Inherited Dysfunctional Nitric Oxide Signaling and the Pathobiology of Atherothrombotic Disease

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Dysfunctional nitric oxide signalling increases risk of myocardial infarction

Erdmann et al

A recent article in Nature by Erdmann et al studies a family with excessive cardiovascular disease in younger adults and identifies mutations in 2 functionally related genes that may be linked to atherothrombotic disease.

Using genome-wide association studies and exome sequencing, large numbers of genetic loci conferring an increased risk of a wide variety of diseases have been identified, revealing new genes and pathways not previously suspected to be relevant in pathobiology. For common complex diseases, such as atherosclerosis, hypertension, diabetes mellitus, and heart failure, single nucleotide polymorphisms associated with increased risk typically have small effect sizes and seem to only account for a small fraction of the genetic risk. These data suggest that the majority of patients likely to develop common diseases, such as atherosclerosis, are unlikely to be identified by currently defined single nucleotide polymorphisms. A recent article in Nature diverges from the population-wide approach to elucidate select variants by studying a family with excessive cardiovascular disease in younger adults and identifies mutations in 2 functionally related genes that may be linked to atherothrombotic disease.

Cardiovascular disease continues to be the major cause of morbidity and mortality in North America and a growing problem in most other parts of the world. Recent data have shown that cardiovascular disease accounts for more than one third of deaths in the United States annually. Thrombus formation within a coronary vessel is the precipitating event in myocardial infarction and unstable angina. Rupture of atheromatous plaque in relatively mildly stenosed vessels and subsequent thrombosis are responsible for most acute coronary syndromes. Both superficial and deep intimal injuries lead to the adherence of platelets to the subendothelium and, subsequently, platelet activation. Common diseases, such as atherosclerosis, are polygenic, with multiple genes causing modest effects that interact with relevant environmental factors. However, cardiovascular disease is also specifically associated with a positive family history of disease. To identify specific mutations relevant to a positive family history, Erdmann et al recently used exome sequencing in a well-phenotyped family with myocardial infarction. These studies led to the discovery of rare variants that may disrupt nitric oxide signaling in the vasculature and lead to accelerated occlusive thrombosis. Using a group identified as part of the German Myocardial Infarction Family Study, the authors found 22 members who had myocardial infarction before the age of 60 years. Genetic studies, resequencing, and validation in independent cohorts led to identification of 2 mutations that they link with cardiovascular disease. The authors surmise that these specific variants contribute to the regulation of nitric oxide and to the pathology associated with atherothrombotic disease.

To identify the novel genetic variants linked with cardiovascular disease, Erdmann et al used a large family with a history of unstable coronary syndromes at a young age. Although microsatellite-based linkage analysis failed to show a casual locus, sequencing of family members who experienced myocardial infarction revealed 2 gene mutations. Specifically, the authors identified the nonsynonymous p.Leu163Phefs*24 and p.Ser525Leu mutations in GUCY1A3 and CCT7, respectively. The first variant found in the guanylate cyclase soluble (sGC) subunit α-3 is an enzyme encoded by the GUCY1A3 gene and is activated by nitric oxide. The second variant was found in the gene CCT7 and encodes a molecular chaperone necessary for folding of newly synthesized cellular proteins and stabilizes soluble guanylyl cyclase. Although they had nonsignificant logarithm of odds scores independently, a 2-locus linkage analysis demonstrated a maximum logarithm of odds score of 5.68. To biologically validate these findings, they show that carriers of both GUCY1A3 and CCT7 variants who all had early cardiovascular disease also had reduced sGC protein levels and decreased cGMP formation. Noncarriers and single mutation carriers had normal sGC formation. Transfected HEK 293 cells with constructs containing GUCY1A3 and CCT7 variants revealed reduced α1-sGC protein expression and cGMP production. Although these cellular studies are supportive of alterations in cGMP expression, the findings do not link the changes with activity that would support the pathobiology seen in unstable coronary disease, such as vascular function, markers of cellular adhesion, or occlusion.

