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Human PON1, a biomarker of risk of disease and exposure

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Abstract

Human paraoxonase 1 (PON1) is a high-density lipoprotein (HDL)-associated serum enzyme that exhibits a broad substrate specificity. In addition to protecting against exposure to some organophosphorus (OP) pesticides by hydrolyzing their toxic oxon metabolites, PON1 is important in protecting against vascular disease by metabolizing oxidized lipids. Recently, PON1 has also been shown to play a role in inactivating the quorum sensing factor *N*-(3-oxododecanoyl)-L-homoserine lactone (3OC12-HSL) of *Pseudomonas aeruginosa*. Native, untagged engineered recombinant human PON1 (rHuPON1) expressed in *E. coli* and purified by conventional column chromatographic purification is stable, active, and capable of protecting *PON1* knockout mice (*PON1*^{-/-}) from exposure to high levels of the OP compound diazoxon. The bacterially-derived rHuPON1 can be produced in large quantities and lacks the glycosylation of eukaryotic systems that can produce immunogenic complications when inappropriately glycosylated recombinant proteins are used as therapeutics. Previous studies have shown that the determination of PON1 status, which reveals both *PON1*₁₉₂ functional genotype and serum enzyme activity level, is required for a meaningful evaluation of PON1's role in risk of disease or exposure. We have developed a new two-substrate assay/analysis protocol that provides PON1 status without use of toxic OP substrates, allowing for use of this protocol in non-specialized laboratories. Factors were also determined for inter-converting rates of hydrolysis of different substrates. PON1 status also plays an important role in revealing changes in HDL-associated PON1 activities in male patients with Parkinson disease (PD). Immunolocalization studies of PONs 1, 2 and 3 in nearly all mouse tissues suggests that the functions of PONs 1 and 3 extend beyond the plasma and the HDL particle.

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Conflict of Interest statement

The authors declare that there are no conflicts of interest.

Keywords

paraoxonase 1; Parkinson disease; organophosphate; therapy for OP poisoning; chlorpyrifos/chlorpyrifos oxon; diazinon/diazoxon

1.1 Genetics of PON1

1.1.1 Early studies

In 1953, Aldridge divided esterases into two categories, those that catalytically hydrolyzed organophosphate substrates (A-esterases) and those that were inhibited by organophosphates (B-esterases) [1]. Plasma paraoxonase 1 (PON1) is an A-esterase. Studies in the 1960s and 1970s demonstrated that PON1 activity was polymorphically distributed in human populations and the frequency of the low activity phenotype varied among populations of different ethnic origins. These studies are summarized in an excellent review by Geldmacher-von Mallinckrodt and Diepgen [2].

1.1.2 Enzyme assays used to establish Q192R phenotype

The early assays used to assess PON1 phenotype varied from assays that measured the ability of serum samples to protect cholinesterase from inhibition to spectrophotometric assays run under varying conditions of pH, salt concentration and presence or absence of EDTA (subsequently shown to be an inhibitor of Ca^{2+} -dependent PON1). These early assay protocols were reviewed by Ortigoza-Ferado et al. [3]. A major improvement in PON1 phenotype resolution was achieved with a two-substrate assay/analysis pioneered in La Du's laboratory [4]. Plotting rates of paraoxon (PO) vs. phenyl acetate hydrolysis clearly resolved low metabolizers from high metabolizers but did not resolve heterozygotes from homozygous high metabolizers. This was achieved using the substrate pair diazoxon (DZO) and PO. Plots of rates of DZO hydrolysis vs. PO hydrolysis provided a clear resolution of all three phenotypes (Fig. 1), which by the time of development of the assay had been shown to be *PON1*_{Q192} homozygotes, *PON1*_{Q/R192} heterozygotes and *PON1*_{R192} homozygotes [5]. It had been shown earlier that it was the amino acid at position 192 that determined catalytic efficiency of PON1 for hydrolysis of some substrates [6-8]. The information gleaned from the two-substrate analysis has been referred to as an individual's PON1 status since it provides both the functional *PON1*₁₉₂ genotype as well as the level of plasma PON1 [9].

