Paraoxonase Gene Polymorphism in South-western Korean Population

Paraoxonase (PON) has anti-atherogenic activity. Considering the important role of polymorphism in the genetic susceptibility to cardiovascular disease and the variability of its allele frequencies in different ethnic groups, the distribution of genotypes and allele frequencies of PON1M55L, PON1Q192R, PON2A148G, and PON2S311C polymorphisms was analyzed in a total 988 South-western Koreans and determined their effects on lipid parameters. The genotype distribution of PON1 at position 55 was LL=0.886, LM=0.114; and at position 192 was QQ=0.406, QR=0.594. The frequencies of the PON1 55L allele and the PON1 192R allele were similar to those seen in Chinese populations and Western populations, respectively. The genetic distribution of PON2 at position 148 was AA=0.619, AG=0.345, GG=0.035; and at position 311 was CC=0.035, SC=0.345, SS=0.619. The frequencies of the PON2 148G and 311S alleles were similar to those seen in Chinese populations. The concentrations of LDL and ApoB were significantly different between the PON2A148G (P<0.05) and PON2 S311C polymorphisms (P<0.01). PON polymorphisms and allele frequencies were described in Koreans living south-western part of Korea. These ethnic variations are considered important in the interpretation of diseases associated with PON polymorphisms.

Key Words : *Paraoxonase; Polymorphisms; Korean*

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INTRODUCTION

The paraoxonase (PON) gene family consists of three members-PON1, PON2, and PON3-located adjacent to each other on the long arm of chromosome 7 in humans. These three human PON genes share approximately 60% identity at the amino acid level and approximately 70% identity at the nucleotide level. However among other mammalian species, these three genes share 79-90% identity at the amino acid level and 81-90% identity at the nucleotide level (1). PON1 is synthesized in the liver and secreted in the blood, where it associates with high-density lipoprotein (HDL). Changes in HDL size and shape can strongly affect the binding affinity and stability of PON1 and result in reduced antioxidative capacity (2). However, PON2 mRNA is more widely expressed in nearly every human tissue including the heart, kidney, liver, lung, placenta, small intestine, spleen, stomach, and testis. Arterial wall Endothelial cells, smooth muscle cells and macrophages of arterial wall are also known to express PON2 (3). PON1 might play an important physiological role in lipid metabolism through protecting against the development of atherosclerosis. Many investigations have provided considerable evidence for PON1 anti-atherogenicity. Studies have shown that PON1 inhibits oxidation of HDL and low-density lipoproteins (LDL) that preserve HDL function, increases cellular cholesterol efflux from macrophages

ameliorates effects of oxidized LDL, and decreases lipid peroxides in atherosclerotic lesions (2).

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 (3). Thus, PON2 plays a similar role to that of PON1 in the metabolism of lipids and lipoproteins (1).

There are two polymorphisms in the PON1 coding region: leucine/methionine at position 55 (M55L) and glutamine/arginine at position 192 (Q192R). PON2 also has two common polymorphic sites in the coding region: alanine/glycine at position 148 (G148A) and cysteine/serine at position 311 (C311S) (4). These polymorphisms are associated with a number of pathophysiological conditions, such as coronary artery disease, Parkinson's disease, stroke, familial hypercholesterolemia, type 2 diabetes, late-onset Alzheimer's disease, and reduced bone mass in postmenopausal women (5-11).

Various population studies have reported inter-ethnic differences in the allele frequencies for 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 the above mentioned diseases. Considering the important contribution of these polymorphisms to genetic susceptibility in atherosclerosis, and the variability in allele

frequencies among different ethnic groups, the aim of this work was to evaluate the distribution of PON polymorphisms and to determine their role association with lipid profiles in a population living in South-western part of Korea.

MATERIALS AND METHODS

Subjects

The allele distribution of the PON polymorphisms was determined in 988 (519 males, 469 females) unrelated volunteers, all of whom were hospital patients or outpatients for health screening from South-west parts of Korea. Written informed consent was obtained from each patient. This study protocol was approved by the Institutional Review Board of Chonbuk National University Hospital.

