Received 8.17.05 | Revisions Received 9.7.05 | Accepted 9.7.05

Gene-Environment Interactions: Paraoxonase (PON1) and Sensitivity to Organophosphate Toxicity

Lucio G. Costa,[1,2] Toby B. Cole,[1,3] Clement E. Furlong[3]

([1]Dept. of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA, [2]Dept. of Human Anatomy, Pharmacology and Forensic Science, University of Parma Medical School, Parma, Italy, [3]Depts. of Medicine (Div. of Medical Genetics) and Genome Sciences, University of Washington, Seattle, WA)

DOI: 10.1309/02OXQ8HQJJTTJDK4

Abstract

Paraoxonase (PON1) is a liver and plasma enzyme that hydrolyzes the active metabolites of several organophosphorus insecticides. PON1 displays several polymorphisms that influence both its level of expression and its catalytic activity, thereby determining the rates at which a given individual will detoxify a specific insecticide. Studies in genetically modified mice have elucidated the role played by PON1 in modulating the toxicity of organophosphates. Low PON1 activity during early development may be involved in the enhanced sensitivity of the young toward organophosphate toxicity. Studies in humans should assess PON1 status of individuals, which takes into account both genetic polymorphisms and plasma activity.

The intrinsic properties of a chemical and its dosage (the level of exposure) are 2 primary determinants of potential adverse health effects. However, risks from exposures to exogenous chemicals can also be influenced by variations in target sites, biotransformation enzymes, and repair responses in the host that are often specified genetically and may have significant genetically determined variations.[1] The identification of genetically conditioned drug reactions paved the way for the development of the field of pharmacogenetics, that has since evolved to include any kind of environmental and xenobiotic agents, not just drugs, yet the term ecogenetics.[2] Specific genetic variations that occur in at least 1% of the population are referred to as genetic polymorphisms. A polymorphism may have no effect or may be considered functional if it results in altered function, stability, and/or level of expression of the resulting protein.[3] As part of the Human Genome Project, the Environmental Genome Project aims at identifying and characterizing genetic polymorphisms that my be involved in environmentally induced diseases.

Pesticides represent a large and important class of environmental chemicals, and are often associated with adverse health effects in non-target species including humans. Among the major classes of pesticides, the insecticides are of most concern with regard to potential adverse effects on the nervous system. This stems from the fact that insecticides attack the nervous system of insects, which shares for the most part the same cellular and biochemical characteristics as mammals. A major class of insecticides is represented by the organophosphates (OP), triesters of phosphoric acid that exert their toxicity primarily by inhibiting the enzyme acetylcholinesterase (AChE).[4] Genetic variations in AChE and in other targets (eg, butyrylcholinesterase) may render an individual more of less susceptible to adverse effects of OPs.[5] In addition, OPs undergo bioactivation and detoxication processes that can be affected by genetic polymorphisms in biotransformation enzymes. Most OP insecticides are organophosphorothioates and require metabolic activation to the corresponding oxons by a process of oxidative desulfuration catalyzed by cytochromes P450 (CYP). Only OPs with a P = O moiety can interact with AChE. CYPs, together with glutathione-S-transferases and several esterases, can also detoxify OPs. As numerous genetic variants exist for all these enzymes, such genetic differences can greatly influence the toxicity and neurotoxicity of OPs. Here, we focus on paraoxonase (PON1), a polymorphic enzyme that is involved in the detoxication of several important OPs.

Paraoxonase (PON1)

Paraoxonase takes its name from the ability to hydrolyze paraoxon (PO), the highly toxic metabolite of the insecticide parathion. Other OP substrates of PON1 include chlorpyrifos oxon (CPO) and diazoxon (DZO), the active metabolites of chlorpyrifos and diazinon, respectively, as well as the nerve agents sarin, soman and VX. However, several other OPs with a P = O moiety (eg, malaoxon, dichlorvos) are not hydrolyzed by PON1, nor are the organophosphorothioates.[6]

PON1 is a member of a family of proteins that also includes PON2 and PON3, the genes for which are clustered in tandem on the long arm of human chromosome 7 (q21, 22). PON1 is synthesized in the liver and a portion of PON1 is secreted into the plasma, where it is closely associated with high density lipoprotein (HDL) particles.[7] This latter finding

