

## Review Article

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# Paraoxonases : Structure, gene polymorphism & role in coronary artery disease

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**Paraoxonases (PONs) *i.e.* PON1, PON2, PON3 are basically lactonases. Of these, PON1 in addition has an efficient esterase activity and can hydrolyze organophosphates. The PONs prevent low density lipoprotein cholesterol (LDL-C) from peroxidation, thereby preventing atherosclerosis. The PON1 is exclusively associated with high density lipoprotein cholesterol (HDL-C) and its antioxidant activity is largely attributed to PON1 located on it. At present, PON1 status *i.e.* its activity and concentration, is considered to be more important than polymorphism alone, in prevention of coronary artery disease (CAD). Its activity has been found to be affected by a number of pharmacological agents, diet and other factors, thereby becoming a promising target for pharmacological intervention. The PON2 prevents cell mediated lipid peroxidation. However, little is known about the role of PON3. This review describes the structure, gene polymorphism, and factors affecting the activity of PONs, and their role in prevention of CAD.**

**Key words** Atherosclerosis - coronary artery disease - paraoxonases - polymorphism

## Introduction

Coronary artery disease (CAD) is a leading cause of morbidity and mortality all over the world including India<sup>1</sup> and its underlying pathogenetic mechanism is atherosclerosis<sup>2</sup>. Atherosclerosis is a progressive disease characterized by the accumulation of lipids and fibrous elements in the large arteries. Various risk factors have been implicated in the development of atherosclerosis and CAD. Of these, obesity, hypertension and dyslipidaemia co-exist more often in these patients than by a chance alone<sup>3,4</sup>. Epidemiological studies have shown an inverse relationship between low density lipoprotein-cholesterol (LDL-C) and high density

lipoprotein cholesterol (HDL-C) concentration as risk factor for the development of CAD and stroke<sup>5,6</sup>. Of all the risk factors for CAD identified from epidemiological studies, low serum HDL-C concentration is the single most important factor<sup>5</sup>. The antioxidant activity of HDL-C is largely due to its enzyme paraoxonase (PON1), located on it<sup>7</sup>. The other enzymes associated with HDL-C and playing role in its anti-oxidant activity are platelet activating factor acetyl hydrolase<sup>8</sup> and lecithin cholesterol acyl transferase<sup>9</sup>. Recently it has been shown that PON1 activity and concentration (PON1 status) may be more important than PON1 polymorphism alone<sup>10,11</sup>. However, limited studies are

available where in addition to gene polymorphism, paraoxonase (PON1) activity and concentration have been considered<sup>12</sup>. Except for some information on serum PON1 activity and development of CAD in North-west Indian Punjabis<sup>13</sup>, little is known about it in other Indian ethnic populations. In this review an attempt is made to describe various paraoxonases and their role in development of CAD.

### Paraoxonases (PONs)

The paraoxonases (PON1, PON2, PON3) are basically lactonases with one of the broadest known substrate specificities<sup>14,15</sup>. All three PONs metabolise 5-hydroxy cicosate traecom acid 1,5 lactone and 4-hydroxy docos ahexaenoic acid which are derived from arachdonic acid. PON3 exclusively hydrolyses lovastatin and spironolactone whereas organophosphates are exclusively hydrolysed by PON1 which has additional esterase activity.

Studies in the early 1990s led to the purification of rabbit and human paraoxonases<sup>16</sup> and subsequent cloning and sequencing of their respective cDNAs<sup>17</sup>. In addition to human PON1 gene, two additional PON genes, designated as PON2 and PON3 have been identified and all these three genes are located on the long arm of chromosome 7q 21.3–22.1<sup>18</sup>. 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 per cent identity at the amino acid level and 70 per cent identity at the nucleotide level<sup>19</sup>. PON1 is the first protein to be identified and is most studied.

### Paraoxonase 1 (PON1)

**PON1 gene:** The PON1 gene has two polymorphisms in the coding region and five in promoter region. PON1 gene substitution of glutamine (Q) by arginine (R) at position 192 and leucine (L) by methionine (M) at position 55 of coding region independently influence the PON1 activity and constitute the molecular basis for interindividual variability<sup>20,21</sup>. In addition to this coding region polymorphism, significant polymorphisms in promoter region have been reported, especially at position 107 which contribute to 22.4 per cent of the variation in paraoxonase gene expression and PON1 serum concentration<sup>22</sup>.

**Structure:** PON1 enzyme has a molecular mass of 43 kDa (355 amino acids). It has three additional nucleotide residues in exon 4 coding for amino acid

105 compared with PON2 and PON3. The PON1 cDNA encodes a protein of 355 amino acids from which only the amino terminal methionine residue is removed during secretion and maturation. The retained leader sequence is required for the association of PON1 with HDL particles<sup>23</sup> and in humans, serum PON1 is entirely associated with HDL-C<sup>7</sup>.

