Prothrombotic genetic markers

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The last decade has been characterized by an explosion of research studies on genetic epidemiology. In particular, as far as ischemic heart disease is concerned, a lot of research was focused on prothrombotic genetic risk factors. Unfortunately, the success of this approach in the field of venous thrombosis has not been replicated in the field of myocardial infarction. In the present editorial, a comment on the studies already available is provided and the possible limitations of the present approach are analyzed.

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Investigations carried out over the last 40 years have demonstrated that coronary artery thrombosis is the critical event underlying myocardial infarction and unstable angina. The existence of a prolonged hypercoagulable state preceding the thrombotic event has been postulated for some time, and significant associations have been established between the plasma concentrations of a number of hemostatic variables and the frequency of myocardial infarction. High plasma fibrinogen, factor VII/VIIa and tissue-type plasminogen activator and plasminogen activator inhibitor levels have been associated with at least as great a risk of developing myocardial (re)infarction or sudden death as high cholesterol levels, especially in the young. However, difficulties in standardizing these assays have limited their application as cardiovascular risk factors in clinical practice. Platelets play a central role in thrombus formation, and platelet aggregates are major constituents of arterial thrombi and provide a catalytic surface for reactions of the hemostatic mechanism; however, it is unclear whether thrombus formation occurs because of the interaction of normal platelets with thrombogenic components, or whether the thrombotic process is enhanced by abnormally active platelets. There is still no consensus as to which laboratory techniques should be used to identify platelet hyperreactivity, largely because of their high degree of variability and lack of standardization. Nevertheless, the results of a large number of clinical studies suggest an association between enhanced platelet function and coronary heart disease.

A recurrent theme of studies of coagulation markers as coronary heart disease risk factors is that the results of one study are often not replicated in others. Although this is partially due to differences in assay methodologies, it may also depend on genetic and population differences, in which case genetic markers could provide highly reproducible assays for cardiovascular risk assessment.

Considerable progress has been made in identifying the genetic abnormalities in coagulation proteins that lead to a change in the systemic balance between procoagulant and anticoagulant factors, but these defects clearly lead to an increased risk of thrombosis only on the venous side of the circulation. Although a number of studies have assessed the role of candidate genes in the occurrence of myocardial infarction, their results have been inconsistent because their sample sizes were insufficient for accurate evaluation of the importance of one or more genes. Complex disorders such as myocardial infarction are likely to involve the effects of multiple genes interacting with environmental exposures (i.e., gene-gene or gene-environment interactions). The effect of any single genetic susceptibility factor for arterial thrombotic disease is likely to be modest, but may take on particular importance in the presence of additional genetic or environmental exposure. Furthermore, most of the studies involved unselected populations with coronary artery disease (including stable and unstable coronary artery disease) of widely varying ages, and this may have con-

founded the possibly age-related effects of a genetic predisposition.

Fibrinogen

Cohort studies have consistently demonstrated that increased plasma fibrinogen levels are associated with an increased risk of myocardial infarction[1-3]. With regard to this, the -455G/A substitution within the promoter region of the fibrinogen beta-chain has been studied. The -455A allele is present in about 20% of the population. Such individuals have fibrinogen levels which are 10% higher than those found in subjects with the GG phenotype[4]. The relationship between the 455GA beta fibrinogen variant and the risk of arterial thrombotic disease is controversial, with some case-control studies observing an association[5-7] and others not[8,9].

Factor VII

Previous studies[10] showed that high factor VII levels were associated with the risk of fatal ischemic events. Plasma factor VII levels are determined by multiple factors (age, gender, body mass index), but a number of intragenic factor VII polymorphisms have also been found to affect them. One Italian study observed an inverse association with homozygosity and the development of myocardial infarction in patients with familial myocardial infarction[11] but other studies did not confirm this association[12-15].

Factor V Leiden resistance to the anticoagulant effect of protein C is most commonly due to a nucleotide G1691A mutation in the gene encoding factor V. Factor V Leiden is the most common genetic factor associated with the risk of venous thromboembolic disease. A relationship between factor V Leiden and myocardial infarction has been suggested in particular subpopulations such as women, but has not been found in the general population[16-28].

Prothrombin gene mutation

An AG to A transition in nucleotide 20210 within the 3' untranslated region of the prothrombin gene is associated with high circulating prothrombin levels and is a risk factor for venous thromboembolic disorders. The relationship between the prothrombin G2'10A mutation and the risk of arterial thrombotic disease is controversial[29-33]. The contrasting results suggest that this prothrombin mutation is not a major risk factor for coronary artery disease, but the statistical power of many of these studies in clearly defining an association is limited by the relatively low incidence of the mutation in the general population. Once again, the results suggest that the prothrombin 20210 mutation may be associated with a modestly increased risk of arterial thrombotic disease but that it may take on particular importance in certain subgroups, such as young women with myocardial infarction.

Glycoprotein IIb/IIIa

Glycoprotein IIb/IIIa is a platelet surface receptor for fibrinogen, von Willebrand factor and a number of other adhesive ligands. The glycoprotein IIIa subunit consists of two common isoforms, PlA1 and PlA2. This dimorphism is due to a T to C nucleotide substitution involving nucleotide 1565 within exon 2 of the glycoprotein IIIa gene, and leads to a Leu33/Pro33 substitution that is associated with a conformational change in the N-terminal disulphide loop of glycoprotein IIIa relative to the fibrinogen binding site[34]. Some studies have shown that PlA2-positive platelets have more *in vitro* platelet reactivity than PlA2-negative platelets[35,36]. The association between PlA2 and an increased thrombotic tendency is also supported by experiments using stable cell lines overexpressing the PlA1 and PlA2 polymorphic forms of glycoprotein IIb/IIIa[37], as well as by a recent autopsy study in which the presence of the PlA2 allele was associated with an increased frequency of acute coronary thrombosis and complicated plaques in men with fatal myocardial infarction[38].

The allele frequency of the PlA2 variant ranges from 10 to 18% in the Caucasian population, and it has been reported that it is associated with an increased risk of unstable angina or myocardial infarction[39] especially in the younger population. A few subsequent studies have confirmed the association between the PlA2 variant and the risk of early coronary artery disease[25,40-42], but the majority has not. Given these conflicting results, it might be expected that the association be confined to younger patients with myocardial infarction, or to specific subgroups such as women[42], cigarette smokers[25], individuals with preexisting atherosclerosis[41] or those with an additional genetic predisposition[43].

Conclusions

The progress in the human genome project and the recent focus on the identification of interindividual genetic variations will greatly increase the potential of evaluating the relationships between functional variants of candidate genes, associated prothrombotic phenotypes, and the risk of arterial thrombotic disease. Molecular epidemiological studies should focus on the genetic mutations that are associated with clearly defined effects on hemostatic function, and which significant-

ly contribute to interindividual phenotype variations. Furthermore, the evaluation of homogeneous populations (young patients, females) and the use of precise, well-defined clinical outcomes (myocardial infarction, sudden death) should enhance the ability to detect associations. Finally, large sample sizes will be required in order to provide enough statistical power to accurately assess the interaction of genetic susceptibility markers with other genetic and environmental risk factors.

Other candidate genes and associated functional polymorphisms involved in myocardial infarction will continue to be identified. The integration of our knowledge on novel hemostatic biomarkers at genetic, biochemical and clinico-epidemiologic levels should lead to significant developments in our understanding of the pathophysiology of myocardial infarction, in the assessment of individual risk, and in the targeting of specific antithrombotic therapies to high-risk individuals.

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