Editorial

Quest for Genes and Mechanisms Linking the Human Chromosome 9p21.3 Locus to Cardiovascular Disease

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In the past several years, advances in molecular genetics have been adopted as tools to dissect the cause and pathophysiology of common complex diseases and their associated risk factors in a hypothesis-free approach, using the genome-wide association study design and high-density, single nucleotide polymorphism (SNP) genotyping arrays. Starting in 2007 with the discovery of the chromosome 9p21.3 (Chr9p21.3) locus (see recent review), major progress has been made in terms of identification of novel susceptibility loci for coronary artery disease (CAD; ie, genomic regions that are strongly and consistently associated with CAD). These CAD susceptibility loci are of major interest because they are expected to harbor genes constituting important nodes in biological pathways underlying CAD and hence may serve as shortcuts to discovery of hitherto unknown disease mechanisms. However, causal variants will only occasionally be among those that are directly typed in genome-wide association scans, and the interval within which the etiologic variant(s) are expected to lie can be sizeable, and often contains several genes. Thus, the challenge for the next several years will be to move from statistical association based on genetic markers to identification and functional characterization of individual risk-associated genes, gene variants, biological pathways, and proteins and to elucidation of their roles in the resulting vascular pathology.

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

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INK4/ARF locus contains 2 genes, CDKN2A and CDKN2B, encoding cyclin-dependent kinase inhibitors, and MTAP, encoding methylthioadenosine phosphorylase. The CDKN2A, CDKN2B, and MTAP genes are important targets in tumor biology because loss of the INK4/ARF locus is a frequent occurrence in the development of cancer, and the Chr9p21 region has appeared as a risk locus in genome-wide association studies for several cancers, including glioma, basal cell carcinoma, breast cancer, and nasopharyngeal carcinoma.

As reported in this issue of Circulation, Kim and colleagues hypothesized that the complex Chr9p21.3 locus regulates the neighboring genes in the INK4/ARF locus, and they set out to examine the mouse region on Chr4 that is orthologous to the human INK4/ARF locus in relation to atherosclerosis in gene-targeted mice that were bred with the hypercholesterolemic APOE3 Leiden transgene. Genes systematically investigated included Mtap, Cdkn2a isoforms p16ink4a and p19arf, and Cdkn2b, of which Cdkn2a and Cdkn2b are established tumor suppressor genes whereas Mtap possesses a putative tumor-promoting action. Of note, there are several proofs of principle that tumor suppressor genes are implicated in atherosclerosis, but previous knockout mouse model studies of the entire orthologous region to the human Chr9p21.3 locus or the p19arf gene have shown contradictory results. Deletion of the region that is orthologous to the human risk locus on Chr9p21.3, which is uniquely enriched with enhancers, resulted in markedly reduced cardiac expression of Cdkn2a and Cdkn2b but no significant alteration in the expression level of Mtap, with accompanying doubling of the proliferative properties of smooth muscle cells when kept under cell culture conditions and a dramatically increased incidence of tumors in the knockout mice. Surprisingly, however, there was no increase in aortic lesion progression, but this study provided convincing evidence that distant-acting gene regulatory functions reside in the noncoding CAD risk interval. In contrast, mice deficient in the 19Arf gene had an increased propensity to develop atherosclerotic lesions in the aorta.

The main findings in the study conducted by Kim and colleagues were that mice that were heterozygous for Mtap, homozgyosity being embryonic lethal, developed larger atherosclerotic lesions than wild-type mice along with changes in levels of metabolites linked to methionine and cysteine processing pathways and perturbations of the global methylation pattern as well as a significant decrease in CD4+ T-cell counts. These are all plausible mechanisms by which Mtap insufficiency might exert an effect on atherosclerosis. Importantly, there was no concomitant counterregulation of other neighboring genes. Accordingly, it was concluded that Mtap...
protects against diet-induced atherosclerosis. However, it should be noted that there was no association between Chr9p21.3 SNP rs4977574 genotype and MTAP expression in vitro in primary human aortic vascular cells, a finding speaking against MTAP as a central player in the Chr9p21.3-associated effect on human atherosclerosis. A further interesting and potentially important observation was the extensive compensatory reciprocal gene regulation existing in the Chr9p21.3 region in all strains except the Mtap heterozygotes. This attests to the importance of this genomic region and renders any effects of the Cdkn2a and Cdkn2b genes hard to evaluate. Nevertheless, knockout of Cdkn2a seemed to protect against atherosclerosis despite compensatory modulation of the expression levels of Cdkn2b and Mtap.

Few human studies have indicated that MTAP is the culprit gene in the Chr9p21.3 locus in relation to atherosclerosis or clinical cardiovascular disease. However, the human and mouse phenotypes studied are materially different. The atherosclerosis phenotype in the APOE/3 Leiden mouse model used in the present study is proliferative in its early stages; hence, it may not be that surprising that a gene influencing cell proliferation, such as Mtap, was indicated as a culprit gene in mice. It is also notable that the lead SNP in the Chr9p21.3 locus has been associated with a wide range of vascular phenotypes in human studies besides clinical manifestations of CAD (except myocardial infarction per se), such as severity and rate of progression of CAD, carotid artery disease, ischemic stroke, abdominal aortic and intracranial aneurysm formation, and peripheral artery disease. This indicates that the Chr9p21.3 locus influences a fundamental mechanism affecting the vasculature, the nature of which remains unknown. It could be speculated that effects on cell proliferation might play a key role, as suggested by the association between Chr9p21.3 SNPs and impaired mechanical properties of the aortic wall.

A significant limitation of the mouse atherosclerosis models is the fact that mice lack a clear ortholog of ANRIL which, because of its position within the Chr9p21.3 haplotype block associated with CAD and expression in tissues and cell types that are affected by atherosclerosis, is currently considered as the most likely CAD susceptibility gene in humans. Also, a majority of studies have demonstrated associations between the CAD-associated SNPs and ANRIL expression, ANRIL splicing, and ANRIL structure and supported the notion that ANRIL regulates INK4/ARF expression. However, studies of CAD SNP associations with the expression of ANRIL and INK4/ARF have not been entirely consistent. Difficulties in measuring ANRIL expression, the presence of a high and growing number of splice variants, and tissue- and cell-specific differences in expression are likely to contribute to the prevailing discrepancies. Alternatively, the CDKN2A, MTAP, and CDKN2B genes might not be ANRIL targets, as indicated by a recent transcriptome and systems biological study showing that the CAD-associated SNP is associated with myocardial expression of a total of 46 genes. Be that as it may, ANRIL appears to be a first-line candidate for modulating the susceptibility to cardiovascular disease at the Chr9p21.3 locus.

In all, although the ambitious study of Kim and colleagues convincingly pinpoints Mtap as a mouse susceptibility gene for diet-induced atherosclerosis and suggests some mechanisms by which its effect might be exerted, the question arises of the extent to which mouse studies will generate substantial information on the CAD-associated region including ANRIL that is of apparent immediate relevance to human atherosclerotic disease. As a result of potentially important differences between human and mouse in this particular locus, a strong emphasis will have to be placed on human studies.

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Disclosures
None.

References


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