Downloaded from http://labmed.oxfordjournals.org/ by guest on January 6, 2017

suggested that endogenous substrates for PON1 may be lipids. Indeed PON1 can metabolize oxidized lipids in both low density lipoprotein (LDL) particles, as well as HDL particles.[8] This antioxidant capacity of PON1 toward oxidized lipids appears to be primarily associated with the Q192 alloform (see below). PON1 knockout mice fed a high-fat diet exhibited larger aortic arteriosclerotic lesions than wild-type mice,[9] while the opposite was found in transgenic animals expressing human PON1 (L55, Q192).[10] A large number of epidemiological studies have revealed a small but significant relative risk of the R192 allele for coronary heart disease.[11] However, most of these studies did not assess PON1 status of individuals (see below), which has been shown to be a better predictor of vascular disease than PON1 genotypes.[12,13] PON1 also displays lactonase activity,[14] which may be involved in its ability to hydrolyze endogenous (eg, 5-HETE lactone) or exogenous (eg, glucocorticoid-γ-lactone) substrates, as well as bacterial-quorum sensing factors.[15]

The Genetic Polymorphisms of PON1

Early studies examining plasma paraoxonase levels in human populations revealed a polymorphic distribution of activity, with either bi- or tri-modal distribution reported, depending on the assay used.[6,16-18] Individuals could thus be classified as low, intermediate, or high metabolizers. The molecular basis of the PON1 activity polymorphisms was elucidated once the human PON1 cDNA was isolated and sequenced.[19] A single nucleotide polymorphism in the coding region was found to cause variability in the amino acid present at position 192, where either glutamine (Q) or arginine (R) could be present.[20,21] PON_{192R} was found to be much more efficient in hydrolyzing PO in vitro than $PON1_{192Q}$.

Another polymorphism was identified in the coding region that resulted in an amino acid substitution at codon 55 [leucine (L)/methionine (M)]. Earlier papers report the same polymorphisms at positions 191 and 54; this apparent discrepancy is due to the numbering of amino acids in lieu of the initiator methionine which is removed during secretion from the liver. A consensus was reached in 1998 in Dubrovnik, Croatia, where it was decided to number the amino acids beginning with the initiator methionine (ie, 192 Q/R and 55 L/M).[22] The published literature also contains references to PON1 A and B alleles; these are the Q and R alleles, respectively. In addition to PO, $PON1_{192R}$ hydrolyzes CPO at a faster rate than $PON1_{192Q}$, while the opposite is true in case of sarin or soman.[23,24] Both alloforms have equivalent catalytic efficiencies of hydrolysis of DZO.[24] With regard to other non-OP substrates, the PON_{192Q} alloform is more efficient at metabolizing lipid-peroxides,[25] while $PON1_{192R}$ hydrolyzes lactones and thiolactones more readily.[26,27]

The PON1 55 L/M polymorphisms has been associated with levels of PON1, with the $PON1_{55M}$ alloform displaying lower activity.[28,29] However, this appears to be due to linkage disequilibrium with a polymorphism in the 5' regulatory region of PON1 (C-108T).[30-33] The C-108T polymorphism has, among other polymorphisms identified in this region, the greatest effect on plasma PON1 levels, with the -108C allele producing about twice the level of plasma PON1 as the -108T allele. The nomenclature for this polymorphism, which is in the middle of a consensus sequence for binding of the transcription factor Sp1, also needs to be standardized, as it is reported at time to be at nucleotide position -107. Almost 200 new polymorphisms have also been identified by sequencing the entire PON1 genes from 47 individuals.[34] Some of these genetic variations can explain the few discrepancies observed between the $PON1_{192Q}$ genotyping of certain individuals and their PON1 status, as determined by the 2-substrate assay.[34] (See also **Figure 1**)

Functional Genomics of PON1: The Determination of PON1 Status

Most studies investigating genetic variability of PON1 rely on measurement of nucleotide polymorphisms Q192R, L55M, and C-108T with polymerase chain reaction-based assays. However, even if an individual were genotyped for all known PON1 polymorphisms, this analysis would not provide the level of plasma PON1 activity, nor the phase of polymorphisms (ie, which polymorphisms are on each of an individual's 2 chromosomes). A functional genomic analysis provides a much more informative approach, as measurement of an individual's PON1 function (plasma activity) takes into account all polymorphisms that may affect activity. It should be noted that in a given population, plasma PON1 activity can vary by more than 40-fold,[16,23,35] and differences in PON1 protein levels up to 13-fold are also present within a single PON_{192} genotype.[36]

