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A *Bal* I RFLP of Dopamine D3 Receptor Gene in Korean Hypertensives

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한국인 고혈압군에서 Dopamine D3 receptor 유전자에 존재하는 *Bal* I 제한절편길이 다형성에 관한 연구

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요 약

고혈압은 다양한 유전적 요인과 환경적 요인들이 상호작용하여 발병하는 질환으로, 기존의 연구에서 dopamine D3 receptor (DRD3)와 고혈압과의 관련성에 관한 보고들이 있었다. 이에, 본 연구에서는 DRD3 유전자에 존재하는 *Bal* I 제한절편길이 다형성이 한국인 집단에서 고혈압과 어떠한 관련성이 있는 지를 조사하였다. 환자-대조군 연구를 수행한 결과 이 유전자에 존재하는 다형성은 한국인 집단에서 고혈압과 유의한 관련성을 나타내지 않았다. 그러나, 이 다형성을 구성하는 대립 유전자의 빈도를 여러 민족집단의 결과들과 비교했을 때, 흑인 집단과 유의한 차이를 나타내었다. 따라서, 이러한 결과는 DRD3 유전자에 존재하는 다형성이 고혈압과의 관련성을 나타내는 지를 정확하게 이해하기 위해서는 흑인 집단을 비롯한 다른 민족집단들을 대상으로 하여 광범위한 연구를 수행할 필요가 있을 것으로 생각된다.

Key words : Dopamine, Hypertension and Korean

INTRODUCTION

Hypertension affects approximately 25% of the adult population and is a major risk factor for heart attack, stroke and kidney failure. Although blood pressure is known to have a strong genetic determination, the genes responsible for susceptibility to hypertension are mostly unknown. From studies in human and animal models it is clear that several genetic loci are involved in regulation of blood pressure and hypertension (Szpirer *et al.*, 1993).

Dopamine plays an important role in the control of renal sodium excretion and rennin, aldosterone, and norepinephrine secretion through specific dopamine receptors (DRs). To date, 5 distinc DRs (D1~D5) have been identified. The DRs are classified into two families: D1 like (includes D1 and D5), which stimulates adenylyl cyclase, and D2 like (includes D2, D3 and D4), which inhibit adenylyl cyclase (Hussain and

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Lokhandwala, 1998; Missale *et al.*, 1998).

Dopamine D3 receptor gene is a candidate gene in hypertension, because it has been known that this receptor is expressed in rat juxtaglomerular cell (Sanada *et al.*, 1997), and might also negatively regulate renin secretion (Soma *et al.*, 2002). In addition, Asico *et al.*, (1998) reported that the DRD3 knockout mice represented high renal rennin activity and resulted in renin dependent hypertension.

Yet, to our knowledge, despite the important role played by DRD3 in the molecular ethiology of hypertension, only one molecular genetic study has attempted to observe putative correlations of a genetic marker in the DRD3 gene and hypertension in human, and it failed to uncover an association between *Bal* I RFLP in the DRD3 gene and hypertension in a Japanese population (Soma *et al.*, 2002). So far, there is no report on a *Bal* I RFLP in Korean hypertensives.

Therefore, the aim of this study was to investigate the relationship between a genetic variation (*Bal* I RFLP) of DRD3 gene and hypertension in subjects of Korean origin.

MATERIALS AND METHODS

Study subjects

A total of 176 unrelated individuals were randomly chosen from the Seoul Hygiene Hospital, Seoul, Korea. We studied 86 subjects with hypertension. Patients were classified as having hypertension if they had systolic blood pressures above 140 mmHg and diastolic blood pressure above 90 mmHg on at least three separate occasions, and had no clinical signs, symptoms and laboratory findings suggestive of secondary hypertension. In additon, a randomly selected normal population (90 individuals) was analyzed as the control groups (blood pressure value, $< 140/90$ mmHg).

Determination of clinical parameters

Blood samples were obtained in EDTA tubes from individuals who had been fasting for 12 ~ 16 hr. Concentration of serum total cholesterol (TC), triglyceride and glucose were measured by enzymatic colorimetry methods with commercial kit (Boehringer Mannheim, Germany) and chemistry analyzer. Serum HDL-cholesterol level was determined by measuring cholesterol in the supernatant after precipitation of the serum with $MgCl_2$ and dextran sulfate, with a Gilford Impact 400E automated analyzer with reagents and calibrators from Boehringer Mannheim. Also, serum low density lipoprotein (LDL)-cholesterol level was calculated by Friedewald's equation (Friedwald *et al.*, 1972).

