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INVITED EDITORIAL
Paraoxonase-Gene Polymorphisms Associated with Coronary Heart Disease: Support for the Oxidative Damage Hypothesis?

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Mechanisms contributing to atherosclerosis, the major cause of coronary heart disease and stroke, are not well understood. Experimental studies with cultured cells and animal models have suggested that oxidative damage could be an important contributing factor, but implicating oxidation in human disease has proved difficult. One potentially powerful approach is to examine polymorphisms of candidate genes in populations. In this issue of the *Journal*, Sanghera et al. (1998) report evidence that common polymorphisms in a cluster of related genes, including that for serum paraoxonase, an enzyme of unknown function, modulate the risk for coronary heart disease. A number of previous genetic epidemiologic studies have yielded consistent findings.

Experimental studies suggest that paraoxonase may lower the risk for coronary heart disease by destroying proinflammatory molecules involved in the initiation and progression of atherosclerotic lesions; these studies support a role for oxidative damage in the human disease. Because paraoxonase is a component of HDL, these data provide a possible explanation for the inverse relationship between HDL levels and coronary heart disease in populations. Taken together with previous studies, these data allow paraoxonase to be added to a short list of genes that exhibit common variations influencing heart disease. As this list grows, improved diagnosis or even risk assessment for coronary heart disease are likely outcomes.

Paraoxonase, an Enzyme without a Function

Paraoxonase is a serum enzyme whose precise physiological role is unknown (Mackness et al. 1996). It hydrolyzes many substrates—including aromatic carboxylic acid—and organophosphates such as paraoxon, a metabolic product of the widely used pesticide parathion. The enzyme has been studied mainly for its ability to break down pesticides and nerve gases, such as sarin. Because insects generally lack paraoxonase activity, they are particularly susceptible to these compounds. The human gene for paraoxonase, PON1, is located on the long arm of chromosome 7, at q21-q22 (Humbert et al. 1993).

The hydrolysis products of paraoxon are relatively nontoxic, in contrast to paraoxon itself, a potent inhibitor of the cholinesterases that break down the neurotransmitter acetylcholine (Mackness et al. 1996). Thus paraoxonase in blood might help prevent paraoxon from reaching the nervous system, where the pesticide would cause acetylcholine to accumulate at cholinergic synaptic junctions and to overstimulate neurons.

Serum levels of paraoxonase activity vary widely among individuals, which may partly account for differences in susceptibility to organophosphate poisoning (Blatter-Garin et al. 1994; Davies et al. 1996). However, activity remains relatively constant for a given individual. The molecular basis for this difference appears to be a polymorphism in the PON1 gene: PON1 A, an isoform with *low* activity toward paraoxon (but *high* activity toward certain other organophosphates), has a glutamine at position 192, whereas the PON1 B isoform, with high activity toward paraoxon, contains an arginine at that position (Davies et al. 1996). Thus, a single amino acid substitution that is readily detected by restriction-enzyme analysis results in an eightfold difference in the enzyme's ability to hydrolyze paraoxon.

The Oxidation Hypothesis of Atherosclerosis

Human and rabbit PON1 genes exhibit a striking degree of sequence similarity (86% at the nucleotide level and 85% at the amino acid level), suggesting that paraoxonase may play an important role in metabolism (Hassett et al. 1991). One intriguing hypothesis is that the enzyme may remove lipid peroxidation products

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Table 1

Association Studies between Paraoxonase-Gene Polymorphisms and Coronary Heart Disease

Study	Polymorphism (Codon)	*P* Value[a]
Sanghera et al. 1998	PON1 (912)	<.0001
Sanghera et al. 1998	PON2 (311)	<.005
Odawara et al. 1997	PON1 (192)	.003
Suehiro et al. 1996	PON1 (192)	NS
Herrmann et al. 1996	PON1 (192)	NS
Garin et al. 1997	PON1 (54)	.03
Antikainen et al. 1996	PON1 (192)	.12
Serrato and Marian 1995	PON1 (192)	.0003
Ruiz et al. 1995	PON1 (192)	.03

[a] NS = not significant.

(Mackness et al. 1991*a*). Such oxidatively damaged macromolecules have been implicated in aging and in diseases ranging from ischemia-reperfusion injury to arthritis to cancer (Ames et al. 1993).

