The 9p21.3 Genetic Region and Coronary Heart Disease

Where Do We Go From Here?*

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An enormous and continually growing body of evidence associates genetic variation at the 9p21.3 locus with various phenotypes of coronary heart disease (CHD). What remains to be discovered is the precise phenotype(s) that 9p21.3 predicts, the underlying specific genetic and pathophysiological mechanisms involved, the possible and optimal application of 9p21.3 genotyping in risk prediction, and the development of therapeutic interventions targeting the 9p21.3 mechanism(s). In keeping with the large universe of 9p21.3 literature, the study of Ardissino et al. confirms an association of an overall CHD phenotype with 9p21.3.

See page 426

We then may ask, what incremental insights does the study provide, and what are its limitations? In this issue of the Journal, Ardissino et al. (1) examined 9p21.3 as a risk predictor from a unique (but restricted) perspective: a very young population (<45 years of age) with a first myocardial infarction (MI) who underwent coronary angiography but did not undergo coronary revascularization for the index event. (This allowed the investigators to assess the probability of subsequent cardiovascular events and the progression of atherosclerosis without the interference of an initial revascularization.) As expected and intended, this youthful population was highly enriched for a family history of CHD (82% prevalence). The population was characterized further by almost universal smoking (>87% rate), an environmental risk factor that may alter the specifics of MI pathological features (2) and genetic susceptibility (3). Of many, mostly highly linked single nucleotide polymorphisms (SNPs) at the 9p21.3 locus, the study focused on 1 specific variant (rs1333040), found to be the most predictive among 5 SNPs in an earlier Italian case-control study. Of concern regarding generalizability, however, is that this variant has been studied less extensively in other populations and was characterized by an unusually high prevalence of the risk allele: 71%.

The study achieved its primary endpoint of associating rs1333040 with the composite of recurrent MI, cardiovascular (CV) death, or revascularization, but this finding was driven almost entirely by revascularization. Although failing to bolster the effect on the primary endpoint, the absence of an impact on MI and CV death actually is entirely consistent with and supported by a growing number of studies, including our own, which are beginning to define the specific 9p21.3-associated phenotype (4–6). In keeping with these other reports, the Ardissino et al. (1) study is confirmatory of an absence of an independent impact of 9p21 on precipitation of MI or CV death after the extent of CV disease is taken into account. In contrast to these negative findings, Ardissino et al. reported a strong positive gradient of CAD prevalence and extent at baseline by 9p21 risk allele. This observation also is consistent with the large body of 9p21 literature (7).

The further suggestion of an impact on disease progression, once present, shares 2 important limitations. First, and understandably, only a symptomatic minority of patients underwent repeat coronary angiography to allow quantitative assessment of CAD progression (405 of 1,508; 27%), raising a concern about unintended selection bias (although genotype presumably did not impact the decision to re-study). Second, and critical to the concern about selection bias, knowledge of baseline angiographic severity of disease, which was a strong 9p21.3-associated finding, undoubtedly entered into the decision to repeat angiography and consider revascularization. With these limitations in mind, rs133340 seemed to be moderately associated with disease progression (p = 0.002). However, baseline CAD presence markedly dominated the rs1333040 allele as an independent predictor of the primary composite of CHD progression (p = 4 × 10−13 vs. p = 0.01). Although confounded in interpretation, these findings seem to support the concept of a large impact of 9p21.3 on CAD initiation and a more modest one on subsequent disease progression (4.8–10). What remains to be discovered is the actual risk-associated genetic allele(s) and its molecular biological basis.

A major reason for our limited mechanistic understanding lies with the fact that the 9p21.3 chromosome is located in a region that is devoid of transcribed genes. Additionally, no prior successes addressing a similar genetic challenge are available to act as role models. However, that does not imply that there are no directions to pursue. Here we propose 5 separate, but related, lines of inquiry that might be attempted. Deep deoxyribonucleic acid sequencing. If identified and associated with the presence of atherosclerosis, deep deoxyribonucleic acid sequencing of the 9p21.3 region may provide additional clues that can isolate the culprit region to a much smaller section of deoxyribonucleic acid. Using data...
from 48 individuals who received deep sequencing of a 200-kb region on chromosome 9p21, Bansal et al. (11) described a novel method of SNP identification from population sequence data that leverages sequence data from a population of individuals to detect SNPs and to assign genotypes to individuals. They demonstrated that their method was highly accurate for detecting variants and was capable of filtering out false SNPs that were attributable to sequencing errors. Although this method of analysis may provide much important information because of the tremendous potential genetic variation within the 9p21.3 region among individual patients, it is likely that thousands of cases and controls will be required to identify the specific important genetic polymorphisms.

**The antisense noncoding ribonucleic acid gene.** Although the much of the 9p21.3 region is devoid of coding genes, it does include a large antisense noncoding ribonucleic acid gene (ANRIL) that may act as a vascular growth regulatory element. Additionally, Burd et al. (12) reported that the SNPs most strongly associated with atherosclerotic vascular disease are 120 kb distant from the nearest coding gene within the ANRIL locus, but they identified novel circular ribonucleic acid (RNA) products emanating from the ANRIL locus and suggested that causal variants at 9p21.3 regulate INK4/ARF expression and atherosclerotic vascular disease risk by modulating ANRIL expression, structure, or both. This preliminary information is promising, but much further study is required.

**Intermediate endpoints.** Although 9p21.3 has been linked directly to atherosclerosis, associations between it and other intermediate but related pathophysiologic processes may be illuminating. One example is the documented association of 9p21.3 with platelet reactivity. In 1,402 asymptomatic Amish adults, Musunuru et al. (13) documented a strong association between the 9p21.3 (rs10965219) genotype, severity of coronary calcification, and measured platelet reactivity that persisted after multivariate adjustment. Further studies associating 9p21.3 with other intermediate processes related to atherosclerosis may be helpful.

**RNA expression.** Finding associations between 9p21.3 and RNA expression also may be an effective way to approach the underlying mechanisms of 9p21’s association with atherosclerosis. To define potential direct links between RNA expression and atherosclerosis, Rosenberg et al. (14) identified a peripheral blood 23-gene RNA expression pattern among 526 symptomatic nondiabetic patients with a clinical indication for coronary angiography that identified those with significant CAD with a sensitivity and specificity of 85% and 43%, respectively. Similar analyses correlating certain RNA gene expression profiles to specific 9p21 genotypes may be helpful in exploring the underlying mechanisms behind its association with atherosclerosis.

**MicroRNA.** MicroRNAs are evolutionarily conserved, small (20 to 25 nucleotides), single-stranded molecules that suppress the expression of protein-coding genes by translational repression, messenger RNA degradation, or both (15). More than 700 microRNAs have been identified in the human genome that participate in a broad range of human expression patterns during development and tissue homeostasis and in the pathogenesis of disease (16). With the added advantage that analysis can be performed on frozen plasma, great potential exists for an association between microRNA expression profiles and 9p21 genotypes.

In summary, the present paper by Ardissino et al. (1) adds further epidemiological evidence that points the effects of the 9p21 genetic polymorphisms toward the initiation and progression of atherosclerotic plaque, rather than plaque rupture. Although useful by itself, much more information regarding the basic mechanisms related to this association are needed before full clinical use of this interesting genetic association can be made.

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Key Words: early-onset myocardial infarction • 9p21.3 genetic variants • outcomes • rs1333040.