EDITORIAL COMMENT

Functional Polymorphisms in a Candidate Gene for Atherothrombosis

Unraveling the Complex Fabric of a Polygenic Phenotype*

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Conventional risk factors for atherothrombotic vascular disease account for approximately 50% of the total attributable risk burden. This fact has led to aggressive approaches to the identification of alternate determinants of risk with mechanistic rationale. In this expanding era of the human genome, epidemiologists in search of other risk factors have been given a very large set of additional targets, that is, polymorphisms or mutations throughout the genome. A polymorphism is a change in the sequence of a normal or "wild-type" gene that is relatively abundant in a population (i.e., ~0.5% to 1%); by contrast, a mutation is a change in the sequence of a wild-type gene that is less common. Moreover, mutations often, and polymorphisms on occasion, have been shown to affect the expression or activity of a gene product, thus making their identification important in discerning possible mechanistic determinants of disease.

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Atherothrombosis is a polygenic disease and is described as a complex genetic trait; as opposed to sickle cell anemia or familial hypercholesterolemia, for example, the disease presentation or phenotype cannot be attributed to a mutation or polymorphism in a single gene. Rather, mutations or polymorphisms in multiple genes define atherothrombotic propensity and phenotype, and these mutations or polymorphisms serve as the intellectual grist of genetic epidemiologists who strive to identify them and quantify the strength of association between them and the risk of disease expression. In some cases, polymorphisms have been identified in the sequence of genes that have no known function; in others, polymorphisms have been identified in genes with well-known functions, and, in some of these genes, polymorphic sequences have been shown to modify the level of expression or the function of the gene product.

In this issue of the Journal, two polymorphisms in the endothelial nitric oxide synthase (eNOS) gene, a gene whose product is important for normal vascular function, were analyzed in two different populations of individuals with vascular disease or dysfunction. Nitric oxide (NO) synthesized by eNOS from L-arginine is a principal mediator of normal endothelial function (1). As a smooth muscle relaxant, it promotes vasodilation; as a platelet inhibitor, it prevents thrombosis; as an inhibitor of smooth muscle migration and proliferation, it limits neointimal hyperplasia after vascular injury; and as a scavenger of reactive oxygen species, it modulates the inflammatory response by suppressing oxidant stress-mediated transcriptional activation of a variety of genes, including those that code for adhesion molecules. As a result of these many effects, eNOS plays a central role in maintaining normal vascular homeostasis and is viewed as atheroprotective.

There are 10 polymorphisms that have been described in the eNOS gene (2), three in the 5′-flanking region of the gene that are in linkage disequilibrium (T-1474A, A-922G, and T-786C), two in the coding sequence (C774T and G894T), and five in intronic regions. Among these base changes, two have been shown to modify expression and activity of the enzyme; the T-786C promoter polymorphism decreases transcription of the gene (3), and the G894T polymorphism leads to a conservative amino acid substitution of an aspartate for a glutamate residue, which is believed to alter the conformation of the enzyme and enhance its susceptibility to proteolytic cleavage (4). Thus, these functional polymorphisms in a candidate gene are prime targets for epidemiological study of possible association with atherothrombotic vascular disease risk.

Early studies of these polymorphisms suggested that there is, indeed, a greater prevalence of the less frequent alleles in diseased (coronary and cerebrovascular) populations than in controls, especially in individuals with arterial vasospasm (3,5–7). As the number of studies increased to include broader, more ethnically diverse populations, however, these associations were not confirmed (8–10). Similarly, two prior studies showed abnormal vascular function in individuals bearing the G894T allele (11,12); however, a third study failed to find such an association (13).

In two studies published in this issue of the Journal, Caucasians (principally southern Europeans) were studied to seek possible associations between the T-786C and G894T polymorphisms and prevalent vascular disease or dysfunction. In one study of 749 patients with angiographically defined coronary artery disease (CAD), Rossi et al. (14) found that the T-786C polymorphism was significantly more frequent than among 449 individuals without CAD; subjects bearing the C allele had a significant increase in the likelihood of having two- or three-vessel CAD than did those who were homozygous for the T allele. No association was found between the G894T polymorphism and the presence of angiographically defined CAD.