PON1 protein is synthesized mostly in liver and is released by a docking process, *i.e.* HDL particles transiently associate with the cell membrane and remove PON1 from the membrane<sup>24</sup>. Unlike PON2 and PON3, it has efficient esterase activity towards many organophosphates (OPs) including paraoxon, parathion and chlorpyrifos as well as nerve agents sarin and soman<sup>25</sup>.

**PON1 coding region polymorphism and CAD:** Of the two coding region polymorphisms, Q192R has been widely investigated as amino acid R192 is an important active site residue<sup>26</sup>. The Q192R polymorphism alters the ability of enzyme to protect LDL from oxidation *in vitro* with Q form being the most protective. The PON L55M polymorphism does not affect the interaction of PON1 with its substrates, but is associated with lower serum activity and concentration of the enzyme<sup>27</sup>. Leviev *et al*<sup>28</sup> found lower PON1 mRNA levels in individuals carrying the M allele. Differences in the ability of the Q192R and L55M polymorphic forms to protect LDL from oxidation have led to numerous case-control studies aimed at determining their contribution to the risk of developing CAD. The results from these studies have not been conclusive<sup>29</sup> as some show an association between PON1 Q192R or PON1 L55M and CAD, whereas other do not<sup>30</sup>. Wheeler *et al*<sup>31</sup> undertook meta-analysis of genetic epidemiological studies and found no association between PON1 55 or -108 or PON2 -310 polymorphism. However, a weak association was found between PON 192 polymorphism and CAD but it was concluded that this association (Q192R) was irrelevant. This meta-analysis did not take ethnicity into consideration and therefore, it is still possible that PON polymorphism may still be a risk factor in some individuals. These conflicting results may be due to inclusion of small number of patients from different populations and/or using different genotyping methods, different sampling strategies and different end points, making the outcomes difficult to interpret<sup>9</sup>. It is likely that other factors such as the large inter-individual variation in serum PON1 activity/concentration also has a significant role to play in the potential protective effect of PON1 against CAD<sup>32</sup>.

**PON1 promoter polymorphism and CAD:** Sequencing of the promoter gene of PON1 has led to the discovery of at least five polymorphisms with varying degrees of influence over its expression. These polymorphisms are located at positions -909/907 (C or G), -832/824 (A or G), -162 (A or G), -126 (C or G) and -108/-107 (C or T)<sup>33</sup>. This variation in the promoter polymorphism has been shown to be physiologically relevant as a good correlation was observed between type of variation and difference in serum PON1 concentration and activity<sup>34</sup>. Polymorphisms have also been detected in the 3' untranslated region of the PON1 gene, but their significance is unknown<sup>35</sup>.

Identification of a clinically significant polymorphism has been hampered by significant linkage disequilibrium between all the promoter polymorphisms. Haplotype analysis of two populations showed that the C (-107) T polymorphism is the main contributor to serum PON1 level variation, accounting for 23-24 per cent of the total variation<sup>33</sup>. Brophy *et al*<sup>35</sup> reported a slight contribution (1.1% total variation) from the A (-162) G site. The site at -909 and -832 makes little or no difference to serum PON1 levels<sup>34</sup>. As the -107 site appears to be most significant contributor to PON1 serum variation, it has been further investigated. A positive association between the low activity of T allele and vascular disease has been observed in young adults<sup>36</sup> and in patients with type II diabetes<sup>37</sup>.

There exists significant linkage disequilibrium between the promoter polymorphisms and the coding region polymorphisms. Brophy *et al*<sup>36</sup> detected linkage disequilibrium between -107 and the R 192 coding region polymorphism, which is associated with a lower level of protection against CAD. The -107 promoter 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 -107 variant<sup>38</sup>. Binding of sp1 to the -107 site is weaker in the presence of T than C, suggesting an effect of the polymorphism on sp1 binding<sup>39</sup>. Co-transfection of the PON1 promoter with a plasmid expressing sp1 resulted in a strong upregulation of promoter activity, supporting further the hypothesis that sp1 is important for PON1 expression<sup>39</sup>.

In an analysis of PON1 genotypes distribution Campo *et al*<sup>40</sup>, revealed a higher percentage of (-107)CC among octogenarians compared with controls and a significant difference in T (-107)C was observed in these with respect to PON1 activity and HDL.

In another study, however, the same authors could not find any significant association between PON1 (-107) T > C or coding polymorphism and early carotid atherosclerosis<sup>41</sup>. In a recent study, the increase in high activity variants G(-907) and G (-107) within the two allelic haplotypes have been found to be reversibly associated with extent of stenosis of coronary arteries. Though authors could not investigate the independent contribution of each of the G(-107) and G (-907) polymorphisms in the extent of stenosis, they could not find any association between A (-162) G polymorphism and extent of stenosis<sup>42</sup>.

