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Paraoxonase 1 (PON1) Activity, Polymorphisms and Coronary Artery Disease

Nidhi Gupta, Kiran Dip Gill and Surjit Singh
*Post Graduate Institute of Medical Education and Research, Chandigarh,
India*

1. Introduction

Cardiovascular disorders (CVD) which include coronary artery disease (CAD), heart failure (HF) and stroke are the leading cause of morbidity and mortality both in developed and developing countries and by 2020 CAD is expected to become the number one cause of death worldwide ¹⁻³. As per WHO, CVD kills nearly 17.5 million persons worldwide each year and is likely to continue to remain number one cause of overall mortality in near future ^{4, 5}.

CAD is the single most important contributor to this increasing burden of CVD. It leads to more deaths than any other disease, including cancer. It can manifest as angina, silent ischemia, unstable angina, myocardial infarction (MI), HF and sudden death. CAD accounts for 52% of 870,000 deaths that occur annually due to CVD in USA i.e. 1 in 5 deaths ⁶ and nearly accounts for 30 % of all deaths globally. Among American Indians in age group (45-74) the incidence of CVD ranges from 1.5%- 2.8% for men and 0.9- 1.5% for women ⁷.

Ethnic and regional variations are known to exist in risk factors for developing CVD. The Asian Indians are 3- 4 times more susceptible to develop CAD than Caucasians, 6- times more than Chinese, and 20- times more than Japanese and tend to develop CAD at a younger age ^{8, 9} as shown by several studies ¹⁰⁻¹³. The study SHARE (Study of Health Assessment and Risk in Ethnic groups) has shown a significant higher risk of cardiovascular events among South Asians as compared to Europeans and Chinese ¹³.

2. Pathophysiology of CAD

The most important underlying pathogenetic mechanism for CVD is atherosclerosis ^{14, 15}. CAD occurs due to atheromatous narrowing and subsequent occlusion of the coronary arteries. Atheroma [from the Greek athera (porridge) and oma (lump)] starts developing in the first decade of life. A mature plaque has a lipid core which comes from necrotic "foam cells" i.e. monocyte derived macrophages which migrate to intima and ingest lipids (fig. I) ¹⁶.

Epidemiological studies have shown an inverse correlation between serum HDL-C levels and risk for developing CAD ^{17, 18}. The protective effect of HDL-C against the development of CAD appears to be complex. A large part of research in this field is centered on the lipid

transport function of HDL-C, particularly in reverse cholesterol transport (RCT). In addition, several studies suggest that HDL-C protects LDL-C from peroxidation, thereby protecting cell membranes from lipid peroxide induced vascular damage. This protection of LDL-C from oxidation by HDL-C possibly potentially impedes the initiation and progression of CAD. Recent studies into the mechanism of the prevention of CAD by HDL-C have revealed that its antioxidant effect is because of its association with an enzyme “paraoxonase”.

3. Paraoxonase (PON1; EC 3.1.8.1)

In 1946, Abraham Mazur was the first to report the presence of an enzyme in animal tissues which could hydrolyze organophosphates¹⁹ which ultimately led to the identification of human serum paraoxonase (PON1) enzyme in early 1950's²⁰. PON1 is a HDL-C associated serum enzyme whose primary role is to protect LDL-C from oxidative modification²¹.

PON1 was first identified in the field of toxicology as it could hydrolyze the organophosphates such as paraoxon, and oxon metabolite of chlorpyrifos, diazinon and nerve gases (e.g., sarin and soman)²². Indeed, the enzyme (EC 3.1.8.1) was initially characterized as organophosphate hydrolase and it still derives its name from its *in vitro* used substrate, paraoxon. Recently, in addition to its role in hydrolyzing organophosphorus compounds, PON1 has been shown to play an important role in lipid metabolism and thus in atherosclerosis and cardiovascular disease.

The PON1 cDNA encodes a protein of 355 amino acids from which only the amino-terminal methionine residue is removed during secretion and maturation²³. The retained leader sequence is required for the association of PON1 with HDL particle²⁴. In human serum, PON1 remains entirely associated with HDL²⁵.

PON1 lowers the risk of CAD by preventing oxidation of LDL-C which is involved in the initiation and progression of atherosclerosis²⁶ (Fig. I). Studies have shown that PON1 can prevent accumulation of oxidized LDL-C *in vitro* and *in vivo*²⁷⁻²⁹. In addition, it has also been shown to hydrolyze the oxidized lipids³⁰. Serum PON1 activity is reduced in diabetes mellitus and familial hypercholesterolemia^{31,32}, diseases which are associated with accelerated atherogenesis.

