Review Article

Indian Journal of Reseach in Anthropology
Volume 1 Number 1, July - December 2015

Ecogenetic Revelations on Paraoxonase 1 Enzyme in Population Genetic Studies

Gautam K. Kshatriya

Author Affiliation: Professor, Department of Anthropology, University of Delhi, Delhi-110007.

Reprint Request: Gautam K. Kshatriya, Professor, Department of Anthropology, University of Delhi, Delhi-110007

E-mail: g26_51@yahoo.co.in

Abstract

Ecogenetics aims at studying the genetics of underlying differential reactions to chemicals in the environment. Genetic modifications due to exposure to foreign substances like toxins due to occupational exposure, dietary constituents, drugs etc. majorly constitute areas studied in the scope of ecogenetics. One of the enzymes that have been at the center-stage of ecogenetic studies is the Paraoxonase 1 (PON1) enzyme. It is one of the oldest enzymes present since the life originated. The presence of an active PON1 enzyme in lower organisms proves that the enzyme's presence predates human origins. It also had a different native activity and not paraoxonase or arylesterase activities that presumably occurred only later in evolution. The enzyme is also characterized by promiscuity in its specificity towards substrates. These features account for the existence of enzyme isoforms of PON1 and their relevance in xenobiotic metabolism has made PON1 an interesting area of research. Previous studies have assessed PON1 variation in different human populations by evaluating them for activity, genetic variation or PON1 status (constituted by gene variations and activity). The variations have been studied in lieu of their toxicological and pharmacological properties and effects on human health. Sensitivity to chronic exposure of chemicals with the organophosphate moiety like pesticides (chlorpyrifos, malathion, parathion) and nerve gases (soman, sarin) is determined by the PON1 catalytic efficiency and abundance. The ethnic variations in PON1 status and gene frequencies are, hence, keys to understand the differential sensitivity among different groups of populations to chronic exposure of OP compounds. We examined selected populations of India for genetic variations of PON1. Findings show an interesting conglomeration of populations having different PON1 gene frequencies that will be important in assessing OP exposure risk among them. Also, the underlying cause of the systemic variation has not been determined. So, a review of previous research was done to find patterns of genetic variation at key loci of PON1 gene to comprehend the extent of differentiation at these loci. The paper aims at outlining the various aspects of PON1 research with special emphasis on the outcomes of toxicogenetic and pharmacogenetic approaches and the leads they provide for conducting population genetic studies. Deciphering the cause of PON1 variation will improve environment risk assessment exercises that help frame public health initiatives.

Keywords: Genetic; Paraoxonase 1; Conglomeration; Enzyme; Metabolism.

Introduction

One of the ways biochemical diversity manifests itself is as varied responses of individuals to environmental agents like pesticides, heavy metals and UV radiation. For instance, drug in one individual differs from the other who has been given the same dose. Three historically significant drugs, consumption of which led to first reports of difference in response of individuals were primaquine, succinylcholine and isoniazid (Meyers, 2004).

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Differences in response towards a group of substances were first reported for drugs but subsequently for a wide range of physical and chemical agents. Human related factors that influence substance-specific differential response are age, sex, physiological and biochemical conditions of the individual and genetic factors. Ecogenetic studies carry out targeted examination of human detoxification systems that with the objective of finding differences in them contributing to variable response. Detoxification systems comprise an enzyme or combination of enzymes functioning in an organized manner to orchestrate the systematic deactivation of a substance foreign and at times toxic to the organism. These substances are called xenobiotics and also represent those substances that are endogenous to but do not exist in an organism under normal conditions. Detoxification systems located in liver were the first to be studied but with discovery of methods with enhanced sensitivity to detect enzyme expression in other tissues, their presence was detected in organs like kidneys, serum, intestine etc. With a more widespread presence, a wider functionality was conjectured for these enzyme systems. As a result the detoxification systems were known to metabolize as well and hence termed drug metabolizing enzymes (the name was suggested as the only known substrate for detoxification enzymes were drugs). Detoxification systems were now considered as xenobiotic metabolizing enzymes or "effector metabolizing enzymes" (the later term was suggested by Nebert, 1997). Considering its wider usage we will be using the term xenobiotic metabolizing enzymes in this paper.

