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The Association between Codon 192 Polymorphism of Paraoxonase/arylesterase Gene and Plasma HDL-cholesterol Level in Korean Population

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ABSTRACT : **Essential hypertension is considered to be a multifactorial disease that is influenced not only by environmental factors but also by genetic factors. Genes involved in lipoprotein synthesis, modification and metabolism are candidates for essential hypertension. The purpose of this study was to estimate gene frequencies of paraoxonase/arylesterase (PON1) gene in Korean population and investigate the relationship between genotypes of this gene and essential hypertension or cardiovascular risk factors. In order to estimate the genotype frequencies, *Alw* I RFLP of PON1 gene was used as genetic marker. There were no significant differences in allele and genotype frequencies between normotensives and essential hypertensives, respectively. However, *Alw* I RFLP of PON1 gene were significantly associated with plasma HDL-cholesterol level in Korean population (one-way ANOVA test, P = 0.008). Therefore, our result suggest that this RFLP of PON1 gene may be protective marker on cardiovascular disease in Korean population.**

Keywords : **Essential Hypertension, Genotype, HDL-cholesterol.**

Introduction

Paraoxonase/arylesterase (PON1: EC 3.1.8.1, aryldialkylphosphatase) is a Ca^{2+}-dependent glycoprotein that is exclusively associated with HDL and hydrolyzes paraoxon, the toxic metabolite of organophosphate anticholinesterases (Mackness *et al.*, 1993). Also, this enzyme has been known to prevent LDL oxidation *in vitro* (Mackness *et al.*, 1991). This property may partly explain the observation that HDL protects LDL from excess oxidation (Parthasarathy *et al.*, 1990).

The PON1 gene is a member of a multigene family which consists of two additional PON1-like genes, designed PON2 and PON3 (Primo-Parmo *et al.*, 1996b), and they are all linked on human chromosome 7 (Primo-Parmo *et al.*, 1996a). Also, PON1 gene is genetically polymorphic, because of the occurance of two common isoforms that differ by substitution of an amino acid (gln → arg) at codon 192 (Adkins *et al.*, 1993; Humbert *et al.*, 1993). Low activity of paraoxonase in serum has been observed in patients with myocardial infarction (McElveen *et al.*, 1986), familial hypercholesterolemia (Mackness *et al.*, 1991) and insulin-dependent diabetes mellitus (Mackness *et al.*, 1991), and there is evidence that the codon 192 polymorphism may be a risk factor for cardiovascular disease in some population (Ruiz *et al.*, 1995; Serrato and Marian, 1995). However, there is no report on the relationship between this polymorphism of PON1 gene and essential hypertension. Therefore, we carried out an association with case-control samples on Korean population aimed at evaluating the relation of the codon 192 polymorphism of the PON1 gene to essential hypertension, and also, investigated the association with cardiovascular risk factors.

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Materials and Methods

Subjects

We obtained 194 blood samples from the outpatients of Seoul Hygiene Hospital, Seoul, Korea. Out of these samples, 97 essential hypertensive Korean individuals were defined as having a blood pressure above 140/90 mmHg. Subjects with secondary forms of hypertension were excluded from the study.

Genotyping

Genomic DNA was prepared from buffy coat of 5ml blood after lysis of red blood cell (Sambrook *et al.*, 1989). The codon 192 polymorphism of PON1 gene was detected

by using polymerase chain reaction (PCR)-*Alw* I digestion (Humbert *et al.*, 1993). The sequence of the sense primer was 5'-TATTGTTGCTGTGGGACCTGAG-3', and antisense primer 5'-CACGCTAAACCCAAATACATCTC-3'. PCR was performed in a final volume of 50 ul (100 ng of genomic DNA, 20 pmol of each primer, 200 uM each of the four dNTPs, 1.5 mM $MgCl_2$, 50mM KCl, and 10 mM Tris-HCl, pH 8.4 and 2.5 unit of *Taq* DNA polymerase). The reactions were denatured at 94°C for 1 min, annealed at 61°C for 45 sec, and extended at 72°C for 45 sec with final extension time of 5 min. Amplified PCR products were digested with restriction enzyme *Alw* I, and then electrophoresed on 10% polyacrylamide gel.

Biochemical assays

Total blood cholesterol (TC), triglyceride (TG) and high-density lipoprotein (HDL)-cholesterol levels were determined enzymatically, and the LDL-cholesterol level was calculated by Friedewalds equation (Friedwald *et al.*, 1972).

