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FUNCTIONAL GENOMICS OF THE PARAOXONASE (PON1) POLYMORPHISMS: Effects on Pesticide Sensitivity, Cardiovascular Disease, and Drug Metabolism

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■ **Abstract** This review focuses on the functional genomics of the human paraoxonase (PON1) polymorphisms. Levels and genetic variability of the PON1 position 192 isoforms (Gln/Arg) influence sensitivity to specific insecticides or nerve agents and risk for cardiovascular disease. A more recent area of investigation, the role of PON1 in drug metabolism, is also discussed. We emphasize the importance of considering both PON1 isoforms and PON1 levels in disease/sensitivity association studies.

INTRODUCTION

Paraoxonase-1 (PON1) is a high-density lipoprotein (HDL)–associated serum enzyme whose primary physiological role is to protect low-density lipoproteins (LDL) from oxidative modifications (1). Early research focused on the observation that PON1 could hydrolyze organophosphorus (OP) compounds, including commonly used insecticides (2). Indeed, the enzyme (EC 3.1.8.1) was initially characterized as an organophosphate hydrolase, and its name derives from one of its most commonly used in vitro substrates, paraoxon. More recently, in addition to its role in lipid metabolism and, hence, in cardiovascular disease and atherosclerosis, PON1 has been shown to play a role in the metabolism of pharmaceutical drugs.

Early studies, which used the single substrate paraoxon to measure PON1 paraoxonase activity in serum from subjects of Caucasian origin, revealed a bimodal or trimodal distribution (3–5). On the basis of enzymatic tests, humans could be divided into three serum PON1 phenotypes: those with low, intermediate, and

high PON1 activity, without a clear demarcation between intermediate and high metabolizers. Studies in the past decade have elucidated the genetics of *PON1* and provided analyses that clearly differentiate among the PON1 phenotypes, as described below. Two polymorphisms in the coding region (6), as well as five in the promoter region, have been identified (7–11). The Q192R polymorphism determines catalytic efficiency of hydrolysis of some substrates (12–16), and some of the promoter polymorphisms, particularly C-108T, help regulate the level of expression of PON1 (7–11). This review focuses on the functional genomics of the *PON1* polymorphisms with respect to their role in determining susceptibilities to the toxicity of certain pesticides and to cardiovascular disease, as well as their recently emerging role in drug metabolism.

PON1 STRUCTURE AND POLYMORPHISMS

Studies in the early 1990s led to the purification of rabbit and human PON1s (6, 18) and subsequent cloning and sequencing of their respective cDNAs (6, 13). The PON1 cDNA encodes a protein of 355 amino acids from which only the amino-terminal methionine residue is removed during secretion and maturation (6). The retained leader sequence is required for the association of PON1 with HDL particles (19), and indeed PON1 is entirely associated with HDL in human serum (20). PON1 protein is synthesized mostly in the liver, from which it is released by a docking process whereby HDL particles transiently associate with the cell membrane and remove PON1 from the membrane (21). Physical mapping placed the human PON1 gene on chromosome 7 q21–22 (14). Two polymorphisms were observed in the PON1 coding sequence: a Gln (Q)/Arg (R) substitution at position 192 and a Leu (L)/Met (M) substitution at position 55 (6, 13, 14). Using the PCR method described by Humbert et al. (14) or modifications thereof, many studies have established PON1 192 and 55 genotypes for individuals in large populations. The polymorphism at position 192 has been the most studied, with gene frequencies for $PON1_{Q192}$ ranging from 0.75 for Caucasians of Northern European origin to 0.3 for some Asian populations (11). On the other hand, the gene frequency for $PON1_{L55}$ ranges from 0.57 in Caucasian populations to 0.99 in an Oji-Cree population. Several studies have shown that the L_{55} and R_{192} alleles are in strong disequilibrium, with ~98% of the R_{192} alleles having L at position 55 (11).

The coding region polymorphisms in the PON1 protein have been studied for effects on the catalytic efficiencies of hydrolysis of specific substrates. The L/M polymorphism at position 55 has not been found to affect catalytic efficiency (13–16) but has been associated with the variability of plasma PON1 levels; $PON1_{M55}$ individuals have lower PON1 levels on average (10, 22, 23). Linkage disequilibrium of the $PON1_{M55}$ allele with promoter polymorphisms appears to explain most of the differences observed (11), although $PON1_{M55}$ has been reported to be less stable than $PON1_{L55}$ (24). On the other hand, the Q/R polymorphism of position

192 significantly affects the catalytic efficiency of PON1, in a substrate-dependent manner (15). Initial studies indicated that the $PON1_{R192}$ isozyme hydrolyzed paraoxon more readily than $PON1_{Q192}$ (13, 14). Further studies suggested that the effects of this polymorphism may be substrate-dependent, as the $PON1_{Q192}$ isoform hydrolyzed diazoxon, sarin, and soman more rapidly than $PON1_{R192}$ with in vitro assays (15).

Additional polymorphisms have been found in the noncoding region of the PON1 gene (7–11). These polymorphisms are at positions –108 (C/T), –126 (G/C), –162 (A/G), –832 (G/A), and –909 (C/G), although reports vary somewhat in assignment of the base positions (10). The most significant of these promoter region polymorphisms is that at position –108, which contributes 22.4% of the variation in PON1 expression, with –108C providing higher levels of plasma PON1. The polymorphism at position –162 also contributes a small (2.4%) amount (10). Contribution of the 3′ polymorphisms (10) to variability of PON1 expression has not yet been investigated.

The existence of PON1 coding-region polymorphisms, which affect catalytic activity toward OPs, and of promoter-region polymorphisms, which affect the levels of PON1 expression, led to the proposal of determining the "PON1 status" of an individual (25, 26). By plotting rates of diazoxon hydrolysis against paraoxon hydrolysis at high salt concentrations (2 M NaCl), an accurate inference of $PON1_{192}$ genotype, as well as PON1 activity levels for individuals, can indeed be made as shown in Figure 1 (15, 25–27).

PON1 AND SENSITIVITY TO ORGANOPHOSPHATE TOXICITY

Many OP compounds are triesters of phosphoric acid. Their major source is from insecticides, although some have found therapeutic applications. Acute exposure to OPs causes neurotoxicity, in the form of a cholinergic syndrome, i.e., an overstimulation of muscarinic and nicotinic acetylcholine receptors in the central and peripheral nervous systems, due to accumulation of acetylcholine in the synaptic cleft resulting from OPs' inhibition of acetylcholinesterase (28).

Only OPs with a P=O moiety can interact with acetylcholinesterase. Commonly used insecticides, such as diazinon and chlorpyrifos, that have a P=S bond, need to be converted to their oxygen analogs. Bioactivation occurs by a process of oxidative desulfuration catalyzed by cytochrome P450 enzymes. A-esterases, such as PON1, can hydrolyze, and hence detoxify, a number of insecticidal organophosphates, including some nerve agents (2, 15).

The existence of polymorphisms in PON1 that confer different hydrolyzing ability toward OPs, as well as different levels of expression, has long inspired the hypothesis that certain individuals may be more sensitive to OP toxicity (29). This hypothesis has received confirmation only in the past decade, primarily from animal studies. Initial evidence for the role of PON1 in OP toxicity came from

cross-species comparisons, from animal experiments using purified PON1, and more recently from studies with PON1 knockout mice. Earlier findings indicated that birds, which have no to very low plasma PON1 activity, were more sensitive than rats to the toxicity of various OPs (30). In turn, rats were more sensitive to the toxicity of OPs than rabbits, whose plasma PON1 activity is seven times higher (31). Although several other factors may contribute to the species differences in OP toxicity, these early findings suggested that low plasma PON1 activity would increase sensitivity to the acute effects of OPs.

