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Association of *PON1* and *PON2* Polymorphisms with PON1 Activity and Significant Coronary Stenosis in a Tunisian Population

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Abstract *PON1* and *PON2* have attracted considerable attention as candidate genes for coronary heart disease because their enzymes function as key factors in lipoprotein catabolism pathways. We studied the distribution of *PON1* and *PON2* polymorphisms, including genotyping, lipid profile, and PON1 activity, and their association with PON1 activity and significant coronary stenosis (SCS) in a Tunisian population. PON1 activity was lower in patients with SCS than in controls. It increased with the R allele (QQ < QR < RR) in *PON1*-192 genotypes and with the L allele (MM < ML < LL) in *PON1*-55 genotypes. In the presence of metabolic syndrome and diabetes, *PON1*-192RR and *PON2*-311CC were associated with an increased risk of SCS and *PON1*-55MM seems to have lower risk. This association was evident among nonsmokers for *PON1*-55MM and among smokers for *PON1*-192RR and *PON2*-311CC. The GTGC haplotype seemed to increase the risk of SCS compared with the wild haplotype in a Tunisian population.

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Introduction

High-density lipoprotein (HDL) has a well-established inverse relationship with coronary artery disease risk (Tsompanidi et al. 2010). The oxidative modification of low-density lipoprotein (LDL) is a key event in the initiation and acceleration of atherosclerosis (Aoki et al. 2012). As an antiatherogenic mediator, HDL, aside from playing an important role in reverse cholesterol transport, protects LDL against oxidation (Farmer and Liao 2011). The antioxidant effect of HDL is determined by its enzymes, in particular paraoxonase 1 (PON1), an HDL-associated enzyme capable of hydrolyzing lipid peroxides (Efrat and Aviram 2010).

The *PON1* gene is clustered in tandem with *PON2* and *PON3* on the long arm of chromosome 7q21.3. Human PON enzymes, particularly PON1 and PON2, have been implicated in the pathogenesis of atherosclerosis (Précourt et al. 2011), and decreased PON activity has been documented in patients with coronary events (Mackness et al. 2003).

Studies have shown that PON1, which is expressed mainly in the liver, inhibits oxidation of LDL, preserves HDL function, increases cellular cholesterol efflux from macrophages, and decreases lipid peroxides in atherosclerotic lesions (Durrington et al. 2001). The antioxidant and anti-inflammatory properties of PON2, along with its intracellular localization, ubiquitous expression, and upregulation in times of oxidative stress, suggest an important physiological role for PON2 in host defense against atherosclerosis (Ng et al. 2006). Thus, PON2 plays a similar role to PON1 in the metabolism of lipids and lipoproteins (Mackness et al. 2002).

Several polymorphisms in the coding region of the *PON1* and *PON2* genes have been investigated in numerous studies for their association with coronary heart disease. The *PON1* gene has two common polymorphisms, which lead to a glutamine/arginine substitution at position 192 (Q192R) and a leucine/methionine substitution at position 55 (L55M). *PON2* also has two common polymorphic sites, which lead to an alanine/glycine substitution at position 148 (A148G) and a serine/cysteine substitution at position 311 (S311C) (Gupta et al. 2009). The association of these polymorphisms with increased risk for coronary heart disease was controversial (Shin et al. 2008; Pasdar et al. 2006).

Various population studies have reported interethnic differences in allele frequencies for the *PON1* and *PON2* polymorphisms. This variability suggests that ethnic differences, gene–gene interactions, and susceptibility to environmental factors might modulate the relationship between *PON* polymorphisms and coronary artery disease. Considering the important contribution of these polymorphisms to genetic susceptibility in atherosclerosis and the variability in allele frequencies among ethnic groups, the aim of the present study was to evaluate the distribution of the *PON1* polymorphisms Q192R (rs662) and L55M (rs854560) and the *PON2* polymorphisms A148G (rs12026) and S311C (rs7493) and to determine their

association with PON1 activity and significant coronary stenosis (SCS) in a Tunisian population.