DNA analysis

Genomic DNA was isolated from buffy coat by the method of Sambrook *et al.* (1989) with slight modification. Polymerase Chain Reaction (PCR) techniques were used for Bal I RFLP of DRD3 gene the primer sequence were as follows;

forward, 5′-GCT CTA TCT CCA ACT CTC ACA -3′ and

backward, 5′-AAG TCT ACT CAC CTC CAG GTA -3′ (Elvidge *et al.*, 2001).

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 2 min denaturation at 94°C followed by 30cycles of denaturing at 94°C for 30 sec, annealing at 54°C for 30 sec, extending at 72°C for 40 sec and a final extension of 5 min at 72°C.

Bal I RFLP analysis

One fifth of the PCR product was digested with 5 U restriction enzyme, *Bal* I (Cat. No. 158S, new England Biolabs, Inc. Belverly, MA, USA) for 18 h at 37 °C, and separated on 2% agarose gel electrophorsis

for 30 min at a constant voltage of 110 V. The gels were stained by 0.5 μg/ml of ethidium bromide. The image was captured on the thermal paper using the Eagle EyeΠ Still Video System (Stratagene, La Jolla, CA, USA).

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 x^2-fitness test with one degree of freedom. The heterozygosity index (H) and the polymorphism information content (PIC) value were calculated according to Bostein *et al.*, (1980) and Ott (1991). The H index is a probability measure of the likelihood that a randomly selected subject is heterozygous for any two alleles at a given gene locus. The PIC value is an indicator of the probability that a marker locus is informative. It is estimated from the frequency of heterozygotes that are determined by the number of marker alleles and their respective frequencies. The significant differences between the total chromosomes for the hypertensives and normotensives were assessed by x^2-independence test with one degree of freedom. The association between genotypes and hypertension was evaluated by x^2-independence test with two degree of freedom. Differences in the clinical parameters among genotypes were assessed by Student's t-test or one-way analysis of variance (one-way ANOVA) test. A P value of less than 0.05 was considered significant. All statistical analysis was performed using the computer program of SPSSWIN (version 9.0).

RESULTS AND DISCUSSION

The PCR amplification of DRD3 gene produced a DNA fragment of 462 bp in length, and the digestion with restriction enzyme, *Bal* I yielded a band of 257 bp denoting allele B1 or two bands of 207 bp and 50 bp denoting allele B2 (Figure 1).

In this study, we tried to estimate the relationship between a *Bal* I RFLP in the DRD3 gene and hypertension in ethnically homogeneous Korean population. Table 1 displays the gene frequencies of DRD3 gene in Korean normotensives and hypertensives, respectively. The frequencies of B1B1, B1B2 and B2B2 genotypes were 53, 30 and 17% in normoten-

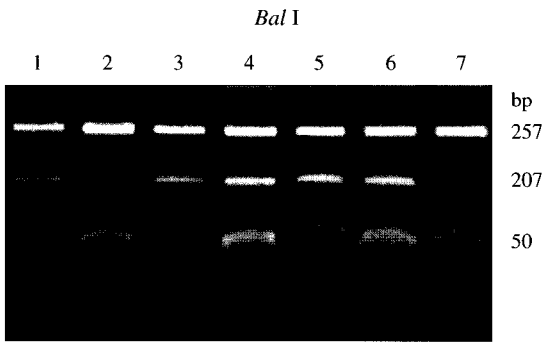

Fig. 1. Analysis of *Bal* I RFLP in the DRD3 gene. Lane 1, 3~6, B1B1 homozygotes; lane 2 and 7, B1B2 heterozygotes.