The variation in response towards xenobiotics among individuals and populations has been attributed to genetic polymorphisms in xenobiotic metabolizing enzymes (XMEs). XME polymorphisms are also termed ecogenetic polymorphisms due to the availability of xenobiotics in varied ecological systems ranging from a soil microbiota to air ecosystem. Ecogenetic polymorphisms affect that affect drug response are studied in the domain of *pharmacogenetics*. Some others that modulate sensitivity towards toxic environmental agents are studied in the expanse of *toxicogenetics*. Genetic studies have also focused on finding the origins of slow metabolizing phenotypes of these enzymes in some populations by studying them in an evolutionary framework. The broad aims of ecogenetic studies are as follows:

(i) Assessing the variation at XME genes and studying the pattern of inheritance

(ii) Examining the pattern of genetic differentiation within and among populations (both at risk and comparatively safe from exposure) at XME genes

(iii) Evaluating the ecological factors responsible for the extent of differentiation observed at these genes

(iv) Examining the contribution of evolutionary forces to explain slow and fast metabolizer phenotypes

PON1: A XME connecting Pseudomonas, Organophosphates and Lipid metabolism

The Paraoxonase1 (PON1) gene has garnered interest of researchers from many different areas of biology. The reason is that it links research areas as diverse as microbial studies, toxicology, pharmacology and HDL-physiology. The promiscuity in substrate specificity or moonlighting property of PON1 is the key for its strategic positioning in animal metabolism. PON1 metabolizes the L-acyl homoserine lactones (AHLs) secreted by the pathogenic bacterium *Pseudomonas aeruginosa* that blocks cell-to-cell communication among the bacterial cells thus inhibits infection. This property of PON1 makes it valuable for maintaining innate immunity and of interest to microbiological research on human pathogens. PON1 was first discovered due to its detoxifying action against the chemical diisopropyl fluorophosphate (DIFP) which is an irreversible cholinesterase inhibitor used in organophosphate insecticides (Mazur, 1946). Thereafter a series of experiments to find out cholinesterase-inhibiting substances hydrolyzed by PON1 followed. The enzyme was found to act against a number of toxic chemicals like nerve gases and organophosphate (OP) compounds used in pesticides. The metabolism of some drugs also requires participation of PON1. These observations lead to inclusion of the enzyme in toxicological and pharmacological studies. The PON1 enzyme was found to prevent accumulation of lipoperoxides (Mackness et al., 1991) in association with high density lipoprotein (HDL) molecules (Blatter et al., 1994) thus preventing increase in oxidative stress. The enzyme is thus capable of metabolizing a vast array of substrates, albeit with varied catalytic efficiencies.

Inter-population level differences in enzyme variants have been subject to clinical and epidemiological investigations. With the advent of molecular biological techniques, the molecular basis of the enzyme variants and their contribution to variation in response to xenobiotics were unraveled. Molecular genetic investigations of PON1 revealed

the complete gene sequences in different species including humans, genetic variants in the coding and regulatory regions of the gene, the presence of two other enzyme paralogs, several orthologs in other species and the molecular genetic basis of differential enzyme concentration, expression and activity. The frequencies of the genetic variants were compared in populations of different ethnicities and the low activity allele was seen to decrease in frequency from Europeans to Africans and East Asians which have the least frequencies for this allele (Geldmacher von-Mallinckrodt et al., 1983). Specificity towards multiple substrates and presence in prokaryotic and eukaryotic genera suggests the antiquity of this enzyme in evolution.

Fig.1: Evolutionary relationship of PON1, PON2 and PON3 genes

The reason why paraoxonase is important to ecogenetic studies is the differential activity of the enzyme that leads to distinct metabolizing capacities. Biochemical and molecular genetic investigations that have thrown light on this are hereby discussed.

