

# The Arg16Gly polymorphism of $\beta_2$ -Adrenergic receptor gene and its association with hypertension in Iranian subjects

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**Abstract:** It was shown that polymorphisms in the gene for the Beta-2 adrenergic receptor ( $\beta_2$ AR) have been linked to hypertension in several studies. Evaluation of the significance of Arg16Gly polymorphism in development of hypertension in Iranian subjects was the aim of present study. In this study, 409 Iranian subjects divided in normotensive (209) and hypertensive (200) groups were analyzed for Arg16Gly polymorphisms through DNA extraction followed by polymerase chain reaction (PCR) - restriction fragment-length polymorphism (RFLP) and statistic analysis. Our findings showed no evidence for significant association between different Arg16Gly polymorphisms in  $\beta_2$ AR and hypertension in Iranian subjects. Arg/Gly genotype itself showed a protective effect for hypertension in comparison with Arg/Arg in position 16 with odds ratio (OR) equal to 0.59 [confidence interval (CI) 95%:0.37-0.96].

**Keywords:** Arg16Gly, Beta 2-adrenergic receptor, hypertension, polymorphism.

## Introduction

Hypertension and cardiovascular diseases are among rapidly growing public health problems of human beings, and it was shown that these disorders have strong genetic as well as environmental determinants.<sup>1-3</sup>

Several studies have taken into account hypertension as related to polymorphisms of  $\beta$ -adrenergic receptor genes in the study population for several years.<sup>4</sup> In humans, the  $\beta_2$ -adrenergic receptor ( $\beta_2$ AR) gene have few polymorphisms at few sites which lead to altered encoded amino acids,<sup>5</sup> means that within the general population, people can have different  $\beta_2$ -adrenergic

receptors. The  $\beta_2$ AR has three sites of variation, at amino acids 16, 27, and 164. It seems that, Gly at

position 16 imparts enhanced agonist-promoted down regulation, regardless of whether position 27 is Glu or Gln based on Green et al study.<sup>6</sup> Several studies were conducted to show associations between  $\beta_2$ AR polymorphisms and hypertension. In 1997, Gly16 was found at increased prevalence among hypertensive African Caribbeans relative to their normotensive counterparts.<sup>7</sup> In the Bergen Blood Pressure Study, the offspring of hypertensive parents were compared with those of normotensive parents in the Northern

European population and it was found that the Arg16 polymorphism was associated with children of hypertensive parents.<sup>8</sup> However, in another study of northern European hypertensive subjects, no evidence was found for association between single nucleotide polymorphisms (SNPs) at amino acids 16, 27, or 164 and essential hypertension in Polish subjects.<sup>9</sup>

In present study, we investigated the relationship between  $\beta$ 2AR gene variant Arg16Gly and hypertension in Iranian subjects.

## Materials and methods

### Subjects

The study was included 409 Iranian subjects living in Tehran and Karaj who underwent medical examination in 2008 (195 males, and 214 female). Among subjects 209 had normal blood pressure and 200 were hypertensive (51.1% normotensive, 48.9% hypertensive). The present study was approved by the ethical committee of Tehran University of medical sciences. The study was explained to all subjects and all of them signed informed consent form for participation.

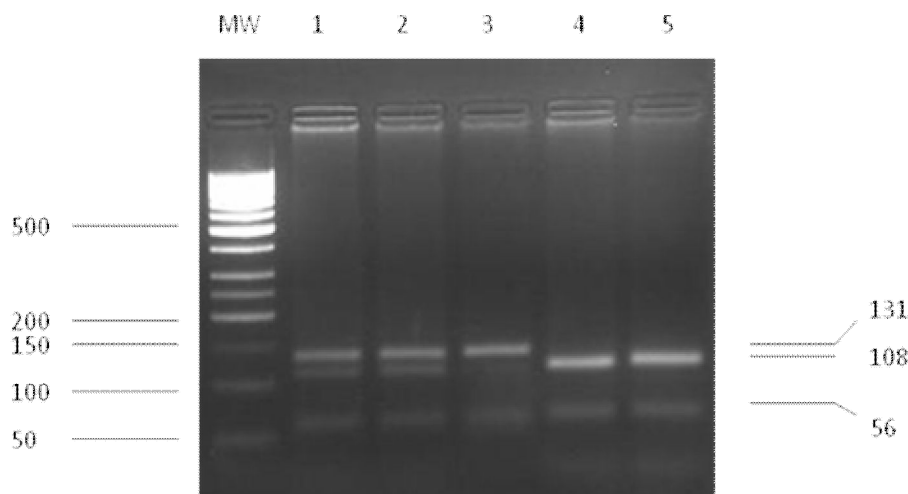
### Laboratory and blood pressure measurement

