Brief Reviews

Genetic Determinants of Arterial Thrombosis

Barbara Voetsch, Joseph Loscalzo

Abstract—Arterial thrombosis is a complex disorder that involves multiple genetic and environmental factors interacting to produce the characteristic phenotype. In the past decades, investigators have focused on the molecular genetics of arterial vascular disorders and have identified numerous polymorphisms and mutations in genes related to the hemostatic system and to enzymes involved in the synthesis and bioavailability of nitric oxide (NO); however, the relation between most polymorphisms and the risk of coronary artery disease, ischemic stroke, and peripheral vascular disease remains highly controversial. In this review, we describe the most common genetic variations involved in the pathogenesis of arterial thrombosis, their functional implications, and their association with disease risk. Specifically, we consider polymorphisms in coagulation factors (fibrinogen, prothrombin, FV Leiden, FVII, and FXIII); fibrinolytic factors (tissue-type plasminogen activator, plasminogen activator inhibitor-1, and thrombin-activatable fibrinolysis inhibitor); platelet surface receptors; methylenetetrahydrofolate reductase; endothelial NO synthase; and the antioxidant enzymes paraoxonase and plasma glutathione peroxidase. Overall, there seems to be a modest contribution of individual genetic variants in the hemostatic and antioxidant systems to the risk of arterial thrombosis. Thus, future research ought to focus on identifying novel genetic determinants and on the interaction of these genetic risk factors with each other and the environment to understand better the pathobiology and susceptibility to arterial thrombotic disease. (Arterioscler Thromb Vasc Biol. 2004;24:216-229.)

Key Words: arterial thrombosis • genetics of cardiovascular disease • coagulation and fibrinolysis • platelets • vascular biology

Arterial thrombosis and its clinical manifestations represent the leading cause of death in the developed world. The pathogenesis of arterial thrombotic disease is complex and involves multiple genetic and environmental factors related to atherosclerosis and thrombosis, as well as their interaction. Classically, acute thrombosis at the site of a ruptured, lipid-rich atherosclerotic plaque is understood as the precipitating event in the transition from stable or subclinical atherosclerotic disease to acute myocardial infarction (MI), ischemic stroke (IS), or peripheral arterial occlusion.1 To date, the prevention of arterial thrombotic disease has consisted of the modification of traditional cardiovascular risk factors that are largely environmental; however, approximately half of all thrombotic events occur in patients without such risk factors,2 and epidemiologic studies increasingly demonstrate that these are insufficient to explain completely the variations in incidence and risk. Twin and sibling studies have shown that inherited risk factors contribute significantly to the development of coronary artery disease (CAD) and IS.3 Thus, in the current era of elucidation of the human genome, investigators have focused on the molecular genetics of thrombosis and atherothrombosis to improve their understanding of the pathobiology of arterial thrombosis, and a range of specific genes contributing to disease risk have been identified. The majority of these are distinct from those involved in venous thrombosis, as these 2 entities have fundamental pathobiologic differences.4

Normal hemostasis is maintained by a careful equilibrium between prothrombotic and antithrombotic processes, which are mediated by cellular components, soluble plasma proteins, and endothelium-derived factors (reviewed by Rosenberg and Aird4). Genetic abnormalities that compromise the production, activity, bioavailability, or metabolism of specific factors can alter this physiologic balance in favor of thrombosis and predispose to premature thromboembolic and atherothrombotic events. In this review, we discuss the role of the most relevant genetic markers associated with arterial thrombosis. These are mutations and polymorphisms in the coding or 5′ regulatory regions of hemostatic factors and enzymes involved in the synthesis and bioavailability of nitric oxide (NO), a principal antithrombotic agent in the vasculature. We describe the functional implications of these genetic variants, as well as their association with disease risk.

Polymorphisms in the Hemostatic System

Fibrinogen

Among the components of the coagulation system, elevated fibrinogen has been most consistently associated with arterial...
independent relative risk of arterial disease is \( \approx 2.0 \) to 2.5 for the highest compared with the lowest quartiles of fibrinogen. Several mechanisms explain the association of increased fibrinogen with arterial thrombotic disease, including increased fibrin formation, blood viscosity, platelet aggregation, and vascular endothelial and smooth muscle cell proliferation. In addition, high fibrinogen concentrations lead to the formation of a fibrin clot with thin and tightly packed fibers that has high thrombogenicity, possibly because the small pore size restricts access of fibrinolytic enzymes. Fibrinogen levels are strongly correlated with traditional vascular risk factors, including age, physical inactivity, hypertension, smoking, and features of the insulin resistance syndrome. Furthermore, fibrinogen is an acute-phase reactant, in part owing to its upregulation via activation of interleukin-6–responsive elements in the promoter of all 3 fibrinogen chains; the acute-phase response arising from viral infection, inflammatory stimuli, and smoking in particular is strongly implicated in the development of arterial disease. Alternatively, elevated fibrinogen might reflect the inflammation associated with atherosclerosis rather than being a causal risk factor.

Genetic factors are estimated to contribute to \( \approx 50\% \) of the total variability in fibrinogen levels. Several polymorphisms have been identified in the genes encoding the 3 pairs of fibrinogen polypeptide chains, \( \alpha, \beta, \) and \( \gamma \); however, because the synthesis of the \( \beta \)-chain is rate-limiting in vitro, most studies have focused on this gene. The main \( \beta \)-chain variants include the Arg448Lys, BcII, \( \sim 148C/T, \sim 455G/A \) (HaeIII), and \( \sim 854G/A \) polymorphisms. The promoter polymorphisms are in strong linkage disequilibrium with each other. The \( \sim 455G/A \) and \( \sim 854G/A \) substitutions are the most physiologically relevant, because the respective alleles have distinct nuclear protein-binding properties, and reporter gene studies in HepG2 cells showed an increased rate of basal transcription in the less common \( \sim 455A \) and \( \sim 854A \) alleles.