Materials and Methods

Study Population

The sampling procedures of this study have been described previously in detail (Rejeb et al. 2008). In brief, 316 study subjects underwent coronary angiography because of myocardial infarction (113 patients), angina (169), thoracic pain (18), or heart failure (16), in the Cardiology Department at Sahloul University Hospital, Sousse, Tunisia. The patients were subdivided into two groups, those with and those without SCS, defined as a luminal narrowing $\geq$50 % of at least one major coronary artery. Metabolic syndrome was defined according to the 2005 International Diabetes Federation definition (Alberti et al. 2005).

Data on lifestyle factors were collected using a questionnaire administered by an interviewer. With informed consent, the participants underwent physical examinations and laboratory tests. Height and weight were measured, and body mass index was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Waist circumference was measured by a trained examiner from the narrowest point between the lower borders of the rib cage and the iliac crest. Blood pressure was measured in a sitting position after a 10 min rest period. Smoking was defined categorically as any positive or negative history of smoking. Hypertension was defined according to the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (Chobanian et al. 2003), and diabetes mellitus was diagnosed according to World Health Organization criteria (Alberti and Zimmet 1998). Patients taking lipid-lowering drugs were excluded. The study was approved by the local medical ethics committee.

Measurement of Lipid Profile

After overnight fasting and before coronary angiography, blood was collected from each subject. Serum total cholesterol, triglyceride, and HDL cholesterol concentrations were determined by a standard method using the Synchrom CX7 Clinical System (Beckman, Fullerton, CA, USA). When the triglyceride concentration was <4 mmol/L, LDL cholesterol concentration was calculated using the Friedewald formula (Friedewald et al. 1972). Otherwise, LDL cholesterol concentration was measured directly using the Synchrom CX7 Clinical System. Serum apolipoprotein (ApoAI and ApoB) concentrations were determined using the Immage Immunochemistry System (Beckman, Fullerton, CA, USA), based on immunonephelometric quantitation. ApoB/ApoAI and total/HDL cholesterol ratios were calculated.

Analysis of PON1 Activity

Serum PON1 activity was measured according to the modified method of Santanam and Parthasarathy (2007) using a Konelab 30 system. PON1 activity toward paraoxon was measured after paraoxon hydrolysis into *p*-nitrophenol and diethyl phosphate catalyzed by the enzyme. In brief, the activity was measured by adding 5 μL serum to freshly prepared Tris–NaOH buffer (0.26 M, pH 8.5) (Promega) containing 0.5 M NaCl (Fluka), 1.2 mM paraoxon (Sigma Aldrich), and 25 mM calcium chloride (Merck). After 30 s incubation at 37 °C, the liberation of *p*-nitrophenol was followed at 405 nm for 6 min.

DNA Extraction and Genotyping

Using a salting-out method (Miller et al. 1988), we extracted genomic DNA from whole blood samples treated with EDTA. The method established by Motti et al. (2001), with some modifications, was used to determine simultaneously the three common polymorphisms of the *PON* cluster (*PON1*-192, *PON1*-55, and *PON2*-311), using a multiplex polymerase chain reaction (PCR) DNA assay with mismatch primers to introduce a unique recognition site for the endonuclease *Hin*fI in the PCR products in the presence of the R allele of *PON1*-192, of the L allele of *PON1*-55, and of the S allele of *PON2*-311.

Three pairs of mismatch primers for genotyping polymorphisms of codon 192 and codon 55 in the *PON1* gene were used as described by Motti et al. (2001). The relevant primer sequences were, for *PON1*-192, 192-forward TTGAATGATATT GTTGCTGTGGGACCTGAG and 192-reverse CGACCACGCTAAACCCAAAT ACATCTCCCAG*a*A; for *PON1*-55, 55-forward GAGTGATGTATAGCCCCAG TTTC and 55-reverse AGTCCATTAGGCAGTATCTCC*g*; and for *PON2*-311, 311-forward GGTTCTCCGCATCCAGAACATT*ga*A and 311-reverse TGTTA AG*a*TATCGCACT*a*TCATGCC. (Lowercase italic letters indicate mismatched nucleotides.) This allowed a restriction site for *Hin*fI (G/ANTC) to be introduced into the DNA amplification products in the presence of the polymorphisms arginine-PON1-192/leucine-PON1-55/serine-PON2-311.