Many lines of evidence implicate oxidized LDL—rather than LDL itself—in atherogenesis (Berliner and Heinecke 1996). Immunohistochemical studies with monoclonal antibodies that specifically recognize protein-bound lipid oxidation products provide direct evidence for LDL oxidation in the artery wall. Moreover, LDL with indications of oxidative damage has been isolated from human and animal atherosclerotic lesions. Also, several chemically unrelated lipid-soluble antioxidants retard or inhibit atherosclerosis in hypercholesterolemic animals (Berliner and Heinecke 1996), and epidemiological studies suggest that a high dietary intake of antioxidants is associated with a decreased risk for coronary artery disease (Steinberg 1995). Most significantly, vitamin E prevents acute coronary events in patients with angiographically established atherosclerotic vascular disease (Stephens et al. 1996), suggesting that oxidative events are of central importance.

The mechanisms that promote LDL oxidation in vivo are uncertain, though many different pathways have been proposed on the basis of in vitro evidence. Because plasma exhibits a powerful array of antioxidant defense mechanisms, however, it is generally thought that LDL oxidation takes place in the artery wall. Recent studies have provided direct chemical evidence that reactive nitrogen species and products of myeloperoxidase may contribute to the oxidative modification of arterial wall lipids and proteins (Heinecke 1997). Metal ions may also be involved late in the disease process.

In contrast to LDL, an elevated level of HDL lowers the risk for atherosclerosis. Because in vitro studies demonstrate that HDL inhibits copper-catalyzed LDL oxidation (Mackness et al. 1991*a*), its antiatherogenic properties may relate in part to its ability to protect LDL from atherogenic modifications. Paraoxonase activity in human serum is strongly associated with HDL. Indeed, it is difficult to separate the enzyme from apolipoprotein AI, the major HDL protein, indicating that HDL carries most of the paraoxonase in serum. Biochemical studies indicate that specific HDL subpopulations contain the paraoxonase protein.

In 1991, Mackness et al. (1991*a*) reported that paraoxonase inhibits the copper-catalyzed oxidation of LDL. This provided the first molecular hypothesis for a specific mechanism whereby HDL might affect LDL oxidation. Navab, Berliner, Fogelman, and coworkers subsequently discovered that paraoxonase can remove oxidized phospholipids from LDL (Watson et al. 1995; Navab et al. 1996, 1997). These lipids activate inflammatory genes and promote the adhesion of monocytes to endothelium, a key event in monocyte recruitment into early atherosclerotic lesions. Thus, the enzyme may alter the risk for vascular disease by cleansing LDL of oxidized lipids in vivo.

Paraoxonase and the Oxidation Hypothesis

The demonstration of links between paraoxonase and LDL oxidation led to an explosion of interest in the enzyme's possible role in atherosclerosis. Serum paraoxonase levels were found to be lower in patients with familial hypercholesterolemia and diabetes; these patients are at greatly increased risk for premature atherosclerotic vascular disease (Mackness et al. 1991*b*). Moreover, some but not all studies have shown that polymorphisms in the PON1 gene associate with an increased risk for coronary artery disease (table 1). The risk varies independently of traditional lipid risk factors, such as HDL and LDL levels, suggesting that, in some populations, additional factors may contribute to a person's risk for developing atherosclerosis.

The influence of paraoxonase on artery disease may be studied in inbred strains of mice, which differ dramatically in their susceptibility to atherosclerosis. For example, C57BL/6J (B6) mice are susceptible to diet-induced aortic fatty streak lesions, whereas C3H/HeJ (C3H) mice are resistant to such lesions. When the two strains received a low-fat diet that fails to promote atherosclerosis, their HDLs protected against LDL oxidation in vitro (Shih et al. 1996). In contrast, HDL isolated from susceptible B6 mice fed a high-fat, high-cholesterol diet was unable to inhibit LDL oxidation, whereas HDL from resistant C3H mice retained this property. Moreover, the ability of the various HDLs to prevent LDL oxidation was strongly associated with serum levels of paraoxonase. Also, liver levels of paraoxonase mRNA declined when susceptible B6 mice, but not resistant C3H mice, were subjected to the high-fat diet, indicating

Table 2

Genes Involved in Common Forms of Coronary Heart Disease

Gene	Polymorphism
Apolipoprotein E	Missense
Apolipoprotein (a)	Number of "kringle" repeats
Angiotensin-converting enzyme	Missense
Paraoxonase	Missense

NOTE.—Reviewed in Lusis et al. (1998).

that liver levels of paraoxonase mRNA are under genetic control. Paraoxonase mRNA levels cosegregated with the extent of atherosclerosis in recombinant inbred strains of mice derived from B6 and C3H mice (Shih et al. 1996). The results of these studies, like those from human studies, support the notion that paraoxonase plays a role in atherogenesis.

