

Original Article

Arg16Gly and Gln27Glu β_2 adrenergic polymorphisms influence cardiac autonomic modulation and baroreflex sensitivity in healthy young Brazilians

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Received October 6, 2014; Accepted November 25, 2014; Epub January 15, 2015; Published January 30, 2015

Abstract: The association between functional β_2 adrenergic receptor (β_2 -AR) polymorphisms and cardiac autonomic modulation is still unclear. Thus, two common polymorphisms in the β_2 -AR gene (Gln27Glu β_2 and Arg16Gly β_2) were studied to determine whether they might affect tonic and reflex cardiac sympathetic activity in healthy young subjects. A total of 213 healthy young white subjects of both genders (53% female), aged 18-30 years (23.5±3.4 y), had their continuous blood pressure curves noninvasively recorded by Finometer at baseline, and other hemodynamic parameters, as cardiac autonomic modulation, baroreflex sensitivity, and allele, genotype, and diplotype frequencies calculated. Associations were made between Arg16Gly β_2 and Gln27Glu β_2 polymorphisms and between β_2 -AR diplotypes and all variables. The heart rate was significantly lower ($P<0.001$) in the presence of homozygous Arg/Arg alleles (60.9±1.5 bpm) than in that of Arg/Gly heterozygotes (65.9±1.0 bpm) or Gly/Gly homozygotes (66.3±1.2 bpm). Homozygous carriers of Arg16 allele had an alpha index (19.2±1.3) significantly higher ($P<0.001$) than that of the subjects with the Gly allele Gly/Gly (14.5±0.7) or Arg/Gly (14.6±0.7). Furthermore, the recessive Gln27Glu and the heterozygous Gln27Glu genotypes had a higher percentage of low-frequency components (LF%) than the homozygous Gln27Gln (15.1% vs. 16.0% vs. 8.2%, $P=0.03$, respectively). In healthy young subjects, the presence of β_2 -AR Arg16 allele in a recessive model was associated with higher baroreflex sensitivity, and increased parasympathetic modulation in studied individuals.

Keywords: β_2 -adrenergic polymorphism, autonomic nervous system, cardiac autonomic balance, heart rate variability

Introduction

β_1 and β_2 adrenergic receptors (β_1 - and β_2 -ARs) have a pivotal role in the sympathetic nervous system, which controls various physiological functions, including energy homeostasis, glucose and lipid metabolism as well as cardiovascular (CV) moment-to-moment regulation [1]. Single nucleotide polymorphisms have been described in the gene encoding human β_2 -AR, and they can affect the function of the receptor in vitro [2]. Functional and common human Arg16Gly and Gln27Glu β_2 -AR polymorphisms

have been examined for association with hypertension, [3] obesity [4, 5], type 2 diabetes [6], and sudden death [7]. The studies reach conflicting results since the great many factors under consideration, including the complexities of the phenotypes and the age of the study sample, may easily confound the researchers and thus hinder data interpretation. The functional relevance of Arg16Gly and Gln27Glu β_2 -AR polymorphisms has been studied mainly in 2 pathways: heart rate (HR) and vasodilators responses. Many studies indicate that, in healthy subjects, HR has only marginal geno-

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Table 1. Baseline characteristics and genotype distribution according to gender in 213 apparently healthy young subjects

