



Genetic Risk Factors for Arterial Thrombosis and Inflammation

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Arterial thrombosis is a central pathologic mechanism contributing to myocardial infarction and stroke, together the leading causes of death in developed countries. This article reviews the current state of knowledge concerning the role of inherited variation in hemostatic and inflammatory factor genes in determining the risk of arterial thrombosis/ischemic heart disease. Despite considerable progress in identifying

important genetic risk factors underlying predisposition to venous thrombosis, the genetic factors contributing to the risk for arterial thrombosis remain largely unknown. However, the rapid development of powerful new genomic resources should facilitate considerably more sophisticated analyses, leading to novel insight into the molecular pathophysiology of this important set of human diseases.

Arterial thrombosis is a central pathophysiologic mechanism contributing to myocardial infarction and stroke, together the leading causes of death in developed countries. Considerable progress has been made over the past 10 years in identifying important genetic risk factors underlying predisposition to venous thrombosis.¹ However, similar insight into the genetic component of arterial thrombosis predisposition has not materialized, despite considerable effort. Though there is a significant genetic component to both forms of thrombosis, there appears to be little overlap among the known genetic risk factors for venous thrombosis and those predisposing to arterial thrombosis.

Factor V Leiden and Other Thrombophilic Mutations

With the identification of factor V Leiden and the prothrombin 20210 mutation as important risk factors for venous thrombosis in 1994,¹ considerable attention was focused on testing the same common genetic variants as factors in the predisposition to arterial thrombosis. Though several small studies have suggested a role in select populations, particularly in children and in young women with myocardial infarction,^{2,3} no effect has been seen in a number of larger studies,⁴ including the Physicians' Health Study.⁵ The general conclusion has been that these common genetic variants are not major risk factors for myocardial infarction, stroke, or any other form of arterial thrombosis. Similarly, other well-established genetic risk factors for venous thrombosis, including protein C defi-

ciency and antithrombin III deficiency, are generally not associated with arterial thrombosis.

Nonetheless, multiple studies demonstrate a major genetic component to the risk for arterial thrombotic/ischemic heart disease. For example, a recent twin study estimated a heritability of ~50% for mortality from coronary heart disease.⁶ Thousands of clinical studies have repeatedly assessed hundreds of potential candidate genetic risk factors with generally confusing and contradictory results. At the end of the day, very few if any of these candidate genetic risk factors can be said to be clearly associated with an increased risk of arterial thrombosis, coronary artery disease, or ischemic stroke.⁴ Data for several of the most prominent candidate risk factors are summarized below.

Genetic Variation in Other Hemostatic Factors

Numerous studies have examined the role of variation in various coagulation factor genes in the risk for coronary artery disease, stroke, and/or arterial thrombosis. Though there are multiple individual reports of suggestive association with one coagulation factor level or another, the most consistent reports in large epidemiologic studies have been seen for factor VII and fibrinogen. The strongest data are for the level of fibrinogen. However, fibrinogen is an acute phase reactant and is elevated in a number of other disease states; thus, it has been difficult to be certain whether the risk associated with elevated fibrinogen levels is causally related to the fibrinogen itself or rather a secondary marker for another underlying pathophysiology. Fibrinogen levels are highly heritable, with an estimated genetic component of ~50%. Most attention has been paid to polymorphisms within the fibrinogen genes, particularly a series of polymorphisms in the β -chain. Association of these DNA sequence polymorphisms with arterial thrombotic risk has been inconsistent, however, as are the data for factor VII and polymorphisms within the factor VII gene (reviewed in⁴).

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Platelet Factors in Arterial Thrombotic Risk

The striking difference in genetic predisposition to venous thrombosis as compared to arterial thrombosis has long been thought to be due to differences in the underlying pathophysiology, with venous thrombosis being primarily dependent on fibrin clot formation, thrombin generation, and the protein C pathway, whereas initiation of thrombosis at high shear in the arterial circulation is more dependent on platelet adhesion. von Willebrand factor and factor VIII levels have been implicated as potential risk factors for both arterial and venous thrombosis, although these data are less convincing for arterial thrombosis.⁷ However, a recent analysis of mothers of hemophiliac patients (most of whom should be carriers with an ~50% reduction in plasma factor VIII) demonstrated a significant reduction in deaths from ischemic heart disease.⁸

Considerable attention has also been focused on platelets and variation in the genes encoding key platelet proteins. Increased risk from generalized platelet hyperreactivity has been proposed, though the existence of such a specific genetic syndrome is still controversial. A common polymorphism in glycoprotein IIIa (Leu33Pro, also known as the PL^{A1}/PL^{A2} antigen) was proposed as an important risk factor for coronary thrombosis.⁹ However, in what has become a familiar theme, this finding could not be confirmed in several larger studies, including the Physicians' Health Study.¹⁰ Similarly conflicting data have been reported for polymorphisms in the GP1a integrin platelet surface receptor.⁴

