Environmental Mutagens & Carcinogens 21-2 : 95-98 (2001)

Association between Arg16Gly Mutation in the β_2-Adrenergic Receptor Gene and Hypertension in the Korean Population

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(Received November 12, 2001 / Accepted December 10, 2001)

ABSTRACT : β_2-adrenergic receptors (β_2-AR) contribute to cardiovascular regulation by influencing several functions and a several studies suggest that a decreased function of the β_2-AR may be involved in essential hypertension. We investigated the Arg16Gly mutation of β_2-AR gene, which show enhanced agonist-promoted downregulation of the receptor and yielded different results in terms of association with essential hypertension. We studied the relationship between genetic variation in the β_2-adrenergic receptor gene and hypertension in a Korean population using *Nde* I restriction fragment length polymorphism (RFLP) analysis. There were significant differences in allele and genotype frequencies between essential hypertensive and normotensive group (Odds ratio(CI) = 1.71 (1.09-2.70)). Therefore, our result suggests that the *Nde* I RFLP of the β_2-adrenergic receptor gene may be useful as a genetic marker in hypertension diagnostics in Korean population.

Keywords : Association, β_2-adrenergic Receptor, Hypertension

Introduction

Hypertension, or persistently elevated blood pressure, is a major risk factor of coronary artery disease, stroke, and renal disease. High blood pressure exacerbates atherogenesis (Alexander *et al.*, 1995; Chobanian *et al.*, 1996) and sustained hypertension increases long-term risk for myocardial infarction to levels comparable to those associated with smoking and elevated serum cholesterol (Wilson 1998). The human β_2-adrenergic receptor (β_2-AR) is encoded by an intronless gene on chromosome 5q31-q32 (Kobika *et al.*, 1987a; Kobika *et al.*, 1987b). Receptor transcripts have a 5 leader region including an open reading frame that encodes a 19-amino acid peptides: this peptide has been shown to modify translation of β_2-AR mRNA (Parola *et al.*, 1994). The deduced amono acid sequence consists of 413 amino acids. The A46→G, leads to the substitution of Gly to Arg at codon 16 of the β_2-AR gene (Reihsaus *et al.*, 1993). This polymorphism in β_2-AR has been reported to have functional significance when exposed to exogenously applied β_2-AR agonists. In transfected Chinese hamster fibroblasts and in primary cultured human airway smooth muscle cells natively expressing this variants, the Gly16 allele possesing cells enhanced agonist-promoted downregulation of receptor number compared with the Arg 16 cells (Green *et al.*, 1994; Green *et al.*, 1995). The Arg16Gly polymorphism may be related to salt sensitivity (Kotanko *et al.*, 1995). The β_2-AR locus on chromosome 5q31-q32 has been implicated as a candidate region for a genetic predisposition to essential hypertension in White Americans, African Americans, African Caribbeans, and northern Europeans (Svetkey *et al.*, 1996; Kotanko *et al.*, 1997; Timmermann *et al.*, 1998). Therefore, we investigated whether the Arg16Gly polymorphsim in the β_2-AR gene is associated with essential hypertension in a Korean population.

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Materials and Methods

Study subjects

One hundred and eighty subjects were recruited from outpatients of Seoul Hygiene Hospital, Seoul, Korea. The essential hypertensives consisted of 90 subjects with higher blood pressure value than 140/90 mmHg, whereas the normotensives consisted of 90 individuals with lower blood pressure value than 140/90 mmHg. In association analysis using genetic markers, subject selection is a critical and difficult problem. Given that blood pressure is influenced by age and sex, we selected closely age- and sex-matched subjects for the normotensive and essential hypertensive groups. Subjects with secondary forms of hypertension

and taking antihypertensive drugs were excluded from the study.

Biochemical assay

Total cholesterol (TC), triglyceride (TG), apolipoprotein AI (ApoA1), lipoprotein(a) (LP(a)), and high-density lipoprotein (HDL)-cholesterol level were determied by enzymatic method and LDL-cholesterol level was calculated by Friedewald's equation (Friedwald *et al.*, 1972).

Genotyping

Genomic DNA was prepared from buffy coats from blood (5 ml) after lysis of the red blood cells (Sambrook *et al.*, 1989). The Arg16Gly polymorphism was detected using a PCR-*Nde* I digestion. The sequence of the upstream primer was 5'-AGCGCCTTCTTGCTGGCACCATAT-3' and the sequence of the downstream primer was 5'-ACAGC-ACATCAATGGAAGTCC-3' (Jia *et al.*, 2000). PCR was performed in a final volume of 50 μl (100 ng genomic DNA, 20 pmol each primer, 200 μM each of the four dNTPs, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl, pH 8.4, and 2.5 units *Taq* DNA polymerase). The reactions were denatured at 94°C for 1 min, annealed at 60°C for 30 s, and extended at 72°C for 1 min for a total of 35 cycles. Amplified PCR products were digested with *Nde* I, separated on 3% agarose gel, and visualized with ethidium bromide staining.