Variables	Women (n=113)	Men (n=110)	Total (n=213)	P-value
Age (years)	23.4 (\pm 3.7)	23.7 (\pm 3.4)	23.5 (\pm 3.4)	0.32
Body mass index (kg/m ²)	22.2 (\pm 3.4)	24.5 (\pm 3.8)	23.3 (\pm 3.8)	<0.0001
AC *(cm)	72.8 (\pm 8.6)	82.0 (\pm 9.9)	77.2 (\pm 10)	<0.0001
Current Smoking (%)	8 (7.0)	9 (9.0)	17 (8.0)	0.61
SBP (mmHg)	112.4 (\pm 8.1)	116.5 (\pm 8.5)	114.3 (\pm 8.5)	0.002
DBP (mmHg)	70.5 (\pm 6.2)	73.4 (7.4)	71.9 (\pm 6.9)	<0.0001
Total cholesterol (mg/dl)	175.6 (\pm 31)	159.4 (\pm 37)	167.9 (\pm 35.3)	0.008
LDL-C cholesterol (mg/dl)	92.8 (\pm 26.2)	91.2 (\pm 32.7)	92 (\pm 29.4)	0.80
HDL-C cholesterol (mg/dl)	65.4 (\pm 14)	51.4 (\pm 10)	58.7 (\pm 14.6)	<0.0001
Triglycerides (mg/dl)	87.2 (\pm 36)	84.2 (\pm 46)	85.8 (\pm 41.6)	0.70
Insuline levels (mU/ml)	7.7 (\pm 3.4)	7.5 (\pm 10.5)	7.7 (\pm 7.5)	0.90
Fasting glucose (mG/dl)	83.5 (\pm 7.8)	84.4 (\pm 7.7)	85.4 (\pm 7.9)	0.004
Creatinine (mg/dl)	0.77 (\pm 0.1)	0.98 (\pm 0.1)	0.9 (\pm 0.2)	<0.0001
Hemoglobin (%)	40.2 (\pm 0.8)	45.6 (\pm 2.7)	42.7 (\pm 3.7)	<0.0001
Hematocrit (g/dl)	13.4 (\pm 0.8)	15.4 (\pm 1.0)	14.4 (\pm 1.4)	<0.0001
Genotype distribution				
Gln27Glu				0.80
Gln/Gln	59 (52.2)	47 (47.0)	106 (49.8)	
Gln/Glu	41 (36.3)	47 (47.0)	88 (41.3)	
Glu/Glu	13 (11.5)	6 (6.0)	19 (8.9)	
Arg16Gly				0.17
Gly/Gly	38 (33.6)	33 (33.0)	71 (33.0)	
Arg/Gly	50 (44.2)	41 (41.0)	91 (42.7)	
Arg/Arg	25 (22.1)	26 (26.0)	51 (23.9)	

For baseline characteristics, values are expressed as mean \pm standard deviation (\pm SD). For genotype distributions, values are expressed as frequency and percentage. AC *=abdominal circumference. All the polymorphisms tested were in Hardy-Weinberg equilibrium ($p > 0.05$). Some proportions might not add up to 100% due rounding.

type-dependent effects on Arg16Gly and Gln-27Glu β_2 -AR polymorphisms [8].

Methods of higher sensitivity such as time domain and spectral analyses of HR variability [9] could be used to dissect the association between cardiac modulation and Arg16Gly and Gln27Glu β_2 -AR polymorphisms since they reflect the sympathetic (adrenergic receptors) and parasympathetic (muscarinic receptors) nerve activity over the sinoatrial node [10, 11].

Furthermore, the baroreflex, the most important autonomic mechanism controlling CV function, may also be affected by β_2 -AR gene variation. In fact, decreased baroreflex sensitivity is associated with increased sympathetic activity and higher cardiovascular (CV) morbidity and mortality [12].

In a published study conducted with young Japanese male subjects, homozygous carriers of the β_2 -AR Arg16 allele had lower sympathetic activity than subjects with the Gly allele, and carriers of β_2 -AR Glu27 allele were linked with higher cardiac sympathetic autonomic activity [13]. However, given the significant differences in genetic profile of different racial/ethnic groups, we carried out a study to test the hypothesis that Arg16Gly and Gln27Glu β_2 -AR polymorphisms influence functional physiological characteristics, such as HR, cardiac sympathetic modulation, and CV reflex control, which are considered intermediate phenotypes with profound implications for the development of CV disease. We studied the correlation of Arg16Gly and Gln27Glu β_2 -AR polymorphisms with hemodynamic parameters, norepinephrine levels, cardiac autonomic modulation, and

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Table 2. Hemodynamic parameters stratified per Arg16Gly genotypes (n=213)