Of the \( \beta \)-chain polymorphisms, the \( \sim 455G/A \) has been the most extensively studied clinically. The \( \sim 455A \) genotype is present in \( \approx 10\% \) to 20\% of the population and is correlated with fibrinogen levels that are 10\% higher than in individuals with the \( GG \) genotype. Nevertheless, the relation between the \( \sim 455G/A \) variant and the risk of arterial thrombotic disease is controversial, with some case-control studies, including the Etude Cas-Temoins sur l’Infarctus du Myocarde (ECTIM) study, indicating an association, while other large studies reported none (reviewed by Endler and Mannhalter). In a pooled analysis of inherited hemostatic risk factors and the risk of acute MI, homozygosity for the fibrinogen \( \sim 455A \) allele was significantly though marginally associated with a decreased risk of MI (odds ratio \( [OR] \), 0.66; 95\% confidence interval \( [CI] \), 0.44 to 0.99). In addition, the \( \sim 455A \) allele has been associated with the progression of atheroma. In a recent cohort of elderly patients with stroke, the presence of the \( \sim 455A \) allele was associated with a 2.5-fold increase in risk of multiple lacunar infarcts but not with large-artery strokes; the authors suggested that elevated fibrinogen levels might predispose to the development of thrombosis primarily in small arteries. The association of other \( \beta \)-chain polymorphisms and arterial thrombosis remains unclear.

In addition to the \( \beta \)-chain polymorphisms, a variant in the \( \alpha \)-chain codes for a Thr312Ala substitution within its carboxy-terminal end, a region important for factor XIII–dependent processes, including \( \alpha/\alpha \)-chain cross-linking. Clots generated in vitro in the presence of the Ala312 fibrinogen isoform have more extensive \( \alpha \)-chain cross-linking and in consequence, thicker fibers. Although in 1 study the Ala312 variant had a gene dose–related influence on poststroke mortality rates in subjects with atrial fibrillation, these findings were not confirmed among patients with MI in the ECTIM study. More recent studies indicate that this \( \alpha \)-chain polymorphism has a more relevant role in the pathogenesis of venous thromboembolism. Taken together, these results suggest that Ala312-induced changes in clot structure predispose to embolization in both the arterial and venous vascular systems. The most common fibrinogen polymorphisms are summarized in Table 1.

**Factor VII**

There has been considerable interest in the influence of the vitamin K–dependent factor VII (FVII) on arterial thrombotic disease owing to its role in the initiation of coagulation. Several prospective studies have examined the association of FVII coagulant activity (FVIIc) and atherothrombotic disease and yielded discrepant results. Although the Northwick Park Heart Study found a positive association between FVIIc and CAD that was stronger than that with cholesterol levels, later reports failed to confirm this finding, most likely because of adjustment for other cardiovascular risk factors. Similar to fibrinogen, various environmental factors influence plasma FVII levels, including age, body mass index, and plasma triglycerides. In fact, the correlation between plasma triglyceride levels and FVIIc might explain in part the close association between hypertriglyceridemia and arterial thrombotic disease.

Seven polymorphisms in the FVII gene have been described, which account for \( \approx 30\% \) of the variation in FVII plasma levels: Arg353Gln and the hypervariable region 4 (HVR4) polymorphism are located in the coding region; a decanucleotide insertion at position \(-323\), and the single box pair changes \(-401G/T, -402G/A, -59T/G, \) and \(-32A/C\), are located in the promoter. The most commonly studied variants are the strongly linked Arg353Gln substitution and the HVR4 polymorphism that involves 37-bp repeats in intron 7. In vitro expression studies suggest that the Arg353-encoding allele and the H7 allele (the longest of the 3 HVR4 alleles) produce higher levels of FVII than do their more common counterparts. In clinical studies, however, the effect of these FVII genotypes on FVII plasma levels and FVIIc has not been as clear, and the relative influence of each polymorphism on FVII levels remains to be clarified fully (reviewed by Lane and Grant). The adjacent \(-401G/T\) and
−402G/A promoter polymorphisms both strongly influence the binding properties of nuclear proteins and affect transcription levels in transfected HepG2 cells; the less common −401T allele is associated with reduced basal rates of transcription and plasma FVII antigen levels, whereas the −402A allele has the opposite effect.26 The −401 variant is completely linked with the 323 decanucleotide polymorphism, which might explain the association of the latter with lower plasma FVII concentrations. The 32C and 59G alleles of the most recently identified FVII polymorphisms, −32A/C and −59T/G, led to a 50% to 80% reduction in activity in reporter gene assays in a minimal promoter construct27; however, these genetic variants were identified in women with bleeding disorders, and their role in arterial thrombosis has not been studied.

Few studies have provided evidence of an association between the FVII polymorphisms and arterial thrombotic disease. The most representative is an Italian case-control study of young adults with familial MI: patients with the 353GlnGln or HVR4 H7H7 genotypes had a significant decrease in the risk of MI (OR, 0.08 and 0.22, respectively) in addition to lower levels of both FVII antigen and FVIIc than did those with the Arg353Arg or HVR4 H6H6 genotypes.28 In contrast, most other studies failed to detect any association of these genotypes with the risk of MI or IS.29,30

![Table 1](https://example.com/table1.png)

**Table 1. Summary of the Association of the Most Common Polymorphisms in the Hemostatic System and Arterial Thrombosis**

