The spectrum of premature atherosclerosis: from single gene to complex genetic disorder
Trip, M.D.

Citation for published version (APA):
Trip, M. D. (2002). The spectrum of premature atherosclerosis: from single gene to complex genetic disorder
Genetic variations of the haemostatic system as risk factors for arterial thrombotic disease

Rendrik F Franco¹, Mieke D Trip², Pieter H Reitsma³

¹Laboratory for Experimental Internal Medicine, and ²Department of Cardiology, Academic Medical Centre, The Netherlands
³Haemophilia and Thrombophilia Centre, School of Medicine of Ribeirão Preto, University of São Paulo, Brazil

Current Genetics 2002; in press
Abstract

Arterial thrombosis (clinically manifested as myocardial infarction, ischaemic stroke, and peripheral occlusive artery disease) represent major health problems that are associated with high rates of morbidity and mortality, particularly in Western societies. Numerous conditions are known to predispose to arterial thrombosis, commonly referred to as risk factors. General accepted risk factors for arterial thrombosis include cigarette smoking, physical inactivity, dyslipidaemia, hypertension, diabetes, obesity, metabolic syndrome, menopause, hyperhomocysteinemia, male gender, and a positive family history of arterial disease. This listing also serves to show that there is not a single cause of arterial thrombosis, and that this is better understood as a complex or multifactorial trait. In addition to the mentioned well-established risk factors for arterial thrombosis, several lines of evidence point to a role of novel genetic risk factors, related to the haemostatic system, in influencing thrombotic risk. In fact, it is becoming increasingly clear that the analysis of genetic risk factors and plasmatic factors, together with private life style and environmental factors may contribute significantly to our understanding of the genetic predisposition to thrombosis. Plasmatic levels of proteins of the haemostatic system have also been alleged to determine the onset and outcome of arterial thrombosis, and several studies examined the relationship between genetic markers of the haemostatic system and arterial disease. The results from such studies are frequently conflicting, and large prospective studies remain needed in order to evaluate the significance of haemostatic gene variations in arterial thrombotic diseases. Such investigations are particularly important in order to reach the ultimate goal of characterising phenotypic and genetic markers for arterial thrombosis, which would enable the establishment of individual profiles of arterial thrombotic risk, and which might eventually result in new and individualised prognostic and therapeutic measures.

Introduction

Vascular thrombotic diseases are major health problems associated with high rates of morbidity and mortality, especially in Western societies. Arterial thrombosis (clinically manifested as myocardial infarction, ischaemic stroke, or peripheral occlusive artery disease) is, in most cases, a consequence of atherosclerosis, a degenerative inflammatory disease of the intima of medium and large arterial vessels.¹,² Arterial
thrombi are primarily composed by platelets and are formed in areas of rapid blood flow. Several factors are known to predispose to atherothrombosis and such factors are usually called "risk factors" or "risk indicators". The well-established risk factors for arterial thrombosis are cigarette smoking, physical inactivity, inherited or acquired dyslipidaemia, hypertension, diabetes, obesity, metabolic syndrome, menopause, hyperhomocysteinemia, male gender, and a positive family history of arterial disease. These lists of risk factors clearly illustrate that there is not a single cause of arterial thrombosis. Indeed, atherothrombosis is better understood as multifactorial (or "complex") disease, in which not only acquired or environmental factors but also inherited factors play a significant role.

The concept that genes contribute to the occurrence of arterial thrombosis may also be inferred from the finding that a familial component is often present in cases presenting with atherothrombosis. In the case of arterial thrombosis, the risk linked to a positive family history cannot be exclusively attributed to familial aggregation of "classical" risk factors. Additionally, the effect of a positive family history seems to be of particular relevance in patients at (apparently) low risk of arterial disease.

In addition to the above-mentioned risk factors and indicators, the blood coagulation system has also been claimed to determine the onset and outcome of atherosclerosis. There are two important features that give support to this presumption. Firstly, cleavage products from the coagulation system (i.e., the activated form of coagulation proteins such as thrombin, factor Xa, factor Vila, and activated protein C), may modulate pro- and anti-inflammatory properties of endothelial cells and leukocytes. Indeed, the inflammatory responses of these cells to blood-borne factors are thought to play a key role in atherogenesis. Secondly, clot formation as occurs in for instance myocardial infarction and ischaemic stroke, is a leading complication of atherosclerosis. Subtle inter-individual differences in the response of the coagulation system may therefore modify the risk of arterial thrombosis.