A more direct approach was provided by studies in which exogenous PON1 was injected into rats or mice. In a pioneering experiment, Main (29) intravenously injected into rats a partially purified PON1 from rabbit serum and noted a decrease in the acute toxicity of paraoxon. More recent studies in rats and mice have confirmed and expanded this early finding. Initially, PON1 purified to homogeneity

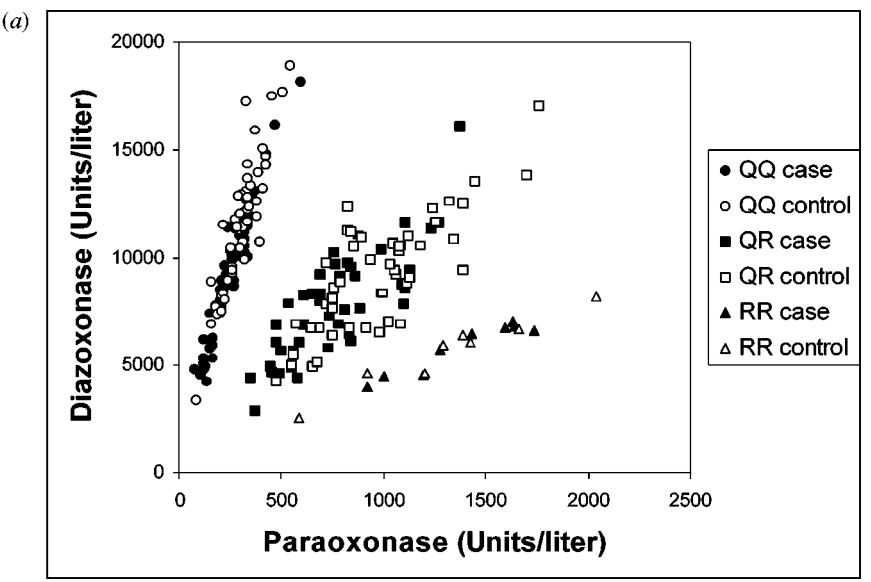

Figure 1 (*a*) Plot of diazoxonase vs. paraoxonase activities (26, 27) for carotid artery disease (CAAD) cases and controls, coded for $PON1_{192}$ genotypes (determined by polymerase chain reaction). Note that the two-substrate assay provides an accurate inference of $PON1_{192}$ genotype as well as the level of plasma PON1 activities (i.e., PON1 status). Reproduced from Reference 27 with permission. (*b*) Diazoxonase levels of CAAD patients and controls separated by position 192 genotypes as indicated. Note the abundance of individuals with low diazoxonase among the PON1QQ cases and the sparcity of cases with high diazoxonase activity. Diazoxonase activity is a relative measure of plasma PON1 protein. Means are indicated by horizontal bars. Reproduced from Reference 27 with permission.

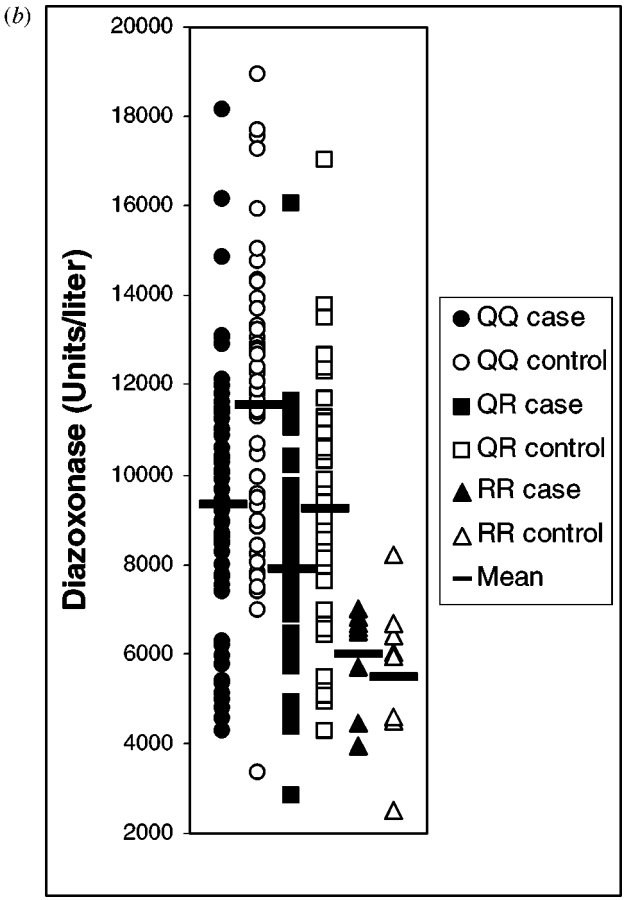

Figure 1 (*Continued*)

from rabbit serum (31a) was given by intravenous injection to rats (32). Administration of the enzyme raised rat serum PON1 activity toward paraoxon and chlorpyrifos oxon by 9- and 50-fold, respectively. When rats were challenged with either of these OPs, a significant protection [assessed by measuring inhibition of acetylcholinesterase (AchE) in different tissues] was observed, particularly against chlorpyrifos oxon. The protection was more prominent in two target tissues (brain and diaphragm), and was also present when OP exposure occurred by the dermal route, as is often the case for occupationally exposed workers (32).

Other experiments, which followed a similar protocol, extended these findings to mice (25). Furthermore, it was found that serum PON1 levels could be increased for extended periods ($t_{1/2} > 30$ h) by administering purified PON1 intravenously and intramuscularly (25). Additional studies indicated that exogenous PON1 could

also afford protection against the toxicity of chlorpyrifos, the parent compound used as an insecticide, when given before or even up to 3 h after OP exposure (33). Recently, recombinant human PON1 (either LR or LQ) expressed from an adenoviral vector was shown to increase serum paraoxonase activity by about 60% and to protect mice against the toxicity of chlorpyrifos (34). Overall, these studies indicated that by artificially increasing serum levels of PON1 it is possible to decrease the toxicity of certain OPs.

More recent experiments have investigated the toxicity of OPs in PON1 knockout ($PON1^{-/-}$) mice, which were produced by targeted disruption of exon 1 of the PON1 gene (35). $PON1^{-/-}$ mice have no plasma or liver hydrolytic activity toward paraoxon and diazoxon, and a very low level of activity toward chlorpyrifos oxon (16). PON1 knockout mice have dramatically increased sensitivity to chlorpyrifos oxon and diazoxon and slightly increased sensitivity to the respective parent compounds chlorpyrifos and diazinon (16, 35). A surprising finding came from the experiments with paraoxon, the OP after which PON1 was named. PON1 knockout mice did not show increased sensitivity to paraoxon, despite the total lack of paraoxonase activity in plasma and liver (16).

Further experiments investigated whether administration of purified PON1 would restore plasma (but not liver) PON1 levels, and thereby OP resistance, in $PON1^{-/-}$ mice. Either human $PON1_{Q192}$ or $PON1_{R192}$ was injected intravenously into $PON1^{-/-}$ mice, and the effects of OPs on brain and diaphragm AChE were determined. In the case of chlorpyrifos oxon, both isoforms were protective, and $PON1_{R192}$ offered about 50% better protection than $PON1_{Q192}$ (16). Both $PON1_{R192}$ and $PON1_{Q192}$ offered equal protection against diazoxon, and neither human PON1 isoform protected against the toxicity of paraoxon, extending the surprising findings described above. The results from the kinetic analysis of substrate hydrolysis by purified human $PON1_{192}$ isoforms provide an explanation for such findings. Although the $PON1_{R192}$ isoform is eight times more efficient than the $PON1_{Q192}$ isoform in hydrolyzing paraoxon (Km/Vm = 6.27 versus Km/Vm = 0.71), neither isoform hydrolyzes paraoxon as efficiently as diazoxon or chlorpyrifos oxon (16). This confirms the hypothesis (36) that PON1 is not efficient at hydrolyzing paraoxon at low concentrations, suggesting that PON1 may not degrade paraoxon efficiently in vivo and that other pathways (e.g., cytochromes P450, carboxylesterases) are primarily responsible for detoxifying paraoxon in vivo (36, 37). The catalytic efficiency of $PON1_{Q192}$ for diazoxon (Km/Vm = 75) was almost the same as that of $PON1_{R192}$ (Km/Vm = 77), and the catalytic efficiency of $PON1_{R192}$ for chlorpyrifos oxon was significantly higher than that of $PON1_{Q192}$ (Km/Vm = 256 versus Km/Vm = 150) (16).

In summary, this series of studies provided evidence that PON1, whose catalytic activity and level of expression are determined by polymorphisms in the coding and 5′-regulatory regions, respectively, plays an important role in modulating the toxicity of some, but not all, OPs, which are in vitro substrates for this enzyme. The catalytic efficiency of hydrolysis of oxons determines whether PON1 provides protection against a specific OP exposure.

An additional issue related to the role of PON1 in determining sensitivity to OP toxicity stems from the possibility that young children may be more sensitive than adults to the toxic effects of certain pesticides (38). The acute toxicity of OPs appears to be influenced by age, with young animals being more sensitive (39, 40), although whether such enhanced sensitivity extends to situations of repeated sublethal exposures is still unclear (41). Furthermore, young animals have been found especially sensitive to the central neurotoxicity of certain OPs (42), whereas they appear resistant to organophosphate-induced delayed polyneuropathy (43). There is an increasing consensus that lower metabolic abilities of young animals are a major determinant of their increased sensitivity to OP toxicity, as suggested a few decades ago (39). In particular, studies with chlorpyrifos have concluded that a lower hydrolytic detoxification by PON1, and perhaps carboxylesterase, accounts for the differential age-related sensitivity in its acute toxicity (44, 45). Indeed, in both rats and mice, liver and serum PON1 activity toward different substrates, as well as liver PON1 mRNA, increases from birth to postnatal day 21 (46). There is also evidence of a low serum paraoxonase activity, measured with paraoxon (4) or phenylacetate (47, 48) as substrates, in human infants, which seems to reach a plateau (whose level is determined by PON1 regulatory-region polymorphisms and the genetic background of the individual) after one year of age (2, 49).