3.1 PON1 structure

The first information about the structure of PON1 was the finding that it retained its signal sequence following secretion from the liver²³. Sorenson *et al.*,²⁴ demonstrated that this signal sequence provided a hydrophobic anchor for attachment of PON1 to HDL. Josse *et al.*,^{33, 34} identified the following amino acid residues which are essential for PON1's catalytic activity: W280, H114, H154, H242, D53, D168, D182, D268, D278, E52, E194 and the two cysteine residues C41 and C352 that are in disulfide linkage.

A major breakthrough in PON1 structural study came with the engineering of a directed evolution³⁵ of a form of PON1 that could be crystallized and subjected to X-ray crystallography³⁶ resulting in determination of crystal structure of the recombinant PON variant, making PON1 the first HDL-associated protein whose three-dimensional structure could be determined³⁶. PON has a six-bladed β -propeller with each blade consisting of four

β -sheets. In the central tunnel of the enzyme there are two calcium atoms which are needed for the stabilization of the structure and catalytic activity³⁶. Three α helices, located at the top of the propeller are involved in its anchoring to the HDL particle³⁶

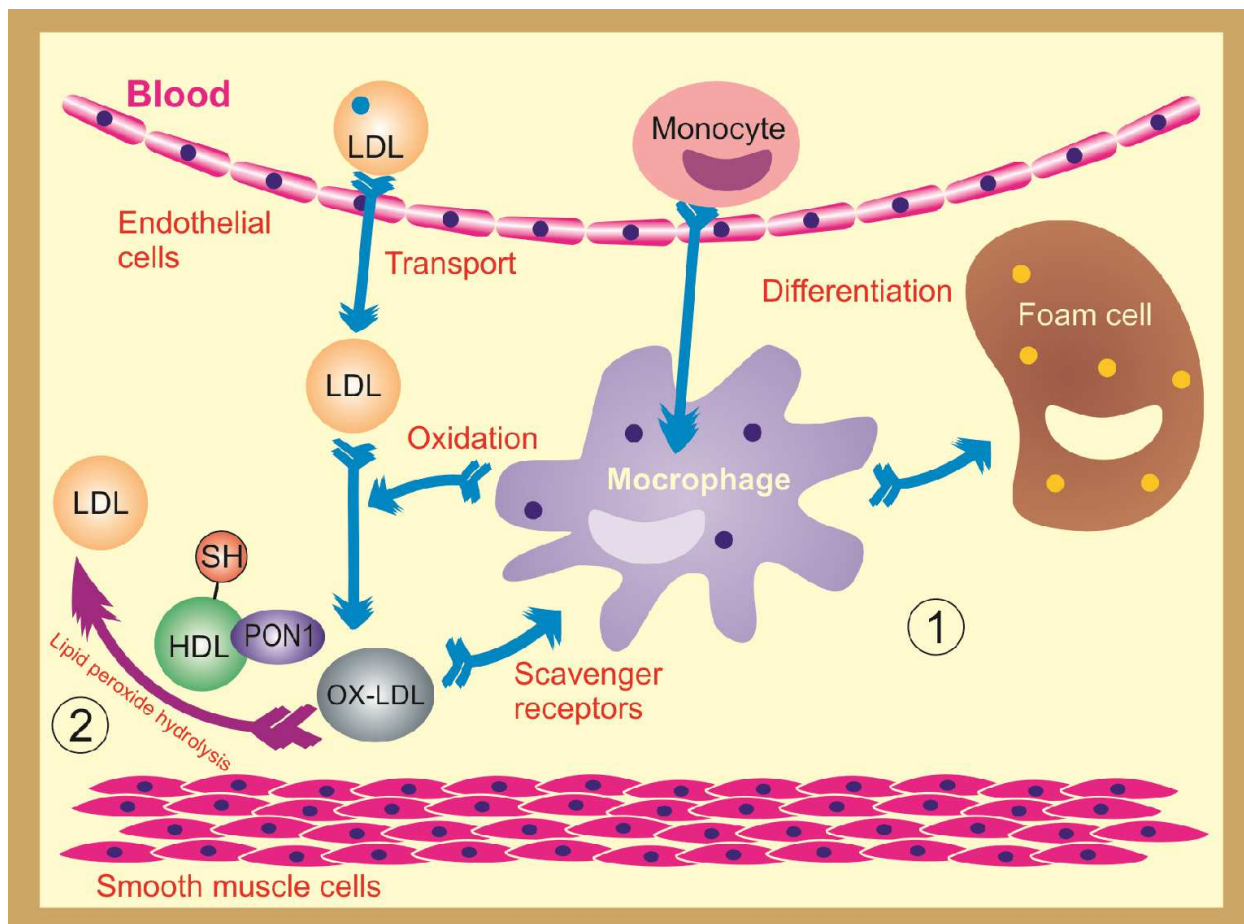


Fig. 1. (1). Normal development of atherosclerosis. (2). Protection from atherosclerosis by PON1.

