Indian J Med Res 132, July 2010, pp 62-66

Polymorphic variants of β_1 adrenergic receptor gene (Ser49Gly & Arg389Gly) in healthy Tamilian volunteers

P. Ramu, K.N. Mahesh Kumar, D.G. Shewade, R.P. Swaminathan*, T.K. Dutta*, J. Balachander** & C. Adithan

*Departments of Pharmacology, *Medicine & **Cardiology, Jawaharlal Institute of Postgraduate Medical Education & Research, Puducherry, India*

Received May 9, 2008

***Background & objectives*: Several studies reported the polymorphisms of β_1-adrenergic receptor gene in healthy volunteers and its influence on cardiovascular disorders. We investigated the genotype and allele frequencies of Ser49Gly and Arg389Gly polymorphism in healthy volunteers of South Indian Tamilian population *vis-à-vis* other major ethnic groups.**

***Methods*: The genetic variants were determined by using Taqman 5' nuclease assay- real time PCR analysis in 533 normal healthy volunteers (18-60 yr; M=290; F=243). The allelic discrimination analysis was done by 7700 SDS software.**

***Results*: The estimated genotype and allele frequencies of Ser49Gly and Arg389Gly polymorphism were compared with other major populations. The frequencies of the variant alleles Gly49 and Gly389 were 15.1 and 25.8 per cent respectively.**

***Interpretation & conclusions*: Our study shows that interethnic variation exists in the polymorphisms of β_1-adrenergic receptor gene and the results generated in this study might serve as a genetic marker for further studies in Tamilian (South India) population.**

Key words β_1-adrenergic receptor - blood pressure - genetic polymorphism - genotype

The β_1-adrenergic receptor (ADRB1) belongs to the family of guanine nucleotide binding regulatory protein coupled receptors (GPCRs). ADRB1 is expressed in the heart and mediates the physiological effects of catecholamines like epinephrine and norepinephrine[1]. The human β_1-adrenergic receptor is encoded by an intronless gene with 45 aminoacids located on chromosome10q24-26[2]. Of the 73 polymorphisms of ADRB1 gene identified so far, 13 of these result in change of amino acid in the ADRB1 protein[3,4]. Among these, two common functional polymorphic variants (Ser49Gly & Arg389Gly) have been identified and extensively studied. Firstly, substitution of nucleotide A→G at 145th position resulted in the change of amino acid serine to glycine[5,6]. This polymorphic variant is localized in the extracellular amino terminus[7]. Secondly, the change of nucleotide C→G at 1165 position resulted in the substitution of amino acid glycine for arginine. This polymorphic variant is localized in the intracellular cytoplasmic tail portion[5]. *In vitro* studies had demonstrated that Arg389 of β_1-adrenergic receptor was reported to have higher affinity in coupling with the Gs protein and increased signal transduction in the presence of agonist when compared to Gly389 polymorphic variant[8]. The polymorphic variant Gly49 of β_1-adrenergic receptor

was shown to have an increased cell expression and affinity towards agonists which leads to desensitization and down-regulation of receptors in contrast to Ser49[9]. The Ser49Gly and Arg389Gly polymorphisms of the β_1-adrenergic receptor gene are the most extensively studied which are known to influence several cardiovascular disorders. The frequency distribution of these polymorphisms varies with ethnicity. Several studies of diverse human population in Mexicans[10], Swedish[11], Chinese[12], Japanese[13], Caucasians[14] and African Americans[15] have been carried out to determine the prevalence of these genetic variants.

To the best of our knowledge, there is no study reporting the genotype and allele frequencies of these two polymorphisms in any of the Indian population. Therefore, we studied the genotype and allele frequency of β_1-adrenergic receptor gene polymorphisms (Ser49Gly and Arg389Gly) among the healthy volunteers in a South Indian Tamilian population.