Variables	Gly/Gly (n=71)	Arg/Gly (n=91)	Arg/Arg (n=51)	P-values
HR (bpm)	66.3 (\pm 1.2)	65.9 (\pm 1.0)	60.9 (\pm 1.5)#	<0.0001
SBP (mmHg)	119.1 (\pm 1.1)	120.7 (\pm 1.1)	118.9 (\pm 1.2)	0.47
DBP (mmHg)	68.1 (\pm 0.6)	69.0 (\pm 0.6)	67.1 (\pm 0.9)	0.16
MBP (mmHg)	87.5 (\pm 0.8)	88.6 (\pm 0.7)	86.4 (\pm 1.1)	0.18
CO (l/minutes)	5.7 (\pm 0.2)	5.8 (\pm 0.1)	5.6 (\pm 0.2)	0.63
TPR (mmHg. s/mL)	0.96 (\pm 0.02)	0.94 (\pm 0.01)	1.0 (\pm 0.03)	0.70
Nor (pg/mL)	70.7 (\pm 5.1)	78.8 (\pm 6.7)	71.6 (\pm 7.1)	0.59

Values are mean and standard deviation (\pm SD). P-values=comparison among groups. HR=Heart Rate; SBP=Systolic Blood Pressure; DBP=Diastolic Blood Pressure; CO=Cardiac Output; TPR=Total Peripheral Resistance; Nor=Norepinephrine. Some proportions might not add up to 100% due rounding. #= p <0.05 Arg/Arg group vs. Arg/Gly and Gly/Gly groups.

baroreflex sensitivity in healthy young male and female Brazilian subjects.

Methods

Studied population

A total of 213 white young subjects of both genders (53% female), aged 18-30 years (mean \pm SE: 23.5 \pm 3.4), were included in the protocol. All subjects underwent a careful clinical examination and had their blood pressure (BP) and anthropometric measurements, such as height, weight, body index mass (BMI), and abdominal circumference (AC), recorded. All subjects were classified as normotensive according to current guidelines (office BP <140/90 mmHg) and were nonobese (BMI <30 Kg/m²). Information about their health conditions, habits, use of medication, and family history was obtained through interviews. Additionally, all subjects took laboratory tests to verify their metabolic status (triglycerides, insulin, total cholesterol, LDL-cholesterol, HDL-cholesterol and glucose levels, and red blood cell count). The exclusion criteria were any chronic diseases, pregnancy, and regular use of any medication (except oral contraceptives). The study protocol was reviewed and approved by the appropriate institutional review committees, the Human Subject Protection Committees of the Heart Institute (InCor) and the Clinic Hospital, University of São Paulo, and was conducted in accordance with World Medical Association International Code of Medical Ethics (Declaration of Helsinki, 1964; revised in 2008). Subjects were informed about

the research protocol and a written informed consent was obtained from each study subject.

Genotyping protocol

Briefly, genomic DNA was extracted from leukocytes in whole blood samples after a standard salting-out technique [14]. Genotypes were detected by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) analysis [15]. Quality control for these assays was carried out by randomly selecting 60 samples

to be re-genotyped by two independent technicians.

Experimental protocol

The experimental protocol was always performed in the morning in a quiet room with temperature control (22°C). Subjects were asked to abstain from alcohol, tobacco, intense exercise and sleep deprivation 24 h before the study, and water intake was stimulated. Each patient rested supine was monitored with an electrocardiogram (ECG) machine, a noninvasive beat-by-beat arterial blood pressure device, and an abdominal impedance plethysmography belt (pneumograph). A venous puncture was also performed.

Continuous BP waveforms were obtained by a digital photoplethysmograph device (*Finometer*[®], *Finapres Medical System BV, Holland*). A software program (*BeatScope*) used BP curves and patient data (age, sex, weight, and height) to calculate systolic and diastolic BP (SBP and DBP), HR, cardiac output (CO), and peripheral vascular resistance (PVR). The waveforms were simultaneously recorded on another computer equipped for acquisition and conversion of the biological signals AT/MCA-CODAS (*DATA Instruments Inc., Akron, Ohio, USA*). The sampling frequency of signals was 1000 Hz. The stored data underwent a routine analysis to provide HR and BP variability.