PON1 AND CARDIOVASCULAR DISEASE

The influence of PON1 and its polymorphisms on cardiovascular disease appears related to its ability to metabolize oxidized lipids and prevent their formation. Oxidized LDL is believed to play a central role in monocyte chemotaxis and macrophage differentiation, which are early events in the progression of atherosclerosis, whereas HDL destroys these biologically active oxidized lipids (reviewed in Reference 50). PON1, the major enzyme responsible for this protective effect, is associated with the HDL particle (51). The HDL particle appears to accept PON1 directly by high-affinity desorption as it is released from the liver, in a process that requires both apolipoprotein and phospholipids (21, 52). Mackness et al. (53) were the first to demonstrate that purified human PON1 could inhibit LDL oxidation in vitro. Other studies have confirmed and extended this finding, demonstrating that PON1 both prevents the formation of oxidized LDL and inactivates LDL-derived oxidized phospholipids once they are formed (51, 54–59). PON1 also protects phospholipids in HDL from oxidation (56). The lipid substrate specificity of PON1 has not yet been fully defined. PON1 hydrolyzes phospholipid and cholesteryl ester hydroperoxides and hydroxides derived from arachidonic acid or linoleic acid (54, 56–58). Purified PON1 can also reduce linoleic acid hydroperoxides to linoleic acid hydroxides (58). Watson et al. (54) suggested that PON1 hydrolysis of phospholipid hydroperoxides produces aldehyde or ketone derivatives that then serve as substrates for platelet-activating factor acetylhydrolase (PAF-AH), generating lysophospholipids such as lysophosphatidyl choline. Ahmed et al.

(60) demonstrated that oxidized HDL lipids are converted by apolipoprotein A-1 to phosphatidylcholine aldehydes that are subsequently hydrolyzed to lysophosphatidylcholine by PON1, without detectable release of lineolate, arachidonate, or their hydroperoxy or hydroxy derivatives. Thus, PON1 appears to have esterase-like, peroxidase-like, and phospholipase-like activities that inhibit the formation of proinflammatory oxidized phospholipids and degrade them once they are formed.

The strongest evidence for the connection between hydrolysis of oxidized lipids by PON1 and increased susceptibility to atherosclerosis comes from a study of $PON1^{-/-}$ mice. Mice without PON1 exhibited significantly larger aortic atherosclerotic lesions than wild-type mice when fed a high-fat diet, and HDL from these mice failed to protect against LDL oxidation and monocyte chemotaxis in an in vitro co-culture model of the artery wall (35). Thus, mice lacking PON1 were more susceptible than wild-type mice to both lipoprotein oxidation and atherosclerosis.

Other studies in mice, humans, and other species demonstrated that low plasma PON1 activity is associated with features of atherosclerosis. In apolipoprotein E (ApoE) knockout mice, LDL receptor knockout mice, and apoAII-overexpressing transgenic mice, plasma PON1 levels are greatly reduced and the mice exhibit hypercholesterolemia and atherosclerosis (61, 62). Furthermore, adding PON1 back to HDL from apoAII transgenic mice restored the protective ability of HDL against lipid hydroperoxide formation (62). HDL isolated from normolipidemic patients with low plasma PON1 activity was unable to protect against LDL lipid oxidation (61). Avian HDL is also deficient in PON1 and unable to prevent LDL oxidation (63). In mice lacking both PON1 and ApoE, very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and LDL contained more oxidized lipid than did VLDL, IDL, or LDL isolated from mice lacking solely ApoE (64). However, clearance of LDL was more rapid in $PON1^{-/-}$ mice. Shih et al. (65) attributed this to increased LDL oxidation, leading to formation of LDL-immunoglobulin complexes that can be removed by the liver. An atherogenic diet decreases plasma PON1 activity (61, 66, 67), at least partly due to changes in liver *PON1* transcription (66). Van Lenten et al. (68) showed that oxidized 1-palmitoyl-2-arachadonoyl-sn-glycero-3-phosphorylcholine (ox-PAPC) reduces hepatic *PON1* expression via an interleukin (IL)–6-mediated pathway.

Based on the studies described above on the role of PON1 in OP detoxification, it is likely that variability among individuals in the PON1-catalyzed hydrolysis of oxidized lipids arises from two sources. The first source of variability is the polymorphism at $PON1_{Q192R}$, which results in different, substrate-specific catalytic efficiencies of hydrolysis for $PON1_{Q192}$ and $PON1_{R192}$. The frequent association of $PON1_{R192}$ with vascular disease predicts that this isoform would have a lower catalytic efficiency of hydrolysis of oxidized lipids than $PON1_{Q192}$. Indeed, this has been shown to be the case experimentally (55, 57, 58, 69). Aviram et al. (58) found that both isoforms reduced total lipid-peroxide content of human atherosclerotic lesion homogenates, with $PON1_{Q192}$ being twice as efficient as $PON1_{R192}$. The second source of variability in PON1 activity is PON1 abundance in the plasma, which has been largely ignored in studies examining the association of PON1

with atherosclerosis or other diseases. The magnitude of individual variability in plasma PON1 levels (up to 13-fold among individuals of the same $PON1_{Q192R}$ genotype) is probably why $PON1_{Q192R}$ genotype alone is not a sufficient predictor of disease (26). Because determining both $PON1_{Q192R}$ genotype and plasma PON1 levels gives a more accurate measure of total PON1 activity, we and others have emphasized strongly the importance of obtaining both measures, i.e., determining an individual's "PON status" (25–27, 70).

As described below, hydrolysis of the cholesterol-reducing drugs lovastatin, mevastatin, and simvastatin by PON1, and altered paraoxonase activity associated with statin drug treatment, add additional layers of complexity to the involvement of PON1 in the progression and treatment of atherosclerosis. Two other paraoxonase family members, PON2 and PON3, are capable of both preventing the formation of mildly oxidized LDL and inactivating mildly oxidized LDL once it is formed (71, 72). Whereas PON3 is produced in the liver and is associated with the HDL particle, PON2 is ubiquitously expressed and does not appear to be part of HDL. It will be important to determine the relative contributions of each of these PON family members to atherosclerosis.

Studies of PON1 genotype as a predictor of human vascular disease have reported mixed results, but overall they suggest that genotype is one of many predictors of vascular disease. The $PON1_{192R}$ allele or $PON1_{192RR}$ genotype have been associated with cardiovascular disease in many (73–80), but not all (81–90), studies. Table 1 summarizes the simple odds ratios derived from these association studies.

Although the results of $PON1_{192}$ genotype-disease association studies are mixed, when a $PON1_{192}$ genotype association with vascular disease was significant, it was consistently the $PON1_{192R}$ allele that was associated with disease. This finding indicates that the association is a true, but weak or variable, positive. It is likely that the studies' results are mixed because of the heterogeneity among the populations studied. There may be more power to detect $PON1_{192}$ genotype effects in studies with Japanese cohorts owing to their higher $PON1_{192R}$ allele frequency (91), despite their lower incidence of vascular disease.

The $PON1_{192R}$ and $PON1_{55L}$ alleles are in strong linkage disequilibrium in multiple ethnic groups (22, 92–93). The $PON1_{55LL}$ genotype predicted CHD in several studies, including a Caucasian sample in which $PON1_{192}$ genotype did not predict disease (94). $PON1_{55}$ genotype did not predict cardiovascular disease in other studied cohorts (70, 77, 88, 95), including an Asian Indian sample in which $PON1_{192}$ genotype did predict cardiovascular disease (92). Newer data suggest that the $PON1_{L55M}$ polymorphism is correlated with the plasma PON1 concentration both through linkage disequilibrium with promoter polymorphisms (10) and possibly through increased protein turnover of the $PON1_{M55}$ isoform (24).

