

Role of hemostatic gene polymorphisms in venous and arterial thrombotic disease

David A. Lane and Peter J. Grant

The development of thrombotic disorders in humans is one of the most common causes of morbidity and mortality in the Western world. Thrombotic disease can be classified in broad terms as that which occurs in the venous system of low flow and pressure or in the high-flow and pressure arterial system. Certain basic distinctions can be drawn between arterial and venous thrombosis, such as the composition of the thrombi (platelet rich in arterial and fibrin rich in venous) and the presence of vascular wall damage (atheroma) in arterial thrombosis. However, the distinctions are not absolute, and there are common underlying mechanisms. Perturbation of hemostasis is central to the pathogenesis of all thrombosis, even though it differs in nature depending on location. Transient or long-lasting environmental influences may play important roles in perturbing hemostasis and influencing risk for thrombosis in both the venous and the arterial systems. The term *environmental* is used in its broadest sense to embrace changes induced by diverse influences such as childbirth, hormone ingestion, surgery, diet, and smoking, by intercurrent disorders such as diabetes mellitus, hypertension and dyslipidemia, and hyperhomocystinemia, and by local changes to the vascular wall. Perturbation of hemostasis may also be in part genetically determined, and, when this is the case, its influence is potentially profound because of its life-long presence. Because of the late onset of the majority of thromboses, it is unlikely that genetic changes could be their *sole* determinant, and this points to the importance of gene–environment interactions in disease.

There is a rapidly growing literature on the relationship between the genetics of hemostatic factors, the environment, and thrombotic occlusion. Most of this literature concerns variation in the genes for blood coagulation factors, inhibitors, fibrinolytic factors, and platelet-membrane receptors. The aim of our review is to summarize this literature, focusing on the common genetic variations (a prevalence approximately 1/100 or greater is used as a rough, not an absolute, guide, and the term *polymorphism* is used regardless of clinical effect) that could potentially have important influence on the health of populations. Certain changes, such as partial or total gene deletions, tend to be of low prevalence and will not be considered. Furthermore, the specific mutations in certain genes predisposing to thrombosis are essentially “private” to a relatively small number of families (such as those in the genes for antithrombin, protein C, and protein S) and will not be considered. At its conclusion, an evaluation will be made of the overall importance of genetics in determining the course of these common disorders, and a perspective on gene–environment interactions will be presented.

Venous thromboembolic disease

Venous thromboembolic disorders form a spectrum of conditions characterized by in situ thrombus formation and the variable presence of embolic manifestations. The clinical presentation depends to a large extent on the site and extent of thrombus formation and on the underlying cause. In the absence of genetic deficiencies, thrombosis occurs in the older population, largely in the context of marked environmental influences such as surgery, obesity, and underlying malignancy. In contrast, at the other extreme, familial thrombosis associated with mutations in genes for protein S, protein C, or antithrombin is associated with younger age of onset, lesser environmental stimulus, and thrombus formation often in unusual sites. There is an increasing awareness of the importance of hemostatic mechanisms in the pathogenesis of venous thrombosis. The hemostatic process comprises coagulation and fibrinolytic systems with activators, zymogens, cofactors, surfaces (such as platelets), and inhibitors. The function and regulation of these composite systems and factors have been extensively studied. Understanding their functional integration in vivo, however, still presents a major challenge. A starting point for a simplified model of integration envisages a balance between procoagulant (clot-promoting) and anticoagulant (clot-preventing) mechanisms. This balance serves to prevent an explosive generation of thrombin when coagulation is triggered. The balance is determined by the (functional) levels of all coagulation and fibrinolytic factors, with increases in the levels of coagulation factors and decreases in the levels of both fibrinolytic factors and natural inhibitors being procoagulant. The role of inhibitory mechanisms in preventing venous occlusion was established after the identification of the link between inherited deficiencies of antithrombin, protein C, protein S, and venous thrombosis during the 1960s and the 1970s. The *general* importance of the anticoagulant pathway involving protein C and protein S was established by the widespread prevalence among white populations and the clinical consequences of the phenotype of activated protein C resistance (APCR) and its main causative gene mutation, factor V G1691A (factor V Arg506Gln, factor V Leiden). There has been slower appreciation of the procoagulant effect of increased levels of coagulation factors in venous disease, but recent work has highlighted the likely importance of prothrombin and factor VIII levels in this regard.

From the Department of Hematology, Imperial College School of Medicine, London, and the Academic Unit of Molecular Vascular Medicine, University of Leeds, Leeds, United Kingdom.

Submitted September 20, 1999; accepted December 16, 1999.

Supported by grants from the British Heart Foundation and the Stroke Association.

Reprints: David A. Lane, Department of Hematology, Imperial College School

of Medicine, Charing Cross Campus, Hammersmith, London W6 8RP, United Kingdom; e-mail: d.lane@ic.ac.uk.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked “advertisement” in accordance with 18 U.S.C. section 1734.

© 2000 by The American Society of Hematology

Genetic polymorphisms and venous thrombosis

Factor V

After the identification of inherited deficiencies of coagulation inhibitors, antithrombin,¹ protein C,² and protein S³ and of the associated high prevalence of thrombosis (thrombophilia), venous thrombosis was commonly regarded as a single gene disorder. This view was conditioned largely by results of studies with small numbers of patients/families with an unusually high prevalence or severity of thrombosis. The recognition of APCR/factor V G1691A, as a prevalent risk factor for venous thrombosis in the general population,⁴⁻⁷ resulted in a clearer view of the episodic nature of thrombosis and of the importance of other genetic and acquired risk factors in the onset of thrombosis. A revised view of genetic predisposition toward venous thrombosis suggests that single gene defects confer increased risks that do not necessarily lead to thrombosis without interaction with other genetic or environmental risk factors.⁸⁻¹⁰ Venous thrombosis is now therefore widely considered a multicausal disorder. The main features distinguishing factor V G1691A from mutations in the genes for antithrombin, protein C, and protein S are the reduced risk associated with its inheritance¹¹ and its high prevalence in white populations.

Factor V is a 300-kd multidomain glycoprotein, the gene of which has 25 exons. The relationship between factor V G1691A and the intermediate phenotype, APCR, is broadly established.¹² The variant factor V Gln506 is activated by thrombin but is less readily inactivated by APC than factor V Arg506. There are 2 primary cleavage inactivation sites, Arg506 and Arg306. Several reports have investigated the kinetics of cleavage inactivation of factor Va. One group found that initial cleavage is at Arg506 and that this promotes more rapid cleavage of Arg306, which is the main inactivation step.¹³ Another group found that cleavage is random at the 2 sites but is much slower at Arg306.¹⁴ Protein S, the cofactor to APC, accelerates cleavage of Arg306.¹⁵ APC inactivation of factor Va Gln506 is delayed. According to the first view, this is because there is no enhanced cleavage at Arg306 by prior cleavage at Arg506, whereas the alternative view is that only the slow rate of cleavage at Arg306 can occur. An additional effect on phenotype is suggested by the finding that factor V functions as an anticoagulant because it is a cofactor in the degradation of factor VIIIa by APC (and protein S).¹⁶ This anticoagulant cofactor function requires cleavage of intact (as opposed to activated) factor V by APC; the C-terminal region of the factor V B domain is necessary for this, and cleavage at Arg506 appears critical.¹⁷ Because factor V Gln506 cannot be cleaved at this position, it cannot act effectively as a cofactor in factor VIIIa degradation. The relative importance of the delayed cleavage of factor Va Gln506 by APC and of the impaired anticoagulant cofactor function of factor V Gln506 in contributing to reduced endogenous anticoagulation in vivo has yet to be defined.

Regardless of the precise pattern of cleavage by which the factor V Arg506Gln substitution causes APCR or which property of the mutant factor V/Va is most important, its inheritance increases the risk for venous thrombosis. This was shown initially using the Leiden Thrombophilia Study, a large population-based case-control study of venous thrombosis,¹⁸ and then in the large prospective US Physicians Health Study¹⁹ and in numerous other reports. There is a high frequency of factor V G1691A in white persons (approximately 5%), and the polymorphism arose from a common source

20,000 to 30,000 years ago (founder effect).²⁰ The high prevalence, coupled with its moderate to high (approximately 8-fold) risk, has enabled its interactions with other relatively uncommon genetic risk factors (in protein C, protein S, and antithrombin)²¹⁻²³ and with environmental factors (such as oral contraceptives)^{24,25} to be studied.

The thrombotic risk of factor V G1691A/APCR is not confined to the deep veins of legs. Cerebral vein thrombosis is more common in carriers.²⁶ However, factor V G1691A/APCR does not appear to be a major risk factor for all venous thromboembolic events, implying that regional factors can dominate risk. For example, hip and knee replacements are associated with a high risk for postoperative venous thrombosis. However, in a large (825-patient) retrospective study, there was no excess thrombosis in carriers of the genotype (31% [confidence interval, CI; 15% to 47%] in carriers; approximately 26% [CI, 22% to 29%] in noncarriers).²⁷ There is evidence that patients with isolated pulmonary embolism do not have a greatly increased risk conferred by factor V G1691A/APCR.²⁸ These studies suggest that certain types or magnitudes of stimuli toward thrombosis, provided by some surgical procedures or by certain coexisting illnesses, might be so dominant that factor V G1691A/APCR provides only a minor contribution to overall risk.

Current outstanding issues concerning the factor V G1691A polymorphism relate to the management of clinically affected and unaffected carriers of factor V G1691A. It is unclear whether factor V G1691A increases the risk for recurrence after a first thrombosis. If this is the case, it might suggest that treatment of the initial event should be more intense in carriers. This is an important issue because of the known increased risk for severe bleeding caused by prolongation of oral anticoagulant treatment. Conflicting results on the frequency of recurrence have been reported²⁹⁻³³ because of either the small number of patients studied or the short period of follow-up. Well-designed studies on the prevalence of recurrence and on optimum duration of anticoagulation are now needed. Additionally, there are no studies that give clear guidance on the optimum management of clinically unaffected carriers.

Some patients have APCR but not factor V 1691A. The frequency of this combined genotype/phenotype has not often been reported and is therefore uncertain. There have, however, been 2 reports of patients with nucleotide changes resulting in alternative amino acid substitutions at factor V Arg306 (to Thr and to Gln).^{34,35} These mutations appear to be private rather than of polymorphic frequency. The population frequency of APCR in the absence of factor V G1691A will depend on the specificity of the functional APCR assay for factor V G1691A. A recent report using the Leiden Thrombophilia Study and an APCR assay nonspecific for the gene change to analyze samples demonstrated that APCR in the absence of factor V G1691A confers a definite risk for thrombosis.³⁶ The carriers of factor V G1691A were excluded from analysis, and normalized APC sensitivity ratios were determined in 337 patients with venous thrombosis and in 455 controls. A dose-response relationship was observed between APCR and risk for thrombosis, with a 4-fold difference between highest and the lowest quartiles of APCR. Elevated factor VIII levels are known to influence APCR, but the relationship remained after adjustment was made for this.

Another approach to elucidate alternative causes of APCR considered the influence of the genetic background of factor V on APCR. A haplotype termed HR2 was defined by 5 restriction enzyme-cutting sites in exon 13 and a single sequence variation in exon 16.³⁷ The presence of this haplotype was found to be

increased in patients heterozygous for factor V G1691A who had severe APCR, and it was increased in noncarriers of factor V Arg506Gln with APCR. One study has suggested that the HR2 haplotype is a mild risk factor for thrombosis.³⁸ In this case-control study of 205 patients with thrombosis and 394 matched controls, the odds ratio (OR) for increased risk associated with the haplotype was 1.8 (CI, 1.1 to 2.8). In a large, retrospective, multicenter cohort study, the combination of the HR2 haplotype with other genetically determined risk factors was assessed.³⁹ Combination of the HR2 haplotype with antithrombin, protein C, and protein S deficiencies did not increase the calculated risk for thrombosis associated with the latter group. However, when persons with factor V G1691A also carried the HR2 haplotype, there was an increase in the risk for thrombosis; compare relative risk (RR) 4.2 (CI, 1.6 to 11.3) for factor V G1691A with RR 10.9 (CI, 2.9 to 40.6) for both. The authors of the latter report add a caution concerning the limited number of thrombotic events in their study. The polymorphic sites within the haplotype do not explain why the haplotype should alter APCR. There are 2 amino acid substitutions coded by the haplotype, 1299His/Arg and 1736Met/Val, but these appear to be neutral. It has been suggested recently in abstract form⁴⁰ that the functional basis for the effects of different haplotypes could arise from an altered distribution of the normal functional isoforms of factor V and may involve differences in glycosylation. The prevalence of this haplotype in Italian, Indian, and Somalian populations has been estimated to be 8% to 10%, suggesting a common ancestral origin.

Prothrombin

There was little in the literature relating prothrombin, a 72-kd zymogen, and venous thrombosis before 1996. A common polymorphism that increases the risk for venous thrombosis was then identified. This is a substitution in the 3'-untranslated region of prothrombin at nucleotide 20 210, G to A,⁴¹ identified by sequencing the coding, 5' and 3'UT regions of the gene in 28 selected patients with familial history of thrombosis. Eighteen percent of these patients had the 20 210A allele compared to 1% of healthy controls. The increased risk for venous thrombosis conferred by the polymorphism was determined in the Leiden Thrombophilia Study. Of the patients, 6.2% had the A allele compared to 2.3% of controls, giving a relative risk for thrombosis of 2.8. The prothrombin 20 210A allele is associated with elevated prothrombin levels, and this appears to be responsible for the increased risk for thrombosis. The precise mechanism by which prothrombin levels are altered has yet to be determined. Increased efficiency of polyadenylation of mRNA transcripts has been suggested. Results of several studies and an overall summary investigation indicate that the prevalence in the white population is 2% (CI, 1.4% to 2.6%)^{42,43} and confirm that the A allele is a risk factor for venous thrombosis, including cerebral vein thrombosis.⁴⁴ The reported variation in population prevalence (0.7 to 4.0) is caused both by the small populations studied and by variations within populations: southern European populations have a higher prevalence of the A allele than northern European populations.⁴³ A perplexing finding was a weak (RR, 1.7; CI, 0.9 to 3.2), nonsignificant association of the A allele with venous thrombosis in the US Physicians Health Study, despite a high population prevalence (3.9%).⁴⁵ Haplotype analysis has shown that this polymorphism also arose from a founder 20 000 to 30 000 years ago.⁴⁶ There is as yet little information on the risk for recurrent thromboembolism in carriers of the prothrombin 20 210A allele. In a single prospective study, 492 patients with (42 patients)

and without (450 patients) the allele were investigated for 24 months.⁴⁷ The probability of recurrence was 8% (CI, 0 to 16.7) and 12.2% (CI, 8.8 to 15.6), respectively, suggesting that long-term treatment with oral anticoagulants is not justified. Although the polymorphism may confer weak risk for thrombosis when an isolated defect, its effect may be more important when in combination with other genetic risk factors. Thus, in a retrospective study of 112 patients with factor V G1691A compared to 17 patients heterozygous for the factor V gene change and for prothrombin 20 210A, the risk for recurrence of thrombosis after a first event was greater (OR, 2.6; CI, 1.3 to 5.1) in those patients with both risk alleles.³³ This finding suggests the need for more prolonged anticoagulation in carriers of both risk alleles after a first thrombotic event.