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Intermediate Phenotype</th>
<th>Association of Phenotype With Disease</th>
<th>Association of Genotype With Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulation factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrinogen β-chain −455 G/A</td>
<td>Elevated plasma fibrinogen levels</td>
<td>Established, possibly not causal</td>
<td>Inconsistent</td>
</tr>
<tr>
<td>Fibrinogen β-chain −854 G/A</td>
<td>Elevated plasma fibrinogen levels</td>
<td>Established, possibly not causal</td>
<td>Inconsistent</td>
</tr>
<tr>
<td>Fibrinogen β-chain Bcl</td>
<td>Elevated plasma fibrinogen levels</td>
<td>Established, possibly not causal</td>
<td>Inconsistent</td>
</tr>
<tr>
<td>Fibrinogen α-chain Thr312Ala</td>
<td>Influences α-chain cross-linking and clot stability</td>
<td>Suggestive</td>
<td>Inconsistent</td>
</tr>
<tr>
<td>FVII Arg353Gln</td>
<td>Elevated plasma FVII levels in vitro</td>
<td>Inconsistent</td>
<td>Inconsistent, possibly only in selected patients</td>
</tr>
<tr>
<td>FVII HVR4</td>
<td>Elevated plasma FVII levels in vitro</td>
<td>Inconsistent</td>
<td>Inconsistent, possibly only in selected patients</td>
</tr>
<tr>
<td>FVII −401G/T, −402G/A</td>
<td>Affects basal levels of FVII transcription</td>
<td>Inconsistent</td>
<td>Unknown</td>
</tr>
<tr>
<td>FV Leiden</td>
<td>Activated protein C resistance</td>
<td>Inconsistent</td>
<td>Possibly only in selected patients and in combination with environmental risk factors</td>
</tr>
<tr>
<td>Prothrombin 20210G/A</td>
<td>Elevated plasma prothrombin levels</td>
<td>Unknown</td>
<td>Possibly only in selected patients and in combination with environmental risk factors</td>
</tr>
<tr>
<td>FXII Val34Leu</td>
<td>Increased FXIII activation by thrombin</td>
<td>Suggestive</td>
<td>Inconsistent protective effect</td>
</tr>
<tr>
<td>Platelet receptors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPIa Leu33Pro</td>
<td>Increased sensitivity to aggregation?</td>
<td>Unknown</td>
<td>Inconsistent, possibly only in selected patients</td>
</tr>
<tr>
<td>GPIb α VNTR</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Inconsistent</td>
</tr>
<tr>
<td>GPIb α Thr145Met</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Inconsistent</td>
</tr>
<tr>
<td>GPIa/IIa α2 807C/T</td>
<td>Increased receptor density</td>
<td>Unknown</td>
<td>Inconsistent, possibly only in selected patients</td>
</tr>
<tr>
<td>GPIa/IIa α2 1648A/G</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Fibrinolytic system</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAI-1 −675 4G/5G</td>
<td>Elevated plasma PAI-1 levels</td>
<td>Relatively consistent</td>
<td>Inconsistent, possibly only in combination with environmental risk factors</td>
</tr>
<tr>
<td>PAI-1 (CA)m</td>
<td>Possibly elevated plasma PAI-1 levels</td>
<td>Suggestive</td>
<td>None as yet</td>
</tr>
<tr>
<td>PAI-1 HinDIII</td>
<td>Possibly elevated plasma PAI-1 levels</td>
<td>Suggestive</td>
<td>None as yet</td>
</tr>
<tr>
<td>t-PA Alu insertion/deletion</td>
<td>Increases t-PA release, probably through linkage disequilibrium with −7351C/T</td>
<td>Paradoxical</td>
<td>Possible</td>
</tr>
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<td>t-PA −7351C/T</td>
<td>Increases t-PA release</td>
<td>Paradoxical</td>
<td>Possible</td>
</tr>
<tr>
<td>TAFI Ala147Thr</td>
<td>Elevated plasma TAFI antigen levels</td>
<td>Suggestive</td>
<td>Inconsistent</td>
</tr>
<tr>
<td>TAFI 1542C/G</td>
<td>Elevated plasma TAFI antigen levels</td>
<td>Suggestive</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

Abbreviations are as defined in text.

Modified with permission from Lane DA, Grant PJ. Role of hemostatic gene polymorphisms in venous and arterial thrombotic disease. Blood. 2000;95:1527.
Several polymorphisms have been identified in the A subunit, the functionally most relevant of which codes for a valine-to-leucine substitution at position 34 of the catalytic subunits (A subunits) and 2 nonenzymatic subunits (B subunits).32 Several polymorphisms have been identified in the A subunit, the functionally most relevant of which codes for a valine-to-leucine substitution at position 34 of the activation peptide.33 Located only 3 amino acid residues from the thrombin cleavage site, residue 34 plays a critical role in the interaction between FXIII and thrombin. The less common 34Leu isoform is activated more rapidly, with a 2.5-fold higher catalytic efficiency and shortened clot-formation time when compared with its 34Val counterpart.34 More rapid activation influences fibrin formation and molecular structure of the fibrin clot: fibrin cross-linked in the presence of the 34Leu isoform does not aggregate laterally, generating clots that consist of thinner fibers, smaller pores, and ultimately, a finer meshwork with altered permeation characteristics.34

Surprisingly, of the recent clinical studies that reported an association between FXIII Val34Leu and arterial thrombosis, most determined that carriers of the 34Leu allele had a decreased risk of MI and IS (reviewed by Ariens and colleagues31). Lim and colleagues35 recently proposed that this apparently paradoxical effect might be due to complex gene-gene or gene-environment interactions. For example, the effect of the Val34Leu polymorphism on clot permeability is modulated by fibrinogen levels, as shown in Figure 1. This helps explain the observation in clinical studies that the presence of the 34Leu allele attenuates the adverse effect of smoking on the risk of cerebrovascular disease36 and MI.37 In addition, the protective effect might reflect alternate activities of FXIII, such as the promotion of angiogenesis.38

**Factor XII**

As described earlier, after activation by thrombin, FXIII catalyzes the formation of covalent bonds between the α- and γ-chains of adjacent fibrin monomers, thereby stabilizing the fibrin clot. The plasma FXIII heterotetramer consists of 2 catalytic subunits (A subunits) and 2 nonenzymatic subunits (B subunits).32 Several polymorphisms have been identified in the A subunit, the functionally most relevant of which codes for a valine-to-leucine substitution at position 34 of the activation peptide.33 Located only 3 amino acid residues from the thrombin cleavage site, residue 34 plays a critical role in the interaction between FXIII and thrombin. The less common 34Leu isoform is activated more rapidly, with a 2.5-fold higher catalytic efficiency and shortened clot-formation time when compared with its 34Val counterpart.34 More rapid activation influences fibrin formation and molecular structure of the fibrin clot: fibrin cross-linked in the presence of the 34Leu isoform does not aggregate laterally, generating clots that consist of thinner fibers, smaller pores, and ultimately, a finer meshwork with altered permeation characteristics.34

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**Factor V/Prothrombin**

The role of the Factor V 1691G/A (FV Leiden) and the prothrombin 20210G/A polymorphisms in arterial thrombotic disease has been examined in numerous studies, and most have yielded negative results, even among patients who suffered vascular events at a young age.39 The studies that have shown an association of these variants with CAD, MI, or IS either have been performed in highly selected populations40 or among children41 or have considered interactions with environmental risk factors. Rosendaal and colleagues42 reported an increased risk of nonfatal MI among young women who carried FV Leiden, with an OR of 2.4. The mutation had little effect in nonsmokers, whereas it led to a significant increase in risk among smokers (OR, 3.6; 95% CI, 0.9 to 14.4), resulting in a 32-fold higher risk of MI in smokers who also carried FV Leiden compared with noncarriers who did not smoke. Furthermore, carriers of the prothrombin 20210A allele had a 4-fold increase in the risk of MI that was again increased >40-fold in smokers.43 In a combined analysis of FV Leiden and the prothrombin 20210A allele in this population, the effect of major coronary risk factors was enhanced 4- to 6-fold by the presence of one of these inherited, prothrombotic risk factors. These interactions were confirmed in 2 case-control studies of men who had an increased risk of MI associated with FV Leiden and the prothrombin 20210G/A variant that was most pronounced in the presence of other cardiovascular risk factors.44,45

