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Title: Paraoxonase-1 Genetic Polymorphisms in Organophosphate Metabolism

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Abstract

Organophosphates (OPs) are a class of chemicals commonly used in agriculture as pesticides, that can often lead to severe toxicity in humans. Paraoxonase-1 (PON1) belongs to a family of A-esterases and hydrolyses several OPs while also serving other biological roles. Two main genetic polymorphisms have been shown to affect enzymatic ability; an A>G transition in the 192nd position (192 Q/R, rs662), and an A>T at codon 55 (55 M/L, rs854560). In this review, we searched PubMed for relevant articles published from its inception till June 2018 and included publications from 1996 to 2018. We aimed to address the distribution of the polymorphisms in various populations, the way they affect enzymatic activity and the possible use of PON1 as a biomarker. The polymorphisms present great heterogeneity between populations, with the data being clearer over 192 Q/R, and this heterogeneity is related to the phylogenetic origins of each population. Concerning enzymatic activity, the different genotypes react better or worse to different OP substrates, with studies presenting a variety of findings. Detecting the "paraoxonase status" of an individual -referring to PON1 function- seems to be important in predicting OP toxicity, as studies have shown that some specific-genotype individuals present symptoms of toxicity in higher rates than others. We are strongly convinced that in order for the scientific community to reach a consensus over which polymorphisms confer susceptibility to toxicity and whether PON1 can eventually be used as a biomarker, more studies need to be carried out, since the data thus far does not seem to reach a universal conclusion.

Abbreviations: OP: organophosphate, OPs: organophosphates, AChE: acetylcholinesterase, BChE: Butyrylcholinesterase, PON1: paraoxonase 1, NTE: neuropathy target esterase.

Keywords: anticholinesterase; organophosphate poisoning; genetic toxicology; paraoxonase 1; polymorphisms.

1. Introduction

Anticholinesterase agents and organophosphates (OPs) in particular, are one of the most commonly used pesticides (Androutsopoulos et al. 2011). They have remained popular because of the positive stance farmers hold after having used them for years, their effectiveness on many pest species, their low cost and general availability, and their excellent environmental profile (Costa 2018). Organophosphorus compounds are not exclusively used in agriculture, though; OPs also have applications as chemical warfare agents (sarin or soman), flame retardants, fuel additives, lubricants and pharmaceuticals among others, such as ecothiophate in glaucoma or trichlorfon in schistosomiasis (Costa et al. 2005; Klaassen et al. 2013). In contrast to their usage as pesticides, warfare agents or pharmaceuticals, OP compounds designed for other uses present little to no anti-AChE activity (Klaassen et al. 2013).

These substances are designed to act on the enzyme acetylcholinesterase (AChE), inhibiting the hydrolysis of acetylcholine and therefore leading to the accumulation of the neurotransmitter in the neuromuscular synapses (Androutsopoulos et al. 2013; Costa 2018; Tsiaoussis et al. 2018). However, OPs are not species-specific, thus they can also present high toxicity to mammals, humans included. They are the main source of human anticholinesterase poisonings, either through accidental exposure, or via suicidal attempts, as they can be highly toxic both via the oral and dermal routes (Costa 2018).

The toxicity can manifest as acute cholinergic syndrome (symptoms include increased sweating and salivation, bronchoconstriction, miosis, muscular twitching, arrhythmias, various CNS effects and even death due to respiratory failure), an intermediate syndrome (marked weakness of respiratory, neck, and proximal limb muscles), appearing several days after the original poisoning, and in some cases, as a delayed polyneuropathy (symptoms include tingling of the hands and feet followed by sensory loss, progressive muscle weakness and flaccidity of the distal skeletal muscles)

that is said to be related to a different esterase present mainly in nerve tissues, called neuropathy target esterase (NTE) (Costa 2018; Lee et al. 2003). There is also some evidence to suggest that their toxicity unfolds over time and involves different metabolic, cellular and molecular mechanisms, affecting various organ systems (Androutsopoulos et al. 2013; Georgiadis et al. 2018a; Georgiadis et al. 2018b), and pesticides are also hypothesized to be implicated in the development of several neurodegenerative diseases, such as ALS (Dardiotis et al. 2018).

Several agents need to be bioactivated as oxygen analogs (oxons) in order to induce toxicity, and this reaction is mediated by CYP/Hepatic Cytochrome P450 variants (Costa 2018; Hernandez et al. 2013). These intermediate oxons are further hydrolysed by paraoxonase-1 (PON1) or conjugated to glutathione, via glutathione-S-transferases (Hernandez et al. 2013).