The procedures for power spectral analysis of R-R interval (heart rate variability-HRV) and

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Table 3. Hemodynamic parameters stratified per Gln27Glu genotypes (n=213)

Variables	Gln/Gln (n=106)	Gln/Glu (n=88)	Glu/Glu (n=19)	P-values
HR (bpm)	64.6 (\pm 1.0)	64.7 (\pm 1.1)	65.8 (\pm 2.8)	0.9
SBP (mmHg)	119.5 (\pm 1.0)	119.9 (\pm 1.0)	120.5 (\pm 1.7)	0.89
DBP (mmHg)	68.2 (\pm 0.6)	68.4 (\pm 0.7)	67.5 (\pm 1.1)	0.84
MBP (mmHg)	87.6 (\pm 0.7)	87.8 (\pm 0.8)	87.1 (\pm 1.3)	0.95
CO (l/minutes)	5.7 (\pm 0.1)	5.9 (\pm 0.1)	5.3 (\pm 0.2)	0.22
TPR (mmHg.s/mL)	1.0 (\pm 0.02)	0.9 (\pm 0.02)	1.0 (\pm 0.04)	0.18
Nor (pg/mL)	76.1 (\pm 6.1)	75.1 (\pm 4.8)	61.1 (\pm 10.1)	0.55

Values are mean and standard deviation (\pm SD). P-values=comparison among groups. HR=Heart Rate; SBP=Systolic Blood Pressure; DBP=Diastolic Blood Pressure; CO=Cardiac Output; TPR=Total Peripheral Resistance; Nor=Norepinephrine. Some proportions might not add up to 100% due rounding.

blood pressure variability (BPV) have been described in the literature. Each heart beat was identified using a specialized algorithm that was implemented for Matlab MT (*MATLAB 6.0, Mathworks, USA*) and that makes the automatic detection of systolic and diastolic pressure wave events. Pulse interval (PI) or R-R interval was calculated as the difference between the beginning and end points of the cycle (t_1-t_0). The power spectral density of the R-R interval and systolic blood pressure were computed using the Fast Fourier Transform and Welch's method over 16,384 points with a Hanning window and 50% overlapping. Spectral bands evaluated for humans were defined according to literature references: very low-frequency (VLF; 0.007-0.04 Hz), low frequency (LF; 0.04-0.15 Hz), high frequency (HF; 0.15-0.4 Hz), and total power [9]. Baroreflex sensitivity was also inferred from the alpha index (ratio of R-R LF ms^2/BP LF mmHg^2) [16].

A peripheral venous blood sample was collected for the measurement of plasma norepinephrine concentration (measured by high-performance liquid chromatography at the Nephrology Laboratory, University of São Paulo) [17] with the patient in supine position.

Statistical analysis

First, a distribution of continuous variables was constructed with the medians (interquartile range) or means (\pm SD) of the baseline characteristics. Absolute and relative frequencies were estimated for categorical variables. Alleles, genotype and diplotype frequencies were calculated and the Hardy-Weinberg equilibrium test using χ^2 -analyses was applied. Pair-

wise linkage disequilibrium (LD) was examined as described by Devlin and Risch. Next, an analysis was made of the association between β_2 -AR genotypes and demographic, anthropometric, and laboratory data, cardiac autonomic balance (HR variability, SBP and DBP, CO, total peripheral resistance, and norepinephrine levels), and hemodynamic parameters.

Chi-square was used to test the association between categorical variables, while ANOVA and the Bonferroni posttest were used

for means of continuous variables. A two-tailed P-value of 0.05 was considered significant. Statistical analyses were made with the SAS version 9.1 and with the SPSS version 16.0. Data were expressed as \pm SEM.