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**Figure 1.** Complex gene-environment interactions modulate vascular risk. Environmental risk factors (smoking, physical inactivity, inflammation) interact with polymorphisms in the fibrinogen-chain genes and determine fibrinogen levels. The FXIII Val34Leu polymorphism further modulates the effect of fibrinogen levels on clot structure; thus, the fibrinogen and FXIII genotypes in combination influence clot formation. Clot permeability is strongly inversely correlated with fibrinogen levels; however, at high fibrinogen concentrations, samples from carriers of the FXIII Val/Val genotype generate clots with denser fibers and a thinner meshwork (with higher thrombogenicity) than clots from homozygous carriers of the Leu allele, revealing a protective effect of the 34Leu allele only above critical fibrinogen levels. (Modified with permission from Lim et al.35)
Thrombomodulin
Thrombomodulin is an endothelial cell surface receptor for thrombin that accelerates thrombin-induced activation of protein C. In a large, prospective, case-control study, decreased plasma thrombomodulin levels were associated with an increased risk of MI.46 Two polymorphisms have been identified in the thrombomodulin gene that code for Ala455Val and Ala25Thr amino acid substitutions. One report suggested an association between the former and MI47 but this has not been confirmed. In the Study of Myocardial Infarctions Leiden (SMILE), the Ala25Thr substitution was found to increase the risk of MI, particularly in smokers (OR, 8.8; 95% CI, 1.8 to 42.2)46; however, there is no biologic evidence that the 25Thr isoform alters protein function.

Platelet Surface Receptors
Glycoprotein IIb/IIIa
Surface membrane glycoproteins (GPs) are essential for adhesion of platelets to exposed subendothelial extracellular matrix components and for platelet-platelet interactions. GPIIb/IIIa (known also as integrin αIIbβ3) is the primary platelet surface receptor for fibrinogen; it also binds von Willebrand factor (vWF) and several other adhesion ligands. The clinically most studied polymorphism in this complex lies in the GPIIIa subunit: a common T/C polymorphism at position 1565 in exon 2 of the GPIIIa gene leads to a substitution of proline for leucine at amino acid 33 (Leu33Pro), resulting in a conformational change in the amino-terminal disulfide loop important for fibrinogen binding.49 This polymorphism is found in ~15% of whites and 5% to 8% of blacks but is virtually absent in Asians; the more common GPIIIa isoform is known as PLA1 (HPA-1a) and the 33Pro allele, as PL A2 (HPA-1b).50 The homozygous A2/A2 form is known to be associated with posttransfusional purpura and neonatal alloimmune thrombocytopenia, conditions in which alloantibodies are formed against the A1 allele. In 1996, Weiss and colleagues51 first published an association of PL A2 with the risk of acute coronary thrombosis, which was strongest in a small subgroup of patients under the age of 60 years, with a relative risk of 6.2. Possible mechanistic associations that were postulated between the PL A2 polymorphism and arterial thrombosis include increased sensitivity of platelet aggregation by various agonists and altered sensitivity to aspirin. Several subsequent studies analyzed the role of the PL A2 allele in CAD and IS, some of which confirmed an association,52,53 but the majority did not. Most of these negative studies, such as the prospective US Physicians’ Health Study54 and the ECTIM study,55 included a large number of patients and controls and also attempted to confirm the findings of Weiss and colleagues51 in subgroup analyses regarding age, without success. In contrast, a case-control study of 200 young survivors of MI found a modest but significant 1.8-fold increase in risk among carriers of the PL A2 allele, which was increased further to 13.7 in carriers who smoked.56 The authors concluded that almost 50% of premature MIs were attributable to the interaction between these 2 risk factors.

Glycoproteins Ia/IIa and Ib/IX/V
GPIa/IIa (integrin αIIbβ3) is the major platelet collagen receptor and is responsible for platelet adhesion to the exposed vessel wall; the GPIb/IX/V complex is the main receptor for vWF. In the gene encoding the GPIbα subunit, a length polymorphism with a variable number of tandem repeats of 39 bp and a linked C/T polymorphism at nucleotide 3550 that leads to a Thr145Met substitution have been of most interest.57 A small number of reports have inconsistently correlated these genetic variants with CAD and IS, particularly in younger patients.56,58 A silent exonic C/T nucleotide substitution at position 807 in the gene coding for the GPIa/IIa α2-peptide has been found to increase receptor density and might be associated with MI, again in younger patients.59 Finally, a second polymorphism in the α2-peptide gene, A1648G, which causes a Glu505Lys substitution, is another recent candidate for an association with vascular disease, although a functional correlation has yet to be identified.

Fibrinolytic System
Tissue-type plasminogen activator (t-PA) is the main endothelium-derived activator of the fibrinolytic system; the major inhibitor of t-PA is plasminogen activator inhibitor-1 (PAI-1). Elevated levels of both t-PA and PAI-1 have been associated with an increased risk of arterial thrombotic disease with relative consistency.23 Negative studies might have resulted from adjustment for confounding vascular risk factors, mainly diabetes mellitus, hypertriglycerideremia, and obesity. PAI-1 exists in the circulation in great excess over t-PA to prevent systemic bleeding while permitting local clot lysis; thus, most circulating t-PA is inactive and complexed with PAI-1. There is a moderate, positive correlation between t-PA and PAI-1 levels (r=0.65), which can complicate the interpretation of fibrinolytic assays and explain the apparently paradoxical association of elevated t-PA levels with cardiovascular disease.39 An imbalance of this fibrinolytic equilibrium is encountered primarily in the insulin resistance syndrome, which leads to increased plasma PAI-1 and t-PA antigen levels (reflecting inactive t-PA/PAI-1 complexes) with a consequent decrease in fibrinolytic activity.60

Plasminogen Activator Inhibitor-1
The most frequently studied of the PAI-1 genetic variants is the 4G/5G insertion/deletion polymorphism located at position −675 of the promoter.61 The 4G allele has been correlated with higher levels of gene transcription and elevated PAI-1 plasma levels compared with its more common 5G counterpart. Interestingly, this promoter site has genotype-specific responses to triglycerides, which leads to the highest levels of PAI-1 in carriers of the 4G/4G genotype who are also hypertriglycerideremic.62 This interaction is explained by a triglyceride-responsive region that has been identified adjacent to the 4G/5G site.63 Although several case-control studies have demonstrated an increased risk of MI, CAD, and IS in carriers of the 4G allele, these findings have not been confirmed in several larger studies (reviewed by Simmonds and colleagues64). A meta-analysis of 9 studies that included ~1500 cases and >2000 controls yielded an overall slight increase in the risk of MI associated with the 4G
allele (OR, 1.23; 95% CI, 1.04 to 1.45), which was, however, confined to subgroups of high-risk populations. In addition to the promoter 4G/5G polymorphism, a CA<sub>n</sub> dinucleotide repeat in intron 3 and a 3′ HindIII site have been identified, for which no role in arterial thrombosis has yet been established.