PON1 contains three Cys residues with one at position 284, having a free sulfhydryl group (Fig. 1). PON1 is the only one whose allozymes are found in serum. A unique feature of PON1 in comparison to the other secreted proteins is the retention of its N-terminal hydrophobic signal-leader sequence. Immunological techniques have revealed that PON1 accumulates in the human arterial wall during the development of atherosclerosis³⁷.

3.2 PON gene cluster

In addition to the known human PON1 gene, two additional PON-like genes, designated as PON2 and PON3 have been identified and all these three genes are located on the long arm of chromosome 7q 21.3–22.1³⁸. These genes share a considerable structural homology and may have arisen from the tandem duplication of a common evolutionary precursor. Within a given mammalian species PON1, PON2 and PON3 share approximately 60% identity at the amino acid level and 70% identity at the nucleotide level. However, between mammalian species each of three genes shares 79–90% identity at the amino acid level and 81–90% identity at nucleotide levels²⁶.

3.3 Paraoxonase 2 (PON2)

PON2 is a widely expressed intracellular protein with a molecular mass of approximately 44kDa ³⁹. PON2 mRNA is ubiquitously expressed in nearly every human tissue with highest expression in liver, lung, placenta, testis, and heart. PON2 is able to lower the intracellular oxidative stress of a cell and prevent the cell-mediated oxidation of LDL. Cells over expressing PON2 are less able to oxidize LDL-C and show considerably less intracellular oxidative stress when exposed to either H₂O₂ or oxidized phospholipids. Since PON2 is ubiquitously expressed not just in cells of the artery but in tissues throughout the body, it is likely that PON2 plays a role in reducing local oxidative stress ³⁹ and thereby protects cells from oxidative stress. However, the mechanism by which this effect is produced is not clearly understood.

3.4 Paraoxonase 3 (PON3)

Human PON3 is an approximately 40kDa protein, synthesized primarily in the liver and is associated with HDL in circulation, albeit at much lower level than PON1 ^{40, 41}. PON3 is interposed between PON1 and PON2 in the PON gene cluster and is the least studied compared to PON1 and PON2. In contrast to PON1, PON3 has very limited arylesterase and no paraoxonase activity but it rapidly hydrolyzes lactones such as statin prodrug.

All the three PON's are thus important players in the maintenance of a low oxidative state in circulating blood, therefore playing role in prevention of atherosclerosis ⁴². However, as exact mechanism of action is not clear, they remain the focus of research in recent years.

4. PONs substrates

PON's native enzyme activity is lactonase ^{43, 44}. Phylogenetic studies have revealed that PON2 is the oldest member of family from which PON3 and PON1 arose ⁴⁵. Draganov *et al.*, ⁴⁶ found that PON's have distinct substrate specificity. Dihydrocoumarin (DHC), long chain fatty acid lactones and acyl- homoserine lactones (AHLs) are hydrolyzed by all the three PONs and represent their natural substrates ⁴⁴. Additionally, PON1 also hydrolyzes organophosphates and aromatic carboxylic acid esters such as paraoxon and phenylacetate respectively ⁴⁷ thereby having paraoxonase (PONase) and arylesterase (AREase) activities ⁴⁸.

4.1 Paraoxonase (PONase) activity

There exists a wide variation in PONase activity in different ethnic groups and within individuals in the same ethnic group ⁴⁹. The PONase activity has been shown to be lower after acute myocardial infarction ⁵⁰. It is also lower in patients with familial hypercholesterolemia and diabetes mellitus, who are more prone to CAD ³¹. This has led to the hypothesis that the lower the PON1 activity, higher is the accumulation of oxidized LDL and risk of CAD.

Nearly 200 single nucleotide polymorphisms (SNPs) of PON1 gene have been identified so far, ⁵¹ of which the most studied are -909G/C [rs854572], -162A/G [rs705381], -108C/T [rs 705379] located in the promoter region and Q192R [rs662], L55M [rs 854560] located in the coding region ⁵². Serum PONase activity has been found to be influenced by the coding Q192R polymorphism ⁵³. The PON1 R192 allozyme hydrolyzes paraoxon more rapidly than

PON1 Q192 allozyme whereas PON1 R192 allozyme hydrolyzes diazoxon, soman and sarin more rapidly than the R192 allozyme ^{54, 55}.