Results

Characteristics of the studied subjects

Table 1 shows baseline characteristics and genotype distribution in 213 healthy young subjects according to gender. All parameters were within the reference range for age. In a gender-stratified analysis, we found that men (n=100) had higher body mass index (BMI), abdominal circumference (AC), systolic (SBP) and diastolic blood pressure (DBP), fasting glucose, serum creatinine, hemoglobin, and hematocrit values than women (n=113), while women had a higher HDL-cholesterol level as compared with men.

In the entire study sample (n=213), allele and genotype frequencies of both Arg16Gly and Gln27Glu genotypes (**Table 1**) were concordant with the Hardy-Weinberg equilibrium ($P>0.05$). At Codon 16, the allele frequency of Gly was 70.3% and that of Arg was 29.7%; at Codon27, the frequencies of Gln and Glu were 54.3% and 45.7%, respectively. The genotype frequencies of Gly16Gly, Arg16Gly, and Arg16Arg were 49.8%, 41.1%, and 9.1%, respectively. The frequencies of Gln27Glu, Gln27Gln, and Glu27Glu were 32.5%, 43.7%, and 23.9%, respectively. It is worth noting that we did not find any male-female differences in genotype frequencies (**Table 1**). Overall, polymorphisms were in modest linkage disequilibrium with one another (normalized Lewontin's $D'=0.72$).

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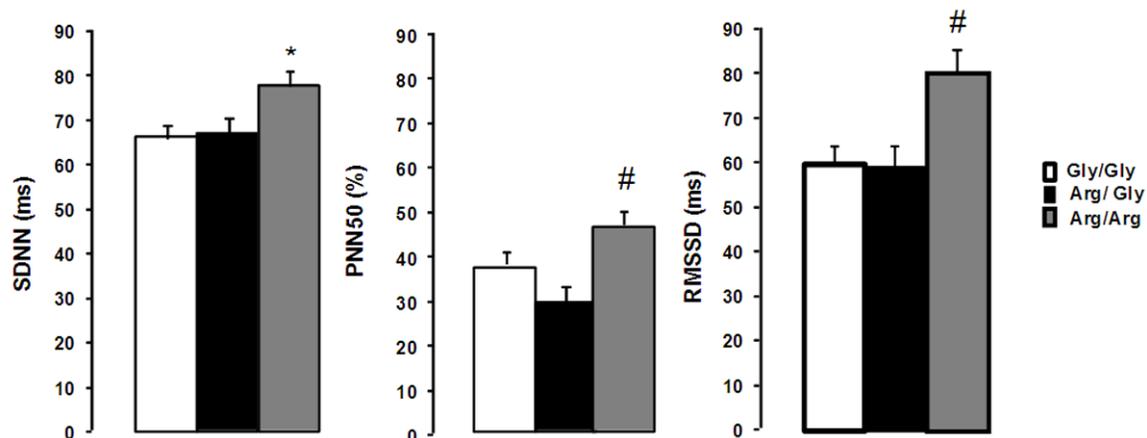


Figure 1. Heart rate variability in time domain stratified per Arg16Gly genotypes. Values are mean and standard deviation (\pm SD). *P*-values=comparison among groups. *= $p < 0.05$ Arg/Arg group vs. Arg/Gly; #= $p < 0.05$ Arg/Arg group vs. Arg/Gly and Gly/Gly groups.