Material & Methods

The study was carried out in JIPMER, Puducherry from February 2005 to March 2008 in 533 (290 men & 243 women) unrelated healthy volunteers of either sex aged 18-60 yr. All of them were residents of Tamilnadu and Puducherry of south Indian Tamilian origin for at least three generations. All the subjects were randomly selected based on the simple randomization procedure from the outpatient clinics without hypertension, diabetes mellitus, hyperlipidemia and other chronic diseases in previous records were recruited. The subjects were examined clinically by measuring height, weight, blood pressure, heart rate, and electrocardiogram. Blood pressure was measured by resting the subjects for 10 min. The measurement was repeated for three times and the average reading was taken for the final analysis. Laboratory investigations were done to evaluate fasting lipid profile and blood sugar level. All the participants were interviewed using a standardized questionnaire with regard to their lifestyle, smoking, alcohol consumption and drug intake. In all subjects, height was measured to the nearest centimeter and weight to the nearest 0.1 kg, which was used for calculation of BMI (kg/m^2). The study protocol was approved by the Institute Research Council and Institutional Human Ethics Committee (JIPMER). Written informed consent was obtained from all the participants.

Genotyping methods: Five ml of venous blood was collected by using EDTA as anticoagulant. Genomic DNA was extracted from peripheral leukocytes using standard phenol: chloroform method. The extracted DNA samples were stored at 4°C. Genotyping for Ser49Gly and Arg389Gly was carried out by Real Time Thermocycler (ABI Prism 7700, Fosters city, CA, USA) using Taqman SNP genotyping assay method which employs the fluorogenic 5'nuclease chemistry (also known as Taqman probe-based chemistry) to enable detection of specific PCR product. The SNP genotyping assay ID used (ABI Prism 7700, Foster city, California, USA) was C__8898508_10 and C__8898494_10 for Ser49Gly and Arg389Gly respectively. The PCR reaction was carried out in duplicate in a 15 µl final volume which contained 10 µl of Taqman Universal PCR master mix (2X), 0.5 µl of 20X working stock of SNP genotyping assay, and 2.5 µl of genomic DNA diluted in DNase free water and 2 µl of deionized water. The thermocycler conditions included 1 cycle at 50ºC; 1 cycle at 95ºC for 10 min to activate the AmpliTaq Gold polymerase followed by 40 cycles of denaturation at 92°C for 15 sec and annealing/extension at 60°C for 1 min. The allelic discrimination analysis was finally performed by 7700 SDS software (ABI Prism 7700, Fosters city, CA, USA).

Statistical analysis: Statistical analysis was done using the Statistical Packages for Social Sciences software. (SPSS, Windows version release 13, SPSS Inc., Chicago, Illinois, USA). Direct gene counting method was used to determine the frequency of genotypes and alleles. The genotype data was analyzed by using χ^2 and Fisher's exact test. The observed genotype frequencies were compared with the expected frequencies and tested for the Hardy-Weinberg equilibrium. Differences in clinical variables were assessed by unpaired *t*-test. 95 per cent confidence interval was analyzed by CIA (version 1). Haplotype frequencies and linkage disequilibrium analysis were estimated by Helix tree software version 6.0.2 (Golden helix, Inc- Bozeman, MT, USA). Continuous variables were expressed as mean ± SEM and $P < 0.05$ was considered as statistically significant.

Results

The baseline characteristics of the study subjects including male and female participants are shown in Table I. There was no significant difference in age, BMI, SBP, DBP, heart rate, LDL cholesterol, triglycerides and VLDL cholesterol but there was a significant difference in total cholesterol and HDL cholesterol among the male and female study participants. The

Table I. Demographic detail of study subjects

Base line characteristics	Male (n = 290)	Female (n = 243)	Total (n = 533)
Age (yr)	41.1 ± 0.6	41.8 ± 0.5	41.3 ± 0.4
BMI (kg/m^2)	22.7 ± 0.2	23.6 ± 0.5	23.1 ± 0.2
SBP (mm Hg)	117 ± 0.6	118.2 ± 0.5	117.5 ± 0.4
DBP (mmHg)	77.9 ± 0.3	78.1 ± 0.3	77.5 ± 0.2
HR (beats/min)	76.4 ± 0.4	75.2 ± 0.4	75.8 ± 0.3
Total cholesterol (mg/dl)	163.6 ± 2.0	168.8 ± 1.9*	165.9 ± 1.4
Triglycerides (mg/dl)	107.2 ± 3.0	108.7 ± 2.8	107.9 ± 2.0
HDL (mg/dl)	40 ± 0.6	42.6 ± 0.6*	41.4 ± 0.4
LDL (mg/dl)	104 ± 1.7	104.6 ± 1.7	104 ± 1.2
VLDL (mg/dl)	21.2 ± 0.6	22.1 ± 0.6	21.5 ± 0.4

*P< 0.05 among male and female subjects. Values are expressed as mean ±SEM. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein

genotype and allele frequencies of the β_1 adrenergic receptor gene polymorphisms are shown in Table II and also compared with other major populations.