Statistical analyses

Allele frequencies were calculated from genotype frequencies, and the deviation from Hardy-Weinberg equilibrium (HWE) was analyzed by a χ^2-test. The polymorphism information content (PIC) was estimated by the methods of Bostein *et al.*, 1980. The relative risk of essential hypertension associated with allelic variation is expressed in terms of an odds ratio (OR) with 95% confidence intervals (CI). Comparison of the variables across genotypes was performed using a parametric one-way ANOVA test with multiple comparison tests by Tukey. All statistical analyses were performed using the SPSSWIN (version 8.0) computer program.

Results

Association between codon 192 polymorphism of PON1 gene and essential hypertension

There is a discrepancy in the numbering of amino acid residues in paraoxonase/arylesterase enzyme. Depending on the amino acid taken to be the N-terminal residue, the gln → arg substitution occurs at either residue 191, as reported by Adkins *et al.*, 1993, or residue 192, as reported by Humbert *et al.*, 1993. At this point, we followed the nomenclature of Humbert *et al.*, 1993.

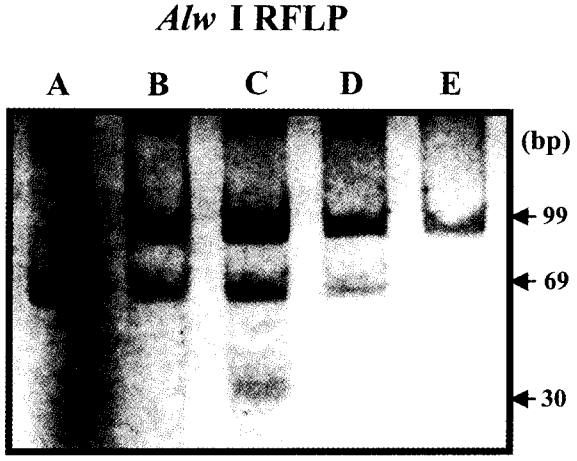

Fig. 1. *Alw* I RFLP of PON1 gene. Lane A, arg/arg genotypes; Lane B, C and D, gln/arg genotypes; Lane E, gln/gln genotypes.

Codon 192 polymorphism of PON1 gene was detected by digestion with restriction enzyme *Alw* I after PCR amplification (Fig. 1). Gln (glutamine) allele yielded a 99 bp band, and arg (arginine) allele gave bands of 69 bp and 30 bp. The genotype and allele frequencies of PON1 gene are displayed in Table 1. The observed genotype distributions of the PON1 gene were not different from those expected for HWE. The genotype frequencies of gln/gln, gln/arg and arg/arg were 26, 42 and 32% in normotensives, and 20, 53 and 28% in essential hypertensives, respectively. There were no significant differences in allele and genotype frequencies between normotensives and essential hypertensives. The PIC of codon 192 polymorphism represented the values of 0.3740 for normotensives and 0.3733 for essential hypertensives, respectively. According to the PIC value, codon 192 polymorphism showed rela-

Table 1. Genotype and allele frequencies of *Alw* I RFLP of the PON 1 gene in normotensives and essential hypertensives

	Genotype No. (%)			Allele No. (%)		H[1]	PIC[2]
	Gln/Gln	Gln/Arg	Arg/Arg	Arg	Gln		
Normotensives	25 (26)	41 (42)	31 (32)	91 (47)	103 (53)	0.5007	0.3740
Hypertensives	19 (20)	51 (53)	27 (28)	89 (46)	105 (54)	0.4992	0.3733
X^2		2.1810		0.0104			
P		0.3369		0.9189			
Odds ratio (CI)[3]			1.04 (0.70-1.55)				

[1]Heterozygosity, [2]Polymorphism Information Content, [3]95% Confidence Interval.
Frequency is given as a percentage in parenthesis.
There were no statistically significant differences in genotype and allele frequencies between normotensives and essential hypertensive subjects.

tively high PIC values in the both groups.

The comparison of anthropometrical data and intermediate phenotypes among genotypes of *Alw* I RFLP at the PON1 gene

Table 2 represents the comparison of anthropometrical data and biochemical parameters across the genotypes of the PON1 gene. There were significant differences in plasma HDL-cholesterol level among the genotypes of codon 192 polymorphism (one-way ANOVA test, P = 0.008). Arg/arg homozygotes (30.2 ± 10.5 mg/dl) represented significantly higher concentration of plasma HDL-cholesterol than gln/arg heterozygotes (24.9 ± 8.1 mg/dl) (multiple comparison by Tukey, P = 0.006), but marginally higher than gln/gln homozygote (25.6 ± 9.5 mg/dl) (multiple comparison by Tukey, P =0 .089). There were no statistically significant differences in plasma HDL-cholesterol level between gln/arg heterozygote and gln/gln homozygote (multiple comparison by Tukey, P = 0.950). Therefore, a recessive model on arg allele of PON1 gene provides the best fit for our data, so the arg/arg genotype could be considered a protecting factor against the susceptibility to cardiovascular diseases.

