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Codon 311 Polymorphism of Paraoxonase-2 Gene and Hypertension in Korean

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한국인에서 Paraoxonase-2 유전자의 Codon 311 다형성에 관한 연구

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요 약

고혈압에서 지질대사 이상은 빈번히 나타나는 현상으로, 지질대사 이상에 관여하는 유전자들은 고혈압의 발병원인을 규명하기 위한 후보 유전자로 인식되어 왔다. 이에 본 연구에서는 paraoxonase 2 (PON2) 유전자에 존재하는 Cys311Ser 다형성을 유전자 표지로 이용하여 한국인 집단에서 이 유전자 표지가 고혈압과 관련성이 있는 지를 조사하고자 하였다. 연구 대상은 총 195명으로, 이들 중에서 82명은 고혈압 환자군이었고, 나머지 113명은 정상 혈압군이었다. PON2 유전자의 Cys311Ser 다형성을 분석하기 위해서 중합효소 연쇄반응과 제한 효소인 *Dde* I 처리를 수행하여 유전자형을 결정하였다. 연구 결과, Cys/Ser 이형접합체를 갖는 사람들이 고혈압군에서 유의하게 높은 빈도로 나타났으며 ($P<0.05$), 다른 신체 계측치 및 혈청내 지질 농도와는 유의한 관련성을 나타내지 않았다. 본 연구에서 관찰된 이러한 관련성이 기능적인 연관인지 혹은 연관불평형에 의한 결과인지에 대해서는 보다 더 많은 연구 대상을 이용한 추시를 통해 밝혀질 수 있을 것으로 생각된다.

Key words : grey mullet, group synchronous, *Mugil cephalus*, offshore migration, reproductive cycle

INTRODUCTION

Hypertension is a complex multifactorial and polygenic disorder that is thought to result from an interaction between an individual's genetic background and various environmental factors (Lifton *et al.*, 2001). Given that hypertension is a major risk factor for coronary artery disease (CAD), stroke, and chronic renal failure, prevention of hypertension is an important public health goal.

One approach to preventing the development of this condition is to identify disease susceptibility gene (Izawa *et al.*, 2003). Genetic linkage and candidate gene association studies have implicated various loci and genes in predisposition to hypertension. Although genetic epidemiological studies have suggested that certain genetic variants, including polymorphisms in the genes encoding angiotensinogen (Jeunemaitre *et al.*, 1992), α-adducin (Cusi *et al.*, 1997), the β3 subunit of G protein (Siffert *et al.*,

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1998), and the β2-adrenergic receptor (Bray *et al.*, 2000), increase the risk of hypertension, the genes that contribute to genetic susceptibility to this condition remain to be identified definitively. In addition, because of ethnic divergence of gene polymorphisms, it is important to construct a database of polymorphisms related to hypertension in each ethnic group.

Among the many candidate genes that are thought to contribute to genetic susceptibility to hypertension, members of paraoxonase (PON) gene family are of particular interest.

PON is a calcium dependent enzyme that is associated with HDL (Blatter *et al.*, 1993), and hydrolyzes some toxic metabolite of organophosphate such as paraoxon (Furlong *et al.*, 1988). Also, this enzyme has been reported to have the anti-oxidant and anti-atherogenic activities (Mackness *et al.*, 1993).

PON gene family consists of three members, PON1, PON2 and PON3 located on the long arm of chromosome 7 between q21.3 and q21.1 in human (Primo-Parma *et al.*, 1996). The genes share considerable structural similarity and appear to have arisen by gene duplication from a common evolutionary precuesor (Primo-Parma *et al.*, 1996; La Du *et al.*, 1999).