4.2 Arylesterase (AREase) activity

Serum enzyme PON1 activity is not affected towards phenylacetate (AREase) substrate across Q192R polymorphism ⁵⁶. Richter *et al.*, ⁵¹ have stated that measurement of AREase activity of PON1 or determination of PON1 protein levels by ELISA are the minimum measure that should be carried out in any epidemiological study. Thus it can act as measure of PON levels and this has been used in a number of studies. ^{48, 51, 56-58}.

4.3 Lactonase activity

Lactonase activity is possibly the common enzymatic activity preserved during evolution of the PON proteins. Recent findings suggest that the name PON is infact a misnomer, since PON2 and PON3 lack any significant paraoxonase activity ³⁹⁻⁴¹. At the molecular level PON1, PON2 and PON3 share an ability to hydrolyze aromatic and long-chain aliphatic lactones, and thus the term lactonase may be more appropriate ^{44, 45}.

Further, the binding of PON1 to HDL particles i.e. the natural carrier of PON1 in blood, has been shown to greatly enhance its lactonase activity. However arylesterase or phosphotriesterase activities are not affected by this. PON1 and PON3 hydrolyze over twenty aromatic and aliphatic lactones with a high degree of overlapping substrate specificity, whereas PON2 lactonase activity is much more restricted.

5. PON1 activity/ levels and CAD

There have been several epidemiological studies to find the relation between PON1 status and CAD. PON1 status can be distinguished into PON1 activity towards paraoxon and PON1 concentration, which is mainly determined in serum by ELISA or can be estimated from phenylacetate hydrolysis activity. The first study on the relation between PON1 activity and CAD was conducted in 1985 ⁵⁰. The outcome of this study indicated that lower the PON1 activity, higher was risk of CAD. Subsequently Navab *et al.*, ⁵⁹ showed that patients with higher HDL-C but low PON1 activity were more susceptible to CAD than patients with low HDL-C but high PON1 activity, suggesting that PON1 activity may be more important than HDL protein for protection against CAD. In subsequent, three studies, investigating the relationship of PON1 status and CAD found that low PON1 activity or levels were associated with an increased risk of CAD ⁶⁰⁻⁶² suggesting that PON1 activity predicted coronary events independent of HDL-C.

There is a wide variation (up to 13 -fold) in PON1 serum concentration and activity between individuals even within the same genotype ^{55, 61}. In addition to genetic polymorphism, PON1 levels can be modified by acquired factors such as diet, lifestyle and disease. It is likely to be the functionality of the enzyme and not simply the genotype that is important in the interaction of PON1 with CAD. A small number of recent studies which include PON1 concentration and/or activity, have found that PON1 levels are reduced in CAD and found this effect is independent of PON1 genotype ^{61, 63}. In a case-control study of CAD, Jarvik *et al.*, ⁶¹ could not find any genotype effect unless PON1 activity was also considered.

A functional genomic analysis as measurement of an individual's PON1 function (serum activity) takes into account all the polymorphisms which might affect its activity. This can be accomplished by use of a high-throughput enzyme assay involving two PON1 substrates usually diazoxon and paraoxon⁵⁵. This, in addition to providing a functional assessment of the serum PON1 192, alloforms also provide the levels of PON1 for each individual, thus encompassing the two factors which affect PON1 levels or activity (position 192 amino acid and serum alloform level). This approach has been referred to as the determination of PON1 'status' of an individual⁵⁵. Measurement of PON1 status, coupled with PCR analysis of codon 192, has been shown to detect genotypes / activity discrepancies that can be explained by the presence of recently discovered mutations in the PON1 gene⁶³.

Fundamental biochemical principle dictates that it is the catalytic efficiency with which PON1 degrades toxic organophosphates and metabolizes oxidized lipids that determines the degree of protection provided by PON1 against insults from physiological or xenobiotic toxins. In addition, the higher concentration of PON1 provides better protection. Thus, for adequate risk assessment it is important to know PON1 level and activity.

The importance of PON1 status in determining susceptibility or protection from toxicity or disease points to the relevance of factors affecting PON1 activity and its levels of expression. Though genetic determinants such as polymorphisms play a primary role in determining an individual's PON1 status, contribution of other factors in modulating PON1 activity and levels is also important^{21, 64}.

