Thromb Haemost 2001; 85: 584–95

Review Articles

Hemostatic Risk Factors and Arterial Thrombotic Disease

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The pathogenesis of arterial thrombotic disease involves multiple genetic and environmental factors related to atherosclerosis and thrombosis. Acute thrombosis at the site of a ruptured, lipid-rich atherosclerotic plaque is the usual precipitating event in the transition from stable or subclinical atherosclerotic disease to acute myocardial infarction (MI), stroke, or peripheral arterial occlusion (1). Pathologic studies of coronary arteries in acute MI suggest that the acute thrombosis likely involves activation of both platelets and the coagulation system.

Following atherosclerotic plaque rupture, platelets initially adhere to exposed subendothelial von Willebrand factor (vWF) and collagen through the glycoprotein Ib/IX receptor complex and the glycoprotein Ia/IIa receptor complex, respectively. The attachment of platelets to the vascular subendothelium (platelet adhesion) induces a series of intracellular signaling events that result in platelet activation. The binding of fibrinogen and vWF to the conformationally active form of the glycoprotein IIb/IIIa receptor results in platelet cohesion or aggregation, and further propagation of the platelet thrombus. Plaque rupture also results in exposure of subendothelial tissue factor, which initiates the coagulation cascade, and leads to the generation of thrombin and formation of a fibrin clot. While arterial thrombi traditionally are considered to be composed predominantly of platelets, accumulating evidence suggests that certain arterial thrombotic disorders (e.g., transmural or "Q-wave" MI) are associated with greater activation of the coagulation system, which results in occlusive thrombi that are relatively rich in fibrin (2).

Prevention of arterial thrombotic diseases, such as myocardial infarction and ischemic stroke, has focused on identification and modification of traditional cardiovascular risk factors, such as smoking, hyperlipidemia, hypertension, and obesity, that are related to atherosclerosis. However, approximately 30% of MI occur in individuals without traditional cardiovascular risk factors (3). Since antithrombotic agents such as aspirin have been shown to be beneficial in primary and secondary prevention of arterial thrombosis (4), attention has shifted in recent years to the characterization and evaluation of novel phenotypic markers of cardiovascular disease, involving lipoprotein metabolism, the coagulation and fibrinolytic systems, and inflammation (5, 6). In addition, the identification of molecular variants within the genes encoding proteins involved in the pathogenesis of thrombosis has led to their evaluation as genetic susceptibility markers in epidemiologic studies of arterial thrombotic disease. In this review, we briefly discuss some general issues related to the studies that examine the associations of both genetic susceptibility markers and intermediate hemostatic phenotypes with arterial thrombotic disease. We then review the evidence pertaining to the role of specific genetic markers and hemostatic phenotypes in arterial thrombosis.

General Issues Related to Study Design and Interpretation of Results

Case-control and cross-sectional studies assess the relationship between phenotypic markers (e.g., plasma coagulation factor levels) and the occurrence of arterial thrombotic disease. However, these studies cannot establish a temporal order, and therefore cannot exclude the possibility that a hemostatic phenotype is a consequence of the disorder (e.g., due to accompanying tissue injury or inflammation). Even in prospective cohort studies, the observed associations may be confounded by subclinical atherosclerosis, which may contribute to both the hemostatic phenotypes as well as the clinical outcomes. In addition, plasma levels of hemostatic factors are sometimes correlated with each other and also are influenced by other cardiovascular risk factors (e.g., obesity, hyperlipidemia, smoking), that may further confound an apparent relationship between the hemostatic marker and risk of disease. For these reasons, the most convincing evidence of a causal relationship between a hemostatic phenotype and the occurrence of arterial thrombotic disease is inferred from clinical trials that demonstrate that altering the phenotype (e.g., lowering plasma levels of the hemostatic factor) reduces disease.

Another means of circumventing some of the spurious conclusions that can result from the use of intermediate hemostatic phenotypes is to analyze genetic variants that are known to be related to phenotypic variation. The advantage of studying genetic variants is that they are "fixed at birth", and therefore clearly precede disease onset and are not confounded by other behavioral and environmental cardiovascular risk factors or subclinical atherosclerosis. However, molecular epidemiologic studies evaluating potential genetic susceptibility markers have their own set of limitations, and different studies evaluating the same genetic marker often yield conflicting results. Comparison of results among studies is hampered by differences in study design and characteristics of case patients and control subjects, variation of allele frequencies among different populations, as well as the complexity and heterogeneity of the clinical endpoints of coronary heart disease, stroke, and peripheral arterial occlusion. These complex disorders likely involve the effects of multiple genes interacting with the environment. The effect of any single genetic susceptibility factor alone for arterial thrombotic disease is likely to be modest, but may assume greater importance in the presence of additional genetic or environmental exposures (i.e., gene-gene or gene-environment interaction).

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...ation between a putative genetic susceptibility marker and arte...rombotic disease may only be demonstrable within a certain sub...ation. As a result, large sample sizes may be required to demon...a statistically stable estimate of effect, particularly for alleles or ...subgroups that are uncommon within the population.