A large number of studies have evaluated polymorphisms in components of the fibrinolytic system, most notably tPA and PAI1 (plasminogen activator inhibitor-1). A polymorphism at -675 of the PAI1 gene promoter (the presence of a run of either 4 or 5 Gs) has received a particularly large amount of attention, with well over 200 citations in Medline for this polymorphism alone! Though in vitro expression data demonstrate higher levels of gene transcription from the 4G allele, consistent with the idea that this polymorphism could confer an in vivo difference in PAI1 expression,¹¹ numerous association studies have given conflicting and inconsistent results⁴ and large epidemiologic studies have failed to confirm a significant effect of the 4G/5G polymorphism on arterial thrombosis risk.¹²

Homocysteinemia

A considerable body of literature has evaluated hyperhomocysteinemia as a risk factor for arterial thrombosis and coronary artery disease (over 700 citations in Medline). Homozygous genetic deficiency of the enzyme cystathionine β -synthase results in the rare genetic syndrome homocysteinuria, which is clearly associated with premature atherosclerosis and arterial thrombosis, including stroke. Thus, it does seem logical that milder but more common genetic variation at loci contributing to homocysteine levels could be important risk factors for the same complications in the general population. Though elevated

plasma levels of homocysteine do appear to be an independent risk factor for atherosclerosis and arterial thrombosis,⁴ the contribution of genetic variation in specific genes contributing to homocysteine level is again controversial.

An extremely common polymorphism, the presence of either a C or T at nucleotide -677 in the coding region of the methylene tetrahydrofolate reductase (MTHFR) gene, has received a remarkable level of attention in the literature (over 1000 Medline citations). This nucleotide change corresponds to the substitution of Val for Ala at amino acid 222 and leads to a decrease in activity and increased thermolability. This is an extremely common polymorphism with the frequency of the T allele as high as 0.35 in Caucasian populations, corresponding to a homozygote frequency of 12%, with 45% of the population being heterozygotes. Though the T allele is associated with higher levels of homocysteine, this elevation is probably largely corrected by folate supplementation, which is currently in place in the commercial food supply in most developed countries. Perhaps partly for this reason, most recent studies have failed to demonstrate any consistent increase in arterial thrombotic risk in association with this polymorphism.¹³

Other Risk Factors

A number of other attractive candidates have recently been examined for contribution to arterial thrombotic risk, including genetic variation in endothelial nitric oxide synthase (eNOS) and the antioxidant enzymes paraoxonase (PON1) and plasma glutathione peroxidase (GPx-3) (reviewed in ⁴). Though suggestive results have been reported in a few selected ethnic groups, results are generally inconsistent and no compelling case for a critical contribution to arterial thrombosis risk has yet emerged for common polymorphisms in any of these genes.

Why Have Genetic Studies for Arterial Thrombosis Risk Factors Been So Unsuccessful?

As noted above, the identification of genetic susceptibility factors for arterial thrombosis and atherosclerosis has received enormous attention and has been the subject of extensive clinical investigation, with surprisingly disappointing results. A number of key factors may contribute to this observation. Many of the reported positive association studies are likely to represent false positives, which are to be expected, given the extremely large number of studies that have been undertaken and the anticipated publication bias toward positive studies. It is also possible that we may often be looking at the wrong gene. Workers in the cardiovascular field generally assume that genetic polymorphisms contributing to variation in gene X will lie in or around the gene X structural gene. However, it is becoming increasingly clear that genetic variation at other loci, for example in critical transcription factors that regulate the expression of gene X, could also be responsible.¹⁴ Such genes will be much more difficult to identify as they may not be obvious candidates at the start.

How to address complex diseases like atherosclerosis/arterial thrombosis is a critical problem at the forefront of modern genetics. The disappointing and frustrating results of previous linkage studies suggest that genetic determinants of this risk are complex, likely to involve multiple genes, and may also be dependent on interactions between these genes. Such small and complex effects may be very difficult to detect by studying complex human populations. There are clearly also major environmental and acquired risk factors of great importance that also interact with the genetic factors, making detection even more difficult. For arterial thrombosis, these include obvious factors such as diet, cholesterol, and cigarette smoking.

There are also many other genes outside of conventional hemostasis that are likely to contribute to arterial thrombosis risk, including genes involving lipid metabolism and inflammation. Indeed, there have been extensive studies looking at polymorphisms in inflammatory genes similar to the studies outlined above (reviewed in ¹⁵). These include polymorphisms in the tumor necrosis factor (TNF) α and β genes, the transforming growth factors β 1 and 2, interleukin 1 and its receptor antagonist, the CD14 receptor, the P and E selectins and PECAM1. As with the factors discussed above, this body of literature is also plagued by conflicting results and inconsistent findings, and to date no single genetic variation in an inflammatory gene has been identified as a clearly significant risk factor for arterial thrombosis or ischemic heart disease.

The field of modern genetics is currently focused on addressing these and other complex diseases, such as type 2 diabetes, that similarly have a major genetic component, but with contributions from many different genes. Powerful new tools being generated by the human genome project, such as the soon-to-be-completed human HapMap, together with continued advances in technologies for high throughput genotyping should lead the way to much more sophisticated analyses.¹⁶ These advances will facilitate the evaluation of all common human genetic variation, and the complex interactions between these variants, in fully determining the genetic predisposition to arterial thrombosis.

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