

Paraoxonase-1 (PON1) promoter region polymorphisms, serum PON1 status and coronary heart disease

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Abstract

Introduction: Serum paraoxonase-1 (PON1) retards the oxidation of low-density lipoprotein and cell membranes and is atheroprotective. Polymorphisms in the promoter region of the PON1 gene have been shown to affect serum PON1 levels and have been related to the presence of coronary heart disease (CHD) in some studies. However, contradictory results have been reported with regard to promoter region polymorphisms and CHD presence; therefore we have re-examined the effects of the C-108T and G-909C promoter polymorphisms on PON1 levels and the presence of CHD in a large case-control study.

Material and methods: Paraoxonase-1 activity, concentration and the C-108T and G-909C polymorphisms were measured in 417 people with CHD and 282 healthy controls, in a case control study.

Results: Paraoxonase-1 activity and concentration were significantly lower in the CHD population compared to controls regardless of their C-108T and G-909C genotype ($p < 0.001$). Paraoxonase-1 activity was significantly different in the C-108T genotypes in both the control and CHD groups in the order TT < TC < CC ($p < 0.01$). Paraoxonase-1 concentration was significantly different in the CHD group only in the G-909C genotype in the order GG > GC > CC ($p < 0.01$). Haplotype analysis revealed no consistent patterns of PON1 activity in the CHD population; however, in the controls PON1 activity differed between haplotypes GGCC > GGTC > GGTT ($p < 0.05$) and GCCC > GCTC > GCTT ($p < 0.02$). Neither promoter polymorphism was associated with CHD presence.

Conclusions: Paraoxonase-1 status was significantly lower in people with CHD and was affected by the promoter region polymorphisms.

Key words: paraoxonase-1, coronary heart disease, genetic polymorphisms, high-density lipid, oxidation.

Introduction

The paraoxonase (PON) multi-gene family comprises 3 members, PON1, PON2 and PON3 [1]. The genes for all 3 members of the family are widely expressed in mammalian tissues [2]; however, PON1 and PON3 are predominantly located in the plasma associated with high-density lipoprotein (HDL) while PON2 is not found in the plasma but has a wide cellular distribution [3]. PON1, PON2 and PON3 all retard the proatherogenic oxidative modification of low-density lipoprotein (LDL) and cell membranes and are therefore considered to be anti-atherogenic [4]. Paraoxonase-1 is now considered to be a major factor in the antioxidative activity of HDL [3].

The transgenic expression of human PON1 in various mouse models of atherosclerosis has been shown to retard or reverse atherosclerosis by mechanisms which include a reduction in circulating and aortic oxidised LDL (ox-LDL), a reduction in macrophage oxidative stress and foam cell formation, an increase in reverse cholesterol transport and a normalisation of endothelial function [5-7]. Several prospective studies have shown that low PON1 is an independent risk factor for new coronary events, independent of all other risk factors including HDL [8-12]. Although this finding is not universal [13, 14], low PON1 is a general feature of people who develop CHD.

The PON1 gene contains 2 exonic polymorphisms at positions 55(L/M) and 192 (Q/R), resulting in 4 possible isoenzymes. These isoenzymes hydrolyse many substrates, including lipid peroxides, at different rates [15, 16]. HDL from different individuals varies in how active it is in retarding the oxidation of LDL according to the particular PON1 isoforms it contains [15, 16].

Recently, several polymorphisms have been reported to be present in the promoter region of the PON1 gene [17, 18]. Research has indicated that these promoter polymorphisms are associated with highly significant differences in serum concentrations and activities of PON1 and are important genetic determinants of PON1 levels. It has also been reported that the promoter polymorphisms are also associated with the presence of CHD in non-diabetic and type 2 diabetic people [19, 20]. However, contradictory results have been reported with regard to promoter region polymorphisms and CHD presence; therefore we have re-examined the effects of the C-108T and G-909C promoter polymorphisms on PON1 levels and the presence of CHD in a large case-control study of non-diabetic patients with CHD and controls.

Material and methods

Subjects

Patients included in the study were all outpatients attending the cardiology departments at either Manchester Royal Infirmary, UK or the Royal Victoria Hospital, Blackpool, UK. All patients had angiographically proven CHD, which was visually assessed. All patients had stenosis severe enough to require intervention either by coronary angioplasty or surgery. No patients had sustained a myocardial infarction (MI) within 6 months prior to taking part in the study. Patients with diabetes, renal and hepatic disease were excluded from the study. Four hundred and seventeen sequential patients who fulfilled the inclusion criteria were studied. Fully informed consent was obtained and the Central Manchester Local Research Ethics Committee approved the study. One hundred and four patients were

receiving β -adrenoreceptor blocking drugs, 89 were taking calcium channel blockers and 154 were on lipid-lowering medication (151 statins, 3 fibrates). All patients were receiving aspirin 75 or 150 mg daily.