6. PON1 gene polymorphisms and CAD

6.1 PON1 coding region polymorphisms and CAD

There is 10-40 -fold interindividual variation in serum PON1 activity^{53, 65} and this variation in part is determined by 2 common polymorphisms in the coding region of the PON1 gene. The first polymorphism involves glutamine ("A genotype") (Q) → arginine ("B genotype") (R) substitution at position 192, giving rise to 2 allozymes^{53, 66}. These allozymes have different activity for different substrates. Some substrates such as paraoxon and fenitroxon are hydrolyzed faster by R allozyme, whereas other substrates such as phenylacetate are hydrolyzed at the same rate by both allozymes²². However, others such as diazoxon and nerve gases soman and sarin are hydrolyzed more rapidly by the Q allozyme²². The Q192R polymorphism may be playing a role in CAD etiology because this genotype is associated with LDL oxidation and hydrolysis of lipid peroxides. The PON1 192R isoform is less effective at hydrolyzing lipid peroxides than Q isoform^{67, 68}. A second polymorphism of the PON1 gene is present at the amino acid position 55, a leucine ("L genotype") (L) → methionine ("M genotype") (M) substitution, independently influences PON1 activity and has been defined as the molecular basis for this interindividual variability^{53, 66}. It is independent of the 192 polymorphism and appears to be the major determinant of the well known biochemical polymorphism in serum PON1 activity towards various organophosphates.

The frequency of PON1 alleles varies greatly across the human population. The distribution of two polymorphisms is significantly different between white and black women. The frequency of the PON1 M55 allele is higher in whites than in blacks, whereas the frequency of the PON1 R192 allele is reverse⁶⁹. The lowest frequency of the PON1 M55 allele has been

reported in Chinese. The relatively high frequency of the PON R192 allele in blacks is similar to that reported in Chinese and Japanese varying from 58% to 65%^{70, 71}. However, Ferre *et al.*,⁷² found no significant differences in genotype and allele frequencies for PON1 polymorphisms at position 55 and 192 between control subjects and patients with myocardial infarction in a Spanish population. The frequencies were similar to those described for other Caucasian populations. These two polymorphisms have significant linkage disequilibrium. Several case-control studies conducted in Caucasians and in Japanese have shown that the Q192R is associated with increased risk of CAD⁷³⁻⁷⁷, although others have failed to replicate this association^{71, 78-82}. Other studies have indicated that Q192R polymorphism is associated with altered PON1 enzyme activity for paraoxon as a substrate^{53, 66}. On the other hand, Garin *et al.*,⁸³ reported that in a French population L55M polymorphism may be a major genetic determinant of PON1 enzyme activity and of increased risk for CAD whereas discordant results were obtained in Singapore in Asian Indians and Chinese⁸⁴ and in Japanese⁷³. In North- West Indian Punjabi's, Q192R was independently associated with CAD (QR (OR: 2.73 (1.57-4.72)) and RR (OR, 16.24 (6.41-41.14))⁵⁸. These results suggest that PON1 R might be an independent risk factor for CAD only in certain populations. Thus, the association between the PON1 polymorphism and CAD is not clear and continues to remain controversial.

The dramatic alteration in enzyme activity caused by this single amino acid change is explained by the structure of the enzyme. Amino acid 192R is an important active site residue³⁶. The Q192R polymorphism alters the enzyme's ability to protect LDL-C from oxidation in vivo with the Q form being the most protective⁶⁷.

The PON1 L55M polymorphism does not affect the interaction of PON1 with its substrates, but is associated with lower serum PON1 activity and concentration of the enzyme⁸³. Leviev *et al.*,⁸⁵ found lower PON1 mRNA levels in individuals carrying the M alleles. Subsequent analysis showed a strong linkage disequilibrium with the C (-108)T polymorphisms in the promoter region of the gene. Clinical reports have demonstrated that PON1 activity is reduced in patients with acute myocardial infarction⁶⁰, fish eye disease⁸⁶ and tangier disease⁸⁷. Lower paraoxonase activity has been observed in Type 2 diabetes mellitus patients with peripheral neuropathy³² and retinopathy⁸⁸⁻⁹⁰.