Emerging candidate genes

Factor XIII

Factor XIII is a transamidase comprised of 2 A and 2 B subunits in a tetrameric structure (A₂B₂) of 320 kd.⁴⁸ The role of activated factor XIII lies mainly in accelerating the formation of shear- and fibrinolysis-resistant cross-linked fibrin; a deficiency of factor XIII is associated with severe bleeding.⁴⁸ Thrombin cleaves a 37-amino acid peptide from the A subunit. The gene for the A subunit codes for a polypeptide of 75 kd.⁴⁹ In excess of 20 mutations have been described in the A subunit gene that relate to factor XIII deficiency by leading to absence of the A subunit.⁵⁰⁻⁵⁵ In addition 4 common coding polymorphic sites have been described at Val34Leu, Pro564Leu, Val650Ile, and Glu651Gln.^{51,54} The polymorphism coding, G/T, for factor XIII Val34Leu produces a coding change in the A subunit only 3 amino acids from the thrombin cleavage activation site at position Arg37-Gly38.

The Val34Leu polymorphism has been associated with a protective effect against venous thrombosis in a case-control study of 221 patients and 254 healthy controls.⁵⁶ Patients with thrombosis had an increased frequency of the Val/Val genotype (63% versus 49%) and a lower frequency of the Val/Leu genotype (31% versus 42%) than controls. In a logistic regression model, the factor XIII genotype was shown to be independent of factor V G1691A as a predictor of thrombosis. A second report⁵⁷ of 189 patients and 187 controls found no association of the heterozygous Val34Leu change with thrombosis, but the homozygous Leu34 was strongly associated with a protective effect (OR, 0.16; CI, 0.05 to 0.5). The proposed protective Leu allele appears to be associated with increased activation of factor XIII⁵⁸ and enhanced fibrin cross-linking in *in vitro* assay systems.^{50,59} This finding of enhanced activation, coupled to an apparent protective effect against venous thrombosis, is intriguing and is the subject of ongoing investigation.

Endothelial cell protein C/activated protein C receptor

Endothelial cell protein C/activated protein C receptor (EPCR) is a 43-kd endothelial cell membrane type I intercalated receptor for protein C.^{60,61} There is a body of evidence accumulating that suggests that this receptor is an important component of the protein C anticoagulant pathway.^{62,63} It is found predominantly on large vessels and serves to ensure high local concentration of protein C for activation by the thrombin-thrombomodulin complex.⁶⁴ The gene for the human EPCR contains 4 exons and has a number of potential polymorphic sites.⁶⁵ An early report (abstract) has identified a 23-bp insertion in exon 3, which duplicates the preceding 23

bases and results in a STOP 6 codons downstream from the insertion point.⁶⁶ The clinical significance of the insertion was examined in 149 patients with venous thrombosis and 404 controls. A crude OR for this mutation was 4.6 (CI, 1.1 to 19.7), suggesting that gene mutation of EPCR may predispose to venous thrombosis.

Thrombomodulin

Intuitively, it might be expected that thrombomodulin gene variation could predispose to thrombosis, given the overall importance of the protein C anticoagulant pathway in maintaining blood fluidity. Thrombomodulin is an endothelial cell membrane intercalated proteoglycan of approximately 100-kd. It functions as a thrombin receptor and transforms the specificity of bound thrombin so that it loses its procoagulant functions and acquires a greatly increased ability to activate protein C. Several coding sequence changes have been identified in families by screening for mutations in the thrombomodulin gene.⁶⁷⁻⁶⁹ However, there is still limited information on the effects on function of these genetic changes, and the families have been too small to provide decisive information on the relationship with thrombosis. The only common polymorphism identified, C/T coding for Ala455Val,⁷⁰ appears not to be associated with thrombosis. It appears that any gene changes altering the function or expression of thrombomodulin are probably private mutations.

Factor VIII(-related)

Increased factor VIII levels have been shown to be associated with thrombosis in several studies. In the Leiden Thrombophilia Study, factor VIII elevated more than 150 IU/dL, measured as factor VIII coagulant activity, was shown to have an associated relative risk of 4.8 (CI, 2.3 to 10).⁷¹ Increased risk for elevated factor VIII has been confirmed by a study of patients referred for unexplained thrombosis who were largely free of markers of inflammation.⁷² To date, no common genetic variation in the factor VIII gene has been identified that might account for this phenotypic variation.⁷³ However, there is good evidence that factor VIII levels may be genetically determined. Von Willebrand factor (vWF) and blood group are well-known determinants of the factor VIII level in plasma, and factor VIII levels have been shown to cluster in families.⁷⁴

Genes other than those of hemostasis

Among the range of factors that can potentially interact with hemostatic genetic determinants, the blood level of homocysteine is of interest in the current context. Homocysteine is a sulfhydryl amino acid arising from the metabolism of methionine. A high level of homocysteine, hyperhomocystinemia, has attracted much recent interest as a potential risk factor for both venous and arterial thrombotic disease, not least because dietary supplementation with folic acid affords a safe and inexpensive therapeutic option. One of the 2 enzymatic pathways of remethylation of homocysteine to methionine involves methylene tetrahydrofolate reductase (MTHFR). The gene for MTHFR contains a common polymorphism, C677T, and this can potentially interact with hemostatic gene polymorphisms. Inconsistent results have been obtained in the numerous published studies, reviewed comprehensively elsewhere.⁷⁵ Hyperhomocystinemia appears to be only a weak risk factor for venous thrombosis,^{76,77} and this may account for the variable outcomes both when the C677T polymorphism has been studied as a sole risk factor and when in combination with hemostatic genetic risk factors.⁷⁵

Arterial occlusive disease

Cardiovascular disease is a generalized disorder of the vascular tree characterized by long-term atheromatous plaque formation and culminating in atherothrombotic obstructive lesions that lead to tissue damage. In many subjects there are coexistent atheromatous lesions in the coronary, carotid, aorto-iliac, and femoropopliteal arteries that can present as various syndromes according to the vessel that is predominantly affected by the process. Angina and acute myocardial infarction (MI) are the clinical manifestations of the chronic development of coronary artery atheroma, with the final pathologic process of plaque rupture and coronary thrombosis. The underlying processes that lead to atheroma formation and coronary thrombosis are complex and involve multiple interrelated systems that regulate vasoactivity, adhesion molecules and their ligands, lipid metabolism, and the coagulation and fibrinolytic pathways. A body of evidence implicates underlying insulin resistance with associated clustering of cardiovascular risk in subjects with and without diabetes as 1 of the common factors linking some of these processes.⁷⁸⁻⁸⁰ The mechanisms involved are multifactorial, and effects of insulin/insulin resistance on nitric oxide production, lipid metabolism, hemostasis, and blood flow are implicated in subjects with diabetes and a proportion of subjects without diabetes.

Although environmental influences arising from a more sedentary and overweight population may account in part for the rise in MI seen in Western societies in the past century, genetic background can also be expected to play a role in influencing disease. An increasing number of studies, many of which are cited below, have been focused on young survivors of MI, and some of these have addressed the possibility of interaction between hemostatic polymorphisms and acquired influences such as smoking, diet, and metabolic changes. The rationale for use of such highly selected patients is that the burden of atherosclerosis in them can be expected to be less. Consequently, dominant pathogenic mechanisms for MI in these patients may more likely involve perturbed hemostatic balance.

Support for the concept of disordered hemostatic balance in MI comes from major prospective and case-control studies of vascular risk that report that elevated concentrations of hemostatic proteins, such as fibrinogen, factor VII, vWF, PAI-1, and tPA relate to vascular risk and cardiovascular outcome.⁸¹⁻⁸⁵ These are clearly different risk factors than those associated with venous thrombosis. Obstruction of a coronary artery involves a thrombus the size of a small pea, whereas there may be a large mass of clot in an obstructed vein. Part of the reason for these differences must lie in the nature of disease in the respective vascular beds. Arterial disease occurs in the context of a high-flow, high-pressure system in which atheroma formation dominates and plaque rupture with exposure of tissue factor probably provides the stimulus for the initiation of clot formation. In a current model, plaque rupture, endothelial cell damage, or both, leads to exposure of the subendothelial layer that facilitates the binding of vWF. This leads to binding of vWF to the glycoprotein (Gp) Ib/IX platelet receptor and platelet adhesion. Alternatively, platelets may directly bind to collagen through their collagen receptors. These reactions lead to a conformational change in the platelet GpIIb/IIIa integrin that facilitates shear rate-dependent binding of both vWF and fibrinogen and platelet aggregation.

Coagulation gene polymorphisms

Fibrinogen

Relationship between plasma levels and disease, influence of polymorphisms on plasma levels. Of all the components of the coagulation system, elevated fibrinogen has been most consistently associated with occlusive vascular disorders. Investigations such as the Northwick Park Heart Study and the Gothenburg and PROCAM studies have prospectively related fibrinogen to MI and stroke outcomes.^{81,82,86} Evidence from the Scottish Heart Health Study indicates that increased fibrinogen also clusters with other cardiovascular risk markers, including hypertension, diabetes, smoking, and peripheral vascular disease.⁸⁷ Fibrinogen provides a link between smoking and the development of arterial disease because of the influence of smoking on fibrinogen levels.⁸⁸

Fibrinogen is a 340-kd glycoprotein consisting of 3 nonidentical polypeptides (α , β , and γ) linked by disulfide bonds. The 3 polypeptides are encoded by 3 fibrinogen genes clustered on the long arm of chromosome 4.⁸⁹ The *cis* and *trans* acting factors that regulate fibrinogen transcription have been extensively studied. The 3 genes contain promoter regions with TATA and CAAT boxes and a number of sequence elements that confer tissue (liver)-specific and enhanced expression.⁸⁹ These include hepatic nuclear factor 1 (HNF 1; 5' to the α and β genes) and IL-6-responsive elements (5' to all genes) (Figure 1).^{90,91} Fibrinogen levels are subject to much biologic variation, with elevated circulating levels arising particularly as a consequence of the acute-phase response. The IL-6 responsive elements are thought to be important for mediating this effect. The acute-phase response arising from viral infection or from smoking is strongly implicated in the development of cardiovascular disease.

Humphries et al^{92,93} were the first to investigate the potential role of genetics in the regulation of fibrinogen levels. Multiple polymorphic sites have since been identified in the 3 genes, but, apart from some more recent interest in the α chain, most studies have focused on β chain polymorphisms. This is because of the suggestion arising from *in vitro* pulse chase studies in Hep G2 cells incubated with L-[³⁵S] methionine that β chain synthesis is rate limiting in the production of mature fibrinogen.⁹⁴ However, no evidence is available to suggest that this might be the case *in vivo*. Studies of the heritability of fibrinogen support a role of genetics, with genetic contributions to variance of 51% reported by Hamsten et al,⁹⁵ 48% in young subjects from the Kibbutzim study,⁹⁶ and 65% in 191 female twin pairs (unpublished data). The 2 most studied polymorphisms are those detected by the *BclI* restriction enzyme (*BclI* polymorphism, located in the 3' region of the β chain) and by the *HaeIII* restriction enzyme, the -455 G/A polymorphism (in the 5' promoter region of the β chain). Of these, the latter (which is in linkage disequilibrium with the former) has been favored by many investigators because of its proximity to the IL-6-responsive element and the HNF 1 element. Humphries et al^{92,93} reported that 9% and 5% of fibrinogen variability could be explained by the β chain polymorphisms detected by *BclI* and *HaeIII*, respectively, whereas 4.2% was determined by an α chain fibrinogen gene polymorphism detected using *TaqI*. A mainly consistent finding of large studies of cardiovascular disease has been confirmation that levels of fibrinogen are associated with these genetic variations. In the ECTIM study⁹⁷ of 565 patients and 668 controls, 10 polymorphisms of the β chain were found to be in linkage disequilibrium. In multivariate analysis, the -455 G/A was found to be indepen-

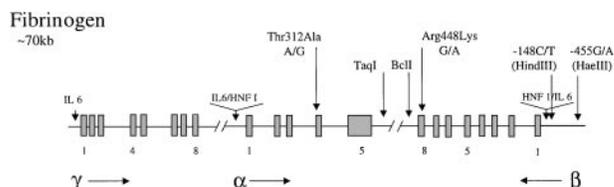


Figure 1. Representation of the organization of the fibrinogen gene locus. The 3 genes for the polypeptides are represented along with the direction in which they are transcribed (horizontal arrows). Exons (blocks) are occasionally numbered. Common polymorphisms discussed in the text are indicated by vertical arrows, as are tissue-specific (HNF 1) and enhancer (IL-6) elements.

dently associated with plasma levels ($P < .0003$). This association was only found, though, in smokers. In the study termed EARS, 585 offspring of fathers with MI and 1106 control were studied.⁹⁸ The -455 A allele was strongly associated ($P < .001$) with elevated levels in males, but the association was also strongly influenced by gender and smoking. The Copenhagen City Heart Study has a large ($n = 9127$) general population sample.⁹⁹ The -455 A allele was associated with fibrinogen level in both genders, and an increase of 1 SD in circulating fibrinogen was associated with a 20% increase in OR for ischemic heart disease. In a study of 923 patients undergoing coronary angiography, this polymorphism was associated with fibrinogen levels ($P < .0002$), and an association between genotype and elevated levels after bypass surgery in 207 patients was also observed.¹⁰⁰ Finally, in 885 male patients with coronary artery disease selected from the study REGRESS, there was a significant ($P < .05$) association between baseline fibrinogen and genotype.¹⁰¹

Polymorphisms and disease. Although there is compelling evidence of associations between fibrinogen level and arterial disease and between fibrinogen level and certain polymorphisms, the relationship between fibrinogen polymorphisms and disease is altogether less clear. Certain studies, some large, suggest an association. In the ECTIM study, a number of associations between fibrinogen and severity of atheroma, assessed by coronary angiography, were documented.⁹⁷ The rarer allele of the 4 β chain polymorphisms exhibiting associations with fibrinogen levels (*BclI*, C448, -455 , and -1420) was significantly more common in subjects with more severe coronary artery disease. No association was found, however, between genotype and MI. Positive findings were reported in a small study of 187 subjects with type 2 diabetes, of whom those with coronary artery disease had a higher prevalence of the G allele of the -455 G/A polymorphism.¹⁰² The -455 G/A polymorphism has also been positively related to the progression of atheroma, though in this case it was the A allele that was associated with deleterious effects.¹⁰¹ The *BclI* polymorphism has been related to MI in a cohort of patients from the GISSI-2 study.¹⁰³ This study was small (102 patients, 173 controls) and unusual because its participants were selected from patients with 1 or more first-degree relatives who had MI or stroke before 65 years of age. There was a significant difference in allele frequency in patients than in controls ($P = .002$). In multivariate analysis, genotype was associated with risk of MI (OR, 2.4; CI, 1.2 to 4.6). The Austrian Stroke Prevention Study examined 399 invited "normal" subjects for associations between the β chain -148 C/T polymorphism, fibrinogen levels, and carotid atherosclerosis.¹⁰⁴ The T/T group ($n = 25$) demonstrated higher grades of atherosclerosis than carriers of the C allele ($P = .003$). The T/T genotype was a significant (OR, 6.17; CI, 1.70 to 22.3) predictor of disease in a multivariate model. Oddly, the fibrinogen level itself was a much weaker

predictor of disease, and there was no association between genotype and fibrinogen level. Carter et al¹⁰⁵ reported a strong association between β 448 genotype and cerebrovascular disease in 149 female patients compared with healthy controls. The β promoter polymorphism G/A -455 has been related to stroke in Japanese subjects, and the C/T 148 has been related to carotid atheroma.¹⁰⁶

In contrast to these positive associations between genotype and disease, 2 very large studies showing the association between genotype and fibrinogen level failed to establish an association between genotype and disease. The Copenhagen City Heart Study⁹⁹ found an association between fibrinogen level and ischemic heart disease, with an increase in 1 SD in level increasing the OR for disease by approximately 20% ($P < .01$ for females, $P < .005$ for males). However, though the -455A allele was associated with fibrinogen levels in both genders (see above), its frequency was not influenced by disease. Either the increase (approximately 4% to 10% of the level associated with AA genotype) is secondary to other more critical influences or it is simply insufficient to influence the course of disease. Similar negative associations were observed in 2 other large studies.^{100,107} Despite the association between genotype and level, noted above in the study by Gardemann et al,¹⁰⁰ no association was found between -455G/A polymorphism and either coronary artery disease or MI. In the study by Wang et al,¹⁰⁷ though fibrinogen was related to the onset and severity of coronary disease, there was no relationship between the polymorphism and disease.