The control population consisted of 282 healthy subjects, who attended a routine health check at a general practice. Absence of CHD in the control population was assessed by use of a health questionnaire. None had a history suggestive of CHD. Subjects with diabetes, renal and hepatic disease were excluded. The demographic details of the patients and controls as well as their lipid and lipoprotein concentrations have been reported previously [21].

Methods

Venous blood was collected from all subjects between 9.00 and 11.00 am following fasting from 10.00 pm the previous day. Serum and plasma were isolated by low speed centrifugation. White cells were removed from the buffy coat of the plasma tube. HDL was isolated immediately by precipitating lower density lipoproteins with heparin/Mn²⁺. Other samples were stored at -20°C for no more than 2 months prior to analysis.

Analysis of PON1 activity

Paraoxonase-1 activity was measured by adding serum to Tris buffer (100 mmol/l, pH 8.0) containing 2 mmol/l CaCl₂ and 5.5 mmol/l paraoxon (O,O-diethyl-O-*p*-nitrophenylphosphate (Sigma Chemical Co.)). The rate of generation of *p*-nitrophenol was determined at 405 nm, 25°C, with the use of a continuously recording spectrophotometer (Beckman DU-68) as described previously [21].

Determination of PON1 concentration

Paraoxonase-1 concentration was determined using our in-house competitive ELISA employing rabbit anti-human PON1 monospecific antibodies as described previously [21].

Determination of PON1 genotype

DNA was extracted from the white cells and PON1 genotype for the 192 and 55 polymorphisms determined by PCR amplification and restriction enzyme digestion. The Q192R polymorphism was detected by Alw I digestion, and the L55M polymorphism was detected by Nla III digestion, as described [21].

The -108 polymorphism was determined by PCR amplification with primers GACCGCAAGCCACGCC-TTCTGTGCACC and TATATTTAATTGCAGCCGAG-CCCTGCTGGGGCAGCG-CCGATTGGCCCCGCCG. These primers create a Bst U I site (New England Biolabs) when a C is present at -108. The products were analysed after digestion by a 3% agarose gel (Sigma).

Presence of a -108C allele results in a digested band of 52 bp and 67 bp (instead of an undigested band of 119 bp). The -909G/C polymorphism was amplified by PCR with primers AACATGCTACTGTGGCATATATAATGCTC and TATTATAATATATATACATT-CACAGTAACAGCAGACAGCAGAGAAAAGA. The primers remove a second Bsm AI site when a G is present at -909, resulting in digested bands of 50 bp and 206 bp.

Statistical analysis

The Wilcoxon sign rank test was used to test for differences in variables with a non-Gaussian frequency distribution, namely PON1 activity towards paraoxon and PON1 concentration. Probabilities of < 0.05 were considered statistically significant. Differences in gene frequency were sought by the χ^2 test. The χ^2 test was also used to test whether genotype frequencies deviated from Hardy-Weinberg equilibrium and to test for linkage disequilibrium between pairs of polymorphisms. ANOVA was used to test for differences in parameters between genotypes.

Results

The PON1 parameters of the study populations are given in Table I. The PON1 activity and concentration were significantly lower in the CHD group as we have previously reported [21]. The gene frequencies of the PON1 exonic polymorphisms were as follows: for Q192R, $Q = 0.74$, $R = 0.26$ in the control subjects, $Q = 0.71$, $R = 0.29$ in the CHD population; and for L55M, $L = 0.64$, $M = 0.36$ in controls and $L = 0.65$, $M = 0.35$ in those with CHD. There

was no statistically significant difference between the frequencies of these polymorphisms in patients and controls.

Paraoxonase-1 activity was significantly different in the C-108T genotypes (Table II) in both the control and CHD groups in the order $TT < TC < CC$. Paraoxonase-1 concentration was not different between genotypes in the control population. However, there were significant differences in the CHD population in the order $GG > GC > CC$ of the -909 genotype (Table II). Both PON1 activity and concentration were significantly lower in the CHD group in all -108 and -909 genotypes. When the populations were divided by -108 and -909 haplotype (Figure 1), there were no consistent patterns of PON1 activity in the CHD population. However, in the controls PON1 activity differed between haplotypes in the order $GGCC > GGTC > GGTT$ ($p < 0.05$) and $GCCC > GCTC > GCTT$ ($p < 0.02$). This pattern was not repeated within the -909CC haplotypes (Figure 1). Neither the -108 nor the -909 genotypes influenced serum PON1 concentration.