**Tissue-Type Plasminogen Activator**

Of the several nucleotide sequence changes that have been identified in the t-PA gene, the most studied is a 311-bp Alu insertion/deletion in intron 8. In a population-based cohort study of almost 8000 subjects, the presence of one insertion allele was associated with an ≈50% increase in risk of MI, whereas homozygous carriers had a >2-fold adjusted increase in risk, suggesting an association between the number of Alu repeats and arterial thrombosis. These results, however, were not confirmed in later studies, including the US Physicians’ Health Study. In addition, the studies found no differences in mean plasma levels of t-PA activity or antigen between carriers of different genotypes.

It has been proposed that local endothelial release rate of t-PA, rather than the steady-state plasma concentration, determines the thrombolytic potential. Net release rates of t-PA in healthy subjects vary markedly between individuals and are in part genetically determined. Jern and colleagues found higher forearm vascular release rates of t-PA in subjects homozygous for the Alu insertion compared with both heterozygotes and homozygotes for the deletion. This prompted the search for other putative genetic variations in the t-PA gene, because it is unlikely that an intronic polymorphism should have a direct effect on protein production. Eight novel polymorphisms were identified, 3 of which were in strong linkage disequilibrium with the Alu polymorphism and consequently, associated with t-PA release: −7351C/T in the upstream enhancer, 20 099T/C in exon 6, and 27 445T/A in intron 10. The −7351 promoter polymorphism is located within an Sp1 binding site, whereas the coding region polymorphisms are silent. A population-based, prospective, case-control study found a >2.5-fold increase in risk of MI among carriers of the −7531T allele, which is in accordance with its reduced binding affinity to Sp1.

**Thrombin-Activatable Fibrinolysis Inhibitor**

Thrombin-activatable fibrinolysis inhibitor (TAFI) is a recently described plasma carboxypeptidase involved in regulating fibrinolysis by removing carboxy-terminal lysine and arginine residues from fibrin, thereby decreasing plasminogen binding to its surface. Activation of TAFI occurs by the thrombin-thrombomodulin complex and results in prolongation of clot lysis time. Both animal and human studies support a physiologic role for this plasma protein in the modulation of fibrinolysis; elevated plasma TAFI levels have been associated with an increased risk of both deep-vein thrombosis and symptomatic or angiographic CAD. In contrast, Juhan-Vague and colleagues found that a TAFI antigen level above the 90th percentile was protective against MI (OR, 0.55; 95% CI, 0.34 to 0.91).

Plasma TAFI concentrations demonstrate high interindividual variability that is poorly explained by environmental factors. In the past few years, several polymorphisms have been described in the TAFI gene, 9 in the promoter region, 2 in the 3′ untranslated region, and 3 in the coding region. Except for one silent coding-region polymorphism, all others are associated with plasma TAFI antigen levels. These polymorphisms are in strong linkage disequilibrium and form 4 main haplotypes. In a multivariate analysis, the Ala147Thr and 1542C/G polymorphisms in combination showed the strongest influence (>60%) on TAFI level variability; however, the mechanism for this effect is not yet understood. In fact, a recent segregation-linkage analysis suggested that these polymorphisms were only markers in linkage disequilibrium with unidentified TAFI-linked quantitative trait loci. To date, only the Ala147Thr polymorphism has been associated with CAD; however, in one study, carriers of the 147 Thr/Thr isoform had an almost 3-fold increase in relative risk of angina pectoris, whereas in another, the 147Thr allele conferred a protective effect against MI.

**Hyperhomocysteinemia**

Extensive epidemiologic evidence has consistently indicated that elevated levels of homocysteine, a sulfur-containing amino acid formed as an intermediary compound during methionine metabolism, is an independent risk factor for atherosclerosis and arterial thrombosis. The adverse effects of homocysteine are manifold but ultimately lead to endothelial dysfunction with associated platelet activation and thrombus formation. Although severe hyperhomocysteinemia as caused by inborn errors of metabolism, such as cystathionine β-synthase deficiency, is rare, mild to moderate elevations in homocysteine levels can be due to homozgyosity of a common 677C/T point mutation in the coding region of the methylenetetrahydrofolate reductase (MTHFR) gene or by nutritional deficiencies in the vitamin cofactors required for homocysteine metabolism (folate, vitamin B<sub>12</sub>, and vitamin B<sub>6</sub>). An inverse correlation between serum levels of these cofactors and homocysteine has been clearly documented.

Despite constituting the most common genetic cause of mild to moderate hyperhomocysteinemia, homozgyosity for the MTHFR 677T allele, which leads to the substitution of valine by alanine in a potential folate-binding site and thermolability of the enzyme, has not been clearly associated with atherothrombotic disease. Studies in certain populations have shown a >3-fold increase in risk of CAD and IS associated with the MTHFR 677T genotype; others, however, found no association (reviewed by Fletcher and Kessler). Brattstrom and colleagues pooled the results of 12 case-control studies and determined that individuals homozygous for the MTHFR 677T allele generally had fasting homocysteine levels that were ≈2 to 4 μmol/L higher than in heterozygous or normal individuals. Nevertheless, 8 of these studies did not report an increased risk of cardiovascular disease associated with the homozygous MTHFR 677TT genotype, and the authors concluded that this polymorphism is, at most, a modest risk factor for arterial thrombosis. Similar results were obtained in a recent meta-analysis of 40 observational studies involving >11 000 CAD patients and 12 000 controls: the overall OR...
TABLE 2. Summary of the Association of Other Genetic Risk Factors and Arterial Thrombosis