PON1 activity level appears to be a better predictor of vascular disease than genotype. In a sample of 212 predominantly Caucasian men, we found lowered PON1 activity in subjects with severe carotid artery disease versus age- and race-matched controls (27). PON1 activity predicted carotid artery disease

TABLE 1 Odds ratios[a] from previous studies of the $PON1_{192}$ polymorphism and vascular disease[b]

Author	Year	Race	Cases/ controls	Outcome[c]	RR v QQ	QR v QQ
Studies reporting a positive association						
Ruiz	1995	French[d]	171/263	CHD	2.48	1.97
Serrato	1995	Caucasian	223/247	CHD	2.52	2.05
Odawara	1997	Japanese[d]	42/122	CHD		10.56[e]
Zama	1997	Japanese	75/115	CHD	5.97	3.06
Sanghera	1997	Indian	368/666	CHD	1.8	2.54
Sanghera	1998	Indian	157/189	CHD	2.83	2.42
Pati	1998	Indian	120/80	CAD	5	11.67
Pfohl	1999	German[d]	170/118	CHD	2.26	1.58
Imai	2000	Japanese	210/431	CAD	3.86	3.21
Ayub	1999	European	50/48	MI	1.44	2.63
Studies reporting no association						
Antikainen	1996	Finnish	380/169	CAD	1.71	0.77
Herrmann	1996	European	642/701	MI	1.07	1.19
Suehiro	1997	Japanese	134/252	CHD	0.85	1.21
Rice	1997	European	165/527	MI	1.04	0.93
Ombres	1998	Italian	310/162	CHD	0.85	1.28
Ko	1998	Chinese	218/218	CHD	1.19	1.28
Hasselwander	1999	Caucasian	103/388	CVD	2.04	0.90
Cascorbi	1999	German	1000/1000	CHD	0.9	0.99
Aubo	2000	Spanish[d]	156/310	MI	0.67	0.89
James	2000	European	137/273	CHD	1.93	1.37

[a]Odds ratios for RR or QR genotype subjects compared with QQ genotype subjects where not reported were calculated from the available data.

[b]Some rounding errors were necessary when the exact numbers of individuals with each genotype were not given, because they had to be reconstructed from genotype proportions.

[c]Abbreviations: CHD, coronary heart disease; CAD, coronary artery disease; MI, myocardial infarction.

[d]Entire sample has type 2 diabetes.

[e]OR for RR + QR vs. QQ.

independently of traditional risk factors. $PON1_{192}$ ($p = 0.75$) and $PON1_{55}$ ($p = 0.83$) genotypes or haplotype ($p = 0.70$) did not predict case-control status unless the activity phenotype was also included as a predictor using logistic regression. This result suggested that the lowered PON1 activity earlier reported in 50 myocardial infarction survivors (95) was a risk factor, rather than a result, of the infarction. The earlier study also failed to detect a significant marginal genotype effect, although the sample was small and, indeed, the odds ratio of the $PON1_{192QR}$ or $PON1_{192RR}$ genotype versus the $PON1_{192QQ}$ genotype was 2.42 (Table 1). Two groups have confirmed an association between lowered PON1 activity and coronary heart disease (CHD) in addition to carotid artery disease (70, 96). James et al. (96) found that PON1 activity in type 2 diabetic patients was lower in those

with CHD than in those without CHD. In a study of 417 cases and 282 controls, Mackness et al. (70) detected significantly lowered PON1 activity in CHD cases.

Like Jarvik et al. (27), Mackness et al. (70) found lowered activity in vascular disease with a marginal association of the $PON1_{192}$ and $PON1_{55}$ coding-region polymorphisms (Table 1). Jarvik et al. (97) subsequently found that $PON1_{-108}$ and $PON1_{-162}$ 5′ promoter-region polymorphisms, like the coding-region polymorphisms, did not predict case-control status. James et al. (96) reported an association of CHD with the lowered-expression, $PON1_{-108TT}$ genotype as well as with the $PON1_{55}$ polymorphism, which was in linkage disequilibrium with the $PON1_{-108}$ site. They did not detect a significant marginal effect of $PON1_{192}$ on CHD in the type 2 diabetics, although the odds ratios for the R-containing genotypes were above 1 (Table 1).

Mackness et al. (70) found lowered plasma PON1 levels associated with lowered PON1 activity in the cases. This suggests that it is the quantity of the enzyme, not its function, that is reduced. Surprisingly, James et al. (96) did not detect a significant decrease in PON1 concentration in cases, despite the significant $PON1_{-107}$ promoter-region polymorphism effect. However, the trend was toward lower concentration in the cases.

Although PON1 activity is a better predictor of disease than genotype, *PON1* genotypes remain important in the study of PON1's role in vascular disease. Careful studies are needed to delineate the roles of both genetic and environmental influences on PON1 activity. Gene-by-environment and gene-by-gene interactions can be expected and will require large sample sizes to detect. One useful method of examining gene-by-gene interactions uses an elegant mouse genetic/proteomic approach (98). Despite the significant linkage disequilibrium across the PON1 gene (10), few recent association studies have considered haplotype as a predictor of disease. Because of the common variants and the strong linkage disequilibrium, *PON1* is amenable to newer methods to assign haplotypes in unrelated individuals (99–101). Haplotype analyses will allow future analyses to consider the joint effects of typed and untyped polymorphic sites. However, PON1 does present the challenge of multiple functional sites leading to a large number of haplotypes. Appropriate haplotype groupings will be essential to improving power to detect haplotype by disease association. Simultaneous consideration of PON1 activity, haplotypes, and environmental covariates will continue to elucidate the role of PON1 in vascular disease. However, at this point in time, the two-substrate assay (diazoxon and paraoxon) for determining PON1 status is the most informative approach for examining the relationship between PON1 status and disease (26, 27).

PON1 AND DRUG METABOLISM

In addition to the oxygen analogs of some OP insecticides, the most commonly used substrate for PON1 has been phenylacetate, which, however, is not a "polymorphic substrate" for the Q192R polymorphism. Recently, the ability of paraoxonase to

hydrolyze some novel substrates, most notably lactones and carbonate esters, has been reported (102, 103). This finding may have several important consequences.

First, PON1 may be involved in the metabolism of pharmaceutical drugs, in which case the PON1 status of an individual would be relevant to a drug's effectiveness and/or side effects. Billecke et al. (103) found that the diuretic spironolactone and the hypocholesterolemic drugs mevastatin, lovastatin, and simvastatin are hydrolyzed by PON1, and that the Q and R isoforms hydrolyze these drugs with approximately the same efficiency. Pravastatin was found to increase serum apolipoprotein A1 and HDL cholesterol to a greater extent in men with the RR genotype of *PON1* than in those with the QQ genotype (104). Simvastatin therapy also increases plasma paraoxonase activity (105), possibly by increasing the transcriptional activity of the PON1 gene (106). The therapeutic response of PON1 activity is independent of $PON1_{192}$ and $PON1_{55}$ polymorphisms (105).

Second, novel classes of so-called pro-drugs may be developed by incorporating a lactone or cyclic carbonate moiety into other molecules, which can thus be inactivated or bioactivated in vivo by PON1. Inactivation has been shown to occur with glucocorticoid γ-lactones (102) and activation with the antibacterial prodrug prulifloxacin (107). The latter is hydrolyzed at a higher rate by type R than by type Q serum. Interestingly, the free cysteine in position 284 of PON1, which is not required for the hydrolysis of paraoxon or phenylacetate, is essential for the lactonase activity of the enzyme (103), as it is essential to protect LDL against oxidation (57).

Third, PON1 may exert other effects on endogenous substrates, in addition to protecting LDL from oxidation (57). It has been proposed that PON1 protects against homocysteinylation by hydrolyzing homocysteine thiolactone, but the catalytic efficiency for this reaction is low (108). Although the Q isozyme is more efficient in protecting against LDL oxidation, the R type hydrolyzes thiolactones more readily (103).

Finally, as noted above, two very toxic organophosphates, paraoxon and diazoxon, are currently used to determine PON1 status (26). The identification of novel PON1 substrates of low or no toxicity, which, unlike phenylacetate, are differentially hydrolyzed by the 192 R and Q alleles, will simplify the application of the two-dimensional assay to the study of large populations. One such compound may be the carbonate ester KB-R4899, which, like paraoxon, is hydrolyzed much more rapidly by the R than the Q isozyme of PON1 (103).

MODULATION OF PON1 BY DIETARY, LIFESTYLE, AND ENVIRONMENTAL FACTORS

As discussed above, polymorphisms in the 5′ regulatory region and coding regions of PON1 determine its level of expression and enzymatic activity toward different substrates. In humans, PON1 serum arylesterase activity increases from birth to 15–25 months of age, when it seems to reach a plateau whose level is determined by the 5′ regulatory-region polymorphisms and the genetic background of the individual

(2). In the adult, PON1 levels are stable and no significant changes have been observed with age (109, 110). However, efficient PON1 regulatory regions do not alone guarantee a high PON1 activity level (10). Enzyme inducers, environmental chemicals, physiological and pathological states, and dietary and lifestyle factors have demonstrated effects on PON1 activity.

Phenobarbital, a classical enzyme inducer, increases paraoxonase activity and mRNA levels in rodent liver but not in serum (2, 111, 112). 3-methylcholanthrene was found to increase both serum and liver PON1 activity in rats (113) but not in mice (2). Administration of lipopolysaccharide, which mimics gram-negative infections, causes a transient decrease in serum and liver PON1 activity and in hepatic mRNA levels (2, 114).