More recently, we have developed a two substrate assay/analysis protocol that clearly resolves the three PON1 phenotypes (functional genotypes) without the use of the highly toxic OP substrates [10-11]. Plots of rates of phenyl acetate hydrolysis at high salt vs. rates of 4-(chloromethylphenyl) acetate (CMPA) at low salt provide a resolution of phenotypes and functional genotypes comparable to the DZO vs. PO plot. Tables to convert rates of hydrolysis of one substrate to rates of hydrolysis of other substrates were included in the two recent publications [10-11]. Rates of *in vivo* detoxication at different oxon levels can also be calculated from the *in vitro* rates and the kinetic parameters of the two *PON1*₁₉₂ alloforms. Since the *PON1*_{Q192R} polymorphism does not affect the rate of hydrolysis of phenyl acetate at low salt concentration, measurement of this rate provides a surrogate measure of plasma PON1 protein level [12].

1.1.3 DNA assays used to establish *PON1* genotypes

The elucidation of the initial human *PON1* cDNA sequences revealed the two common coding region polymorphisms that have since been studied extensively [13]. Since both the L55M and Q192R polymorphisms included restriction sites for one allele, it was easy to genotype individuals for these two polymorphisms [6]. Single nucleotide polymorphisms

(SNPs) were also found in the promoter and 3' untranslated regions of the *PON1* gene. Five of the promoter region polymorphisms have been characterized by three research groups [14-16]. The functionality of the 3' untranslated region polymorphisms has yet to be studied. Of the promoter region polymorphisms, the *C-108T* polymorphism appears to have the most significant effect on the regulation of PON1 expression [14,17]. On average, the *PON1_{C-108}* allele expresses approximately twice the level as the *PON1_{T-108}* allele [14]. It is interesting that this polymorphism occurs in an Sp1 transcription factor binding site [17]. Sequencing the entire *PON1* gene from 47 individuals revealed nearly 200 additional SNPs in the *PON1* gene (Fig. 2). There is a relatively low level of linkage disequilibrium across the *PON1* gene with non-recombinant blocks of only several thousand bases observed; thus haplotype analysis is not highly informative [18-19].

1.1.4 PON1 genetic variability and risk of OP exposure

1.1.4.1 Rodent models—Early experiments by Main [20] demonstrated that the injection of partially purified rabbit PON1 provided some protection against exposure to PO. This observation was followed up by our laboratory, initially in rats [21] and more extensively in a mouse model system with wild-type mice and genetically modified mice. Injection of purified rabbit PON1 provided some protection against dermal exposure of wild-type mice to PO, but much better protection against exposure to chlorpyrifos oxon [22]. The injected rabbit PON1 also protected against chlorpyrifos exposure [23]. These experiments prompted us to propose the use of PON1 as a therapeutic for OP poisoning [23].

Experiments with *PON1*^{-/-} mice illustrated the importance of catalytic efficiency of hydrolysis for protecting against OP exposure [24]. Injection of the purified PON1_{Q192} or PON1_{R192} alloform into the *PON1*^{-/-} mice restored resistance to CPO and DZO and provided information on the ability of each alloform to protect against OP exposure. In the case of CPO, the PON1_{R192} alloform protected better than the PON1_{Q192} alloform, whereas both alloforms protected equally as well against DZO. Surprisingly, neither alloform protected against PO exposure. Determination of the catalytic efficiency of OP hydrolysis by the two PON1₁₉₂ alloforms provided a clear explanation for these results. PON1_{R192} had a higher catalytic efficiency than PON1_{Q192} for hydrolyzing CPO, whereas both alloforms had nearly equivalent catalytic efficiencies for hydrolyzing DZO. Even though human PON1_{R192} has approximately 9-times the catalytic efficiency of PON1_{Q192} for the hydrolysis of PO, it was not high enough to provide protection against exposure. The early *in vitro* assays that revealed the PON1 Q192R polymorphism was responsible for the large difference in rates of PON hydrolysis between the PON1₁₉₂ alloforms, leading to the incorrect assumption that high PO hydrolytic activity would protect against PO and parathion (PS) exposure [2].

Experiments with transgenic mouse lines that expressed either recombinant human PON1_{R192} (rHuPON1_{R192}) or PON1_{Q192} (rHuPON1_{Q192}) were in complete accord with the results of the injection experiments [25]. Mice expressing tgHuPON1_{R192} were more resistant to CPO exposure than mice expressing rHuPON1_{Q192}. At high CPO concentrations, the rHuPON1_{Q192} mice were as sensitive as the *PON1*^{-/-} mice. This is a significant finding, since approximately one-half of individuals of Northern European origin are homozygous for PON1_{Q192}.