2. Paraoxonase 1

2.1 The family of paraoxonases

PON1, PON2 and PON3 belong to the family of paraoxonases (Aldridge 1953; Dardiotis et al. 2013). In 1953, Aldridge et al. (1953), classified OP hydrolases as follows: the A-esterases which catalytically hydrolyzed OPs; the B-esterases which were irreversibly inihibited by OPs; and finally, the C-esterases which did not interact with OPs (Aldridge 1953). PON1 was proven to be an A-esterase and received its name based on its ability to hydrolyse paraoxon, the first studied substrate (Androutsopoulos et al. 2011; Costa et al. 2013; Costa et al. 1999). The products of the linked genes of PON1 were named, after their discovery, PON2 and PON3 (Primo-Parmo et al. 1996).

These enzymes possess several common properties. PON1, PON2 and PON3 have anti-oxidative properties (Ng et al. 2001), inhibit the proliferation of *Pseudomonas* via the hydrolysis of its quorum sensing factor N-(3-oxododecanoyl)-L-homoserine lactone (3OC12-HSL) (Ozer et al. 2005; Schweikert et al. 2012) and have lactonase activity, on different substrates (Draganov et al. 2005). However, although some of their action is overlapping, the differences between the three are

considerable. For example, unlike PON1 and PON3, PON2 exerts its activity at a cellular level (Ng et al. 2001) and is not present in the plasma (Marsillach et al. 2008), as it is synthesized and detected in several tissues, such as the kidneys and the brain (Moya and Manez 2018); there, it associates with the endoplasmic reticulum membrane and core, where it reduces oxidative stress as a caspase (Moya and Manez 2018) and presents anti-apoptotic properties (Altenhofer et al. 2010). The difference that particularly interests us in terms of toxicology, is the fact that neither PON2 nor PON3 metabolize OPs (Dardiotis et al. 2018; Draganov and La Du 2004; Draganov et al. 2005), therefore we focused our attention on PON1. The discussion over renaming those two enzymes- since they have no actual paraoxonase ability- is not new, but it has been tabled until their natural substrates are found (La Du et al. 1999).

A list of PON1, PON2 and PON3 biological roles can be found in Table 1.

2.2 Biosynthesis and biological role of PON1

PON1 is a glycoprotein consisting of 354 amino acids and having an approximate molecular mass of 43KDa (Mackness and Mackness 2015). PON1 is, as mentioned before, an A-esterase concerning OP metabolism, and is produced in the liver (Costa et al. 2013; Dardiotis et al. 2018; Mackness and Mackness 2015; Ng et al. 2001; Sunay et al. 2015; Tsatsakis et al. 2009; Zafiropoulos et al. 2010). When secreted into the plasma, it binds with high-density lipoproteins and is dependent on serum calcium, like PON3 (Costa et al. 2013; Lu et al. 2006; Tsatsakis et al. 2009). It is thought to serve plenty of functional roles, such as protecting against lipid peroxidation and postprandial oxidative stress, detoxifying homocysteine-thiolactone, inhibiting cholesterol biosynthesis in macrophages while increasing its efflux in the same cells, modulating lipid metabolism in adipose tissue and even preventing infection by gram negative bacteria such as *Pseudomonas* (Costa et al. 1999; Ferretti and Bacchetti 2012; Mackness and Mackness 2015; Ozer et al. 2005; Tsatsakis et al. 2009).

Despite its plethora of attributed functions, the enzyme has received its name from its ability to hydrolyse paraoxon, the first studied substrate, as also mentioned previously (Androutsopoulos et al. 2011; Costa et al. 2013; Costa et al. 1999). However, PON1 hydrolyses the active metabolites of several other pesticides as well, such as diazoxon and chlorpyrifos, and nerve agents, such as sarin (Costa et al. 2013; Dardiotis et al. 2018; Furlong et al. 2016; Klaassen et al. 2013; Lee et al. 2003), and is even more potent in deactivating them than it is towards paraoxon (Chambers 2008). Remarkably, chlorpyrifos-oxon is more potent than paraoxon as a cholinesterase inhibitor, but is considered less toxic because of the greater efficacy PON1 presents in hydrolysing it (Pond et al. 1998).

PON1 is currently classified as “aryldialkylphosphatase”, but the term paraoxonase has prevailed (Mackness and Mackness 2015). As Moya et al. (2018) noted, PON1 qualifies best as a lipolactonase, which serves as arylesterase, phosphotriesterase and lactonase(Moya and Manez 2018). It detoxifies oxons derived from pesticide phase-I metabolism in the liver; more precisely it hydrolyses paraoxon, chlorpyrifos-oxon and diazoxon into diethylphosphate and p-Nitrophenol, 3,5,6-trichloro-2-pyridinol and 2-isopropyl-4-methyl-6-hydroxypyrimidine respectively (Androutsopoulos et al. 2011). However, its crystal form has not been determined yet and several questions concerning its activity and mechanism remain unanswered, as scientific groups try to create a highly-potent PON1 analog as a possible OP-antidote (Tripathy et al. 2017).