PON1 activity depends on calcium, and the calcium chelator EDTA abolishes its activity. Other cations, however, have shown an inhibitory effect on PON1 activity. Barium, lanthanum, copper, zinc, and mercurials were found to inhibit PON1 activity from rat or human liver in vitro (115). In vitro experiments with purified human enzymes showed that $PON1_{R192}$ was more sensitive than $PON1_{Q192}$ to inhibition by cadmium, zinc, mercury chloride, and iron, whereas $PON1_{Q192}$ displayed a higher sensitivity to inhibition by lead (116). In vivo, however, when mice were treated with cadmium or methylmercury to achieve environmentally relevant blood concentrations, no inhibition of PON1 activity was found (116).

PON1 activity can vary depending on physiological conditions or pathological states. For example, serum PON1 activity is significantly decreased during pregnancy (5, 117). Low PON1 activity has been found in renal disease (118), diabetes mellitus (23, 119), various HDL deficiencies (120), and liver cirrhosis (121).

Dietary and lifestyle factors can also affect PON1 activity. Smoking has been shown to decrease serum PON1 levels and activity (27, 122–124), both of which appear to normalize relatively soon after cessation. In vitro experiments found that inhibition of PON1 activity by a cigarette-smoke extract was antagonized by reduced glutathione (GSH), N-acetylcysteine, and 2-mercaptoethanol, suggesting that free thiols are central to the inhibitory effects (122). Ethanol and other aliphatic alcohols have been shown to inhibit serum PON1 activity (125); however, a study in middle-aged men indicated that daily moderate alcohol consumption increased serum PON1 activity, with no differences between wine, beer, and spirits (126). This increase may be due to the consumption of alcohol itself or to that of antioxidants, as similar results were obtained after consumption of red wine (127) or pomegranate juice (128, 129). A high-fat diet was shown to reduce serum PON1 levels in mice (66, 67); in rats, dietary supplementation with fish oil also decreased serum PON1, whereas supplementation with the triglyceride triolein increased PON1 activity (130). Meals rich in thermally stressed olive oil, but not safflower oil, were found to increase postprandial serum PON1 activity in middle-aged women but not in men (131).

The hypocholesterolemic drug simvastatin was found to increase serum PON1 activity (105, 106). As discussed above, statins and other lactones are hydrolyzed by PON1. On the other hand, the lactams, isosteric forms of lactones in which the ring oxygen is replaced by nitrogen, inhibit PON1. Compounds such as

δ-valerolactam or ε-caprolactam inhibit PON1 activity, with IC50s in the micromolar range (103).

These limited studies suggest that PON1 levels and activity can be modulated by dietary, lifestyle, and possibly environmental factors. Although individuals with inefficient regulatory regions appear unable to express very high levels of PON1 (10), the contribution of exogenous factors to an individual's PON1 status should not be discounted.

CONCLUSIONS AND FUTURE PROSPECTS

Polymorphisms in the PON1 gene influence both the quantity and quality of PON1 (i.e., PON1 status) (16, 26, 27). The quality of PON1 is governed by the Q192R polymorphism, which determines substrate-dependent differences in the catalytic efficiency of hydrolysis of specific OP substrates, including insecticide metabolites and several nerve agents. Recent studies indicate that the Q192R polymorphism also governs the efficiency of detoxification of biologically active oxidized lipids (54), and hence, the risk for vascular disease (27, 70, 96). Recent data on the role of PON1 in the metabolism of specific drugs also indicate that PON1 status is important in determining the pharmacokinetics of metabolism (102, 103, 107).

The solid conclusion from all of these studies is that PON1 levels in all cases and the Q192R polymorphism in some cases determine the rates at which a given individual will detoxify a specific insecticide, metabolize harmful oxidized lipids, and activate or inactivate specific drugs.

Interesting questions remain. It is clear that the 5′ regulatory-region polymorphisms influence but do not fully determine PON1 levels. Other as-yet-unidentified genes must also contribute to the large variability of plasma PON1 levels observed among individuals (15, 26, 27). These influences are often referred to as genetic background contributions. The recent mouse proteomic research described by Klose et al. (98) points to a direction for elucidating these influences. Klose et al. were able to map mouse genes that influenced the levels of specific proteins.

The alignment of the three PON1 genes, *PON1*, *PON3*, and *PON2*, on chromosome 7 provides an excellent system for examining questions of linkage disequilibrium in the human genome. Considerable linkage disequilibrium with the PON1 gene has already been described (10). It will be interesting to determine how far outside the PON1 gene the blocks of linkage disequilibrium extend.

Last, but not least, the therapeutic possibilities of PON1, e.g., in treating individuals for exposure to nerve agents or insecticides or for reducing the risk of vascular disease, need to be examined. The animal studies described above (2, 16, 25, 33, 35) indicate that the injection of recombinant PON1 will provide an efficient catalytic agent for detoxifying specific compounds in humans. The studies on the importance of catalytic efficiency (16, 36, 37) provide guidelines for engineering recombinant PON1 variants with catalytic efficiencies that would be efficacious for treating exposure to specific OP compounds.

Following the 1995 sarin releases in Japan, we determined a population distribution of hydrolysis rates for sarin and soman (15). The data showed that, like the rates of hydrolysis of some OP insecticide metabolites, the rates of agent hydrolysis were significantly affected by the $PON1_{R192Q}$ polymorphism. Plasma from $PON1_{R192}$ homozygotes demonstrated very low rates of sarin hydrolysis, whereas plasma from $PON1_{Q192}$ homozygotes exhibited much better rates of sarin hydrolysis, depending on the level of PON1 present in the plasma. Heterozygotes exhibited intermediate rates of sarin hydrolysis. The rates of soman hydrolysis were also somewhat higher for the $PON1_{Q192}$ homozygotes than for the $PON1_{R192}$ homozygotes.

These observations led to the assumption that individuals with higher rates of agent hydrolysis might be more resistant to exposure than individuals with low rates of hydrolysis, i.e., $PON1_{Q192}$ homozygotes might be more resistant to sarin exposure than $PON1_{R192}$ homozygotes. Following our studies on the importance of catalytic efficiency in providing protection against dermal exposures (16), we concluded that the catalytic efficiency of agent hydrolysis by PON1 is probably not adequate for significant protection against nerve agent exposure, in contrast to protection against diazoxon or chlorpyrifos oxon exposures. This conclusion is consistent with the studies by Yamada et al. (132) on the $PON1_{192}$ genotypes of the individuals exposed in the Tokyo subway sarin attack. Based on the current state of knowledge about PON1 status and possible resistance to nerve agent exposure, it would not make sense to screen plasma from individuals for high or low rates of agent hydrolysis. For PON1 to be effective in treating agent exposure, recombinant PON1 with higher catalytic efficiencies of agent hydrolysis will need to be engineered.

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LITERATURE CITED

1. Durrington PN, Mackness B, Mackness MJ. 2001. Paraoxonase and atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* 21:473–80
2. Costa LG, Li WF, Richter RJ, et al. 2002. PON1 and organophosphate toxicity. See Ref. 2a, pp. 165–83

2a. Costa LG, Furlong CE. 2002. *Paraoxonase (PON1) in Health and Disease: Basic and Clinical Aspects*. Norwell, MA: Kluwer Acad.

3. Eckerson HW, White CM, La Du BN. 1983. The human serum paraoxonase/arylesterase polymorphism. *Am. J. Hum. Genet.* 35:1126–38
4. Mueller RF, Hornung S, Furlong CE, et al. 1983. Plasma paraoxonase polymorphism: a new enzyme assay, population,

