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Association between Genetic Variation of the Insulin Receptor Gene and Essential Hypertension in the Korean Population

Byung Yong Kang, Ki Tae Kim, Hyun Seon Eo[1], Kyung Ho Lee[1], Sung Soo Hong[1], Jung Hee Shin[1], and Chung Choo Lee[1]*

Seoulin Research Institute of Life Sciences, Seoulin Bioscience, Co., Ltd, Seoul 134-030, Korea;
[1]School of Biological Sciences, Seoul National University, Seoul 151-742, Korea

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Essential hypertension is a multifactorial disease, and has been shown to be associated with insulin resistance. The relationship between the genetic variation of the insulin receptor (INSR) gene and essential hypertension in Korean population was investigated by the *Nsi* I restriction fragment length polymorphism (RFLP) pattern of this gene. The observed genotype frequencies of INSR gene were not deviated from those expected for the Hardy-Weinberg equilibrium (HWE), but a significant association was observed between essential hypertension and *N1* allele of *Nsi* I RFLP at the INSR gene (χ^2-test; $P<0.05$). Moreover, the frequency of *N1* allele was significantly different between normotensives and essential hypertensives in subgroups that were not obese (χ^2-test; $P<0.05$). These data suggest that the *Nsi* I RFLP of INSR gene may be a useful genetic marker for essential hypertension in Korean population.

Essential hypertension is a multifactorial disease in which both genetic and environmental factors play important roles (Hamet et al., 1998; Lifton, 1995). Insulin resistance is frequently observed in patients with essential hypertension as well as obesity (Lucas et al., 1985; Modan et al., 1985; Reaven, 1991), and a role for inherited defects within the insulin receptor (INSR) gene in the etiology of insulin-resistant syndromes was reported (Yoshimasa et al., 1988; Awata et al., 1994). Thus, INSR gene has been considered as a candidate gene in the pathogenesis of essential hypertension.

The INSR gene is located in chromosome 19, and consists of 22 exons separated by 21 introns (Seino et al., 1989). The genetic polymorphisms at the INSR gene have been identified (Elbein et al., 1986; Takeda et al., 1986), and used as genetic markers for clinical association studies (Li et al., 1988; McClain et al., 1988; Raboudi et al., 1989). Positive association of INSR gene and essential hypertension have been established on Caucasian population (Morris et al., 1993; Morris et al., 1994; Schrader et al., 1996; Ying et al., 1991; Zee et al., 1993; Zee et al., 1994), but little is known about non-Caucasian groups. In view of the functional importance of INSR gene as a genetic marker for essential hypertension, the present case-control study was designed to investigate the association between the *Nsi* I restriction fragment length polymorphism (RFLP) of INSR gene and essential hypertension in Korean population.

* To whom correspondence should be addressed.
Tel: 82-2-880-6691, Fax: 82-2-872-1993
E-mail: chunglee@plaza.snu.ac.kr

Materials and Methods

Subjects

Two hundreds and twenty subjects were recruited from outpatients of Seoul Hygiene Hospital, Seoul, Korea. The essential hypertensives consisted of 86 subjects with higher blood pressure value than 140/90 mmHg, whereas the normotensives consisted of 134 individuals with lower blood pressure value than 140/90 mmHg. Subjects with secondary forms of hypertension and taking antihypertensive drugs were excluded from the study.

Biochemical assay

Plasma glucose, total cholesterol (TC), triglyceride (TG) and high-density lipoprotein (HDL)-cholesterol levels were determined by enzymatic methods, and LDL-cholesterol level was calculated by Friedewald's equation (Friedewald et al., 1972).

Genotyping

Genomic DNA was prepared from buffy coats of 5 ml blood after lysis of red blood cell (Sambrook et al.,

1989). The exon 8 polymorphism of the INSR gene was detected by using a PCR-*Nsi*I digestion (Hanis and Bertin, 1990). The sequence of the upstream primer was 5′-CGGTCTTGTAAGGGTAACTG-3′ and the sequence of downstream primer was 5′-GAATTCACA TTCCCAAGACA-3′ (Seino et al., 1990). PCR was performed in a final volume of 50 μl (100 ng of genomic DNA, 20 pmol of each primers, 200 μM each of the four dNTPs, 1.5 mM $MgCl_2$, 50 mM KCl, and 10 mM Tris-HCl, pH 8.4 and 2.5 unit of *Taq* DNA polymerase). The reactions were denatured at 94℃ for 1 min, annealed at 55℃ for 1.5 min, and extended at 70℃ for 2.5 min for a total of 25 cycles. Amplified PCR products were digested with restriction enzyme *Nsi*I, and then electrophoresed on 1% agarose gel.