Although not subject to confounding by other behavioral and envi...mental risk factors, population studies of genetic variants are subject ...confounding by ethnicity, i.e., population admixture or "population ...tification". Thus, a spurious association may arise if the frequency ...genetic marker and the frequency of arterial thrombotic disease ...vary according to genetic ancestry. This is most likely to be the ...in ethnically heterogeneous populations, and can be accounted for ...specific analyses that assess genetic linkage as well as association. ...other possible reason for a positive association in some studies but ...in others is that the genetic marker of interest is not directly in...ved in disease susceptibility, but rather is in linkage disequilibrium ...the actual causative mutation located in the same gene or another ...nearby. Depending on the population history and the recombina...fraction, the genetic marker may be associated with the causative ...ation in some populations but not in others. Therefore, it is prefer...le to assess genetic markers that are well-characterized with respect ...the functional consequences of the molecular defect and that con...ute significantly to phenotypic variation within the population.

...gulation Factors

...ogen

...Plasma fibrinogen levels have been consistently and independently ...ted with an increased risk of MI and stroke in prospective ...of both apparently healthy persons and patients with preexisting ...rombotic disease (7-9). Subjects with plasma fibrinogen levels ...highest tertile have ~2-fold increased risk compared to those in ...lowest tertile. Fibrinogen may contribute to arterial thrombotic ...via a number of mechanisms, including increased fibrin forma...plasma viscosity, platelet aggregation, and vascular endothelial ...smooth muscle proliferation (7). On the other hand, fibrinogen is an ...phase reactant, and the association of elevated fibrinogen and ...tive protein levels with risk of arterial thrombotic disease sug...that the inflammation that accompanies atherosclerosis may con...te to increased fibrinogen levels (10). Plasma fibrinogen levels are ...highly correlated with other cardiovascular risk factors, such as ...moking, diabetes, and estrogens (7, 11). Bezafibrate, an agent that ...es both fibrinogen-lowering and lipid-modifying properties, was ...ociated with an overall reduction of arterial thrombotic events in ...t clinical trial of patients with coronary heart disease (12).

...man fibrinogen is composed of 3 pairs of polypeptide chains, α, ...d γ, encoded by 3 separate genes clustered on chromosome 4q. ...the synthesis of the fibrinogen β-chain is rate-limiting (13), ...eral β-chain polymorphisms have been associated with inter...dual variation in plasma fibrinogen levels. The -455 G/A substi...within the promoter region of the fibrinogen β-chain has been ...most extensively. The –455A allele is present in about 20% of ...ulation, who have ~10% higher fibrinogen levels compared to ...with the GG genotype (14). The relationship between the –455 ...fibrinogen variant and risk of arterial thrombotic disease is ...versial, with some case-control studies observing an association ...), and other large studies finding no association (18, 19). These ...sistent findings cast doubt on a direct cause-and-effect relation...between fibrinogen levels and arterial thrombotic disorders.

Factor VII

Factor VII is a vitamin-K dependent coagulation protein, which upon activation, binds to tissue factor present in damaged vascular subendothelium and initiates coagulation. In the Northwick Park Heart Study, a prospective study of over 1,300 middle-aged men, plasma factor VII activity levels were associated with fatal, but not non-fatal, ischemic heart disease (20). However, several subsequent prospective studies have failed to confirm the association between factor VII levels and risk of coronary heart disease (21-23). Factor VII is also unrelated to risk of venous thrombotic disease (24). Plasma factor VII levels are determined by multiple environmental factors, including age, gender, body mass index, dietary fat, and triglyceride level (25). In addition, several intragenic factor VII polymorphisms which influence plasma factor VII levels have been described. These include an Arg353Gln substitution that is associated with decreased factor VII secretion in vitro and lower factor VII levels (26). An Italian case-control study of young adults with familial MI observed an inverse association with homozygosity of the factor VII Gln353 allele (OR = 0.08, 95% CI = 0.01–0.9) (27). In contrast, several other case-control studies involving either young men (28) or a broader age range (29-31) found no association between the Arg353Gln polymorphism and risk of MI or stroke. Thus, the evidence to date does not support factor VII at either the phenotypic or genotypic level as an important risk factor for arterial thrombotic disease.

Factor VIII and von Willebrand Factor

Increased plasma levels of factor VIII activity, von Willebrand factor (vWF) antigen, and vWF activity have been associated with incident fatal and non-fatal arterial thrombotic events in prospective studies involving healthy middle-aged (22, 23, 32, 33) and elderly (34) populations. In some studies, the associations were independent of other cardiovascular risk factors. In most prospective studies of patients with underlying atherosclerotic disease, vWF levels have been independently associated with risk of acute thrombotic events (10, 35, 36). Factor VIII and vWF circulate as a complex, and plasma levels of these two factors are highly correlated with each other. Thus, elevated factor VIII/vWF levels may be either a marker of atherosclerosis (i.e., endothelial dysfunction and vascular injury) or contribute directly to fibrin formation (factor VIII) or shear-induced platelet adhesion and aggregation (vWF). The demonstration that congenital factor VIII deficiency is associated with decreased mortality from ischemic heart disease (37, 38), as well as the association of elevated factor VIII levels with venous thrombotic disease (39), support the hypothesis of a direct effect of factor VIII concentration on the occurrence of thrombotic events. Factor VIII levels are also influenced by ABO blood group, and individuals with blood group non-O have both higher factor VIII levels and higher incidence of coronary heart disease compared to individuals with blood group O (32, 40). Further evaluation of factor VIII and cardiovascular risk awaits elucidation of other genetic determinants of increased factor VIII levels (41).

