Paraoxonase and Coronary Heart Disease Risk
Language Misleads, Linkage Misinforms, Function Clarifies

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Organophosphates were first synthesized in the 1930s as insecticides and were subsequently shown to have direct neurotoxic effects in mammals, as well. The neurotoxicity is derived from their ability to inhibit acetylcholinesterase by covalently modifying the active-site serine group in the enzyme. Mazur first demonstrated the presence of an organophosphate-hydrolyzing enzyme in mammalian tissue, an observation that ultimately led to the identification of a human paraoxonase in serum in 1953. Paraoxonase—so named because of its ability to hydrolyze the toxic metabolite of parathion, paraoxon—was also shown early after its identification to manifest arylesterase activity, an effect that was underappreciated until the enzyme was found to play a role in modulating vascular oxidant stress many years later.

The paraoxonase story is a good example of the power of language to mislead. There is no teleological reason for mammals to have evolved an enzyme that can hydrolyze synthetic organophosphates; yet, paraoxonase was isolated in an effort to understand the endogenous metabolism of these exogenous neurotoxins. The enzymatic activity for which the enzyme is named is screened by using synthetic substrates without regard for the native substrate or its role in human (patho)biology. This focus on one aspect of the enzyme’s function, overemphasized by its denotation, delayed an appreciation of other potential—and perhaps more relevant—roles. In 1991, Mackness et al. showed that paraoxonase could limit the accumulation of lipid hydroperoxides in low-density lipoprotein, thereby ushering in an era of interest in its relationship to atheroprotection. Although the preferred endogenous substrate of paraoxonase remains unknown, lactones (including homocysteine thiolactone) comprise one possible candidate class (see below).

The paraoxonases comprise 3 isoforms (PON1, PON2, and PON3) that attenuate oxidant stress; among these, only PON1 hydrolyzes organophosphates, and thus, reference to the other 2 isoforms as paraoxonases is a misnomer. As stated, PON1 protects low-density lipoprotein from oxidation; it binds to high-density lipoprotein in a calcium-dependent manner and inhibits its oxidation, as well. These in vitro effects are reflected in the pathophenotype of the PON1-null mouse, which manifests increased susceptibility to diet-induced atherosclerosis.

These mechanistic studies suggesting an atheroprotective effect of PON1 led to a host of clinical studies designed to assess the relationship of PON1 genetic variants, PON1 activity, or both to coronary heart disease risk. Two major coding variants of PON1, Q192R and L55M, and 5 promoter polymorphisms have been reported to date. Epidemiological association studies have yielded conflicting results, however, with a recent meta-analysis of 43 studies showing no effect of the L55M and C-107T polymorphisms but a slightly increased risk of the 192R allele for coronary heart disease. The extraordinary work required to perform the studies that led to this meta-analysis showing a modest effect for 1 variant allele highlights the ability of genetic linkage analysis to misinform when performed without expression studies or functional studies. This point is particularly relevant to PON1 because of the 10- to 40-fold interindividual variability in its serum activity. A wide variety of factors is known to modulate PON1 gene expression, including oxidized low-density lipoprotein, oxidized low-molecular-weight lipids, interleukin (IL)-6, IL-1β, and tumor necrosis factor-α, all of which downregulate gene expression, and IL-6 and simvastatin (acting through sterol regulatory element-binding protein 2), both of which upregulate gene expression. Smoking and trans-fats have been shown to decrease serum paraoxonase activity, whereas polyphenols, moderate alcohol consumption, ascorbate, and α-tocopherol have been shown to increase serum paraoxonase activity in humans. Thus, simple knowledge of the types of genetic variants cannot possibly be sufficiently informative to ascertain the relationship of these variants to disease risk. To do so fully requires a measure of gene expression (mRNA or protein) and enzyme activity.

Clinical studies of serum paraoxonase activity use organophosphate substrates to determine enzyme activity. In that the organophosphate-hydrolyzing activity of paraoxonase need not reflect its activity toward an endogenous (as yet unknown) substrate, variability in this activity and its implications for atheroprotection are uncertain. Illustrating this point and confounding the problem of a lack of knowledge of the endogenous substrate is the observation that the less prevalent 192R variant has a ~9-fold greater catalytic efficiency (kcat/KM) than the 192Q variant toward an organophosphate substrate. That the 192R allele confers a greater (albeit modest) risk of coronary heart disease than the 192Q allele is difficult to reconcile with the assumption that paraoxonase...
activity is a surrogate for enzymatic activity toward an endogenous substrate that promotes atherogenesis.

In the study by Richter et al in this issue of Circulation: Cardiovascular Genetics, the authors develop alternate assays to measure paraoxonase activity in serum, taking advantage of its arylersterase activity. Using 2 different aryl esters and different ionic strengths of the assay medium, they have developed a method that permits discrimination among QQ, RQ, and RR alleles at position 192 and have done so using nontoxic substrates. They show that this new method correlates well with the conventional 2-substrate organophosphate profiling, suggesting that it may serve as a viable alternative to current activity assays in population screening studies.

Now armed with known variant alleles that can influence function, a knowledge of the determinants of gene expression and its wide interindividual variability, and a safe and reproducible assay for enzyme activity, geneticists can ascertain the relation of this antioxidant enzyme to coronary heart disease risk in a rigorous and meaningful way. The remaining unknown, however, is the relationship between the catalytic efficiencies of the enzyme variants toward the natural substrate and the artificial substrate (organophosphate or aryl ester). Without this knowledge, we have a functional assay in search of a biologically relevant functional correlate. The lactonase activity of paraoxonase may be an important consideration here, especially if one endogenous substrate is the naturally occurring homocysteine thiolactone. As this homocysteine derivative can adversely affect protein function by posttranslational modification and, as a result, vascular cell phenotype, studies will be required to determine the catalytic efficiency of the 192R and 192Q variant alleles toward homocysteine thiolactone. If this is an important atheroprotective function of the enzyme, one would expect lower thiolactonase activity of the R variant than of the Q variant.

These data demonstrate that genetic determinants of complex phenotypes must ideally be assessed at multiple levels for completeness. Genetic linkage analysis is only the beginning and must be followed by studies of expression and function. The functional phenotype should correlate with an understanding of its relevance to disease mechanism. One can view these principles as essential for rigorous validation of any genetic association study. Paraoxonase clearly illustrates the importance of this range of approaches at every level. This interesting enzyme remains an uncertain determinant of atherosclerotic risk at this time, supportive preclinical data notwithstanding.

Disclosures
None.

References

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