2.3 PON1 Genetics

The PON1 gene is located on the long arm of chromosome 7 (q.21.22), close to AChE, and all paraoxonase genes share a similarity of greater than 60% (Androutsopoulos et al. 2011; Costa et al. 2013; Mackness and Mackness 2015; Tsatsakis et al. 2009). PON1 is critical to the metabolism of cholinesterase inhibitors and approximately 400 SNPs have been found in its gene (Sunay et al. 2015). However, most of the literature has so far focused on two non-synonymous exonic polymorphisms; an A>G transition in the 192nd position (192 Q/R, rs662), and an A>T at codon 55

(55 M/L, rs854560) (Costa et al. 2013; Mackness and Mackness 2015). The 192 Q/R polymorphism affects the catalytic efficiency (Dardiotis et al. 2018; Klaassen et al. 2013; Tsatsakis et al. 2011; Zafiropoulos et al. 2010); more specifically, the isoform with arginine (R allele) at position 192 is generally thought to better metabolize paraoxon, while the glutamine isoform (Q allele) sarin, soman and diaxozon (Costa et al. 1999; Dardiotis et al. 2018; Ginsberg et al. 2009; Macharia et al. 2014; Mohamed Ali and Chia 2008). In general, the RR, RQ, and QQ genotypes are associated with high, intermediate, and low serum paraoxonase activities, respectively (Lee et al. 2003). The 55 M/L polymorphism is considered to influence PON1 plasma levels (Dardiotis et al. 2018; Ginsberg et al. 2009; Tsatsakis et al. 2011; Zafiropoulos et al. 2010), alongside the C-108T polymorphism (rs705379) in a non-coding region (Costa et al. 2013; Dardiotis et al. 2018; Klaassen et al. 2013); the C allele seems to provide levels of PON1 twice as high as the T one (Costa et al. 2013). Alongside the discussion of the role PON1 has in OP metabolism, plenty of interest has also been shown in the way PON1 polymorphisms are implicated in other diseases as well, such as hypertension and diabetes (Tsatsakis et al. 2009), and even neurodegenerative diseases (Androutsopoulos et al. 2011; Dardiotis et al. 2018).

The distribution of the polymorphisms presents great heterogeneity between populations and between individuals, up to 10- or 40-fold (Lee et al. 2003; Sunay et al. 2015; Tsatsakis et al. 2009; You et al. 2013), and several publications have described the association between enzyme efficiency concerning OPs and the polymorphisms. The aim of the current review is to categorize and concisely present data concerning the frequency of the polymorphisms and the way they affect enzymatic activity, and finally discuss the possible use of PON1 as a biomarker of susceptibility to toxicity, based on a plethora of studies assessing various aspects of PON1 and OP toxicity.

3. Polymorphism Frequencies

There is great variability in polymorphism frequencies between individuals and populations (Lee et al. 2003; Macharia et al. 2014; Scacchi et al. 2003; Sunay et al. 2015; Tsatsakis et al. 2009;

You et al. 2013; Zafiropoulos et al. 2010). Ginsberg et al. (2009), summing up the data from various ethnical studies, reported that the 192R variant occurred more commonly, with a frequency of 25-64% in the populations analysed, while the 55M allele was found to be rarer, occurring in 5-40% of the individuals (Ginsberg et al. 2009). It is thus evident from the range of the percentages, that great interethnic and inter-individual differences exist. The data grouped below are presented consicely in Table 2.

In studies performed on African populations, such as Egyptians and citizens of mixed African decent, the 192R-containing-genotypes seem to prevail (Ellison et al. 2012; Lee et al. 2003; Macharia et al. 2014; Scacchi et al. 2003). The 192R was found in higher frequencies in African Americans as well (Luu et al. 2011; Mohamed Ali and Chia 2008). Concerning the 55 M/L polymorphism, the results seem to be slightly conflicting; in a review by Mohamed Ali et al. (2008), the L polymorphism appeared in higher frequencies (Mohamed Ali and Chia 2008), but newer studies report a higher prevalence of M (Macharia et al. 2014).

Further supporting the claims of heterogeneity between population groups, studies from Asian countries have shown the different polymorphisms that dominate in each region. Japanese and Chinese populations present higher frequencies of 192R and 55L (Mohamed Ali and Chia 2008; Sanghera et al. 1997; Sato et al. 2016; Zhang et al. 2014), whereas studies from India and Thailand report higher frequencies of 192Q and 55M (Pati and Pati 1998; Phuntuwate et al. 2005; Sanghera et al. 1997).