Biochemistry of Paraoxonase 1 enzyme

The biochemistry of PON1 enzyme is explained by its catalytic properties: substrate specificity and hydrolytic activity. PON was first known as an esterase possessing both paraoxonase and arylesterase activities (Smolen, 1991). Numerous studies have been conducted ever since to find out the substrates acted upon by the enzyme and catalytic efficiencies towards them. Organophosphate compounds were the first identified substrates of PON1. Studies revealed that chemicals with aromatic carboxylic ester and lactone based moieties are also PON1 substrates (Billecke et al., 2000; Rochu et al., 2003).

The first structure-activity study on PON1 indicated that a lactone like structure is necessary for hydrolysis by PON1 (Augustinsson and Ekedahl, 1962). PON1 activity is characterized by wide substrate-specificity and high inter-individual variability. But no difference was observed between sexes (Simpson, 1971). Also the activity increased with age. The PON1 activity was found to vary among distinct ethnic groups. A polymorphic distribution was observed with populations exhibiting bi-modal and tri-modal distribution depending on the substrates used for assaying the activity (Eckerson et al., 1983; Mueller et al., 1983; Geldmacher von-Mallinckrodt and Dipegen, 1988). When hydrolytic activity was evaluated with paraoxon and aryl-ester compounds, three levels of activity were found: low, intermediate and high and the difference in levels of activity were 30-40 folds (Furlong et al., 1988). Molecular basis of the polymorphic activity was found by Humbert et al. (1993) through an *in-situ* hybridization technique.

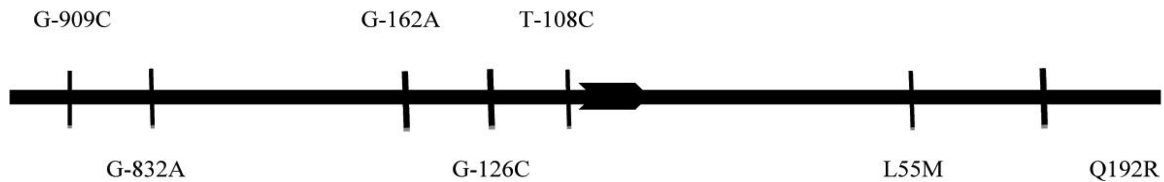
Paraoxonase 1 was earlier described to possess a paraoxonase and an arylesterase activity. Activity studies using different substrates pointed that lactonase might have been the native activity of the PON family as it was found in different genera (Billecke et al., 2000). Structure-activity relationship (SAR) studies confirmed the native activity of the enzyme to be that of a lactonase (Khersonsky and Tawfik, 2005) where the native activity was defined to be the basic function for which the enzyme and its structure had evolved. The native lactonase activity is characterized by robustness (specificity) that is lesser in the other promiscuous activities (arylesterase and paraoxonase) and so the latter active sites are more responsive to mutations (Aharoni et al., 2005). PON1 has been studied both for its native and promiscuous activities.

PON1 structure

The cDNA of PON1 mRNA codes for a protein which has one methionine amino acid less at the N-terminal end (Hassett et al., 1991; Adkins et al., 1993). This residue is removed when the enzyme is secreted from liver into blood and subsequently matures. The leader sequence is retained post-translation and facilitates PON1's attachment with an HDL particle through a signal peptide which modulates HDL function (La Du et al., 1993). The matured, functional enzyme is 354 amino acids long with a molecular weight of 43kda (Mackness et al., 1996). The human PON1 enzyme is coded by nine exons (Clendenning

et al., 1996). It was found that in humans, total serum PON1 is associated with HDL-C (Vekic et al., 2007). The structure of the PON1 gene has also been studied for its variations that have important role in the protein's function. In all about 200 Single Nucleotide Polymorphisms (SNPs) have been found (Seattle SNPs, http://pga.gs.washington.edu/). The ones mostly studied (Figure2) are found in the exons (exonic) and the promoter region (5' UTR). They have been discussed here.

