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Linkage Disequilibrium of Dopamine D2 Receptor Gene in the Korean Population

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ABSTRACT. The genetic basis of hypertension is complex, and has been considered to be associated with the dopamine D2 receptor gene (DD2R). Because association studies using the candidate gene approach may provide important clues regarding the pathogenesis of hypertension and establish basis for further study, we performed the association study on the relationship between genetic polymorphisms in the DD2R gene and hypertension in Koreans. Eighty nine patients with hypertension and 86 age-matched subjects with normal blood pressure were enrolled. Genomic DNA was extracted from peripheral blood leukocytes. PCR-RFLP analysis was performed to detect the three polymorphic *Taq* I sites in the DD2R gene. There were no significant differences in genotype, allele and haplotype distributions of any polymorphisms in the DD2R gene between two groups, respectively ($P>0.05$), although significant linkage disequilibriums among these polymorphic sites were detected by pair-wise analysis ($P<0.05$). Therefore, our negative result suggest that the three *Taq* I RFLPs in the DD2R gene were not significantly associated with hypertension in Koreans.

Keywords: Blood pressure, Dopamine D2 receptor, Korean population.

INTRODUCTION

Hypertension affects about 25% of most adult populations, and is an important risk factor for death from stroke, myocardial infarction, and congestive heart failure (Mosterd *et al.*, 1999). The genetic basis of hypertension is very complex, and today, genetic association study has been widely performed to search for the causative gene of hypertension.

Pharmacological data suggest that the dopamine D2 receptor (DD2R) can modulate blood pressure. The DD2R belongs to the D2-like (D2 to D4) family of dopamine receptors. The remaining dopamine receptors, of the 5 currently identified, fall into the D1-like (D1 and D5) family (Hussain and Lokhandwala, 1998; Sibley and Monsma, 1992). The DD2R has been localized to the cerebral medulla, kidneys, and systemic arteries (Hussain and Lokhandwala, 1998; Sibley and Monsma, 1992). Dopamine has both central and peripheral neurotransmitter roles, and the stimulation of the DD2R has been shown to inhibit sympathetic neuronal norepinephrine release. Dopamine also acts as an intrarenal natriuretic hormone through the D1 receptor and to a lesser extent, the DD2R (Hussain and Lokhandwala, 1998; Jose *et al.*, 1997; Mannelli *et al.*, 1997).

The DD2R gene has been cloned, and mapped to chromosome 11q22-23 (Grandy *et al.*, 1989a, b). This gene includes several polymorphisms, and some of them have been associated with receptor activity (Cravchik *et al.*, 1996), several psychiatric disorders related to stimulation of the reward pathway including substance abuse (Blum *et al.*, 1993; Comings *et al.*, 1993; OHara *et al.*, 1993) or hypertension (Rosmond *et al.*, 2001; Thomas *et al.*, 2000).

The objective of our present study were: firstly to estimate the gene frequencies of three *Taq*I RFLPs in the DD2R gene for hypertensive and normotensive subjects of Korean origin, secondly to investigate the influence of these polymorphisms on anthropometrical and biochemical parameters, and finally to measure the degree of linkage disequilibrium among these RFLP sites in the DD2R gene.

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Table 1. Clinical characteristics of the subjects

Parameter	Subjects		Probability
	Normotensives	Hypertensives	
Age (year)	56.4 ± 9.6	63.2 ± 11.9	<0.05
SBP(mmHg)[1]	117.5 ± 9.8	162.5 ± 38.6	<0.05
DBP(mmHg)[2]	77.5 ± 5.1	115.0 ± 34.2	<0.05
BMI (kg/m^2)[3]	23.5 ± 2.4	24.0 ± 2.6	
Tg (mg/dl)[4]	127.3 ± 82.6	133.3± 66.2	
TC (mg/dl)[5]	150.9 ± 40.2	152.3 ± 32.4	
LDL-chol (mg/dl)[6]	96.1 ± 39.5	100.8 ± 31.3	
HDL-chol (mg/dl)[7]	29.1 ± 9.8	24.8 ± 9.8	<0.05
Glucose (mg/dl)	91.7 ± 76.4	89.0 ± 52.1	

[1]Systolic blood pressure, [2]Diastolic blood pressure, [3]Body Mass Index, [4]Triglyceride, [5]Total cholesterol, [6]LDL-cholesterol and [7]HDL-cholesterol.
There were statistically significant differences in age, SBP, DBP and HDL-cholesterol level between two groups, respectively (P<0.05).