A polymorphism in the coding region of the α chain (Thr312Ala) has been described¹⁰⁸ that is of some interest because of its proximity to the factor XIII cross-linking site at position 328.¹⁰⁹ Additionally, it lies within the region of the fibrinogen α chain that is involved in promoting the dissociation of the factor XIII A and B subunits and that enhances factor XIII activation.¹¹⁰ It has been reported that the substitution polymorphism at α Thr312Ala is associated with more rigid and less porous fibrin gel structures,¹⁰⁹ suggesting this polymorphism as a potentially important candidate for involvement in thrombotic disorders. However, in a recent study by ECTIM of 585 patients with MI and 658 controls, there was no difference in the frequency of the genotypes.¹¹¹ In a study of mortality rates after stroke, though, patients with the Ala312 coding allele had a dose-related significant reduction in survival than subjects homozygous for the Thr312 allele.¹¹² This study also describes an increased frequency of the Ala312 allele in patients with pulmonary embolus compared to those with DVT alone or with normal controls. These findings do lend some support for the hypothesis that the Ala312 allele influences clot stability, though further clinical and in vitro studies are required to clarify this issue.

The most striking point about these association studies is their inconsistency in relating genotype, fibrinogen level, and arterial disease. In the study¹⁰³ in which there are universally clear associations of *BcI* polymorphism and MI (GISSI-2), the unambiguous associations almost certainly arose because of the unusual selection criteria (familial cardiovascular disease) for the small number of patients (also see below).

Factor VII

Relationship between plasma levels and disease, influence of polymorphisms on plasma levels. Interest in the relationship between factor VII and cardiovascular disease was stimulated by the finding from the Northwick Park Heart Study that elevated levels were related to fatal but not to nonfatal MI. In this prospective study of white males ($n = 1511$) aged 40 to 64 years at

the time of recruitment, 109 developed a first major coronary artery disease event. Factor VIIC (ie, factor VII determined by clotting assay using factor VII-deficient plasma) was found to be strongly associated with coronary risk (117% compared with 107%; $P < .001$) with a 1 SD increase in factor VII associated with a 62% increase in risk over the first 5 years of the study.⁸¹ These results are often reported to have been supported by a second prospective study, PROCAM.^{86,113} In PROCAM, 130 coronary heart disease events occurred in 8 years of follow-up. However, in this study the results for factor VII were not decisive. Factor VIIC was elevated in patients who had coronary events (112% compared to 109%; $P < .023$), but in multiple logistic regression analysis, factor VIIC was not an independent risk factor for coronary events. A third prospective study, the Edinburgh Artery Study, also failed to confirm factor VII as an independent predictor of coronary disease.¹¹⁴ Interestingly, in the ECTIM study, the controls had uniformly higher levels of factor VII than the patients, which tended to militate against its major role as a cardiovascular risk marker.¹¹⁵ A number of other cross-sectional studies have provided conflicting conclusions about the importance of factor VII.¹¹⁶⁻¹¹⁸ A particular complication with factor VII is the methodology of its assay. The clotting assay, factor VIIC, reflects the total factor VII antigen, but it can also be influenced by the amount of factor VII that is activated (factor VIIa) in the plasma. The latter can be measured directly with an assay that utilizes truncated tissue factor as stimulant.¹¹⁹ It has been suggested that the factor VIIC assay used in the Northwick Park Heart Study is particularly sensitive to factor VIIa. There is, however, no good evidence that factor VIIa is an informative marker with respect to the prediction of cardiovascular disease.¹²⁰

Factor VII is an inactive vitamin K-dependent zymogen synthesized in the liver and secreted as a single-chain glycoprotein of 48-kd. On contact with tissue factor, it is converted to the 2-chain active form by selective proteolysis by factor Xa, and the factor VIIa/tissue factor complex then activates factor X and factor IX. The gene coding for factor VII has 5 identified polymorphic sites that may associate with circulating levels of the gene product (Figure 2), accounting for up to 30% of the variance in factor VII levels in plasma.¹²¹⁻¹²⁵ In the promoter, a decanucleotide insertion at position -323 is in strong linkage disequilibrium with a single base substitution (A/G) at codon 353 (Arg/Gln) in exon 8. Two additional promoter polymorphisms arise at -401 (G to T) and -402 (G to A). A further common polymorphism has been described in hypervariable region 4 of intron 7, HVR4, with 3 different length alleles termed H5, H6, and H7. The relative influence of each polymorphic site on factor VII levels has yet to be fully resolved. The Arg353Gln site was noted initially to associate with a 20% to 30% variance in factor VII levels in males and females and in different ethnic groups.¹²⁶ Many subsequent studies have confirmed that carriers of the allele coding for Gln353 have lower factor VII levels. It has been demonstrated recently¹²⁷ that factor VII Gln353 is secreted with lower efficiency in vitro. It can be concluded that the Arg/Gln substitution is responsible for significant variation in level, with factor VII Gln353 approximately 20% lower. An association of triglyceride with factor VII level has been reported in several studies.^{128,129} A study of 215 pairs of twins has shown that genetic influences account for 57% of variation in factor VII levels and that there is a significant genetic correlation between factor VII and triglycerides.¹²⁹ The association between factor VII and triglycerides has been attributed to the presence of the factor VII Arg353.¹²⁸ In the Rotterdam Study, a large population-

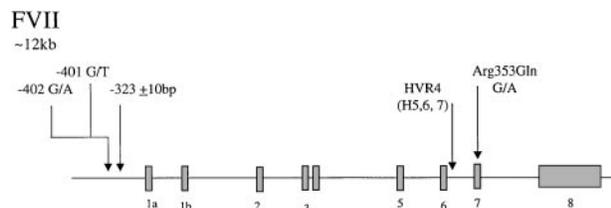


Figure 2. Representation of the organization of the factor VII gene, together with the location of common polymorphisms discussed in the text.

based study, an association of triglycerides with factor VII Arg353 was observed only in females.¹³⁰ Returning to the promoter polymorphisms, the effects of the -401 and -402 polymorphisms have been studied using reporter gene analysis and in relation to the levels of factor VII in Swedish males.¹²⁵ Both polymorphisms strongly influence the binding properties of nuclear proteins and alter transcriptional activity in vitro. The rare $-401T$ allele is associated with reduced transcription and reduced plasma factor VII levels. The rare $-402A$ allele, in contrast, confers increased transcriptional activity and is associated with elevated levels in plasma. Together, they are associated with up to 30% variation in the factor VII/factor VIIa levels.

Polymorphisms and disease. The relationship of certain of these polymorphisms to disease is highly controversial. In a study of a cohort of 453 patients with MI and 476 controls from the ECTIM study, the Arg353Gln polymorphism was found to relate strongly to levels of factor VII in cases and controls.¹¹⁵ There was, however, no difference in genotype frequencies between these 2 large groups. Similar results were obtained in a case-control study of 270 patients investigated for chest pain by coronary angiography.¹¹⁷ A case-control study in Sweden of 94 men with MI before the age of 45 years was reported by Moor et al.¹²⁰ Using data from this study, Doggen et al.¹³¹ calculated that there was a nonsignificant trend for an association of Arg353 with MI (OR, 1.81; CI, 0.79 to 4.13). The largest published case-control study, SMILE, in the Netherlands, included 560 patients and 644 controls.¹³¹ It found the expected association of factor VII levels with Arg353Gln in the control samples. However, Arg353 was associated with a reduced, rather than an increased, risk for MI (OR, 0.80; CI, 0.60 to 1.06), which was more pronounced in patients younger than 50 (OR, 0.49; CI, 0.28 to 0.84). Conclusions of this large study are that a genetic propensity to high factor VII levels is not associated with a risk for MI and that an elevated factor VII level itself is not a causal determinant. In contrast to these studies with largely negative results, a study of 165 Italian subjects with familial MI and 225 controls showed that there are higher risks associated with the common coding allele for both the Arg353Gln polymorphism and the hypervariable region in intron 7.¹³² The protection from MI associated with the homozygous Gln allele was large (OR, 0.08; CI, 0.01 to 0.9). It is interesting to note again (see "Fibrinogen") that this small study derived from the GISSI 2 population provided strong associations between genotype and disease, whereas larger studies failed. Why should the same mutation be highly associated with MI in Italy but be apparently protective of disease in a study that was 3 times larger in the Netherlands? The issue of the unique manner of selection of patients in the Italian study must be considered as a possible influencer of outcome. Because these patients all had familial arterial disease, it is highly likely that there are associated important genetic factors that strengthened any associated risk. Similar overestimates of strengths of associated

risks by family-based studies have been noted in venous thrombotic disease.¹³³

There has been single study of factor VII genotype and levels in relation to atherothrombotic stroke in 317 patients, confirmed by computed tomography.¹³⁴ The results again demonstrate the consistent finding of a strong relationship between genotype and levels of factor VII, but there is no indication that either is related to disease.

Emerging candidate genes

Factor XIII

Relatively little information is available relating changes in factor XIII level or activity to human arterial disease. Two animal studies have provided evidence for a role for circulating factor XIII in the pathogenesis of both MI and thromboembolic disease.^{135,136} In a canine electrically induced MI model treated with a factor XIII inhibitor, fibrinolytic therapy led to increased clot lysis compared to animals not pretreated with the inhibitor.¹³⁵ In a ferret model of pulmonary embolus, factor XIII cross-linking is associated with increased resistance to exogenous tPA therapy.¹³⁶ Both studies conclude that activated factor XIII has an important role in fibrinolysis-resistant clot formation and may affect outcomes in these 2 pathologically distinct syndromes that have fibrin formation as a common feature.

Two published case-control studies report associations between factor XIII Val34Leu and a history of MI. In a study of subjects with angiographically proven coronary artery disease and a history of MI determined by World Health Organization criteria, factor XIII Leu34 was reported to be significantly less common in those with a history of MI.¹³⁷ In those subjects with Leu34 who had had a previous MI, there were higher levels of the fibrinolytic inhibitor PAI-1, the PAI-1 4G/4G genotype was more common, and there was evidence of interactions with other clotting factors and with features of the insulin-resistance syndrome.^{138,139} In the same population there was no evidence of relationships between other coding polymorphisms in the A subunit and cardiovascular disease.¹⁴⁰ A mixed postmortem and case-control study of survivors of MI in Finnish subjects again suggested a protective effect of Leu34, but it was unable to reproduce the PAI-1 4G/4G genotype associations.¹⁴¹ Interestingly this study found the prevalence of the protective allele to be lowest in the Eastern Kainuu region, which has the highest prevalence of MI, similar to that reported in the high-risk Asian community in England.¹⁴²

A single study of factor XIII Val34Leu in computed tomography-confirmed stroke was carried out in 612 patients.¹⁴³ There were no differences in the prevalence of Leu34 in subjects with atherothrombotic stroke, though there was a marginally significant increase in Leu34 in subjects with a diagnosis of primary intracerebral hemorrhage. These results are consistent with the findings in relation to MI in that Leu34 may be protective against thrombosis though involved in the pathogenesis of hemorrhagic disorders. However, the small numbers in this study require that these results be viewed as preliminary.

Factor V/prothrombin

The factor V G1691A and prothrombin G20210A polymorphisms in arterial disease have been subjects of numerous reports. Many of these—some large—concerned with their association with arterial disease in young, middle-aged, and elderly populations have had negative results.^{19,43,45,144-148} In contrast, some studies report positive associations, particularly when the interaction of these polymor-

phisms with environmental factors has been formally evaluated. For example, a study of factor V G1691A in 88 young women with MI and 388 controls found an OR for MI of 2.4 (CI, 1.0 to 5.9).¹⁴⁹ The variant had little effect on nonsmokers, whereas it led to a large-risk increase in smokers (OR, 3.6; CI 0.9 to 4.4), resulting in a 32-fold increase in OR for carriers who smoked compared to noncarriers who did not smoke. Furthermore, the prothrombin G20210A polymorphism was associated with a high risk for MI in this population (OR, 4.0; CI, 1.1 to 15.1).¹⁵⁰ This risk was again amplified in smokers (OR, 43.3; CI, 6.7 to 281). In a large case-control study of primarily middle aged men with MI (560 patients and 646 controls; SMILE), a small increase in risk for MI was associated with both polymorphisms.¹⁵¹ The risk associated with these polymorphisms was amplified in smokers (OR, 6.1; CI, 3.0 to 12.5) and in those with metabolic risk factors for MI (OR, 3.2; CI, 1.5 to 6.7). A large (n = 826) population study of males and females undergoing ultrasound imaging of femoral and carotid arteries found associations between APCR and stenosis at either location.¹⁵² Decreases in APCR were associated stepwise with increased stenosis in the presence and the absence of the factor V polymorphism.

EPCR/thrombomodulin

The 23-bp insertion in the EPCR (see above) has been investigated in a single study of MI and reported in abstract form.⁶⁶ It was found in 4 of 203 patients and 0 of 195 controls, suggesting an association with MI. Until recently, there has been little published work relating changes in thrombomodulin expression and arterial thrombosis. A large (n = 14,170) case-cohort prospective study of coronary heart disease and atherosclerosis, the ARIC study, has now found an inverse relationship between plasma thrombomodulin levels and risk for incident coronary heart disease.¹⁵³ This suggests that low thrombomodulin levels could be associated with increased risk for coronary heart disease. Support for genetically determined levels of thrombomodulin has been provided by a recent study of a family with a private mutation in this gene.¹⁵⁴ There are 2 polymorphisms in the thrombomodulin gene, the C/T change coding for Ala455Val (mentioned above) and G/A change coding for Ala25Thr.^{155,156} One report has suggested an association between Ala455Val and MI,¹⁵⁷ but this has not been confirmed.¹⁵⁸ The Ala25Thr dimorphism has been studied in the SMILE study, and the data are suggestive that it is associated with increased risk for MI, particularly when it interacts with smoking (OR, 8.8; CI, 1.8 to 42.2) or with metabolic risk factors for the disease (OR, 4.4; CI, 0.9 to 21.3).¹⁵⁶ There is no biochemical evidence showing that Thr25 alters expression or function of the protein.