Factor XIII

Factor XIII is a transglutaminase that forms covalent bonds between adjacent fibrin monomers, and thus stabilizes a fibrin clot. Factor XIII circulates as a zymogen composed of 2 catalytic A subunits and 2 carrier B subunits. Thrombin cleavage of a 37-amino-acid N-terminal peptide results in activation of the catalytic subunit. A Val34Leu poly-

morphism of the catalytic subunit is located 3 amino acid residues from the thrombin cleavage site, and the less common Leu34 allele has been associated with increased plasma factor XIII activity levels (42, 43). Paradoxically, several recent studies suggest that the "high activity" Leu34 allele may be associated with a decreased risk of MI (44, 45), ischemic arterial stroke (46), and venous thrombotic disease (47), as well as an increased risk of hemorrhagic stroke (48). The mechanism for the apparent antithrombotic effect of the factor XIII Val.Leu34 substitution may involve an increased rate of conversion of factor XIII to activated factor XIII by thrombin that results in an altered cross-linked fibrin clot structure (49).

Levels of other Coagulation Factors

Recently, elevated levels of factor V, but not factor X or prothrombin, were associated with occurrence of MI after adjusting for other risk factors (50). Because of the retrospective nature of this study, one can not distinguish whether increased factor V activity is a cause rather than a consequence of MI. To our knowledge, the associations of levels of coagulation factors XI, X, and IX, V, and prothrombin with arterial thrombotic disease have not been assessed in prospective studies.

Inherited Thrombophilic Disorders

Protein C, Protein S, Antithrombin Deficiency

It is well-established that congenital deficiencies of protein C, protein S, and antithrombin are associated with an increased risk of venous thrombotic disease. Whether congenital deficiencies of these naturally-occurring coagulation inhibitors are associated with increased risk of arterial thrombotic disease is less clear (51, 52). In a pooled analysis of 8 small series of arterial cerebral infarction in predominantly young patients (n = 311), deficiencies of protein S, protein C, and antithrombin were noted in 11.9%, 2.2%, and 2.2% of cases, respectively (53). However, in many of these studies, interpretation of results is limited due to small numbers of subjects, lack of controls, lack of follow-up or family studies to document inherited vs. acquired deficiency, and population admixture. More recent studies of unselected young adults with acute cerebral ischemia suggest that acute, transient or acquired deficiencies of the naturally-occurring anticoagulants are not uncommon, but that true congenital deficiencies are rare (54-56). Several recent pediatric series suggest that protein C or protein S deficiency may be present in as many as 5–15% of children with ischemic stroke (57-59). However, in some cases true congenital deficiency has not been clearly documented, and other pediatric studies have not confirmed these associations (60, 61). Taken together, the evidence suggests that deficiencies of natural anticoagulants are likely to be associated with arterial thrombotic disease, particularly in very young individuals with acute cerebral ischemia. However, since the prevalences of these deficiencies are low even among young patients, they account for a small proportion of cases.

Activated Protein C Resistance and Factor V Leiden

Resistance to the anticoagulant effect of activated protein C is most commonly due to a nucleotide G1691A mutation in the gene encoding factor V that results in an Arg506Gln mutation (factor V Leiden). This mutation occurs at the initial activated protein C cleavage site within the factor Va heavy chain and results in a prothrombotic state because of a reduced rate of factor Va inactivation. Factor V Leiden is the most common genetic factor associated with risk of venous thromboembolic disease, and is found in approximately 3–5% of the Caucasian population (62, 63).

A relationship between factor V Leiden and arterial thrombotic disease was first suggested by Holm et al. (64), who reported two young women (ages 33 and 34 years) with MI and homozygosity for factor V Gln506. However, most subsequent studies have failed to show an association between factor V Leiden and coronary disease or nonfatal MI (65-71), even among patients who developed acute coronary syndromes at a young age (72-74). In contrast, Rosendaal et al. (75) reported a higher risk of non-fatal MI among young female carriers of factor V Leiden (OR 2.4, 95% CI 1.0–5.9) that was particularly high among smokers (32-fold increased risk compared to nonsmoking women without the factor V mutation). In a large, case-control study of middle-aged-to-elderly Dutch men, Doggen et al. (76) also reported an increased risk of MI associated with heterozygosity for factor V Leiden (OR 1.4; 95% CI 0.8–2.2) that was most pronounced in men with other cardiovascular risk factors.