The multiplex PCR was carried out using a DNA thermal cycler (LP×2 Thermal Cycler, Thermo Electron Corp., Milford, NE, USA). Each amplification was performed with 100 ng genomic DNA in a 30 μL volume containing 200 μM dNTPs, 2 mM $MgCl_2$, 0.13 μM of both *PON1*-192 primers, 0.13 μM of both *PON1*-55 primers, 0.26 μM of both *PON1*-311 primers, and 2 U EuroTaq DNA polymerase (EuroClone, Italy). DNA was amplified with an initial step of 94 °C for 5 min; followed by 40 cycles of 1 min at 94 °C, 45 s at 55 °C, and 45 s at 72 °C; with a final extension step of 5 min at 72 °C. The multiplex PCR products were separated by electrophoresis on a 2 % agarose gel and visualized by ethidium bromide staining. Multiplex amplification products (15 μL) were digested with 2 U *Hin*fI ((Promega, Madison, WI, USA) in a total volume of 20 μL at 37 °C for 24 h. The digested products were separated by electrophoresis on a 4 % agarose gel and visualized by ethidium bromide staining on a UV transilluminator.

The *PON2*-148 polymorphism was genotyped by PCR restriction fragment length polymorphism analysis. Primers for genotyping this polymorphism were used as described by Oliveira et al. (2004). The 20 μL reaction for the single *PON2*-148 amplification contained 0.1 μM each primer, 100 μM dNTPs, 1.5 mM $MgCl_2$, and 1 U EuroTaq DNA polymerase (EuroClone). The PCR products of 232 base pairs (bp) PCR products were digested with 2 U *Fnu*4HI (New England BioLabs, Ipswich, MA, USA) at 37 °C for 24 h. Digested products were resolved by gel electrophoresis (4 % agarose gel) and visualized by ethidium bromide staining.

Statistical Analysis

Statistical analysis was performed by SPSS 16.0 for Windows. The biological variables were compared using one-way analysis of variance, then with Student's *t*-test or Fisher's exact test, and their values were reported as the mean ± standard deviation. A chi-square analysis was performed to determine Hardy–Weinberg equilibrium of each polymorphism studied in both groups with one degree of freedom. Genotype and allele frequencies were compared using a chi-square test. A single nucleotide polymorphism analyzer program (Yoo et al. 2005) was used to estimate linkage disequilibrium (LD) and to perform haplotype analyses. Pairwise LD coefficients were expressed as D′, which is the ratio of unstandardized coefficient to its minimal/maximal value. The odds ratio (OR) was calculated as a measure of the association of each *PON* genotype and haplotype with the phenotype. For each OR, the two-tailed p value and 95 % confidence interval (CI) were calculated; p was considered significant when it was <0.05. Adjusted ORs for potential confounders were determined using logistic regression analysis, and corresponding p values were reported.

Results

Population Characteristics

Patients with SCS had significantly lower HDL cholesterol ($p = 0.040$) and ApoAI ($p = 0.009$) concentrations, significantly higher triglyceride concentrations ($p = 0.022$), and a higher ApoB/ApoAI ratio ($p = 0.036$) than patients without SCS (Table 1). All variables with a p value <0.25 between the two groups were considered confounding factors for further OR adjustment.

Genotype Frequencies

Based on the multiplex PCR with mismatch primers, the three *PON* polymorphisms could be identified simultaneously. The multiplex PCR amplification yielded products of 111 bp for *PON1*-192, 144 bp for *PON1*-55, and 196 bp for *PON2*-311. After incubation with *Hin*fI, the presence of *PON1*-192R resulted in digestion of the 111 bp product into fragments of 77 and 34 bp, and the presence of *PON1*-55L resulted in digestion of the 144 bp product into fragments of 122 and 22 bp. The

Table 1 Clinical and biochemical characteristics of the study population with and without significant coronary stenosis