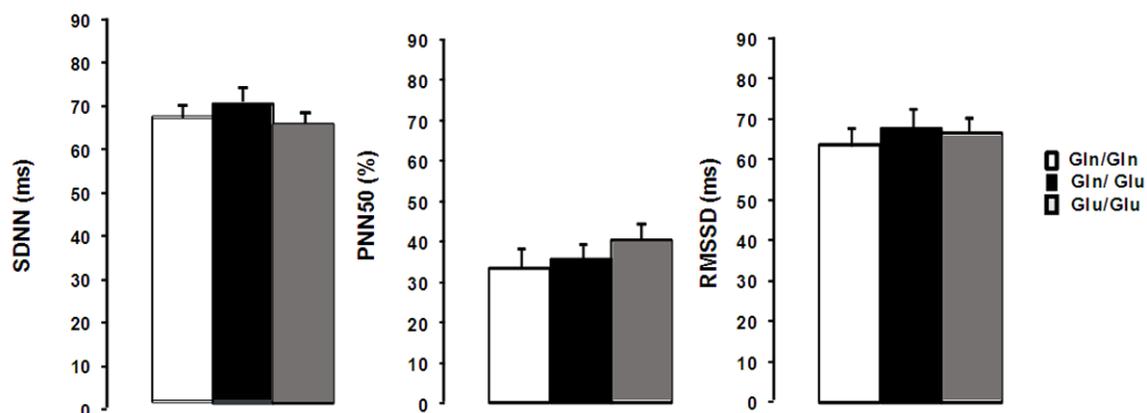


Figure 2. Heart rate variability in time domain stratified per Gln27Glu genotypes. Values are mean and standard deviation (\pm SD). *P*-values=comparison among groups.

Association of β_2 gene polymorphisms with hemodynamic parameters, autonomic modulation, and baroreflex sensitivity

Association of heart rate, BP, cardiac output, total peripheral resistance values, and norepinephrine levels of all of the study subjects with Arg16Gly and Gln27Glu genotypes are displayed in **Tables 2** and **3**, respectively. Examination of Arg16Gly genotypes shows the heart rate was significantly lower ($P < 0.001$) in the presence of homozygote Arg/Arg alleles (60.9 ± 1.5 bpm) than in that of heterozygote Arg/Gly (65.9 ± 1.0 bpm) or homozygote Gly/Gly (66.3 ± 1.2 bpm) (**Table 2**). Other hemodynamic parameters, as well as norepinephrine levels, were similar across Arg16Gly genotypes at baseline. No significant differences were detected

in hemodynamic and norepinephrine levels at baseline across Gln27Glu genotypes, as observed in **Table 3**.

Analysis of HRV and BPV were evaluated in time and frequency domain. The evaluation of Arg16Gly genotypes shows that SDNN index was increased in Arg/Arg (SDNN= 78 ± 3 ms) alleles as compared with heterozygote Arg/Gly (SDNN= 67 ± 3 ms) (**Figure 1**). RMSSD and pNN50 were higher in the presence of homozygote Arg/Arg alleles (rMSSD= 80 ± 6 ms; pNN50= $46 \pm 3\%$) than homozygote Gly/Gly (rMSSD= 50 ± 3 ms; pNN50= $37 \pm 2\%$) and heterozygote Arg/Gly (rMSSD= 59 ± 5 ms; pNN50= $30 \pm 3\%$) (**Figure 1**). As observed in **Figure 2**, heart rate variability in time domain was not different between Gln27Glu genotypes subjects.

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Table 4. Heart rate and blood pressure variability stratified per Arg-16Gly genotypes (n=213)

Variables	Gly/Gly (n=71)	Arg/Gly (n=91)	Arg/Arg (n=51)	P-values
Var RR	4876 (\pm 423)	5479 (\pm 563)	6884 (\pm 896)	0.10
LF (ms ²)	1346 (\pm 132)	1679 (\pm 155)	1856 (\pm 176)	0.09
HF (ms ²)	1432 (\pm 132)	1721 (\pm 87)	1899 (\pm 132)†	<0.05
LF (%)	49 (\pm 3.4)	49 (\pm 4.1)	49 (\pm 3.9)	0.99
HF (%)	51 (\pm 3.4)	50 (\pm 4.1)	51 (\pm 3.9)	0.99
LF/HF	0.94 (\pm 0.11)	1 (\pm 0.1)	1 (\pm 0.1)	0.89
Var SBP	53 (\pm 3)	47 (\pm 4)	50 (\pm 10)	0.45
LF (mmHg ²)	6 (\pm 1.0)	8 (\pm 1.2)	5 (\pm 0.8)	0.15
α index (ms/mmHg)	15 (\pm 0.6)	14 (\pm 0.7)	19.26 (\pm 1)#	<0.05

Values are mean and standard deviation (\pm SD). P-values=comparison among groups. †=p<0.05 Arg/Arg group vs. Gly/Gly group; #=p<0.05 Arg/Arg group vs. Arg/Gly and Gly/Gly groups.