Determination of PON1 status for an individual is accomplished through the use of a 2-substrate enzyme assay, utilizing PO and DZO (**Figure 1**). For this assay, which has been described in detail elsewhere,[37] plasma is isolated from heparin-collected blood. Citrate plasma (or serum) can also be used, though consistency is necessary among samples. EDTA plasma is not useful, as PON1 activity is calcium-dependent, and EDTA irreversibly inhibits PON1. Initial rates of DZO and of PO hydrolysis are measured by monitoring the formation of 2-isopropyl-4-methyl-6-hydroxypirimidine and p-nitrophenol,

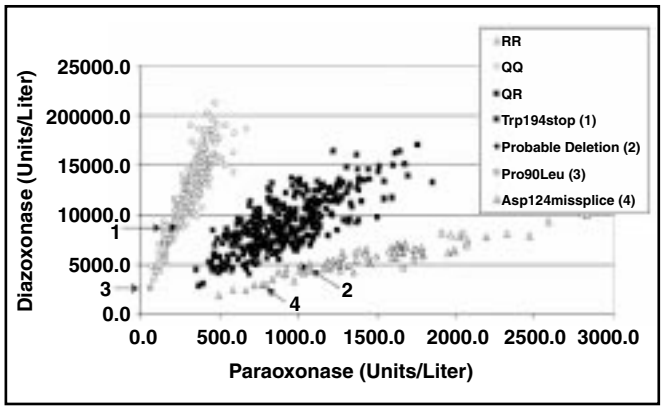

Figure 1_Determination of *PON1* Status. Plot of diazoxonase vs. paraoxonase activities in plasma of carotid artery disease cases and controls, coded for $PON1_{Q192R}$ genotype (determined by PCR) (refs. 34, 35). Note that the 2-substrate assay provides an accurate inference of $PON1_{192}$ genotype as well as the level of plasma *PON1* activities (*PON1* status). Because it is a functional analysis, it provides a 100% accurate determination of the functional genomics of *PON1* status. Newly-discovered SNPs explain why some individuals have lower *PON1* activity than would be predicted by Q192R genotype alone. Individuals 1-4 genotyped as heterozygotes (Q/R192); however, their enzyme analysis indicated homozygosity for Q or R. Complete sequencing of the *PON1* genes revealed mutations in one allele, resulting in only one alloform of *PON1* in their serum. The mutations revealed are noted in the figure. (Note the large variability in *PON1* levels, even among individuals of the same Q192R genotype) (from Ref. 34).

Downloaded from http://labmed.oxfordjournals.org/ by guest on January 6, 2017

respectively. This method separates individuals into the 3 phenotypes of $PON1_{192}$ activity, (ie, $PON1_{192QQ}$, $PON1_{192QR}$ and $PON1_{192RR}$). In addition, PON activities within a genotype provide information on the levels of PON1 in the plasma of a given individual within that genotype group. The PON1 genotype can be accurately inferred by this analysis, and can be verified by genotyping the individuals DNA.[37] However, with very few exceptions, this functional genomics analysis is very accurate, and does not require verification.[12] An example of possible discordance is an individual who genotypes as a $PON1_{QR}$ heterozygote, but whose PON1 status functional analysis indicates a $PON1_{192Q}$ or $PON1_{192R}$ homozygote; this would indicate that one of the alleles is nonfunctional, because of additional mutations (eg, W194X; 34), and underlines the advantage of the PON1 status analysis over straight genotyping. It is also important to note that several recent studies investigating the role of PON1 in coronary heart disease, have indeed provided evidence that PON1 status (encompassing genotype and activity levels) is a much better predictor of disease than PON1 genotype alone.[12,13]

Modulation of PON1 Levels

The importance of PON1 status in determining susceptibility or protection from toxicity or diseases, points to the relevance of factors affecting PON1 activity and levels of expression. Clearly, genetic determinants play a primary role in determining an individual's PON1 status; nevertheless, the contribution of other factors in modulating PON1 activity and levels should not be overlooked. The potential role of environmental, pharmacological, life-style, and dietary factors in modulating PON1, as well as the role of age, gender, and certain physiological and pathological conditions have been recently reviewed.[38,39] Among the factors discussed, one should mention the lipid-lowering pharmaceutical drugs statins, which may upregulate PON1 through an interaction with the Sp1 binding site, though other sites may also be involved.[40] Low doses of ethanol may also increase PON1 activity and levels by a similar mechanism,[41] while smoking is associated with reduced serum PON1 activity.[42] Lipid-rich diets have differential effects on PON1, depending on the lipid composition (eg, monoenoic vs. polyenoic fatty acids), while antioxidants seem to increase PON1 activity, possibly by protecting the enzyme from oxidative stress-induced inactivation, though the findings remain controversial.[43] Among other conditions found to affect PON1 activity, 2 should be mentioned: both insulin-dependent (type 1) and non-insulin dependent (type 2) diabetes have been consistently associated with lower serum PON1 activity, independently from the genotype. Age is another major determinant of PON1 activity. A progressive decrease in PON1 activity has been found in elderly subjects, while neonates and young children have low PON1 activity, which may be even lower in fetuses.[44,45] Low PON1 activity during development is believed to represent a relevant risk factor for increased susceptibility to the toxicity of certain OPs, as discussed below.