The search for haemostatic candidate genes and polymorphisms involved in the predisposition to arterial thrombosis became an important step towards a more comprehensive understanding of the pathogenesis of cardiovascular disease. In fact, an enormous number of polymorphisms in genes coding for proteins of the haemostatic system have been claimed to influence thrombotic risk. Coagulation gene variations, however, are not consistently and unequivocally linked to arterial thrombosis and phenotypic or genotypic analyses of the haemostatic system have no part to play in the current routine management of subjects with arterial thrombotic disease.
A review of current data on candidate haemostatic risk factors is quite complicated in arterial thrombosis. There are several reasons for this. The first problem is inherent to the two putative roles of the coagulation system, i.e. modulating the inflammatory response and determining the rate of clot formation, and which are not always easily distinguished in an investigation of patients with atherothrombotic disease. Secondly, sources of data are heterogeneous, and both descriptive and analytic investigations have yielded findings which are often contradictory. Diversity of study design may contribute to the discrepancies, making direct comparisons between studies complicated. Many studies also suffer from a sample size which is too small to confirm or rule out the presence of a relevant epidemiological association between specific polymorphisms and arterial disease. Moreover, the lack of data showing a clear functional consequence on protein phenotype of several gene variations hampers the interpretation of data derived from many studies aimed at testing associations. In addition, it is unlikely that specific haemostatic gene variations are, in isolation, very strong risk factors for arterial disease. More probable is the scenario where haemostatic gene polymorphisms influence atherosclerosis or arterial thrombotic risk by interacting with other established genetic or acquired risk factors. Such interactions may not be apparent in all investigations due to the influence of factors such as sample size, ethnicity, selection and recall bias in case-control studies, and clinical end-points. Finally, several candidate polymorphisms may be in linkage disequilibrium with actual disease causing polymorphisms (a variation located somewhere in the regulatory or coding region of the same gene or even in another gene). The degree of disequilibrium may vary from population to population, a fact that may also be responsible for some of the discrepancies between studies analysing the same gene variation as a risk factor for arterial thrombosis.

In spite of these difficulties, which are inherent to genetic association studies, the objective of defining which gene haemostatic abnormalities are actual risk factors for thrombosis remains important. Indeed, if the ultimate goal of characterising phenotypic and genetic markers for arterial thrombosis was reached, it would enable one to establish individual profiles of thrombotic risk, which might eventually result in new and individualised prognostic and therapeutic measures. In this paper, we shortly review the published data that relate gene abnormalities of the haemostatic system with arterial thrombosis.
Polymorphisms in genes coding for platelet surface proteins

Glycoprotein la-IIa
Gp la-IIa, also referred to as $\alpha_2\beta_1$ integrin, is the major constitutive collagen receptor on the platelet surface, which is involved in platelet adhesion to the subendothelial matrix. A silent dimorphism at nucleotide position 807 (C807T) of the Gpla-IIa gene was reported to affect $\alpha_2\beta_1$ integrin density on platelets and collagen receptor activity. The C807T polymorphism is in tight linkage disequilibrium with a second Gp la-IIa polymorphism (G873A) and with other variations in the same gene.30,31 The 807T allele (linked to high $\alpha_2(31$ density) was associated with a significant 1.6-fold increased risk of MI in a large study investigating male patients undergoing coronary angiography.31 The odds ratios for MI linked to the 807T allele increased with decreasing age, pointing to a more relevant role of this polymorphism in determining arterial thrombotic risk in young patients.31 Subsequent reports also found the 807T allele to be a risk factor for MI and stroke, particularly in young patients,32-34 but others did not confirm these findings.35-38 Thus, there are data suggesting that the C807T Gp la-IIa polymorphism may be a risk factor for arterial thrombosis, but the issue is controversial, pointing to the need of additional studies dealing with larger patient populations to resolve this issue.39

Glycoprotein Ib-V-IX
Gp Ib-V-IX is a constitutively active platelet receptor for von Willebrand factor (vWF), and mediates platelet binding to vWF present in the perivascular matrix. Two polymorphisms in the gene coding Gp Ib$\alpha$ were identified which affect the phenotype (HPA-2 and a VNTR).40-42 The HPA-2 alloantigen system is defined by the presence of a C to T substitution at nucleotide position 3550 (which results in a Thr145Met substitution). The VNTR is a repeat of 39-bp in the macro-glycopeptide region of the Gp Ib$\alpha$, and the following alleles are known: D (four repeats), C (three repeats), B (two repeats), and A (one repeat). Each repeat results in the addition of 13 amino acids to the protein, and it is thought that the addition of amino acids modifies the distance between the vWF-binding domain and the platelet surface. The exact functional consequence of this alteration on platelet function is, however, poorly defined.40-42 The HPA-2 and the VNTR polymorphisms are in tight linkage disequilibrium: HPA-2a (Thr at position 145) is linked to the C and D VNTRs, whereas HPA-2b (Met at position 145) is associated with the A and B VNTR alleles.
The HPA-2 and the VNTR Gp Ibx polymorphisms were examined as risk factors for arterial thrombotic diseases in several investigations. Both positive associations (between HPA-2b and VNTR B allele and increased risk of MI and stroke) and negative findings were reported. The contribution of Gp Ib( gene variations to arterial thrombosis is therefore unclear.