Fibrinolysis gene polymorphisms

tPA

tPA is the main endothelial cell-derived blood activator of the fibrinolytic system. It is a 70-kd serine proteinase activator of plasminogen whose catalytic efficiency is greatly increased by the presence of fibrin.¹⁵⁹ For many years it was believed that impaired fibrinolysis caused by the decreased function of tPA might be a risk factor for thrombosis. Recent interest has been on elevated, rather than reduced, levels of tPA as a risk marker.¹⁶⁰ Both the US Physicians Health and the ECAT prospective studies have found an increased risk for future MI in persons with elevated levels of tPA.^{85,161} Possible explanations for this apparently paradoxical

association are that elevated tPA levels may reflect preexisting disease or that they may be caused by increased PAI-1, with which tPA forms an inactive complex. Certainly, the predictive influence of tPA in patients with angina is greatly influenced by adjustments for insulin resistance, inflammation, and endothelial cell markers.¹⁶²

Numerous nucleotide sequence differences have been identified within the tPA gene locus. That which has been studied most in respect to cardiovascular disease is the Alu insertion/deletion within the intron between exons 8 and 9.¹⁶³ A relationship between the polymorphism and the phenotype has yet to be established; a study of genetically typed umbilical endothelial cells found no difference in basal tPA expression over 24 hours between different genotypes.¹⁶⁴ It has been suggested that there may be an association between the Alu repeats and thrombosis.¹⁶⁵ However, no association was found in the US Physicians Health Study or in 114 Italian patients selected for a familial history of cardiovascular events.^{166,167}

PAI-1

Relationship between plasma levels and disease, influence of polymorphisms on plasma levels. PAI-1 is a member of the SERPIN family, and its main function is as the fast-acting inhibitor of tPA.⁶⁸ PAI-1 exists in the circulation in molar excess over tPA, and it is generally accepted that this prevents the development of systemic fibrinogenolysis and permits local clot lysis without systemic bleeding.⁶⁸ It appears that fibrin shields fibrinolytic activators from the inhibitory effects of PAI-1, and it has been suggested that in this way fibrin facilitates its own destruction. In spite of the inherent difficulties that this concept introduces, it seems that elevated concentrations of PAI-1 are fairly consistently associated with vascular risk. High concentrations of PAI-1 occur in the atheromatous plaque,^{169,170} and these changes are more marked in patients with diabetes. Circulating PAI-1 has been related to vascular outcome in a number of studies.^{80,171,172} These associations occur during clustering of other vascular risk markers in the presence of insulin resistance with or without diabetes mellitus.^{78,79,173} It remains to be determined whether the associations result from any causal effect of elevated PAI-1.

The gene for PAI-1 codes for a protein of 50-kd. It has several polymorphic loci (Figure 3), including a 3' HindIII site, a CA(n) dinucleotide repeat in intron 3,¹⁷⁴ and a 4G/5G insertion/deletion –675 bp from the start site of the promoter.¹⁷⁵ The latter showed differential transcriptional responses to IL-1 in HepG2 cells with higher rates of PAI-1 synthesis in cells containing the 4G/4G genotype. It was suggested that the 4G site only binds an enhancer whereas the 5G allele binds both an enhancer and a suppressor, leading to lower rates of transcription with the 5G genotype.⁷⁵ In a population of 145 young patients with MI and 95 healthy controls, Dawson et al¹⁷⁴ found the dinucleotide repeat and the HindIII sites to be associated with variability in PAI-1 concentrations which, at the HindIII locus, appeared to be triglyceride dependent. Subsequently, the 4G/5G promoter site was reported to exhibit genotype-specific responses to triglyceride, with the highest levels of PAI-1 in 4G/4G persons with elevated triglyceride levels.^{76,177} These findings have been supported by laboratory studies in which a triglyceride-responsive region has been identified adjacent to the 4G/5G site.¹⁷⁸ Considerable interest has been generated by these observations, and several studies have attempted to tease out the relative contributions of gene and environment to circulating PAI-1 levels. In a small study of 25 healthy twins (12 monozygotic), additive genetic influences accounted for 71.4% of variance in PAI-1 levels, but environment only contributed 13.2%.¹⁷⁹ Oddly, in

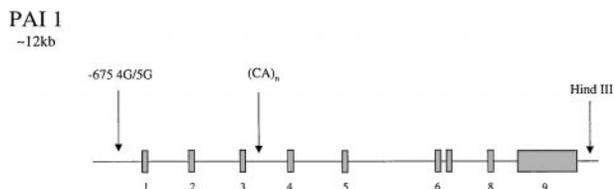


Figure 3. Representation of the organization of the PAI 1 gene, together with the location of common polymorphisms discussed in the text.

this study, none of the genetic influence was attributed to the 4G/5G locus, and it was suggested that either further PAI-1 gene polymorphisms or a quantitative trait locus was responsible for the genetic contribution to levels. However, a study of 5 common polymorphisms in the PAI-1 gene in a French population reported no association with PAI-1 levels.¹⁸⁰ In support of the latter study, it has been reported that metabolic features of the insulin-resistant state are more important than genetic influences, accounting for 49% of PAI-1 variance in men.¹⁸¹ Numerous studies have reported that levels of PAI-1 in homozygous 4G persons are approximately 25% higher than they are in 5G/5G subjects.⁸²⁻¹⁸⁷

Polymorphisms and disease. In a small study of young Swedish males with premature cardiovascular disease and controls, there were no differences in prevalence of either the dinucleotide repeat or the *HindIII* genotypes.¹⁷⁴ Identification of the promoter 4G/5G site led to a flurry of publications with a mixture of results. In this study, a higher prevalence of the 4G allele was observed in the group of young Swedish males with MI than in controls.⁸⁴ Similar results were obtained in a small number of subjects with type 2 diabetes and myocardial infarction.⁷⁷ In a mixed-gender population of 453 patients undergoing coronary angiography, of whom 166 had a history of MI, the 4G allele was significantly associated with PAI-1 levels and with MI; the relationship with MI and the genotype were strengthened in those with established atheroma.¹⁸³ In a study of 1179 healthy subjects and their first-degree relatives, the 4G allele was associated with significantly higher risk for MI (OR, 1.62; CI, 1.17 to 2.25).¹⁸⁸ A small study has reported that the 4G allele is related to the development of acute coronary syndromes.¹⁸⁹ However, other studies, including the large ECTIM,¹⁸² the US Physicians Health Study,¹⁹⁰ and SMILE,¹⁹¹ failed to show any relationship between genotype and MI. A recent large study of 2565 patients undergoing coronary angiography has reported an association between the 4G allele and coronary artery disease.⁹² In the total sample, there was elevated risk associated with the 4G/4G genotype for coronary artery disease (OR, 1.31; CI, 1.04 to 1.65). The polymorphism was also a risk for the severity of disease when groups at high risk (smokers, persons with hypertension) were selected.

Despite a number of publications on this topic, many of the reports are conflicting and there is no consistently clear evidence that PAI-1 polymorphisms are associated with MI. A meta-analysis of many studies suggested a weak, significant effect of PAI-1 genotype on MI.¹⁹³ In the context of the obvious reservations regarding meta-analysis, these findings indicate that, at most, PAI-1 genotype has a minor influence on the course of atherothrombotic disorders.

A single, large case-control study of more than 600 subjects with cerebrovascular disease, confirmed by computed tomography, failed to demonstrate any differences in genotype frequency compared than in a control group.¹⁹⁴ Additionally, in this study there was no relationship between genotype and PAI-1 levels.

Platelet-membrane glycoproteins

The normal functional activity of platelets is dependent on the presence of platelet-membrane glycoproteins that have an important role in platelet adhesion and aggregation.^{195,196} Glycoprotein (Gp) IIb/IIIa (also known as integrin α IIb/ β 3) and Gp Ib-IX-V are membrane receptors of this family, which bind a variety of circulating ligands under differing shear conditions to promote clot formation.¹⁹⁷ The importance of these receptors was originally noted by their absence in Glanzmann thrombasthenia (Gp IIb/IIIa deficiency) and Bernard Soulier syndrome (Gp Ib-IX-V deficiency).¹⁹⁸

The importance of different ligands in platelet activation differs at different flow shear rates. Gp Ib-IX-V binds predominantly to the vWF molecule, which is itself tethered to damaged and exposed subendothelial collagen to promote platelet adhesion. This process activates the platelet and alters the conformation of the Gp IIb/IIIa receptor; at high shear rates it binds vWF and leads to platelet aggregation, but at lower shear rates fibrinogen is more efficient in promoting this process.¹⁹⁷

Certain platelet surface proteins and receptors are involved in the direct interaction of platelets with collagen. The most important of these appear to be the Gp Ia/IIa complex (also known as VLA-2 and integrin α 2/ β 1) and Gp VI.¹⁹⁵ Both receptors were found to be deficient in patients with mild bleeding diatheses whose platelets exhibited impaired responses to collagen.^{199,200}

Gp IIb/IIIa

Gp IIb is a disulfide-linked protein of 150-kd that associates with the 90-kd polypeptide Gp IIIa. Many coding polymorphisms in Gp IIb/IIIa have been identified in normal populations and approximately 18 mutations thought to be involved in the pathogenesis of Glanzmann thrombasthenia have been described. In Gp IIIa a common point mutation results in the substitution of Pro to Leu at position 33. The wild-type Leu (PLA1) is found in approximately 85% of the white population, whereas the Pro33 substitution (PLA2) is present in 15%.²⁰¹

In April 1996 Weiss et al²⁰² published the first report of an association between the Gp IIIa PLA2 allele and risk for myocardial infarction. In a small study of 71 patients with a diagnosis of MI or unstable angina admitted to a coronary care unit, it was reported that the PLA2 allele was overrepresented in subjects with coronary artery disease than in 68 normal controls (39.4% versus 19.1%, respectively). In the total population, PLA2 was associated with a 2.8-fold increase in risk for coronary artery disease, whereas in a subgroup of 21 subjects younger than 60 with disease, an OR of 6.2 was reported. This study was shortly followed by a case report of the death from MI of an Olympic athlete who was found to be homozygous for the PLA2 allele.²⁰³ In the 2 to 3 years after the publication of this association study, there have been a number of largely negative reports.²⁰⁴⁻²⁰⁹ Of particular note among these are the results of the prospective US Physicians Health Study of 374 patients with MI and 704 controls²⁰⁴ and the ECTIM study of 620 MI and 700 controls,²⁰⁵ both of which showed no suggestion of an association between the PLA2 allele and MI. Most of these studies with negative results carried out subgroup analysis to try to confirm the findings of Weiss et al²⁰² in relation to age; again, most results were negative. Exceptions were a cohort analyzed by Carter et al²¹⁰ that showed a statistically significant increase in the PLA2 allele in a small group of subjects younger than 47 and a comprehensive

study of a number of prothrombotic genetic risk factors in young survivors of MI.²¹¹ In the latter case-control study of 200 survivors of MI younger than 45, none of the common polymorphisms of coagulation and fibrinolysis (factor V G1691A, prothrombin G20210A, factor VII Arg355Gln, PAI 1 4G/5G) were strongly associated with MI. In contrast, the OR for carriers of PLA2 was 1.84 (CI, 1.12 to 3.03), and this was increased in 51 carriers who smoked, OR 13.7 (CI, 6.41 to 31.2). It was concluded that in smokers who carried the PLA2 allele, approximately 50% of early onset MI could be attributed to interaction of these 2 risk factors.

Two studies have reported an association between PLA2 and extent of coronary atheroma estimated by coronary angiography. In 1 study this association was weak (OR, 1.5; CI, 1.01 to 2.26) for the presence of more than 50% stenosis in more than 1 vessel,²⁰⁶ and in the other PLA2 was associated with severe coronary artery disease in patients at low risk.²¹² Several similar studies have reported no association with coronary atheroma.^{209,213,214}

Two studies have addressed whether PLA2 influences outcome in patients undergoing coronary artery procedures. Walter et al²¹⁵ studied 318 patients followed up for 30 days and reported an OR of 5.26 for stent occlusion (CI, 1.55 to 17.8) for subjects with the PLA2 allele. Mamotte et al²¹³ investigated the risk for coronary artery restenosis after angioplasty and reported no relationship to PLA2.

Four major studies (including the prospective US Physicians Health Study) of a total of 1140 patients with cerebrovascular disease and 1612 controls have failed to show a relationship between the PLA2 allele and disease.^{204,216,217} Carter et al²¹⁶ looked at stroke in patients younger than 47 years from their large case-control study and reported a prevalence of the PLA2 allele of 50% in this group, findings similar to those reported by the same group in young patients with MI.²⁰⁶ These results must be confirmed in a separate study of young patients with stroke.

Gp Ib-IX-V complex

Four gene products comprise the Gp Ib-IX-V receptor complex.¹⁹⁶ Gp Ib α , approximately 140-kd, is disulfide bonded to Gp Ib β , approximately 25-kd, and these polypeptides are noncovalently associated with Gp IX, approximately 22-kd and Gp V, approximately 82-kd. Two polymorphisms have attracted interest. A length polymorphism in Gp Ib α results from a variable number of tandem repeats (VNTR) of 39-bp coding for 13 amino acids in the glycosylated region (macroglycopeptide). Up to 4 polymorphic forms result, designated D, C, B, A, in order of increasing number of repeats (1, 2, 3, or 4 repeats). The function of the macroglycopeptide is thought to be that of a spacer, keeping the ligand-binding region well above the platelet surface. The second polymorphism, C to T at position 3550, results in a Thr145Met substitution and is linked to the HPA-2 alloantigen system.

A study of 101 patients with acute coronary disease, 104 patients with cerebrovascular disease, 95 patients with venous thrombosis—with matched controls—has reported an association of genotypes with arterial but not venous disease.²¹⁸ For coronary disease, the associated risk of the C/B genotype was given by the OR of 2.84 (CI, 1.28 to 6.41); for cerebrovascular disease, the OR was 2.83 (CI, 1.16 to 7.07). Similarly, there was an association of Met145 with coronary disease (OR, 2.09; CI, 0.98 to 4.49). In contrast to these positive associations, no association was found between MI and Thr145Met in a case-control study of 200 young patients.²¹¹

Gp Ia/IIa complex

This collagen receptor consists of an approximately 167-kd (α 2) and an approximately 130-kd (β 1) polypeptide. What has been of great interest is the finding that though the receptor is expressed at low density on the platelet surface (1000 to 3000 copies), there is a wide, approximately 10-fold, variation within normal persons that results in variability of response to collagen.²¹⁹ A genetic association of the density variation with linked sequence polymorphisms within the α 2 gene has been reported. The variation is particularly associated with a silent exonic dimorphism at position 807, C/T. In a study of patients with von Willebrand disease (vWD), it was found that the 807C allele, associated with low receptor density, was higher in patients with-type I vWD, suggesting it might increase the tendency for bleeding.²²⁰

The significance of this polymorphism to arterial disease has been evaluated in a large study (n = 2237) of male patients undergoing coronary angiography.²²¹ An association was found between the T allele (predicting high-receptor density) and MI in patients younger than 62 years mean age (OR, 1.57; CI, 1.14 to 2.13). Conversely, a large (n = 546) case-control study of MI was unable to establish an association of the T allele, (OR, 0.88; CI, 0.74 to 1.05), even for homozygous carriers.²²² In a small (n = 45) case-control study of patients younger than 50 years with stroke, an association of the T allele with disease was found (OR, 3.02; CI, 1.20 to 7.61).