PON1 Modulates Sensitivity to OP Insecticide Toxicity

The existence of polymorphisms in PON1 that infer different hydrolyzing ability toward several OPs, as well as different levels of expression, have long suggested that certain individuals may be more sensitive to OP toxicity. This hypothesis has received confirmation from animal studies, though a direct satisfactory verification in humans is still lacking. Furthermore, PON1 may represent a relevant factor conferring increased susceptibility to OP toxicity in the young.

Earlier studies showed that animals with low PON1 activity, such as birds, displayed higher sensitivity to OPs.[46] Subsequent studies indicated that administration of exogenous purified PON1 to rats or mice increased their resistance to the toxic effects of OPs such as CPO.[47,48] Of practical interest is that exogenous PON1 could also offer protection against the toxicity of the parent compound chlorpyrifos, when given before or even after (up to 3 hours) OP exposure.[49] Recombinant human PON1 (either LR or LQ) expressed from an adenoviral vector was also shown to protect mice against the toxicity of chlorpyrifos.[50] More recently, it was reported that administration to mice of recombinant naked DNA (pcDNA/PON1) protected them from the acute toxicity of soman.[51] Overall, these studies indicate that by artificially increasing serum levels of PON1, it is possible to decrease the toxicity of certain OPs.

Experiments in genetically modified mice have provided further information on the role of PON1 in modulating OP toxicity. PON1 knockout mice have dramatically increased sensitivity to CPO and DZO toxicity, and a slight increased sensitivity to the respective parent compounds, chlorpyrifos and diazinon.[9,24] Administration of purified human PON1 to PON1 knockout mice to restore plasma PON1, conferred protection toward CPO and DZO.[24] In case of DZO, both $PON1_{192R}$ and $PON1_{192Q}$ provided equal protection, while in case of CPO, $PON1_{192R}$ provided about 50% better protection.[24] These latter findings were later confirmed in a transgenic mouse model in which murine PON1 was substituted with either $hPON1_{192R}$ or $hPON1_{192Q}$. Though both mouse lines expressed similar PON1 levels, $hPON1_{192R}$ mice were much more resistant to CPO toxicity.[52] **Table 1** shows a summary of the CPO experiments. All these findings can be explained by an analysis of in vitro catalytic activity of PON1 toward CPO or DZO. Both human $PON1_{192}$ alloforms hydrolyze DZO with similar catalytic efficiency; however, in case of CPO, $PON1_{192R}$ is about twice as efficient as $PON1_{192Q}$.[24] A surprising finding was obtained when testing PO, the enzyme

Table 1_Toxicity of CPO in Genetically Modified Mice

	Brain AChE Activity (% of control)
Wild-type	90
PON1 KO	18
PON KO + $hPON1_{192Q}$	25
PON1 KO + $hPON1_{192R}$	85
$hPON1_{192Q}$-TG	35
$hPON1_{192R}$-TG	60

Mice were given CPO (2 mg/kg) by dermal exposure and sacrificed after 4h. This dose of CPO caused only a 10% inhibition of brain AChE in wild-type mice, but over 80% inhibition in PON1 knockout (KO) mice. When mice were injected ip with purified hPON1 (916 U of arylesterase activity) 4h before CPO, $hPON1_{192R}$ provided significant greater protection (only 15% brain AChE inhibition) than $hPON1_{192Q}$. Similarly, "humanized" transgenic (TG) mice (expressing either $hPON1_{192Q}$ or $hPON1_{192R}$ on a KO background) were differentially sensitive to CPO, despite expressing similar enzyme levels. CPO caused a 40% inhibition of brain AChE in $hPON1_{192R}$-TG mice, but 65% inhibition in hPON1192Q-TG mice. Data adapted from Shih et al. (ref. 9), Li et al. (ref. 24) and Cole et al. (ref. 52).