family, biochemical and linkage studies. *Am. J. Hum. Genet.* 35:393–408
5. Geldmacher-Von Mallinckrodt M, Diepgen TL. 1988. The human serum paraoxonase polymorphism and specificity. *Toxicol. Environ. Chem.* 18:79–196
6. Hassett C, Richter RJ, Humbert R, et al. 1991. Characterization of DNA clones encoding rabbit and human serum paraoxonase: the mature protein retains its signal sequence. *Biochemistry* 30:10141–49
7. Leviev I, James RW. 2000. Promoter polymorphisms of human paraoxonase PON1 gene and serum paraoxonase activities and concentrations. *Arterioscler. Thromb. Vasc. Biol.* 20:516–21
8. Suehiro T, Nakamura T, Inoue M, et al. 2000. A polymorphism upstream from the human paraoxonase (PON1) gene and its association with PON1 expression. *Atherosclerosis* 150:295–98
9. Brophy VM, Hastings MD, Clendenning JB, et al. 2001. Polymorphisms in the human paraoxonase (PON1) promoter. *Pharmacogenetics* 11:77–84
10. Brophy VH, Jampsa RL, Clendenning JB, et al. 2001. Effects of 5′ regulatory-region polymorphisms on paraoxonase-gene (PON1) expression. *Am. J. Hum. Genet.* 68:1428–36
11. Brophy VH, Jarvik GP, Furlong CE. 2002. PON1 polymorphisms. See Ref. 2a, pp. 53–77
12. Smolen A, Eckerson HW, Gan K, et al. 1991. Characteristics of the genetically determined polymorphic forms of human serum paraoxonase/arylesterase. *Drug. Metab. Disp.* 19:107–12
13. Adkins S, Gan KN, Mody M, et al. 1993. Molecular basis for the polymorphic forms of human serum paraoxonase/arylesterase: glutamine or arginine at position 191, for the respective A or B allozymes. *Am. J. Hum. Genet.* 53:598–608
14. Humbert R, Adler DA, Disteche CM, et al. 1993. The molecular basis of the human serum paraoxonase activity polymorphism. *Nat. Genet.* 3:73–76
15. Davies HG, Richter RJ, Keifer M, et al. 1996. The effect of the human serum paraoxonase polymorphism is reversed with diazoxon, soman and sarin. *Nat. Genet.* 14:334–36
16. Li WF, Costa LG, Richter RJ, et al. 2000. Catalytic efficiency determines the *in vivo* efficacy of PON1 for detoxifying organophosphates. *Pharmacogenetics* 10:1–13
17. Deleted in proof
18. Gan KN, Smolen A, Eckerson HW, et al. 1991. Purification of human serum paraoxonase/arylesterase. Evidence for one esterase catalyzing both activities. *Drug Metab. Dispos.* 19:100–6
19. Sorenson RC, Bisgaier CL, Aviram M, et al. 1999. Human serum paraoxonase/arylesterase's retained hydrophobic N-terminal leader sequence associates with HDLs by binding phospholipids: apolipoprotein A-1 stabilizes activity. *Arterioscler. Thromb. Vasc. Biol.* 19: 2214–25
20. Blatter MC, James RW, Messmer S, et al. 1993. Identification of a distinct human high-density lipoprotein subspecies defined by a lipoprotein-associated protein, K-45. Identity of K-45 with paraoxonase. *Eur. J. Biochem.* 211:871–79
21. Deakin S, Leviev I, Gomaraschi M, et al. 2002. Enzymatically active paraoxonase-1 is located at the external membrane of producing cells and released by a high-affinity, saturable, desorption mechanism. *J. Biol. Chem.* 277(6):4301–8
22. Blatter Garin MC, James RW, Dussoix P, et al. 1997. Paraoxonase polymorphism Met-Leu 54 is associated with modified serum concentrations of the enzyme. A possible link between the paraoxonase gene and increased risk of cardiovascular disease in diabetes. *J. Clin. Invest.* 99:62–66
23. Mackness B, Mackness MI, Arrol S, et al. 1998. Serum paraoxonase (PON1) 55 and 192 polymorphism and paraoxonase activity and concentration in non-insulin

dependent diabetes mellitus. *Atherosclerosis* 139:341–49
24. Leviev I, Deakin S, James RW. 2001. Decreased stability of the M54 isoform of paraoxonase as a contributory factor to variations in human serum paraoxonase concentrations. *J. Lipid Res.* 42(4):528–35
25. Li WF, Costa LG, Furlong CE. 1993. Serum paraoxonase status: a major factor in determining resistance to organophosphates. *J. Toxicol. Environ. Health* 40: 337–46
26. Richter RJ, Furlong CE. 1999. Determination of paraoxonase (PON1) status requires more than genotyping. *Pharmacogenetics* 9:745–53
27. Jarvik GP, Rozek LS, Brophy VH, et al. 2000. Paraoxonase (PON1) phenotype is a better predictor of vascular disease than is $PON1_{192}$ or $PON1_{55}$ genotype. *Arterioscler. Thromb. Vasc. Biol.* 20:2441–47
28. Lotti M. 2000. Organophosphorus compounds. In *Experimental and Clinical Neurotoxicology*, ed. PS Spencer, H Schaumburg, AC Ludolph, pp. 898–925. Oxford, UK: Oxford Univ. Press
29. Main AR. 1956. The role of A-esterase in the acute toxicity of paraoxon, TEPP and parathion. *Can. J. Biochem. Physiol.* 34:197–216
30. Brealey CB, Walker CH, Baldwin BC. 1980. A-Esterase activities in relation to the differential toxicity of pirimiphos-methyl to birds and mammals. *Pestic. Sci.* 11:546–54
31. Costa LG, Richter RJ, Murphy SD, et al. 1987. Species differences in serum paraoxonase correlate with sensitivity to paraoxon toxicity. In *Toxicology of Pesticides: Experimental, Clinical and Regulatory Perspectives*, ed. LG Costa, CL Galli, SD Murphy, pp. 262–66. Heidelberg: Springer-Verlag

31a. Furlong CE, Richter RJ, Chapline C, et al. 1991. Purification of rabbit and human serum paraoxonase. *Biochemistry* 30:10133–40

32. Costa LG, McDonald BE, Murphy SD, et al. 1990. Serum paraoxonase and its influence on paraoxon and chlorpyrifos oxon toxicity in rats. *Toxicol. Appl. Pharmacol.* 103:66–76
33. Li WF, Furlong CE, Costa LG. 1995. Paraoxonase protects against chloropyrifos toxicity in mice. *Toxicol. Lett.* 76:219–26
34. Cowan J, Sinton CM, Varley AW, et al. 2001. Gene therapy to prevent organophosphate intoxication. *Toxicol. Appl. Pharmacol.* 173:1–6
35. Shih DM, Gu L, Xia YR, et al. 1998. Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis. *Nature* 394:284–98
36. Pond AL, Chambers HW, Chambers JE. 1995. Organophosphate detoxication potential of various rat tissues via A-esterases and aliesterase activities. *Toxicol. Lett.* 78:245–52
37. Chambers JE, Ma T, Boone JS, et al. 1994. Role of detoxication pathways in acute toxicity levels of phosphorothionate insecticides in the rat. *Life Sci.* 54:1357–64
38. Eskenazi B, Bradnam A, Castorina R. 1999. Exposure of children to organophosphate pesticides and their potential adverse health effects. *Environ. Health. Perspect.* 107(Suppl. 3):409–19
39. Benke GM, Murphy SD. 1975. The influence of age in the toxicity and metabolism of methylparathion and parathion in male and female rats. *Toxicol. Appl. Pharmacol.* 31:254–69
40. Pope CN, Liu J. 1997. Age-related difference in sensitivity to organophosphorus pesticides. *Environ. Toxicol. Pharmacol.* 4:309–14
41. Sheets LP. 2000. A consideration of age-dependent differences in susceptibility to organophosphorus and pyrethroid insecticides. *NeuroToxicology* 21:57–64
42. Dam K, Seidler FJ, Slotkin TA. 2000. Chlorpyrifos exposure during a critical neonatal period elicits gender-selective

deficits in the development of coordination skills and locomotor activity. *Dev. Brain Res.* 121:179–87
43. Moretto A, Capodicasa E, Peraica M, et al. 1991. Age sensitivity to organophosphate-induced delayed polyneuropathy. Biochemical and toxicological studies in developing chicks. *Biochem. Pharmacol.* 10:1497–504
44. Mortensen SR, Chanda SM, Hooper MJ, et al. 1996. Maturational differences in chlorpyrifos-oxonase activity may contribute to age-related sensitivity to chlorpyrifos. *J. Biochem. Toxicol.* 11:279–87
45. Padilla S, Buzzard J, Moser VC. 2000. Comparison of the role of esterases in the differential age-related sensitivity to chlorpyrifos and metamidophos. *NeuroToxicology* 21:49–56
46. Li WF, Mathews C, Disteche CM, et al. 1997. Paraoxonase (PON1) gene in mice: sequencing, chromosomal localization and developmental expression. *Pharmacogenetics* 7:137–44
47. Augustinsson KB, Barr M. 1963. Age variation in plasma arylesterase activity in children. *Clin. Chim. Acta* 8:568–73
48. Augustinsson KB, Brody S. 1962. Plasma arylesterase activity in adults and newborn infants. *Clin. Chim. Acta* 7:560–65
49. Ecobichon DJ, Stephens DS. 1972. Perinatal development of human blood esterases. *Clin. Pharmacol. Ther.* 14:41–47
50. Lusis AJ. 2000. Atherosclerosis. *Nature* 407:233–41
51. Mackness MI, Arrol S, Abbott C, et al. 1993. Protection of low-density lipoprotein against oxidative modification by high-density lipoprotein associated paraoxonase. *Atherosclerosis* 104:129–35
52. Noto H, Hashimoto Y, Satoh H, et al. 2001. Exclusive association of paraoxonase 1 with high-density lipoprotein particles in apolipoprotein A-I deficiency. *Biochem. Biophys. Res. Commun.* 289:395–401
53. Mackness MI, Arrol S, Durrington PN. 1991. Paraoxonase prevents accumulation of lipoperoxides in low-density lipoprotein. *FEBS Lett.* 286:152–54
54. Watson AD, Berliner JA, Hama SY, et al. 1995. Protective effect of high density lipoprotein associated paraoxonase. Inhibition of the biological activity of minimally oxidized low density lipoprotein. *J. Clin. Invest.* 96:2882–91
55. Mackness B, Mackness MI, Arrol S, et al. 1998. Effect of the human serum paraoxonase 55 and 192 genetic polymorphisms on the protection by high density lipoprotein against low density lipoprotein oxidative modification. *FEBS Lett.* 423:57–60
56. Aviram M, Rosenblat M, Bisgaier CL, et al. 1998. Paraoxonase inhibits high-density lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase. *J. Clin. Invest.* 101:1581–90
57. Aviram M, Billecke S, Sorenson R, et al. 1998. Paraoxonase active site required for protection against LDL oxidation involves its free sulfhydryl group and is different from that required for its arylesterase/paraoxonase activities: selective action of human paraoxonase allozymes Q and R. *Arterioscler. Thromb. Vasc. Biol.* 18:1617–24
58. Aviram M, Hardak E, Vaya J, et al. 2000. Human serum paraoxonases (PON1) Q and R selectively decrease lipid peroxides in human coronary and carotid atherosclerotic lesions: PON1 esterase and peroxidase-like activities. *Circulation* 101:2510–17
59. Navab M, Hama SY, Anantharamaiah GM, et al. 2000. Normal high density lipoprotein inhibits three steps in the formation of mildly oxidized low density lipoprotein: steps 2 and 3. *J. Lipid Res.* 41:1495–508
60. Ahmed Z, Ravandi A, Maguire GF, et al. 2001. Apolipoprotein A-I promotes the formation of phosphatidylcholine core aldehydes that are hydrolyzed by paraoxonase (PON-1) during high density