Genes other than those of hemostasis

A consistent finding in case-control and cross-sectional studies has been an association between hyperhomocysteinemia and atherothrombotic disease.^{223,224} This association has not, however, always been found in prospective studies, for reasons that could include nutritional or genetic differences between the populations studied.⁷⁵ The MTHFR C677T polymorphism has been studied extensively as a possible genetic determinant of the increased risk associated with the phenotype. A meta-analysis of 13 studies of this polymorphism in 3281 patients with cardiovascular disease (and their controls) has failed to support this link between genotype, phenotype, and disease.²²⁵

Conclusions and perspectives

A casual glance at a rapidly expanding literature could easily lead to the conclusion that we are well on the way to establishing the genetic basis for vascular disorders, such is the number of polymorphisms that have been investigated (see summary Table 1). There has clearly been appreciable progress in relation to venous thromboembolic disease whereby the functional and clinical consequences of certain polymorphisms are accepted. Venous disease occurs in the context of a low-pressure, low-flow system in which atheroma does not occur. The most common genetic risk determinant for venous disease alters the function of the protein C anticoagulant pathway, whereas other genetic risk factors alter levels or activities of certain coagulation factors. Although environmental influences are undoubtedly important, genetic changes can play a crucial role and even sometimes lead to *apparently* spontaneous venous disease.

In contrast, there is little clarity in relation to arterial disease (see Table 1). The initial promise that genetic risk factors might contribute appreciably to an explanation of the development of arterial disease has largely been unfulfilled, and the expectations

Table 1. Summary of the outcome of investigations of hemostatic polymorphisms

Polymorphism	Phenotype	Relationship of Phenotype to Disease	Association of Genotype With Disease
Venous thromboembolic disease			
Factor V G1691A, Arg506Gln	APCR	Clearly established	Clear risk factor alone or in combination with other risk factors, genetic or acquired
Prothrombin G20210A	Altered levels, mechanism uncertain	Most likely	Moderate risk factor alone or in combination with other risk factors
Factor V HR2 haplotype	Slight APCR; glycosylation isoforms altered?	Suggestive	Weak risk factor alone or in combination with factor V G1691A?
Factor XIII Val34Leu	Increased activation rate	Suggestive	Preliminary results suggest 34Leu is protective against thrombosis
EPCR 23-bp repeat	Predicted altered expression on cell surface	Unknown	Preliminary results suggest it a weak/moderate risk factor
Arterial occlusive disease			
Fibrinogen β chain -455G/A	Associated with altered level	Established, but causal?	Inconsistent
Fibrinogen β chain Bcl	Associated with altered level	Established, but causal?	Inconsistent
Fibrinogen α Thr313Ala	Altered clot structure?	Unknown	Inconsistent
Factor VII Arg353Gln	Altered level	Inconsistent	Inconsistent
Factor VII HVR4	Probably altered level	Inconsistent	Unknown
Factor VII -402G/A	Altered level	Inconsistent	Unknown
Factor VII -401G/T	Altered level	Inconsistent	Unknown
Factor VII -323 O/10	Altered level	Inconsistent	Unknown
Factor XIII Val34Leu	Increased activation rate	Unknown	Preliminary results suggest 34Leu is protective against MI
Factor V G1691A, Arg506Gln	APCR	Inconsistent	Possibly in selected patients and in combination with acquired risk factors
Prothrombin G20210A	Altered level	Unknown	Possibly in selected patients, and in combination with acquired risk factors
EPCR 23-bp repeat	Predicted altered expression on cell surface	Unknown	Preliminary results suggest it may be a weak risk factor
Thrombomodulin Ala455Thr	Unknown	Suggestive	Inconsistent
Thrombomodulin Ala25Thr	Unknown	Suggestive	Preliminary results suggest 25Thr associated with MI
tPA Alu insertion/deletion	Unlikely	Paradoxical	Inconsistent
PAI-1 -675 4G/5G	Inconsistent	Suggestive	Inconsistent
PAI-1 (CA) _n	Suggestive of altered level	Suggestive	Unknown
PAI-1 <i>Hind</i> III	Suggestive of altered level	Suggestive	Unknown
Gp IIIa Pro33Leu	No effect on fibrinogen binding, but increased sensitivity to activation?	Unknown	Inconsistent
Gp IαVNTR	Unknown	Unknown	Preliminary results show association
Gp Ib; Iα C3550T, Thr145Met	Unknown	Unknown	Inconsistent
Gp Ia/Ia, α2 C807T	Associated with altered surface expression of receptor, altered collagen binding	Unknown	Inconsistent

Data on each polymorphism are summarized in terms of the nature of its phenotype, what is known of the relationship between the phenotypic change (not necessarily genetically determined) and the disease, and the direct association of the genotype to the disease.

raised by early reports of positive associations have been tempered by inconsistent results with almost all genes studied. Arterial disease occurs in a high-pressure, high-flow system with atheromatous disease as a dominant feature. Hemostatic factors have been extensively studied as possible risk determinants for disease. The most consistent associations have been found for fibrinogen. Although it is formally possible that fibrinogen is merely a marker of another underlying process, such as the acute-phase reaction,²²⁶ a strong case can be made that fibrinogen is causally involved in disease. For each of the other hemostatic factors, though a causal role is highly plausible, the evidence for this is less strong. It is interesting to note that hemostatic risk factors for atherothrombotic disorders are largely those that have been associated to some extent with classical risk markers such as features of insulin resistance. These observations indicate the importance of environmental influences and emphasize the complexity of the processes involved in vascular disease. When individual hemostatic factors do contribute to risk, it is in concert with myriad other metabolic factors. This understanding further increases the importance of lifelong risk interactions and may suggest an explanation for some of the inconsistencies in case-control studies of arterial disease. The

available studies have often been too small to be informative in terms of the interactions between environment and genetic polymorphisms. Additionally, some of the problems in identifying causal genetic markers are related to difficulties associated with the precise definition of the clinical phenotype under study. These have been compounded by inadequate consideration of issues surrounding the distribution of polymorphisms in the population. Examples of factors that may be responsible for some of the observed inconsistencies are as follows. (1) The effects of common single-gene changes are often minor; study size and power are therefore critical issues. (2) There is plurality in clinical endpoints (MI, unstable angina, coronary artery disease, progression of arterial disease, stroke), and these have been often selected in a post-data collection search for significance. (3) Genetic polymorphisms may vary appreciably within and between racial groups. (4) Numerous studies have paid insufficient attention to minimizing recruitment bias in the selection of patients, controls, or both. (5) It is likely that the greatest effect of the gene polymorphism will most often be in association with (a) specific (set of) environmental changes that may vary between study cohorts and that will require very large studies to define.

Additionally, the importance of heritability of risk factors has generally not been fully appreciated. The apparent paradox of a firm relationship between gene and protein and protein and disease, but inconsistent gene-disease relationships, must be related to the quantitative contribution of heritability to the phenotype (circulating protein levels or density of expression in the case of receptors). Taking fibrinogen as an example of a plasma protein, there is a clear link between circulating levels and all forms of atherothrombotic disorders. Although most studies indicate that fibrinogen polymorphisms are related to levels, the relationship between fibrinogen genotype and disease remains uncertain. Most reports have estimated the contribution of individual polymorphisms on the variance of plasma levels to be approximately 5%. Formal heritability studies suggest that the *total* genetic influence on levels is approximately 50%. It follows from this that any currently known polymorphism in the fibrinogen gene locus can only contribute to a small (less than 10% of the effect of total level) extent to vascular risk and may therefore be undetectable in most sample sizes. If the genetic components that contribute 100% of the heritable variance in fibrinogen could be identified, they would be expected to have a predictive power for disease similar to fibrinogen levels. A key issue for the future, therefore, must be the identification of all the major heritable influences on variance. Some of these may lie within or immediately around the gene of interest and have to date evaded detection, but others may lie outside the gene loci. Fibrinogen is a strong case for further study, for the reasons mentioned above. For those factors in which the relationship between circulating levels of a protein (or its activity, or its receptor density) and disease is weak, establishing relationships between genetic determinants of variance and disease is likely to remain difficult even if the major genetic influences on heritability are identified. It can be concluded that the study of population genetics of polygenic disorders, such as arterial thrombotic disease, would *ideally* require prior knowledge of the relationship between the protein level/receptor density and disease,

the degree of heritability of variance in the plasma levels of the protein/receptor density, and the genetic determinants of heritability.

In future studies, attention should be paid to identifying more clearly the optimum clinical phenotype and, in general, to applying a more rigorous approach to the clinical aspects. Because it is highly unlikely that single genetic polymorphisms will be sole determinants of disease, future studies should be designed specifically to investigate interactions between potential genetic polymorphisms and acquired risk factors. They should be designed at their outset to minimize recruitment bias and to have clear and precisely defined clinical endpoints. Because of these requirements, they should be larger than are currently used, unless of course they are early hypothesis-forming studies. A realistic position with regard to radical treatments for MI must be adopted. It must be clear from the above, and from the slow progress developing gene delivery systems, that gene therapy to alter hemostatic polymorphic phenotypes is a weak option. The immediate value of the genetic approach lies in increasing the understanding of mechanisms of disease and thereby giving the real opportunity for developing novel and selective drug-based therapies for the prevention of disease.

There is a growing number of potential genetic risk factors for occlusive thrombotic disease. This review has concentrated on hemostatic genes implicated in disease. The perspective in this field may change because of the international drive to complete the sequence of the human genome. There may be many novel genes involved in blood vessel development or in blood cell function awaiting identification and characterization that could play important roles in modifying the disease process. It might be that the hemostatic genes have to interplay with other genetic influences before they can influence significantly disease progression. Consequently, though there will no doubt continue to be expanding interest in this area, substantial further development may benefit from a clearer understanding of the pathophysiologic processes involved in the development of vessel disease in atherothrombotic disorders.

References

- Egeberg O. Inherited antithrombin III deficiency causing thrombophilia. *Thromb Diath Haemorrh*. 1965;13:516.
- Griffin JH, Evatt B, Zimmerman TS, Kleiss AJ, Wideman C. Deficiency of protein C in congenital thrombotic disease. *J Clin Invest*. 1981;68:1370.
- Comp PC, Nixon RR, Cooper MR, Esmon CT. Familial protein S deficiency is associated with recurrent thrombosis. *J Clin Invest*. 1984;74:2082.
- Dahlback B, Carlsson M, Svensson PJ. Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C: prediction of a cofactor to activated protein C. *Proc Natl Acad Sci U S A*. 1993;90:1004.
- Dahlback B. Inherited resistance to activated protein C, a major cause of venous thrombosis, is due to a mutation in the factor V gene. *Haemostasis*. 1994;24:139.
- Bertina RM, Koeleman BP, Koster T, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature*. 1994;369:64.
- Voorberg J, Roelse J, Koopman R, et al. Association of idiopathic venous thromboembolism with single point-mutation at Arg506 of factor V. *Lancet*. 1994;343:1535.
- Majerus PW. Human genetics: bad blood by mutation. *Nature*. 1994;369:14.
- Lane DA, Mannucci PM, Bauer KA, et al. Inherited thrombophilia, part 1. *Thromb Haemost*. 1996;76:651.
- Rosendaal FR. Venous thrombosis: a multicausal disease. *Lancet*. 1999;353:1167.
- Martinelli I, Mannucci PM, de Stefano V, et al. Different risks of thrombosis in four coagulation defects associated with inherited thrombophilia: a study of 150 families. *Blood*. 1998;92:2353.
- Kalafatis M, Mann KG. Factor V Leiden and thrombophilia. *Arterioscler Thromb Vasc Biol*. 1997;17:620.
- Kalafatis M, Rand MD, Mann KG. The mechanism of inactivation of human factor V and human factor Va by activated protein C. *J Biol Chem*. 1994;269:31,869.
- Nicolaes GAF, Tans G, Thomassen MCLGD, et al. Peptide bond cleavages and loss of functional activity during inactivation of factor Va and factor Va R506Q by activated protein C. *J Biol Chem*. 1995;270:21,158.
- Rosing J, Hoekema L, Nicolaes GAF, et al. Effects of protein S and factor Xa on peptide bond cleavages during inactivation of factor Va and factor Va R506Q by activated protein C. *J Biol Chem*. 1995;270:27,852.
- Shen L, Dahlback B. Factor V and protein S as synergistic cofactors to activated protein C in degradation of factor VIIIa. *J Biol Chem*. 1994;269:18,735.
- Thorelli E, Kaufman RJ, Dahlback B. The C-terminal region of the factor V B-domain is crucial for the anticoagulant activity of factor V. *J Biol Chem*. 1998;273:16,140.
- Koster T, Rosendaal FR, de Ronde H, Briet E, Vandenbroucke JP, Bertina RM. Venous thrombosis due to poor anticoagulant response to activated protein C: Leiden Thrombophilia Study. *Lancet*. 1993;342:1503.
- Ridker PM, Hennekens CH, Lindpaintner K, Stampfer MJ, Eisenberg PR, Miletich JP. Mutation in the gene coding for coagulation factor V and the risk of myocardial infarction. *N Engl J Med*. 1995;332:912.
- Zivelin A, Griffin JH, Xu X, et al. A single genetic origin for a common Caucasian risk factor for venous thrombosis. *Blood*. 1997;89:397.
- Koeleman BP, Reitsma PH, Allaart CF, Bertina RM. Activated protein C resistance as an additional risk factor for thrombosis in protein C-deficient families. *Blood*. 1994;84:1031.
- Zoller B, Berntsdotter A, Garcia de Frutos P, Dahlback B. Resistance to activated protein C as