The Arg389Gly polymorphism was found to be in Hardy-Weinberg equilibrium whereas the Ser49Gly polymorphism was not found to be in Hardy-Weinberg equilibrium. Both the polymorphisms were found to be in linkage disequilibrium (D' value 0.6, LD correlation=0.14). Haplotype frequencies were estimated for both the polymorphisms by expectation maximization algorithm method (EM). The observed haplotype distributions were Ser49/Arg389 60.7, Ser49Gly389 24.2, Gly49Ser389 13.5 and Gly49Gly389 1.6 per cent.

Discussion

This is the first study describing the genotype and allele frequency of β1 adrenergic receptor gene polymorphisms in any of the Indian population. The Tamilians are Dravidian people from the Indian subcontinent living in southern parts of India and north-eastern Sri Lanka. They are ethnically, linguistically and culturally related to other Dravidian people of

Table II. Comparison of Ser49Gly and Arg389Gly genotype and allele frequencies of Tamilian population with other major populations†

	% Frequency distribution				
Ser49Gly Genotypes	Tamilian (n=533) (Present study)	Mexicans (n=190)[10]	Swedish (n=265)[11]	Chinese (n=400)[12]	Japanese (n=240)[13]
Ser/Ser	76.5 (73.0-80.1)	54.2*** (47.1-61.3)	72.8 (67.5-88.2)	70.5* (66.0-75.0)	73.3 (67.7-78.9)
Ser/Gly	16.7 (13.5-19.9)	35.8*** (29.0-42.6)	24.9** (19.7-30.1)	28.0*** (23.6-32.4)	26.3** (20.7-31.8)
Gly/Gly	6.8 (4.7-9.2)	10.0 (6.1-15.2)	2.3** (0.8-4.9)	1.5*** (2.1-3.2)	0.4*** (0.6-2.3)
Alleles					
Ser	84.9	84.5	85.3	72.1***	86.5
Gly	15.1	15.5	14.7	27.9***	13.5
Arg389Gly Genotypes	Tamilian (n=533) (Present study)	Caucasians (n=230)[14]	African Americans (n=194)[15]	Chinese (n=400)[12]	Japanese (n=237)[13]
Arg/Arg	54.8 (50.6-59.0)	53.1 (45.3-58.2)	30.9*** (24.4-37.4)	57.0 (52.1-62.0)	60.3 (54.1-66.6)
Arg/G/y	38.8 (34.7-43.0)	39.1 (32.8-45.4)	53.1** (46.1-60.1)	38.2 (33.5-43.8)	33.3 (27.3-39.3)
Gly/Gly	6.4 (4.4-8.8)	7.8 (5.7-13.6)	16.0*** (10.8-21.0)	4.8 (2.8-7.3)	6.4 (4.0-10.2)
Alleles					
Arg	74.2	72.6	52.3***	76.1	77.0
Gly	25.8	27.4	47.6***	23.9	23.0

* P<0.05, ** P<0.01, *** P<0.001 when compared to Tamilians. Values given in parenthesis are 95 per cent confidence intervals
† Data compiled from previous studies

the sub-continent. An estimated 77 million Tamilians reside in India and around the world[16].

In the present study, Ser49Gly polymorphism significantly deviated from Hardy-Weinberg equilibrium. The evolution of Ser49Gly polymorphism might have accumulated over generations that led to substantial changes in Tamilian population. The frequency of homozygous Ser49Ser genotype in our population was almost similar to that of Swedish and Japanese population[11,13] but it was significantly different from Mexican population[10] (Table II). The frequency of heterozygous variant genotype Ser49Gly was significantly higher in other populations when compared to our population[10-13]. The homozygous variant genotype Gly49Gly in our population was significantly different from Swedish, Chinese and Japanese population[11-13]. The allele frequency of Gly49 from the present study matches with the other population except Chinese population[12]. The genotype and allele frequency of Arg389Gly polymorphism of Tamilian population matches with Chinese, Japanese, and Caucasian population[12-14]. African Americans were shown to have highest variant allele Gly389 frequency (Table II) which did not match with any other population[15].