1.1.4.2 Development and OP sensitivity—It was shown early on that the level of plasma arylesterase, since shown to be PON1, in newborns was low at birth and increased over time [26-27]. A study that tracked PON1 activity over time in individual children showed that plasma PON1 levels reach a plateau between 6 and 24 months of age [28]. However, a more recent study reported that PON1 levels can continue to increase beyond 5

years of age [29]. To examine the significance of variable plasma PON1 levels during development, a dose response (sq) to CPO was carried out with *PON1*^{-/-} and wild-type mice at postnatal day 4. The wild-type mice were already 2.5-times more resistant to CPO than the *PON1*^{-/-} mice (Cole et al. unpublished data).

1.1.4.3 Human epidemiological studies—Relatively few epidemiological studies in humans relating PON1 status or levels to OP sensitivity have been published. Some studies, e.g., [30] have examined only SNP data which provided no measure of plasma level of PON1, the most important factor in determining OP resistance. As noted above, it is important to determine PON1 status for individuals to obtain an accurate estimate of their ability to detoxify DZO and CPO, the only two OPs for which PON1 status appears to physiologically relevant. Genotyping alone will not provide this information.

A recent study by Hofmann et al. [31] of pesticide handlers in Washington State reported that individuals homozygous for *PON1*_{Q192} in the lowest tertile of plasma PON1 levels were the most sensitive to butyrylcholinesterase inhibition during the spraying season, as would be expected. A determination of PON1 status in farm worker mothers and their babies (cord bloods) predicted a range of sensitivity to DZO of 65-fold from the baby with the lowest PON1 status to the mother with the highest PON1 status. Due to the contribution of both PON1 levels and the *PON1*₁₉₂ polymorphism for CPO detoxication, the range was even greater, i.e., ~131- to 164-fold [12]. Other studies have also examined PON1 levels and OP susceptibility [32]. Some of these investigators have interpreted the lower V_{\max} of the *PON1*_{R192} homozygous phenotype as a risk for higher sensitivity to diazoxon [32-33]. This is not the case. While the V_{\max} of *PON1*_{R192} is lower than that of *PON1*_{Q192}, the affinity of *PON1*_{R192} is higher than that of *PON1*_{Q192} resulting in a slightly higher catalytic efficiency (V_{\max}/K_m) of the *PON1*_{R192} alloform [8,24,34].

1.1.4.4 Ethics of testing for PON1 status among agricultural workers—The study of the Washington State agricultural workers by Hofmann et al. [31] raised the important ethical question of how to inform a worker that they would be predicted to be highly sensitive to DZO/diazinon (DZ) and CPO/chlorpyrifos (CPF) exposure based on the functional PON1 status assays. One approach to this issue would be to provide anonymous testing through the workers' union with results provided to the workers along with educational materials in the native language of the workers, written at a level understandable to the worker. It might also be necessary in cases of illiterate workers to provide a verbal explanation and interpretation of the test results by an appropriate spokesperson. Since low PON1 status is also a risk factor for carotid artery disease [35] as well as other vascular disease [36] (see next section) and at-risk individuals can implement protective measures against vascular disease, knowledge of one's PON1 status may have other health benefits.

2.1 PON1 genetic variability and risk of disease

2.1.1 Diseases associated with PON1 variability

In recent years, there have been approximately 500 papers published related to the association or lack of association of *PON1* with specific diseases (PubMed searches). The largest number of studies has been carried out on vascular disease. Other diseases studied for their possible association with genetic variability in PON1 include Parkinson disease (PD), amyotrophic lateral sclerosis (ALS), kidney disease, eye diseases, systemic lupus erythematosus, abdominal aortic aneurysms, asthma, chronic idiopathic pancreatitis, diabetes and associated kidney disease, Chron's disease, sarcoidosis, erectile dysfunction, systemic vasculitis, glomerulonephritis, breast cancer, prostate cancer, brain tumors, multiple myeloma, dementia and multiple chemical sensitivity, There have been too many studies to cover in this brief review. The reader is referred to recent reviews [37-38].

However some important comments are relevant to all of these studies as well as studies that examine PON1 activity variability as a risk of exposure, infection or other physiological function.