When stratified by blood pressure status, there were the significant difference in the plasma TC level (one-way ANOVA test, P = 0.037) and HDL-cholesterol level (one-way ANOVA test, P = 0.029) across the genotypes in only normotensive group (Table 3). With respect to essential

Table 2. Clinical characteristics of subjects according to genotypes of the PON 1 gene

Variables	Genotypes		
	Gln/Gln (No.)[6]	Gln/Arg (No.)	Arg/Arg (No.)
Age (year)	58.3 ± 10.8 (43)[7]	60.6 ± 12.1 (90)	59.1 ± 9.9 (58)
BMI (kg/m^2)[1]	24.2 ± 2.3 (39)	23.9 ± 2.4 (77)	23.4 ± 2.1 (58)
Tg (mg/dl)[2]	124.5 ± 84.5 (28)	139.9 ± 89.1 (70)	116.3 ± 45.7 (50)
TC (mg/dl)[3]	146.9 ± 40.9 (28)	146.0 ± 33.4 (70)	158.6 ± 35.5 (50)
LDL-chol (mg/dl)[4]	96.5 ± 34.9 (28)	92.8 ± 36.6 (70)	105.2 ± 34.0 (50)
[a]HDL-chol (mg/dl)[5]	25.6 ± 9.5 (28)	24.9 ± 8.1 (70)	30.2 ± 10.5 (50)

[1]Body Mass Index, [2]Triglyceride, [3]Total cholesterol, [4]LDL-cholesterol, [5]HDL-cholesterol and [6]Number. [7]Values are mean ± SD (Standard Deviation).
[a]Statistically significant association (One-way ANOVA test, P = 0.008).

Table 3. Clinical characteristics of control group according to genotypes of the PON 1 gene

Variables	Genotypes		
	Gln/Gln (No.)[6]	Gln/Arg (No.)	Arg/Arg (No.)
Age (year)	55.3 ± 9.3 (25)[7]	57.4 ± 8.9 (40)	56.4 ± 10.5 (31)
BMI (kg/m^2)[1]	23.8 ± 2.2 (25)	23.3 ± 2.0 (40)	23.6 ± 1.8 (31)
Tg (mg/dl)[2]	121.4 ± 88.2 (21)	138.6 ± 104.5 (33)	112.3 ± 47.1 (29)
[a]TC (mg/dl)[3]	140.4 ± 44.0 (21)	142.5 ± 36.3 (33)	164.4 ± 34.5 (29)
LDL-chol (mg/dl)[4]	91.2 ± 38.1 (21)	87.1 ± 42.3 (33)	110.2 ± 32.7 (29)
[b]HDL-chol (mg/dl)[5]	24.9 ± 10.2 (21)	27.1 ± 8.6 (33)	31.7 ± 9.3 (29)

[1]Body Mass Index, [2]Triglyceride, [3]Total cholesterol, [4]LDL-cholesterol, [5]HDL-cholesterol and [6]Number. [7]Values are mean ± SD (Standard Deviation).
[a]Statistically significant association (One-way ANOVA test, P = 0.037).
[b]Statistically significant association (One-way ANOVA test, P = 0.029).

Table 4. Clinical characteristics of essential hypertensive group according to genotypes of the PON 1 gene

Variables	Genotypes		
	Gln/Gln (No.)[6]	Gln/Arg (No.)	Arg/Arg (No.)
Age (year)	62.4 ± 11.6 (18)[7]	63.2 ± 13.7 (50)	62.3 ± 8.3 (27)
BMI (kg/m^2)[1]	24.8 ± 2.7 (14)	24.4 ± 2.7 (37)	23.2 ± 2.3 (27)
Tg (mg/dl)[2]	133.6 ± 77.2 (7)	141.1 ± 74.2 (37)	121.8 ± 44.2 (21)
[a]TC (mg/dl)[3]	166.4 ± 21.7 (7)	149.2 ± 30.8 (37)	150.7 ± 36.3 (21)
LDL-chol (mg/dl)[4]	112.2 ± 16.7 (7)	97.9 ± 30.4 (37)	98.3 ± 35.3 (21)
[b]HDL-chol (mg/dl)[5]	27.6 ± 7.7 (7)	23.1 ± 7.1 (37)	28.0 ± 11.7 (21)

[1]Body Mass Index, [2]Triglyceride, [3]Total cholesterol, [4]LDL-cholesterol, [5]HDL-cholesterol and [6]Number. [7]Values are mean ± SD (Standard Deviation).

hypertensive group, there were no significant differences in the any anthropometrical parameters and plasma lipid level across the genotypes (Table 4).

