Ericsson CG, et al. (2012). Relationships of plasma factor VIIa-antithrombin complexes to manifest and future cardiovascular disease. *Thromb. Res.* 130: 221-225.

Singh A, Foster GD, Gunawardana J, McCoy TA, et al. (2012). Elevated circulating tissue factor procoagulant activity, factor VII, and plasminogen activator inhibitor-1 in childhood obesity: evidence of a procoagulant state. *Br. J. Haematol.* 158: 523-527.

Spronk HM, van der Voort D and Ten Cate H (2004). Blood coagulation and the risk of atherothrombosis: a complex relationship. *Thromb. J.* 2: 12.

Steppich BA, Braun SL, Stein A, Demetz G, et al. (2009). Plasma TF activity predicts cardiovascular mortality in patients with acute myocardial infarction. *Thromb. J.* 7: 11.

Turfan M, Poyraz F, Kaymak AÖ, Ergun MA, et al. (2014). Plasma levels and distribution of gene polymorphisms of factor VII in Turkish population. *Clin. Appl. Thromb. Hemost.* 20: 164-168.

Undas A, Stepien E, Potaczek DP and Tracz W (2009). Tissue factor +5466A>G polymorphism determines thrombin formation following vascular injury and thrombin-lowering effects of simvastatin in patients with ischemic heart disease. *Atherosclerosis* 204: 567-572.

Zakai NA, Lange L, Longstreth WT Jr, O'Meara ES, et al. (2011). Association of coagulation-related and inflammation-related genes and factor VIIc levels with stroke: the Cardiovascular Health Study. *J. Thromb. Haemost.* 9: 267-274.

Zhang Z, Xu G, Liu D, Fan X, et al. (2012). Angiotensin-converting enzyme insertion/deletion polymorphism contributes to ischemic stroke risk: a meta-analysis of 50 case-control studies. *PloS One* 7: e46495.

Zhou RF, Liu Y, Wang YX, Mo W, et al. (2011). Coagulation factor III (tissue factor) is required for vascularization in zebrafish embryos. *Genet. Mol. Res.* 10: 4147-4157.