European studies and reports on Caucasian populations seem to reach the same conclusion; the 192Q genotype dominates (Antikainen et al. 1996; Costa et al. 2015; Gardemann et al. 2000; Helbecque et al. 1999; Janicsek et al. 2015; Mackness et al. 1997; Scacchi et al. 2003; Tsatsakis et al. 2011; Tsatsakis et al. 2009; Zafiropoulos et al. 2010). Concerning the 55 M/L polymorphism, a few studies seem to hint towards 55L being the most common (Gardemann et al. 2000; Janicsek et al. 2015; Tsatsakis et al. 2011; Tsatsakis et al. 2009; Zafiropoulos et al. 2010).

In Hispanic populations, the 192Q polymorphism seems to be encountered more frequently (Bernal-Hernandez et al. 2014; Rainwater et al. 2009; Zuniga-Venegas et al. 2015), with small deviations in studies towards R (Martinez-Salazar et al. 2011; Scacchi et al. 2003), while a clear affinity towards 55L has also been shown (Bernal-Hernandez et al. 2014; Martinez-Salazar et al. 2011; Rainwater et al. 2009).

This phylogenetic heterogeneity was also shown in studies comparing different populations living in the same region. Sanghera et al. (1997) compared Chinese and Indian subjects living in Singapore and found considerable differences in their polymorphism frequencies(Sanghera et al. 1997). Accordingly, Janicsek et al. (2015), compared Hungarians with Roma living in Hungary (Roma form a large population of 4-10 million scattered around Europe, in which a plethora of single gene diseases and susceptibility genes have been found), and also came across considerable differences, such as a significant decrease in the R allele and the QQ+MM genotype combination to be 2.5 times higher in Roma (Janicsek et al. 2015). Similarly, Mitra et al. (2015) studied four Indian populations and reported that the high genetic differentiation at the 192Q/R locus, expressed by the difference in the polymorphism frequencies of the groups under study, is indicative of the role of the populations' history, such as migration and phylogenetic origins, and other evolutionary forces (Mitra et al. 2015).

4. Enzymatic Activity

4.1 Effect of 192 Q/R

PON1 activity seems to be strongly influenced by the 192 Q/R polymorphisms, mainly in terms of substrate specificity and affinity towards them. The studies focusing on this matter can be found summarized in Table 3.

Lee et al. (2003) considered the RR genotype carriers as "fast" metabolizers, the QR as "intermediate" and the QQ as slow. In their study, the prevalence of chronic toxicity increased, from

58.8% in pesticide applicators with “fast” metabolism to 75% among applicators with “slow” metabolism (Lee et al. 2003). Sirivarasai et al. (2007) presented findings that agree with Lee et al.(2003), reporting that serum paraoxonase activity differed significantly between genotypes, with that of RR carriers being higher than QR, which was in turn higher than QQ; a finding that was not replicated when they assessed the arylesterase and diazonase activities (Sirivarasai et al. 2007). Other studies also concur with these findings, naming R carriers as those with the highest serum paraoxonase activity (Mackness et al. 2003; Phuntuwate et al. 2005; Sato et al. 2016; Singh et al. 2011; Sunay et al. 2015). Additionally, Costa et al. (2015) found lower advanced glycation end products and advanced oxidation protein products in farmers exposed to pesticides including OPs, that carried the RR genotype (Costa et al. 2015). Therefore, the 192Q polymorphism could increase the risk of OP toxicity (You et al. 2013).

However, as also mentioned in the introduction and above, the various genotypes react better or worse to different substrates. The 192R polymorphism seems to hydrolyse diazoxon at lower rates than-or at least not better than- Q (Mackness et al. 2003; Phuntuwate et al. 2005; Sato et al. 2016). Mackness et al. (2003) reported that farmers with the R polymorphism, exposed to diazoxon, presented chronic ill health at higher rates than their Q counterparts, a finding that was also noted in controls in the same study (Mackness et al. 2003).

Rainwater et al. (2009) studied the effect of the polymorphisms on PON1’s various roles and found that QQ individuals presented lower activity than RR individuals in both, paraoxon hydrolysis, and as an arylesterase, but higher levels than RR individuals when PON1 was assessed as a lactonase (Rainwater et al. 2009). Macharia et al. (2014) also found lower paraoxonase activity in 192Q carriers, but lower oxidative stress markers in the QQ individuals when evaluating the arylesterase role of PON1. In the same study, the Q allele also seemed to elevate the concentration of PON1 (Macharia et al. 2014). More recently, Sato et al. (2016) found PON1 activity towards chlorpyrifos-methyl and PON1 activity as an arylesteraseto be lower in RR individuals (53.8% and 18.2%

respectively, when compared to QQ) (Sato et al. 2016), but Zúñiga-Venegas et al. (2015) came to the conclusion that the Q allele, in their study subjects, was not as efficient as the R allele in metabolizing chlorpyrifos (Zuniga-Venegas et al. 2015), a fact also supported by in-vivo studies in mice (Li et al. 2000); therefore the available data seem to be quite inconclusive so far.