While factor V Leiden is not a risk factor for ischemic stroke in middle-aged to elderly patients, (66, 68, 71, 77), some studies in younger adults (< 45-50 years) indicate a higher prevalence of factor V Leiden in stroke cases than controls (56), but most show no association (78-80). In an Italian study (56), the risk of stroke associated with the factor V Leiden mutation was stronger in women than men, although a smaller North American study of exclusively young women found no association (80). In children, recent data suggests that factor V Leiden may account for approximately 20% of cases of ischemic stroke (58-60, 81).

The relationship between factor V Leiden and peripheral arterial occlusive disease has been examined in three case-control studies. In two studies, the frequency of factor V Leiden carriership was similar among cases, which is higher than the population prevalence (82, 83). In contrast, a larger Austrian study of 336 patients with peripheral arterial occlusion and 300 controls observed a similar frequency of the factor V Leiden mutation between cases (8%) and controls (9%) (84).

Taken together, the cumulative results of studies examining the relationship of factor V Leiden and incident arterial thrombotic disease suggest that factor V Leiden is not a major cardiovascular risk factor, but may assume increased importance in certain patient subgroups, particularly children with arterial stroke and young women with other cardiovascular risk factors. Alternatively, the positive findings among only a few of the published studies suggests the possibility of false-positive association due to population admixture. Additional association studies involving larger numbers of subjects and family-based studies that assess genetic linkage will be required to address these issues.

There is some evidence that activated protein C resistance may influence the risk of arterial thrombotic disease even in the absence of the factor V Leiden mutation (85, 86). In a cross-sectional study of 1,391 men and women, carotid and femoral artery atherosclerosis as well as prevalent athero-thrombotic disease were associated with decreased response to APC (86). In this study, less than 50% of the subjects with APC resistance were carriers of the factor V Leiden mutation, suggesting that other genetic and environmental factors may contribute to the APC resistance phenotype as well as to the risk of arterial thrombotic disease (86, 87). However, the evaluation of a phenotypic abnormality in the context of a cross-sectional study raises the issue of whether the observed resistance is a consequence rather than a cause of the atherothrombosis.

...bin G20210A Mutation

...rombin is the circulating precursor of thrombin, which plays a ... role in fibrin formation and thrombosis. A G to A transition in ...ide 20210 within the 3' untranslated region of the prothrombin ... associated with elevated circulating prothrombin levels, and ... initially identified as a risk factor for venous thromboembolic ... (88). This mutation is present in about 2% of Caucasians, but ... prevalence increases from Northern to Southern Europe (89).

... with factor V Leiden, the relationship between the prothrombin ...210A mutation and risk of arterial thrombotic disease is controver... In most of the published studies, there was no association between ... presence of the 20210A allele and risk of acute MI or coronary ... disease (90-94). While these results suggest that the prothrombin ... is not a major risk factor for coronary heart disease, the statis... power of many of these studies to detect an association is limited ... the relatively low frequency of the mutation in the general popula... In a pooled analysis of 1,115 patients and 1,888 controls from ...dies, Franco et al. noted a significant association in the studies that ... confined to patients with MI (OR = 2.5; 95% CI 1.5–4.3), but no ...ciation in the pooled analysis of studies that included cases with a ...der range of athero-thrombotic disease (95). In the largest single ...ted case-control study of patients with MI (560 patients under ...e of 70), the prothrombin 20210A allele was associated with a ... (50%) increased risk of MI that increased substantially to ... in the presence of traditional cardiovascular risk factors such as ... and hypertension (76). Similarly, in a small case-control study ... women <45 years old with acute MI, Rosendaal et al. reported a ...increased risk of MI associated with prothrombin 20210A that ...ally enhanced in the presence of smoking or other metabolic ...tors (96).

... majority of studies examining the relationship between pro... G20210A and ischemic arterial stroke in young adults (56, ...) and older subjects (90, 94) have been negative. The exception is ...-control study of 72 Italian young men and women (<50 years ... with ischemic arterial stroke who did not have traditional cardio...ular risk factors (OR = 5.1, 95% CI = 1.6–16.3) (78). In a case-...ol study of 148 pediatric patients, the prothrombin 20210A allele ... associated with a 5-fold increased risk of spontaneous ischemic ... stroke (58), but two smaller pediatric studies found no associa... (61; 81). A recent case-control study found no association of the ...rombin 20210A allele with risk of peripheral arterial occlusion in ...erly patient population (84).

...aken together, the results of these studies suggest that the pro...bin 20210A mutation may be associated with a modestly in...ed risk of arterial thrombotic disease that may assume particular ...rtance in certain subgroups, such as young women with MI or ...erebrovascular events in children.