Statistical analysis

Allele frequencies were calculated from genotype frequencies, and the deviation from Hardy-Weinberg equilibrium (HWE) was analyzed by the χ^2-test. The polymorphism information content (PIC) was measured by the methods of Bostein et al. (1980). The relative risk of essential hypertension associated with allelic variation was expressed in terms of an odds ratio (OR) with 95% confidence interval (CI). The Kolmogorov-Smirnov test was used to assess the normality of anthropometrical data and intermediate phenotypes. The comparison of the variables that were normally distributed, was performed by the one-way analysis of variance (ANOVA) test. Kruskal-Wallis test was used about variables that were not normally distributed. All statistical analyses were performed by the computer program of SPSSWIN (version 8.0).

Results

*Association between Nsi*I *polymorphism of INSR gene and essential hypertension*

A polymorphism of INSR gene was detected by digestion with restriction enzyme *Nsi*I after PCR amplification (Fig. 1). *N1* allele yielded a 324 bp band, and *N2* allele gave bands of 239 bp and 85 bp. The genotype and allele frequencies of the *Nsi*I RFLP at the INSR gene are displayed in Table 1. The observed genotype distributions of the INSR gene were not different from those expected for HWE. The genotype frequencies of *N1N1*, *N1N2* and *N2N2* were 58, 38 and 4% in normotensives, and 73, 27 and 0% in essential hypertensives, respectively. The *N2N2* genotype was not observed in essential hypertensives. Frequencies of the *N1* allele at the INSR gene were 0.77 for normotensives and 0.87 for essential hypertensives, respectively. The PIC of *Nsi*I RFLP showed values of 0.29 for normotensives and 0.20 for essential hypertensives, respectively. According to the PIC value, polymorphism detected with restriction enzyme *Nsi*I was reasonably informative in only normotensives. There

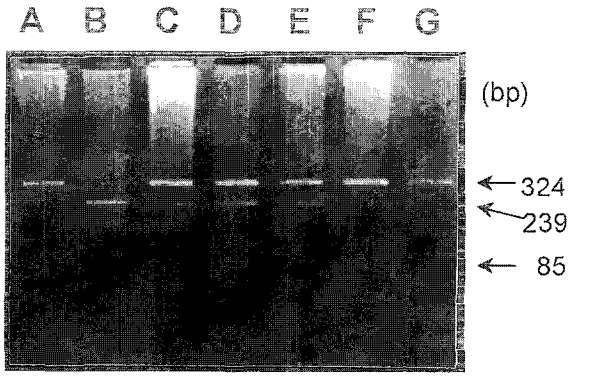

Fig. 1. *Nsi*I RFLP of INSR gene. Lane A, F and G, *N1N1* genotypes; Lane B, *N2N2* genotypes; Lane C, D and E, *N1N2* genotypes

were significant differences in allele and genotype frequencies between two groups (odds ratio=1.91, P<0.05). When stratified by BMI (body mass index), this difference persists in subgroups that were not obese (BMI<26 kg/m^2) (χ^2-test; P<0.05) (Table 2).

*The comparison of anthropometrical data and intermediate phenotypes among genotypes of Nsi*I *RFLP at INSR gene*

Table 3 represents the comparison of anthropometrical data and biochemical parameters according to genotypes of the INSR gene in Korean population. All of the parameters except for plasma TC and LDL-cholesterol levels were not normally distributed by the Kolmogorov-Smirnov test. Therefore, plasma TC and LDL-cholesterol levels were compared by the one-way ANOVA test, whereas age, BMI, systolic blood pressure (SBP), diastolic blood pressure (DBP), plasma TG and glucose levels were compared by the Kruskal-Wallis test. There were marginal differences in DBP among the genotypes of the INSR *Nsi*I RFLP (Kruskal-Wallis test, P=0.055). The *N1N1* homozygote (87.2±15.3 mmHg) had higher value of DBP than *N1N2* heterozygote (83.3±10.2 mmHg) and *N2N2* homozygote (74.8±7.3 mmHg). On the other hand, the *N2N2* homozygote was associated with significantly higher values of age and plasma TG level (Kruskal-Wallis test, P<0.05).

Discussion

The existence of a genetic component in the etiology

Table 1. Genotype and allele frequencies of a *Nsi*I RFLP at the INSR gene from normotensive and essential hypertensive groups

	Genotypes (%)			Alleles (%)	
	N1N1	*N1N2*	*N2N2*	*N1*	*N2*
Normotensives	78 (58)	51 (38)	5 (4)	207 (77)	61 (23)
Hypertensives	63 (73)	23 (27)	0 (0)	149 (87)	23 (13)
χ^2		5.153		5.979	
P		**0.023**		0.014	
Odds ratio			**1.91** (1.13 to 3.22)*		

* 95% confidence interval

Table 2. Comparison of allele frequencies of *Nsi*I RFLP between normotensive (NT) and hypertensive (HT) subgroups of individuals who were not obese and obese

Group	*N1*	*N2*	χ^2	*P*
HT, *BMI < 26 kg/m^2	113	17	4.181	< **0.05**
NT, BMI < 26 kg/m^2	183	51		
HT, BMI ≥ 26 kg/m^2	36	6	2.587	> 0.05
NT, BMI ≥ 26 kg/m^2	24	10		

*Body mass index

of multifactorial diseases such as essential hypertension is now firmly established, and one approach for examining the contribution of genes is to test for association between genetic markers and disease (Herrera and Ruiz-Opazo, 1994). The present study has found the significant association between essential hypertension and *Nsi*I RFLP of INSR gene in Korean population. The *N1* allele was more frequent in essential hypertensives than normotensives. Moreover, the observed genotype distribution of this gene was also marginally associated with variation in DBP, confirming the significant association between essential hypertension and *Nsi*I RFLP of the INSR gene.