Recently, a gene polymorphism in the Kozak sequence of the Gp Ibx receptor has been described. This variation is a T/C dimorphism at nucleotide -5, which is located in the translation initiation codon of the Gp Ibx gene. The -5C allele is associated with more efficient mRNA translation and increased levels of the Ibx receptor on the platelet surface. In a recent study, Frank et al. investigated the -5C allele as a risk factor for MI and stroke in a population-based study involving relatively young women. The polymorphism had a neutral effect on the risk of stroke, and, paradoxically, a trend towards protection against MI by the -5C allele (odds ratio 0.53) was observed. Further studies are certainly warranted to better delineate the role of this polymorphism in arterial thrombotic disease.

**Glycoprotein IIb-IIIa**

Gp IIb-IIIa (also referred to as integrin \( \alpha_{\text{II}B}\beta_3 \)) mediates platelet aggregation by functioning as the fibrinogen receptor on the platelet surface. In platelets at rest, Gp IIb-IIIa exhibits a low affinity for fibrinogen, but upon activation this affinity is markedly increased. Gp IIb-IIIa may also serve as a receptor for vWF and other soluble ligands. A number of Gp IIb-IIIa gene variations have been described in the general population. The polymorphism which received particular attention regarding its association with arterial disease is a common variation in the Gp IIIa gene: a T to C transition at nucleotide 1565, which results in a Pro to Leu substitution at amino acid position 33. The Pro33 allele (also referred to as PIA2) is known to exhibit a heterogeneous ethnic distribution and was claimed to result in increased platelet aggregability, but the exact functional consequences of this polymorphism are still unclear.

PIA2 was associated with a 2.8-fold increased risk of MI in an initial report. Since then numerous studies investigated the PIA2 allele as a risk factor for coronary thrombotic disease, and most of these studies did not confirm the hypothesis that this polymorphism modifies thrombotic risk. In most studies aimed at exploring the relationship between the PIA2 allele and stroke, a negative result was found, but at least one investigation suggested that this polymorphism increases the risk of cerebral vascular disease in relatively young subjects. In a recent study by Renner et al. the PIA2 allele was found with similar frequency in 815 patients with documented
Genetic variation of the haemostatic system

Peripheral artery disease and in two groups of controls. A recently published meta-analysis comparing the frequency of the PIA2 polymorphism in 4839 patients with myocardial infarction with the frequency in 5799 controls yielded no significant association. The conflicting results regarding the relationship between the PIA2 polymorphism and arterial disease are difficult to judge, and only will be clarified when more data from larger samples become available.

Another Gp IIb-IIIa gene variation that has been investigated in its association with arterial thrombotic disease is the Gp IIb Ile843Ser polymorphism, with most studies yielding negative results. One study suggested that the Ser843 allele is associated with increased risk of MI in young women (aged less than 45 years), in the presence of other major risk factors for atherosclerosis such as smoking, positive family history of MI and high cholesterol levels. Further investigations are warranted to confirm these findings.