Fig. 2: Locations of most studied PON1 gene polymorphisms



The glutamine (*Gln*, Q) to arginine (*Arg*, R) mutation occurring at 192 amino acid position (Figure2) was found to produce the A and B alloforms of the enzyme (Adkins et al., 1993) with low and high paraoxonase activity respectively. The substitution is due to an A to G transition in exon 6 of the PON1 gene. Designated as Q192R, the polymorphism is the more investigated among two coding region polymorphisms and the most studied among all the PON1 polymorphisms considering its non-synonymous nature that results in the two alloforms having different activity. The polymorphism has a substrate-dependent effect on the enzyme activity which is determined by measuring hydrolytic activity and catalytic efficiency (*Vmax/Km* ratio, where *Vmax* is the initial velocity of the enzyme catalyzed reaction and *Km* is a constant specific for the enzyme-substrate complex). The wild type allele Q192* shows greater hydrolytic activity towards diazoxon, soman and sarin while *192R rapidly hydrolyzes paraoxon and chlorpyrifos-oxon (Furlong et al., 1988). Both the alleles exhibit equal activity towards phenylacetate (Humbert et al., 1993; Davies et al., 1996; Li et al., 2000). Resultantly, serum paraoxonase activity of PON1 was greater in individuals homozygous for *192R allele in comparison with those homozygous for Q192*. The Q192* homozygotes showed increased activity towards nerve gases, tabun, sarin and soman. Similar patterns were observed with different organophosphate chemicals among varying ethnic groups (Brophy et al., 2000; Furlong et al., 2006). *In vitro* studies show inconsistency in the hydrolytic activities of the same allele with varying concentrations of the same substrate (Furlong et al., 1991; Davies et al., 1996; Brophy et al., 2000; Li et al., 2000; O'Leary et al., 2005; Sirivasarai et al., 2007). The population studies of PON1 activity are in accordance with the results from the *in vitro* kinetic studies (Davies et al., 1996). Studies on transgenic mice showed higher resistance to acetyl cholinesterase (AChE) inhibition in *192R expressing mice (Furlong et al., 2005).

The second coding region polymorphism included in this study designates a nucleotide substitution (T to A) in exon 3 at codon 55 that produces a non-synonymous mutation of leucine (*Leu*, L) to methionine (*Met*, M). This mutation causes decreased serum enzyme concentrations. Individuals homozygous for *55M allele were found to have decreased serum enzyme concentration but normal activity (Blatter-Garin et al., 1997). The crystal structure of PON1 suggested that L55M mutation lead to distortion in packing and a destabilized structure (Harel et al., 2004). Population studies also suggest that L55M polymorphism alters gene expression (Blatter-Garin et al., 1997; Brophy et al., 2000). A lower expression in presence of *55M allele was initially conjectured to be due to an unstable transcript but later studies described the differential expression to be a result of linkage disequilibrium with the promoter region polymorphisms (Brophy et al., 2000; Leviev et al., 2001).

The low gene expression due to *55M allele and low catalytic efficiency due to Q192* together (QQ192*/*55MM) show the lowest activity towards paraoxon while individuals with *192RR/LL55* genotype have the highest activity (Blatter-Garin et al., 1997). Substrate-specific variations are observed among genotype combinations (Ginsberg et al., 2009). It was also noted that the *192R allele is in linkage disequilibrium with the *55L.

Five polymorphisms have been identified in the promoter region of the *PON1* gene (Figure2) positioned at -108, -126, -162, -832, -909 nucleotide positions from the transcription start site (Leviev & James, 2000; Suehiro et al., 2000). These

polymorphisms affect the expression pattern of *PON1*. Elevated PON1 activity was observed in -108C, -162A and -909G polymorphisms in comparison to individuals with -108T, -162G and -909C (Brophy et al., 2000). -108 polymorphism contributed to 23% of the observed variance in PON1 expression in a Caucasian sample (Suehiro et al., 2000) and the variation was consistent with change in phenotype in different samples (Brophy et al., 2001; Chen et al., 2003). Leviev and James (2000), however, did not find any effect of -108 on PON1 activity but that of -832 instead. -108 polymorphism was found to be the principal cause of the association of *55 polymorphism with PON1 gene expression due to the linkage disequilibrium between the two positions (Brophy et al., 2001; Chen et al., 2003). -126 and -162 polymorphisms did not modify the serum PON1 activity. Carlson et al. (2006) found 26 different polymorphisms affecting PON1 function but none of these have been characterized through animal models or kinetic studies on humans.