However, this RFLP did not result in amino acid substitution, because the mutation in the *Nsi*I cutting site of INSR gene were produced at the third base of the triplet coding for alanine at amino acid 523. Therefore, this association between *Nsi*I polymorphism RFLP of the INSR gene and essential hypertension may be due to linkage disequilibrium between the *N1* allele and a significant causative allele.

With respect to BMI, *Nsi*I RFLP of INSR gene was not associated in essential hypertensives who were obese (BMI ≥ 26 kg/m^2) in this study. The reason why the association was not confirmed in obese hypertensives may be explained by the fact that this effect was masked in this group by the influence of high BMI. In other words, the association between *Nsi*I RFLP of INSR gene and essential hypertension seems to be diluted by the presence of obesity, since obesity itself is known to be caused by various genetic and

Table 3. Comparison of the anthropometrical data and intermediate phenotypes according to *Nsi*I genotypes of INSR gene

Variable	Genotype		
	N1N1	*N1N2*	*N2N2*
[a]Age (yr)	45.1 ± 12.4	46.3 ± 11.5	59.6 ± 7.4
BMI (kg/m^2)[1]	23.3 ± 3.9	23.5 ± 2.8	23.3 ± 2.4
SBP (mmHg)[2]	134.8 ± 22.0	130.2 ± 18.7	118.8 ± 10.5
[b]DBP (mmHg)[3]	87.2 ± 15.3	83.3 ± 10.2	74.8 ± 7.3
[c]TG (mg/dl)[4]	240.2 ± 138.3	211.1 ± 141.9	264.8 ± 117.9
TC (mg/dl)[5]	202.3 ± 46.4	201.7 ± 38.8	242.4 ± 47.7
LDL-chol (mg/dl)[6]	103.3 ± 38.3	107.9 ± 34.4	134.6 ± 45.3
HDL-chol (mg/dl)[7]	48.9 ± 11.4	52.1 ± 11.7	54.8 ± 18.9
Glucose (mg/dl)	90.9 ± 30.6	86.6 ± 27.0	100.0 ± 37.5

[1]Body mass index, [2]Systolic blood pressure, [3]Diastolic blood pressure, [4]Triglyceride, [5]Total cholesterol, [6]LDL-cholesterol, [7]HDL-cholesterol. Values are x ± SD.
[a]Statistically significant association (Kruskal-Wallis test, P=0.031),
[b]Statistically marginal association (Kruskal-Wallis test, P=0.055),
[c]Statistically significant association (Kruskal-Wallis test, P=0.045)

Table 4. Comparison of allele frequencies of *Nsi*I RFLP at the INSR gene from various populations

Population	N	Allele frequency		Reference
		N1	N2	
Mexican American	179	0.76	0.24	Hanis and Bertin, 1990
Caucasian Australian	126	0.73	0.27	Schrader et al., 1996
Asian Korean	220	0.81	0.19	Present study

environmental factors, and strongly associated with insulin resistance and essential hypertension (Chen et al., 1998; Kroke et al., 1998; Wannamethee et al., 1998). Therefore, these results strongly suggest that the *Nsi*I RFLP of INSR gene is associated with essential hypertension, independently of obesity.

Of particular interest is that *N1N1* homozygote of INSR gene was significantly associated with younger age and lower plasma TG level than *N1N2* heterozygote and *N2N2* homozygote. Because age and plasma TG concentration are the potential risk factors for essential hypertension (Cornoni-Huntley et al., 1989; Fuh et al., 1987; Klang et al., 1990; Lever and Harrap, 1992; Rebbeck et al., 1996; Schorr et al., 1998), these results indicate that the observed association between the *Nsi*I RFLP of INSR gene and essential hypertension are not mediated through the risk factors such as age and plasma TG concentration.

The hypertension-associated *N1* allele frequency (0.81) in Korean population was slightly higher than those previously reported in the Mexican-American population (0.76) (Hanis and Bertin, 1990), and Caucasian population (0.73) (Schrader et al., 1996) (Table 4). However, there were no statistically significant differences in the allele frequency among each ethnic groups.

In conclusion, the *Nsi*I RFLP of INSR gene appears to increase the risk for essential hypertension by 1.91 times in Korean population, and its effect is independent of obesity. Therefore, *Nsi*I RFLP of INSR gene may be an useful genetic marker for the pathogenesis of essential hypertension in Korean population.

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