Many of the reported studies have examined *PON1* SNPs and ignored plasma PON1 levels, the most important determinant of the rates of metabolism of endogenous or exogenous toxins. Not surprisingly, the results from these studies have provided conflicting information. A number of papers have reviewed these studies [19,36,39-42]. Studies that examined either PON1 levels or PON1 status (plasma PON1 activity levels and Q192R genotype) have shown that low PON1 levels are a risk factor for vascular disease [35-36,43-44]. It stands to reason that higher levels of a protein involved in metabolizing oxidized lipids or toxic OP compounds would be more protective and the level of protection should be related to the activity level of the protein. Conversely, low PON1 levels would be a risk factor for disease or exposure. In some cases, such as the metabolism of CPO, the *PON1*₁₉₂ genotype is also important [24], but in no case would the activity level of PON1 be unimportant [19]. The recently-developed high throughput protocol for determining PON1 status without the use of the highly toxic OP substrates PO and DZO should make it convenient for epidemiologists to do a proper study of the relationship of PON1 genetic variability (PON1 status) to CVD and other diseases [11,45-46].

If new coding region polymorphisms that generate mutant PON1s are discovered, they will most likely be found through the current next-generation DNA sequencing efforts that examine entire exomes, or focused sequencing of *PON1* exons (or complete genes) in cases where risk of CVD or other disease maps to the *PON1* gene. Promoter mutations that affect expression level would be reflected in the PON1 status analysis as low plasma activity levels. Among individuals that genotype as heterozygotes for position 192, defects in one allele can be detected by comparing Q192R genotyping with the PON1 status analysis [47]. Samples that are discrepant between DNA analyses and PON1 status analyses or which have very low PON1 levels are candidates for defects in one *PON1*₁₉₂ allele. For example, if the genotyping indicates a *PON1*₁₉₂ heterozygote and the functional PON1 status analysis indicates a position *PON1*_{192Q} or *PON1*_{192R} homozygote, this would be presumptive evidence for a defective *PON1*₁₉₂ allele that can be verified by DNA sequencing [47].

3.1 PON1 status as a possible indicator of defects in the modulation of oxidative stress

Several recent studies have suggested linkage between Parkinson disease (PD) and genetic variability in the PON region of chromosome 7 [48-51], while others have not [52-55]. Based on our knowledge of the importance of PON1 status in risk of disease or exposure, we felt that it would be important to carry out a PON1 status analysis on a sizeable cohort of patients with PD. We expected to find lower PON1 levels in PD patients compared with control subjects as we had seen in our earlier study of PON1 status and carotid artery disease (CAAD) [35]. We did not find such differences; however, we did find differences in ratios of rates of substrate hydrolysis in two dimensional plots of activity rates with different substrates. Plots of arylesterase activity vs. paraoxonase activity (POase) showed differences in males with PD compared with control subjects, but not in females with PD (Furlong et al. unpublished data). A likely explanation of these data is that the environment of the HDL particle in which PON1 is localized is affected by defects in the modulation of oxidative stress that in turn are reflected in subtle differences in rates of hydrolysis of different substrates. This explanation is reasonable since many of the mutations that lead to PD are related to defects in mitochondrial function [56-58].

4.1 PONs and quorum sensing

Ozer et al. reported that all three PONs hydrolyzed the quorum sensing factor of *Pseudomonas aeruginosa* N-3-oxododecanoyl homoserine lactone (3OC12-HSL) [59]. Exposure experiments with *PON1*^{-/-} mice were inconclusive in demonstrating a protective effect for PON1 due to the abilities of PON2 and PON3 to inactivate 3OC12-HSL [59]. Experiments with *PON2*^{-/-} mice were more convincing with respect to demonstrating a protective role of PON2 against *P. aeruginosa* infection [60]. The ability of PON1 to protect against the lethality of *P. aeruginosa* infection was demonstrated by expressing human PON1 in *Drosophila* [61]. These flies were also resistant to chlorpyrifos exposure. These observations led the authors to speculate about the roles for PON1 in human-pathogen interactions, including a role for PON1 as a regulator for normal bacterial flora, and a link between infection/inflammation and cardiovascular disease. The potential for therapy was also noted. Thus, in addition to the role of PON1 in protecting against OP exposure and the importance of all three PONs in modulating oxidative stress, all three PONs appear to function in innate immunity via their abilities to inactivate bacterial quorum sensing factors.

5.1 PON1 as a potential therapeutic

Our early studies [22-23] following those of Main [20] indicated that it would be possible to use PON1 purified from human plasma to treat cases of CPF/CPO and DZ/DZO exposure, with the PON1_{R192} alloform being the best choice of alloforms, since it hydrolyzes both CPO and DZO efficiently [24]. However, it will be necessary to engineer recombinant human PON1 for higher catalytic efficiency for use in treating exposure to OPs hydrolyzed with low catalytic efficiency [24,62-63].

