Frequency distribution of the single-nucleotide -108C/T polymorphism at the promoter region of the *PON1* gene in Asian Indians and its relationship with coronary artery disease

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Received: 17 September 2010 / Accepted: 21 January 2011 / Published online: 7 February 2011
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Abstract A single-nucleotide promoter region polymorphism (-108C/T) of the paraoxonase (*PON1*) gene had been suggested to influence an individual's susceptibility to coronary artery disease (CAD). No data is available on this polymorphism from India. One hundred seventy-eight healthy individuals and 204 angiographically proven CAD patients were recruited to get baseline data on the frequency distribution of the -108C/T polymorphism in normal people of Asian Indian ethnicity and its relation with the risk of CAD. Polymerase chain reaction followed by restriction fragment length analysis was used as the method for genotyping. Blood samples were used for DNA isolation. In the normal subjects, the genotypes were distributed as CT (43.26%)>CC (30.34%), >TT (26.4%). The allele frequency of the C allele was 0.52, and that of the T allele was 0.48. The patients showed a similar pattern, but the TT genotype was about two times more frequent in the controls than in patients. Odds ratios for developing CAD for individuals with CT, TT, and CT + TT genotypes were 0.89 (0.50–1.59), 0.56 (0.27–1.08), and 0.76 (0.44–1.29), respectively (at 95% confidence

interval), when compared to CC homozygous people (age- and sex-adjusted, $p=0.114$, all genotypes compared). This suggested a trend for the T allele as protective against CAD. This first report on the frequency distribution of the -108C/T polymorphism in people of Asian Indian ethnicity suggests that the normal distribution is similar to that observed for the Chinese, Japanese, and Latino people, but the disease association is unique. The TT genotype and the T allele which are widely found associated with the risk of CAD showed a protective trend in this study.

Keywords PON1-108C/T · Coronary artery disease · Asian Indians

Introduction

Serum paraoxonase (PON, arylalkylphosphatase) is a glycoprotein which circulates in the dense HDL subfraction that also contains Apo-E and Apo-J (Bergmeier et al. 2004). Human serum paraoxonase (E.C.3.1.1.2) catalyzes the hydrolysis of the organophosphate paraoxon to the nontoxic products, p-nitrophenol and diethylphosphoric acid (Aldridge 1953a, b; Erdos and Boggs 1961). Much of the early interest in this enzyme was related to its role in organophosphate pesticide toxicity. Paraoxonase has also been recognized as a key contributor to the anti-LDL oxidant functions of HDL (Garner et al. 1998). It is involved in the prevention of LDL oxidation and destruction of pro-inflammatory oxidized phospholipids present in oxidized LDL (Durrington et al. 2001). It counteracts the transition metal ion-mediated oxidation of LDL and also catalyzes the breakdown of compounds in oxidized LDL. It reduces the

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lipid peroxide content in arterial lesions and prevents the oxidation of HDL itself, which would otherwise lead to HDL losing function (Aviram et al. 2000). Transgenic mice deficient in this enzyme showed significantly higher rates of atherosclerosis when fed a high cholesterol diet (Shih et al. 1998). Variations in paraoxonase activity accounted for 20% of the variation in the prevention of lipid peroxidation by HDL in one study (Mackness and Durrington 1995). Paraoxonase activity may thus be an independent risk-determining factor for atherosclerotic vascular disease.

There are at least five known polymorphisms in the area upstream of the *PON1* structural gene, -108C/T, -126 G/C, -162A/G, -832 G/A, and -909 G/C (Brophy et al. 2001a, b). The numbering of these polymorphisms may show a slight variation among groups, though numbering begins at the base immediately preceding the translation start codon in all studies.

Earlier studies on *PON1* polymorphisms have demonstrated substantial variations in the genotype and allele distribution between individuals of different races (Allebrandt et al. 2002; Sanghera et al. 1998; Garin et al. 1997; Srinivasan et al. 2004; Pati and Pati 1998; Agrawal et al. 2009), which in turn could have a significant impact on the risk assessment for coronary artery disease (CAD) in a population.