Discussion

The exact pathogenesis of essential hypertension remains unknown. A number of risk factors including genetic defects are known to predispose to essential hypertension. Therefore, identification of the genetic risk factors is expected to enhance our understanding of the molecular basis for essential hypertension. Allelic association studies are commonly used to identify the susceptibility genes for complex traits such as essential hypertension (Landers and Schork, 1994).

The activity of serum paraoxonase in human has been reported to have up to 10-40 fold interindividual variation (Furlong *et al.*, 1988; Humbert *et al.*, 1993). Genetic variation in PON1 gene has been found to be the major determinant of interindividual variation of paraoxonase activity (Adkins *et al.*, 1993). The actual molecular basis for the PON1 polymorphism is a glutamine → arginine substitution in codon 192 of PON1 gene (Adkins *et al.*, 1993; Humbert *et al.*, 1993). By the study of Humbert *et al.*, 1993, paraoxonase allozyme that has glutamine at residue 192 has low enzyme activity, while the second paraoxonase allozyme, which has arginine at residue 192, has high enzyme activity. Several study have reported that codon 192 polymorphism of PON1 gene may be a genetic risk factor for cardiovascular diseases including coronary artery disease (Serrato and Marian, 1995), coronary heart disease in type 2 diabetes (Ruiz *et al.*, 1995) or Parkinson's disease (Akhmedova *et al.*, 1999). To our knowledge, the information on the association between codon 192 polymorphism of PON1 gene and essential hypertension is scanty.

This study evaluated the association of essential hypertension with codon 192 polymorphism at the PON1 gene in Korean population. Our result indicated the lack of association between the codon 192 polymorphism of PON1 gene and essential hypertension in the population under study. Thus, this data suggest that amino acid change due to glutamine or arginine alleles of PON1 gene does not exert a major effect on the pathogenesis of essential hypertension among in part Korean population. It should not excluded, however, that this polymorphism could have small effect for the pathogenesis of this disease because a small gene effect may expected in the case of a disease as complex as essential hypertension. Furthermore, these types of study design (association studies of case-control type) are prone to type II errors. In other word, negative findings generated by retrospective case-control studies can in no way be advocated to rule out gene effects in clinical phenotypes under investigation. Therefore, further studies using larger sample size will require to understand the influence of the PON1 gene in the pathogenesis of essential hypertension.

Numerical epidemiological studies have established a strong inverse relationship between plasma HDL-cholesterol level and cardiovascular disease (Buring *et al.*, 1992; Fuh *et al.*, 1987; Jayakumari *et al.*, 1993). In our study, the codon 192 polymorphism of PON1 gene was significantly associated with plasma HDL-cholesterol level. Especially, arg/arg homozygotes showed the elevated plasma HDL-cholesterol level compared with other two genotypes. As a possible explanation for this, codon 192 polymorphism of PON1 gene may affect some function *in vivo* and this in turn affects plasma HDL metabolism and concentration. Second, this polymorphism studied might have been in linkage disequilibrium with another unidentified functional variant in the PON gene family or at another gene on chromosome 7.

When stratified by blood pressure status, the association between codon 192 polymorphism of PON1 gene and plasma HDL-cholesterol level was maintained in only normotensive group. In addition, there was the significant difference in plasma TC level across the genotype in this group, reflecting higher plasma TC level in arg/arg homozygote than other two genotypes. Arg/arg homozygote also showed higher plasma LDL-cholesterol level than that of other two genotypes, but this difference was not statistically significant.

In the case of essential hypertensive group, any association between this genetic marker and plasma lipid parameters was not detected across the genotypes. Since essential hypertension is known to be caused by both genetic and environmental factors and essential hypertensive status influences the plasma lipid or lipoprotein components of each individuals, this distortion of the association between codon 192 polymorphism of PON1 gene and plasma lipid level by blood pressure status may be due to unknown gene-gene interaction or gene-environment interaction.

Whatever the mechanism of the association between codon 192 polymorphism and plasma HDL-cholesterol level, however, this result suggests that arg/arg genotype may be useful as a protective marker on cardiovascular disease in Korean population.

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