Watson *et al.*,²⁹ have shown that purified PON1 can prevent the pro-inflammatory effects of oxidized LDL when incubated in a vascular cell co-culture system, probably due to the mechanism of oxidized-arachidonic acid derivatives in the Sn-2 position of LDL-phospholipids. Of the PON1 192 allozymes the R allozyme proved to be more efficient at protecting LDL-C from oxidation. Numerous case-control studies have therefore been conducted to determine whether the PON1 192R polymorphism is more closely associated with CAD than the Q polymorphism. Some studies have shown association whereas others have not²¹. However, a recent meta-analysis has revealed a statistically significant increased likelihood of CAD with the PON1 192R allele. Some studies suggest that the PON1 R allele may increase susceptibility to other established CAD risk factors, such as diabetes mellitus⁸⁸, cigarette-smoking⁹¹ and age⁹². The PON1 55L allozyme is also more effective in vivo in protecting LDL-C against oxidation than M allozyme. Few case-control studies of the 55 polymorphism have been done. Some have shown an association between the PON1 55L allele and atherosclerosis^{83, 93}, but others have not^{84, 94}. However, no prospective studies of CAD and PON1 polymorphisms are available. Moreover the association between CAD and

PON1 genotype although largely confirmatory is not the only test for hypothesis that PON1 protects against CAD. This may be due to acquired factors acting either on the composition of the lipid environment of HDL, in which PON1 operates, or on the promoter region of the PON1 gene or in some manner as yet to be identified. When PON1 activity is measured directly in patients with CAD, it is about half that of the disease-free controls^{50, 60}. Ayub *et al.*,⁶⁰ have observed low PON1 activity within few hours of the onset of myocardial infarction, suggesting a low serum PON1 activity to precede the event. Low serum PON1 activity independent of genotype has been reported in several other disorders, which are known to be associated with CAD. These include experimental and clinical diabetes mellitus^{31, 32, 88, 95, 96}, hypercholesterolemia³¹ and renal failure⁹⁷.

Interestingly, in addition to preventing LDL-C oxidation, PON1 may also stimulate cellular cholesterol efflux, the first step in reverse cholesterol transport. Thus, PON1 might affect the efficiency of lipid transfer between HDL-C and LDL-C²⁶. Also, Rodrigo *et al.*,⁹⁸ demonstrated that PON1 may play a role in protecting against bacterial endotoxins and may have a stabilizing property for cellular membranes that undergo either acute or chronic exposure to oxidative agents and free radicals.

6.2 PON1 promoter region polymorphisms and CAD

Sequencing of the promoter PON1 gene led to the discovery of at least five polymorphisms with varying degree of influence over gene expression. These polymorphisms are located at -909(G/C), -832 (A/G), -162 (A/G), -126(C/G) and -108 (C/T)⁹⁹⁻¹⁰¹ of PON1 gene. Promoter containing polymorphisms GAAC, as opposed to CGGT, at positions -909,-832, -162 and -108 respectively, are up to two times more active⁹⁹⁻¹⁰¹. These variations in promoter activity have been shown to be physiologically relevant as they correlate with significant differences in serum PON1 concentration and activity⁹⁹⁻¹⁰¹.

Identification of clinically significant polymorphisms has been hampered by the fact that there is significant linkage disequilibrium between all the promoter polymorphisms. Haplotype analysis of two populations showed that the C(-108)T polymorphism was the main contributor to the serum PON1 variation, accounting for 23-24% of the total variation¹⁰⁰. Brophy *et al.*,¹⁰⁰ also reported a slight contribution (1.1% total variation) from the A (-162) G site. The sites at -909 and -832 made little or no difference to serum PON1 levels^{100, 102}.

Reporter gene assays using promoter regions of varying length have shown that approximately 200 base pair region covering the -108 and -162 polymorphism is sufficient for transcription of the PON1 gene¹⁰²⁻¹⁰⁴. Deleting this region completely abolishes promoter activity, indicating that it is an essential regulatory region of the promoter¹⁰² site of PON1 gene.

As the -108 site appears to be the most significant contributor to the PON1 serum variation, it has been the subject of further investigation. The polymorphism is located in the center of a consensus binding site for the ubiquitous transcription factor sp1 and sp3. This consensus site is abolished by the presence of the -108T variant^{99, 100}.

Binding of the sp1 to the -108 site is weaker in the presence of T than C, suggesting an effect of the polymorphism on sp1 binding¹⁰². There are multiple Sp1 sites in this region of the

PON1 promoter, so the effect of the polymorphism is likely to be positional. The -162 polymorphism lies over a potential NF-1 (nuclear factor-1) binding site, with the high activity A variant forming the site and the low-activity G variant disrupting it¹⁰³. This may explain the effect of the change at -162 on gene expression. Other polymorphisms in the promoter region (-162A/G, and -909G/C) may have less significant effect on PON1 expression. They are in strong disequilibrium with C-108T¹⁰⁵.

The increasing role attributed to PON1 in assuring protective mechanisms associated with HDL-C underlines the need to clarify fully the factors which control gene expression and thus modulate the serum PON1 concentration.