The -108C/T polymorphism (sometimes denoted as -107) is believed to be the most important of the promoter region polymorphisms of *PON1*, and it alters the expression of this enzyme (Brophy et al. 2001a, b; Leviev and James 2000). The locus lies within the GGCGGG binding site for transcription factor Sp1 that has been shown in several studies to affect promoter activity (Leviev and James 2000; Sun et al. 1995; Osaki et al. 2004). The binding of the transcription factor is weaker in the presence of T than with C (Osaki et al. 2004).

Asian Indians are at a very high risk of coronary artery disease in comparison to other ethnic groups because of genetic predisposition and departure from traditional lifestyles. The normal level of serum HDL has been found to be lower in Asian Indians than in other ethnic groups, and low serum HDL has been demonstrated as a major risk factor for CAD in this population (Chhabra et al. 2005; Mahajan and Bermingham 2004). Since paraoxonase is associated with HDL and is pivotal to the cardio-protective functions of HDL, the relationship of the *PON1* gene polymorphisms with coronary artery disease needs to be explored in the Asian Indian subjects.

Thus far, studies on Asian Indians have focused on the coding-region polymorphisms (Agrawal et al. 2009; Pati and Pati 1998; Sanghera et al. 1998). This study aimed at assessing the distribution of the -108C/T polymorphism in normal individuals of Asian Indian ethnicity living in the city of Delhi and surrounding areas

(of approximately 100-km radius) and its relationship with the risk for developing coronary artery disease.

Methods

Two hundred-four patients, chosen randomly from among those people visiting the outpatient clinics or those admitted in the wards of the Department of Cardiology at AIIMS for CAD, and 178 CAD-free controls, chosen from families of patients, friends, and volunteers were included in the study after giving informed consent and completing a questionnaire. Persons receiving statin treatment or prophylaxis for CAD were excluded from both patient and control groups. Other coexisting cardiac pathology, major communicable and non-communicable diseases, endocrine dysfunction, drug abuse, and pregnancy were also grounds for exclusion. Patients with hypertension or diabetes, however, were not excluded. Assessment of putative patients through coronary angiography was done, and only those with stenosis greater than or equal to 70% of the diameter of one or more coronary arteries, or their primary branches, were included in the study.

Venous blood samples were drawn after an overnight fast by the study subjects. Genomic DNA was isolated from Leukocytes derived from whole blood.

The genotype of the -108C/T polymorphism was determined by PCR amplification with forward primer 5'-GACCG CAAGCCACGCCTTCTGTGCACC-3' and reverse primer 5' TATATTTAATTGCAGCCGCAGCCCTGCTGGGG CAGCGCCGATT-GGCCCCGCCG-3' with 5% dimethyl sulfoxide and *Taq* polymerase (Fermentas). The thermocycling consisted of denaturation at 94°C for 45 s, annealing at 64°C for 1 min, and extension at 72°C for 45 s for 35 cycles. The reverse primer creates a *Bst*UI site when a "C" is present at -108. For detection of -108C/T polymorphism, about 10 µl of PCR product was digested at 60°C overnight with 5 units of *Bst*UI restriction enzyme (New England Biolabs) in the presence of 1.5 µl of 10× restriction buffer provided with the restriction enzyme and 3 µl of water. The digested PCR product was run on 12% non-denaturing polyacrylamide gel using 1× TBE buffer at 250 V for at least 1 h and 30 min. The digested PCR fragments were visualized by silver staining of the gels. DNA sequencing and polymorphism analysis was based on PCR followed by purification by PCR purification kit (Fermentas) according to the manufacturer's protocol. The purified product was sequenced at MWG Biotech (Bangalore, India) by ABI Prism 3730 (Applied Biosystem; Foster City, CA, USA) as per manufacturer's instructions in an automated sequencing machine using the dye terminator labeling method. The sequence analysis was carried out using Sequencing Analysis (version 5.1, Applied Biosystems) and SeqMan (version 5.07,

DNASTAR) softwares. The homozygous and heterozygous alleles were scored manually.