Genotyping of DNA

Peripheral blood samples were collected after the consent. DNA was extracted using Nucleon® DNA extraction kits. DNA samples were quantified using a GeneQuant II RNA/DNA calculator (Amersham-Pharmacia Biotech, Uppsala, Sweden), and 5-10 ng of DNA form each patient was amplified through a Hot Start polymerase chain reaction (PCR) protocol. The primers applied for PON1 55 PCR and PON1 192 PCR were PON1-55F, PON1-55R, PON1-192F, and PON1-192R, determining a 138 bp and a 151 bp PCR amplicon size, respectively. The primers applied for PON2 148 PCR and PON2 311 PCR were PON2-148F, PON2-148R, PON2-311F, and PON2-311R, determining a 165 bp and a 225 bp PCR amplicon size, respectively. The detection oligonucleotide sets applied for PON1 55, PON1 192, PON2 148, and PON2 311 genotyping consisted of a 3´ fluorescein-labeled anchor and either a 5´-LC Red 705-labeled (PON1 192 mutation, PON2 148 mutation) or a 5´-LC Red 640-labeled (PON1 55 mutation, PON2 311 mutation) sensor probe. The sequences of primers and probes, as well as the applied fluorophores and nucleotide sets applied for the PON genotyping with melting point analysis, are shown in Table 1. Both primers and fluorescently labeled probes were synthesized by TIB MolBiol (Berlin, Germany). PCR and melting curve analysis were performed in 20 μL volumes in glass capillaries (Hoffmann-La Roche, Alameda, CA, U.S.A.). During PCR, the LightCycler automate (software version 4.5) continuously displays the current fluorescence and fluorescence history of each capillary. Analysis of PCR products on agarose gel and sequencing confirmed the presence of the specific PCR products (data not shown). The observed melting point temperature in PON1 55 MM was 63.0°C, PON1 55 LL was 58°C, PON1 192 QQ was 63°C, PON1 192 RR was 57°C, PON2 148 AA was 64°C, PON2 148 GG was 58°C, PON2 311 SS was 59°C, and PON2 311 CC was 53°C (5).

Table 1. Sequences of primers and probes, as well as the applied fluorochromes used for PON1 and PON2 genotyping through melting point analysis on the LightCycler

Primers/probes	Sequences
Primers	
PON1-55F	5´-CCTGCAATAATATGAAACAACCTG-3´
PON1-55R	5´-CTAGAACACAGAAAAGTGAAAGAAAAC-3´
PON1-192F	5´-TATTGTTGCTGTGGGACCTGAG-3´
PON1-192R	5´-CCTTCTGCCACCACTCGAAC-3´
PON2-148F	5´-ATGAGCAGCTTCCCATCA-3´
PON2-148R	5´-CATGTACGGTGGTCTTATATTCATAC-3´
PON2-311F	5´-TTCAACAGCATGTCCCCTTAA-3´
PON2-311R	5´-CTTCCCATCATACACTGAGGCTA-3´
Probes	
55-Sensor	5´-CTCTGAAGACATGGAGATACTGCC-FL-3´
55-Anchor	5´-LCRed640-ATGGACTGGCTTTCATTAGCTCTG-TGAGGT-3´
192-Sensor	5´-CCCCTACTTACAATCCTGGGAGAT-FL-3´
192-Anchor	5´-LC705-ATTTGGGTTTAGCGTGGTCGTATGTTG-3´
148-Sensor	5´-GAACATTCTATCTGAGAAGCCTACAGTG-FL-3´
148-Anchor	5´-LC705-CTACAGTTTATGCCAACAATGGGTCTG-TTC-3´
311-Sensor	5´-CATCCAGAACATTCTATGTGAGAA-FL-3´
311-Anchor	5´-LC640-CCTACAGTGACTACAGTTTATGCCAAC-3´

F, forward primer; R, reverse primer; LC Red 705, fluorophore LightCycler Red 705; LC Red 640, fluorophore LightCycler Red 640.