MATERIAL AND METHOD

Study Subject

A total of 175 unrelated subjects were randomly chosen from the Dept. of Clinical Pathology, Seoul Hygiene Hospital, Seoul, Korea. Subjects were classified as having hypertension if they exhibited the systolic blood pressure (SBP) > 140 mmHg and diastolic blood pressure > 90 mmHg and had no clinical signs, symptoms and laboratory findings suggestive of secondary hypertension. Among them, 89 subjects were patients with hypertension. The body mass index (BMI) value was calculated by the body weight (kg) divided by the square of the height (m^2). Serum triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL) cholesterol and glucose concentrations were measured using enzymatic methods and a HITACHI 7150 automatic analyzer. Low density lipoprotein (LDL) cholesterol concentration was calculated by Friedewalds formula (Friedewald *et al.,* 1972). The clinical characteristics of the subjects included in the study were shown in Table 1.

Genetic Analysis

Genomic DNA was prepared from buffy coats of 5 ml blood after lysis of red blood cell. Three polymorphisms in the DD2R gene were assayed by means of PCR-*Taq*I RFLP. Briefly total 50 ul of the reaction mixture contained 200~400 ng of genomic DNA, 10 pmol of each primer, 200 μM of each dNTP, 0.25 U of *Taq* DNA polymerase (Promega Co. Ltd., Madison, WI, USA) and buffers recommended by the manufacturer. The primer sequences for DD2R gene polymorphisms studied were displayed in Table 2. PCR amplification was performed in an automatic MJ Research PTC-200 thermal cycler. The reaction mixture was denatured at 94°C for 1 min, annealed at 60°C for 1 min, and extended at 72°C for 1 min in a total of 30 cycles.

After PCR amplification, the amplified product was digested with restriction enzyme, *Taq* I, and analyzed by 2% agarose gel electrophoresis. *Taq* I alleles were visualized as fragments of 236 bp (*Taq* I "absence": A1) or 124 bp and 112 bp (*Taq* I "presence": A2) for *Taq* IA RFLP, 459 bp (*Taq* I "absence": B1) or 267 bp and 192 bp (*Taq* I "presence": B2) for *Taq* IB RFLP, and 300 bp (*Taq* I "absence": D1) or 212 bp and 88 bp (*Taq* I "presence": D2) for *Taq* ID RFLP (Nam and Lee, 1997).

Statistics

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS), version 11.0 for Windows. Allele frequency was calculated from observed genotype frequency, and the difference in observed genotype or allele frequencies between populations studied was tested using the χ^2-test. The heterozygosity (H) and polymorphism information content (PIC) values were calculated using the method by Bostein *et al.* (1980). Maximum likelihood estimate (MLE) of haplotype frequency was calculated by EH program (version 1.14). The degree of linkage disequi-

Table 2. List of the sequences of oligonucleotide primers and PCR conditions to detect three *Taq* I RFLPs in the DD2R gene

Polymorphic site	Sequence of oligonucleotide primer	Amplification condition	Size of amplified DNA
DD2R			
Taq IA	5'-CCTTCCTGAGTGTCATCAAC-3'	94°C 30 sec	236 bp
	5'-ACGGCTCCTTGCCCTCTAG-3'	60°C 30 sec	
		72°C 1 min	
Taq IB	5'-GATGTGTAGGAATTAGCCAGG-3'	94°C 30 sec	459 bp
	5'-GTACCCACTTCAGGAAGTC-3'	60°C 30 sec	
		72°C 1 min	
Taq ID	5'-CCTCTGAGGCTTACTGTCTG-3'	94°C 30 sec	300 bp
	5'-AAAACTAGGGAGGGTCAGAG-3'	60°C 30 sec	
		72°C 1 min	

Table 3. Comparison of genotype frequencies in three *Taq* I RFLPs of the DD2R gene in normotensive and hypertensive groups

Polymorphic site	Genotype	Normotensive			Hypertensive		
		Freq.[1]	H^2	PIC[3]	Freq.	H	PIC
Taq IA	A1A1	0.14	0.4642	0.3565	0.14	0.4680	0.3585
	A1A2	0.45			0.47		
	A2A2	0.41			0.39		
Taq IB	B1B1	0.13	0.4790	0.3643	0.19	0.4858	0.3678
	B1B2	0.54			0.45		
	B2B2	0.33			0.36		
Taq ID	D1D1	0.01	0.1107	0.1046	0.00	0.1774	0.1616
	D1D2	0.10			0.20		
	D2D2	0.89			0.80		