...let Receptor Polymorphisms

...protein IIb/IIIa

...ycoprotein IIb/IIIa (integrin $\alpha_{IIb}\beta_3$) is the platelet surface receptor ...rinogen, von Willebrand factor (vWF), and several other adhe... ligands. The glycoprotein IIIa subunit consists of two common ...orms, PLA^1 (HPA-1a) and PLA^2 (HPA-1b). This dimorphism is due ... T to C nucleotide substitution involving nucleotide 1565 within ...2 of the glycoprotein IIIa gene, and results in a Leu33/Pro33 substitution that is associated with a conformational change in the N-terminal disulfide loop of glycoprotein IIIa relative to the fibrinogen binding site (98). The allele frequency of the PLA^2 variant ranges from ~10 to 18 percent in Caucasian populations, ~8% in African populations, and is virtually absent in Asian populations.

In 1996, Weiss et al. reported that the frequency of carrying at least one copy of the PLA^2 allele was twice as high in 71 hospitalized patients with MI or unstable angina (39.4%) compared with 68 hospitalized control subjects (19.1%) (OR = 2.8; 95% CI = 1.2–6.4) (99). When the analysis was restricted to the subgroup of patients under the age of 60, the relative risk of acute coronary disease associated with the PLA^2 allele increased to 6.2 (95% CI = 1.8–22.4). Since this initial report, there have been over 20 additional observational studies that have examined the relationship between the PLA^1/PLA^2 polymorphism of glycoprotein IIIa and risk of acute or stable coronary artery disease. While a few of these studies have confirmed the association between the PLA^2 allele and increased risk of early-onset coronary heart disease (73, 100-102), the majority have not. The negative studies include the 5 involving at least 300 cases (103-107). However, comparison of the results among studies is complicated by differences in definition of coronary disease, selection of control subjects, and study design. Thus, negative results observed in some studies might be expected if the association is confined to younger patients with MI and/or to specific subgroups, such as women (102), cigarette smokers (73), individuals with pre-existing coronary atherosclerosis (101), or individuals with additional genetic predispositions (108).

Of the published studies that have assessed the relationship between PLA^2 and ischemic stroke, one (109) observed an association only in subgroups of patients under the age of 50 (OR = 1.68; 95% CI 1.00–2.82) and in non-smokers (OR = 2.37; 95% CI 1.19–4.74), and another only in a small subgroup of women (n = 18) who were white and had an identifiable etiology (110). In contrast, two other studies involving a broader age range of both men and women (111, 112), and a study of male physicians (106) found no association between the PLA^2 variant and risk of ischemic stroke.

The assessment of the relationship between the PLA^2 genetic variant of glycoprotein IIIa and risk of arterial thrombotic disease is also hampered by the lack of a clearly defined effect on platelet function. While some studies have demonstrated that PLA^2-positive platelets display increased in vitro platelet reactivity compared to PLA^1-negative platelets (113, 114), others have not (115, 116). The association between PLA^2 and an increased thrombotic tendency is also supported by experiments using stable cell lines overexpressing the PLA^1 and PLA^2 polymorphic forms of glycoprotein IIb/IIIa (117), as well as a recent autopsy study in which the presence of the PLA^2 allele was associated with an increased frequency of acute coronary thrombosis and complicated atherosclerotic plaques in men with fatal MI (118).

In summary, both the clinical and experimental data assessing the role of the PLA^2 variant of glycoprotein IIIa in arterial thrombotic disease have yielded conflicting results. Additional molecular genetic and functional analysis of the platelet glycoprotein IIb/IIIa receptor, as well as studies involving larger numbers of subjects that allow stratification by other cardiovascular risk factors, will be required to resolve these issues.

Glycoprotein Ia/IIa

Glycoprotein Ia/IIa (integrin $\alpha_2\beta_1$) is the major platelet collagen receptor, and is responsible for platelet adherence to exposed vascular subendothelium. Several single nucleotide polymorphisms within the

glycoprotein Ia (α_2 integrin) subunit of the glycoprotein Ia/IIa receptor have been described. Two silent single nucleotide polymorphisms, C807T and G873A are in complete linkage disequilibrium with each other, as well as with several other single nucleotide polymorphisms located in the adjoining introns (119, 120). Among normal individuals, there is a 5-10 fold variation in the surface level of glycoprotein Ia/IIa that correlates with collagen-induced platelet adhesion and aggregation in vitro. The nucleotide 807T variant of glycoprotein Ia is associated with increased platelet glycoprotein Ia/IIa receptor levels and increased collagen-induced platelet adhesion compared to the 807C allele (119-121). The mechanism of the association between the 807T allele and increased receptor density is unknown. The 807T allele may be linked to another polymorphism within the glycoprotein Ia gene involving a regulatory element that controls gene transcription levels or mRNA stability.