Table 1. Genotype and allele frequencies of the *Bal* I RFLP in the DRD3 gene between normotensives and hypertensives

	Genotype No. (%)			Allele No. (%)		H^1	PIC^2
	B1B1	B1B2	B2B2	B1	B2		
Normotensives	48 (53)	27 (30)	15 (17)	123 (68)	57 (32)	0.4328	0.3391
Hypertensives	46 (53)	35 (41)	5 (6)	127 (74)	45 (26)	0.3864	0.3117
X^2		5.9870		1.0410			
P		0.0501		0.3076			
Odds ratio (CI)[3]			1.31 (0.82~2.08)				

[1]Heterozygosity, [2]Polymorphism Information Content,
[3]95% Confidence Interval.
Frequency is given as a percentage in parenthesis.

Table 2. Clinical parameters of subjects according to genotypes of the *Bal* I RFLP in the DRD3 gene

Variables	Genotypes		
	B1B1 (No.)[6]	B1B2 (No.)	B2B2 (No.)
Age (year)	59.1±10.2 (90)	61.5±11.5 (62)	56.7±11.1 (20)
BMI (kg/m^2)[1]	23.8±2.2 (83)	23.3±3.0 (57)	24.6±2.4 (20)
Tg (mg/dl)[2]	119.4±56.5 (70)	142.0±100.6 (50)	119.8±57.3 (19)
TC (mg/dl)[3]	147.4±38.0 (70)	152.9±29.1 (50)	165.4±39.9 (19)
LDL-chol (mg/dl)[4]	96.6±36.0 (70)	98.6±30.5 (50)	112.6±36.9 (19)
HDL-chol (mg/dl)[5]	26.2±9.7 (70)	26.5±8.7 (50)	28.8±6.4 (19)
Glucose (mg/dl)	74.0±54.2 (48)	115.2±105.3 (27)	85.0±42.0 (13)

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

sives, and 53, 41 and 6% in hypertensives, respectively. There was a marginally negative association between a *Bal* I RFLP in the DRD3 gene and hypertension in our subjects ($P>0.05$). Thus, this RFLP may not be useful as a genetic marker to explain the ethiology of hypertension in Korean population as well as Japanese population (Soma *et al.*, 2002).

The heterozygosity index and PIC values of a *Bal* I RFLP showed the values of 0.4328 and 0.3391 in normotensives, and 0.3864 and 0.3117 in hypertensives, respectively. According to the heterozygosity index and PIC value, this RFLP indicated the relatively higher degree of polymorphism.

Table 2 represents the comparison of various clinical parameters according to a *Bal* I RFLP in the DRD3 gene among our study subjects. There were no significant differences in any clinical parameters across three genotypes (one-way ANOVA test, $P>0.05$). It is unlikely that this RFLP may not influence any cardiovascular risk factors studied in our subjects.

Allelic distributions of a *Bal* I RFLP in different ethnic populations are relatively consistent (Table 3). B1 allele (0.62~0.78) was prevalent than B2 allele in all Asian and European populations studied. However, B1 allele frequency (0.12) in African population (Crocq *et al.*, 1996) was very lower than those in Asian and European populations. These results emphasize the importance of studying newly discovered DNA polymorphism both for ethnic distribution and disease association within each ethnic group. Association of DNA polymorphisms with disease observed in one population may not apply others (Bae *et al.*, 2001). Until now, the relationship between a genetic marker in the DRD3 gene and hypertension has been performed in only two Asian populations (Japanese and Korean). Thus, association studies in other ethnic groups, especially African populations will be of interest, because the allele distribution in African population indicated the different aspect among populations studied.

Table 3. Genotype frequencies of *Bal* I RFLP in the DRD3 gene among different ethnic groups

Population	Number	B1	B2	Reference
African				
Congo	56	0.12	0.88	Crocq *et al.*, 1996
European				
Austria	57	0.65	0.35	Williams *et al.*, 1998
Belgium	62	0.73	0.27	Elvidge *et al.*, 2001
Canada	66	0.74	0.26	Williams *et al.*, 1998
France	101	0.67	0.33	Crocq *et al.*, 1996
France	52	0.64	0.36	Williams *et al.*, 1998
France	86	0.66	0.34	Elvidge *et al.*, 2001
Germany	60	0.69	0.31	Williams *et al.*, 1998
Germany	100	0.70	0.30	Elvidge *et al.*, 2001
Ireland	235	0.64	0.36	Williams *et al.*, 1998
Italy	55	0.62	0.38	Williams *et al.*, 1998
Spain	100	0.67	0.33	Williams *et al.*, 1998
Spain	53	0.66	0.34	Elvidge *et al.*, 2001
Sweden	53	0.72	0.28	Williams *et al.*, 1998
UK	77	0.73	0.27	Williams *et al.*, 1998
UK	225	0.67	0.33	Elvidge *et al.*, 2001
Asian				
China	48	0.78	0.22	Williams *et al.*, 1998
China	115	0.70	0.30	Elvidge *et al.*, 2001
Japan	90	0.73	0.27	Williams *et al.*, 1998
Japan	100	0.71	0.29	Elvidge *et al.*, 2001
Japan	181	0.67	0.33	Soma *et al.*, 2002
Singapore	137	0.69	0.31	Williams *et al.*, 1998
Korea	90	0.68	0.32	Present study