**PON1 and CAD:** The most convincing link between PON1 activity and CAD comes from transgenic mouse studies<sup>43,44</sup>. HDL-C from PON1 'knockout' mice cannot prevent oxidation of LDL-C in a co-culture model simulating the arterial wall. These mice are more prone to atherosclerosis than the wild type as their macrophages contain more oxidized lipids due to reduced capacity to oxidize LDL-C. The apolipoprotein E (apo-E) knockout (apoE<sup>-/-</sup>) mouse is a well known model of atherosclerotic lesion development. However, the double knockout apoE<sup>-/-</sup>/PON1<sup>-/-</sup> has increased susceptibility to atherosclerosis over and above that caused by the lack of apoE, suggesting a role for PON1 in the prevention of the disease<sup>44</sup>. In contrast, mice overexpressing PON1 gene show protection against atherosclerosis in both wild type and apoE<sup>-/-</sup> background<sup>45</sup>.

The PON1 activity and concentration are highly variable in humans. The quality and quantity of enzyme in serum is likely to be more important in an individual for risk of developing cardiovascular disease. There is a wide variation (up to 13-fold) of PON1 serum concentration and activity between individuals and even within the same genotype groups. In addition to genetic polymorphisms, PON1 activity 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 and it is essential that PON1 serum concentration and/or activity (PON1status) be also measured in addition to gene polymorphism while evaluating its association with CAD<sup>46</sup>. A few studies which have included PON1 concentration and/or activity data, have found that PON1 concentration was reduced in CAD patients and that this reduction was independent of PON1 genotype. In a case-control study of CAD, Jarvik *et al*<sup>47</sup> found no effect of genotype on CAD unless PON1 activity was taken into consideration.

**Paraoxonase -1 gene expression:** Currently little is known about PON1 gene expression. The PON1 gene coding region polymorphism affects the catalytic activity of PON1 whereas that of promoter region affects the PON1 gene expression<sup>48</sup>. The association of these polymorphisms and factors modulating serum PON 1 activity determine the PON1 status.

**Pharmacological agents - PON 1 gene expression** has been shown to be affected in *in vitro* and *in vivo* studies by fenofibrate and statins<sup>49</sup>. Consumption of polyphenol (resveratrol) rich diets is associated with the beneficial cardiovascular effects because of the antioxidant properties of polyphenols. In addition to their antioxidant properties, polyphenols (resveratrol) also have been shown to modulate gene expression of PON1 leading to increased PON1 activity. Pharmacologically relevant concentrations of resveratrol (in the micromolar range) have been reported in plasma after moderate wine intake<sup>50</sup>. Biologically, resveratrol displays antioxidant and anti-inflammatory properties by inhibiting lipid peroxidation, thereby decreasing serum triglycerides and LDL levels *in vivo*<sup>51</sup>.

**Diabetes mellitus -** Various studies have found that PON1 activity is reduced in type 1 and type 2 diabetic patients<sup>52</sup>. The mechanism by which PON1 activity is reduced in diabetics is poorly understood, but could be a result of increased blood glucose concentration. Glycation can both inactivate PON1 and increase lipid peroxidation in HDL<sup>53</sup>. Lower PON1 activity has been observed in patients with type 2 diabetics having neuropathy and retinopathy. It has been postulated that decreased serum PON1 activity associated with diabetes may be playing a role in development of premature atherosclerosis and thereby CAD.

**Dietry and life style factors -** In both rabbit and transgenic mice models, pro-atherogenic diet caused a significant fall in PON1 activity which correlated with a reduction in HDL-C. In contrast, oleic acid in olive oil is associated with increased activity. Meals rich in cooking fat with higher amount of oxidized lipids, have been shown to lead to a significant fall in PON1 activity in healthy women<sup>54</sup> which is in agreement with *in vitro* studies where PON1 was found to be inactivated by oxidized lipids and oxidized LDL<sup>55</sup>. It has been shown that anti-oxidant vitamins such as vitamins C and E intake may influence the PON1 activity<sup>56</sup>. However, a recent study has shown that other than tobacco smoking, dietary and lifestyle factor have little role in modulating PON1 activity<sup>57</sup>.

**Alcohol -** The mechanism by which alcohol may lower the risk of developing CAD is poorly understood. Van der Gaag *et al*<sup>58</sup> showed that drinking 40 g/day of alcohol increased both PON1 activity and mass. There was no difference between red wine, beer or spirits, suggesting that it is not the red wine polyphenols alone that cause the effect. Similar results were obtained in a study<sup>59</sup>, which examined the effect of drinking alcoholic beer compared with non-alcoholic beer revealing that only alcoholic beer had a positive effect on PON1 activity. Moderate drinking significantly increases serum HDL-cholesterol and apoAI concentrations, which may account for the observed increase in PON1 concentration. Increased serum PON1 activity may be one of the factors which may be contributing to reduced CAD risk in moderate drinkers.