The prothrombotic tendency of the glycoprotein Ia 807T allele is supported by four recent case-control studies. In a study of 2,237 consecutive German men undergoing coronary angiography, Santoso et al. (122) reported a higher frequency of the 807T allele in individuals who had suffered an MI compared to those without MI (OR 1.57; 95% CI 1.14–2.13) that was most pronounced in obese men under the age of 49 (OR 4.92; 95% CI 1.71–14.2). In a smaller case-control study, Moshfegh et al. reported a 3-fold increased risk of MI in individuals who carried two copies of the 807T allele compared to individuals who possessed the CC or CT genotype (OR 3.3; 95% CI 1.23–8.83) (123). Similarly, the 807T allele was associated with a 2-3 fold increased risk of ischemic stroke in men under the age of 50 (124) and women under the age of 45 (125). In contrast to these 4 positive studies, Croft et al. reported no association between glycoprotein C807T and risk of MI, either overall (OR 0.88, 95% CI 0.74–1.05), in younger individuals, or in subgroups defined by other risk factors (126). Corral et al. also reported no association between the 807T allele and risk of either acute coronary heart disease or ischemic stroke in a middle-aged to elderly study population (121). Furthermore, it should be noted that the frequency of 807TT homozygotes among the control group of one of the positive studies (5.6%) was considerably lower than other published population studies (123). In summary, preliminary results suggest that the 807T variant of glycoprotein Ia may be a genetic risk factor for early-onset arterial thrombotic disease, but further studies involving larger numbers of subjects, as well as further characterization of the mechanism responsible for increased receptor levels, are required to settle this issue.

Glycoprotein Ib/IX

Glycoprotein Ib/IX receptor is the major platelet receptor for von Willebrand factor. Two polymorphisms that affect the amino acid sequence of the glycoprotein Ib heavy chain (glycoprotein Ibα) have been described. The first, an ACG to ATG substitution at codon 145, results in a Thr/Met dimorphism that is responsible for the human platelet antigen (HPA)-2 system. The second is a size polymorphism that results from a variable number of tandem repeats (VNTR) of a 13-amino-acid sequence (Ser399–Thr411) that is present in either one (D), two (C), three (B), or four (A) copies (127). The HPA-2 and VNTR polymorphisms are in strong linkage disequilibrium with each other. The HPA-2 Thr145Met dimorphism results in a protein conformational change in a region adjacent to the vWF binding region of glycoprotein Iba, but an effect on in vitro platelet function or von Willebrand factor binding has not been demonstrated (128, 129). The VNTR polymorphism involves the macroglycopeptide region of glycoprotein Ibα, with the addition of each repeat resulting in an increased distance between the ligand binding region and the platelet surface. While it has been hypothesized that this distance variation may affect platelet susceptibility to shear-induced activation (127), this has yet to be demonstrated experimentally.

Despite the lack of an associated prothrombotic phenotype, several case-control studies have noted a 2-3 fold increased risk of coronary heart disease or stroke associated with the Met145 (HPA-2b) or VNTR-B allele, particularly in younger patients (130-132). In contrast, Ardissino et al. did not find an association between the HPA-2 polymorphism and premature MI in a case-control study involving predominantly men (73). Two other studies did not observe an association between HPA-2 or VNTR genotypes and risk of ischemic stroke in older patient populations (109, 112).

Other Hemostatic Factors

Fibrinolytic System

The fibrinolytic system consists of the circulating proenzyme plasminogen, which is converted to plasmin by the plasminogen activators, tPA and urokinase. Plasminogen activator inhibitor-1 (PAI-1) is a major inhibitor of the fibrinolytic system, and is synthesized by a variety of cell types, including platelets, endothelial cells, and vascular smooth muscle cells. Data from several prospective studies indicate an association between plasma levels of various fibrinolytic markers (tPA antigen, PAI-1 activity and antigen levels, and fibrin degradation products) and risk of arterial thrombotic disease (133-137). The presence of increased levels of PAI-1 mRNA in atherosclerotic plaques (138) and the association of elevated plasma PAI-1 levels with subclinical atherosclerosis (139) also suggest a role for impaired fibrinolysis in the occurrence of athero-thrombotic disease. However, this role is complex; inhibition of fibrinolysis may promote thrombosis, but also may inhibit matrix metalloendopeptidase activation and vascular smooth muscle proliferation, which may alter atherosclerotic plaque stability (140).

The interpretation of cardiovascular epidemiologic studies of fibrinolytic markers is complicated by several other factors. Plasma tPA antigen and PAI-1 activity levels are highly correlated, in part because the tPA antigen assay measures both free tPA and tPA complexed to PAI-1, which increases in patients with high PAI-1 levels due to delayed clearance (141). Measurement and interpretation of plasma PAI-1 activity levels are particularly problematic due to diurnal variation, spontaneous transformation of PAI-1 to an inactive or "latent" form at neutral pH, and the potential for release of PAI-1 from platelets; these necessitate special measures during blood collection and sample processing (142). PAI-1 is also an acute phase reactant, and circulating levels are influenced by a number of hormones and cytokines (143). Increased PAI-1 levels are also correlated with obesity, hyperinsulinemia and hypertriglyceridemia, (i.e., the "insulin resistance syndrome"). Thus, in most epidemiologic studies, the association between PAI-1 or tPA levels and risk of arterial thrombotic disease may be confounded by these other cardiovascular risk factors.