lipoprotein oxidation with a peroxynitrite donor. *J. Biol. Chem.* 276:24473–81
61. Navab M, Hama-Levy S, Van Lenten BJ, et al. 1997. Mildly oxidized LDL induces an increased apolipoprotein J/paraoxonase ratio. *J. Clin. Invest.* 99:2005–19
62. Castellani LW, Navab M, Lenten BJV, et al. 1997. Overexpression of apolipoprotein AII in transgenic mice converts high density lipoproteins to proinflammatory particles. *J. Clin. Invest.* 100:464–74
63. Mackness B, Durrington PN, Mackness MI. 1998. Lack of protection against oxidative modification of LDL by avian HDL. *Biochem. Biophys. Res. Commun.* 247:443–46
64. Shih DM, Xia YR, Wang XP, et al. 2000. Combined serum paraoxonase knockout/apolipoprotein E knockout mice exhibit increased lipoprotein oxidation and atherosclerosis. *J. Biol. Chem.* 275: 17527–35
65. Shih DM, Reddy S, Lusis AJ. 2002. CHO and atherosclerosis: human epidemiological studies and transgenic mouse models. See Ref. 2a, pp. 93–123
66. Shih DM, Gu L, Hama S, et al. 1996. Genetic-dietary regulation of serum paraoxonase expression and its role in atherogenesis in a mouse model. *J. Clin. Invest.* 97:1630–39
67. Hedrick CC, Hassan K, Hough GP, et al. 2000. Short-term feeding of atherogenic diet to mice results in reduction of HDL and paraoxonase that may be mediated by an immune mechanism. *Arterioscler. Thromb. Vasc. Biol.* 20:1946–52
68. Van Lenten BJ, Wagner AC, Navab M, et al. 2001. Oxidized phospholipids induce changes in hepatic paraoxonase and ApoJ but not monocyte chemoattractant protein-1 via interleukin-6. *J. Biol. Chem.* 276:1923–29
69. Mackness B, Durrington PN, Mackness MI. 1999. Polymorphisms of paraoxonase genes and low-density lipoprotein lipid peroxidation. *Lancet* 353:468–69
70. Mackness B, Davies GK, Turkie W, et al. 2001. Paraoxonase status in coronary heart disease: Are activity and concentration more important than genotype? *Arterioscler. Thromb. Vasc. Biol.* 21:1451–57
71. Reddy ST, Wadleigh DJ, Grijalva V, et al. 2001. Human paraoxonase-3 is an HDL-associated enzyme with biological activity similar to paraoxonase-1 protein but is not regulated by oxidized lipids. *Arterioscler. Thromb. Vasc. Biol.* 21:542–47
72. Ng CJ, Wadleigh DJ, Gangopadhyay A, et al. 2001. Paraoxonase-2 is a ubiquitously expressed protein with antioxidant properties and is capable of preventing cell-mediated oxidative modification of low density lipoprotein. *J. Biol. Chem.* 276:44444–49
73. Ruiz J, Blanche H, James RW, et al. 1995. Gln-Arg192 polymorphism of paraoxonase and coronary heart disease in type 2 diabetes. *Lancet* 346:869–72
74. Serrato M, Marian AJ. 1995. A variant of human paraoxonase/arylesterase (HUMPONA) gene is a risk factor for coronary artery disease. *J. Clin. Invest.* 96:3005–8
75. Pfohl M, Koch M, Enderle MD, et al. 1999. Paraoxonase 192 Gln/Arg gene polymorphism, coronary artery disease, and myocardial infarction in type 2 diabetes. *Diabetes* 48:623–27
76. Zama T, Murata M, Matsubara Y, et al. 1997. A 192Arg variant of the human paraoxonase (HUMPONA) gene polymorphism is associated with an increased risk for coronary artery disease in the Japanese. *Arterioscler. Thromb. Vasc. Biol.* 17:3565–69
77. Sanghera D, Saha N, Aston CE, et al. 1997. Genetic polymorphism of paraoxonase and the risk of coronary heart disease. *Arterioscler. Thromb. Vasc. Biol.* 17:1067–73
78. Sanghera D, Aston CE, Saha N, et al. 1998. DNA polymorphisms in two paraoxonase genes (PON1 and PON2) are associated with the risk of coronary heart disease. *Am. J. Hum. Genet.* 62:36–44

79. Pati N, Pati U. 1998. Paraoxonase gene polymorphism and coronary artery disease in Indian subjects. *Int. J. Cardiol.* 66:165–68
80. Imai Y, Morita H, Kurihara H, et al. 2000. Evidence for association between paraoxonase gene polymorphisms and atherosclerotic diseases. *Atherosclerosis* 149:435–42
81. Heijsmans B, Westendorp RG, Lagaay AM, et al. 2000. Common paraoxonase gene variants, mortality risk and fatal cardiovascular events in elderly subjects. *Atherosclerosis* 149:91–97
82. Antikainen M, Murtomaki S, Syvanne M, et al. 1996. The Gln-Arg191 polymorphism of the human paraoxonase gene (HUMPONA) is not associated with the risk of coronary artery disease in Finns. *J. Clin. Invest.* 98:833–35
83. Rice G, Ossei-Gerning N, Stickland MH, et al. 1997. The paraoxonase Gln-Arg 192 polymorphism in subjects with ischaemic heart disease. *Coron. Artery Dis.* 8:677–82
84. Herrmann S, Blanc H, Poirier O, et al. 1996. The Gln/Arg polymorphism of human paraoxonase (PON 192) is not related to myocardial infarction in the ECTIM Study. *Atherosclerosis* 126:299–303
85. Suehiro T, Nakauchi Y, Yamamoto M, et al. 1996. Paraoxonase gene polymorphism in Japanese subjects with coronary heart disease. *Int. J. Cardiol.* 57:69–73
86. Ombres D, Pannitteri G, Montali A, et al. 1998. The Gln-Arg192 polymorphism of human paraoxonase gene is not associated with coronary artery disease in Italian patients. *Arterioscler. Thromb. Vasc. Biol.* 18:1611–18
87. Ko YK, Wang SM, Hsu LA, et al. 1998. The Gln-Arg 191 polymorphism of the human paraoxonase gene is not associated with the risk of coronary artery disease among Chinese in Taiwan. *Atherosclerosis* 141:259–64
88. Cascorbi I, Laule M, Mrozikiewicz PM, et al. 1999. Mutations in the human paraoxonase 1 gene: frequencies, allelic linkages, and association with coronary artery disease. *Pharmacogenetics* 9:755–61
89. Hasselwander O, Savage DA, McMaster D, et al. 1999. Paraoxonase polymorphisms are not associated with cardiovascular risk in renal transplant recipients. *Kidney Int.* 56:289–98
90. Aubo C, Senti M, Marrugat J, et al. 2000. Risk of myocardial infarction associated with Gln/Arg 192 polymorphism in the human paraoxonase gene and diabetes mellitus. The REGICOR Investigators. *Eur. Heart J.* 21:33–38
91. Odawara M, Tachi Y, Yamashita K. 1997. Paraoxonase polymorphism (Gln192-Arg) is not associated with coronary heart disease in Japanese noninsulin-dependent diabetes mellitus. *Clin. Endocrinol. Metab.* 82:2257–60
92. Sanghera D, Saha N, Kamboh MI. 1998. The codon 55 polymorphism in the paraoxonase 1 gene is not associated with the risk of coronary heart disease in Asian Indians and Chinese. *Atherosclerosis* 136:217–23
93. Boright A, Connelly PW, Brunt JH, et al. 1998. Genetic variation in paraoxonase-1 and paraoxonase-2 is associated with variation in plasma lipoproteins in Alberta Hutterites. *Atherosclerosis* 139:131–36
94. Schmidt H, Schmidt R, Niederkorn K, et al. 1998. Paraoxonase PON1 polymorphism leu-met54 is associated with carotid atherosclerosis: results of the Austrian Stroke Prevention Study. *Stroke* 29:2043–48
95. Ayub A, Mackness MI, Arrol S, et al. 1999. Serum paraoxonase after myocardial infarction. *Arterioscler. Thromb. Vasc. Biol.* 19:330–35
96. James RW, Leviev I, Ruiz J, et al. 2000. Promoter polymorphism T(–107)C of the paraoxonase PON1 gene is a risk factor for coronary heart disease in type 2 diabetic patients. *Diabetes* 49:1390–93