Downloaded from http://labmed.oxfordjournals.org/ by guest on January 6, 2017

after which PON1 was named. PON1 knockout mice were not more sensitive than wild-type animals to its toxicity, despite the total lack of PON1 activity in plasma and liver. Furthermore, neither human $PON1_{192}$ alloform given to PON1 knockout mice afforded protection against PO toxicity. These results, too, can be explained by in vitro kinetic analysis. In vitro, under physiological conditions, the efficiency of $PON1_{192R}$ is 8 times greater than that of $PON1_{192Q}$ at hydrolyzing PO; however, the overall catalytic efficiency is very low, compared to efficiencies observed for CPO or DZO hydrolysis.[24] Thus, PON1 is not efficient at hydrolyzing PO at low concentrations, indicating that PON1 may not degrade PO efficiently in vivo, as first suggested by Pond and colleagues.[53]

This series of studies provide strong evidence that PON1, whose catalytic activity and level of expression are determined in humans by polymorphisms in the coding and promoter regions, plays an important role in modulating the toxicity of some, but not all, OPs, which are in vitro substrates for this enzyme. Specifically, levels of PON1, independently of genotype, are important to confer protection toward DZO; PON1 levels and Q192R genotype are relevant in case of CPO, with $PON1_{192R}$ providing greater protection; and PON1 does not play a role in modulating PO toxicity in vivo.

Studies in mice expressing either human $hPON1_{Q192}$ or $hPON1_{R192}$ provide a convincing case of extrapolating the results obtained with animal models to humans; however, direct and conclusive confirmation in humans of the relevance of PON1 status in determining relative sensitivity to OP toxicity is still lacking. In recent years, different situations in Japan, the Middle East, and the United Kingdom, have called for investigations on the role of PON1 in OP toxicity in humans. The studies, which dealt with exposure to sarin in the Tokyo subway system following a terrorist attack,[54] potential exposure to sarin and/or other OPs in American and British veterans of the 1991 Gulf War,[55,56] and sheep dippers exposed to diazinon in the United Kingdom[57] have been discussed in detail elsewhere.[58] Overall, they are suggestive of a role of PON1 polymorphisms in modulating sensitivity to OPs in humans, but do not allow any firm conclusion. It appears that more and better-designed studies are needed in human populations to address directly the issue of whether an individual's PON1 status may confer protection or increased sensitivity to the toxicity of specific OPs. In particular, the level and nature (ie, the specific OP involved) of exposure, and the early consequences of exposure (eg, blood AChE activity), need to be documented.[59] Furthermore, our studies[24] clearly point out the importance of catalytic efficiency in determining whether or not PON1 can contribute significantly to modulating toxicity associated with a specific exposure.

An important aspect of these experimental and epidemiological studies lies in the potential role of PON1 in modulating the developmental toxicity and neurotoxicity of OPs. The toxicity of OPs appears to be influenced by age, with young animals being more sensitive to the effects of acute exposure.[60] Lower metabolic abilities of young animals are believed to be a major determinant of their increased sensitivity to OP toxicity. In rodents, PON1 levels are very low at birth and increase postnatally, reaching a plateau around 3 weeks.[45,61] Low PON1 activity is also seen in human infants, where a plateau is reached between 6 and 15 months.[16,44,45] PON1 levels may even be lower before birth, as suggested by the finding that premature babies (33 to 36 weeks of gestation) have 25% lower PON1 activity compared to term babies.[44] In addition, an expectant mother with low PON1 status would be predicted not to be able to provide protection for the fetus against exposure to some OPs. Indeed, offspring of mothers with low PON1 status, exposed in utero to chlorpyrifos, were reported to have significant smaller head circumference compared to those born to mothers with high PON1 activity or those not exposed to chlorpyrifos.[62] As small head size has been found to predict subsequent cognitive ability, prenatal exposure to chlorpyrifos may have long term detrimental effects in offspring of mothers with low PON1 activity.