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Intermediate Phenotype</th>
<th>Association of Phenotype With Disease</th>
<th>Association of Genotype With Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperhomocysteinemia</td>
<td>Mild to moderate hyperhomocysteinemia</td>
<td>Established</td>
<td>Possibly in patients with low folate levels</td>
</tr>
<tr>
<td>MTHFR 677C/T</td>
<td>eNOS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intron 4 (27)n/(27)m</td>
<td>Possible reduction in nitrate/nitrite</td>
<td>Suggestive</td>
<td>Inconsistent, possibly only in certain ethnic groups</td>
</tr>
<tr>
<td>Intron 13 (CA)n</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Inconsistent</td>
</tr>
<tr>
<td>−786T/C, −922A/G, −1468T/A</td>
<td>Reduced eNOS expression</td>
<td>Suggestive</td>
<td>Inconsistent, possibly only in certain ethnic groups</td>
</tr>
<tr>
<td>Exon 7 Glu298Asp</td>
<td>Alters primary structure of protein</td>
<td>Suggestive</td>
<td>Inconsistent, possibly only in certain ethnic groups</td>
</tr>
<tr>
<td>PON1</td>
<td>192Q/R</td>
<td>Elevated PON1 levels and activity</td>
<td>Suggestive</td>
</tr>
<tr>
<td>55L/M</td>
<td>Elevated PON1 levels and activity</td>
<td>Suggestive</td>
<td>Inconsistent</td>
</tr>
<tr>
<td>−107C/T, −824G/A</td>
<td>Increased PON1 gene expression</td>
<td>Suggestive</td>
<td>Suggestive (few studies)</td>
</tr>
<tr>
<td>GPx-3</td>
<td>Gpx-3 −68A/T, −622A/T, −688T/C, −703A/C</td>
<td>Presumably reduced plasma GPx-3 levels</td>
<td>Suggestive</td>
</tr>
</tbody>
</table>

Abbreviations are as defined in text.

for carriers of the 677TT genotype was 1.16 (95% CI, 1.05 to 1.18); most studies, however, did not yield statistically significant risk estimates. The heterogeneity of findings was interpreted as being secondary to the interaction of the MTHFR 677CT polymorphism and folate status. Evidence from previous reports supports this hypothesis: Jacques and colleagues determined that among individuals homozygous for the MTHFR 677T allele, homocysteine levels were only elevated when plasma folate concentrations were low (<15.4 nmol/L), yet there was no significant difference in plasma homocysteine levels between 677TT homozygotes who had normal folate concentrations and individuals with the 677CC genotype. Similarly, in the US Physician’s Health Study, homozygotes with the lowest plasma folate concentrations had the highest homocysteine levels. Thus, folate levels can confound the interpretation of genotype association studies. The hypothesis that folate supplementation might be a means of preventing atherothrombotic events in these patients is currently being assessed in prospective trials.

A second common variant in MTHFR 1298A/C is associated with decreased enzyme activity in vitro and in vivo, especially when occurring simultaneously with the 677 C/T polymorphism. Fasting homocysteine is significantly higher in individuals heterozygous for both substitutions compared with individuals who carry only the 677CT variant. These polymorphisms are in linkage disequilibrium, rarely forming homozygous states. They are functional, with the −1298C allele having a lower enzyme activity. The role of the MTHFR 677/1298 haplotypes in arterial thrombosis has not been studied.

**Endothelial NO Synthase**

NO, a product of the normal endothelium, has a variety of physiologic effects that converge to maintain normal endothelial function and an antithrombotic intravascular milieu. NO is a smooth muscle relaxant and regulator of vascular tone; it limits vascular smooth muscle cell proliferation and leukocyte adhesion to the endothelium; it is a scavenger of reactive oxygen species (ROS); and it inhibits the adhesion, activation, and aggregation of platelets. NO-dependent endothelial dysfunction is now accepted as a key initial step in atherothrombogenesis. NO is produced in the vasculature by the constitutive endothelial isoform of the nitric oxide synthases (NOSs) as a by-product of the conversion of arginine to citrulline. The endothelial NOS gene (eNOS) is located on chromosome 7q35–36 and comprises 26 exons.

Numerous polymorphic sites have been identified in the eNOS gene, most of which are intronic. The clinically most relevant eNOS variants are listed in Table 2. Two distinct variable nucleotide tandem repeats (VNTRs) in introns 4 and 13 have been examined in association with vascular disease. The intron 4 VNTR is characterized by the presence of either 4 (minor allele) or 5 (major allele) copies of a 27-bp repeat. A mild but significant reduction in plasma levels of nitrogen oxides had been observed in homozygotes of the minor allele. A few Japanese studies have found an association of the minor allele with MI; however, this association has not been confirmed in other populations. In intron 13, between 17 to 44 copies of a CA repeat have been described, and the presence of a minimum of 38 repeats has been associated with an independent 2.2-fold increase in the risk of CAD in one study.

Three strongly linked, single base pair changes, −786T/C, −922A/G, and −1468T/A, have been identified in the promoter region of the eNOS gene and associated with coronary spasm in the Japanese population. The authors determined that the substitution at position −786 resulted in a significant reduction in eNOS gene promoter activity, as assessed by luciferase reporter gene assays, whereas the other 2 polymorphisms had no effect. Japanese carriers of the −786CC genotype have also been shown to have reduced cerebral blood flow, yet this effect was only observed among smokers. Recently, 2 Italian studies found an association of
the −786C allele with angiographically defined CAD, as well as with endothelial dysfunction, among hypertensive individuals, as measured by forearm flow-mediated dilation99,100; however, numerous other studies have failed to confirm these observations.

A G/T base-pair change at position 894 in exon 7 predicts a Glu298Asp substitution, which is the only polymorphism that alters the primary structure of the protein.101 This amino acid change influences enzyme stability, the 298Asp isoform being degraded more rapidly than its 298Glu counterpart. Numerous studies have attempted to find a role for this polymorphism in atherothrombotic disease, and similar to findings with the promoter −786 polymorphism, most positive studies have been carried out in the Japanese population, where an ∼2-fold increase in risk of MI and hypertension was found in carriers of the 298Asp isoform.