**Smoking -** Cigarette smoking has been found to be detrimental to PON1 activity. James *et al*<sup>60</sup> showed that PON1 serum concentration and activity were reduced in smokers compared with non smokers. Ex-smokers had PON1 activity and concentrations comparable to those of non smokers, suggesting reversibility of effect of cigarette smoke on its activity. Interestingly, smokers who also drank moderately or exercised regularly had PON1 activity similar to those of non smokers, suggesting that these activities can attenuate the effect of smoking on PON1 activity.

**Age -** A number of studies have shown that PON1 activity decreases with age<sup>61</sup>. It has been found that elderly have an increased susceptibility of HDL to oxidation. The PON1 Q192R polymorphism appears to play a role in this loss of activity due to ageing. The QQ homozygotes have been found to have further loss in enzyme activity with age<sup>62</sup>.

**Disease states -** Decreased PON1 activity has also been observed in a number of disorders such as alcoholic liver disease<sup>63</sup>, hepatitis-C<sup>64</sup> and human immunodeficiency virus infection<sup>65</sup>, neurological disorders like ALS<sup>66</sup>, Gulf War Syndrome and anxiety states<sup>67</sup>.

**APO-E polymorphisms -** Murphy *et al*<sup>67</sup> found significant interaction between APOE and PON 55 on PON activity. The MTMFR C677T polymorphism has been reported to be a risk factor for various diseases exclusively because of its adverse effects on homocysteine metabolism. The study revealed that this polymorphism adversely affected lipid metabolism<sup>68</sup>.

## **Paraoxonase 2 (PON2)**

There is little information available on PON2. PON2 mRNA is ubiquitously expressed in every human tissue,



with the 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 overexpressing PON2 are less able to oxidize LDL and show considerably less intracellular oxidative stress when exposed to either H<sub>2</sub>O<sub>2</sub> or oxidized phospholipid<sup>69</sup>.

**PON2 polymorphisms:** PON2 is the second member of the PON gene cluster on chromosome 7q21.3–22.1. The human PON2 gene has two common polymorphisms due to amino acid substitutions and these are designated as G/A148 and C/ S311<sup>70</sup>.

**PON2 polymorphism and CAD:** There are a few published reports of an association of PON2 gene polymorphisms and CAD. Pan *et al*<sup>71</sup> showed that the PON2 Ser311 polymorphism is associated with CAD. However, in another study between PON2 polymorphisms and CAD, no association was observed between increased risk of myocardial infarction except in smokers<sup>72</sup>. In another study it has been shown that PON2 expression increases in monocytes during their maturation into macrophages as a result of NADPH oxidase activation and this process is partly regulated by the transcription factor AP-1. The stimulation of PON2 may represent a compensatory mechanism against the superoxide dismutase radicals accumulation in cells and there by protection against atherogenesis<sup>73</sup>.

### Paraoxonase 3 (PON3)

PON3 is interposed between PON1 and PON2 in the PON gene cluster and is the least studied compared to PON1 and PON2. PON3 is a 40 kDa protein associated with the HDL. In contrast to PON1, PON3 has limited arylesterase activity. However, it rapidly hydrolyzes lactones such as statin prodrugs. PON3 is synthesized primarily in the liver. PON3 may provide a basal constitutive atheroprotective function, while the protective effect of PON1 is more variable<sup>74</sup>. Further studies such as the generation of PON3 knockout and transgenic mice models, may determine whether PON3 activity is required *in vivo* for the prevention of atherosclerosis.

### Future directions in clinical research

Human epidemiological and experimental studies provide convincing evidence that PONs 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 PONs and role in CAD and other related

diseases. Since nutritional and environmental factors explain some of the individual variations in serum PON1 activity, the enzyme is considered as a promising target for pharmaceutical intervention. Therefore, pharmacological modulation of PON1 activity or PON1 gene expression could constitute a useful approach for the prevention of CAD. Moreover, now 3-D structure of PON 1 has been determined<sup>75</sup>. The crystal structure unfolds both the overall fold of PON family and details of PON1 structure. It permits the postulation of the catalytic mechanism of the esterase and lactonase function of PONs. The mutagenesis data indicated these two activities of PON1 are catalyzed by HIS 115 and HIS 134 dyad mutations (G2E6) while the paraoxonase activity is not affected by dyad mutations and hence must be catalyzed by different residues. These need to be determined in future.

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