Allele and genotype frequencies in the study subjects were estimated by gene-counting method. Chi-square goodness-of-fit was used to verify that the genotype and allele distributions for each of the polymorphisms were in agreement with those expected from a population in Hardy–Weinberg equilibrium. Chi-square test was also applied to compare genotypic frequencies between the patient and control groups. Contingency table approach (Fisher's RxC test) was used to determine if there are significant differences in allele frequencies among the groups of individuals. Multiple logistic regression analysis was used to evaluate the effect of genotypes on CAD severity after adjusting for age and sex. Odds Ratios (OR) and 95% confidence intervals (CI) were calculated from the β coefficients to their standard errors. The statistical analysis was performed using STATA 9.0 (Texas, USA). Statistical significance level was set at $p < 0.05$.

Results

In all, 178 control and 204 patient samples were analyzed. The mean age of the controls was 45.93 ± 10.43 years, while that of the patients was 55.62 ± 8.62 years. Males formed 64% and 86% of the controls and patients, respectively. The proportion of persons with histories of smoking, diabetes mellitus, and hypertension was higher in the patients than in the controls but was a minority in both groups. Within the

patient group, there were 88 individuals with single vessel disease (SVD), 55 with double vessel disease (DVD), and 61 with triple vessel disease (TVD) (data not tabulated). Of the 174 normal subjects studied, the heterozygous CT genotype was most frequent ($n=77$, 43.26%), followed by the homozygous CC ($n=54$, 30.34%), and TT ($n=47$, 26.4%) genotypes. The allele frequency of the C allele was 0.52, and that of the T allele was 0.48 (Table 1). The observed genotype frequencies were in agreement with those expected from the Hardy–Weinberg principle (Table 1). The distribution of PON1-108C/T genotypes and alleles in the 204 patients with CAD was in agreement with the Hardy–Weinberg equilibrium. When compared to the normal distribution (Table 1), the frequency of the TT genotype was 33% lower in patients. Logistic regression was used to assess whether people carrying the T allele are at lower risk of developing CAD as compared to those with the CC genotype. The age- and sex-adjusted odds ratio (OR) for developing CAD for individuals with TT genotype, when compared to CC homozygotes, was 0.56 (95% CI 0.27–1.08). The adjusted odds ratio for all T allele carriers (CT + TT) was 0.76 (95% CI 0.44–1.29), while that for CT genotype individuals was 0.89 (95% CI 0.50–1.59). Odds ratios suggested only a trend for T allele to be protective against CAD, which was not statistically significant. The genotype distribution among patients in SVD, DVD, and TVD groups did not deviate significantly from the Hardy–Weinberg equilibrium (SVD, $\chi^2=0.14$, $p=0.232$; DVD, $\chi^2=0.560$, $p=0.454$; TVD, $\chi^2=1.188$, $p=0.275$; SVD +

Table 1 Genotype and allele distribution of the PON1-108C/T polymorphism in patients and controls

Group	Number and percentage of individuals with particular genotype			Number and proportion of particular allele	
	CC	CT	TT	C	T
Controls	54 (30.34%)	77 (43.26%)	47 (26.4%)	185 (0.52)	171 (0.48)
Patients	67 (32.84%)	101 (49.50%)	36 (17.64%)	235 (0.58)	173 (0.42)
Genotypes					
Odds ratios to develop CAD (95% CI)	CC 1 (Referent)	CT 0.89 (0.50–1.59)	TT 0.56 (0.27–1.08)	CT + TT 0.76 (0.44–1.29)	

Hardy–Weinberg equilibrium calculations (Fisher's exact test):

Controls: $\chi^2=3.171$, $p=0.074$

Patients: $\chi^2=0.037$, $p=0.845$

Genotype comparison: patients compared to controls

All genotypes compared: $\chi^2=4.341$, $df=2$, $p=0.114$, non-significant

Pooled genotypes: CC compared to CT + TT: $\chi^2=0.275$, $df=1$, $p=0.599$, non-significant

Allele comparison: patients compared to controls: $p=0.11$

Genotype comparison: controls, SVD, DVD, and TVD compared simultaneously

All genotypes compared: $\chi^2=8.24$, $df=6$, $p=0.22$, non-significant

Pooled genotypes: CC compared to CT + TT: $\chi^2=2.74$, $df=3$, $p=0.433$, non-significant

DVD + TVD, $\chi^2=0.064$, $p=0.799$; $df=1$ for all groups) (data not tabulated). Logistic regression was used to calculate the odds of T allele carriers to develop one or more diseased vessels. The OR of T-allele carriers, as compared to that of CC individuals, to develop SVD, DVD, and TVD were 0.59 (95% CI 0.32–1.08), 1.01 (95% CI 0.48–2.11), and 0.9 (95% CI 0.44–1.85) after adjusting for age and sex. This again suggested a trend for the T allele to have a protective role against developing SVD.