There were no differences in allele frequencies between the controls and patients with CHD for either the -108 or the -909 polymorphism (Table III). However, all the polymorphisms studied, including the 55 and 192 coding region polymorphisms, were in linkage disequilibrium with each other (a minimum of $p < 0.01$). In a further analysis of the relationship between the PON1, -108 and -909 promoter polymorphisms and the presence of CHD, we calculated the odds of having CHD according to promoter polymorphism haplotype (Table IV). Again, we

Table I. PON1 parameters in the populations studied

Parameter	Healthy control	CHD
N	282	417
PON1 activity [nmol/min/ml serum]	214.6 (26.3-620.8)	122.8* (3.3-802.8)
PON1 concentration [μ g/ml]	89.1 (16.8-527.4)	71.6* (11.4-489.3)

Data are median (range). Significantly different from control * $p < 0.001$

Table II. PON1 activity and concentration in the control and CHD populations according to the PON1 promoter polymorphisms

PON1 polymorphism	PON1 activity [mmol/min/ml]		PON1 concentration [μ g/ml]		
	Controls	CHD	Controls	CHD	
-108	TT	159.8 (26.3-518.7)	85.9 ³ (24.6-494.1)	86.4 (25.1-527.4)	71.5 ³ (14.7-267.1)
	TC	211.8 (51.8-592.5)	131.9 ^{2,3} (8.3-802.8)	91.3 (16.8-509.5)	73.6 ³ (12.5-433.7)
	CC	245.3 ¹ (78.6-620.8)	145.6 ^{2,3} (41.7-653.9)	90.4 (27.3-464.7)	65.3 ³ (11.4-489.3)
-909	GG	211.8 (89.9-574.9)	156.6 ³ (56.0-663.4)	86.9 (27.3-464.7)	68.4 ³ (16.4-342.7)
	GC	227.0 (51.8-620.8)	113.6 ³ (8.3-802.8)	88.5 (16.8-490.5)	73.4 ³ (17.7-489.3)
	CC	191.4 (26.3-592.5)	101.0 ^{3,4} (30.3-653.9)	91.4 (25.1-527.4)	72.8 ³ (11.4-433.7)

Figures are median (range). ¹Significantly different from TT $p < 0.001$; ²significantly different from TT $p < 0.01$; ³significantly different from control $p < 0.001$, ⁴significantly different from GG $p < 0.01$

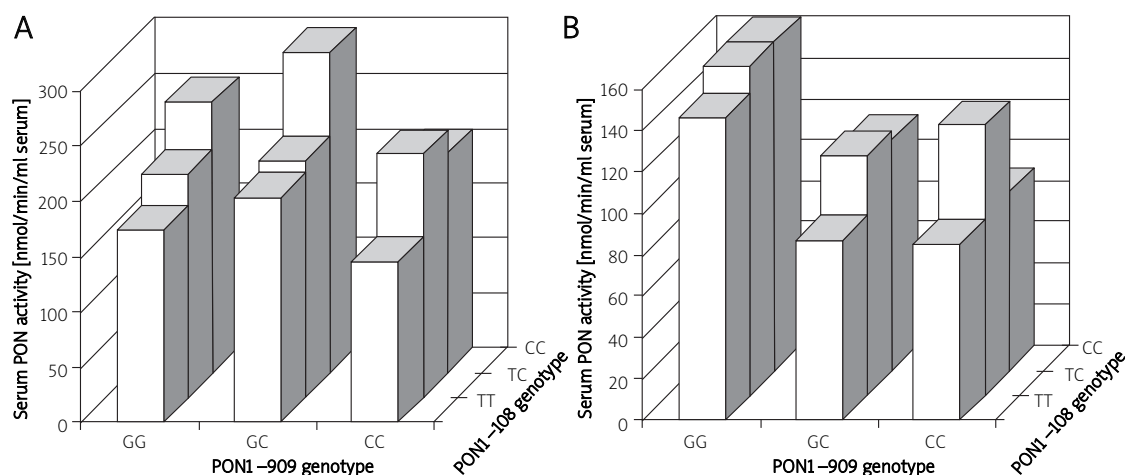


Figure 1. PON1 activity according to the -108, -909 polymorphism haplotypes: **A** – controls, **B** – CHD

could find no significant association between the promoter polymorphisms and the presence of CHD.