6.3 PON1 haplotypes and CAD

Determination of haplotypes is gaining attention because multiple linked SNP's have the potential to provide significantly more power to genetic analysis than individual SNP's¹⁰⁶. Information is lacking regarding PON1 haplotypes and CAD risk. In North-West Indian Punjabi's L-T-G-Q-C (carrying 4 variant and 1 wild type allele) and L-T-G-R-G (carrying 4 variant and 1 wild type allele) haplotypes are associated with 3.2 and 2.8 fold increase in the risk of CAD whereas haplotypes M-C-A-Q-G (carrying all wild type allele), L-T-A-Q-G (carrying 2 variant and 3 wild type allele) and L-C-A-Q-G (carrying 1 variant and 4 wild type allele) which are more prevalent in controls could be protective of CAD⁵⁸.

7. PON1 polymorphisms and lipoproteins

Many studies have suggested that variation in serum PON1 activity is associated with variation in serum lipoprotein concentration including the serum apoA1, LDL-C and HDL-C. Several studies have been conducted to determine the relationship between PON1 gene polymorphisms and serum lipoproteins in Hutterite North American population genetically isolated by religious belief. Further analysis of this population using several candidate genes led¹⁰⁷ to reveal that PON1 was one of nine genes which was responsible for between 3.2 and 7.8% of the total variation in plasma lipoproteins in them. The PON1 genotypes were significantly associated with variation in the plasma concentration of HDL-C, LDL-C, TG and apo B¹⁰⁷. Homozygotes for the low activity variant of PON1 had significantly lower levels of plasma triglycerides, LDL and apo-B than heterozygotes and homozygotes for the high activity variant. Furthermore, homozygotes for the low-activity variant had significantly lower ratios of total cholesterol/HDL-C, LDL -C/HDL- C and apo B/apo A1 indicating that homozygotes for the low activity allele had a less atherogenic lipoprotein profile than heterozygotes and homozygotes for the high activity allele.

More recently, Leus *et al.*,¹⁰⁸ found that a significant difference in mean TC and LDL-C levels between subjects with the PON1 LL55 and MM55 genotype, and PON1 MM55 had a better plasma lipoprotein profile.

Watson *et al.*,²⁹ (the Fogelman group) have reported that PON1 in HDL may block inflammatory response by preventing the oxidation of LDL. The same group has also shown that during an acute-phase response, there was a significant loss of the PON1 activity, thus accounting for the failure of HDL to protect LDL from oxidation during it¹⁰⁹. More recently the same group⁵⁹ have reported a failure of HDL to protect LDL from oxidation in patients with CAD, which they propose is due to low serum PON1 activity in them.

8. Modulation of PON1 by exogenous compounds

8.1 Environmental chemicals

PON1 activity is completely dependent upon Ca^{++} and EDTA, irreversibly abolishes its activity. Other cations, also, have been shown to have an inhibitory effect on PON1 activity. The barium, lithium, copper, zinc and mercurials have been found to inhibit PON1 activity in rat and human liver ¹¹⁰. In case of mercurials and copper, studies suggest that a free thiol group on the Cys 285 residue may be the molecular target ^{110, 111}. More recent experiments have revealed that cadmium, iron, zinc and mercurials are highly potent in vitro inhibitors of PON1 192R activity and can inhibit upto 80% of activity. However, in vivo the PON Q192 appears to be less sensitive to inhibition by metals, with the exception of lead ¹¹². In vivo exposures of mice to cadmium, methylmercury or dietary iron leading to metal serum concentration of higher than $1\mu\text{M}$ has failed to alter PON1 activity in plasma and liver ¹¹². This is probably due to binding of metal to proteins in plasma leading to protection of PON1.

8.2 Classical inducers

A few studies have investigated whether PON1 is an inducible enzyme. Phenobarbital, a classical enzyme inducer which is particularly effective toward certain isozymes of cytochrome P450 (e.g. CYP2B), caused a modest (20-150%) increase in hepatic PON1 activity ¹¹³, with a concomitant increase in liver RNA levels ¹¹⁴. However, serum PON1 activity has been found to be decreased in patients on (40-50%) phenobarbital treatment ¹¹³⁻¹¹⁶.

9. Modulation of PON1 by life-style factors

Enzyme inducers, environmental chemicals, physiological and pathological states, and dietary and lifestyle factors have shown their effects on PON1 activity.

9.1 Age

In humans, PON1 serum arylesterase activity increases from birth to 15-25 months of age, when it seems to reach a plateau whose level is determined by the 5' regulatory-region polymorphisms and the genetic background of the individual ¹¹⁴. In an adult, PON1 levels remain stable as no significant changes have been observed with age ^{117, 118}.