Both melting point temperatures were noted in heterozygotes (PON1 55 LM, PON1 192QR, PON2 148 AG, PON2 311SC).

Statistical analysis

Differences in demographic characteristics were compared by univariate analysis using Student's t test. Allele and genotype frequencies were calculated by gene counting. The chi-square test was used both to estimate the Hardy-Weinberg equilibrium and to compare the genotype and allele frequencies between the sexes. Allele frequencies observed in the Korean population were compared with those reported in other populations using the Mantel-Haenszel chi-square test. One-way analysis of variance (ANOVAR) was used to compare the mean levels of lipid parameters among the different genotypes. A value of $P<0.05$ was considered statistically significant.

RESULTS

Clinical characteristics of study subjects are shown in Table 2. The mean age was 66.18±12.2 yr, with no age difference between the sexes (male, n=519, 64.13±12.2 yr; female, n=469, 67±12.4 yr; P=0.561). With respect to vascular disease risk factors, a large proportion of individuals had hypertension. Genotype and allele frequencies for the PON1M55L,

Table 2. Study population characteristics (n=988)

	Mean±SD
Age (yr)	66.18±12.20
Male (n [%])	518 (52.5%)
Hypertension (n [%])	451 (45.7%)
Diabetes mellitus (n [%])	182 (18.4%)
Cholesterol (mg/dL)	190.0±42.04
logcholesterol	2.3±0.10
Triglyceride (mg/d)	133.1±98.28
logTG	2.0±0.24
HDL (mg/dL)	43.7±11.42
logHDL	1.6±0.11
LDL (mg/dL)	116.7±35.87
logLDL	2.0±0.13
Lpa (mg/dL)	35.1±28.09
logLPa	1.5±0.50
ApoAI (g/d)	1.2±0.26
ApoB (g/L)	0.9±0.25
HbA1C	6.2±1.26
Total Homocysteine (μM/L)	2.0±0.27
Glucose (mg/dL)	146.8±59.98

Values are presented as mean±SD.
HDL, high density lipoprotein; Apo, apolipoprotein; Lpa, lipoprotein A.

PON1Q192R, PON2A148G, and PON2S311C polymorphisms are summarized in Table 3. Observed and expected frequencies for the polymorphisms were at Hardy-Weinberg equilibrium. In the case of the PON1 55 polymorphism, the most frequent allele was L (94.3%), and the most common genotype was the homozygote LL (88.6%). The most frequent PON1 192 allele was Q (70.3%) and the most common genotype was heterozygote QR (59.4%). In the case of the PON2 148 polymorphism, the most frequent allele was A (79.2%), and the most common genotype was the homozygote AA (61.9%). The most frequent PON2 311 allele was S (79.2%), and the most common genotype was homozygote SS (61.9%). There was no significant difference between the sexes in terms of genotype or allele frequency. The alle frequencies of PON1M55L, PON1Q192R, PON2A148G, and PON2S311C were compared in the Korean population to those previously described in other Asian (12-18), American (19-23) and European (7, 8, 24-31) groups (Table 4). The PON1 55 L allele frequencies in Asian populations were higher than in other ethnic groups ($P<0.05$), and the allele frequency in Koreans was higher than those seen in Thai, Iranian, and Pakistani populations ($P<0.05$). The Korean population showed a PON1 192Q allele frequency similar to that seen in other ethnic groups, except for those of China, Taiwan, Mexico and Latin America ($P<0.05$). The PON2 148 A allele frequency in the Korean population was similar to that seen in other populations, except for those of Pakistan and Brazil ($P<0.005$). The PON2 311S allele frequency in the Korean population was different compared to that of populations in Pakistan and the U.S.A. ($P<0.05$).