With respect to PON1 gene, two common (codon 55 and 192) polymorphisms were described in this genetic locus (Serrato *et al.*, 1995; Malin *et al.*, 1999), and some studies reported the significant association between these two polymorphisms of this gene and cardiovascular disease (CVD), while conversely, others reported a lack of association between these polymorphisms and risk for CVD (Mackness *et al.*, 2002). In the previous study, our study group reported that codon 192 polymorphism of PON1 gene was significanly associated with plasma HDL-cholesterol level in Korean population, but not with hypertension (Kang *et al.*, 2001).

A comon polymorphism in codon 311 of the PON2 gene has also described, and is associated with CVD in Asian Indians (Sanghera *et al.*, 1988). Though, there is no report on the relationship between codon 311 polymorphism in the PON2 gene and hypertension. Thus, we estimated the relationship between this polymorphism of the PON2 gene and hypertension in Korean population.

MATERIALS AND METHODS

1. Study subjects

Total 195 Korean subjects were recruited from clinical pathology of the Seoul Hygiene hospital, Seoul, Korea. Of these individuals, 82 subjects were diagnosed with hypertension. Subjects were classified as having hypertension if they exhibited the systolic blood pressure (SBP) above 140 mmHg and diastolic blood pressure above 90 mmHg and had no clinical signs, symptoms and laboratory findings suggestive of secondary hypertension. Normotensives consisted of 113 indivials having systolic blood pressure (SBP) under 140 mmHg and diastolic blood pressure under 90 mmHg. Male /female (M/F) ratio was not statistically different between two groups (For normotensives, the M/F ratio was 46.4.7%/53.6%; for hypertensives, the M/F ratio was 34.2%/65.8%; $\chi^2 = 2.3839$, df = 1, P = 0.1226).

2. Determination of clinical parameters

Blood samples were obtained in EDTA tubes from individuals who had been fasting for 12~16 hour. Serum lipid parameterd including total cholesterol (TC), triglyceride and high density lipoprotein (HDL)-cholesterol was determined by using a Hitachi 7150 automatic chemistry analyzer. Serum low density lipoprotein (LDL)-cholesterol level was calculated by using the formula of Friedewald *et al.*, (1972) as follows: Serum LDL-cholesterol level = serum TC level-serum TG level/5-serum HDL-cholesterol level (unit: mmHg).

The demogrphic characteristics of our study subjects are displayed in Table 1. There were the significant differences in age and serum HDL-cholesterol level between normotensives and hypertensives, respectively ($P < 0.05$).

Table 1. Basic demographics of study subjects

Variables	Mean ± SD[1](Number)		Student's t-test
	Normotensives	Hypertensives	P-value
Age (year)	56.5±9.3(112)	63.8±12.0(79)	<0.001**
BMI (kg/m^2)[2]	23.5±2.4(112)	24.0±2.7(68)	0.215
TG (mg/dl)[3]	125.6±78.4(92)	133.8±69.0(56)	0.520
TC (mg/dl)[4]	151.8±40.2(92)	156.1±31.2(56)	0.491
LDL-chol (mg/dl)[5]	97.6±38.7(92)	105.2±39.2(56)	0.290
HDL-chol (mg/dl)[6]	28.8±9.4(92)	24.2±7.5(56)	0.002*

Abbreviations: [1]Standard deviation, [2]body mass index, [3]triglyceride, [4]total cholesterol, [5]low density lipoprotein cholesterol and [6]high density lipoprotein cholesterol. *P<0.05 and **P<0.001

3. DNA analysis

DNA was extracted from buffy coats as described by Sambrook *et al.* (1989). Codon 311 polymorphism in the PON2 gene was detected by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique described by Sanghera *et al.* (1998) with the following primers;

sense, 5'-ACA TGC ATG TAC GGT GGT CTT ATA-3' and

anti-sense, 5'-AGC AAT TCA TAG ATT AAT TGT TA-3'.