Conclusions

Polymorphisms in the PON1 gene influence both the quantity and the quality of PON1 (ie, PON1 status). Strong evidence indicates that PON1 levels and, in some cases, the Q192R polymorphism, determine the efficiency with which an individual will detoxify a specific OP. Direct proof of this in human populations still requires carefully conducted studies where PON1 status is correlated with the degree of exposure and with early indicators of toxicity. In all cases, human studies should rely on assessment of PON1 status, rather than straight genotyping. LM

Acknowledgements: *Research by the authors was supported by grants from the National Institute of Health (ES04696; ES07033; ES09883; ES09601/EPA-R826886).*

1. Costa LG, Eaton, DL (editors). *Gene environment Interactions: Fundamentals of Ecogenetics.* New York: John Wiley & Sons, 2005.
2. Costa LG. The emerging field of ecogenetics. *Neurotoxicology.* 2000;21:85-90.
3. Kelada SN, Eaton DL, Wang SS, et al. Applications of human genome epidemiology to environmental health. In: Khoury MG. Little J. Burke W, editors. *Human Genome Epidemiology.* Oxford: Oxford University Press, 2004; pp. 145-167.
4. Lotti M. Organophosphorus compounds. In: Spencer PS, Schaumburg HH, Ludolph AC, editors. *Experimental and Clinical Neurotoxicology.* Oxford: Oxford University Press, 2000; pp. 898-925.
5. Costa LG. Pesticide exposure: Differential risk for neurotoxic outcomes due to enzyme polymorphisms. *Clinics Occup Environm Med.* 2001;1:511-523.
6. Goldmacher von Mallinckrodt M, Diepgen TL. The human serum paraoxonase – polymorphism and specificity. *Toxicol Environ Chem.* 1988; 18: 79 – 196.
7. Sorenson RC, Bisgaier CL, Aviram M, et al. Human serum paraoxonase/arylesterase's retained hydrophobic N-terminal leader sequence associates with HDLs by binding phospholipids: aopoprotein A-1 stabilizes activity. *Arterioscler Thromb Vasc Biol.* 1999;19:2214-2225.
8. Mackness MI, Arrol S, Durrington PN. Paraoxonase prevents accumulation of lipoperoxides in low-density lipoproteins. *FEBS Lett.* 1991;286:152-154.
9. Shih DM, Gu L, Xia YR, et al. Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis. *Nature.* 1998;392:284-287.
10. Tward A, Xia SR, Wang SP, et al. Decreased arteriosclerotic lesions formation in human serum paraoxonase transgenic mice. *Circulation.* 2000;106:484-490.
11. Wheeler JG, Kearney BD, Watkins H, et al. Four paraoxonase polymorphisms in 11,212 cases of coronary heart disease and 12,786 controls: Meta-analysis of 43 studies. *Lancet.* 244;363:689-695.
12. Jarvik GP, Rozek LS, Brophy VH, et al. Paraoxonase (PON1) phenotype is a better predictor of vascular disease than is $PON1_{192}$ or PON_{155} genotype. *Arterioscler Thromb Vasc Biol.* 2000;20:2441-2447.
13. Mackness M, Mackness B. Paraoxonase 1 and atherosclerosis: is the gene or the protein more important? *Free Rad Biol Med.* 2004;37:1317-1323.
14. Draganov DJ, La Du BN. Pharmacogenetics of paraoxonase: a brief review. *Naunyn-Schmiedeberg's Arch Pharmacol.* 2004;369:78-88.
15. Draganov DJ, Teiber JF, Speelman A, et al. Human paraoxonases (PON1, PON2, PON3) are lactonases with overlapping and distinct specificities. *J Lip Res.* 2005;46:1239-1247.