Characteristic	With SCS ($n = 212$)	Without SCS ($n = 104$)	p value
Sex ratio, men/women	1.97	1.26	0.010*
Age, in mean years ± SD	60.6 ± 10.6	59.4 ± 11.9	0.380
Smokers (%)	120 (56.6)	47 (45.2)	0.013*
Diabetics (%)	73 (34.4)	23 (22.1)	0.001*
Hypertension (%)	97 (45.7)	46 (44.2)	0.353
History of myocardial infarction (%)	91 (42.9)	13 (12.5)	<0.001*
Dyslipidemia (%)	33 (15.6)	9 (8.6)	0.027*
Cholesterol, mean mmol/L ± SD			
Total	5.029 ± 1.192	5.025 ± 1.087	0.970
HDL	0.961 ± 0.283	1.030 ± 0.276	0.040*
LDL	3.440 ± 1.167	3.330 ± 1.039	0.370
Total/HDL cholesterol	5.630 ± 2.040	5.410 ± 1.890	0.430
Triglyceride, mean mmol/L ± SD	1.614 ± 1.117	1.340 ± 0.647	0.022*
Apolipoprotein, mean g/L ± SD			
ApoAI	1.160 ± 0.379	1.290 ± 0.437	0.009*
ApoB	1.153 ± 0.376	1.110 ± 0.413	0.460
ApoB/ApoAI	1.057 ± 0.493	0.920 ± 0.290	0.036*

* Statistically significant ($p < 0.05$)

196 bp product of *PON2*-311 was digested into 173 and 23 bp in the presence of the S allele. For *PON2*-148, the 232 bp product was digested into 161 and 71 bp in the presence of the A allele (Fig. 1).

The polymorphisms of Q192R and L55M were distributed at the *PON1* locus, and the S311C and A148G polymorphisms at the *PON2* locus (Table 2). All genotypes, at each locus, followed Hardy–Weinberg equilibrium, with all chi-square values <3.84 and $p > 0.05$. The prevalence of homozygous individuals for the *PON1*-55M allele was higher in the group without SCS. Carriers of the *PON2*-311C allele were significantly higher in the SCS group. No mutated genotype for the *PON2*-148 polymorphism was found in the two groups. No difference in allele frequency was observed between the two groups for any polymorphism.

Associations Between *PON1* and *PON2* Polymorphisms, Lipid Profile, and PON1 Activity

In our study population, there was no difference in lipid profile among *PON1* and *PON2* polymorphisms (data not shown). We reported that PON1 activity was lower in the SCS group than in those without SCS; however, this difference was not statistically significant (307.041 ± 206.685 U I/L vs. 353.494 ± 200.392 U I/L, $p = 0.571$). The analyses of serum PON1 activity for *PON1* and *PON2* genotypes (Table 3) revealed statistically significant ($P < 0.000$) differences in PON1 activity

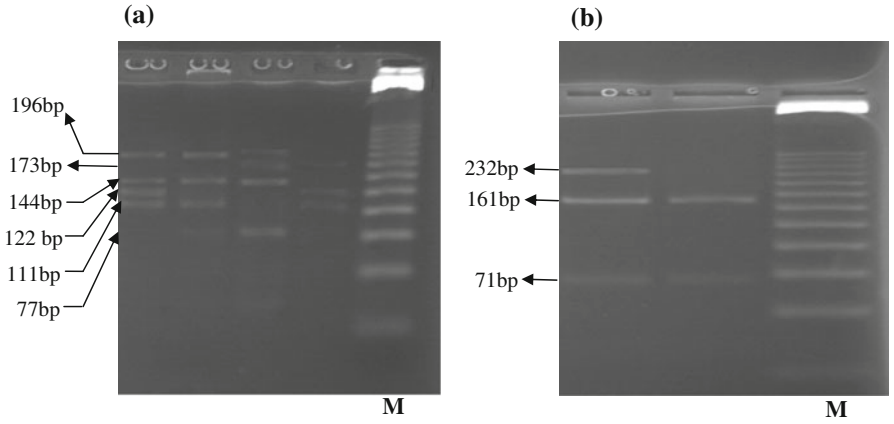

Fig. 1 Separation of (**a**) *PON* multiplex PCR products after digestion with *Hin*fI by 4 % agarose gel electrophoresis. The combined genotypes (*PON1*-192, *PON1*-55, *PON2*-311) resulting from the analysis are (*left* to *right*) QQ-LM-CC; QR-MM-CS; RR-MM-CS; QQ-LL-SS. Separation of (**b**) single PCR products relative to the *PON2*-148 polymorphism after digestion with *Fnu*4HI by 4 % agarose gel electrophoresis. Genotype analysis (*left* to *right*), AG (three bands of 232, 161, and 71 bp), PON2-148, AA (two bands of 161 and 71 bp). M, 25 bp DNA Step Ladder (Promega). Length of digestion products is reported on the *left*

among genotypes for the coding region polymorphisms, *PON1*-L55M and *PON1*-Q192R. The highest PON1 activities were detected in LL and RR genotypes, intermediate activities in LM and QR genotypes, and the lowest activities in MM and QQ genotypes. No difference was observed between PON1 activity and the two polymorphisms S311C and A148G of the *PON2* gene.