- an additional genetic risk factor in hereditary deficiency of protein S. *Blood*. 1995;85:3518.
23. van Boven HH, Reitsma PH, Rosendaal FR, et al. Factor V Leiden (FV R506Q) in families with inherited antithrombin deficiency. *Thromb Haemost*. 1996;75:417.
 24. Vandenbroucke JP, Koster T, Briet E, Reitsma PH, Bertina RM, Rosendaal FR. Increased risk of venous thrombosis in oral-contraceptive users who are carriers of factor V Leiden mutation. *Lancet*. 1994;344:1453.
 25. Rosing J, Tans G, Nicolaes GAF, et al. Oral contraceptives and venous thrombosis: different sensitivities to activated protein C in women using second- and third-generation oral contraceptives. *Br J Haematol*. 1997;97:233.
 26. Martinelli I, Landi G, Merati G, Cella R, Tosetta A, Mannucci PM. Factor V gene mutation is a risk factor for cerebral venous thrombosis. *Thromb Haemost*. 1996;75:393.
 27. Ryan DH, Crowther MA, Ginsberg JS, Francis CW. Relation of factor V Leiden genotype to risk for acute deep venous thrombosis after joint replacement surgery. *Ann Intern Med*. 1998;128:270.
 28. Desmarais S, de Moerloose P, Reber G, Minazio P, Perrier A, Bounameaux H. Resistance to activated protein C in an unselected population of patients with pulmonary embolism. *Lancet*. 1996;347:1374.
 29. Rintelen C, Pabinger I, Knobl P, Lechner K, Manthaler C. Probability of recurrence of thrombosis in patients with and without factor V Leiden. *Thromb Haemost*. 1996;75:229.
 30. Ridker PM, Miletich JP, Stampfer MJ, Goldhaber SZ, Lindpaintner K, Hennekens CH. Factor V Leiden and risks of recurrent idiopathic venous thromboembolism. *Circulation*. 1995;92:2800.
 31. Simioni S, Prandoni P, Lensing AWA, et al. The risk of recurrent venous thromboembolism in patients with an Arg506 to Gln mutation in the gene for factor V (factor V Leiden). *N Engl J Med*. 1997;336:399.
 32. Eichinger S, Pabinger I, Stumpfen A, et al. The risk of recurrent venous thromboembolism in patients with and without factor V Leiden. *Thromb Haemost*. 1997;77:624.
 33. De Stefano V, Martinelli I, Mannucci PM, et al. The risk of recurrent deep venous thrombosis among heterozygous carriers of both factor V Leiden and G20210A prothrombin mutation. *N Engl J Med*. 1999;341:801.
 34. Chan WP, Lee CK, Kwong YL, Lam CK, Liang R. A novel mutation of factor V in Hong Kong Chinese. *Blood*. 1998;91:1135.
 35. Williamson D, Brown K, Luddington R, Baglin C, Baglin T. Factor V Cambridge: a new mutation (Arg306 to Thr) associated with resistance to activated protein C. *Blood*. 1998;91:1140.
 36. de Visser MCH, Rosendaal FR, Bertina RM. A reduced sensitivity for activated protein C in the absence of factor V Leiden increases the risk of venous thrombosis. *Blood*. 1999;93:1271.
 37. Bernardi F, Faioni EM, Castoldi E, Castaman G, Sacchi E, Mannucci PM. A factor V genetic component differing from factor V R506Q contributes to the activated protein C resistance phenotype. *Blood*. 1997;90:1552.
 38. Alhenc-Gelas M, Nicaud V, Gandrille S, et al. The factor V gene A4070G mutation and the risk of venous thrombosis. *Thromb Haemost*. 1999;81:193.
 39. Faioni EM, Franchi F, Bucciarelli P, et al. Coinheritance of the HR2 haplotype in the factor V gene confers an increased risk of venous thrombosis to carriers of factor V R506Q (factor V Leiden). *Blood*. 1999;94:3062.
 40. Castoldi E, Rosing J, Lunghi B, et al. Factor V gene mutations (r2 gene) are associated with coronary artery disease in elderly people [abstract]. *Thromb Haemost*. 1999;509.
 41. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood*. 1996;88:3698.
 42. Rosendaal FR, Doggen CJM, Zivelin A, et al. Geographic distribution of the prothrombin 20210 G to A prothrombin variant. *Thromb Haemost*. 1998;79:706.
 43. Ferraresi P, Marchetti G, Legnani C, et al. The heterozygous 20210 G/A prothrombin genotype is associated with early venous thrombophilias and is not increased in frequency in arterial disease. *Arterioscler Thromb Vasc Biol*. 1997;17:2418.
 44. Martinelli I, Sacchi E, Landi G, Taioli E, Duca F, Mannucci PM. High risk of cerebral-vein thrombosis in carriers of a prothrombin-gene mutation and in users of oral contraceptives. *N Engl J Med*. 1998;338:1793.
 45. Ridker PM, Hennekens CH, Miletich JP. G20210A mutation in prothrombin gene and risk of myocardial infarction, stroke, and venous thrombosis in a large cohort of US men. *Circulation*. 1999;99:999.
 46. Zivelin A, Rosenberg N, Faier S, et al. A single genetic origin for the common prothrombotic G20210A polymorphism in the prothrombin gene. *Blood*. 1998;92:1119.
 47. Eichinger S, Minar E, Hirschl M, et al. The risk of early recurrent venous thrombosis after oral anti-coagulant therapy in patients with the G20210A transition in the prothrombin gene. *Thromb Haemost*. 1999;81:14.
 48. Ichinose A. The physiology and biochemistry of factor XIII. In: Bloom AL, Forbes CD, Thomas DP, Tuddenham EGD, eds. *Haemostasis and Thrombosis*. 3rd ed. Edinburgh: Churchill Livingstone; 1994:531.
 49. Tuddenham EGD, Cooper DN. *The Molecular Genetics of Haemostasis and Its Inherited Disorders*. Oxford: Oxford University Press; 1994.
 50. Anwar R, Gallivan L, Edmonds SD, Markham AF. Genotype/phenotype correlations for coagulation factor XIII: specific normal polymorphisms are associated with high or low factor XIII specific activity. *Blood*. 1999;93:897.
 51. Suzuki K, Iwata M, Ito S, Matsui K, Uchida A, Mizoi Y. Molecular basis for subtype differences of the "a" subunit of coagulation factor XIII with description of the genesis of the subtypes. *Hum Genet*. 1994;94:129.
 52. Anwar R, Stewart AD, Miloszewski KJA, Losowsky MS. Molecular basis of inherited factor XIII deficiency: identification of multiple mutations provides insights into protein function. *Br J Haematol*. 1995;91:728.
 53. Mikkola H, Syrjala M, Rasi V, et al. Deficiency in the A-subunit of coagulation factor XIII: two novel point mutations demonstrate different effects on transcript levels. *Blood*. 1994;84:517.
 54. Suzuki K, Henke J, Iwata M, et al. Novel polymorphisms and haplotypes in the human coagulation factor XIII A-subunit gene. *Hum Genet*. 1996;98:393.
 55. Kamura T, Okamura T, Murakawa M, et al. Deficiency of coagulation factor XIIIa subunit caused by dinucleotide deletion at the 5'-end of exon 3. *J Clin Invest*. 1992;90:315.
 56. Catto AJ, Kohler HP, Coore J, Mansfield MW, Stickland MH, Grant PJ. Association of a common polymorphism in the factor XIII gene with venous thrombosis. *Blood*. 1999;93:906.
 57. Franco RF, Reitsma PH, Lourenco D, et al. Factor XIII Val34Leu is a genetic factor involved in the aetiology of venous thrombosis. *Thromb Haemost*. 1999;81:676.
 58. Kangsadalampai S, Board PG. The Val34Leu polymorphism in the A subunit of coagulation Factor XIII contributes to the large normal range in activity and demonstrates that the activation peptide plays a role in catalytic activity. *Blood*. 1998;92:2766.
 59. Kohler HP, Ariens RAS, Whitaker P, Grant PJ. A common coding polymorphism in the Factor XIII A-subunit gene (FXIIIVal34Leu) affects cross linking activity. *Thromb Haemost*. 1998;80:704.
 60. Bangalore N, Drohan WN, Orthner CL. High affinity binding sites for activated protein C and protein C on cultured human umbilical vein endothelial cells independent of protein S and distinct from known ligands. *Thromb Haemost*. 1994;72:465.
 61. Fukudome K, Esmon CT. Identification, cloning, and regulation of a novel endothelial cell protein C/activated protein C receptor. *J Biol Chem*. 1994;269:26,486.
 62. Fukudome K, Kurosawa S, Sterns-Kurosawa DJ, He X, Rezaie AR, Esmon CT. The endothelial cell protein C receptor: cell surface expression and direct ligand binding by the soluble receptor. *J Biol Chem*. 1996;271:17,491.
 63. Sterns-Kurosawa DJ, Kurosawa S, Mollica JS, Ferrell GL, Esmon CT. The endothelial cell protein C receptor augments protein C activation by the thrombin-thrombomodulin complex. *Proc Natl Acad Sci U S A*. 1996;93:10,212.
 64. Lazik Z, Mitro A, Tatlor FBJ, Ferrel G, Esmon CT. Human protein C receptor is primarily present on endothelium of large blood vessels: implications for the control of the protein C pathway. *Circulation*. 1997;96:3633.
 65. Simmonds RE, Lane DA. Structural and functional implications of the intron/exon organization of the human endothelial cell protein C/activation protein C receptor (EPCR) gene: comparison with the structure of CD1/major histocompatibility complex a1 and a2 domains. *Blood*. 1999;94:632.
 66. Merati G, Biguzzi F, Oganessian N, et al. A 23bp insertion in the endothelial protein C receptor (EPCR) gene in patients with myocardial infarction and deep vein thrombosis [abstract]. *Thromb Haemost*. 1999;507.
 67. Ohlin A-K, Marlar RA. The first mutation identified in the thrombomodulin gene in a 45 year old man presenting with thromboembolic disease. *Blood*. 1995;85:330.
 68. Ohlin A-K, Marlar RA. Mutations in the thrombomodulin gene associated with thromboembolic disease. *Thromb Haemost*. 1995;73:1096.
 69. Ohlin AK, Norlund L, Marlar RA. Thrombomodulin gene variations and thromboembolic disease. *Thromb Haemost*. 1997;78:396.
 70. van der Velden PA, Krommenhoek-Van Es T, Allaart CF, Bertina RM, Reitsma PH. A frequent thrombomodulin amino acid diamorphism is not associated with thrombophilia. *Thromb Haemost*. 1991;65:511.
 71. Koster T, Blann AD, Briet E, Vandenbroucke JP, Rosendaal FR. Role of clotting factor VIII in effect of von Willebrand factor on occurrence of deep vein thrombosis. *Lancet*. 1995;345:152.
 72. O'Donnell J, Tuddenham EGD, Manning R, Kemball-Cooke G, Johnson D, Laffan M. High prevalence of elevated factor VIII levels in patients referred for thrombophilia screening: role of increased synthesis and relationship to acute phase reaction. *Thromb Haemost*. 1997;77:825.
 73. Mansvelt EPG, Laffan M, McVey JH, Tuddenham EGD. Analysis of the F8 gene in individuals with high plasma factor VIII:C levels and associated venous thrombosis. *Thromb Haemost*. 1998;80:561.