The mutations identified in the study by Erdmann et al could impair sGC-dependent nitric oxide signaling; a relevant target as the function of this pathway is connected to the pathogenesis of cardiovascular disease. Nitric oxide is a potent vasodilator produced by endothelial cells that mediates vessel relaxation in many vascular beds including the coronary arteries. Nitric oxide also inhibits platelet activation and thrombus formation, and platelet release of nitric oxide may be attenuated in the setting.

The opinions expressed in this Commentary are not necessarily those of the editors or of the American Heart Association.

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Circulation Research is available at http://circres.ahajournals.org
DOI: 10.1161/CIRCRESAHA.114.303976
of unstable coronary syndromes. Nitric oxide synthase has been identified in human platelets, and platelet-derived nitric oxide has been shown to inhibit platelet recruitment after aggregation. Importantly, data suggest that the lack of platelet-derived nitric oxide alters in vivo hemostatic response by increasing platelet recruitment and altering the regulation of hemostasis and may be clinically relevant to acute thrombotic disorders. In this regard, the data presented in the study by Erdmann et al1 have some limitations. In this study, only platelet sGC expression and activity were measured without direct correlation with platelet function either by standard functional assays or by flow cytometry. Platelet levels or activity of cGMP have not been robust measurements of platelet function or activity particularly in the setting of acute coronary disease, limiting the assumptions that can be made by the findings. Platelet function itself was only measured using mice deficient for α1-sGC and demonstrated increased thrombus formation in vivo in the arteriole bed. Again, these studies are restricted to only measurement of small vessels and without direct study of platelet function. Because no targeted deletion, bone marrow transplantation, or platelet reinfusion studies were performed, only limited conclusions can be drawn because one cannot deduce whether the thrombotic changes were because of the platelets themselves. Finally, vascular reactivity, an important component of cGMP/nitric oxide–dependent coronary function, was not investigated.

Despite the limitations in the functional studies, the concept that platelet activity in the setting of thrombosis may be heritable is relevant and lacking extensive data. In one of the largest population-based studies of genomics and platelet function, measured covariates accounted for only 4% to 7% of the overall variance in platelet aggregation, and heritable factors accounted for 20% to 30%. However, predicted functional variants, such as the platelet glycoprotein IIIa Pl (A2) polymorphism and the fibrinogen Hind III β-148 polymorphism, contributed <1% to the overall variance. Further investigation tested the association of 2.5 million single nucleotide polymorphisms with platelet aggregation in ~4000 individuals and identified associations of 7 loci with platelet aggregation near or within specific genes. Six of these loci replicated at P<0.05 in an additional black cohort. With further investigation, the relevance of the GUCY1A3/CCT7 mutations to vascular function and thrombosis may be more clearly defined.

An important question raised by this study is how widely can the results be applied to a common disease? Studies of human genetics are filled with examples of single gene mutations that account for disease in families; however, relatively few examples use a 2-locus model such as in the study by Erdmann et al. To assess the linkage between GUCY1A3/CCT7 and cardiovascular disease beyond the index family, the authors clearly confirmed that both mutations were absent in large cohorts of control patients. Unfortunately, they were unable to identify GUCY1A3 and CCT7 mutations in families other than the index family, it could not be extended into other families with clusters of young myocardial infarction.

In summary, the recent article in Nature by Erdmann et al reports select variants using a family with excessive cardiovascular disease in younger adults and identifies mutations in 2 functionally related genes that may be linked to atherothrombotic disease. Although these data suggest that the GUCY1A3 and CCT7 mutations contribute to myocardial infarction risk in the index family, in the absence of validation of digenic inheritance in additional independent cases, it is still possible that unrecognized mutations in the family contributed to disease in a polygenic manner, highlighting the importance of understanding complex diseases by focusing on how genes work together in groups rather than singly. Genetic testing for cardiovascular disease remains distant, and these studies, although biologically relevant and plausible, do not immediately further our ability to predict who is at risk for acute myocardial infarction.

Sources of Funding
Dr Freedman is supported by P01-HL085381, RFA-HL-12-008, and RFA-RM-12-013NH from Common Fund, through the Office of Strategic Coordination/Office of the National Institutes of Health Director.

Disclosures
None.

References
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Circ Res. 2014;114:1372-1373
doi: 10.1161/CIRCRESAHA.114.303976

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