Ecogenetic studies on PON1

Early population genetics studies made worldwide comparisons of the pattern of the distribution of PON activity (Geldmacher von-Mallinckrodt, 1983; Mueller et al., 1983; Goedde et al., 1984). The percentage of low activity group was observed to be less in African and Asian populations and even absent in some tribes. European populations however had a higher percentage of the low activity group. Basing on the populations screened, the low, intermediate and high activity type populations were found in 'Indio-German', 'Oriental' and 'Negroid' ethnic groups (Geldmacher von-Mallinckrodt, 1983). These observations encouraged further investigations that were required to find out the molecular and genetic basis of the differential distribution of PON1 activity. The gene frequency patterns found later enunciate the observations of the PON1 activity pattern reported in earlier studies for some populations. But for some populations like East Asians the allele frequencies do not support the observations in activity measurements. This emphasizes the importance of restriction fragment length polymorphism (RFLP) analysis for studying PON1 polymorphism. The distribution of both *192 and *55 polymorphisms in populations from different ethnicities have been reported by several groups (Serrato and Marian, 1995; Antikainen et al., 1996; Davies et al., 1996; Hermann et al., 1996; Blatter-Garin et al., 1997; Mackness et al., 1997; Zama et al., 1997; Sanghera et al., 1998; Aynacioglu et al., 1999; Richter and Furlong, 1999; Fanella et al., 2000; Imai et al., 2000; Hong et al., 2001; Jakubowski et al., 2001; Malin et al., 2001; Allebrandt et al., 2002; Scachhi et al., 2003; Rojas-Garcia et al.,

Table 1: Substrates of PON1 enzyme (Adapted from Billecke et al., 2000; Hioki et al., 2011; Ishizuka et al., 2012)

Substrate classification	PON1 substrate
Drugs	Ampicillin
	Aspirin
	Ciprofloxacin
	Cisplatin
	Cyclophosphamide
	Cyclosporin A
	Fluvastatin
	Glucocorticoid δ - lactones
	Levonorgestrel
	Lovastatin
	Mevastatin
	Mithramycin
	Olmesartan medoxomil (OM)
	Paracetamol
	Pilocarpine
	Pitavastatin
	Prulifloxacin
	Simvastatin
	Spironolactone
Nerve agent (NA)	DFP
	Tabun
	Soman
	Sarin
Pesticide	Parathion
	Diazinon
	Chlorpyrifos
	Diisopropyl fluorophosphates
	Phenytoin
	Dihydrocoumarin

2005; Sirivasarai et al., 2007). The gene frequencies reported for various groups of populations for the Q192R polymorphisms are given in Table 2 and Figure 3.

Genetic studies have also helped understand the role of PON1 polymorphisms in human health due to the enzyme's antioxidant and antiorgano-

Table 2: Global low activity allele Q192* frequencies among different populations of the world