Polymorphisms in coagulation factors

Fibrinogen

Fibrinogen is the precursor of fibrin and its levels influence platelet aggregation, blood viscosity, and endothelial cell injury, mechanisms which all play a role in atherosclerosis and arterial thrombosis. Thus, it is conceivable that, as a determinant of hypercoagulability, plasma fibrinogen level may influence the risk. Based on this hypothesis, several studies have addressed the risk of cardiovascular disease for high in comparison with low fibrinogen levels in plasma. A meta-analysis of 22 of these studies (13 prospective, 5 cross-sectional and 4 case-control) showed that elevated plasma fibrinogen levels (within the highest tertile) are associated with an overall two-fold increased risk of cardiovascular disease both in healthy subjects and in high-risk subjects. The effect of fibrinogen as a determinant of arterial thrombotic risk seems to be independent from other classical risk factors for arterial disease. Fibrinogen exhibits interactive effects with other risk factors, mainly smoking and hypertension. Moreover, high fibrinogen levels appear to improve prediction of recurrence of arterial thrombotic events by 8%, pointing to a possible relevant prognostic role of measuring fibrinogen levels in patients with arterial thrombotic disease. It should be mentioned however that fibrinogen is an acute phase reactant and therefore the observed effect of elevated fibrinogen levels may at least in part reflect the degree of the inflammation that accompanies atherosclerotic disease.
The fibrinogen molecule is a glycoprotein containing two copies of three polypeptide chains (α, β, and γ) encoded by three distinct genes located on the long arm of chromosome 4 (q23-32). Various polymorphisms have been identified in all three genes, mainly in the locus encoding the β chain of fibrinogen. Most attention has been paid to two dimorphisms in the β-chain gene: the HaeIII polymorphism (a G to A substitution at position -455 in the 5'- promoter region) and the BclI polymorphism in the 3'-untranslated region. These two polymorphisms are in linkage disequilibrium with each other. The 5'- promoter polymorphism is of particular interest because of its location nearby an interleukin-6 and a hepatocyte nuclear factor I responsive element. Indeed, the -455G/A substitution was found to be a determinant of plasma fibrinogen levels in different investigations. These findings prompted several authors to look for an association between this fibrinogen gene variation and risk of arterial thrombosis, but conflicting results were reported. In fact, the results from studies addressing this issue have not been consistent and it could be argued that there is hardly any evidence that the predicted genotype-disease relationship indeed exists for arterial disease. Another fibrinogen gene variation that has drawn some attention is the α-chain Thr312Ala polymorphism in the coding region. It was claimed that the 321Ala allele increases clot stability, and this polymorphism has been investigated as a risk factor for arterial disease in a few studies. In the ECTIM study, Thr312Ala did not influence the risk of MI. In another investigation, the 312Ala allele was associated with decreased survival after stroke and with increased risk of pulmonary embolism. The exact role of Thr312Ala in thrombotic disorders remains to be clarified.

Factor II
In 1996, a novel genetic factor involved in the aetiology of VTE was described: a G→A transition at nucleotide position 20210 in the 3'-untranslated region of the coagulation factor II gene (FII, prothrombin) causing increased mRNA accumulation and protein synthesis. This mutation is found in 1-3% of subjects in the general population, and in 6-18% of patients with VTE. These studies established that FII G20210A is linked to a 2- to 5-fold increased risk of VTE. Conflicting results were reported regarding the role of FII G20210A as a risk factor for arterial thrombotic disease, and the issue remains undecided. It has been claimed that in particular subgroups of patients (for instance, young subjects, and smokers), FII G20210A may amplify arterial thrombotic risk by
interacting with established risk factors for athero-thrombosis.\textsuperscript{110-112} Recently, Psaty et al. reported an interactive effect between FII G20210A carriership and use of hormone replacement therapy in determining the risk of MI in postmenopausal women.\textsuperscript{113}

**Tissue factor**

The major initiator of the blood coagulation cascade is tissue factor (TF). A recent study asked the question whether individual differences in TF gene expression would predispose to thrombosis.\textsuperscript{114} Sequencing of the promoter region of the TF gene yielded six novel polymorphisms that were distributed over two haplotypes with equal frequencies (designated 1208 D and 1208 I). Although the haplotype was claimed to determine plasma levels of TF, the 1208 D/I genotype did not influence the risk of coronary thrombotic disease in a case-control study involving 2354 individuals.\textsuperscript{115}

**Factor V**

In 1993 Dahlbäck and colleagues described an abnormality that was highly prevalent in patients with venous thrombosis.\textsuperscript{116} In a modified APTT assay, the authors observed that addition of activated PC to the plasma of patients with VTE did not result in the expected prolongation of the clotting time, a phenomenon that was referred to as “activated protein C resistance” (APCR).