From a wider perspective, Monroy-Noyola et al. (2015) raised an interesting point; the alloforms of the enzyme, depending on the 192 Q/R polymorphisms, all seemed to present a stereoselective hydrolyzing action on the S-enantiomer of an OP compound, while presenting low hydrolysis rates of the R-enantiomer, which is thought to cause OPIDP (Monroy-Noyola et al. 2015). Therefore, several other aspects, apart from the genetic polymorphisms, need to be considered when assessing susceptibility and toxicity of OPs.

Finally, Hunter Coombes et al. (2014) raised a second interesting point. They reported that although the "fast" metabolism allele carriers (R) did present higher activity towards chlorpyrifos-oxon (CPO), the same difference between genotypes could not be detected when CPO was tested in lower, more environmentally relevant concentrations. This could be probably attributed to the fact that the low concentrations could not saturate the enzyme and therefore PON1 could not display its maximal catalytic capacity (Coombes et al. 2014). Consequently, the question of whether PON1 genotypes influence the metabolism of OPs in the extent that the median citizen is exposed still remains unanswered and more studies should be performed towards this direction.

4.2 Effect of 55 M/L

Concerning the 55 M/L polymorphism, several studies agree that it considerably affects the enzymatic activity. Mackness et al. (1997) had initially reported that regardless of the 192Q/R polymorphism, MM homozygotes had lower activity towards paraoxon compared to the LM and LL genotypes (Mackness et al. 1997), a finding that was further confirmed by later studies (Ferretti and Bacchetti 2012; Phuntuwate et al. 2005; Rainwater et al. 2009; Singh et al. 2011). Concerning the different substrates, Sato et al. (2016) report lower activity in M carriers towards chlorpyrifos (Sato

et al. 2016), while Rainwater et al. (2009), in the study described above, found higher activities for LL individuals in all three paraoxonase "roles" (as a paraoxonase, arylesterase and lactonase) (Rainwater et al. 2009). Phuntuwate et al. (2005) and Bernal-Hernandez et al. (2014) also reported that LL individuals presented a higher activity towards phenylacetate when compared to LM/MM individuals (Bernal-Hernandez et al. 2014; Phuntuwate et al. 2005). In an attempt to dilute any confusion on the matter, You et al. (2013) performed a meta-analysis of case-control studies with patients with OP toxicity and concluded that the 55L seems to transfer susceptibility to toxicity, but mainly in Caucasian populations (You et al. 2013). It should be noted, however, that despite the numerous studies showing that 55 M/L does influence PON1 activity, some researchers have presented different findings, such as Macharia et al. (2014), who came to the conclusion that only 192Q/R influences the activity and the serum concentration (Macharia et al. 2014). Therefore some scepticism concerning the matter is advised.

5. PON1 as aPredictor for Toxicity and a Biomarker of Susceptibility

5.1 Prevalence of Polymorphisms in Toxicity Cases

Several studies have so far attempted to elucidate whether the different polymorphisms transfer susceptibility to OP toxicity, mainly by comparing polymorphism prevalences in cases and controls, or between exposed individuals that either presented symptoms of toxicity or not; a concise presentation can be found in Table 4.

Mackness et al. (2003) found that farmers reporting chronic ill health due to OP exposure had a higher proportion of 192R and 55L alleles, associated with lower enzymatic activity towards diazoxon, in cases and controls (Mackness et al. 2003). Zayed et al. (2015) also found a higher prevalence of the RR genotype and the R allele in symptomatic patients of acute OP toxicity when compared to controls and asymptomatic patients of acute OP toxicity (Zayed et al. 2015), a finding further supported by other studies on workers exposed to pesticides (Tawfik Khattab et al. 2016; Zhang et al. 2014). Furthermore, Hernandez et al. (2013), studied the effect of PON1 polymorphisms

on the liver damage caused by OPs and reported that the RR genotype was significantly associated with a decrease in BChE levels (a bioscavenger for OPs) and elevated levels of ALT, AST, and lower levels of ALP (Hernandez et al. 2013). Lee et al. (2003) however, showed an increasing rate of chronic toxicity symptoms from pesticide applicators with the RR genotype to pesticide applicators with the QR/QQ genotypes (Lee et al. 2003).

Accordingly, the 55L allele seemed to be more frequent in cases-over-controls studies on toxicity (Mackness et al. 2003; Zhang et al. 2014), and was shown to confer susceptibility in the meta-analysis by You et al. (2013), especially in Caucasians (You et al. 2013).