Discussion

The *PON1* gene has various polymorphisms that affect plasma paraoxonase enzyme levels and function. Two coding-region polymorphisms at p55 (L- > M, leucine- > methionine) and p192 (Q- > R, glutamine- > arginine) have been studied extensively in various populations, including Asian Indians (Humbert et al. 1993; Sanghera et al. 1998; Pati and Pati 1998; Agrawal et al. 2009). The promoter region polymorphisms are less studied. Of these, the single-nucleotide-108C/T polymorphism appears to have a major impact on the expression of *PON1* and hence has been studied in different populations, with the CT genotype being the most common in most studies. Brophy et al. had hypothesized earlier that this may indicate some evolutionary pressure to maintain the -108C/T allele frequencies close to equal (Brophy et al. 2001b). However, the relative frequencies of the CT and TT genotypes and thus of the C and T alleles were different in different populations. The frequency of the T allele was higher than that of the C allele in Swiss, Italian, male Japanese, Mexican, and US Caucasian populations (Leviev and James 2000; Campo et al. 2004b; Suehiro et al. 2000; Inoue et al. 2000; Rojas-Garcia et al. 2005; Chen et al. 2003). Recent studies on healthy individuals from Hispanic/Latino and African-American populations have shown a dominant presence for the C allele (Chen et al. 2003; Catano et al. 2006). In mothers and neonates from these populations, it was CC, not CT, which was the most common genotype (Chen et al. 2003). There is no information available so far on the normal distribution of the -108 *PON1* promoter region polymorphism among Asian Indians. We studied 178 healthy volunteers from the northern plains of India to get this baseline data. The residents of this part of India, mostly Indo-Aryans, do not represent an ethnic homogeneity but do share common lifestyles and socio-cultural heritage. The genotype prevalence in this group was in the order of CT > CC > TT, with C > T, this pattern being very similar to that reported for Japanese, Chinese, and Latino-American populations (Holland et al. 2006; Yamada et al. 2002; Wang et al. 2003). This broad similarity in allele frequency and genotype distribution for the -108 polymor-

phism between different ethnic groups is quite unlike the wide variations seen between races for the *PON1* gene's L55M and Q192R polymorphisms (Allebrandt et al. 2002; Sanghera et al. 1998; Garin et al. 1997; Srinivasan et al. 2004; Pati and Pati 1998; Agrawal et al. 2009; Brophy et al. 2001b).

It has been found that the -108C/T polymorphism has a dominant effect on serum paraoxonase levels (Brophy et al. 2001a, b; Leviev and James 2000). Since the level of paraoxonase activity has been found to be associated with the risk of coronary artery disease, this polymorphism is envisaged to have a causal relationship with CAD. Studies on the relationship of *PON1*-108C/T with CAD are not many, and the findings are quite divergent. A large study on Chinese men by Wang et al. (2003) did not find any association of the -108 polymorphism with CAD, though it did suggest a role for other promoter polymorphisms (Wang et al. 2003). A meta-analysis by Wheeler et al. (2004), covering four studies, found no significant link of the -108 polymorphism with CAD (Wheeler et al. 2004). Two studies on European populations with hypercholesterolemia could not find any association between -108 genotype and carotid intima-media thickness (Roest et al. 2005; Campo et al. 2004a). Inoue et al. (2000) noted no significant difference in the -108 allele distribution between normal individuals and those with type II diabetes mellitus (Inoue et al. 2000).