Inherited APCR is, in most cases, the result of a gain-of-function point mutation in coagulation factor V: a G→A transition at nucleotide position 1691, leading to the substitution of arginine (R) by glutamine (Q) at amino acid position 506 (a cleavage site for activated PC in the factor V molecule). This point mutation was first described in 1994 and is currently referred to as factor V Leiden (FVL).\textsuperscript{117} In contrast with the clear propensity towards veno-occlusive disorders, the association of FVL with arterial thrombosis is less clear. Most studies failed to demonstrate that APCR or FVL is a risk factor for arterial disease.\textsuperscript{48,118-134} As is the case for the FII G20210A polymorphism, some investigations claimed that FVL may contribute to athero-thrombosis by acting synergistically when other major risk factors are also present.\textsuperscript{133-135} For instance, an increased risk for myocardial infarction was present in young women carrying FVL, who also reported cigarette smoking.\textsuperscript{133} These data suggest that FVL is not per se a major risk factor for arterial thrombotic disease, but the mutation may increase the risk conferred by classical risk factors.
Factor VII
Coagulation factor VII (FVII) is a vitamin K-dependent glycosylated plasma protein that circulates as an inactive zymogen and is activated by proteolysis mediated by factor IXα, Xα and XIα. FVII was one of the first coagulation genes for which relationships between common DNA polymorphisms and plasma levels have been reported. The interest in this coagulation protein was aroused by findings in the Northwick Park Heart Study that elevated levels were prospectively associated with arterial occlusive disease. These findings have been partly confirmed in another large prospective study, the PROCAM, although the results concerning the association between FVII levels and arterial disease were not clear-cut since it was not found to be an independent risk factor for MI after adjustment for potential confounders. Furthermore, additional studies did not confirm elevated plasma FVII levels to be a risk factor for arterial thrombotic disease.

Several polymorphisms in the FVII gene have been described and shown to influence plasma FVII levels. Most attention has been focused on the Arg353Gln mutation in exon 8, which is located in the catalytic domain of FVII. The Gln353 allele is linked to lower FVII antigen and coagulant activity in plasma, and has been investigated as a risk factor for arterial thrombosis in several studies. In a case-control study involving 165 relatively young patients with familial MI, the Gln353 allele was reported to confer a strong protective effect against the occurrence of MI. Other larger case-control studies however failed to confirm these findings, since the allelic distribution of this polymorphism was the same in patients with arterial disease and controls.

Given the doubtful association between plasma FVII levels and athero-thrombosis, and the contradictory findings concerning the association of FVII polymorphisms with arterial disease, the role of FVII is determining arterial thrombotic risk is more than questionable at present.

Factor VIII
Plasma factor VIII concentrations reflect the combined influence of inherited and acquired factors. For example, genes encoding ABO blood groups and von Willebrand factor influence factor VIII levels. Additionally, familial aggregation of elevated factor VIII (not linked to blood group or vWF) was also described, pointing to the existence of unknown genetic components determining factor VIII concentrations.

Elevated plasma FVIII activity levels have been associated with increased risk of arterial thrombosis. The causative nature of this risk relationship, however,
may be questioned, since FVIII is known to be a phase-acute reactant. In addition, no specific molecular abnormality in FVIII gene has been identified that explains the higher levels or the increased risk of arterial thrombosis.

**Von Willebrand factor**

Similarly to FVIII, elevated plasma vWF levels have been linked to increased risk of arterial thrombotic events in several studies assessing the issue, but independent associations have not been consistently reported. Moreover, no gene polymorphisms in the vWF have been so far demonstrated to influence atherothrombotic risk.

**Factor XIII**

FXIII Val34Leu was found to protect against the occurrence of myocardial infarction and stroke in several studies. Such a protective effect was, however, not confirmed in other investigations. As is the case for its relationship with VTE, prospective studies will be necessary to define the role of FXIII Val34Leu in arterial thrombosis.

**Mutations and polymorphisms in the anticoagulant system**

**Antithrombin, protein C and protein S deficiency**

During activation of the coagulation system, serine proteases with procoagulant activity are sequentially generated, eventually resulting in the formation of a stable fibrin clot. The activity of these proteases is down-regulated by a group of proteins usually referred to as natural anticoagulants or as physiological coagulation inhibitors, of which the main components are antithrombin (AT), protein C (PC) and protein S (PS).

Genetic defects in these coagulation inhibitors are very rare in the general population, but result in a prothrombotic state and increased risk of venous thrombosis. However, the role of AT, PC and PS inherited deficiencies in arterial disease is much unclear and it is unlikely that such defects importantly contribute to arterial thrombotic risk. A possible exception is that in children presenting with stroke of apparently unidentified aetiology anticoagulant deficiencies may contribute to the thrombotic event, but even in this clinical context some controversy exists.
**Thrombomodulin**

Thrombomodulin is an integral membrane protein of endothelial cells and monocytes. When thrombin binds to thrombomodulin, it loses its procoagulant activity but becomes capable of activating protein C. Given this crucial function in the protein C pathway, a hereditary deficiency of thrombomodulin might very well play a role as a risk factor for thrombotic disease.