5.2 Polymorphisms and OP Cellular Damage

It has also been demonstrated both in vivo, as well as in vitro models that OPs can lead to the malignant transformation of cells (Vakonaki et al. 2013), while studies have shown that exposure to OP leads to DNA damage and oxidative stress (Atherton et al. 2009; Kisby et al. 2009). Following this line of thought, when studying DNA damage between OP workers and controls, Singh et al. (2011) showed that the individuals with low paraoxonase activity, having the QQ and MM genotypes, presented higher DNA damage, indicating that those individuals are more susceptible to genotoxicity (Singh et al. 2011). Similarly, Costa et al. (2015) found a significant decrease in oxidative stress products in exposed farmers exhibiting the 192RR genotype, claiming that the genotype could be a genetic predictor of toxic OP effects, and that measuring AOPP could even be a biomarker for oxidative damage in exposed individuals (Costa et al. 2015).

An interesting study performed by Engel et al. (2011) addressed the effect of prenatal OP exposure on the neurodevelopment of children. The level of a prenatal OP metabolite level was associated with a decline in mental development among blacks and Hispanics and this association was found greater in children whose mothers carried the 192 QR/RR genotype. Later, the aforementioned level was associated with a decrement in perceptual reasoning in the QQ genotype mothers' children. In general, prenatal exposure to OPs negatively affects cognitive development,

and the researchers believe that PON1 may consist an important susceptibility factor (Engel et al. 2011). As far as pregnant women are concerned, Huen et al. (2012) showed that maternal PON1 activity was inversely associated with the odds of detecting pesticides in cord blood; higher maternal enzymatic activities seemed to protect the fetus from diazinon and chlorpyrifos (Huen et al. 2012).

5.3 Assessing the PON1 Status

Given the importance of PON1 in OP toxicity, the question of whether individuals should be "screened" for their PON1 profiles in order to predict toxicity, and whether PON1 can be used as a biomarker, is raised. In humans, the term biomarker refers to a biological, biochemical and molecular marker than can be measured by chemical, biochemical or molecular techniques, while having been acquired from ethically obtainable tissues such as blood, urine or buccal cells (Costa et al. 2005). For OPs, most of the exposure measurements include OP metabolites in urine, BChE activity in plasma, AChE activity in red blood cells and NTE in lymphocytes (Costa et al. 2005; Hofmann et al. 2010). PON1 could possibly serve as a biomarker of susceptibility to OPs' toxicity.

Commonly, studies evaluate PON1 for its polymorphisms via polymerase chain reaction (PCR). However, it seems that a functional genomic analysis is more accurate, as it includes all of the polymorphisms that may affect its activity (Costa et al. 2005; Costa et al. 2013). This is commonly performed via a spectrophotometric enzyme assay involving two of its substrates, paraoxon and diazoxon (Ceron et al. 2014; Costa et al. 2005; Costa et al. 2013; Hofmann et al. 2010). This is called the determination of one's PON1 "status" (Costa et al. 2005) and studies have shown an excellent correlation between the predicted 192 Q/R genotype via the assay and the genotype found via PCR (Hofmann et al. 2010). The "status" appears to be very important, as the degree to which PON1 degrades toxic OPs and other substances, and its concentration both correlate to the protection it offers against adverse toxic effects (Costa et al. 2005; Ellison et al. 2012); as a result, PON1 phenotype could potentially be a biomarker of susceptibility (Bernal-Hernandez et al. 2014). PON1 can be directly quantified with immunological methods (Ceron et al. 2014) and the level of its

activity can be indirectly evaluated by measuring arylesterase activity using phenylacetate as a substrate; it is considered to be an adequate substitute for PON1 concentration, as it is not affected by the 192 Q/R polymorphism (Bernal-Hernandez et al. 2014; Hofmann et al. 2010).

So far, studies seem to indicate that the PON1 status influences the susceptibility for OP toxicity, although there are often conflicting results as to which allele transfers greater susceptibility. If PON1 genotype/status is to be used to predict toxicity or as a biomarker of susceptibility, more research towards this direction is necessary. The currently available studies are not consistent with each other; different pesticides used, different evaluation methods, different criteria and so forth. Therefore, we are of the opinion that although considerable progress has been made, the polymorphisms need to be systematically evaluated in large groups of subjects, against particular OP substances, taking into consideration various covariates, following the same protocols and criteria, so as to reach a consensus, given the high genotype heterogeneity between populations and the variation in activity towards different substrates.

In conclusion, though additional studies are necessary to validate the utility of PON1 as a biomarker of susceptibility, progress has been noted on its possible therapeutic use. Nachon et al. (2013) report that human PON1 is under development as a bioscavenger, with researchers seeking to improve its catalytic efficiency toward some of the most toxic isomers of nerve agents (Nachon et al. 2013). Furthermore, several PON1 variants are being tested in mice against OP pesticides, and some of these substances, particularly the VII-D11, as Mata et al. concluded (2014), seems fairly promising (Mata et al. 2014).