James et al. (2000) found the T allele to be a risk marker for CAD in a Swiss population of diabetics (James et al. 2000). However, that study looked at risk association in a universe of patients who already had type II diabetes mellitus, and this might have been a modifying factor. Leviev et al. (2001), also studying a Swiss population, found the -107C allele to be associated with a lower risk of coronary artery disease in persons younger than 60 (Leviev et al. 2001). However, in the overall population and in persons older than 60, there was no relationship of the polymorphism to CAD. They hypothesized that the lower paraoxonase activity in older individuals nullifies the protective effect of the C allele. However, Campo et al. (2004a, b), who studied a population of healthy octogenarians of both sexes in Sicily, found the proportion of C-allele carriers to be significantly higher in the octogenarians as against that of younger controls (Campo et al. 2004b). This suggested a protective role for the C allele in aging individuals, though the overall frequency of the C allele was lower as compared to that of other ethnic groups. In most of the studies, the T allele was found associated with low levels of serum paraoxonase.

In our study, the frequency of the TT genotype was higher in controls than in patients. For individuals with TT genotype, as compared to CC homozygotes, the OR to develop CAD was 0.56 after age and sex adjustment. Carriers of the "T" allele (CT + TT), when compared to CC homozygotes, had an OR

for developing CAD of 0.76 after age and sex adjustment. These results suggest a protective trend of the T allele against CAD. While studying the influence of this polymorphism on the severity of CAD, odds ratio did suggest a protective trend of the T allele against SVD but not against DVD and TVD. This trend, however, is very different from the role suggested in the literature for the T allele, that it increases the risk of CAD.

At present, we are unable to explain our findings; but we can speculate on differences in the nature of gene–gene interactions and gene–environment interactions in people of Asian Indian ethnicity, which may be predisposing them to a higher risk of CAD. The strength of linkage among *PON1* loci varies between different ethnic groups (Chen et al. 2003). Therefore, further studies on the linkage of the -108C/T polymorphism with other loci of the *PON1* gene in Asian Indians are required, as they may be able to explain the findings we obtained. Although ours was a random sample, it was constrained by the number of subjects recruited.

Conclusion

To conclude, to the best of our knowledge, this is the first report on the PON1-108C/T polymorphism in Asian Indians. In healthy volunteers, the heterozygous CT was the major genotype, as had been noted previously in Japanese, Chinese, Caucasian, and Mexican populations. However, the comparative frequencies of the CC and CT genotypes and C and T alleles resembled only the Chinese, Japanese, and Latino–American populations. Our findings suggested a trend for T allele as protective against CAD. This finding was novel, different from what has been suggested by studies on other population groups. Variations among our study subjects in the extent of gene–gene and gene–environment interactions may be responsible for this difference. A larger-scale study on the association of this polymorphism with other loci, levels of paraoxonase enzyme, lipid profile, and other CAD risk factors is warranted.

Acknowledgments We thank the Council of Scientific and Industrial Research (CSIR), Government of India, and the Indian Council of Medical Research for funding our research. We also thank Ms. Roopa Bandooni for the technical assistance rendered. We also would like to express our gratitude to all the persons who volunteered to donate their blood samples for the purpose of our study.

Competing interests None.

References

Agrawal S, Tripathi G, Prajnya R, Sinha N, Gilmour A, Bush L, Mastana S (2009) Paraoxonase 1 gene polymorphisms contribute to coronary artery disease risk among north Indians. *Indian J Med Sci* 63(8):335–344