A case control study in Japanese population revealed that males homozygous for Gly389Gly genotype exhibited protective effect against hypertension[17]. A sib-pair study conducted in Swedish population showed a significant increase in diastolic blood pressure and heart rate in the carriers homozygous for Arg389 allele when compared to Gly389 carriers[11]. In another study, males homozygous for Gly16Gly/Gly389Gly genotype showed a higher increase in BMI from childhood to adulthood when compared to Arg389Arg/Arg16Arg carriers. This suggested an interaction of Arg16Gly and Arg389Gly polymorphism in men and significant interaction between β_1 and β_3 genes were noticed in women[18]. Over a long period of time Ser49Gly polymorphism influences the increase in BMI in female subjects with 49Gly polymorphism[19]. Inclusion of subjects with higher BMI in our study would have been a strong evident to confirm the previous findings in our population. Ser49Gly polymorphism was found to be associated with coronary artery disease in Caucasians and resting heart rate in Asians (Ranade). Borjesson *et al*[6] reported a cardio-protective role of Gly49 variant in heart failure patients and Ser49 homozygotes showing increased susceptibility to cardiovascular mortality. Patients homozygous for Arg389 and Ser49 required significant increase in concomitant heart failure therapy as compared with the variant genotypes[20].

Linkage disequilibrium between the two polymorphisms was reported in different populations[19,21]. In the present study, both Ser49Gly and Arg389Gly were found to be in moderate LD. Contrast to our study, it was observed to be in weak LD in Swedish population[19]. Another study in Caucasians and African-Americans revealed a strong LD between the two polymorphisms[22]. The haplotype combinations Gly49/Gly389 were found to be higher in our population with 1.6 per cent as compared to 0.6 per cent in Italian Caucasians[22] and 1 per cent in Australian Caucasian population[23]. An interethnic variability in genotype and allele frequencies, physiological response as well as response to beta blockers and disease susceptibility were reported[10,15,24,25].

To conclude, the present study established the genotype and allele frequencies of β_1 adrenergic receptor gene polymorphisms in a South Indian Tamilian population. Further case-control, response to beta blockers, gene-gene and gene-environment interaction and haplotype studies are required to elucidate the role of β1 adrenergic receptor gene polymorphisms.

Acknowledgement

The study was funded by Department of Biotechnology, New Delhi.

References

1. Yang-Feng TL, Xue FY, Zhong WW, Cotecchia S, Frielle T, Caron MG, *et al*. Chromosomal organization of adrenergic receptor genes. *Proc Natl Acad Sci* 1990; *87* : 1516-20.
2. Frielle T, Collins S, Daniel KW, Caron MG, Lefkowltc RJ, Kobilka BK. Cloning of the cDNA for the human beta 1 adrenergic receptor. *Proc Natl Acad Sci USA* 1987; *84* : 7920-4.
3. NCBI database [home page on the internet]. Available from: *http://www.genecards.org*, accessed on August 21, 2008.
4. Taylor MR. Pharmacogenetics of the human beta-adrenergic receptors. *Pharmacogenomics J* 2007; *7* : 29-37.
5. Maqbool A, Hall AS, Ball SG, Balmforth AJ. Common polymorphisms of beta1- adrenoreceptor: identification and rapid screening assay. *Lancet* 1999; *353* : 897.
6. Borjesson M, Magnusson Y, Hjalmarson A, Andersson B. A novel polymorphism in the gene coding for the beta$_1$-adrenergic receptor associated with survival in patients with heart failure. *Eur Heart J* 2000; *21* : 1853-8.
7. Taylor MR, Bristow MR. The emerging pharmacogenomics of the beta-adrenergic receptors. *Congest Heart Fail* 2004; *10* : 281-8.

8. Mason DA, Moore JD, Green SA, Liggett SB. A gain-of-function polymorphism in a G-protein coupling domain of the human beta$_1$-adrenergic receptor. *J Biol Chem* 1999; *274* : 12670-4.

9. Levin MC, Marullo S, Muntaner O, Andersson B, Magnusson Y. The myocardium protective Gly-49 variant of the beta1-adrenergic receptor exhibits constitutive activity and increased desensitization and down-regulation. *J Biol Chem* 2002; *277* : 30429-35.

10. Fragoso JM, Rodriguez-Perez JM, Perez-Vielma N, Martinez-Rodriguez N, Vargas- Alarcon G. Beta1 adrenergic receptor polymorphisms Arg389Gly and Ser49Gly in the Amerindian and Mestizo populations of Mexico. *Hum Biol* 2005; *77* : 515-20.