The 4G allele of the 4G/5G polymorphism located within the promoter region of the PAI-1 gene has been associated with increased cytokine-induced gene transcription in vitro (143) due differential binding of a transcriptional repressor (144), and increased plasma PAI-1 levels in most studies (140). While several case-control studies have demonstrated an increased risk of MI or coronary artery disease and

with the 4G allele (144-146), these findings have not been confirmed in several larger studies (147-149). A meta-analysis of nine studies that included 1,521 cases and 2,120 controls indicated an overall slightly increased risk of MI associated with the 4G allele (OR 1.23, CI 1.04-1.45) (150). However, the increased risk was confined to a subgroup of case-control studies in which both cases and controls were taken from "high-risk" populations.

Homocysteine

Homocysteine is a sulfur-containing amino acid that is formed as an intermediary compound during methionine metabolism. Congenital homocystinuria, a rare autosomal recessive metabolic disorder, is associated with very high levels (>100 μmol/L) of fasting plasma total homocysteine, and is often accompanied by premature thrombotic disease involving the coronary, cerebral, and peripheral arteries. Mild hyperhomocysteinemia (i.e., >15 μmol/L) is not uncommon in the general population, and is associated with a variety of acquired and genetic factors, including increasing age, male sex, cigarette smoking, reduced consumption of nutritional factors (folate, vitamin B_6, vitamin B_{12}), renal failure, and heterozygous deficiencies of enzymes involved in homocysteine metabolism (151, 152). The adverse effects of homocysteine include vascular endothelial injury, smooth muscle proliferation, oxidation of low-density lipoprotein cholesterol, and inhibition of a prothrombotic vascular endothelial microenvironment (151, 152).

Since the very high levels of plasma homocysteine that accompany congenital homocystinuria are strongly related to risk of arterial thrombotic disease, some degree of association with mildly elevated levels is quite plausible. There have been a number of case-control and cross-sectional studies that indicate that mild hyperhomocysteinemia is associated with an increased risk of coronary heart disease, cerebrovascular disease, and peripheral vascular disease (153-155). However, it should be noted that homocysteine levels may increase following acute arterial thrombosis (156, 157), which complicates the interpretation of studies in which levels are measured retrospectively. Prospective studies assessing the risk of arterial thrombotic disease associated with elevated homocysteine levels have yielded mixed results with most studies showing a weak or no association (154, 155). The strongest associations have tended to occur in studies involving subjects with pre-existing cardiovascular disease. This raises the issue of whether the elevated homocysteine levels are a consequence rather than a cause of subclinical atherosclerosis.

Mild hyperhomocysteinemia has been associated with a thermolabile variant of MTHFR that is due to a C to T transition at nucleotide 677. Homozygosity for the C677T MTHFR variant is present in 5% to 15% of Caucasians. A recent meta-analysis of 23 case-control studies including over 12,000 subjects concluded that homozygosity for the thermolabile MTHFR variant is not associated with risk of cardiovascular disease (158). This absence of an association raises doubt about the role of mild hyperhomocysteinemia as a cause of arterial thrombotic disease. However, based on the association of MTHFR and homocysteine levels, the predicted relative risk of the thermolabile MTHFR genotype is small (possibly no more than a 20-percent risk increase), and even a meta-analysis may have insufficient power to detect a risk of this magnitude. Ongoing randomized clinical trials of folate and B vitamin supplementation to reduce plasma homocysteine levels should provide more definitive answers to the public health questions surrounding homocysteine and risk of athero-thrombotic disease.

Lipoprotein (a)

Lp(a) is a complex serum lipoprotein composed of an LDL particle linked by its surface apolipoprotein (apo) B-100 via a disulfide bond to a unique and highly polymorphic glycoprotein, apo(a) (159). Apo(a) shares extensive structural homology with plasminogen, and consists of a non-functional serine protease domain, a variable number of multiple repeats of kringle IV, and a single copy of kringle V. The variable number of kringle IV repeats is due to varying number of copies within the apo(a) gene, and results in at least 35 different molecular weight isoforms.

Plasma Lp(a) levels are largely genetically determined (160), and vary inversely with apo(a) isoform size. Levels increase in patients with renal disease, and are lower in white populations compared to African Americans (161). In contrast to plasma LDL levels, Lp(a) levels are unaffected by diet, physical activity, and most lipid-lowering agents. On the other hand, estrogen replacement therapy and high-dose niacin can lower plasma Lp(a) levels.

Experimental evidence suggests that Lp(a) may contribute to the occurrence of arterial thrombotic disease through effects on both atherosclerosis and thrombosis (159). Oxidized Lp(a) can be internalized by macrophages and contribute to delivery of cholesterol to sites of vessel injury. Because of its structural similarity to plasminogen, Lp(a) inhibits plasminogen binding to fibrin and endothelial cells (162), and thereby interferes with fibrinolysis and promotes thrombosis (163). Lp(a) accumulates in atherosclerotic lesions, and has been implicated in the progression of coronary atherosclerosis (164). Lp(a) may also impair endothelial function and induce smooth muscle proliferation.