Association of *PON1* and *PON2* Polymorphisms with the Presence of SCS

Odds ratios were calculated for SCS of variant genotypes of the *PON* polymorphisms (Table 4). After adjustment for confounding variables, the OR of SCS associated with the MM genotype of *PON1*-55 polymorphism was 0.668 (95 % CI 0.43–0.93; $p = 0.042$), suggesting that the MM genotype is associated with a 33 % lower risk of SCS. This protective effect seemed to be particularly significant in patients with metabolic syndrome (46 % lower risk; OR 0.556, 95 % CI 0.18–0.894; $p = 0.035$), in diabetic patients (64 % lower risk; OR 0.359, 95 % CI 0.14–0.94; $p = 0.034$), and in nonsmokers (35 % lower risk; OR 0.648, 95 % CI 0.36–0.95; $p = 0.044$). In the present study, we also reported that the RR genotype of the *PON1*-192 polymorphism and the CC of *PON2*-311 were associated with an increased risk of SCS (OR 1.83, 95 % CI 1.02–3.29, $p = 0.043$ for *PON1*-192RR; OR 1.76, 95 % CI 1.04–3.10, $p = 0.035$ for *PON2*-311CC), particularly in diabetics, smokers, and patients with metabolic syndrome (Table 4). No significant association was observed with the risk of SCS for the *PON2*-148 polymorphism and the *PON1*-192QR, *PON1*-55LM, *PON2*-311SC genotypes, either in the study population or based on smoking or diabetic status or the presence of metabolic syndrome.

Table 2 Genotype and allele frequencies of paraoxonase polymorphisms among patients with and without significant coronary stenosis

Polymorphism	With SCS ($n = 212$)			Without SCS ($n = 104$)			p
	% (n)	Variant allele frequency	χ^2	% (n)	Variant allele frequency	χ^2	
PON1-Q192R							
QQ	40.6 (86)	0.379	1.67	47.1 (49)	0.341	2.86	0.534
QR	42.9 (91)			37.5 (39)			
RR	16.5 (35)			15.4 (16)			
PON1-L55M							
LL	38.7 (82)	0.403	3.46	28.8 (30)	0.51	3.83	0.05
LM	42 (89)			40.4 (42)			
MM	19.3 (41)			30.8 (32)			
PON2-S311C							
SS	33 (70)	0.453	3.27	50.9 (53)	0.313	3.07	0.006*
SC	43.4 (92)			35.6 (37)			
CC	23.6 (50)			13.5 (14)			
PON2-A148G							
AA	92.4 (196)	0.038	0.326	91.3 (95)	0.043	0.212	0.731
AG	7.6 (16)			8.7 (9)			
GG	0 (0)			0 (0)			

χ^2 Hardy–Weinberg. p χ^2 test between the two groups

* Statistically significant ($p < 0.05$)

Linkage Disequilibrium and Haplotype Analysis

In our population, significant LD was observed between *PON1*-55 and *PON1*-192 ($D' = -0.44$, $p < 0.000$). Haplotype reconstruction for the four *PON* polymorphisms under study resulted in 14 haplotypes, possessing *PON1*-192 (A672G), *PON1*-55 (T260A), *PON2*-311 (C1053G), and *PON2*-148 (C564G) polymorphisms. We compared frequencies of different haplotypes with different ORs among patients with and without SCS (Table 5). When the four *PON* polymorphisms were combined, the GTGC haplotype model seemed to be the most atherogenic. It occurred more frequently in patients in the SCS group than in those without SCS (16.7 vs. 11 %; $p = 0.028$). In the haplotype analysis, after adjustment for confounding variables, the risk of SCS was higher in the GTGC haplotype than in the wild haplotype (possessing all common alleles: ATCC) (OR 1.58, 95 % CI 1.050-4.55; $p = 0.039$).