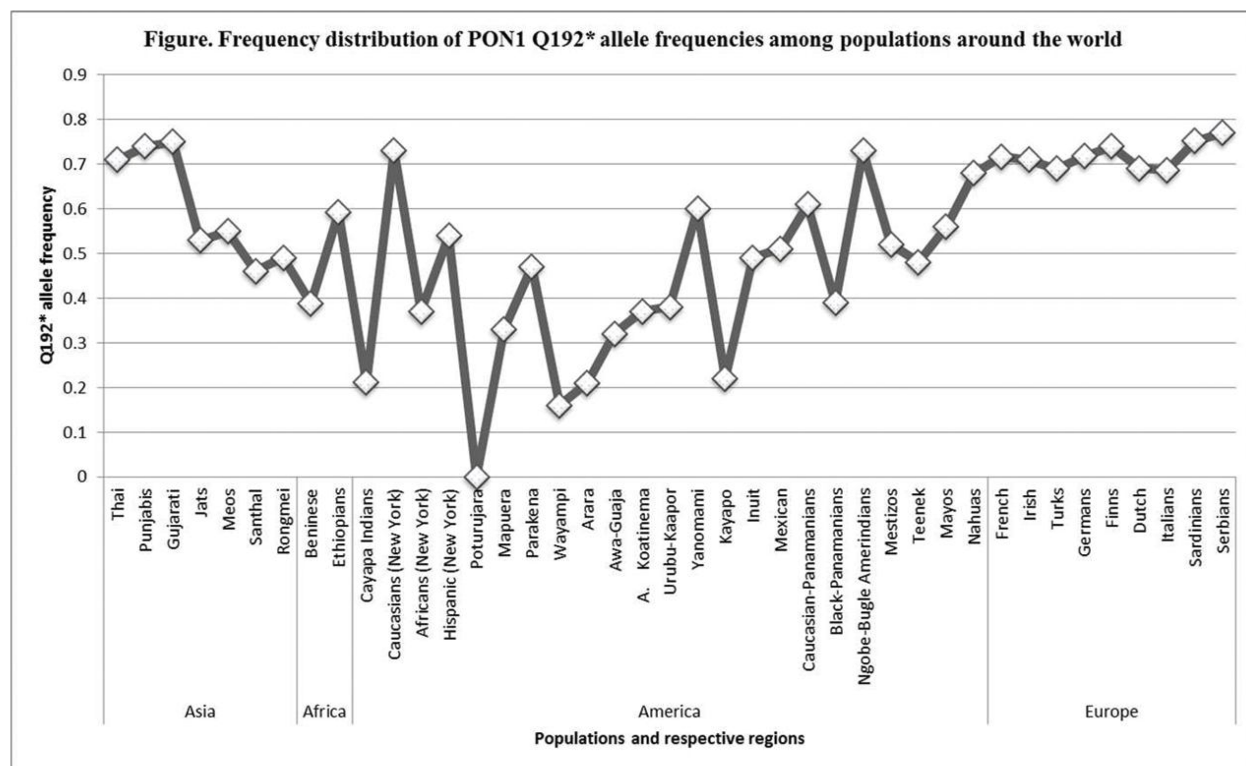
Region	Population	Allele Frequency		N	Reference
		Q	R		
Asia (7)	Thai	0.710	0.290	202	Phuntuwate et al., 2005
	Punjabis	0.740	0.260	300	Gupta et al., 2011
	Gujarati	0.750	0.250	223	Patel et al., 2007
	Jats	0.530	0.470	100	Unpublished results
	Meos	0.550	0.450	83	Unpublished results
	Santhal	0.460	0.540	95	Unpublished results
	Rongmei	0.490	0.510	70	Unpublished results
Africa (2)	Beninese	0.388	0.612	98	Scacchi et al., 2003
	Ethiopians	0.592	0.408	169	Scacchi et al., 2003
America (23)	Cayapa Indians	0.211	0.789	83	Scacchi et al., 2003
	Caucasians (New York)	0.730	0.270	82	Chen et al., 2003
	Africans (New York)	0.370	0.630	117	Chen et al., 2003
	Hispanic (New York)	0.540	0.460	203	Chen et al., 2003
	Poturujara	0	1	47	Santos et al., 2005
	Mapuera	0.330	0.670	29	Santos et al., 2005
	Parakena	0.470	0.530	17	Santos et al., 2005
	Wayampi	0.160	0.840	16	Santos et al., 2005
	Arara	0.210	0.790	35	Santos et al., 2005
	Awa-Guaja	0.320	0.680	28	Santos et al., 2005
	A. Koatinema	0.370	0.630	15	Santos et al., 2005
	Urubu-Kaapor	0.380	0.620	26	Santos et al., 2005
	Yanomami	0.6	0.4	19	Santos et al., 2005
	Kayapo	0.22	0.780	27	Santos et al., 2005
	Inuit	0.490	0.510	793	Lahiry et al., 2007
	Mexican	0.510	0.490	214	Rojas-Garcia et al., 2005
	Caucasian-Panamanians	0.610	0.390	50	Tejada et al., 2010
	Black-Panamanians	0.390	0.610	40	Tejada et al., 2010
	Ngobe-Bugle Amerindians	0.730	0.270	48	Tejada et al., 2010
	Mestizos	0.520	0.480	208	Gamboa et al., 2006
	Teenek	0.480	0.520	57	Gamboa et al., 2006
	Mayos	0.560	0.440	55	Gamboa et al., 2006
	Nahuas	0.680	0.320	56	Gamboa et al., 2006
Europe (9)	French	0.717	0.283	796	Ruiz et al., 1995; Hermann et al., 1996
	Irish	0.710	0.290	170	Hermann et al., 1996
	Turks	0.690	0.310	381	Aynacioglu et al., 1999
	Germans	0.718	0.282	2784	Gardemann et al., 2000
	Finns	0.740	0.260	169	Antikainen et al., 2000
	Dutch	0.691	0.309	815	Heijmans et al., 2000; Leus et al., 2000
	Italians (mainland Italy)	0.687	0.313	179	Scacchi et al., 2003
	Sardinians	0.752	0.248	161	Scacchi et al., 2003
	Serbians	0.770	0.230	122	Pejin-Grubisa et al., 2010