5.4 PON1 and Other Biomarkers

The available data suggest PON1 might be a suitable candidate for predicting toxicity and whether an individual will develop a serious reaction when exposed to OPs. However, it does not seem to be of much help in terms of locating exposed individuals or the level of their exposure.

Other substances in the scientific literature fill this gap and may accompany PON1 in completing a full set of biomarkers for OPs in the future.

Two human cholinesterases are widely recognized; AChE and BChE, or pseudocholinesterase (Assis et al. 2018). Both of them are considered satisfactory markers of OP exposure(Costa et al. 2005) and AChE, the main OP target, is considered to be a better marker of toxicity (Hofmann et al. 2010). It is mostly located in tissues of the nervous system but it also appears in erythrocyte membranes, and erythrocyte AChE is currently examined to be used in the biological monitoring of OP exposure (Assis et al. 2018; Costa et al. 2005). According to Assis et al. (2018), hemoglobulin-free erythrocyte AChE has several advantages over methods that have been endorsed by WHO since many years ago, as it presents significant correlation to the AChE of the nervous system and may even be better at some aspects than BChE (Assis et al. 2018).

BChE is a stoichiometric scavenger of OPs and protects from toxicity by binding to OPs and not allowing them to inhibit AChE; although its main physiological function has not been elucidated yet, it is even being considered as a pharmaceutical agent against OP poisonings (Klaassen et al. 2013; Nachon et al. 2013). BChE is thought to be more sensitive than AChE in detecting exposure, as it is more effectively inhibited by most OPs, such as chlorpyrifos and diazinon, and is found significantly lower in groups after both, low and high exposure periods, when compared to controls (Hofmann et al. 2010; Lockridge et al. 2016; Sirivarasai et al. 2007).

Several studies have also attempted to associate BChE with PON1. Although Bernal-Hernandez et al. (2014) did not find any significant association between PON1 genotypes and BChE, Nam et al. (2016) did; BChE inhibition was found higher in RR genotypes than in QQ (Bernal-Hernandez et al. 2014; Nam et al. 2016). Similarly, Hernandez et al. (2013) reported significantly higher levels of ALT and AST, and lower levels of ALP for each 100 U/l decrease in BChE activity for RR genotype carriers (Hernandez 2013). However, Hofmann et al. (2009) reported the opposite finding; QQ genotype carriers showed higher BChE inhibition and the researchers came to the

conclusion that low PON1 plasma activity (the 192Q polymorphism) was tied to BChE inhibition (Hofmann et al. 2009). More studies are needed to shed light into this matter more properly, though.

Finally, serum albumin has been shown to have OP-hydrolysing abilities that, unlike PON1, are independent of serum calcium (Vilanova and Sogorb 1999). Albumin seems to cut ester bonds in OP compounds and scientific interest concerning which amino-acids offer this esterase ability has peaked lately; Tyr150, Tyr411 and Ser193 are thought to be implicated (Belinskaia et al. 2014). New approaches involve monoclonal antibodies against nerve agent–phosphonylated peptides of human serum albumin and this compound may make albumin another excellent biomarker of exposure (Chen et al. 2013).

6. Conclusions

PON1 plays a crucial role in the metabolism of several OP agents and in several other pathways as well. The two polymorphisms most widely studied, 192 Q/R and 55 M/L, both seem to considerably influence its activity and its serum concentrations, but a universal consensus as to the extent of this influence and its particulars has yet to be reached.

The distribution of the 192 Q/R and 55 M/L heavily varies around the globe, with some populations presenting very low prevalence of an allele, and others very high. Similarly, there is great inter-individual variance concerning enzymatic activity and activity towards different substrates. Studies seem to also contradict each other when it comes to pointing a particular allele responsible for susceptibility against OPs, and although the measurement of PON1 and the assessment of the genotype can be done very efficiently, much is still needed to be done before PON1 can be used as a biomarker of susceptibility.

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Figures and table

Table 1. Biological roles of PON1, PON2 and PON3.

Biological role	**PON1**	**PON2**	**PON3**	**Reference**
Organophosphate metabolism	✔	-	-	*Hernandez et al., 2013, Furlong et al., 2016*
Protection against *Pseudomonas*	✔	✔	✔	*Ozer et al., 2005*
Lipid metabolism	✔	✔	✔	*Ng et al., 2001; Dardiotis et al., 2018*
Protection against oxidation	✔	✔	✔	*Ng et al., 2001*
Protection against apoptosis	-	✔	-	*Altenhöfer et al., 2010*
Protection against inflammation	✔	✔	✔	*Furlong et al., 2016*

Table 2. Genotype prevalence of 192 Q/R and 55 M/L in different continents.