- Aldridge WN (1953a) Serum esterases. I. Two types of esterase (a and b) hydrolysing p-nitrophenyl acetate, propionate and butyrate, and a method for their determination. *Biochem J* 53(1):110–117
- Aldridge WN (1953b) Serum esterases. II. An enzyme hydrolysing diethyl p-nitrophenyl phosphate (e600) and its identity with the a-esterase of mammalian sera. *Biochem J* 53(1):117–124
- Allebrandt KV, Souza RL, Chautard-Freire-Maia EA (2002) Variability of the paraoxonase gene (PON1) in Euro- and Afro-Brazilians. *Toxicol Appl Pharmacol* 180(3):151–156
- Aviram M, Hardak E, Vaya J, Mahmood S, Milo S, Hoffman A, Billicke S, Draganov D, Rosenblat M (2000) Human serum paraoxonases (PON1) q and r selectively decrease lipid peroxides in human coronary and carotid atherosclerotic lesions: PON1 esterase and peroxidase-like activities. *Circulation* 101(21):2510–2517
- Bergmeier C, Siekmeier R, Gross W (2004) Distribution spectrum of paraoxonase activity in HDL fractions. *Clin Chem* 50(12):2309–2315
- Brophy VH, Hastings MD, Clendenning JB, Richter RJ, Jarvik GP, Furlong CE (2001a) Polymorphisms in the human paraoxonase (PON1) promoter. *Pharmacogenetics* 11(1):77–84
- Brophy VH, Jampsa RL, Clendenning JB, McKinsty LA, Jarvik GP, Furlong CE (2001b) Effects of 5' regulatory-region polymorphisms on paraoxonase-gene (PON1) expression. *Am J Hum Genet* 68(6):1428–1436
- Campo S, Sardo MA, Trimarchi G, Bonaiuto M, Castaldo M, Fontana L, Bonaiuto A, Bitto A, Saitta C, Saitta A (2004a) The paraoxonase promoter polymorphism (-107)t>c is not associated with carotid intima-media thickness in Sicilian hypercholesterolemic patients. *Clin Biochem* 37(5):388–394
- Campo S, Sardo MA, Trimarchi G, Bonaiuto M, Fontana L, Castaldo M, Bonaiuto A, Saitta C, Bitto A, Manduca B, Riggio S, Saitta A (2004b) Association between serum paraoxonase (pon1) gene promoter t(-107)c polymorphism, PON1 activity and HDL levels in healthy Sicilian octogenarians. *Exp Gerontol* 39(7):1089–1094
- Catano HC, Cueva JL, Cardenas AM, Izaguirre V, Zavaleta AI, Carranza E, Hernandez AF (2006) Distribution of paraoxonase-1 gene polymorphisms and enzyme activity in a Peruvian population. *Environ Mol Mutagen* 47(9):699–706
- Chen J, Kumar M, Chan W, Berkowitz G, Wetmur JG (2003) Increased influence of genetic variation on PON1 activity in neonates. *Environ Health Perspect* 111(11):1403–1409
- Chhabra S, Narang R, Lakshmy R, Das N (2005) APOA1-75G to A substitution associated with severe forms of CAD, lower levels of HDL and apoA-I among northern Indians. *Dis Markers* 21(4):169–174
- Durrington PN, Mackness B, Mackness MI (2001) Paraoxonase and atherosclerosis. *Arterioscler Thromb Vasc Biol* 21(4):473–480
- Erdos EG, Boggs LE (1961) Hydrolysis of paraoxon in mammalian blood. *Nature* 190:716–717
- Garin MC, James RW, Dussoix P, Blanche H, Passa P, Froguel P, Ruiz J (1997) Paraoxonase polymorphism Met-Leu54 is associated with modified serum concentrations of the enzyme. A possible link between the paraoxonase gene and increased risk of cardiovascular disease in diabetes. *J Clin Invest* 99(1):62–66
- Garner B, Waldeck AR, Witting PK, Rye KA, Stocker R (1998) Oxidation of high density lipoproteins. II. Evidence for direct reduction of lipid hydroperoxides by methionine residues of apolipoproteins AI and AII. *J Biol Chem* 273(11):6088–6095
- Holland N, Furlong C, Bastaki M, Richter R, Bradman A, Huen K, Beckman K, Eskenazi B (2006) Paraoxonase polymorphisms, haplotypes, and enzyme activity in Latino mothers and newborns. *Environ Health Perspect* 114(7):985–991
- Humbert R, Adler DA, Distech CM, Hassett C, Omiecinski CJ, Furlong CE (1993) The molecular basis of the human serum paraoxonase activity polymorphism. *Nat Genet* 3(1):73–76