To test this hypothesis, we expressed three recombinant human PON1 alloforms in an *E. coli* expression system, rHuPON1_{R192}, rHuPON1_{Q192} and the engineered variant rHuPON1_{K192}. The replacement of Q/R-192 with lysine was suggested from our earlier experiments where we observed high catalytic efficiency of chlorpyrifos oxon with rabbit PON1, which has lysine at position 192 [64] and experiments with recombinant GSTHuPON1 constructs that showed increased OP hydrolase activity for PO, CPO and DZO when lysine was present at position 192 [65]. We were able to express and purify untagged rHuPON1 variants in the *E. coli* expression system using ion exchange and hydrophobic interaction chromatography [62]. The rHuPON1_{K192} variant was about twice as efficient as the rHuPON1_{R192} protein for hydrolyzing CPO, PO and DZO.

The requirements for a protein to be useful as a therapeutic are that it must be non-toxic and non-immunogenic when injected into an animal or human, it should persist in the system for some time, it should protect against or be useful in treating OP exposures and should have a useful shelf-life. Injection intraperitoneally (ip) of the purified rHuPON1_{K192} (1.2 U) into *PON1*^{-/-} mice showed that the rHuPON1_{K192} was non-toxic and persisted in plasma beyond two days following injection. A second experiment where 3.91 U of rHuPON1_{K192} were injected intraperitoneally (ip) into the *PON1*^{-/-} mice followed by a dermal challenge of 1 mg/kg DZO demonstrated that the PON1 still in the plasma 48 h post-injection was able to protect against this exposure, demonstrating that the rHuPON1_{K192} could be used prophylactically to prevent ChE inhibition by an OP exposure. These mice showed no ill effects from the injected PON1, and anti-PON1 antibodies were not detectable by ELISA 4 months postexposure. The mice survived more than 12 months post-exposure. To test the ability of rHuPON1 to be used as a therapeutic post OP exposure, two *PON1*^{-/-} mice were exposed to 2 and 3 LD₅₀ dermal doses of DZO followed 10 min later by ip and intramuscular (im) injections of a total of 3.91 U of rHuPON1_{K192}. The injected rHuPON1_{K192} protected the mice against these lethal exposures of OP. Since these doses

would have killed a *PON1*^{-/-} mouse, the control group, that also received DZO, was only given 1.5 mg/kg. The two mice that received the high-dose exposures and the injected rHuPON1_{K192} post-exposure not only survived, but showed less severe symptoms of intoxication when compared to the control group which received the lower DZO exposure. They did not demonstrate any symptoms related to the injection of the rHuPON1_{K192}. Thus, these experiments demonstrated that 1) it was possible to engineer human PON1 for higher catalytic efficiency on the native rHuPON1 scaffold in an *E. coli* expression system, 2) the injected rHuPON1 persisted for more than 2 days post exposure, 3) the injected rHuPON1 protected against high levels of OP exposure, 4) the injected rHuPON1 was not immunogenic in a mouse that lacked PON1, 5) the lack of glycosylation did not change the half-life of the injected PON1 significantly, nor the activity of the rHuPON1_{K192} and 6) the purified rHuPON1_{K192} was stable for more than two months at 4° C. These experiments also indicate that it should be possible to generate rHuPON1 for use in treating individuals whose vascular disease may result from very low PON1 levels [35,66-67] and individuals who may be susceptible to infection by *Pseudomonas aeruginosa* due to low PON1 levels [61,67]. PON1 may also be useful for treating other diseases that result from low PON1 levels.