Several variations have been identified in the thrombomodulin gene. The Ala455Val polymorphism was claimed to influence the risk of arterial thrombosis because the A allele was found to be more frequent in survivors of myocardial infarction (MI) in an initial report, but this was not confirmed in a second study.\(^\text{160,161}\) Recently, Wu and colleagues reported that the 455Val allele is linked to a 6.1-fold increased risk of coronary heart disease in Blacks but does not significantly influence risk among Whites.\(^\text{162}\)

Another TM polymorphism that has been investigated in its relation with arterial disease is the G to A substitution at nucleotide position 127 in the gene, which predicts an Ala25Thr substitution in the protein. The 25Thr allele was found to be more prevalent among 560 male patients with MI, than in 646 controls in the “Study of Myocardial Infarctions Leiden” (SMILE), yielding an odds ratio for MI of 2.0.\(^\text{163}\) A particularly high risk (6.5-fold increase) was observed in relatively young patients (aged below 50) and in the presence of additional risk factors such as smoking and metabolic risk factors (9-fold increased risk). In contrast, in two subsequent investigations the Ala25Thr polymorphism was not found to be a risk factor for coronary artery disease\(^\text{164}\) or stroke.\(^\text{165}\)

Recently, Li et al reported that a polymorphism in the thrombomodulin gene promoter (-33 G/A) influences plasma soluble thrombomodulin levels and is linked to a 1.8-fold increased risk for coronary heart disease in the Chinese population.\(^\text{166}\)

The same group also reported a significant association between carriership of the -33A allele and a 2.4-fold increased risk for the occurrence of carotid atherosclerosis in subjects aged less than 60 years.\(^\text{167}\)

The potential role of thrombomodulin mutations in MI recently also received further support from the documentation of a frameshift mutation in a family with arterial disease.\(^\text{168}\) Again, the data are not definitive, but add further to the notion that thrombomodulin mutations may be more important in arterial than in venous thrombotic disease.

**Tissue-factor pathway inhibitor**

TFPI is a so-called Kunitz-type inhibitor that plays a major role in the inhibition of
the extrinsic coagulation pathway. Inhibition takes place by the formation of a quaternary complex between tissue factor, factor VIIa, factor Xa and TFPI. Because of its inhibitory function, TFPI is a candidate risk factor for thrombotic disease; however, with plasmatic assays, no clear qualitative or quantitative deficiency states have been found.

Recently, systematic sequencing of the TFPI gene has yielded four different polymorphisms (Pro151Leu, Val264Met, T384C exon 4, and C-33T intron 7), but their association with thrombotic disease is doubtful. The recently described -287 T/C polymorphism in the 5'-untranslated region did not alter TFPI levels in a control population analysed, and does not appear to influence the risk of coronary athero-thrombosis. Data on the role of TFPI in arterial thrombosis are still scarce, and the role of TFPI gene polymorphisms as potential risk factors remains poorly explored.

**Endothelial protein C receptor**

In a preliminary report, the 23-base-pair insertion in exon 3 of the EPCR gene was linked to an increased risk of athero-thrombotic disease. However, a recent study did not confirm the EPCR insertion to be a risk factor for myocardial infarction. Recent data demonstrated that the insertion is linked to impaired function of the receptor. It is possible that a significant relationship between carriehership of the mutation and arterial thrombosis has not been demonstrated heretofore on account of the rarity of the insertion in the general population. If this is the case, only large studies will unravel a significant risk modification associated with the EPCR insertion.

**Polymorphisms in the fibrinolytic system**

**Plasminogen activator inhibitor (PAI-1)**

PAI-1 is a member of the serpin (serine protease inhibitor) superfamily. PAI-1 functions as an important inhibitor of plasminogen activation thereby regulating fibrinolysis. Augmented PAI-1 mRNA expression in macrophages and vascular smooth muscle cells was found in human atheroma plaques. Increased plasma PAI-1 levels have been also related to increased risk of arterial thrombosis in a number of investigations, although not uniformly. The putative relationship of plasma PAI-1 levels with the risk of arterial disease is thought to be influenced by other risk modifiers in the presence of insulin resistance.
Several gene variations are known for the PAI-1 gene, including a variation at a CA(n) dinucleotide repeat in intron 3, a *HindIII* VNTR polymorphism in the 3'-untranslated region, and a 4G/5G deletion/insertion promoter polymorphism.\(^{189,193}\) The latter dimorphism has been more widely investigated with respect to putative physiopathologic and clinical importance. The 4G allele was reported to exhibit increased mRNA transcription in comparison with the 5G allele, in *in vitro* experiments using HepG2 human hepatoma cell lines transiently transfected with the two alleles and stimulated with interleukin-1.\(^{194}\) In addition, the 4G/5G polymorphism is also known to determine PAI-1 levels, the 4G genotype being associated with higher plasma concentrations.\(^{195-198}\) The 4G/5G promoter polymorphism has been also investigated as a risk factor for arterial thrombotic disease in numerous studies, and controversial results emerged from these analyses. Several investigations claim that the 4G genotype is a risk factor for coronary thrombosis in subgroups of patients with cardiovascular disease,\(^{196,199-202}\) but other studies did not confirm these findings.\(^{157,186,203-205}\) A recent meta-analysis found that the 4G allele is associated with a mild increase (1.2-fold) in the risk of MI.\(^{206}\)