74. Kamphuisen PW, Houwing-Duistermaat JJ, van Houwelingen HC, Eikenboom JCJ, Bertina RM, Rosendaal FR. Familial clustering of factor VIII and von Willebrand factor levels. *Thromb Haemost.* 1998;79:323.
75. Cattaneo M. Hyperhomocysteinemia, atherosclerosis and thrombosis. *Thromb Haemost.* 1999;81:165.
76. Cattaneo M, Tsai MY, Bucciarelli P, et al. A common mutation in the methylenetetrahydrofolate reductase gene (C677T) increases the risk for deep vein thrombosis in patients with mutant factor V (factor V:Q506). *Arterioscler Thromb Vasc Biol.* 1997;17:1662.
77. den Heijer M, Koster T, Blom HJ, et al. Hyperhomocysteinemia as a risk factor for deep vein thrombosis. *N Engl J Med.* 1996;334:759.
78. Juhan-Vague I, Alessi MC, Vague P. Increased plasma plasminogen activator inhibitor 1 levels: a possible link between insulin resistance and atherothrombosis. *Diabetologia.* 1991;34:457.
79. Juhan-Vague I, Thompson SG, Jespersen J, on behalf of the ECAT Angina Pectoris Study Group I. Involvement of the hemostatic system in the insulin resistance syndrome: a study of 1,500 patients with angina pectoris. *Arterioscler Thromb.* 1993;13:1865.
80. Mansfield MW, Stickland MH, Grant PJ. Circulating levels of factor VII, fibrinogen and von Willebrand factor and features of insulin resistance in first degree relatives of patients with NIDDM. *Circulation.* 1996;94:2171.
81. Meade TW, Mellows S, Brozovic M, et al. Haemostatic function and ischaemic heart disease: principal results of the Northwick Park Heart Study. *Lancet.* 1986;2:533.
82. Wilhelmsen L, Svardsudd K, Korsan-Bengtson K, Larsson B, Welin L, Tibblin G. Fibrinogen as a risk factor for stroke and myocardial infarction. *N Engl J Med.* 1984;311: 501-505.
83. Jansson JK, Nilsson TK, Johnson O. von Willebrand factor in plasma: a novel risk factor for recurrent myocardial infarction and death. *Br Heart J.* 1991;66:351.
84. Hamsten A, De Faire U, Walldius G, et al. Plasminogen activator inhibitor in plasma: risk factor for recurrent myocardial infarction. *Lancet.* 1987;ii:3.
85. Ridker PM, Hennekens CH, Stampfer MJ, Manson JE, Vaughan DE. Prospective study of endogenous tissue plasminogen activator and risk of stroke. *Lancet.* 1994;343:940.
86. Heinrich J, Balleisen L, Schulte H, Assmann G, van de Loo J. Fibrinogen and Factor VII in the prediction of coronary risk. *Arterioscler Thromb.* 1994;14:54.
87. Lee AJ, Lowe GDO, Woodward M, Tunstall Pe-doe H. Fibrinogen in relation to a personal history of prevalent hypertension, diabetes, stroke, intermittent claudication, coronary heart disease and family history: the Scottish Heart Health Study. *Br Heart J.* 1993;69:338.
88. Meade TW, Imeson J, Stirling Y. Effects of changes in smoking and other characteristics on clotting factors and the risk of ischaemic heart disease. *Lancet.* 1987;ii:986.
89. Kant JA, Furnace AJ, Saxe D, Simon MI, McBride OW, Crabtree GR. Evolution and organization of the fibrinogen locus on chromosome 4: gene duplication accompanied by transcription and inversion. *Proc Natl Acad Sci U S A.* 1985;82:2344.
90. Courtois G, Morgan JG, Campbell LA, Fourel G, Crabtree GR. Interaction of a liver specific nuclear factor with the fibrinogen and α -1 antitrypsin promoters. *Science.* 1987;238:688.
91. Dalmon J, Laurent M, Courtois G. The human b-fibrinogen promoter contains hepatocyte nuclear factor 1-dependent interleukin-6-responsive element. *Mol Cell Biol.* 1993;13:1183.
92. Humphries SE, Cook M, Dubowitz M, Stirling Y, Meade TW. Role of genetic variation at the fibrinogen locus in determination of fibrinogen concentrations. *Lancet.* 1987;ii:1452.
93. Humphries SE, Green FR, Temple A, et al. Genetic factors determining thrombosis and fibrinolysis. *Ann Epidemiol.* 1992;2:371.
94. Yu S, Sher B, Kudryk B, Redman CM. Intracellular assembly of human fibrinogen. *J Biol Chem.* 1984;259:10,574.
95. Hamsten A, Iselius L, De Faire U, Blombäck M. Genetic and cultural inheritance of plasma fibrinogen concentrations. *Lancet.* 1987;2:988.
96. Friedlander Y, Elkana Y, Sinnreich R, Kark JD. Genetic and environmental sources of fibrinogen variability in Israeli families: The Kibbutzim Study. *Am J Hum Genet.* 1995;56:1194.
97. Behague I, Poirier O, Nicaud V, et al. b fibrinogen gene polymorphisms are associated with plasma fibrinogen and coronary artery disease in patients with myocardial infarction. *Circulation.* 1996;93:440.
98. Humphries SE, Ye S, Talmud P, Bara L, Wilhelmsen L, Tiret L. European Atherosclerosis Research Study: genotype at the fibrinogen locus (G₄₅₅-A b-gene) is associated with differences in plasma fibrinogen levels in young men and women from different regions in Europe: evidence for gender-genotype-environment interaction. *Arterioscler Thromb Vasc Biol.* 1995;15:96.
99. Tybjaerg-Hansen A, Agerholm-Larsen B, Humphries SE, Abildgaard S, Schnohr P, Nordestgaard BG. A common mutation (G₄₅₅-A) in the b-fibrinogen promoter is an independent predictor of plasma fibrinogen, but not of ischemic heart disease: a study of 9,127 individuals based on the Copenhagen City Heart Study. *J Clin Invest.* 1997;99:3034.
100. Gardemann A, Schwartz O, Haberbosch W, et al. Positive association of the b fibrinogen H1/H2 gene variation to basal fibrinogen levels and to increase in fibrinogen concentration during acute phase reaction but not to coronary artery disease and myocardial infarction. *Thromb Haemost.* 1997;77:1120.
101. de Maat MPM, Kastelein JJP, Jukema JW, et al. -455G/A polymorphism of the b-fibrinogen gene is associated with the progression of coronary atherosclerosis in symptomatic men: proposed role for an acute phase reaction pattern of fibrinogen. *Arterioscler Thromb Vasc Biol.* 1998;18:265.
102. Carter AM, Mansfield MW, Stickland MH, Grant PJ. b-fibrinogen gene -455 G/A polymorphism and fibrinogen levels: risk factors for coronary artery disease in subjects with NIDDM. *Diabetes Care.* 1996;19:1265.
103. Zito F, Di Castelnuovo A, Amore C, D'Orazio A, Donati MB, Iacoviello L. Bcl I polymorphism in the fibrinogen b-chain gene is associated with the risk of familial myocardial infarction by increasing plasma fibrinogen levels: a case-control study in a sample of GISSI-2 patients. *Arterioscler Thromb Vasc Biol.* 1997;17:3489.
104. Schmidt H, Schmidt R, Niederkorn K, et al. b-fibrinogen gene polymorphism (C₁₄₈ to T) is associated with carotid atherosclerosis. *Arterioscler Thromb Vasc Biol.* 1998;18:487.
105. Carter AM, Catto AJ, Bamford JM, Grant PJ. Gender-specific associations of the fibrinogen Bb 448 polymorphism, fibrinogen levels, and acute coronary disease. *Arterioscler Thromb Vasc Biol.* 1997;17:589.
106. Nishiuma S, Kario K, Yakushiji K, et al. Genetic variation in the promoter region of the b-fibrinogen gene is associated with ischemic stroke in a Japanese population. *Blood Coagul Fibrinolysis.* 1998;9:373.
107. Wang XL, Wang J, McCredie RM, Wilken DEL. Polymorphisms of factor V, factor VII, and fibrinogen genes: relevance to severity of coronary artery disease. *Arterioscler Thromb Vasc Biol.* 1997;17:246.
108. Baumann RE, Henschen AH. Human fibrinogen polymorphic site analysis by restriction endonuclease digestion and allele-specific polymerase chain reaction amplification: identification of polymorphisms at position Aa312 and Bb448. *Blood.* 1996;82:2117.
109. Muszbek L, Adany R, Mikkola H. Novel aspects of blood coagulation factor XIII, I: structure, distribution, activation and function. *Crit Rev Clin Lab Sci.* 1996;33:357.
110. Credo RB, Curtis CG, Lorand L. a-chain domain of fibrinogen controls generation of fibrinolyticase (factor XIIIa): calcium ion regulatory aspects. *Biochemistry.* 1981;20:3770.
111. Curran JM, Evans A, Arveiler D, et al. The a-fibrinogen T/A 312 polymorphism in the ECTIM study. *Thromb Haemost.* 1998;79:1057.
112. Carter AM, Catto AJ, Grant PJ. Association of the a-fibrinogen Thr 312Ala polymorphism with post stroke mortality in subjects with atrial fibrillation. *Circulation.* 1999;99:2423.
113. Junker R, Heinrich J, Schitte H, van der Loo J, Assman G. Coagulation factor VII and the risk of coronary heart disease in healthy men. *Arterioscler Thromb Vasc Biol.* 1997;17:1539.
114. Smith FB, Lee AJ, Fowkes FGR, Price JF, Rumley A, Lowe GDO. Hemostatic factors as predictors of ischemic heart disease and stroke in the Edinburgh Artery Study. *Arterioscler Thromb Vasc Biol.* 1997;17:3321.
115. Lane A, Green F, Scarabin PY, et al. Factor VII Arg/Gln353 polymorphism determines factor VII coagulant activity in patients with myocardial infarction (MI) and control subjects in Belfast and in France but is not a strong indication of MI risk in the ECTIM study. *Arteriosclerosis.* 1996;119:119.
116. Hoffman CJ, Miller RH, Lawson WE, Hultin MB. Elevation of factor VII activity and mass in young adults at risk of ischemic heart disease. *J Am Coll Cardiol.* 1989;14:941.
117. Heywood DM, Ossei-Gerning N, Grant PJ. Association of factor VII: C levels with environmental and genetic factors in patients with ischaemic heart disease and coronary atherosclerosis characterised by angiography. *Thromb Haemost.* 1996;76:161.
118. Vaziri ND, Kennedy SC, Kennedy D, Gonzales E. Coagulation, fibrinolytic, and inhibitory proteins in acute myocardial infarction. *Am J Med.* 1992;93:651.
119. Morrissey JH, Macic BG, Neuenschwander PF, Comp PC. Quantitation of activated factor VII levels in plasma using a tissue factor mutant selectively deficient in promoting factor VII activation. *Blood.* 1993;81:734.
120. Moor E, Silveira A, van't Hooft F, et al. Coagulation factor VII mass and activity in young men with myocardial infarction at a young age: role of plasma lipoproteins and factor VII genotype. *Arterioscler Thromb Vasc Biol.* 1995;15:655.
121. Green F, Kelleher C, Wilkes H, Temple A, Meade T, Humphries S. A common genetic polymorphism associated with lower coagulation factor VII levels in healthy individuals. *Arterioscler Thromb.* 1991;11:540.
122. Marchetti G, Patracchini P, Papischini M, Ferrati M, Bernardi F. A polymorphism in the 5' region of coagulation factor VII gene (F7) caused by an inserted decanucleotide. *Hum Genet.* 1993;90:575.
123. Bernardi F, Marchetti G, Pinotti M, et al. Factor VII gene polymorphisms contribute about one third of the factor VII level variation in plasma. *Arterioscler Thromb Vasc Biol.* 1996;16:72.
124. Pollak ES, Hung HL, Godin W, Overton GC, High KA. Functional characterization of the human factor VII 5'-flanking region. *J Biol Chem.* 1996;271:1738.

125. van't Hooft FM, Silveira A, Tornvall P, et al. Two common functional polymorphisms in the promoter region of coagulation factor VII gene determining plasma factor VII activity and mass concentration. *Blood*. 1999;93:3432.
126. Lane A, Cruikshank JK, Mitchell J, Henderson A, Humphries S, Green FR. Genetic and environmental determinants of factor VII coagulant activity in ethnic groups at different risk of coronary heart disease. *Atherosclerosis*. 1992;94:43.
127. Hunault M, Arbini AA, Lopaciuk S, Carew JA, Bauer KA. The Arg353Gln polymorphism reduces the level of coagulation factor VII: in vivo and in vitro studies. *Arterioscler Thromb Vasc Biol*. 1997;17:2825.
128. Humphries SE, Lane A, Green FR, Cooper J, Miller GJ. Factor VII coagulant activity and antigen levels in healthy men are determined by factor VII genotype and plasma triglyceride concentration. *Arterioscler Thromb Vasc Biol*. 1994;14:193.
129. Hong Y, Pedersen NL, Egberg N, de Faire U. Genetic effects for plasma factor VII levels independent of and in common with triglycerides. *Thromb Haemost*. 1999;81:382.
130. Mennen LI, de Maat MPM, Schouten EG, et al. Coagulation factor VII, serum-triglycerides and the R/Q353 polymorphism: differences between older men and women. *Thromb Haemost*. 1997;78:984.
131. Doggen CJM, Cats VM, Bertina RM, Reitsma PH, Vandenbroucke JP, Rosendaal FR. A genetic propensity to high factor VII is not associated with the risk of myocardial infarction in men. *Thromb Haemost*. 1998;80:281.
132. Iacoviello L, Di Castelnuovo A, de Knijff P, et al. Polymorphisms in the coagulation factor VII gene and the risk of myocardial infarction. *N Engl J Med*. 1998;338:79.
133. Lensen RPM, Rosendaal FR, Koster T, et al. Apparent different thrombotic tendency in patients with factor V Leiden and protein C deficiency due to selection of patients. *Blood*. 1996;88:4205.
134. Heywood DM, Carter AM, Catto AJ, Bamford JM, Grant PJ. Polymorphisms of the factor VII gene and circulating FVIIc levels in relation to acute cerebrovascular disease and post stroke mortality. *Stroke*. 1997;28:816.
135. Shebuski RJ, Sitko GR, Claremon DA, et al. Inhibition of factor XIIIa in a canine model of coronary thrombosis: effect of reperfusion and acute reocclusion after recombinant tissue-type plasminogen activator. *Blood*. 1990;76:1455.
136. Reed GL, Houg AK. The contribution of activated Factor XIII to fibrinolytic resistance in experimental pulmonary embolus. *Circulation*. 1999;99:299.
137. Kohler HP, Stickland MH, Ossei-Gerning N, Carter A, Mikkola H, Grant PJ. Association of a common polymorphism in the factor XIII gene with myocardial infarction. *Thromb Haemost*. 1998;79:8.
138. Kohler HP, Grant PJ. Clustering of haemostatic risk factors with FXIII Val34Leu in patients with myocardial infarction [letter]. *Thromb Haemost*. 1998;80:862.
139. Kohler HP, Mansfield MW, Clark PS, Grant PJ. Interaction between insulin resistance and factor XIII Val34Leu in patients with coronary heart disease. *Thromb Haemost*. 1999;82:1202.
140. Kohler HP, Futers T, Grant PJ. Prevalence of three common polymorphisms in the a-subunit gene of factor XIII in patients with coronary artery disease: association with FXIII activity and antigen levels. *Thromb Haemost*. 1999;81:511.
141. Wartiovaara U, Perola M, Mikkola H, et al. Association of FXIII Val34Leu with decreased risk of myocardial infarction in Finnish males. *Atherosclerosis*. 1999;142:295.
142. McCormack L, Kain K, Catto AJ, Kohler HP, Stickland MH, Grant PJ. Prevalence of FXIII V34L in populations with different cardiovascular risk. *Thromb Haemost*. 1998;80:523.
143. Catto AJ, Kohler HP, Bannan S, Stickland M, Carter A, Grant PJ. Factor XIII Val34Leu: a novel association with primary intracerebral haemorrhage. *Stroke*. 1998;29:813.
144. Emmerich J, Poirier O, Evans A, et al. Myocardial infarction, Arg 506 to Gln factor V mutation, and activated protein C resistance [letter]. *Lancet*. 1995;345:321.
145. Ardissino D, Peyvandi F, Merlini PA, Colombi E, Mannucci PM. Factor V (Arg506 to Gln) mutation in young survivors of myocardial infarction. *Thromb Haemost*. 1996;75:701.
146. Cushman M, Rosendaal FR, Psaty BM, et al. Factor V Leiden is not a risk factor for arterial vascular disease in the elderly: results from the Cardiovascular Health Study. *Thromb Haemost*. 1998;79:912.
147. Catto A, Carter A, Ireland H, et al. Factor V Leiden gene mutation and thrombin generation in relation to acute stroke. *Arterioscler Thromb Vasc Biol*. 1995;15:783.
148. Longstreth WT, Rosendaal FR, Siscovick DS, et al. Risk of stroke in young women and two prothrombotic mutations: factor V Leiden and prothrombin gene variant (G20210A). *Stroke*. 1998;29:577.
149. Rosendaal FR, Siscovick DS, Schwartz SM, et al. Factor V Leiden (resistance to activated protein C) increases the risk of myocardial infarction in young women. *Blood*. 1997;89:2817.
150. Rosendaal FR, Siscovick DS, Schwartz SM, et al. A common prothrombin variant (20210 G to A) increases the risk of myocardial infarction in young women. *Blood*. 1997;90:1747.
151. Doggen CJM, Cats VM, Bertina RM, Rosendaal FR. Interaction of coagulation defects and cardiovascular risk factors: increased risk of myocardial infarction associated with factor V Leiden or prothrombin 20210A. *Circulation*. 1998;97:1037.
152. Kiechl S, Muigg A, Santer P, et al. Poor response to activated protein C as a prominent risk predictor of advanced arterial disease. *Circulation*. 1999;99:614.
153. Salomaa V, Matei C, Aleksic N, et al. Soluble thrombomodulin as a predictor of incident coronary heart disease and symptomless carotid artery atherosclerosis in the Atherosclerosis Risk in Communities (ARIC) Study: a case-cohort study. *Lancet*. 1999;353:1729.
154. Kunz G, Ireland H, Stubbs PJ, Kahan M, Coulton GC, Lane DA. Identification and characterization of a thrombomodulin gene mutation coding for an elongated protein with reduced expression in a kindred with myocardial infarction. *Blood*. 2000;95:569.
155. Norland L, Zoller B, Ohlin AK. A novel thrombomodulin gene mutation in a patient suffering from sagittal sinus thrombosis. *Thromb Haemost*. 1997;78:1164.
156. Doggen CJM, Kunz G, Rosendaal FR, et al. A mutation in the thrombomodulin gene, 127G to A coding for Ala25Thr, and the risk of myocardial infarction. *Thromb Haemost*. 1998;80:743.
157. Norland L, Holm J, Zoller B, Ohlin AK. A common thrombomodulin amino acid dimorphism is associated with myocardial infarction. *Thromb Haemost*. 1997;77:248.
158. Ireland H, Kunz G, Kyriakoulis K, Stubbs PJ, Lane DA. Thrombomodulin gene mutations associated with myocardial infarction. *Circulation*. 1997;96:15.
159. Collen D. The plasminogen (fibrinolytic) system. *Thromb Haemost*. 1999;82:259.
160. Jansson JH, Olofsson BO, Nilsson TK. Predictive value of tissue plasminogen activator mass concentration on long term mortality in patients with coronary artery disease. *Circulation*. 1993;88:2030.
161. Thompson SG, Kienast J, Pyke SDN, Haverkate F, van de Loo JCW. Hemostatic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. *N Engl J Med*. 1995;332:635.
162. Juhan-Vague I, Alessi MC. PAI-1, obesity, insulin resistance and the risk of cardiovascular events. *Thromb Haemost*. 1997;78:656.
163. Ludwig M, Wihn K-D, Scheuning W-D, Olek K. Allelic dimorphism in the human tissue-type plasminogen activator (tPA) gene as a result of an Alu insertion/deletion event. *Hum Genet*. 1992;88:388.
164. van den Eijnden-Schrauwen Y, Lakenberg N, Emeiss JJ, de Knijff P. Alu-repeat polymorphism in the tissue-type plasminogen activator gene does not affect basal endothelial tPA synthesis [letter]. *Thromb Haemost*. 1995;74:1202.
165. van der Bom JG, de Knijff P, Haverkate F, et al. Tissue plasminogen activator and the risk of myocardial infarction: the Rotterdam Study. *Circulation*. 1997;95:2623.
166. Ridker PM, Baker MT, Hennekens CH, Stampfer MJ, Vaughan DE. Alu-repeat polymorphism in the gene coding for tissue-type plasminogen activator (t-PA) and risks of myocardial infarction among middle-aged men. *Arterioscler Thromb Vasc Biol*. 1997;17:1687.
167. Iacoviello L, Di Castelnuovo A, De Knijff P, et al. Alu-repeat polymorphism in the t-PA gene, tPA levels and the risk of familial AMI. *Fibrinolysis*. 1996;10:13.
168. Booth NA. The natural inhibitors of fibrinolysis. In: Bloom AL, Forbes CD, Thomas DP, Tuddenham EGD, eds. *Haemostasis and Thrombosis*. 3rd ed. Edinburgh: Churchill Livingstone; 1994:699.
169. Lupu F, Bergonzelli GE, Heim DA, et al. Localization and production of plasminogen activator inhibitor-1 in human healthy and atherosclerotic arteries. *Arterioscler Thromb*. 1993;13:1090.
170. Sobel BE, Woodcock-Mitchell J, Schneider DJ, Holt RE, Marutsuka K, Gold H. Increased plasminogen activator inhibitor type 1 in coronary artery atherectomy specimens from type 2 diabetic compared with non-diabetic patients: a potential factor predisposing to thrombosis and its persistence. *Circulation*. 1998;97:2213.
171. Margaglione M, Di Minno G, Grandone E, et al. Abnormally high circulation levels of tissue plasminogen activator and plasminogen activator inhibitor-1 in patients with a history of ischemic stroke. *Arterioscler Thromb*. 1994;14:1741.
172. Thogersen AM, Jansson JH, Boman K, et al. High plasminogen activator inhibitor and tissue plasminogen activator levels in plasma precede a first acute myocardial infarction in both men and women: evidence for the fibrinolytic system as an independent primary risk factor. *Circulation*. 1998;98:2241.
173. Mansfield MW, Stickland MH, Grant PJ. PAI-1 concentrations in first degree relatives of patients with non-insulin-dependent diabetes mellitus: metabolic and genetic associations. *Thromb Haemost*. 1997;77:357.
174. Dawson S, Hamsten A, Wiman B, Henney A, Humphries S. Genetic variation at the plasminogen activator inhibitor-1 locus is associated with altered levels of plasminogen activator inhibitor-1 activity. *Arterioscler Thromb*. 1991;11:183.
175. Dawson SJ, Wiman B, Hamsten A, Green F, Humphries S, Henney AM. The two allele sequences of a common polymorphism in the promoter of the plasminogen activator inhibitor-1 (PAI-1) gene respond differently to interleukin-1 in HepG2 cells. *J Biol Chem*. 1993;268:10,739.
176. Panahloo A, Mohamed-Ali V, Lane A, Green F, Humphries SE, Yudkin J. Determinants of plasminogen activator inhibitor-1 activity in treated