All subjects were classified as being either hypertensive (n=200) or normotensive (n=209) by the definition of hypertension as a Systolic Blood Pressure (SBP)  $\geq$  140 mmHg and/or Diastolic Blood Pressure (DBP)  $\geq$  90 mmHg. The subjects recruited were healthy volunteers with SBP  $\leq$  135 mmHg and DBP  $\leq$  85 mmHg. Height, body weight, waist-to-hip ratio and blood pressure were determined. The body mass index (BMI) was calculated as weight in Kg divided by height in m<sup>2</sup>. Blood pressure was measured twice on the right arm with the participants in a sitting position, using a random-zero mercury sphygmomanometer, with a 5-minutes rest for the

subject before and between readings. A venous blood sample was obtained from an antecubital vein for the extraction of DNA.

### PCR and RFLP analysis

Genomic DNA was extracted from leukocyte in sample of whole blood with DNG-plus<sup>TM</sup> solution (CinnaGen-Tehran, Iran). The PCR amplification of the DNA segments containing codon 16 of the  $\beta$ 2AR gene was performing with thermocycler apparatus (Eppendorf-Hamburg, Germany). In brief, PCR amplification of the DNA segments was carried out in a volume of 25  $\mu$ l. Containing 100–300 ng DNA, 0.5  $\mu$ l of dNTP mix 10 mM, 2.5  $\mu$ l of 10X PCR buffer, 0.75  $\mu$ l of MgCl<sub>2</sub> 50 mM, 1  $\mu$ l of each primer with OD=0.06 and 1U of Taq DNA polymerase (CinnaGen-Tehran, Iran). The forward primer was 5'-CTTCTTGCTGGCACGCAAT-3' and the reverse primer was 5'-CCAGTGAAGTGATGAAGTAGTTGG-3'. PCR was started with denaturation at 94°C for 4 minutes, followed by 34 cycles of denaturation (94°C for 30 seconds), annealing (63°C for 20 seconds) and extension (72°C for 20 seconds), with a final extension at 72°C for 10 minutes. The PCR product size from these primers is 201 base pairs (bp). The amplified product was digested at 65°C for 1 hour with 2U of BseMI (Fermentas-Vilnius, Lithuania). The fragments were resolved on 3.5 % agarose gel with TAE buffer (40 mmol L<sup>-1</sup> Tris acetate, 2 mmol L<sup>-1</sup> EDTA) and visualized under ultraviolet after staining with ethidium bromide. This digestion produced fragments of following size: 14 , 56 , 131 bp in Arg 16 homozygous, 14 , 23 , 56 , 108 and 131 bp in Arg 16 Gly 16 heterozygous and 14 , 23 , 56 and 108 bp in Gly 16 homozygous (Figure -1).



**Figure -1: Size marker 50 bp , Lanes 1, 2 Arg 16 Gly heterozygote, Lane 4 Arg homozygote, Lanes 4, 5 Gly homozygote.**

**Table 1. Genotype distribution of the  $\beta_2$ AR polymorphisms Arg16Gly for hypertensive and normotensive individuals**

Genotype	Groups		P Value	Odds Ratio (CI 95%)
	hypertensive	normotensive		
Arg/Arg % (number)	34.0% (68)	26.8% (56)	-	1 (Reference)
Arg/Gly % (number)	32.0% (64)	42.6% (89)	0.031	0.59 (0.37-0.96)
Gly/Gly % (number)	34.0% (68)	30.6% (64)	0.594	0.87 (0.54-1.43)
Gly/Gly+Arg/Gly % (number)	66.0% (132)	73.2 % (153)	0.113	1.41 (0.92-2.15)
Total number	200	209		

**Table 2. Baseline characteristic of the hypertensive and normotensive subjects**

Characteristics	Hypertensive subjects (n=200)	Normotensive subjects (n=209)	P Value*
Age (Years)	45.0±12.2	43.6±10.9	0.216
Male (%)	46.0	43.5	0.623
BMI (Kg/m <sup>2</sup> )	29.6±5.5	27.4±5.5	0.001
SBP (mmHg)	154.2±14.4	119.5±12.6	0.000
DBP (mmHg)	94.4±9.2	75.3±8.2	0.000

Data presented as mean±SD or as percentage

\* Student's t test and chi-square test.