Despite these experimental data, clinical data supporting the role of Lp(a) as a cardiovascular risk factor are controversial. A number of studies have shown an association between elevated Lp(a) levels and increased risk of coronary, cerebrovascular, and peripheral arterial disease (161, 165, 166). However, like homocysteine, Lp(a) levels increase following an acute thrombotic event. Thus, retrospective studies may not be able to discern whether elevated Lp(a) levels are the cause or consequence of cardiovascular disease, and prospective studies have yielded conflicting results (167-174). Based on the cumulative epidemiologic data, if an association between Lp(a) levels and cardiovascular exists, the effect is likely to be small and confined to a small proportion of the population with the very highest serum levels (175), onset at a young age (170) or those with additional dyslipidemias (167, 174, 176, 177). Further evaluation of the significance of apo(a) isoform size on the occurrence of athero-thrombotic disease (171, 177) as well as other genetic mechanisms that underlie Lp(a) heterogeneity (178) may lead to improvements in the use of Lp(a) as a determinant of cardiovascular risk.

Thrombomodulin

Thrombomodulin is an endothelial cell surface receptor for thrombin that functions as an anticoagulant by greatly accelerating thrombin-induced activation of protein C. A soluble, truncated form of thrombomodulin circulates in plasma, but its significance is unknown. In a large prospective case-cohort study, decreased plasma thrombomodulin levels were associated with an increased risk of MI (179). Some reports have suggested that several rare polymorphisms within the thrombomodulin promoter and coding sequence may be related to risk of MI (180-182).

Recommendations for Screening

Despite the proliferation of epidemiologic studies, increased fibrinogen level remains the only hemostatic biomarker that has been clearly identified as a risk factor for arterial thrombotic disease. And even for fibrinogen one may question the causality of the association. The decision whether to perform screening for a hemostatic marker, however, should be based on whether screening offers a cost-effective way of identifying individuals who would benefit from treatment. Therefore, screening of plasma fibrinogen is generally not advocated because levels are favorably affected by modification of other risk factors (e.g., smoking cessation, exercise, and post-menopausal hormone replacement therapy) and because a beneficial effect of lowering fibrinogen level on cardiovascular risk has yet to be demonstrated. There is some epidemiologic and biologic evidence that elevated plasma levels of factor VIII, homocysteine, and Lp(a), as well as several genetic hemostatic variants (factor V Leiden, factor XIII Leu34, prothrombin G20210A, the PL^{A2} variant of glycoprotein IIIa, and glycoprotein Ia 807T) are related to the occurrence of arterial thrombosis. However, there is currently no evidence that screening for any of the above hemostatic markers either within the general population or among subgroups, such as individuals who develop arterial thrombotic disease at a young age, would have any prognostic or therapeutic consequences. Several inherited conditions associated with venous thrombosis (deficiencies of protein C, protein S or antithrombin, factor V Leiden, and prothrombin G20210A) are not as important in the overall occurrence of arterial thrombosis, except possibly in subgroups such as the very young or premenopausal women. Thus, evaluation of these disorders in the setting of arterial thrombosis may be considered in young patients once more likely candidates (lipid disorders, hyperhomocystenemia, lupus anticoagulant/antiphospholipid antibody syndrome) have been excluded, or in MI patients without significant coronary atherosclerosis (183). In addition, a thrombin time and fibrinogen level should be obtained in these young patients to exclude congenital dysfibrinogenemia, another rare cause of both venous and arterial thrombophilia (184).

Conclusion

With the progression of the human genome project and the recent focus on identification of inter-individual genetic variation, the potential for evaluating the relationship between functional variants of candidate genes, associated prothrombotic phenotypes, and risk of arterial thrombotic disease will greatly increase. Several guidelines for future studies that evaluate novel hemostatic markers should be considered. First, molecular epidemiologic studies should focus on genetic mutations that are associated with clearly defined effects on hemostatic function and that contribute significantly to inter-individual phenotypic variation. Second, examination of homogenous populations and precise, well-defined clinical outcomes should enhance the ability to detect associations. For example, hereditary determination by as yet unidentified genetic factors may be particularly important in MI that occurs at younger ages and in women (185). Furthermore, restriction to ethnically homogeneous populations or eliciting detailed information regarding genetic ancestry from study subjects should minimize the problem of false positive associations arising from population admixture. In addition, any preliminary associations observed in population-based studies should be complemented with family-based studies that provide evidence that the marker of interest is genetically linked to the disease (186). Third, large sample sizes will be required to provide enough statistical power to assess the interaction of genetic susceptibility markers with other genetic and environmental risk factors. Undoubtedly other candidate genes and associated functional polymorphisms involved in arterial thrombotic disease will continue to be identified. Integration of knowledge involving novel hemostatic biomarkers at the genetic, biochemical, and clinical-epidemiologic levels should lead to significant developments in understanding the pathophysiology of arterial thrombotic disease, the assessment of cardiovascular risk, and the targeting of specific antithrombotic therapies to high-risk individuals.

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Received July 24, 2000 Accepted after revision September 13, 2000