97. Jarvik GP, Furlong CE, Hatsukami TS. 2002. Vitamin C and E intake predict increased paraoxonase activity. *J. Am. Coll. Cardiol.* 39:373B
98. Klose J, Nock C, Hermann M, et al. 2002. Genetic analysis of the mouse brain proteome. *Nat. Genet.* 30:385–93
99. Clark AG, Weiss KM, Nickerson DA, et al. 1998. Haplotype structure and population genetic inferences from nucleotide-sequence variation in human lipoprotein lipase. *Am. J. Hum. Genet.* 63:595–612
100. Fullerton SM, Clark AG, Weiss KM, et al. 2000. Apolipoprotein E variation at the sequence haplotype level: implications for the origin and maintenance of a major human polymorphism. *Am. J. Hum. Genet.* 67:881–900
101. Stephens M, Smith NJ, Donnelly P. 2001. A new statistical method for haplotype reconstruction from population data. *Am. J. Hum. Genet.* 68:978–89
102. Biggadike K, Angell RM, Burges CM, et al. 2000. Selective plasma hydrolysis of glucocorticoid γ-lactones and cyclic carbonates by the enzyme paraoxonase: an ideal plasma inactivation mechanism. *J. Med. Chem.* 43:19–21
103. Billecke S, Dragonov D, Counsell R, et al. 2000. Human serum paraoxonase (PON1) isozymes Q and R hydrolyze lactones and cyclic carbonate esters. *Drug Metab. Dispos.* 28:1335–42
104. Malin R, Laaksonen R, Knuuti J, et al. 2001. Paraoxonase genotype modifies the effect of pravastatin on high-density lipoprotein cholesterol. *Pharmacogenetics* 11:625–33
105. Tomàs M, Sentì M, Garcia-Faria F, et al. 2000. Effect of simvastatin therapy on paraoxonase activity and related lipoproteins in familial hypercholesterolemic patients. *Arterioscler. Thromb. Vasc. Biol.* 20:2113–19
106. Leviev I, James R. 2000. Simvastatin increases plasma levels of the antioxidant enzyme paraoxonase by PON1 gene activation. *Atherosclerosis* 151:41
107. Tougou K, Nakamura A, Watanabe S, et al. 1998. Paraoxonase has a major role in the hydrolysis of prulifloxacin (NM441), a prodrug of a new antibacterial agent. *Drug Metab. Dispos.* 26:355–59
108. Jakubowski H. 2000. Calcium-dependent human serum homocysteine thiolactone hydrolase: a protective mechanism against protein N-homocysteinylation. *J. Biol. Chem.* 275:3957–62
109. Playfer JR, Powell C, Evans DA. 1977. Plasma paraoxonase activity in old age. *Age Ageing* 6:89–95
110. Zech R, Zurcher K. 1974. Organophosphate splitting serum enzymes in different mammals. *Comp. Biochem. Physiol.* 48:427–33
111. Hernandez AF, Gonzalvo MC, Gil F, et al. 1997. Divergent effects of classical inducers on rat plasma and microsomial fraction paraoxonase and arylesterase. *Environ. Toxicol. Pharmacol.* 3:83–86
112. Kaliste-Korhonen E, Tuovinen K, Hanninen O. 1998. Effects of phenobarbital and γ-naphtoflavone on activities of different rat esterases after paraoxon exposure. *Gen. Pharmacol.* 31:307–12
113. Rodrigo L, Hernandez AF, Lopez-Caballero JJ, et al. 2001. Immunohistochemical evidence for the expression and induction of paraoxonase in rat liver, kidney, lung and brain tissue. Implications for its physiological role. *Chem. Biol. Interact.* 137:123–37
114. Feingold KR, Memon RA, Moser AH, et al. 1998. Paraoxonase activity in the serum and hepatic mRNA levels decrease during the acute phase responses. *Atherosclerosis* 139:307–15
115. Gonzalvo MC, Gil F, Hernandez AF, et al. 1997. Inhibition of paraoxonase activity in human liver microsomes by exposure to EDTA, metals and mercurials. *Chem. Biol. Interact.* 105:169–79
116. Cole TB, Li WF, Richter RJ, et al. 2002. Inhibition of paraoxonase (PON1) by heavy metals. *Toxicologist* 66(1-S): 312

117. Weitman SD, Vodicnick MJ, Lech TJ. 1983. Influence of pregnancy on parathion toxicity and disposition. *Toxicol. Appl. Pharmacol.* 71:215–24
118. Hasselwander O, McMaster D, Fogarty DG, et al. 1998. Serum paraoxonase and platelet-activating factor acetylhydrolase in chronic renal failure. *Clin. Chem.* 44:179–81
119. Abbott CA, Mackness MI, Kumar S, et al. 1995. Serum paraoxonase activity, concentration and phenotype distribution in diabetes mellitus and its relationship to serum lipids and lipoproteins. *Arterioscler. Thromb. Vasc. Biol.* 15:1812–18
120. Mackness MI, Walker CH, Carlson LA. 1987. Low A-esterase activity in serum of patients with fish-eye disease. *Clin. Chem.* 33:587–88
121. Ferré N, Camps J, Cabré M, et al. 2001. Hepatic paraoxonase activity alterations and free radical production in rats with experimental cirrhosis. *Metabolism* 50:997–1000
122. Nishio E, Watanabe Y. 1997. Cigarette smoke extract inhibits plasma paraoxonase activity by modification of the enzyme's free thiols. *Biochem. Biophys. Res. Commun.* 236:289–93
123. James RW, Leviev I, Righetti A. 2000. Smoking is associated with reduced serum paraoxonase activity and concentration in patients with coronary artery disease. *Circulation* 101:2252–57
124. Jarvik GP, Tsai N, McKinstry LA, et al. 2002. Vitamin C and E intake is associated with increased paraoxonase activity. *Arterioscler. Thromb. Vasc. Biol.* 22:1329–33
125. Debord J, Dantoine T, Bollinger JC, et al. 1998. Inhibition of arylesterase by aliphatic alcohols. *Chem. Biol. Interact.* 113:105–15
126. Van der Gaag MS, van Tol A, Scheek LM, et al. 1999. Daily moderate alcohol consumption increases serum paraoxonase activity: a diet-controlled randomized intervention study in middle-aged men. *Atherosclerosis* 1471:405–10
127. Hayek T, Fuhrman B, Vaja J, et al. 1997. Reduced progression of atherosclerosis in apolipoprotein E-deficient mice following consumption of red wine, or its polyphenol quercetin or catechin, is associated with reduced susceptibility of LDL to oxidation and aggregation. *Arterioscler. Thromb. Vasc. Biol.* 17:2744–52
128. Aviram M, Dornfeld L, Rosenblat M, et al. 2000. Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL, and platelet aggregation: studies in humans and in atherosclerotic apolipoprotein E-deficient mice. *Am. J. Clin. Nutr.* 71:1062–76
129. Kaplan M, Hayek T, Raz A, et al. 2001. Pomegranate juice supplementation to atherosclerotic mice reduces macrophage lipid peroxidation, cellular cholesterol accumulation and development of atherosclerosis. *J. Nutr.* 131:2082–89
130. Kudchodkar BJ, Lacko A, Dory L, et al. 2000. Dietary fat modulates serum paraoxonase 1 activity in rats. *J. Nutr.* 130:2427–33
131. Wallace AJ, Sutherland WHF, Mann JI, et al. 2001. The effect of meals rich in thermally stressed olive oil and safflower oils on postprandial serum paraoxonase activity in patients with diabetes. *Eur. J. Clin. Nutr.* 55:951–58
132. Yamada Y, Takatori T, Nagao M, et al. 2001. Expression of paraoxonase isoform did not confer protection from acute sarin poisoning in the Tokyo subway terrorist attack. *Int. J. Legal Med.* 115:82–84