### Restriction fragment polymorphism

Based on digestion with BseMI restriction enzyme, a distinct pattern for the three genetic variant was found; homozygosity for Arg16 (considered the 'wild' form) or Gly16 (mutated form), and heterozygosity Arg16Gly16.

### Genotype examination in codon 16

Genotyping of  $\beta_2$ AR was performed on DNA from subjects to delineate variant in amino acid 16. Table 1 shows the distribution of the  $\beta_2$ AR gene polymorphism in hypertensive and normotensive subjects.

### Statistical Analysis

All of the statistical analysis was performed using the SPSS software program version 11.5 for Windows (SPSS Inc., Illinois, USA) and Stata 8 Software Package (Stata Corporation, Texas, USA). Data are expressed as the mean ± SD and frequencies are expressed in proportion (percentage). The difference level below 5% was considered to be significant. Frequency distribution of  $\beta_2$ AR polymorphisms Arg16Gly genotypes among two groups was examined by chi-square test. The logistic regression analysis was

used to model the effect of each single polymorphism on the risk of hypertension with adjustment for sex, age, diabetes and BMI.

### Results

Demographic characteristics of normotensive and hypertensive groups are presented in table 2. There was no significant difference between age and gender distributions and these two groups were completely comparable on these variables. However, there was a significant difference in BMI (Body Mass Index) that was higher in hypertensive subjects ( $29.6 \pm 5.5$  vs.  $27.4 \pm 5.5$ ;  $p=0.001$ ).

The proportion of individuals carrying the three possible genotypes (Arg/Arg, Arg/Gly, and Gly/Gly) was not significantly different between normotensive and hypertensive subjects ( $P=0.075$ ) (Table 1). Overall, no significant association of the  $\beta_2$ AR genotype with hypertension risk was observed (Crude OR, 0.95; CI 95%, 0.74-1.20 and Adjusted OR, 0.94; CI 95%, 0.69-1.33 with logistic regression method for age, gender, diabetes and BMI). Even, Considering Arg/Arg as wild genotype and other two genotypes (Arg/Gly and Gly/Gly) as mutated forms, there was no significant association of the  $\beta_2$ AR genotype with

hypertension risk (Crude OR, 1.41 ; CI 95%, 0.92-2.15 and Adjusted OR, 1.29 ; CI 95%, 0.72-2.31 with logistic regression method for age, gender, diabetes and BMI) (Table 1). However, Arg/Gly genotype itself showed a protective effect on hypertension with an OR equal to 0.59 (CI 95%: 0.37-0.96) in comparison with wild genotype Arg/Arg.

### Discussion

The primary goal of the current study was evaluation of the significance of Arg16Gly polymorphisms of the  $\beta_2$ AR gene in incidence of hypertension in Iranian population as it could be a risk factor for cardiovascular disorders. According to our findings, there was no association between Arg16Gly  $\beta_2$ AR gene polymorphisms and hypertension. Our findings are in accordance with Tomaszewski *et al*<sup>9</sup> as well as Iaccarino *et al*<sup>10</sup> who showed no evidence for association of  $\beta_2$ AR gene and hypertension. In present study, a protective effect on hypertension was shown in Arg/Gly genotype. In contrast, two other studies conducted by Kotanko *et al*<sup>7</sup> and Gratze *et al*<sup>11</sup>, were shown association of Arg16 and Gly16 alleles with

hypertension, respectively. As long as the results of genetic association studies strongly depend on several factors such as the homogeneity of the population sampled, polymorphism-disease relationships shown in one population or study may fail to be shown in another, particularly for diseases like hypertension where several different factors are known participants. This was also shown by a single study of Wu *et al*<sup>12</sup> examining two non-Han Chinese minority populations for  $\beta_2$ AR SNPs and haplotypes associated with hypertension. In mentioned study, only in one of two studied populations, both SNPs at positions 16 and 27 demonstrated variants associated with hypertension. This also may suggest that Arg16Gly polymorphism is not per se responsible for the association but some polymorphism on linkage disequilibrium with Arg16Gly could be involved instead.

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