**Antioxidant Enzymes**

**Paraoxonase**

Serum paraoxonase (PON1) is a calcium-dependent esterase synthesized by the liver and bound exclusively to HDL in plasma. Until the 1990s, this enzyme was of interest mainly to toxicologists, owing to its ability to detoxify organophosphate insecticides and nerve gases. Recently, a role for PON1 in the pathogenesis of atherosclerosis and arterial thrombosis has been proposed. PON1 has been shown to preserve HDL function and to protect LDL from oxidative modification by hydrolyzing lipid peroxides.102 In addition, PON1 protects against the induction of monocyte-endothelial interactions in the artery wall by metabolizing biologically active lipids in oxidized LDL.103 Both peroxidation of LDL and the secondary inflammatory responses are key steps in the initiation of atherogenesis. Thus, PON1 is now thought to be responsible, at least in part, for the cardioprotective properties of HDL. Furthermore, Jakubowski104 demonstrated that PON1 has the ability to metabolize homocysteine thiolactone and is in fact, biochemically identical to homocysteine thiolactonase. Homocysteine thiolactone is a metabolite of homocysteine thiolactone and adversely affects endothelial function by reacting with lysine residues in proteins and impairing their activities. Thus, decreased PON1 levels might be associated with increased protein homocysteinylination. In fact, HDL and LDL are vulnerable to homocysteinylination, rendering them resistant to the protective effect of PON1 and more prone to be taken up by subendothelial macrophages.105 Conversely, in a murine model of hyperhomocysteinemia (cystathionine β-synthase–deficient mice), the expression of PON1 in the liver was downregulated 3-fold.106 PON1-deficient mice are more susceptible to diet-induced atherosclerosis than are their wild-type littermates, and HDL isolated from these animals is unable to prevent LDL oxidation in vitro.107

Clinical reports have demonstrated that PON1 activity is reduced in patients with acute MI, hypercholesterolemia, and diabetes mellitus.108 There is a 10- to 40-fold interindividual variability in serum PON1 activity, as measured by rates of hydrolysis of the exogenous substrate paraoxon. Two common polymorphisms in the coding region of the PON1 gene, which lead to a Gln/Arg substitution at position 192 and a

![Figure 2. The association among GPx-3, peroxides, and NO. GPx-3 reduces H2O2 and lipid peroxides (LOOH) to water and the corresponding lipid alcohols (LOH), respectively, with glutathione (GSH) as a source of reducing equivalents. If GPx-3 activity is reduced, these peroxides can then react with NO or transition metals, ultimately leading to the formation of nitrous acid (HNO2) or lipid peroxynitrite (LOONO). (Reproduced with permission from Loscalzo. Nitric oxide insufficiency, platelet activation, and arterial thrombosis. Circ Res. 2001;88:756–762.)](image)

Leu/Met substitution at position 55, independently influence PON1 activity.108 In addition, 5 polymorphic sites in the promoter have been described recently: −107C/T, −126G/C, −160G/A, −824G/A, and −907G/C.109 Reporter gene constructs and genotyping studies in healthy populations established that, in particular, the −107C/T and −824G/A substitutions have a strong impact on gene expression and serum concentrations of the enzyme. Several case-control studies have investigated the association between the PON1 coding-region polymorphisms and CAD, yielding conflicting results. Although some reports have shown an increased susceptibility to CAD, carotid intima-media thickness, and IS among carriers of the 192R allele, with risk estimates ranging between 1.7 and 8.8, others have reported a lack of association.110,111 Homozygosity for the PON1 55L allele has also been demonstrated to increase the risk of CAD independently,112 yet results of several later studies failed to confirm this association. With respect to the promoter polymorphisms, 2 studies have analyzed the prevalence of the −107C/T substitution in CAD and found that the −107TT low-expressor genotype is associated with a moderately increased risk in selected populations.113,114 We have investigated the role of the promoter polymorphisms in a group of young patients with IS and found that although the −107T allele had a modest influence on the risk of IS when analyzed individually, its interaction with the paraoxonase 192RR genotype led to a 17-fold increase in risk compared with individuals carrying neither variant.115

**Plasma Glutathione Peroxidase**

There is strong evidence indicating that antioxidant status is crucial in normal platelet function and the prevention of thrombosis.116 Plasma glutathione peroxidase (GPx-3), the only extracellular member of the GPx family, scavenges ROS produced during normal metabolism or after oxidative insult, as shown in Figure 2. As a major antioxidant enzyme in plasma, GPx-3 maintains the bioavailability of NO in the vasculature. Deficiencies of the cellular and plasma isoforms of GPx have been associated with CAD in clinical reports.117–119 Friedman and colleagues120 studied the plasmas of 2 brothers with idiopathic childhood stroke and no known hypercoagulable defect and proposed a novel prothrombotic mechanism. Both children had hyperreactive platelets, as determined by whole-blood platelet aggregometry and flow cytometric measurements of platelet surface expression of P-selectin, re-
Reduced plasma NO concentrations, decreased platelet cGMP content, and increased plasma H$_2$O$_2$ generation. Mixing experiments showed that the patients’ platelets behaved normally in control plasma; however, control platelets resuspended in the patients’ plasmas were not inhibited by exogenous NO donors. Further investigation of the brothers’ plasmas revealed that these effects were a consequence of a reduction in GPx-3 activity. The investigators proposed that a deficiency of GPx-3 reduces bioavailable NO, owing to the impaired metabolism of ROS, and increases the risk of thrombosis. Interestingly, the reduction in GPx-3 activity was also present in the clinically unaffected mother. In a second study including 7 families with childhood stroke, Kenet and colleagues confirmed the familial distribution of these observations, suggesting a heritable trait.

In an attempt to identify the molecular basis of this defect, we recently studied the entire GPx-3 gene of young IS patients and healthy age- and gender-matched controls by single-strand conformational polymorphism analysis and sequencing of fragments with electrophoretic shifts. We identified 4 novel, linked polymorphisms in the promoter of the GPx-3 gene: H1100268AT, H11002622A/T, H11002688T/C, and H11002703A/C. Carriers of the haplotype combining nucleotides H1100268T, H11002622T, H11002688C, and H11002703C had a 2-fold increase in risk of IS compared with noncarriers (OR, 2.1; 95% CI, 1.1 to 3.9). These risk estimates remained unchanged after adjustment for inherited prothrombotic and conventional vascular risk factors. Interestingly, in individuals simultaneously exposed to vascular risk factors that enhance oxidative stress, such as smoking and hypertension, the risk...
associated with the GPx-3 polymorphisms was amplified >4-fold. Expression studies showed that basal activity of the risk haplotype was lower than that of the more common haplotype, especially under hypoxic condition.\(^{123}\) Studies are currently underway in our laboratory to characterize further the functional effect of the GPx-3 haplotypes.

**Potential Novel Genetic Markers**

Novel polymorphisms associated with arterial thrombotic disease are continually being identified. A recent large-scale association study that examined 112 polymorphisms of 71 candidate genes in almost 3000 Japanese patients with MI found a significant association with a 1019C/T polymorphism in the human gap junctional protein connexin 37 gene in men and a \(-1171\) 5A/6A repeat polymorphism in the metalloproteinase stromelysin-1 gene in women, in addition to the earlier described \(-675\) 4G/5G polymorphism in the PAI-1 gene.\(^{124}\) The connexin 37 \(1019C/T\) allele has previously been associated with atherosclerotic plaque formation\(^{125}\); however, the risk allele in that study was the C allele, whereas Yamada and colleagues\(^{124}\) found the T allele to confer risk.