Statistical analysis

Allele frequencies were estimated by the gene counting method. Deviation in genotype distribution from that expected for Hardy-Weinberg equilibrium (HWE) was estimated by a χ^2-fitness test. The heterozygosity and polymorphism information content (PIC) was measured by the method of Bostein *et al.* (1980). The significance of differences in genotype or allele frequencies between populations was also estimated by χ^2-independence test. To understand the structure of significance of χ^2-test, we calculated the adjusted residuals. The relative risk of essential hypertension associated with allelic variation was expressed in terms of an odds ratio (OR) with a 95% confidence interval (CI). The association between genotypes and quantitative variables was assessed by one-way analysis of variance (ANOVA) with multiple comparison tests by Tukey. All statistical analyses were performed using the SPSSWIN (version 8.0) compute program.

Results

The Arg16Gly polymorphism of β_2-AR gene was detected by digestion with the restriction enzyme *Nde* I after PCR amplification (Fig. 1). This digestion produced fragments of the following sizes: 325 bp in the Arg16 homozygotes; 325 bp, 304 bp, and 21 bp in the Arg16Gly heterozygotes; 304 bp, and 21 bp in the Gly16 homozygotes. The genotype and allele frequencies of the Arg16Gly polymorphism are given in Table 1. The observed genotype distribution of β_2-AR gene was not deviated from those expected Hardy Weinberg Equilibrium (HWE) (hypertensive, $\chi^2 = 3.455$, $P = 0.063$; normotensive, $\chi^2 = 2.888$, $P = 0.089$). The observed genotype frequencies of Arg/Arg, Arg/Gly, and Gly/Gly were 31.1%, 39.2%, and 29.7% respectively, in the hypertensive

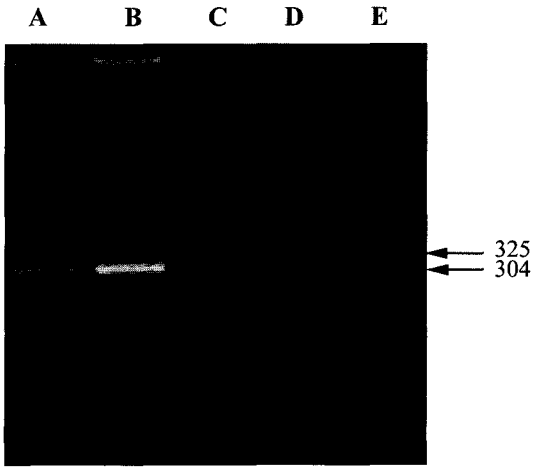

Fig. 1. *Nde* I RFLP of β_2-AR gene. Lanes A and B: the Arg16 allele genotype; lanes C: the heterozygote genotype; lanes D and E: the Gly16 allele genotype.

Table 1. Genotype and allele frequencies of *Nde* I RFLP of the β_2-AR gene in normotensives and essential hypertensives.

	No of genotype %)			Total	No of allele (%)			
	Arg/Arg	Arg/Gly	Gly/Gly		Arg	Gly	H[1]	PIC[2]
Normotensives	29 (36.2)	44 (55.0)	7 (8.8)	80	102 (0.64)	58 (0.36)	0.461	0.355
Hypertensives	23 (31.1)	29 (39.2)	22 (29.7)	74	75 (0.51)	73 (0.49)	0.500	0.375
X^2		11.317			5.377			
P		0.003			0.02			
Odds ratio (CI)[3]			1.71 (1.09-2.70)					

[1]Heterozygosity, [2]Polymorphism Information Content, [3]95% Confidence Interval. Frequency is given as a percentage in parenthesis. There were statistically significant differences in genotype and allele frequencies between normotensives and essential hypertensive subjects.

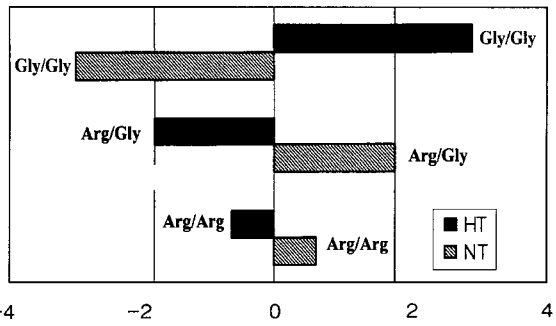

Fig. 2. Adjusted residuals of *Nde* I RFLP at the β_2-adrenergic receptor gene. Abbreviation: HT, Hypertensives; NT, Normotensives.

Table 2. Comparison of the anthropometrical data and intermediate phenotypes according to *Nde* I genotypes of β_2-AR gene.