6.1 Immunolocalization of the PON family of enzymes

Circulating PON1 is synthesized in the liver [13,68] and the secreted PON1 circulates in plasma tightly bound to HDL [68], using its unprocessed hydrophobic signal sequence as an anchor into the lipoprotein particles [13,69]. Some authors have reported PON1 protein expression in kidney and aorta [70-71]. Immunolocalization of PON1 has been described in nearly all the tissues of mice [72]. Specifically, PON1 was found in nearly all the studied epithelia, such as chondrocytes, enterocytes, eye lens and retinal layers, skin epidermis, stomach, tongue and trachea. Since PON1 metabolizes toxic agents such as oxidized lipids, it would be logical to find the protein where its function is needed. In this vein, PON1 was also found in the muscle fibers of both skeletal and cardiac muscle, areas where free radicals are produced as a consequence of energy metabolism. PON1 also plays a protective role against lipid peroxidation, so it is not surprising to find this protein in adipocytes, and acini from exocrine pancreas, submandibular gland and sebaceous gland. Consistent with this concept, PON1 is also expressed in cells where oxidative stress occurs and lack of detoxication could result in significant disease in liver, kidney proximal tubules, fiber tracts of the encephalon and the spinal cord. Finally, inability to modulate oxidative stress could contribute to infertility, emphasizing the importance of finding PON1 in ovary follicular fluid, seminiferous tubules and spermatozoa.

Similar results were reported in human tissues by the Swedish Human Protein Atlas Database (http://proteinatlas.org/tissue_profile.php?antibody_id=1610). Recently, PON1 has also been localized in macrophages, endothelial cells and smooth muscle cells of human aorta with or without atherosclerosis (Marsillach, unpublished data), and in human lens tissues [73]. These findings open a debate about where PON1 is synthesized and its possible functions.

Sorenson et al. [69] were the first to demonstrate that phospholipids competitively remove PON1 from HDL, suggesting that PON1 may migrate between HDL and cell membranes. Some years later, Deakin et al. [74] described the transfer of PON1 from membrane to HDL, supporting the idea of the transfer process. More recently, Efrat et al. [75] demonstrated that PON1 is able to bind to macrophages and be internalized in them. The authors speculated that the binding could be via the union of HDL to macrophage scavenger receptor B1 (SR-B1) and the anchor of PON1 to cell membrane phospholipids. These observations suggest that although synthesized by the liver and tightly bound to HDL, PON1 may also have an important role outside the HDL complex.

These findings also raise the possibility of local PON1 synthesis in these cells. In the mouse study [71], authors did not find co-localization of PON1 with apo A-I in tissues. This could result from local synthesis of PON1 or a transfer of PON1 from HDL without a co-transfer of apo A-I. However, as yet, no PON1 gene expression has been reported for other tissues. Additional studies should be carried out to address these questions. The finding of PON1 protein in so many tissues [72-73]

[http://proteinatlas.org/tissue_profile.php?antibody_id=1610] [Marsillach et al. unpublished data] not only suggests the transfer of PON1 from the liver to the other tissues, but may also reveal an important role for this enzyme in preventing oxidative stress and inflammation in other tissues, making it an important candidate to be studied in the development of other diseases. PON2 and PON3 are the other members of the paraoxonase family and *PON* gene cluster. The three share common activities of modulating oxidative stress and inactivating quorum sensing factors. Like PON1, PON3 is also found in HDL while PON2 seems to be a ubiquitously expressed intracellular enzyme, not found in circulation. Unlike the gene expression results obtained for PON2 in humans [76], PON2 appears to be poorly expressed in mouse tissues studied by Marsillach *et al.* [72] who reported a very light staining of PON2 in some tissues where PON1 was found, and only strong staining in skeletal and cardiac muscle endomysium, ovary follicular fluid and ducts from submandibular glands. PON3 protein expression paralleled that of PON1. The antibodies used in these experiments were raised against peptides from specific sequence of each mature PON, so they lack of cross-reactivity between the 3 enzymes.

Summary

The many studies carried out on the PON family of enzymes have provided convincing evidence for the protective role of PON1 in modulating exposures to CPO/CPF and DZO/DZ, but not in modulating exposure to PS/PO or nerve agents. To protect against the latter OPs, PON1 will need to be engineered for increased catalytic efficiency. All three PONs appear to be important in modulating oxidative stress and protecting against vascular disease and perhaps other diseases that result from deficiencies in modulating oxidative stress. The recent findings that PONs hydrolyze microbial quorum sensing factors and that PON1 can protect against the lethality of infections from *Pseudomonas aeruginosa* show that they are also important components of the innate immunity system. Space does not permit a discussion of the roles of the PONs in the pharmacokinetics of drug metabolism, however, the papers published to-date suggest that this will be a fertile area for future investigation. Again, it is important to note that investigations of the role of PON1 in risk of disease of exposures should at a minimum determine PON1 status for the study subjects. Knowing only the *PON1*₁₉₂ genotype or SNP haplotype provides wholly inadequate information for an epidemiological study. Convenient high-throughput assays have been developed for determining PON1 status. All of the nearly 200 SNPs in the *PON1* gene could be characterized and one would not be able to accurately predict PON1 activity levels for an individual, the most important factor in resistance to exposure or disease [19].