Abbreviation; [1] Frequency, [2]Heterozygosity and [3]polymorphism information content.

librium was determined by calculation of delta (Δ) (Hill and Robertson, 1968) and D' (Lewontin, 1964) values between the polymorphic sites in the DD2R gene. To test the significance of linkage disequilibrium, $n\Delta^2$ value was used as the χ^2 distribution with 1df. The mean value of continuous data according to genotypes was analyzed by One-Way ANOVA test or Indepentent Samples T-test. Statistical significance was tested at the P=0.05 level.

RESULTS

Genotype Distribution

The genotype frequencies of three *Taq* I RFLPs in the DD2R gene were shown in Table 3. In *Taq* IA RFLP, the observed genotype distribution was not deviated from Hardy-Weinberg equilibrium. The observed genotype frequencies of A1A1, A1A2 and A2A2 were 14, 45 and 41% in normotensives, and 14, 47 and 39% in hypertensives, respectively. Frequencies of the A1 allele were about 0.37 for normotensives and hypertensives, respectively. The PIC of *Taq* IA RFLP showed values of about 0.36 in the both groups, respectively. There was no statistically significant difference between normotensives and hypertensives in genotype and allele frequencies, respectively.

In *Taq* IB RFLP, the observed genotype distribution was also in Hardy-Weinberg equilibrium. The observed genotype frequencies of B1B1, B1B2 and B2B2 were 13, 54 and 33% in normotensives, and 19, 45 and 36% in hypertensives, respectively. Frequencies of the B1 allele were 0.40 for normotensives and 0.42 for hypertensives, respectively. The PIC of *Taq* IB RFLP showed values of about 0.36 for normotensives and 0.37 for hypertensives, respectively. There was also no statistically significant difference between two groups in genotype and allele frequencies, respectively.

Like *Taq* IA and *Taq* IB RFLPs, the observed genotype distribution of *Taq* ID RFLP was also in Hardy-Weinberg equilibrium. The observed genotype frequencies of D1D1, D1D2 and D2D2 were 1, 10 and 89% in normotensives, and 0, 20 and 80% in hypertensives, respectively. The D1D1 genotypes was observed in only one normotensive subject. Frequencies of the D1 allele were 0.06 for normotensives and 0.10 for hypertensives, respectively. The PIC of *Taq* ID RFLP showed values of about 0.10 for normotensives and 0.18 for hypertensives, respectively. There was also no statistically significant difference between two groups in genotype and allele frequencies, respectively.

The Comparison of Anthropometrical and Biochemical Parameters Among Genotypes of Three *Taq* I RFLPs in the DD2R Gene

The comparison of anthropometrical or biochemical parameters according to genotypes of three *Taq* I RFLPs in the DD2R gene was displayed in Table 4. None of parameters was significantly associated with genotypes of all RFLPs investigated in our subjects.

Table 4. The comparison of anthropometrical and biochemical parameters among genotypes of three *Taq* I RFLPs in the DD2R gene

Parameter	P-value by statistical test		
	Taq IA	*Taq* IB	*Taq* ID
Age (year)	0.651	0.954	0.762
BMI (kg/m^2)[1]	0.536	0.914	0.474
Tg (mg/dl)[2]	0.409	0.758	0.600
TC (mg/dl)[3]	0.895	0.999	0.098
LDL-chol (mg/dl)[4]	0.610	0.987	0.176
HDL-chol (mg/dl)[5]	0.732	0.998	0.600
Glucose (mg/dl)	0.359	0.730	0.436

[1]Body Mass Index, [2]Triglyceride, [3]Total cholesterol, [4]LDL-cholesterol and [5]HDL-cholesterol.

Table 5. Extended haplotype distribution of three *Taq* I RFLPs in the DD2R gene

Polymorphic Site			Subject	
Taq IA	*Taq* IB	*Taq* ID	Normotensive	Hypertensive
A1	B1	D2	0.321033	0.404255
A1	B2	D2	0.023411	0.000000
A2	B1	D1	0.000000	0.000256
A2	B1	D2	0.045633	0.052935
A2	B2	D1	0.022222	0.095488
A2	B2	D2	0.587700	0.447065
Chromosome number			90	94
P[1]			>0.05	

[1]The significant difference between normotensives and essential hypertensives was detected in haplotype frequencies (Monte-Carlo simulation, T_4=4.9795, df=3, P=0.1733, simulation number=10,000).