11. Bengtsson K, Melander O, Orho-Melander M, Lindblad U, Ranstam J, Rastam L, *et al*. Polymorphism in the beta (1)-adrenergic receptor gene and hypertension. *Circulation* 2001; *104* : 187-90.

12. Liu ZQ, Liu J, Xiang ZH, Hu MY, Mo W, Wang LS, *et al*. Distributive characteristics of Ser49Gly and Gly389Arg genetic polymorphisms of beta1-adrenoceptor in Chinese Han and Dai populations. *Acta Pharmacol Sin* 2006; *27* : 254-8.

13. Inagaki Y, Mashima Y, Fuse N, Funayama T, Ohtake Y, Yasuda N, *et al*. Polymorphism of beta-adrenergic receptors and susceptibility to open-angle glaucoma. *Mol Vis* 2006; *12* : 673-80.

14. Covolo L, Gelatti U, Metra M, Nodari S, Picciche A, Pezzali N, *et al*. Role of beta1- and beta2-adrenoceptor polymorphisms in heart failure: a case-control study. *Eur Heart J* 2004; *25* : 1534-41.

15. Xie HG, Dishy V, Sofowora G, Kim RB, Landau R, Smiley RM, *et al*. Arg389Gly beta 1-adrenoceptor polymorphism varies in frequency among different ethnic groups but does not alter response *in vivo*. *Pharmacogenetics* 2001; *111* : 191-7.

16. Tamil people [home page on the internet]. [cited 2009, 29th January]. Available from: *http:/en.wikipedia.org/wiki/Tamil_people*.

17. Shioji K, Kokubo Y, Mannami T, Inamoto N, Morisaki H, Mino Y, *et al*. Association between hypertension and the alpha-adducin, beta1-adrenoreceptor and G-protein beta3 subunit genes in the Japanese population; the Suita study. *Hypertens Res* 2004; *27* : 31-7.

18. Ellsworth DL, Coady SA, Chen W, Srinivasan SR, Boerwinkle E, Berenson GS. Interactive effects between polymorphisms in the beta-adrenergic receptors and longitudinal changes in obesity. *Obes Res* 2005; *13* : 519-26.

19. Linne Y, Dahlman I, Hoffstedt J. Beta1-adrenoceptor gene polymorphism predicts long- term changes in body weight. *Int J Obes (Lond)* 2005; *29* : 458-62.

20. Terra SG, Pauly DF, Lee CR, Patterson JH, Adams KF, Schofield RS, *et al*. beta-Adrenergic receptor polymorphisms and responses during titration of metoprolol controlled release/extended release in heart failure. *Clin Pharmacol Ther* 2005; *77* : 127-37.

21. Belfer I, Buzas B, Evans C, Hipp H, Phillips G, Taubman J, *et al*. Haplotype structure of the beta adrenergic receptor genes in the US Caucasians and African Americans. *Eur J Hum Genet* 2005; *3* : 341-51.

22. Defoor J, Martens K, Zielinska D, Matthijs G, Nerum HV, Schepers D, *et al*. The CAREGENE study: polymorphisms of the bata1-adrenoceptor gene and aerobic power in coronary artery disease. *Eur Heart J* 2006; *27* : 808-16.

23. Chen L, Meyers D, Javorsky G, Burstow D, Lolekha P, Lucas M, *et al*. Arg389Gly beta1-adrenergic receptors determine improvement in left ventricular systolic function in nonischemic cardiomyopathy patients with heart failure after chronic treatment with carvedilol. *Pharmacogenet Genomics* 2007; *7* : 941-9.

24. Wilk JB, Myers RH, Pankow JS, Hunt SC, Leppert MF, Freedman BI, *et al*. Adrenergic receptor polymorphisms associated with resting heart rate: The HyperGEN study. *Ann Hum Genet* 2006; *70* : 566-73.

25. Kurnik D, Li C, Sofowara GG, Friedman EA, Muszkat M, Xie HG, *et al*. Beta-1- adrenoceptor genetic variants and ethnicity independently affect response to beta- blockade. *Pharmacogenet Genomics* 2008; *18* : 895-902.

Reprint requests: Dr C. Adithan, Director-Professor & Head, Pharmacogenomics Laboratory, Department of Pharmacology
Jawaharlal Institute of Postgraduate Medical Education & Research, Puducherry 605 006, India
e-mail: jipgene@jipmer.edu