Downloaded from http://labmed.oxfordjournals.org/ by guest on January 6, 2017

16. Mueller RF, Hornung S, Furlong CE, et al. Plasma paraoxonase polymorphism: a new enzyme assay, population, family, biochemical and linkage studies. *Am J Hum Genet.* 1983;35:393-408.
17. Eckerson HW, Wyte CM, La Du BN. The human serum paraoxonase/arylesterase polymorphism. *Am J Hum Genet.* 1983;35:1126-1138.
18. Ortigoza-Ferado J, Richter RJ, Hornung SK, et al. Paraoxon hydrolysis in human serum mediated by a genetically variable arylesterase and albumin. *Am J Hum Genet.* 1984; 36: 295-305.
19. Hassett C, Richter RJ, Humbert R, et al. Characterization of DNA clones encoding rabbit and human serum paraoxonase: the mature protein retains its signal sequence. *Biochemistry.* 1991;30:10141-10149.
20. Humbert R, Adler DA, Distech CM, et al. The molecular basis of the human serum paraoxonase activity polymorphism. *Nat Genet.* 1993;3:73-76.
21. Adkins S, Gan KN, Mody M, et al. Molecular basis for the polymorphic forms of human serum paraoxonase / arylesterase: glutamine or arginine of position 191, for the respective A or B allozymes. *A J Hum Genet.* 1993;53:598-608.
22. La Du BN, Furlong CE, Reiner E. Recommended nomenclature system for the paraoxonases. *Chem Biol Interact.* 1999;119:599-601.
23. Davies H, Richter RJ, Kiefer J, et al. The human serum paraoxonase polymorphisms is reversed with diazoxon, soman and sarin. *Nat Genet.* 1996;14:334-336.
24. Li WF, Costa LG, Richter RJ, et al. Catalytic efficiency determines the in vivo efficacy of PON1 for detoxifying organophosphates. *Pharmacogenetics.* 2000;10:767-779.
25. Aviram M, Hardak E, Vaya J, et al. Human serum paraoxonase (PON1) Q and R selectively decrease lipid peroxides in human coronary and carotid arteriosclerotic lesion; PON1 esterase and peroxidase-like activities. *Circulation.* 2000;101:2510-2517.
26. Billecke S, Draganov D, Counsel R, et al. Human serum paraoxonase (PON1) isozymes Q and R hydrolyze lactones and cyclic carbonate ester. *Drug Metab Dispos.* 2000;28:1335-1342.
27. Tougou K, Nakamura A, Watanabe S, et al. Paraoxonase has a major role in the hydrolysis of prulifloxacin (NM441), a prodrug of a new antibacterial agent. *Drug Metab Dispos.* 1998;26:355-359.
28. Blatter Garin MC, James RW, Dussoix P, et al. Paraoxonase polymorphisms 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.* 1997;99:62-66.
29. Mackness B, Mackness MI, Arrol T, et al. Effect of the human serum paraoxonase 55 and 192 genetic polymorphisms on the protection by high density lipoprotein against low density lipoprotein oxidative modifications. *FEBS Lett.* 1998;423:57-60.
30. Brophy VH, Hastings MD, Clendenning JB, et al. Polymorphisms in the human paraoxonase (PON1) promoter. *Pharmacogenetics.* 2001;11:77-84.
31. Brophy VH, Jampsa RL, Clendenning JB, et al. Effects of 5' regulatory-region polymorphisms on paraoxonase gene (PON1) expression. *Am J Human Genet.* 2001;68:11428-1436.
32. Leviev J. James RW. Promoter polymorphisms of human paraoxonase PON1 gene and serum paraoxonase activites and concentrations. *Atheroscler Thromb Vasc Biol.* 2000:20:516-521.
33. Suehiro T, Nakamura T, Inoue M, et al. A polymorphism upstream from the human paraoxonase (PON1) gene and its association with PON1 expression. *Atherosclerosis.* 2000;150:296-298.
34. Jarvik GP, Jampsa R, Richter RJ, et al. Novel paraoxonase (PON1) nonsense and missense mutations predicted by functional genomic assay of PON1 status. *Pharmacogenetics.* 2003;13:291-295.
35. Richter RJ, Furlong CE. Determination of paraoxonase (PON1) status requires more than genotyping. *Pharmacogenetics.* 1999;9:745-753.
36. Costa LG, Cole TB, Jarvik GP, et al. Functional genomics of the paraoxonase (PON1) polymorphisms: effects on pesticide sensitivity, cardiovascular disease, and drug metabolism. *Annu Rev Med.* 2003;54:371-392.
37. Richter RJ, Jampsa RL, Jarvik BP, et al. Determination of paraoxonase 1 status and genotypes at specific polymorphisms sites. In: Maines M, Costa LG, Reed DJ, Hodgson E, editors. *Current Protocols in Toxicology.* New York: John Wiley and Sons, 2004; pp. 4.12.1 – 4.12.19.