REFERENCE

Asico LD, Ladines C, Fuchs S, Accili D, Carey RM, Semer-

aro C, Pocchiari F, Felder RA, Eisner GM and Jose PA. Disruption of the dopamine D3 receptor produces renin-dependent hypertension, J. Clin. Invest. 1998;102: 493-498.

Bae JS, Kang BY, Kim KT, Shin JH and Lee CC. Association study on the genetic variation of the dopamine D1 receptor gene in Korean essential hypertensives, Korean J. Genet. 2001; 23: 221-226.

Bostein D, White RL, Skolnick M and Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms, Am. J. Hum. Genet. 1980; 32: 314-331.

Crocq M-A, Buguet A, Bisser S, Burgert E, Stanghellini A, Uyanik G, Dumas M, Macher J-P and Mayerova A. *Bal* I and *Msp* I polymorphisms of the dopamine D3 receptor gene in African Blacks and Caucasians, Hum. Hered. 1996; 46: 58-60.

Elvidge G, Jones I, McCandless F, Asherson P, Owen MJ and Craddock N. Allelic variation of a *Bal* I polymorphism in the DRD3 gene does not influence susceptibility to bipolar disorder: results of analysis and meta-analysis, Am. J. Med. Genet. 2001; 105: 307-311.

Friedwald WT, Levy LI and Fridrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without the use of the preparative ultracentrifuge, Clin. Chem. 1972; 18: 499-502.

Hussain T and Lokhandwala MF. Renal dopamine receptor function in hypertension, Hypertension 1998; 32: 187-197.

Missale C, Nash SR, Robinson SW Jaber M and Caron MG. Dopamine receptors: from structure to function, Physiol. Rev. 1998; 78: 189-225.

Ott J. Genes and genetic polymorphisms. In: Analysis of Human Genetic Linkage. Baltimore, MD: The Jone Hopkins University Press, pp. 23-38, 1991.

Sambrook J, Fritsch EF and Maniatis T. Molecular Cloning-A Laboratory Manual. 2nd ed. Cold Spring Harbor NY, pp. 9.16-9.23, 1989.

Sanada H, Yao L, Jose PA, Carey RM and Felder RA Dopamine D3 receptor in rat juxtaglomerular cells, Clin. Exp. Hypertens. 1997; 19: 93-105.

Soma M, Nakayama T, Rahmutula D, Uwabo J, Sato M, Kunimoto M, Aoi N, Kosuge K and Kanmatsuse K. Ser9Gly polymorphism in the dopamine D3 receptor gene is not associated with essential hypertension in the Japanese, Med. Sci. Monit. 2002; 8: CR1-4.

Szpirer C, Riviere M, Szpirer J, Levan G, Guo DF, Iwai N and Inagami T. Chromosomal assignment of human and rat hypertension candidate genes: type 1 angiotensin II receptor genes and the S_A gene, J. Hypertens. 1993; 11: 919-925.

Williams J, Spurlock G, Holmans P, Mant R, Murphy K, Jones L, Cardno A, Asherson P, Blackwood D, Muir W, Meszaros K, Aschauer H, Mallet J, Laurent C, Pekkarinen P, Seppala J, Stefanis CN, Papadimitriou GN, Macciardi F, Verga M, Pato C, Azevedo H, Crocq M-A, Gurling H, Kalsi G, Curtis D, McGuffin P and Owen MJ. A meta-analysis and transmission disequilibrium study of association between the dopamine D3 receptor gene and schizophrenia, Mol. Psyciatry 1998; 3: 141-149.