Variable	Genotype		
	Arg/Arg	Arg/Gly	Gly/Gly
Age (year)	60.8±10.7[8]	57.6± 9.6	59.2±14.5
BMI (kg/m^2)[1]	23.6± 3.2	23.5± 2.5	24.2± 2.0
ApoA1 (mg/dl)[2]	92.8±35.6	95.0±30.8	104.7±34.8
LP(a) (mg/dl)[3]	15.6±12.3	17.2±12.1	16.2± 8.8
TG (mg/dl)[4]	125.6±65.1	138.9±94.4	139.2±66.9
TC (mg/dl)[5]	150.2±31.8	157.7±35.3	156.9±39.9
LDL-chol (mg/dl)[6]	98.9±32.7	102.2±35.6	103.7±46.7
HDL-chol (mg/dl)[7]	26.2± 7.3	28.3± 8.5	25.4± 9.5

[1]Body mass index, [2]Apolipoprotein AI, [3]Lipoprotein(a), [4]Triglyceride, [5]Total cholesterol, [6]LDL-cholesterol, [7]HDL-cholesterol, [8]Standard deviation.

group, and 36.2%, 55.0%, and 8.8% respectively, in the normotensive group. The frequency of the Gly allele at codon 16 of the β_2-AR gene was 0.49 for the hypertensive group and 0.36 for the normotensive group. The heterozygotsity and polymorphism information content (PIC) values are 0.500 and 0.375 respectively, for hypertensive group and 0.461 and 0.355, respectively, for normotensive group. There were very significant differences between the hypertensive and normotensive groups in allele and genotype frequencies (genotype frequency, $P = 0.003$; allele frequency, $P = 0.02$). Figure 2 showed the adjusted residuals of *Nde* I RFLP at the β_2-AR gene. The Gly/Gly genotype indicated the very high frequency value in hypertensive group (Adjusted residuals of Gly/Gly = 3.3). Comparisons of anthropometrical data and biochemical parameters according to the Arg16Gly genotypes of the β_2-AR gene in the Korean population are given in Table 2. No clinical parameters were significantly associated with genotypes of the β_2-AR gene in this group.

Discussion

Essential hypertension ia a heterogeneously multifactorial disease in which blood pressure is harmfully high without overt cause. It affects a large number of individuals in many populations. Both genetic and environmental factors have been implicated in its etiology. In a previous study, this Arg→Gly mutation was shown to decrease the receptor number by down-regulation with specific molecules (Liggett *et al.*, 1999).

The association of the amino-termainal polymorphism with the receptor pharmacological phenotype *in vitro* has been supported by a series of genotype-phenotype clinical studies based on the role of β_2-AR in modulating bronchial or vascular smooth muscle relaxation. The homozygous Gly16, which becomes more downregulated and desensitized by β_2-agonists *in vitro*, was significantly more prone to bronchodilator tolerance after administration of formoterol (Tan *et al.*, 1997) or albuterol (Martinez *et al.*, 1997). Such an impaired smooth muscle tone response to circulating β_2-agonists is probably due to the receptor downregulation and desensitization observed in *in vitro* studies. The Gly16 allele was also associated with steroid-dependent (Reihsaus *et al.*, 1993), nocturnal (Turki *et al.*, 1995) and severe asthma (Weir *et al.*, 1998). A recent study has demonstrated that homozygous Gly16 subjects have increased basal mean BP and decreased salbutamol-induced *in vivo* vasodilatation compared with Arg16Arg sujects (Gratze *et al.*, 1999).

In this study, we investigated the possible significance of the Arg16Gly polymorphism of the β_2-AR gene for hypertension. We have demonstrated that the Gly16 allele is very significantly associated with hypertension in the Korean population. Especially, the frequency of Gly/Gly homozygote was significantly higher in hypertensive group than normotensives by adjusted residual analysis. Thus, a recessive model on Gly allele of β_2-AR gene provides the best fit for this data, so the Gly/Gly genotype could be considered a genetic marker on the risk for hypertension in Korean population.

However, the results of the hypertension-associated Gly allele in Korean population was opposited with a case of the African-American, White, Black African and East Anglian Caucasian population studies (Geoff *et al.*, 2000; Jia *et al.*, 2000; Volker *et al.*, 2000). Therefore, this association between Gly allele and essential hyperetension in Korean population may be due to linkage disequilibrium with a "causative" variant nearby in the same chromosome.

With respect to anthropometrical data and biochemical parameters, no parameters were significantly associated with the genotypes of the β_2-AR gene in both hypertensive and normotensive groups. Therefore, this genetic variation may not influence any cardiovascular risk factors.

In conclusion, essential hypertension in Korean population is associated with an increased frequency of the Gly16

allele of the β_2-AR. Therefore, the Arg16Gly polymorphism of the β_2-AR gene may be useful as a genetic marker for the pathogenesis of hypertension in the Korean population.

Acknowledgement

The human blood samples were kindly supplied by Dr. Seung Hee Cho, Clinical Pathology, Seoul Hygiene Hospital, Seoul, Korea.

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