Discussion

The *PON* gene family has drawn much attention in recent years. In a series of association studies between *PON1* and *PON2* polymorphisms and coronary heart

Table 3 Serum PON1 activity in genotype groups

Polymorphism	PON1 activity (IU/L)	*p*
PON1-Q192R		
QQ (135)	200.314 ± 133.460	<0.000*
QR (130)	367.984 ± 178.526	
RR (51)	554.597 ± 220.449	
PON1-L55M		
LL (112)	426.348 ± 219.237	<0.000*
LM (131)	291.947 ± 195.197	
MM (73)	229.703 ± 138.924	
PON2-S311C		
SS (123)	368.279 ± 219.914	0.360
SC (129)	276.571 ± 130.379	
CC (64)	333.069 ± 226.779	
PON2-A148G		
AA (291)	320.442 ± 203.880	0.353
AG (25)	370.437 ± 233.928	

p Anova test for PON1 activity according to genotype

* Statistically significant ($p < 0.05$)

Table 4 Association of paraoxonase variant genotypes with significant coronary stenosis

Population group	Polymorphism					
	PON1-192RR		PON1-55MM		PON2-311CC	
	OR (95 % CI)	*p*	OR (95 % CI)	*p*	OR (95 % CI)	*p*
Study population	1.833 (1.02–3.29)	0.043*	0.668 (0.43–0.93)	0.042*	1.76 (1.04–3.10)	0.035*
Metabolic syndrome						
With	2.044 (1.05–3.98)	0.036*	0.556 (0.18–0.89)	0.035*	2.37 (1.12–7.47)	0.031*
Without	1.31 (0.18–9.5)	0.787	0.673 (0.38–1.67)	0.297	1.28 (0.83–8.57)	0.203
Diabetes						
With	1.64 (1.098–3.38)	0.038*	0.359 (0.14–0.94)	0.034*	2.34 (1.08–5.06)	0.031*
Without	1.18 (0.41–3.08)	0.728	0.691 (0.36–1.3)	0.256	1.5 (0.32–7.12)	0.609
Smoking						
Smokers	2.39 (1.09–6.37)	0.041*	0.942 (0.38–2.32)	0.897	2.88 (1.24–13.1)	0.024*
Nonsmokers	1.063 (0.205–3.62)	0.840	0.648 (0.36–1.15)	0.044*	1.55 (0.75–3.22)	0.237

* Statistically significant ($p < 0.05$)

disease, the majority of the reports have revealed significant associations, although some exceptions exist. In the present study, the relationship of *PON1*-Q192R and -L55M polymorphisms and *PON2*-S311C and -A148G polymorphisms with lipid profile, PON1 activity, and SCS was investigated.

Table 5 Frequency of haplotypes among patients with and without significant coronary stenosis

Haplotype	With SCS, % (*n*)	Without SCS, % (*n*)	Adjusted OR (95 % CI)	*p* value
ATCC	11.8 (50)	8.6 (18)	–	–
GTCC	8.5 (36)	12.5 (26)	1.42 (0.565–3.61)	0.429
ATGC	17 (72)	17.8 (37)	0.86 (0.475–1.54)	0.616
GTGC	16.7 (71)	11 (23)	1.58 (1.050–4.55)	0.039*
AAGC	19.6 (83)	20.7 (43)	0.67 (0.104–4.52)	0.694
GAGC	3.3 (14)	4.8 (10)	1.05 (0.612–1.80)	0.863
AACC	13 (55)	15.8 (33)	0.56 (0.076–4.14)	0.568
GACC	6.4 (27)	4.8 (10)	1.43 (0.725–2.81)	0.34
GTCG	0.9 (4)	0.5 (1)	2.14 (0.404–11.35)	0.26
AACG	0 (0)	1 (2)	0.4 (0.016–10.02)	0.571
GACG	0.9 (4)	1 (2)	0.826 (0.089−0.92)	0.932
AAGG	0.2 (1)	1 (2)	0.80 (0.044−14.64)	0.88
GAGG	0.5 (2)	0 (0)	0.72 (0.44−1.14)	0.537
GTGG	1.2 (5)	0.5 (1)	0.714 (0.45−1.14)	0.391

Subjects with SCS = 212; without SCS = 104

* Statistically significant ($p < 0.05$)

Associations Between *PON1* and *PON2* Polymorphisms, Lipid Profile, and PON1 Activity

This study found no association of the *PON1* and *PON2* genotypes with lipid profile (data not shown). Our results agree with many studies (Watzinger et al. 2002; Sen-Banerjee et al. 2000) but contradict others (Shin et al. 2008; Rios et al. 2007; Gluba et al. 2010). This inconsistency may be due to ethnic differences.