PCR was carried out in 50 μL total volume containing 100 ng genomic DNA, 0.5 μM of each primer, 0.2 mM of each dATP, dDDP, dCTP and dGTP, 50 mM KCl, 20 mM Tris-HCl, pH 8.0, 15 nM $MgCl_2$, 2.5 U of *Taq* DNA polymerase (Cat. No. N 808-0160, Perkin-Elmer, Foster City, CA, USA). Thermal cycling was carried out in a Perkin-Elmer GeneAmp PCR system 9700 Thermal Cycler with an initial 4 min denaturation at 94°C followed by 30 cycles of denaturing at 94°C for 1 min, annealing at 46°C for 1 min 30 sec, extending at 72°C for 2 min and a final extension of 10 min at 72°C.

4. Dde I RFLP analysis

Ten uL of the PCR product was digested with 10 U restriction enzyme, *Dde* I (Promega, Co. Ltd., USA) for 18 h at 37°C, and separated on 3% Nusieve agarose gel electrophoresis for 20 min at a constant voltage of 100 V. The gels were stained by 0.5 μg/mL of ethidium bromide. The image was captured on the thermal paper using the Eagle Eye∏ Still Video System (Stratagene, La Jolla, CA, USA).

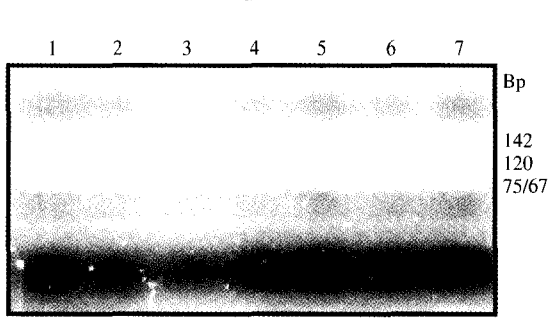

Fig. 1. Analysis of codon 311 polymorphism in the PON2 gene. Lane 1~3, Cys/Ser heterozygotes; lane 4~7, Ser/Ser homozygotes.

The PCR amplification of PON2 gene produced a DNA fragment of 262 bp in length, and the digestion with restriction enzyme, *Dde* I revealed the existence of codon 311 polymorphism (Fig. 1). By restric-tion digestion, Cys allele consists of two bands of 142 and 120 bps, while Ser allele has three bands of 120, 75 and 67 bps, respectively (Fig. 1).

5. Statistical Anaysis

Data are presented as mean±standard deviation (SD). Allele frequencies were calculated from the genotypes of all subjects. Hardy-Weinberg equilibrium was assessed by (χ^2-fitness test with one

degree of freedom (df). The heterozygosity index (H) and the polymorphism information content (PIC) value were calculated according to the methods described by Bostein *et al.* (1980). Comparisons of genotype distribution and allele frequencies were assessed by (χ^2-statistics with 2 and 1 df, respectively. One-way ANOVA test was performed to compare the mean values of clinical parameters among different genotypes. All statistical analysis was performed using the computer program of SPSSWIN (version 11.0).

RESULTS AND DISCUSSION

The distributions of codon 311 polymorphism in the normotensives and hypertensives are shown in Table 2. The frequencies of Cys/Cys, Cys/Ser and Ser/Ser genotypes were 4, 22 and 74% in normotensives, and 2, 39 and 51% in hypertensives, respectively. Observed genotype distribution in the both groups was in Hardy-Weinberg equilibrium ($P > 0.05$). The heterozygosity index and PIC values of a codon 311 polymorphism showed the values of 0.2618 and 0.2275 in normotensives, and 0.3427 and 0.2839 in hypertensives, respectively. According to the heterozygosity index and PIC value, this polymorphism indicated the relatively higher degree of polymorphism in only hypertensives.