To determine the of PON gene polymorphisms on the lipid

Table 3. Genotype and allele frequencies of PON gene cluster polymorphisms

Genotypes	Group			χ^2	P
	Male (n=519)	Female (n=469)	Total (n=988)		
PON1M55L					
LL	88.4%	88.7%	88.6%		
LM	11.4%	11.3%	11.3%	0.91	0.64
MM	0.1%	0.0%	0.1%		
Allele					
L	94.1%	94.3%	94.3%		
M	5.8%	5.7%	5.7%		
PON1Q192R					
QQ	41.0%	40.1%	40.6%		
QR	59.0%	59.9%	59.4%	0.09	0.76
RR	0.0%	0.0%	0.0%		
Allele					
Q	70.5%	70.1%	70.3%		
R	29.5%	29.9%	29.7%		
PON2A148G					
AA	60.7%	63.3%	61.9%		
AG	35.1%	33.9%	34.5%	1.87	0.39
GG	4.2%	2.8%	3.5%		
Allele					
A	78.3%	80.3%	79.2%		
G	21.8%	19.8%	20.8%		
PON2S311C					
CC	4.2%	2.8%	3.5%		
SC	35.1%	33.9%	34.5%	1.87	0.39
SS	60.7%	63.3%	61.9%		
Allele					
C	21.8%	19.8%	20.8%		
S	78.3%	80.3%	79.2%		

PON, paraoxonase.

profile, the lipid profile was classified as high, normal or low according to the previously defined normal ranges (cholesterol, 0-200 mg/dL; triglyceride, 0-200 mg/dL; HDL cholesterol, 48.9-73.5 mg/dL; LDL cholesterol, 0-140 mg/dL; ApoAI, 1.08-2.24 g/L, ApoB 0.60-1.17 g/L; lipoprotein A, 0-30 mg/dL; total homocysteine 5-20 μM/L). There was no difference in lipid profile among PON 1 polymorphisms, but the PON2 A148G and PON2S311C polymorphisms showed significant differences in LDL and ApoB concentrations ($P=0.023$, $P<0.001$) (Table 5). Homozygotes for PON2 148G and PON2 311 C showed higher concentrations of LDL cholesterol and higher ApoB levels (Table 6).

DISCUSSION

Numerous studies have been conducted on the relation between PON polymorphisms and genetic susceptibility to coronary heart disease (CHD). PON1 has the capacity to inhibit LDL oxidation. The PON1 192R allele is positively

Table 4. PON polymorphism allele frequencies in various world populations compared with those found in the present study