phosphate action. The role of gene polymor- phisms in regulation of PON1 activity and their subsequent effect on disease, toxicological and pharmacological aspects of health are discussed next.

Paraoxonase 1 enzyme metabolizes chemicals at different rates in individuals (La Du, 1996). Some of these chemicals are known carcinogens while for others therapeutic or toxic properties are well established. This formed the basis of the toxicogenetic and pharmacogenetic studies of PONs. The lactones have been observed in all phyla from the unicellular bacteria to the complex multicellular animal and plant kingdoms. Lactones constitute many phytochemicals and are used to enhance flavor of food products. Besides, some drugs comprise lactone and cyclic carbonate structures that are targeted by the PON1 enzyme. A list of different chemicals metabolized by PON1 enzyme is given in Table1.

Molecular genetic studies gave interesting revelations that went on to have huge repercussions in toxicological and pharmacological studies involving PON1. The important ones were i. The signal peptide sequence by the PON1 during secretion is retained and ii. The Q192R

Fig. 3: Graphical representation of PON1*Q192 allele frequencies in populations around the world. Allele frequency at rs662 position of PON1 is given for 40 populations. The populations are grouped into four geographical regions Asia, Africa, America and Europe.



polymorphism is the main contribution of differential PON1 activity. The coding region polymorphism L55M and promoter region polymorphisms have also been studied for their roles in modulating PON1 levels and expression that affect PON1-mediated xenobiotic sensitivity.

Serum paraoxonase protects an organism from the cholinesterase inhibiting organophosphate pesticides. The organophosphate (OP) compounds that are metabolized by PON1 are the main derivatives of a group of OP pesticides. The OP compounds undergo oxidative desulfuration by cytochrome P450 enzymes to produce oxon derivatives of the compound. The PON1 enzyme then hydrolyzes the highly toxic oxon derivative into non-toxic by-products that are secreted out of the system. The level of activity of the enzyme determines the rates of hydrolysis of OP pesticides. Lower oxon hydrolysis causes higher sensitivity towards OP exposure as bioavailability of OP will be high for cholinesterase inhibition. The OP pesticides that are widely used are chlorpyrifos, diazinon, malathion and parathion (Table1). The respective oxon derivatives of the pesticides mentioned are chlorpyrifos oxon, diazoxon, malaoxon and paraoxon. PON1 also hydrolyzes nerve gases like soman and sarin. The toxicogenetic studies involving PON1 have examined the inter-individual, inter-population and inter-species differences in catalytic efficiency of the enzyme towards these compounds and the role of genetic polymorphisms. It was found that *R192 allele better hydrolyzed paraoxon while Q192* showed higher activity for diazoxon, soman and sarin. Effects of OP exposure on PON1 activity has been studied in different cases. Allelic variation-induced differential sensitivity to OP exposure was noted in some. Some of these studies recruited the Gulf war veterans, sheep dip workers, farm workers and victims of the Tokyo subway attack. An important observation in these studies was that the hydrolytic activity of PON1 was more effective during chronic exposure to low doses of poisoning and not acute exposure which again emphasizes the fact that organophosphatase activities are promiscuous to the enzyme and hence less effective at high exposure of toxins. PON1 polymorphisms modulate risk in environments of chronic exposure like among agriculturists who are exposed to pesticides on a regular basis.