Increased levels of P-selectin have been observed in various arterial thrombotic disorders\(^{126}\) and have been shown to be predictive of future vascular events in healthy women.\(^{127}\) Two common substitutions in the 5′ flanking region \((-2123C/G\) and \(-1969A/G\)) and a Thr715Pro polymorphism that might affect mRNA stability have been described.\(^{128}\) Although these polymorphisms are correlated strongly with P-selectin levels, most association studies with arterial thrombotic disease have been negative.\(^{129}\)

The NADPH oxidase system is a major source of the ROS superoxide in the vasculature. This complex comprises a group of heteromeric, membrane-associated enzymes, one of which is p22 PHOX. Of 3 polymorphisms that have been reported in the coding region of this enzyme,\(^{129}\) a 242C/T variant that predicts a histidine-to-tyrosine substitution at residue 72 near a putative heme-binding site has been implicated in arterial vascular disease. The few studies that analyzed the role of this polymorphism in arterial disease and endothelial dysfunction had conflicting results; most were negative, but one recent study reported a protective effect of the 242T allele among patients with CAD,\(^{130}\) while another found an \(\approx\)2-fold increase in risk of IS associated with the T allele.\(^{131}\)

**Conclusion**

The past decade has been marked by rapidly expanding efforts to characterize the genetic basis of arterial thrombotic disease. Numerous polymorphisms have been identified; however, as described in this review and summarized in Tables 1 and 2, the relation between most polymorphisms and disease is controversial. In contrast to venous thromboembolic disease, wherein the role of certain thrombophilia markers is well established, there is little clarity in relation to arterial thrombotic disease. Although early reports in the literature revealed positive associations, numerous negative studies have followed, and the initial hope that inherited risk factors might contribute significantly to the development of atherothrombotic disease remains largely unconfirmed. Perhaps the most striking aspect of several studies is the inconsistency in associating the genotype with the intermediate phenotype and clinical outcome, as shown in Tables 1 and 2. The most consistent associations have been found for fibrinogen. Nevertheless, there is currently no recommendation to screen for any of the herein discussed genetic markers, because there is no evidence to indicate any prognostic or therapeutic consequence, either within the general population or among subgroups of individuals (eg, patients who develop premature atherothrombotic disease).\(^{39}\)

The results of genetic association studies should be interpreted cautiously, taking several issues into consideration (reviewed by Cardon and Bell\(^{132}\)). First, the study design and characteristics of the patient and control groups vary greatly among different reports; eg, among reports on cerebrovascular disease, some authors included patients with transient ischemic attacks, whereas others limited the study population to patients with a neuroimaging study showing an ischemic lesion appropriate to their symptoms. Similarly, some investigators analyzed carotid intima-media thickness while others only enrolled cases with severe carotid stenosis. Different clinical end points render the comparisons difficult, because the pathobiology of these disorders have subtle yet relevant specific differences. Examination of homogeneous patient populations and well-defined clinical outcomes should enhance the ability to detect true associations.

Second, many of these polymorphisms or mutations have low prevalences in the general population. As a result, large sample sizes are required to provide statistical power to demonstrate an effect, particularly when subgroup analyses are performed, and when the interaction of genetic susceptibility markers with other genetic and environmental factors is assessed. Third, the prevalence of genetic polymorphisms might vary greatly within and between ethnic groups, as has been clearly described for FV Leiden, the prothrombin variant, the MTHFR C677T, and eNOS polymorphisms. In the case of populations with ethnically heterogeneous backgrounds, false-positive associations might arise if the frequency of the genetic marker varies because of population admixture rather than as a consequence of a true association with the disease phenotype. Finally, the genetic marker of interest might not be directly involved in disease susceptibility but rather be in linkage disequilibrium with the actual functional polymorphism or mutation located in the same or a nearby gene.

There is compelling evidence that atherothrombosis is a complex disorder and is multifactorial and that its pathogenesis involves multiple gene-gene and gene-environment interactions. The identification of inherited thrombophilia factors that are strongly associated with a high risk of venous thromboembolism led investigators to believe arterial thrombosis would have a similar molecular basis defined by not more than a few mutations; however, most carriers of these genetic markers never experience arterial vascular events, demonstrating that the effect of any single genetic susceptibility factor alone for arterial thrombotic disease is likely to be modest but might assume importance in the presence of other genetic and acquired risk factors. For several polymorphisms, the contribution to risk is only detectable in
association with other environmental risk factors. FV Leiden and the prothrombin variant influenced the risk of atherothrombosis only in a subgroup of female smokers.\textsuperscript{32,44} The increase in plasma fibrinogen levels associated with genetic variants of the \( \beta \)-fibrinogen gene is enhanced in smokers.\textsuperscript{13} Japanese carriers of the ~786CC genotype were shown to have reduced cerebral flow velocity, yet this effect was only observed among smokers.\textsuperscript{88} Elevated plasma homocysteine concentrations in patients homozygous for the MTHFR 677TT genotype have a synergistic effect on risk among smokers and patients with hypertension. The demonstration that individuals who are homozygous for this mutation had elevated plasma homocysteine levels only in the setting of folic acid depletion further indicates the importance of environmental influences on the genetic risk of vascular disease. An equally intriguing concept is that of gene-gene interactions as the basis of arterial thrombotic disorders; these interactions might be due to the presence of multiple polymorphisms within one gene or might occur between different genes whose products are part of pathways that ultimately converge to regulate hemostasis, as shown in Figure 3. Studies have shown evidence that individual polymorphisms within a gene might not be correlated with disease risk, whereas the analysis of haplotypes clearly demonstrates an influence on risk. The unique interaction of multiple polymorphisms within a haplotype might affect the biologic phenotype and outcome, but individual polymorphisms might not play a relevant role in the determination of disease. In the future, subgroups of patients might be selected on the basis of their environmental risk factor profile and screened for specific genetic variants. Conversely, patients carrying certain genetic markers might be advised to avoid certain environmental risk factors that might potentiate their risk of thrombosis; however, these conclusions are as yet speculative. Future studies will need to focus on these gene-gene and gene-environment interactions to unravel the complex pathobiology that underlies the development of atherothrombosis and arterial thrombotic disorders.

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References


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