**Tissue-plasminogen activator (t-PA)**

t-PA, also referred to as tissue-type PA, is a plasma serine protease which mediates plasmin generation and clot lysis by specifically cleaving clot-bound plasminogen.\(^{178}\) Increased plasma t-PA levels have been (paradoxically) associated with increased risk of coronary thrombotic disease.\(^{207,208}\) Numerous polymorphisms have been reported for the t-PA gene.\(^{209}\) It has been reported that an Alu insertion/deletion polymorphism in the t-PA gene influences release rates of total t-PA.\(^{210}\) Homozygosity for the Alu insertion polymorphism has been associated with a two-fold increased risk of MI in the Rotterdam study,\(^{211}\) but this finding could not be confirmed in other investigations.\(^{212-215}\) Thus, the role of this variation in arterial disease is uncertain. Recently, eight novel t-PA gene polymorphisms have been described.\(^{215}\) Three of the polymorphisms are in strong linkage disequilibrium with the Alu polymorphism, and influence t-PA release rates. The other variations were silent and without apparent effect on t-PA release.\(^{215}\) The relation of these polymorphisms with arterial disease is as yet unknown.
### Table 1. Haemostatic gene variations in arterial thrombotic disease

<table>
<thead>
<tr>
<th>Haemostatic system component</th>
<th>Gene symbol</th>
<th>Chromosome Localization</th>
<th>&quot;Candidate&quot; gene variation for arterial disease</th>
<th>Functional consequence</th>
<th>Association of the gene with arterial disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Platelets</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP Ia-IIa (α2 subunit)</td>
<td>ITGA2</td>
<td>5q23-31</td>
<td>α2.C807T</td>
<td>Determinant of integrin density and collagen receptor activity</td>
<td>Disputed</td>
</tr>
<tr>
<td>GP Ibb-V-IX (β3b subunit)</td>
<td>GP1BA</td>
<td>17p12-p12</td>
<td>C357UT (Thr145Met), 39-bp VNTR, T-5C (Kosak polymorphism)</td>
<td>Unclear</td>
<td>Disputed</td>
</tr>
<tr>
<td>GP IIb-IIIa (β3a subunit)</td>
<td>ITGB3</td>
<td>17q21-32</td>
<td>T1565C (Pro33Leu)</td>
<td>Unclear</td>
<td>Unconfirmed (single study)</td>
</tr>
<tr>
<td>GP IIb-IIIa (β1b subunit)</td>
<td>ITGA2B</td>
<td>17q21-32</td>
<td>Ile843Ser</td>
<td>Unclear</td>
<td>Disputed</td>
</tr>
<tr>
<td><strong>Procoagulant factors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrinogen (β-chain)</td>
<td>FGB</td>
<td>4q31</td>
<td>G-455A (Asp11 polymorphism), Bcll polymorphism (3'-UT region)</td>
<td>Determinant of plasma level</td>
<td>Disputed</td>
</tr>
<tr>
<td>Fibrinogen (α-chain)</td>
<td>FGA</td>
<td>4q31</td>
<td>Thr312Ala</td>
<td>Increased clot stability</td>
<td>Disputed</td>
</tr>
<tr>
<td>Factor II</td>
<td>F2</td>
<td>11p11-q11.2</td>
<td>G20210A (3'-UT region)</td>
<td>Determinant of plasma level</td>
<td>Disputed</td>
</tr>
<tr>
<td>Tissue factor</td>
<td>F3</td>
<td>1p22-21</td>
<td>1208 D/I promoter haplotypes</td>
<td>Determinant of plasma level</td>
<td>Unconfirmed (single study)</td>
</tr>
<tr>
<td>Factor V</td>
<td>F5</td>
<td>1p23</td>
<td>GI691A (Arg566Gln)</td>
<td>Activated protein C resistance</td>
<td>Disputed</td>
</tr>
<tr>
<td>Factor VII</td>
<td>F7</td>
<td>13q34</td>
<td>Avg353Gln</td>
<td>Determinant of plasma level</td>
<td>Disputed</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>F8C</td>
<td>Xq28</td>
<td>None</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>on Willebrand factor</td>
<td>WF</td>
<td>12p13</td>
<td>None</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Factor XIII (α-subunit)</td>
<td>F13A1</td>
<td>6p24-25</td>
<td>Val34Leu</td>
<td>Influences enzyme activity</td>
<td>Disputed</td>
</tr>
<tr>