38. Deakin SP, James RW. Genetic and environmental factors modulating serum concentrations and activities of the antioxidant enzyme paraoxonase-1. *Clin Sci.* 2004;107:435-447.
39. Costa LG, Vitalone A, Cole TB, et al. Modulation of paraoxonase (PON1) activity. *Biochem Pharmacol.* 2005;69:541-550.
40. Deakin S, Leviev I, Guernier S, et al. Simvastatin modulates expression of the PON1 gene and increases serum paraoxonase: a role for sterol regulatory element-binding protein-2. *Arterioscler Thromb Vasc Biol.* 2003;23:2083-2089.
41. Rao MN, Marmillot P, Gong M, et al. Light, but not heavy, alcohol drinking, stimulates paraoxonase by upregulating liver mRNA in rats and humans. *Metabolism.* 2003;52:1287-1294.
42. James RW, Leviev I, Righetti A. Smoking is associated with reduced serum paraoxonase activity and concentration in patients with coronary artery disease. *Circulation.* 2000;101:2252-2257.
43. Nguyen SD, Sok DE. Beneficial effect of oleoylated lipids on paraoxonase-1: protection against oxidative inactivation and stabilization. *Biochem J.* 2003; 375: 275-285.
44. Ecobichon DJ, Stephens DS. Perinatal development of human blood esterases. *Clin Pharmacol Ther.* 1973;14:41-47.
45. Cole TB, Jampsa RL, Walter BJ, et al. Expression of human paraoxonase (PON1) during development. *Pharmacogenetics.* 2003;13:357-364.
46. Brealey CB, Walker CH, Baldwin BC. A-Esterase activities in relation to the differential toxicity of pirimiphos-methyl to birds and mammals. *Pest Sci.* 1980;11:546-554.
47. Costa LG, McDonald BE, Murphy SD, et al. Serum paraoxonase and its influence on paraoxon and chlorpyrifosoxon toxicity in rats. *Toxicol Appl Pharmacol* 1990;103:66-76.
48. Li WF, Costa LG, Furlong CE. Serum paraoxonase status: A major factor in determining resistance to organophosphates. *J Toxicol Environ Health.* 1993;40:337-346.
49. Li WF, Furlong CE, Costa LG. Paraoxonase protects against chlorpyrifos toxicity in mice. *Toxicol Lett.* 1995;76:219-226.
50. Cowan J, Sinton CM,Varley AW, et al. Gene therapy to prevent organophosphate intoxication. *Toxicol Appl Pharmacol.* 2001;173:1-6.
51. Fu AL,Wang YX, Sun MJ. Naked DNA prevents soman intoxication. *Biochem Biophys Res Commun.* 2005;328:901-905.
52. Cole TB, Walter BJ, Shih DM, et al. Toxicity of chlorpyrifos and chlorypyrifos oxon in a transgenic mouse model of the human paraoxonase (PON1) Q192R polymorphism. *Pharmacogenet Genomics.* 2005;15:589-598.
53. Pond AL, Chambers HW, Chambers JE. Organophosphate detoxication potential of various rat tissues via A-esterases and aliesterase activities. *Toxicol Lett.* 1995;78:245-252.
54. Yamada Y, Takatori T, Negao M, et al. Expression of paraoxonase isoforms did not confer protection from acute sarin poisoning in the Tokyo subway terrorist attack. *Int J Leg Med.* 2001;115:82-84.
55. Haley RW, Billecke S, La Du BN. Association of low PON1 type Q (type A) arylesterase activity with neurologic symptoms complexes in Gulf War veterans. *Toxicol Appl Pharmacol.* 2000;157:227-233.
56. Mackness B, Durrington PN, Mackness MI. Low paraoxonase in Persion Gulf War veterans self-reporting Gulf war Syndrome. *Biochem Biophys Res Commun.* 2000;276:729–733.
57. Mackness B, Durrington P, Povey A, et al. Paraoxonase and susceptibility to organophosphorus poisoning in farmers dipping sheep. *Pharmacogenetics.* 2003;13:81-88.
58. Costa LG, Cole TB, Furlong CE. Polymorphisms of paraoxonase (PON1) and their significance in clinical toxicology of organophosphates. *J Toxicol Clin Toxicol.* 2003;41:37-45.
59. Costa LG, Cole TB, Vitalone A, et al. Measurement of paraoxonase (PON1) status as a potential biomarker of susceptibility to organophosphate toxicity. *Clin Chim Acta.* 2005;352:37-47.
60. Pope CN, Liu J. Age-related difference in sensitivity to organophosphorus pesticides. *Environ Toxicol Pharmacol.* 1997;4:309-314.
61. Li WF, Mathews C, Disteche CM, et al. Paraoxonase (PON1) gene in mice: sequencing, chromosomal localization and developmental expression. *Pharmacogenetics.* 1997;7:137-144.
62. Berkowitz GS. Wetmur JF, Birman–Deych E, et al. In utero pesticide exposure, maternal paraoxonase activity and head circumference. *Environ Health Perspect.* 2004;112:388-391.

Downloaded from http://labmed.oxfordjournals.org/ by guest on January 6, 2017