Continent	**Country/Decent**	**Author and Year**	**192 Q/R(rs662) prevalence**	**55 M/L(rs854560) prevalence**
Africa	South Africa	*Lee et al., 2013*	R genotypes	-
	Egypt	*Zayed et al., 2015*	R genotypes	-
	Egypt	*Khattaba et al., 2016*	R genotypes	-
	Egypt	*Ellison et al., 2012*	QR genotype	-
	African American	*Luu et al., 2011*	R genotypes	-
	Mixed African	*Macharia et al., 2014*	R genotypes	M genotypes
	Benin	*Scacchi et al., 2003*	R genotypes	-
Asia	China	*Zhang et al., 2014*	R genotypes	L genotypes
	Japan	*Mohamed Ali and Chia, 2008*	R genotypes	L genotypes
	China	*Mohamed Ali and Chia, 2008*	R genotypes	L genotypes
	Japan	*Sato et al., 2016*	R genotypes	L genotypes
	Indian, Singapore	*Sanghera et al., 1997*	Q genotypes	L genotypes
	Chinese, Singapore	*Sanghera et al., 1997*	R genotypes	L genotypes
	Thailand	*Phuntuwate et al., 2005*	Q genotypes	L genotypes
	India	*Pati and Pati, 1998*	Q genotypes	L genotypes
Europe	Britain	*Mackness et al., 1997*	Q genotypes	L genotypes
	Finland	*Antikainen et al., 1996*	Q genotypes	-
	Greece	*Tsatsakis et al., 2009*	Q genotypes	L genotypes
	Greece	*Zafiropoulos et al., 2010*	Q genotypes	L genotypes
	Greece	*Tsatsakis et al., 2011*	Q genotypes	L genotypes
	Hungary	*Janicsek et al., 2015*	Q genotypes	L genotypes
	Germany	*Gardemann et al., 2000*	Q genotypes	L genotypes

	Italy	*Costa et al., 2015*	Q genotypes	-
	Iitaly	*Scacchi et al., 2003*	Q genotypes	-
America	Chile	*Zúñiga-Venegas et al., 2015*	Q genotypes	-
	Ecuador	*Scacchi et al., 2003*	R genotypes	-
	Mexican	*Rainwater et al., 2009*	Q genotypes	L genotypes
	Mexico indigenous	*Hernandez et al., 2014*	Q genotypes	L genotypes
	Mexico	*Martinez-Salazar et al., 2011*	R genotypes	L genotypes

Table 3. Genotypes and Alleles with the Highest Enzymatic Activity in PON1's Various Roles.

Enzyme Role	**Author/Year**	**Highest Activity Genotype/Allele**
Paraoxonase	*Mackness et al., 1997*	-/55L
	Lee et al., 2003	192RR/-
	Mackness et al., 2003	192R/55L
	Sirivarasai et al., 2007	192RR/-
	Phuntuwate et al., 2005	192RR/55LL
	Rainwater et al., 2009	192RR/55LL
	Singh et al., 2011	192RR/55LL
	Ferretti and Bacchetti, 2012	55L/-
	Macharia et al., 2014	192R/-
	Sunay et al., 2015	192R/-
	Costa et al., 2015	192RR/-
	Sato et al., 2016	192RR/-
Arylesterase	*Phuntuwate et al., 2005*	-/55LL
	Sirivarasai et al., 2007	No difference found
	Rainwater et al., 2009	192RR/55LL
	Bernal-Hernandez et al., 2014	-/55LL
	Macharia et al., 2014	192QQ/-
	Sato et al., 2016	192QQ/-
Diazonase	*Sirivarasai et al., 2007*	No difference found
	Phuntuwate et al., 2005	192QQ/-
	Mackness et al., 2003	192Q/55M
	Hunter Coombes et al., 2014	192R/-
Chlorpyrifos oxonase	*Zúñiga-Venegas et al., 2015*	192R/-
	Sato et al., 2016	192QQ/55LL
Lactonase	*Rainwater et al., 2009*	192QQ/55LL
	Ferretti and Bacchetti, 2012	-/55L

Table 4. Studies on subjects exposed to organophosphates.

Author/Year	**Study Subjects**	**Prevalent Genotype/Alleles**

Mackness et al., 2003	Farmers with chronic ill health due to OP exposure	192R, 55L
Lee et al., 2003	Pesticide applicators with chronic toxicity symptoms	192QR/QQ
Hernandez et al., 2013	Farm workers tested for BChE inhibition and elevation of liver enzymes	192RR
Zhang et al., 2014	Workers exposed to OPs and controls	192R, 55L
Zayed et al., 2015	Symptomatic patients of acute OP toxicity	192R/192RR
Tawfik Khattab et al., 2016	Farm workers chronically exposed to OPs	192R/192RR