Population	PON1-55			PON1-192			PON2-148			PON2-311			References
	L	M	P	Q	R	P	A	G	P	S	C	P	
Korea (n=988)	0.941	0.058	–	0.705	0.295	–	0.7825	0.2175	–	0.7825	0.2175	–	Present study
Japan (n=2,210)	0.927	0.073	0.774	0.666	0.334	0.614	–	–		0.802	0.198	0.728	Yamada et al., 2003 (13)
China (n=475)	1	0	0.013	0.352	0.648	<0.001	0.826	0.174	0.372	0.827	0.173	0.372	Wang et al., 2003 (12)
Taiwan (n=597)	0.972	0.028	0.306	0.359	0.641	<0.001	–	–	-	-	-	-	Li et al., 2006 (14)
Thailand (n=202)	0.95	0.005	0.756	0.71	0.29	0.913	–	–	-	-	-	-	Phuntuwate et al., 2005 (15)
Iran (n=132)	0.59	0.41	<0.001	0.69	0.31	0.841	–	–	-	–	–		Sepahvand et al., 2007 (16)
Pakistan (n=370)	0.783	0.217	0.001	0.672	0.328	0.614	0.51	0.49	0.001	0.388	0.612	<0.001	Saeed et al., 2007 (17)
Austria (n=303)	0.644	0.356	<0.001	0.746	0.254	0.455	–	–	–	–	–	–	Watzinger et al., 2002 (18)
U.S.A. (n=2,553)	0.637	0.363	<0.001	0.72	0.28	0.79	0.766	0.234	0.866	0.233	0.767	<0.001	Ranade et al., 2005 (19)
Canada (n=324)	0.64	0.36	<0.001	0.73	0.27	0.671	0.76	0.24	0.737	–	–	–	McKeown-Eyssen et al., 2004 (20)
Mexico (n=214)	0.84	0.16	0.024	0.51	0.49	0.005	–	–	–	–	–	–	Rojas-Garcia et al., 2005 (21)
Brazil (n=376)	0.64	0.36	<0.001	0.66	0.34	0.513	0.6	0.4	0.006	0.71	0.29	0.256	Oliveira et al., 2004 (22)
Latin America (n=260)	0.82	0.18	0.009	0.48	0.52	0.001	-	–	–	–	–	–	Holland et al., 2006 (23)
U.K. (n=405)	0.63	0.37	<0.001	0.71	0.29	0.913	0.73	0.27	0.411	0.74	0.26	0.508	Pasdar et al., 2006 (8)
Netherlands (n=201)	0.627	0.373	<0.001	0.677	0.323	0.724	–	–	–	–	–	–	Leus et al., 2001 (24)
Ireland (n=388)	0.65	0.35	<0.001	0.69	0.31	0.841	–	–	–	–	–	–	Hasselwander et al., 1999 (25)
Germany (n=535)	0.644	0.3566	<0.001	0.723	0.277	0.79	–	–	–	–	–	–	Gardemann et al., 2000 (26)
Italy (n=273)	0.601	0.399	<0.001	0.711	0.289	0.913	–	–	–	0.802	0.198	0.728	Martinelli et al., 2005 (27)
Poland (n=437)	0.64	0.36	<0.001	0.743	0.257	0.5578	–	–	–	0.76	0.24	0.737	Slowik et al., 2007 (7)
Turkey (n=116)	0.702	0.298	<0.001	0.6214	0.3785	0.214	–	–	–	–	–	–	Agachan et al., 2005 (28)
Israel (n=193)	0.583	0.417	<0.001	0.65	0.35	0.422	–	–	–	0.842	0.158	0.279	Karban et al., 2007 (29)
Spain (n=388)	0.626	0.374	<0.001	0.701	0.299	0.963	–	–	–	–	–	–	Ferre et al., 2003 (30)
Switzerland (n=199)	0.666	0.334	<0.001	0.666	0.334	0.614	–	–	–	–	–	–	Garin et al., 2005 (31)

Statistical significance between the world populations and the Korean population in this study was determined by means of the Mantel-Haenszel chi-square test. PON, paraoxonase.

Table 5. Association between PON2 polymorphisms and lipid profile (n=988)

	PON2A148G				PON2S311C			
	AA	AG	GG		SS	SC	CC	
LDLC								
Normal	469 (47.5)	275 (27.9)	19 (1.9)	P=0.023	469 (47.5)	275 (27.9)	19 (1.9)	P=0.023
High	140 (14.2)	69 (7.0)	15 (1.5)		140 (14.2)	69 (7.0)	15 (1.5)	
ApoB								
Low	42 (4.2)	21 (2.1)	7 (0.7)	P<0.001	42 (4.2)	21 (2.1)	7 (0.7)	P<0.001
Normal	501 (50.8)	295 (29.9)	15 (1.5)		501 (50.8)	295 (29.9)	15 (1.5)	
High	67 (6.8)	28 (2.8)	11 (1.1)		67 (6.8)	28 (2.8)	11 (1.1)	

PON, paraoxonase; LDLC, low-density lipoproteins cholesterol; Apo, apolipoprotein.

associated with CHD while the Q allele is protective against atherosclerosis (1). The majority of clinicians have studied the PON1 Q192R polymorphism. On meta-analysis of PON Q192R polymorphisms and CHD, a highly significant positive association was confirmed in the population with type 2 diabetes (32). Fewer studies have been conducted on the PON1 L55M, PON2 A148G, and PON2 S311C polymorphisms in CHD, but the results have proved conflicting (2, 33). The major factor contributing of these differences is a completely different distribution of PON polymorphisms among different ethnic populations.