In a second study of 137 essential hypertensive and 50...
normotensive subjects without clinical evidence of atherothrombotic vascular disease, Rossi et al. (15) found a greater prevalence of the T-786C polymorphism among hypertensive individuals with endothelial dysfunction than among those without endothelial dysfunction; subjects bearing the C allele had a significant reduction in the maximal forearm blood flow response to acetylcholine compared with those who were homozygous for the T allele. Importantly, while there was a significant difference between hypertensives and normotensives in their response to acetylcholine (confirming reports by others), there was no relationship between T-786C allele frequency and forearm blood flow response among normotensives. These results suggest that either the T-786C allele is an important determinant of the hypertensive diathesis or is a determinant of endothelial dysfunction in individuals who have or are prone to hypertension for other genetic reasons. Again, these investigators found no association between the prevalence of the G894T polymorphism and the dysfunctional endothelial phenotype; however, they did detect a significant interaction between the T-786C and G894T polymorphisms and the associated endothelial dysfunction (15).

These observations confirm the potential functional importance of these polymorphisms in atherothrombogenesis. Owing to their relationship to abnormally low eNOS activity, these polymorphisms lead to a state of relative NO deficiency and promote endothelial dysfunction. Nitric oxide deficiency is not only a manifestation of endothelial dysfunction, but also limits the endothelial reserve needed to combat the adverse effects of exogenous risk factors that lead to a state of endothelial dysfunction. It is, therefore, not surprising that, in the study of patients with CAD, smoking, obesity, hypercholesterolemia, and older age were additive to the effect of the T-786C polymorphism in predicting the prevalence of atherothrombosis. A similar result was recently reported between the T-786C polymorphism and smoking as a risk for vascular abnormalities in the cerebral circulation (16).

The quantitative strength of the association of the T-786C polymorphism with the prevalence of CAD deserves comment. This association is weak, albeit statistically significant, with an odds ratio of approximately 1.7. This result suggests either that the association is not biologically relevant or that this polymorphic allele alone is insufficient to account for enhanced susceptibility to vascular disease or dysfunction. Rather it requires other risk factors to manifest adverse effects, that is, it is a disease-modifying allele. This view is consonant with the polygenic nature of atherothrombosis in which multiple alleles interact with each other and with environmental factors to produce the disease phenotype.

There are multiple limitations of these two studies that fall into study design and biological categories. In the study of CAD (14), the use of two different control populations, exclusion of some important risk factors from the logistic regression model, the lack of correlative measures of eNOS function, and the lack of a more quantitative measure of atheroma burden are clear shortcomings. In the study of endothelial function (15), the many significant baseline differences between the hypertensive and normotensive subjects and the lack of measurement of NO metabolites or bioactivity are both important limitations. The lack of a graded risk response to adverse allele abundance can either be interpreted to mean that a threshold effect is all that is required to promote disease expression, or that the association is not biologically relevant. If the latter were the case, one might interpret the results to mean that the eNOS polymorphism is not itself related to disease progression but, rather, genetically linked to another, as yet unidentified, disease-determining genetic locus.

These observations taken together with previously published results suggest that the eNOS polymorphisms do, indeed, promote endothelial dysfunction and atherothrombogenesis. To be sure, these data are small steps on a long journey of discovery, but important, nonetheless, in gaining a clear understanding of a complex pathobiology. In addition, these observations suggest that therapies tailored to enhance endogenous NO production, to substitute exogenous NO donors for limited endogenous stores, or to decrease the oxidative inactivation of NO may prove beneficial in individuals with eNOS polymorphisms that limit the generation of bioactive, atheroprotective NO.

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REFERENCES