9.2 Enzyme inducers and environmental chemicals

3-Methylcholanthrene has been found to increase both serum and liver PON1 level in rats ⁹⁸ but not in mice ¹¹⁴. Administration of lipopolysaccharide, which mimics gram-negative infection, causes a transient decrease in serum and liver PON1 activity and in hepatic mRNA levels ^{114, 119}. The phytoalexin resveratrol is considered to be a major biologically active component contributing to the beneficial effect of wine ¹²⁰ and is known to modulate gene expression.

PON1 activity can vary depending on physiological conditions or pathological states. Serum PON1 activity is significantly decreased during pregnancy ¹²¹.

9.3 Smoking

Cigarette smoke extract is known to inhibit PON1 activity in vitro ¹²², suggesting that smoking may be detrimental to enzyme activity in vivo. James *et al.*,⁹¹ showed that PON1 serum concentration and activity were reduced in smokers compared with non-smokers. Ex-smokers had activities and concentrations comparable with those of non-smokers, suggesting a reversible influence of smoking on PON1. In vitro experiments found that inhibition of PON1 activity by a cigarette-smoke extract was antagonized by reduced glutathione (GSH), N-acetylcysteine, and 2-mercaptoethanol, suggesting that free thiols are central to the inhibitory effects ¹²².

9.4 Alcohol

Moderate wine consumption appears to have potential beneficial effects related to the prevention of CAD ¹²³. Wine consumption increases serum PON1 activity ^{124, 125}. Ethanol and other aliphatic alcohols have been shown to inhibit serum PON1 activity ¹²⁶; however, in middle aged men daily moderate alcohol consumption increased serum PON1 activity, with no differences between wine, beer, and spirits ¹²⁷. This increase may be due to the consumption of alcohol itself or to that of antioxidants, as similar results were obtained after consumption of red wine ¹²⁸ or pomegranate juice ^{129, 130}.

9.5 Diet

In both rabbit and transgenic mouse model, a proatherogenic diet caused a significant fall in PON1 activity, which correlated with a reduction in HDL-cholesterol ^{89, 131-133}. Diets with a high trans-unsaturated fat content can reduce PON1 activity ¹³⁴. In contrast, oleic acid from olive oil is associated with increased activity ^{135, 136}. Meals rich in used cooking fat which contains a high content of oxidized lipids, is followed by a significant fall in PON1 activity when fed to healthy men ¹³⁷.

PON1 is highly susceptible to inactivation by oxidation. In vitro, PON1 activity is protected by the anti-oxidant polyphenols quercetin and glabridin ¹³⁸, suggesting that dietary antioxidants may play a similar role in vivo. Some studies have shown that consumption of pomegranate juice which is rich in polyphenols and other antioxidants, can raise PON1 activity up to 20% in both humans and apoE knockout mice ¹³⁰.

10. Conclusions and future prospects

Human epidemiological studies and experimental work carried out so far provides convincing evidence that PON(s) play an important role in protection against atherosclerosis. Studies are required to elucidate the role of the PON genetic polymorphisms in this potentially important function of PON(s) and role in CAD and other related diseases. Since nutritional and environmental factors explain some of the individual variations in serum PON 1 activity, the enzyme is considered as a promising target for pharmaceutical intervention. Therefore, pharmacological modulation of PON1 activity or PON 1 gene expression could constitute a useful approach for the prevention of CAD.

11. Acknowledgement

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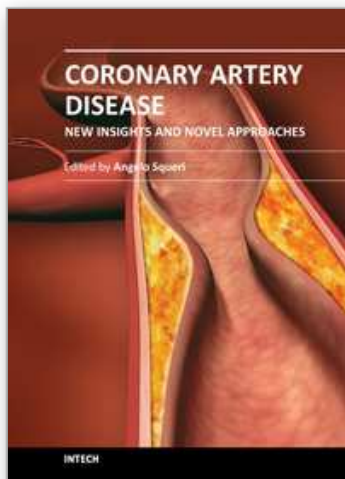
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Coronary Artery disease is one of the leading causes of death in industrialized countries and is responsible for one out of every six deaths in the United States. Remarkably, coronary artery disease is also largely preventable. The biggest challenge in the next years is to reduce the incidence of coronary artery disease worldwide. A complete knowledge of the mechanisms responsible for the development of ischaemic heart disease is an essential prerequisite to a better management of this pathology improving prevention and therapy. This book has been written with the intention of providing new concepts about coronary artery disease pathogenesis that may link various aspects of the disease, going beyond the traditional risk factors.

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University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
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InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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