It has been shown that PON1 enzymatic activity is decreased in coronary heart disease patients (Mackness et al. 2003; Azarsiza et al. (2003). In our study, we report that PON1 activity was lower in the SCS group; however, this difference was not statistically significant. Our result is in agreement with Azarsiza et al. (2003) and Mackness et al. (2001).

Putative mechanisms leading to decreased PON1 activity could be the inactivation of enzyme by increased oxidative stress (Aviram et al. 1999). The decrease in PON1 activity could be the result of lower HDL concentrations in patients with coronary artery disease. Lipid peroxides, which are substrates for PON1 (Camps et al. 2009) and which have been shown to be raised in people with coronary artery disease, are inhibitors of PON1. The role of oxidative stress in decreased PON1 activity might be confirmed by the inverse association between lipid peroxidation and PON1 activity (Mackness et al. 2001).

Between individuals, PON1 activity varies approximately 10–40 fold. Part of this variability is explained by two polymorphisms of the *PON1* gene. The R allele of the *PON1*-Q192R polymorphism has higher activity toward paraoxon hydrolysis than the Q allele (Humbert et al. 1993). Regarding lipid peroxidation, preliminary

studies suggest that the R/R isoform is less effective in hydrolyzing lipid peroxides than the Q/Q isoform (Mackness et al. 1997; Regieli et al. 2009). On the other hand, the polymorphic site at position 55 (L/M) has been related with differences in PON1 activity, with higher activity for the 55L isoenzyme than for the 55M isoenzyme (Blatter-Garin et al. 1997). The L55M polymorphism occurs in the NH_2-terminal region of the peptide, where a highly hydrophobic sequence may facilitate binding of PON to HDL (Brophy et al. 2000). In our study, we reported that significant association was found between both the *PON1*-192 and -55 polymorphisms and PON1 activity toward PON, which increased with the presence of the R allele (QQ < QR < RR) in the *PON1*-192 genotype and with the L allele (MM < ML < LL) in the *PON1*-55 genotype.

Association of *PON1* and *PON2* Polymorphisms with the Presence of SCS

The association of *PON* polymorphisms with cardiovascular events is a controversial issue. Our data show that the *PON1*-192RR genotype was associated with an increased risk of SCS ($p = 0.043$), consistent with the findings of some studies (Ranade et al. 2005; Gluba et al. 2010; Wang et al. 2011) but not others (Kaman et al. 2009; Bhattacharyya et al. 2008; Pasdar et al. 2006). Despite this high activity, atherogenicity of the *PON1*-192RR genotype may be explained by less ability to protect LDL from oxidative modification than the *PON1*-192QQ genotype (Aviram et al. 1999). We report that the *PON1*-55MM genotype is associated with a 33 % lower risk of SCS. Several reports showed similar results (Oliveira et al. 2004; Kaman et al. 2009); others did not (Can Demirdöğen et al. 2008; Arca et al. 2002). Concerning the *PON2* gene, our study and other reports failed to show a significant role for the *PON2*-A148G variation in atherogenicity (Shin et al. 2008; Oliveira et al. 2004; Ranade et al. 2005). In contrast, Shin (2009) reported an association. Our result, in accordance with other studies (Jalilian et al. 2008; Yang et al. 2006), found that the *PON2*-311CC genotype was associated with increased risk of SCS. This association may be explained by a possible coding change in the *PON2* gene, which could cause a change from serine to cysteine at the active site of PON2, impairing its antioxidation function and consequently changing the metabolic potency of the cell (Qu et al. 2008). However, controversies were found in many studies (Gluba et al. 2010; Guxens et al. 2008; Ranade et al. 2005). Inconsistent association of *PON* polymorphisms with coronary artery disease may be attributed to differences between studies in several factors, including ethnic factors, the type of population studied, dietary habits, and environmental differences.