Table 6. Pair-wise linkage disequilibrium statistic (Δ, D') among three *Taq* I RFLP pairs of DRD2 gene

D' Δ	*Taq* IA	*Taq* IB	*Taq* ID
Taq IA		0.9663[1]	-0.9977[2]
Taq IB	0.9092[1]		-0.6738[3]
Taq ID	-0.1975[2]	-0.1549[3]	

[1]The significant linkage disequilibrium were detected (χ^2=226.50, df=1, *P*<0.0001).
[2]The significant linkage disequilibrium were detected (χ^2=8.43, df=1, *P*=0.0037).
[3]The significant linkage disequilibrium were detected (χ^2=5.42, df=1, *P*=0.0199).

The Haplotype Distribution and Linkage Disequilibrium Analysis of Three *Taq* I RFLPs in the DD2R Gene

The haplotype frequency constructed by three *Taq* I RFLPs in the DD2R gene was represented in Table 5. Because the existence of double heterozygotes disturbed the construction of haplotype in our subjects, we calculated the maximun likelihood estimates of haplotype with EH program. By Monte-Carlo simulation, there was no statistically significant difference in haplotype frequency between two groups.

The degree of linkage disequilibrium of three *Taq* I RFLPs in the DD2R gene was shown in Table 6. Significant linkage disequilibrium was detected among all polymorphic sites studied (*P*<0.05).

DISCUSSION

The search for susceptibility genes involved in hypertension has mainly focussed on the use of family-based linkage and population-based case-control association studies. In particular, the latter can be used to test for the involvement of potential candidate genes. One such gene thought to have a plausible role in hypertension is the DD2R gene located on chromosome 11q22-23 (Grandy *et al.*, 1989a, b). Animal model study suggested that genetic variation within or near the DD2R gene may be involved in blood pressure variability in spontaneously hypertensive rat model (Kren *et al.*, 1997). In the case of human, Thomas *et al.* (2000) reported a positive association between hypertension and *Taq* IA RFLP of the DD2R gene in Hong Kong population of Han Chinese origin. However, our study showed the lack of association between three *Taq* I RFLPs of DD2R gene and hypertension in Korean population. The reason for this discrepancy observed between two Asian populations is difficult to interpret, but differences in disease classification, sampling design and/or ethnic background of study subjects may explain inconsitent findings. Another possibility is that these differences can be influenced by the different prevalence rates of hypertension.

We also investigated the relationship between three *Taq* I RFLPs in the DD2R gene and anthropometrical or biochemical parameters in Koreans. However, none of three *Taq* I RFLPs in the DD2R gene was not significantly associated with any parameters, respectively. It is unlikely that these polymorphisms contribute to the genetic component of cardiovascular risk factors studied in our subjects.

The comparison of haplotype frequencies between normotensive and hypertensive groups may be more sensitive than analysis of individual RFLP sites, since the testing of hypotheses of genetic association between the genetic polymorphisms in the DD2R gene and complex disease in human would be facilitated by increasing the PIC value in the DD2R gene (Di Lella *et al.*, 1986). Accordingly, we constructed haplotypes of three *Taq* I RFLPs, and investigated the relationship between haplotypes of these polymorphisms and hypertension in Koreans. However, haplotype frequencies in our subjects were not significantly different between two groups. Thus, no evidence for a significant association was detected in the present sample.

The presence of linkage disequilibrium among RFLPs in any gene influence the choice of genetic markers used in linkage or association studies. Our findings indicated that significant linkage disequilibrium exist among three *Taq* I RFLPs in the DD2R gene. The presence of strong linkage disequilibrium among three *Taq* I RFLPs decreases the genetic diversity of the haplotypes in our subjects, while it did not require the large sample size to perform the association study. Therefore, in the case of our study, the association study may be better choice than linkage analysis in detecting the causative gene of

hypertension.

In summary, we conclude that three *Taq* I RFLPs in the DD2R gene are not important factors in the susceptibility to hypertension in koreans. It remains possible, however, that other sequence variation(s) within or near this gene could play a role.

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