<td><strong>Anticoagulant proteins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antithrombin</td>
<td>AT3</td>
<td>1q23-25</td>
<td>&gt; 250 loss-of-function mutations</td>
<td>AT deficiency</td>
<td>Unknown</td>
</tr>
<tr>
<td>Protein C</td>
<td>PC</td>
<td>2q13-14</td>
<td>&gt; 160 loss-of-function mutations</td>
<td>PC deficiency</td>
<td>Unknown</td>
</tr>
<tr>
<td>Protein S</td>
<td>PROS1</td>
<td>3p11.1-q11.2</td>
<td>&gt; 130 loss-of-function mutations</td>
<td>PS deficiency</td>
<td>Unknown</td>
</tr>
<tr>
<td>Thrombomodulin</td>
<td>THBD</td>
<td>20p11.2</td>
<td>Ala455Val, Ala257Thr, G-33A</td>
<td>Determinant of plasma soluble TM level</td>
<td>Disputed</td>
</tr>
<tr>
<td>Tissue factor pathway inhibitor</td>
<td>TFPI</td>
<td>2p31-32.1</td>
<td>Pro151Leu, Val264Met, T384C, exon 4, and C-33T intron 7 T-287C</td>
<td>Val264Met is a determinant of plasma level</td>
<td>Unconfirmed (single study)</td>
</tr>
<tr>
<td><strong>Endothelial protein C receptor</strong></td>
<td>EPCR</td>
<td></td>
<td>23-bp insertion in exon 3</td>
<td>Receptor with impaired function</td>
<td>Disputed</td>
</tr>
<tr>
<td><strong>Fibrinolytic proteins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasminogen activator inhibitor 1</td>
<td>PLANH1</td>
<td>7q22.1-22.3</td>
<td>-675 4G/5G (D/I polymorphism)</td>
<td>Determinant of plasma level</td>
<td>Disputed</td>
</tr>
<tr>
<td>Tissue-type plasminogen</td>
<td>PLAT</td>
<td>8p12-q11.2</td>
<td>Alu insertion/deletion</td>
<td>Unclear</td>
<td>Disputed</td>
</tr>
</tbody>
</table>
Concluding remarks

A comparative analysis of data on haemostatic gene polymorphisms in arterial thrombosis yields several important observations. It seems clear that levels of some haemostatic factors predict disease risk. A notable example is plasma fibrinogen level. However, the causal role of haemostatic plasma markers as predictors of arterial disease is still uncertain, because frequently inconsistent and conflicting results from the genetic studies. Uncertainty is further augmented by the fact that positive results were more often than not found in post-hoc subgroup analyses (Table 1). Given that the relationship between genetic determinants of the haemostatic system and arterial disease remains elusive, it is our opinion that there is no need for clotting factor analysis in the routine management of patients with atherothrombotic cardiovascular disease. Consequently the main challenge presented to researchers is to conduct sufficiently large and well designed investigations that will not only identify haemostatic variations playing a significant role as risk modifiers but also will decipher relevant synergistic effects between such genetic markers and the well established cardiovascular risk factors.

In conclusion, no convincing data exist for haemostatic gene variations playing a significant role in arterial thrombosis. Investigations in this area are particularly important because if the ultimate goal of characterising phenotypic and genetic markers for thrombosis was reached, it would enable one to establish individual profiles of thrombotic risk, and might eventually result in new and individualised prognostic and therapeutic measures.

Acknowledgements

R.F. Franco was supported by Fapesp (Grants N. 98/02821-0, 98/14247-6, and 00/02623-5). The continuing support of Prof. M.A. Zago is also gratefully acknowledged.

References


32. Carlsson LE, Santoso S, Spitzer C, et al. The \( \alpha_2 \) gene coding sequence T\(_{807}/A\(_{873}\) of the platelet collagen receptor integrin \( \alpha_2 \beta_1 \) might be a genetic risk factor for the development of stroke in younger patients. *Blood* 1999;93:3583-6.