Effect of Metabolic Syndrome, Diabetes, and Smoking Status

Because diabetic and metabolic syndrome patients are at greater risk of coronary heart disease and oxidative stress (Sentí et al. 2003; Mackness et al. 2000; Rejeb et al. 2010), we investigated the effect of *PON* polymorphisms on SCS in relation to these clinical conditions. There are many conflicting genetic results among such studies (Osei-Hyiaman et al. 2001; Martinelli et al. 2005; Ergun et al. 2011). We reported that the *PON1*-192RR and *PON2*-311CC genotypes were associated with

an increased risk of SCS in diabetics and in patients with metabolic syndrome, and that the *PON1*-55MM genotype seems to have lower risk. Our results were in accordance with several studies (Aubó et al. 2000; Mackness et al. 2005).

In association with diabetes or metabolic syndrome, which increases peroxide formation, one may hypothesize that the R allele of *PON1*-192 and the C allele of *PON2*-311 may promote oxidative stress. This, in turn, might increase cardiovascular risk in diabetic or metabolic syndrome patients. In this context, it has been suggested that the L isoform of *PON1*-55 protects less well from oxidative stress than the M isoform (Mackness et al. 1997).

Like diabetes and metabolic syndrome, smoking is an established risk factor for coronary artery disease in which oxidative mechanisms play an important role. Thus, we studied the effect of smoking status on the association of these polymorphisms with SCS. According to Nishio and Watanabe (1997), cigarette smoke extract inhibits PON activity in a dose- and time-dependent manner. This inhibition might be caused by steric hindrance resulting from the introduction of a large substituent near a region of the molecule critical for substrate binding or the maintenance of an active enzyme conformation (Kuo and La Du 1995). We reported that the *PON1*-MM genotype was associated with lower risk in nonsmokers but not in smokers. Smokers may lack the protective effect of the MM genotype because cigarette smoke extract decreases PON activity against nonphysiological substrates (Nishio and Watanabe 1997), and it may also reduce PON1 activities that are involved in cardioprotection. Thus, the deleterious effects of cigarette smoke may equalize or outweigh the differences in potentially positive enzyme activities conferred by the *PON1* genotype.

On the other hand, the *PON1*-192RR and *PON2*-311CC genotypes were associated with an increased risk of SCS in smokers. Our findings were similar to previous studies (Osei-Hyiaman et al. 2001; Martinelli et al. 2004), but not all (Sen-Banerjee et al. 2000; Rios et al. 2007). It is possible that these results can be explained by large amounts of free radicals in cigarette smoke (Church and Pryor 1985) and by lower antioxidative capacity of PON from the RR and CC genotypes (Princen et al. 1992), so that oxidized LDL particles from smokers with these genotypes generate more lipid peroxidation products than LDL from nonsmokers (Scheffler et al. 1992). In fact, cigarette extracts decrease PON activity from the RR and CC genotypes, which is initially less effective in hydrolyzing lipid peroxidation than that from the QQ and SS genotypes.

In haplotype analysis, after adjustment for confounding variables, the haplotype model of GTGC, with the polymorphisms *PON1*-192 (A672G), *PON1*-55 (T260A), *PON2*-311 (C1053G), and *PON2*-148 (C564G), seemed to increase the risk of SCS compared with the wild haplotype (possessing all common alleles, ATCC). Our result was confirmed by some studies (Kallel et al. 2010; Arca et al. 2002).

Some limitations of our study should be noted. We studied only four polymorphisms in the *PON* gene. It is possible that haplotype analysis with additional genotyping might increase the power to find these potential associations. In addition, the study population consisted of a relatively small number of subjects.

In conclusion, the *PON1*-192RR and -55LL genotypes increased PON1 activity. In the presence of metabolic syndrome and diabetes, *PON1*-192RR and

PON2-311CC were associated with an increased risk of SCS, and *PON1*-55MM appears to be protective against SCS. This association was evident among nonsmokers for *PON1*-55MM and among smokers for *PON1*-192RR and *PON2*-311CC. The haplotype GTGC was associated with an atherogenic effect.

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