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Abbreviations

CPF	chlorpyrifos
CPO	chlorpyrifos oxon
DZ	diazoxon
DZO	diazoxon
PO	paraoxon
PD	Parkinson disease
PON1	paraoxonase 1
PON2	paraoxonase 2
PON3	paraoxonase 3
PCR	polymerase chain reaction
tgHuPON1_{R192}	transgenic human PON1 _{R192}
tgHuPON1_{Q192}	transgenic human PON1 _{Q192}
PS	parathion

CVD	cardiovascular disease
CAAD	coronary artery disease
POase	paraoxonase activity
3OC12-HSL	<i>N</i> -(3-oxododecanoyl)-L-homoserine lactone
sq	subcutaneous
ip	intraperitoneally
im	intramuscularly
SNP	single nucleotide polymorphism
OP	organophosphorus compound
EDTA	ethylenediaminetetraacetate
rHuPON1_{K192}, rHuPON1_{R192}, rHuPON1_{K192}	recombinant human PON1 ₁₉₂ variants
HDL	high density lipoprotein
SR-B1	macrophage scavenger receptor B1

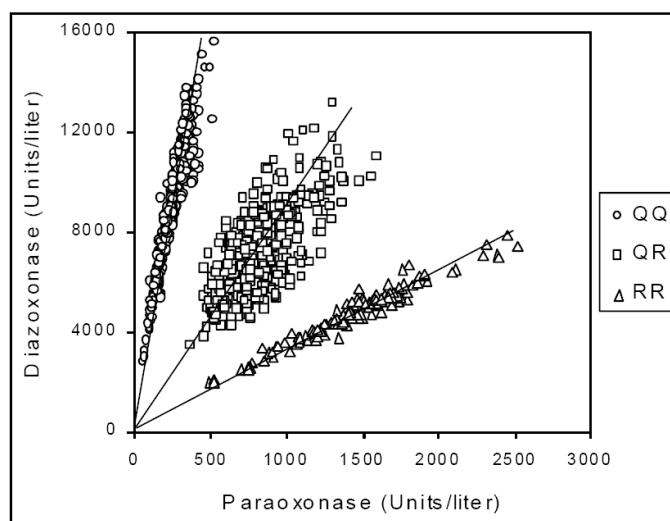


Figure 1. Determination of the functional genomics of plasma PON1 using citrate stored plasma. Plotting the rates of hydrolysis of diazoxon vs. paraoxon for plasma (or serum) samples from a population divides the population into 3 distinct groups, individuals functionally homozygous for PON1_{Q192}, heterozygotes (PON1_{Q/R192}) and individuals homozygous for PON1_{R192}. While PCR analysis of the position 192 polymorphism may reveal that an individual possesses one copy of each allele, this analysis will pick up alleles in heterozygotes that are inactivated by any number of mutations, i.e., the analysis provides the functional status of an individual's PON1 genomics. Reproduced from [77] with permission.

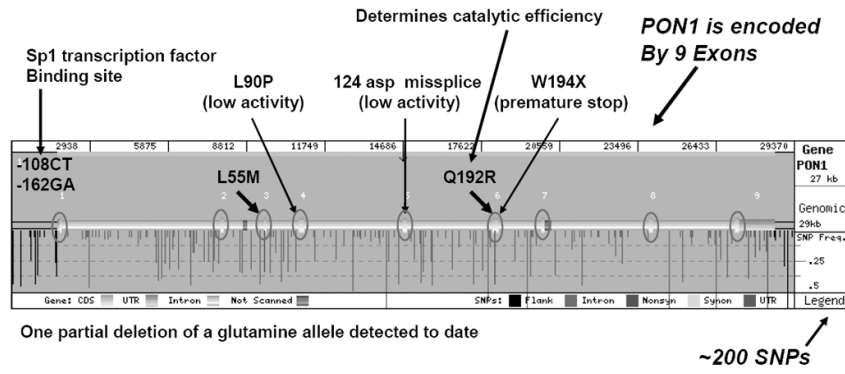


Figure 2. The human *PON1* gene with known polymorphisms and their frequencies. The 5' end of the gene is on the left. (SeattleSNPs, <http://pga.gs.washington.edu/>)