Paraoxonase Polymorphisms Are Associated with Coronary Heart Disease

The report by Sanghera et al. (1998) is the latest in a series of association studies between paraoxonase-gene polymorphisms and coronary heart disease (table 1). The majority of these studies have revealed significant association, though several have not. However, association studies have a number of potential problems. For example, population admixture can result in apparent associations that are due to differences in the frequencies of alleles in different ethnic groups. Certain analytic procedures, such as transmission-distortion analysis, can minimize this problem, but such methods have not yet been applied to studies of PON1. Another problem that has undermined confidence in association studies is the adoption of insufficiently stringent thresholds for establishing significant findings. For example, many studies include multiple phenotypes and group the data in various ways for analysis. When such multiple comparisons are performed, the threshold for significance should be adjusted, though the degree of adjustment is often complex. Because relatively few association studies consider the problem of multiple comparisons, many findings reported to be significant are in fact type 1 errors. Probably the most convincing evidence for an association is reproducibility in separate studies and in separate populations.

Associations between paraoxonase-gene polymorphisms and coronary heart disease have now been repeatedly reproduced (table 1), and the negative results of two studies could be explained by such factors as population differences, ascertainment differences, or simply chance. The conclusion that paraoxonase-gene expression affects the development of coronary heart disease is supported by several lines of evidence. As discussed above, paraoxonase can destroy certain oxidized lipids that may contribute to the inflammatory aspects of atherosclerosis. Also, a variety of studies have suggested that low paraoxonase levels are associated with atherosclerosis, hyperlipidemia, and type 2 diabetes (Mackness et al. 1991*b*, 1996; Navab et al. 1996). Finally, genetic studies of mice have revealed a relationship between paraoxonase expression and fatty streak lesions in mice (Shih et al. 1996).

A Cluster of Paraoxonase Genes

PON1 appears to be clustered with at least two other closely related genes, PON2 and PON3. The latter appear to be expressed in a variety of tissues, and their products are likely to be intracellular rather than secreted (Primo-Parmo et al. 1996). This raises the possibility that the associations observed for the PON1 polymorphism result from linkage disequilibrium with a closely linked PON gene. Sanghera et al. (1998) have explored this issue by typing a polymorphism of PON2, as well as the 192 polymorphism of PON1. The results indicate that both polymorphisms are significantly associated with coronary heart disease, though the PON1 association is much stronger (table 1). The data on the distributions of genotypes at the two genes suggest an interaction affecting the risk of coronary heart disease, though this requires confirmation. It remains possible that the observed association is due to linkage disequilibrium with either PON3 or another nearby gene. Also, since haplotypes for the PON1 and PON2 polymorphisms were not rigorously analyzed by use of families, it remains possible that the PON2 association is due to linkage disequilibrium with the PON1 polymorphism.

Toward an Understanding of the Biochemical and Genetic Basis of Coronary Heart Disease

The study reported by Sanghera et al. (1998) has important mechanistic implications. In vitro studies suggest that paraoxonase inhibits LDL oxidation by decomposing peroxidized lipids, such as the oxygenated phospholipids implicated in monocyte attachment to endothelium. However, the allele most commonly associated with an increased risk of coronary heart disease, PON1 B, elevates paraoxonase activity in plasma. Whether this allele alters the activity of the enzyme toward the natural substrate(s) is not yet established. Moreover, the biochemical mechanisms by which the enzyme degrades oxidized lipids have not been clearly identified. It is intriguing, however, that Sanghera et al. find that both PON1 B and PON2*S appear to increase the risk of atherosclerosis. This raises the interesting possibility that the two paraoxonase proteins act in concert to influence the

formation of atherogenic molecules. Alternatively, this combination of genotypes may be less effective at removing oxidative damage from lipoproteins.

Another important implication of the study reported by Sanghera et al. (1998) relates to our understanding of the genetic contribution to coronary heart disease. Although a number of Mendelian disorders contributing to atherosclerosis, such as familial hypercholesterolemia, have been characterized and are now relatively well understood, such disorders explain only a small fraction of cases of coronary heart disease, the vast majority of which result from the interactions of multiple genetic and environmental factors. However, epidemiologic studies of candidate genes are slowly adding to our understanding. The PON-locus polymorphisms should now be added to a short list of other genes for which there is significant evidence for contributions to common forms of coronary heart disease (table 2). A number of other genetic variations are very likely to affect risk, since they influence factors such as homocysteine levels (the methylenetetrahydrofolate reductase gene) or HDL cholesterol (the hepatic lipase gene). Several very interesting candidate genes, such as the scavenger receptors that mediate cellular uptake of cholesterol to give rise to foam cells, have yet to be examined. As this list grows, it may be possible to subdivide the different forms of coronary heart disease to tailor treatments to specific genetic defects or even to predict an individual's risk.

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