Pharmacogenetic investigations examine the effect of allelic variations on the within and between population dissimilarities in drug metabolism. Drug metabolizing enzymes (DMEs) transform a drug into an active metabolite which then passes on into the

circulation before acting on the target tissues. A similar course of action is followed in metabolism of phytochemicals that are consumed by animals as nutrients. Enzyme isoforms produced due to allelic variants in *PON1* gene differ in their efficiencies of biotransformation thus influencing drug (or other chemical) kinetics and consequently response. A subset of PON1 variants have been investigated in pharmacogenetic studies due to their interaction with drugs and drug derivatives. The Q192R polymorphism has been studied for its role in the bio-activation of the combination therapy with clopidogrel and aspirin in patients with coronary artery disease. Xenobiotic response elements (XREs) with a consensus sequence GCGTG in the promoter region of PON1 have been found to regulate the gene expression in presence of different chemical substances. Also, the promoter polymorphisms are putative binding sites for transcriptional factors which are often targeted by xenobiotics. Pharmacogenetic interactions have been studied between the promoter polymorphisms of PON1 gene and statins which are used to inhibit the HMG-CoA reductase enzyme to treat atherosclerotic plaques (Goudedard et al., 2003; Deakin and James, 2004; Deakin et al., 2007). The effect of dietary polyphenol, quercetin on PON1 gene expression was studied and a reduction in promoter up-regulation was observed when a mutation occurred in a 20bp sequence of the promoter region. This implies a mutation in this region will affect the metabolism of the drugs and polyphenols that act through the promoter region to impress upon the enzyme activity.

The distribution of gene frequencies at these loci will help determine the potential risks the populations are associated with. Studying individual populations will help customize group specific interventions to ward off episodes of toxicity caused by OP chemicals metabolized by PON1. One of the outcomes of the population genetic analysis was the finding that *192R is the ancestral allele at the Q192R polymorphism (Scacchi et al., 2003). This indicates that most of the populations carry this allele in a higher frequency. *192R codes for the high activity phenotype of PON1 and hence decreases susceptibility of an individual towards poisoning by oxon derivatives of parathion and chlorpyrifos. In contrast, it would make an individual more susceptible to poisoning by diazoxon. Thorough population screening programs can thus help in introducing suitable pesticides that would pose less danger to the human population due to chronic exposure while performing the function of pest-killing. Population screening is more pertinent in countries where the use of OP based pesticides is more prevalent.

Population genetic studies have, hitherto, targeted description of PON1 to give a rich resource of data on gene frequencies among different populations. A complete picture describing the worldwide genetic diversity at PON1 loci will be useful in answering questions ecogenetic relevance. The questions that need to be answered by population geneticists are the roles played by drift and selection forces in maintaining the allele frequencies of PON1 for the low activity alleles. Recent genome wide scans for detecting selection pressures also found signs of positive selection at the coding region polymorphisms of PON1 gene. Examining the apportionment of PON1 variation among worldwide populations will be helpful to find out which of the factors, geography, ethnicity or subsistence acts as the proxy for PON1 status.

Conclusion

An evolutionary understanding of the health problems posed by increased exposure (both intended and non-intended) to xenobiotics will help reduce its burden. Population genetic studies targeting xenobiotic-metabolizing enzymes have helped to gain insights on their evolutionary history. These findings can be useful for genetic medicine to leverage the ills of toxic xenobiotics or adverse events caused due to drugs and lowered drug efficacy. Albeit they are few in number, clear evidences of toxicogenetic and pharmacogenetic relevance have been reported. Population genetic studies have revealed the genetic variants in the PON1 gene that affect the enzyme's activity and concentration in populations of different ethnic groups. Further understanding of the geographical, climatic, and dietary variations in these groups will help provide clues to explain the patterns observed in the genetic variation in this gene. An improved understanding of the factors influencing PON1 activity and expression including genetic factors will help unravel the relationship between PON1 genotypes and variability in response to environmental toxins and drugs. Populations having the low activity alleles for specific substrate should be finely screened for comprehending the population risk towards the substrate in case it is a toxin. If the substrate is a drug, the low activity alleles might be accountable for lower efficacy towards the drug. This might be important for formulating group-specific drug regimes.

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