- NIDDM and its relation to a polymorphism in the plasminogen activator inhibitor-1 gene. *Diabetes*. 1995;44:37.
177. Mansfield MW, Stickland MH, Grant PJ. Plasminogen activator inhibitor-1 promoter polymorphism and coronary artery disease in non-insulin dependent diabetes. *Thromb Haemost*. 1995;74:1032.
178. Eriksson P, Nilsson L, Karpe F, Hamsten A. Very-low-density lipoprotein response element in the promoter region of the human plasminogen activator inhibitor-1 gene implicated in the impaired fibrinolysis of hypertriglyceridemia. *Arterioscler Thromb Vasc Biol*. 1998;18:20.
179. Cesari M, Sartori MT, Patrassi GM, Vettore S, Rossi GP. Determinants of plasma levels of plasminogen activator inhibitor-1: a study of normotensive twins. *Arterioscler Thromb Vasc Biol*. 1999;19:316.
180. Henry M, Chomiki N, Scarabin PY, et al. Five frequent polymorphisms of the PAI-1 gene: lack of association between genotypes, PAI activity, and triglyceride levels in a healthy population. *Arterioscler Thromb Vasc Biol*. 1997;17:851.
181. Henry M, Tregouet DA, Alessi MC, et al. Metabolic determinants are much more important than genetic polymorphisms in determining the PAI-1 activity and antigen plasma concentrations: a family study with part of the Stanislas Cohort. *Arterioscler Thromb Vasc Biol*. 1998;18:84.
182. Ye S, Green FR, Scarabin PY, et al. The 4G/5G genetic polymorphism in the promoter of the plasminogen activator inhibitor-1 (PAI-1) gene is associated with differences in plasma PAI-1 activity but not with risk of myocardial infarction in the ECTIM study. *Thromb Haemost*. 1995;74:837.
183. Ossei-Gerning N, Mansfield MW, Stickland MH, Wilson IJ, Grant PJ. Plasminogen activator inhibitor-1 (PAI-1) promoter 4G/5G genotype and levels in relation to a history of myocardial infarction in patients characterised by coronary angiography. *Arterioscler Thromb Vasc Biol*. 1997;17:33.
184. Eriksson P, Kallin B, van't Hooft FM, Bavenholm P, 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.
185. Burzotta F, Di Castelnuovo A, Amore C, et al. 4G/5G promoter PAI-1 gene polymorphism is associated with plasmatic PAI-1 activity in Italians: a model of gene-environment interaction. *Thromb Haemost*. 1998;79:354.
186. Stegner M, Uhrin P, Peternel P, et al. The 4G/5G sequence polymorphism in the promoter of plasminogen activator inhibitor-1 (PAI-1) gene: relationship to plasma PAI-1 level in venous thromboembolism. *Thromb Haemost*. 1998;79:975.
187. Margaglione M, Grandone E, Vecchione G, et al. Plasminogen activator inhibitor-1 (PAI-1) antigen plasma levels in subjects attending a metabolic ward: relation to polymorphisms of PAI-1 and angiotensin converting enzyme (ACE) genes. *Arterioscler Thromb Vasc Biol*. 1997;17:2082.
188. Margaglione M, Cappucci G, Colaizzo D, et al. The PAI-1 gene locus 4G/5G polymorphism is associated with a family history of coronary artery disease. *Arterioscler Thromb Vasc Biol*. 1998;18:152.
189. Iwai N, Shimoike H, Nakamura Y, Tamaki S, Kinoshita M. The 4G/5G polymorphism of the plasminogen activator inhibitor gene is associated with the time course of progression to acute coronary syndromes. *Atherosclerosis*. 1998;136:109.
190. Ridker PM, Hennekens CH, Lindpaintner K, Stampfer MJ, Miletich JP. Arterial and venous thrombosis is not associated with the 4G/5G polymorphism in the promoter of the plasminogen activator inhibitor gene in a large cohort of US men. *Circulation*. 1997;95:59.
191. Doggen CJM, Bertina RM, Manger Cats V, Reitsma PH, Rosendaal FR. The 4G/5G polymorphism in the plasminogen activator inhibitor-1 gene is not associated with myocardial infarction. *Thromb Haemost*. 1999;82:115.
192. Gardemann A, Lohre J, Katz N, Tillmanns H, Hehrlein FW, Haberbosch W. The 4G4G genotype of the plasminogen activator inhibitor 4G/5G gene polymorphism is associated with coronary atherosclerosis in patients at high risk for this disease. *Thromb Haemost*. 1999;82:1121.
193. Iacoviello L, Burzotta F, Di Castelnuovo A, Zito F, Marchioli R, Donati MB. The 4G/5G polymorphism of PAI-1 promoter gene and the risk of myocardial infarction: a meta-analysis. *Thromb Haemost*. 1998;80:1029.
194. Catto AJ, Carter AM, Stickland MH, Bamford JM, Davies JA, Grant PJ. Plasminogen activator inhibitor-1 4G/5G promoter polymorphism and levels in subjects with cerebrovascular disease. *Thromb Haemost*. 1997;77:730.
195. Peerschke EIB, Lopez JA. Platelet membranes and receptors. In: Loscalzo J, Schafer AL, eds. *Thrombosis and Hemorrhage*. 2nd ed. Baltimore: Williams & Wilkins; 1998:229.
196. Andrews RK, Shen Y, Gardiner EE, Dong JF, Lopez JA, Berndt MC. The glycoprotein Ib-IX-V complex in platelet adhesion and signaling. *Thromb Haemost*. 1999;82:357.
197. Ruggeri ZM. New insights into the mechanisms of platelet adhesion and aggregation. *Semin Hematol*. 1994;31:229.
198. Bray PF. Inherited diseases of platelet glycoproteins: considerations for rapid molecular characterisation. *Thromb Haemost*. 1994;72:492.
199. Moroi M, Jung SM, Okuma M, Shinmyozu K. A patient with platelets deficient in glycoprotein VI that lack both collagen-induced aggregation and adhesion. *J Clin Invest*. 1989;84:1440.
200. Nieuwenhuis HK, Akkerman JWN, Houdijk WPM, Sixma JJ. Human blood platelets showing no response to collagen fail to express surface glycoprotein Ia. *Nature*. 1985;318:470.
201. Newman PJ. Platelet alloantigens: cardiovascular as well as immunological risk factors. *Lancet*. 1997;349:370.
202. Weiss EJ, Bray PF, Tayback M, et al. A polymorphism of a platelet glycoprotein receptor as an inherited risk factor for coronary thrombosis. *N Engl J Med*. 1996;334:1090.
203. Goldschmidt-Clermont PJ, Shear WS, Schwartzberg J, Varga CF, Bray PF. Clues to the death of an Olympic champion. *Lancet*. 1996;348:485.
204. Ridker PM, Hennekens CH, Schmitz C, Stampfer MJ, Lindpaintner K. PIA1/A2 polymorphism of platelet glycoprotein IIIa and risks of myocardial infarction, stroke and venous thrombosis. *Lancet*. 1997;349:385.
205. Herrmann SM, Poirier O, Marques-Vidal P, et al. The Leu 33/Pro polymorphism (PIA1/PIA2) of the glycoprotein IIIa (GPIIIa) receptor is not related to myocardial infarction in the ECTIM Study. *Thromb Haemost*. 1997;77:1179.
206. Carter AM, Ossei-Gerning N, Wilson IJ, Grant PJ. Association of the platelet P(A) polymorphism of glycoprotein IIb/IIIa and the fibrinogen Bb448 polymorphism with myocardial infarction and extent of coronary artery disease. *Circulation*. 1997;96:1424.
207. Samani NJ, Lodwick D. Glycoprotein IIIa polymorphism and risk of myocardial infarction. *Cardiovasc Res*. 1997;33:693.
208. Marian AJ, Brugada R, Kleiman NS. Platelet glycoprotein IIIa PIA polymorphism and myocardial infarction. *N Engl J Med*. 1996;334:1071.
209. Durante-Mangoni E, Davies GJ, Ahmed N, Ruggiero G, Tuddenham EG. Coronary thrombosis and the platelet glycoprotein IIIa gene PIA2 polymorphism. *Thromb Haemost*. 1998;80:218.
210. Carter AM, Ossei-Gerning N, Grant PJ. Platelet glycoprotein IIIa PIA polymorphism in young men with myocardial infarction. *Lancet*. 1996;348:485.
211. Ardissino D, Mannucci PM, Merlini PA, et al. Prothrombotic genetic risk factors in young survivors of myocardial infarction. *Blood*. 1999;94:46.
212. Gardemann A, Humme J, Stricker J, et al. Association of the platelet glycoprotein IIIa PIA1/A2 gene polymorphism to coronary artery disease but not to nonfatal myocardial infarction in low risk patients. *Thromb Haemost*. 1998;80:214.
213. Mamotte CD, van Bockxmeer FM, Taylor RR. PIA1/A2 polymorphism of glycoprotein IIIa and risk of coronary artery disease and restenosis following coronary angioplasty. *Am J Cardiol*. 1998;82:13.
214. Zotz RB, Winkelmann BR, Nauck M, et al. Polymorphism of platelet membrane glycoprotein IIIa: human platelet antigen Ib (HPA-Ib/PIA2) is an inherited risk factor for premature myocardial infarction in coronary artery disease. *Thromb Haemost*. 1998;79:731.
215. Walter DH, Schachinger V, Elsner M, Dimmeler S, Zeiher AM. Platelet glycoprotein IIIa polymorphisms and risk of coronary stent thrombosis. *Lancet*. 1997;350:1217.
216. Carter AM, Catto AJ, Bamford JM, Grant PJ. Platelet GPIIIa PIA and Gplb variable number tandem repeat polymorphisms and markers of platelet activation in acute stroke. *Arterioscler Thromb Vasc Biol*. 1998;18:1124.
217. Carlsson LE, Greinacher A, Spitzer C, Walther R, Kessler C. Polymorphisms of the human platelet antigens HPA-1, HPA-2, HPA-3 and HPA-5 on the platelet receptors for fibrinogen (GPIIb/IIIa), von Willebrand factor (GPIb/IX) and collagen (GPIa/IIa) are not correlated with an increased risk for stroke. *Stroke*. 1997;28:1392.
218. Gonzalez-Conejero R, Lozano ML, Rivera J, et al. Polymorphisms of platelet membrane glycoprotein Iba associated with arterial thrombotic disease. *Blood*. 1998;92:2771.
219. Kunicki TJ, Orzechowski R, Annis D, Honda Y. Variability of integrin $\alpha 2 \beta 1$ activity on human platelets. *Blood*. 1993;82:2693.
220. Di Paola J, Federici AB, Mannucci PM, et al. Low platelet $\alpha 2 \beta 1$ levels in type I von Willebrand disease correlate with impaired platelet function in a high shear stress system. *Blood*. 1999;93:3578.
221. Santoso S, Kunicki TJ, Kroll H, Haberbosch W, Gardemann A. Association of the platelet glycoprotein Ia C807T gene polymorphism with nonfatal myocardial infarction in younger patients. *Blood*. 1999;93:2449.
222. Croft SA, Hampton KK, Sorrell JA, et al. The GplA C807T dimorphism associated with platelet collagen receptor density is not a risk factor for myocardial infarction. *Br J Haematol*. 1999;106:771.
223. Boushey CJ, Beresford SAA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease: probable benefits of increasing folic acid intakes. *JAMA*. 1995;274:1049.
224. Graham IM, Daly LE, Refsum HM, et al. Plasma homocysteine as a risk factor for vascular disease: the European Concerted Action Project. *JAMA*. 1997;277:1775.
225. Brattstrom L. Common mutation in the methylenetetrahydrofolate reductase gene offers no support for mild hyperhomocysteinemia being a causal risk factor for cardiovascular disease. *Circulation*. 1997;96:3805.
226. Tracy RP. Epidemiological evidence for inflammation in cardiovascular disease. *Thromb Haemost*. 1999;82:826.