In the present study, the allele and genotype frequencies

Table 6. Comparison of concentrations of LDLC and ApoB in PON2 polymorphisms

PON2A148G	AA	AG	GG	
LDLC	112.2±30.9	114.4±29.3	135.1±28.5	P=0.017
ApoB	0.877±0.248	0.852±0.223	1.373±0.241	P=0.014
PON2S311C	SS	SC	CC	
LDLC	112.2±30.9	114.4±29.3	135.1±28.5	P=0.017
ApoB	0.877±0.248	0.852±0.223	1.373±0.241	P=0.014

Data are reported as mean±SD. Homozygotes for PON2A148G and PON2S311 C show higher concentrations of LDL cholesterol and higher ApoB levels.
PON, paraoxonase; LDLC, low-density lipoproteins cholesterol (mg/dL); Apo, apolipoprotein (g/L).

of PON polymorphisms were described in a south-western Korean population. The frequencies were compared with previously described frequencies in other populations. We found no significant difference between the sexes with respect to PON genotype distribution or allele frequency. Frequency distribution showed that the Korean population had a similar distribution of POM1 55 polymorphisms compared to the Asian population, with the exception of an increased frequency of the L allele in the Korean population. The Korean population had important differences in PON1 distribution when compared to American and European population (P<0.05). With respect to the PON1 192 polymorphism, the Korean population showed a frequency pattern similar to that of other ethnic groups, with increased frequency of the Q allele. However, this was significantly different compared to populations from China and Taiwan. The genotype distribution of PON2 polymorphism was narrower than that seen in the PON1 polymorphism. PON2 A148G and PON2 S311C polymorphisms seen in the Korean population exhibited a pattern similar to those seen in other ethnic groups, with increased frequencies of A and S alleles. However, there were significant distribution differences compared with populations from Pakistan, Brazil, and the U.S.A. PON serum concentration and activity are considered more important than PON polymorphisms in the risk of atherosclerotic disease, and gene-gene and gene-environmental interaction may increase or decrease susceptibility to the disease. Therefore, these ethnic variations in PON polymorphisms could help us to understand the role of PON as a marker for the interpretation of data correlated with a specific disease.

In order to determine the role of PON polymorphism in the lipid profile of this study population, the lipid levels were determined and the association of the PON polymorphisms and lipid profiles was evaluated. In this analysis, no association was found between PON1 genotypes and lipid concentration (data not shown). However the PON2 polymorphism was found to affect concentrations of LDL and ApoB. Homozygotes for PON2 148G and PON2 311 C showed higher concentrations of LDL cholesterol and higher ApoB levels (P=0.017, 0.014, respectively). These findings differ from

previous reports. Homozygotes for PON2 148A and PON2 311S had significantly higher plasma total cholesterol and LDL cholesterol and higher ApoB levels than homozygotes for PON2 148G and PON2 311C and heterozygotes for PON2 A148G and PON2 S311C (4). Another report found that homozygotes for PON2 148G had the highest plasma concentrations of total cholesterol, HDL cholesterol, and ApoAI (34). Homozygotes of for PON2 311C was reported to have the highest concentration of total cholesterol and LDL cholesterol (24). These discrepancies suggest that polymorphisms in the PON gene are associated with significant variations in intermediate traits in plasma lipoprotein metabolism, through an unknown mechanism. Additional studies using large population based cohorts will be needed to determine the general applicability of the present results for Korean population.

In this study, the PON polymorphisms and allele frequencies were described in a Korean population, and compared with other ethnic groups. These ethnic variations are considered important factors in the interpretation of diseases associated with PON polymorphism.

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