- Inoue M, Suehiro T, Nakamura T, Ikeda Y, Kumon Y, Hashimoto K (2000) Serum arylesterase/diaxoxonase activity and genetic polymorphisms in patients with type 2 diabetes. *Metabolism* 49 (11):1400–1405
- James RW, Leviev I, Ruiz J, Passa P, Froguel P, Garin MC (2000) Promoter polymorphism t(-107)c of the paraoxonase PON1 gene is a risk factor for coronary heart disease in type 2 diabetic patients. *Diabetes* 49(8):1390–1393
- Leviev I, James RW (2000) Promoter polymorphisms of human paraoxonase PON1 gene and serum paraoxonase activities and concentrations. *Arterioscler Thromb Vasc Biol* 20(2):516–521
- Leviev I, Righetti A, James RW (2001) Paraoxonase promoter polymorphism t(-107)c and relative paraoxonase deficiency as determinants of risk of coronary artery disease. *J Mol Med* 79 (8):457–463
- Mackness MI, Durrington PN (1995) HDL, its enzymes and its potential to influence lipid peroxidation. *Atherosclerosis* 115 (2):243–253
- Mahajan D, Bermingham MA (2004) Risk factors for coronary heart disease in two similar Indian population groups, one residing in India, and the other in Sydney, Australia. *Eur J Clin Nutr* 58 (5):751–760
- Osaki F, Ikeda Y, Suehiro T, Ota K, Tsuzura S, Arai K, Kumon Y, Hashimoto K (2004) Roles of sp1 and protein kinase c in regulation of human serum paraoxonase 1 (PON1) gene transcription in hepg2 cells. *Atherosclerosis* 176(2):279–287
- Pati N, Pati U (1998) Paraoxonase gene polymorphism and coronary artery disease in Indian subjects. *Int J Cardiol* 66(2):165–168
- Roest M, Jansen AC, Barendrecht A, Leus FR, Kastelein JJ, Voorbij HA (2005) Variation at the paraoxonase gene locus contributes to carotid arterial wall thickness in subjects with familial hypercholesterolemia. *Clin Biochem* 38(2):123–127
- Rojas-Garcia AE, Solis-Heredia MJ, Pina-Guzman B, Vega L, Lopez-Carrillo L, Quintanilla-Vega B (2005) Genetic polymorphisms and activity of PON1 in a Mexican population. *Toxicol Appl Pharmacol* 205(3):282–289
- Sanghera DK, Saha N, Kamboh MI (1998) The codon 55 polymorphism in the paraoxonase 1 gene is not associated with the risk of coronary heart disease in Asian Indians and Chinese. *Atherosclerosis* 136(2):217–223
- Shih DM, Gu L, Xia YR, Navab M, Li WF, Hama S, Castellani LW, Furlong CE, Costa LG, Fogelman AM, Lusis AJ (1998) Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis. *Nature* 394(6690):284–287
- Srinivasan SR, Li S, Chen W, Tang R, Bond MG, Boerwinkle E, Berenson GS (2004) Q192r polymorphism of the paraoxonase 1 gene and its association with serum lipoprotein variables and carotid artery intima-media thickness in young adults from a biracial community. The Bogalusa Heart Study. *Atherosclerosis* 177(1):167–174
- Suehiro T, Nakamura T, Inoue M, Shiinoki T, Ikeda Y, Kumon Y, Shindo M, Tanaka H, Hashimoto K (2000) A polymorphism upstream from the human paraoxonase (PON1) gene and its association with PON1 expression. *Atherosclerosis* 150(2):295–298
- Sun XM, Neuwirth C, Wade DP, Knight BL, Soutar AK (1995) A mutation (t-45c) in the promoter region of the low-density-lipoprotein (LDL)-receptor gene is associated with a mild clinical phenotype in a patient with heterozygous familial hypercholesterolaemia (FH). *Hum Mol Genet* 4(11):2125–2129
- Wang X, Fan Z, Huang J, Su S, Yu Q, Zhao J, Hui R, Yao Z, Shen Y, Qiang B, Gu D (2003) Extensive association analysis between polymorphisms of PON gene cluster with coronary heart disease in Chinese Han population. *Arterioscler Thromb Vasc Biol* 23 (2):328–334
- Wheeler JG, Keavney BD, Watkins H, Collins R, Danesh J (2004) Four paraoxonase gene polymorphisms in 11,212 cases of coronary heart disease and 12,786 controls: meta-analysis of 43 studies. *Lancet* 363(9410):689–695
- Yamada Y, Izawa H, Ichihara S, Takatsu F, Ishihara H, Hirayama H, Sone T, Tanaka M, Yokota M (2002) Prediction of the risk of myocardial infarction from polymorphisms in candidate genes. *N Engl J Med* 347(24):1916–1923