By case-control comparison, there was significant association in genotype frequency between codon 311 polymorphism and hypertension in our subjects ($P < 0.05$). Especially, individulas with Cys/Ser heterozygotes indicated the high hypertensive risk than those with other genotypes. Thus, it is likely that this polymorphism is useful as a genetic marker to explain the pathogenesis of hypertension in Korean

Table 2. Genotype and allele frequencies of the codon 311 polymorphism in the PON2 gene between normotensives and hypertensives

	Genotype No. (%)			Allele No. (%)			
	Cys/Cys	Cys/Ser	Ser/Ser	Cys	Ser	H[1]	PIC[2]
Normotensives	5 (4)	25 (22)	83 (74)	35 (15)	191 (85)	0.2618	0.2275
Hypertensives	2 (2)	32 (39)	48 (51)	36 (22)	128 (78)	0.3427	0.2839
χ^2		6.7386		2.2506			
P		0.0344		0.1336			
Odds ratio(CI)[3]			1.53 (0.92~2.57)				

[1]Heterozygosity was calculated as $H = 1 - \Sigma pi^2$ (p: allele frequency).

[2]Polymorphism Information Content was calculated as $PIC = 1 - \Sigma pi^2 - \Sigma\Sigma 2pi^2pj^2$ (p: allele frequency).

[3]95% Confidence Interval.

Frequency is given as a percentage in parenthesis.

Table 3. Clinical parameters of subjects according to genotypes of the codon 311 polymorphism in the PON2 gene

Variables	Genotypes		
	Cys/Cys (No.)[6]	Cys/Ser (No.)	Ser/Ser (No.)
Age (year)	57.1±10.7 (7)	60.8±11.5 (56)	59.1±10.9 (128)
BMI (kg/m^2)[1]	23.9±2.4 (7)	24.2±2.5 (51)	23.5±2.5 (122)
Tg (mg/dl)[2]	75.6±45.4 (5)	136.9±68.0 (41)	128.0±77.9 (102)
TC (mg/dl)[3]	124.0±74.2 (5)	157.9±38.5 (41)	153.0±33.7 (102)
LDL-chol (mg/dl)[4]	90.9±58.8 (5)	101.5±36.1 (41)	100.5±34.3 (102)
HDL-chol (mg/dl)[5]	18.0±11.4 (5)	27.8±8.4 (41)	27.2±9.0 (102)

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

population.

Table 3 represents the comparison of various clinical parameters according to codon 311 polymorphism among our study subjects. There was no significant association between each genotype in codon 311 polymorphism and any cardiovascular risk factors (one-way ANOVA test, $P>0.05$). It is unlikely that this polymorphism is one of the genetic components for cardiovascular risk.

Although we observed the sifnificant association between codon 311 polymorphism in the PON2 gene and hypertension in Korean population, this study does not provide a mechanism by which Cys/Ser genotype predisposes to hypertension. Until now, the biological role of PON2 gene is unclear, and it is still unknown whether this polymorphism is related to paraoxonase enzyme activity. Of course, it could not excluded the possibility that chance effect by modest sample size results in the type I error. However, the distribution of Ser allele (0.82) in our subjects was similar to those reported by other studies including Indian (0.61) (Sanghera *et al.*, 1988), Chinese (0.76) (Shi *et al.*, 2004), Korean (0.74~0.77) (Choi *et al.*, 1999; Hong *et al.*, 2001), Italian (0.65) (Motti *et al.*, 2001) and Dutch (0.74) (Leus *et al.*, 2001). Altogether, Ser allele frequency was always significantly higher than that of Cys allele in all ethnic groups investigated. It is likely that codon 311 polymorphism arose before the divergence of different racial group in human population. Because of the probable absence of selective forces at this locus, neither Cys nor Ser alleles may progressed to fixation. If this hypothesis is right, this association between codon 311 polymorphism and hypertension in our study may be explained by the linkage disequilibrium between this polymorphism and other mutation in the PON2 gene or PON3-like other genes close to PON2 gene (Campo *et al.*, 2004). Further studies using large sample size and other genetic markers close to PON2 gene will be required to clarify the precise role of PON2 gene in the ethiology for hypertension.

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