Discussion

Previous studies have shown a significant effect of the PON1 -108 and -909 promoter region polymorphisms of both PON1 activity towards phenylacetate and PON1 concentration [17, 18]. Using paraoxon as a substrate for PON1 we have been able to confirm a significant effect of the -108 polymorphism on PON1 activity in both controls and subjects with CHD, but we could only find an effect of the -909 polymorphism on PON1 activity in the group with CHD. The -108 polymorphism lies within a potential transcription factor Sp1 binding site and influences promoter activity resulting in different serum PON1 activities, the -108C polymorphism having greater promoter activity than the -108T polymorphism [22].

Neither the -108 nor the -909 promoter polymorphism was related to the presence of CHD. Results from other laboratories have reported a relationship between the presence of CHD and the PON1 -108 polymorphism [19, 20]. However, some contradictory results have been reported with elevated CHD risk being found in both -108C and -108T carriers in different populations [23, 24]. The reasons for these differences are unclear. However,

the gene frequencies reported in this study are consistent with those previously published [17-24]. Differences in the results presented here and those of other researchers could be due to different methodologies employed between studies and the use of different substrates (paraoxon as opposed to phenylacetate). Differences in PON1 activity, concentration and genotype distribution have long been known to occur between different populations throughout the world [25]. We and others have shown that subtle but significant differences exist in all PON1 parameters between European populations [26]. Genetic epidemiological studies of the relationship between PON1 coding region polymorphisms and the presence of CHD have been inconsistent. However, a recent meta-analysis showed a small but significantly increased odds ratio for the association of the PON1-192R allele and CHD, a relationship which was confounded by reporting bias [27]. Therefore, differences between populations

Table III. Allele frequencies for the PON1 promoter polymorphisms in the control and CHD populations

PON1 polymorphism		Control	CHD
-108	T	0.47	0.49
	C	0.53	0.51
-909	G	0.44	0.45
	C	0.56	0.55

Table IV. The odds of having CHD by promoter polymorphism haplotype

		-909 Polymorphism		
		GG	GC	CC
-108 Polymorphism	CC	1.29 (0.87-1.42)	0.42 (0.37-0.45)	1.01 (0.89-1.11)
	TC	1.01 (0.83-1.09)	0.98 (0.81-1.05)	1.21 (0.92-1.32)
	TT	2.06 (0.98-2.24)	1.06 (0.92-1.12)	0.97 (0.89-1.07)

Figures are odds (95% CI)

from differing geographical regions may be the major reason for any difference between this study and those reported earlier. It is interesting to note, in this context, that a recent study of the Han Chinese has indicated that the PON1-55L/M polymorphism does not exist in this population [28], although it is known to exist in other oriental populations [29]. It is also possible that the PON1 promoter polymorphisms only have a small effect on CHD development, which may be overwhelmed by competing influences such as dietary and environmental factors influencing CHD and PON1 activity more in some populations than in others. It is also worth noting that this (and the other studies) were cross sectional in design and as such have limitations such as the difficulty in proving causality, which may also have contributed to differences in results.

Given the putative role of PON1 in protecting against the development of atherosclerosis by hydrolysing lipid peroxides generated during the oxidation of LDL, it is surprising that such large differences in activity between the PON1 –108T/C alleles are not reflected in a genetic association with CHD. We were also unable to show any interactions between the promoter polymorphisms and plasma lipid/lipoprotein concentrations as has been recently suggested [30]. Correcting for any possible influence of the –909 polymorphism in the haplotype analysis failed to strengthen any association. This analysis did, however, show that in people with CHD, the –909 polymorphism negates the influence of the –108 polymorphism on PON1 activity (Figure 1), which may be the reason why there is no relationship between the PON1 promoter polymorphisms studied here and CHD. A similar analysis of the odds of having CHD by –108/55 and –108/192 haplotypes also showed no significant genetic association with CHD (result not shown). Such problems of inconsistency in results will only be clarified when large prospective studies of PON1 promoter polymorphisms and CHD are reported.

In conclusion, low PON1 activity (rather than promoter region polymorphisms) is associated with CHD presence. Developing strategies to raise PON1 activity in people at high risk of developing CHD may be beneficial in preventing/retarding the development of CHD.

Acknowledgments

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