The Var RR and absolute LF component of HRV were similar among the studied Arg16Gly genotypes (Table 4). The absolute HF component of HRV was higher in homozygote Arg/Arg than homozygote Gly/Gly; and similar between homozygote Gly/Gly and heterozygote Arg/Gly. The LF%, HF% components of HRV, and the LF/HF ratio reveal that Arg16Gly polymorphisms had a similar cardiac autonomic modulation at baseline (Table 4).

The Var SBP and absolute LF component of SBP were similar among the groups. However, homozygous carriers of Arg16 allele had a higher alpha index than in subjects with the Gly allele (Table 4).

The parameters of HRV and BPV in the time and frequency domains, stratified per Gln27Glu genotypes (Gln/Gln, Gln/Glu and Glu/Glu), were similar among studied groups (Table 5).

Discussion

The main findings of the present study suggest that healthy young white Brazilian subjects, who were homozygous for the β_2 -AR Arg16 allele, presented a lower resting HR, higher vagal modulation, and higher baroreflex sensitivity than carriers of the Gly16 allele. However, it was not observed in Gln27Glu genotypes. These data thus indicate that, in a healthy young population, the β_2 -AR Gly16Arg polymorphisms can influence intermediate phenotypes, such as HR, cardiac parasympathetic modulation, and baroreflex sensitivity. These results are important because such intermediate phys-

iological traits in healthy individuals may be predictive of future disease or a distant phenotype [18]. Furthermore, to our knowledge, this is the first study conducted in Brazilian health population.

Resting HR has been shown to be positively correlated with subsequent development of hypertension and CV disease [19, 20]. Reduced HRV and higher sympathetic activity have been considered strong and independent mortality predictors in individuals with myocardial infarction, heart failure, or other morbidities that increase CV risk like diabetes mellitus. In addition, a decrease in baroreflex sensitivity is also an independent predictor of CV events [12].

In the present study, subjects with the β_2 -AR Arg16 allele had protective intermediate traits characterized by increased cardiac parasympathetic modulation and higher baroreflex sensitivity than carriers of the Gly16 allele. We did not detect differences among groups concerning blood pressure and cardiac output at rest. Previous studies have demonstrated an association of Gly16 allele with elevated blood pressure [21, 22] and plasma norepinephrine than Arg16 carriers [23]. Differences in population [21, 22] or in experimental conditions [23] may have influenced the discrepancy in the results.

β_2 -AR Gly16Arg and Gln27Glu polymorphisms are localized in the extracellular amino-terminus region of the protein, and *in vitro* studies have indicated that they have a subtle effect on the agonist-promoted down regulation of the receptor expression when studied in a cell-based system where transfections of vectors containing only the open reading frames [24]. The *in vitro* impact of β_2 -AR gene polymorphisms has been extensively reviewed [25]. Taken together, the results of different studies it has been demonstrated that the β_2 -AR Gly16Arg genotypes appear to influence the degree of agonist-induced receptor desensitization [26], with Gly16 showing increased agonist-induced desensitization compared with Arg16 [24]. On the other hand, the presence of Glu27, rather than Gln27, is associated with resistance to desensitization [26].

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Table 5. Heart rate and blood pressure variability stratified per Gln-27Glu genotypes (n=213)

Variables	Gln/Gln (n=106)	Gln/Glu (n=88)	Glu/Glu (n=19)	P-values
Var RR (ms)	5738 (\pm 526)	5500 (\pm 544)	5353 (\pm 887)	0.91
LF (ms ²)	1296 (\pm 129)	1593 (\pm 321)	1564 (\pm 210)	0.67
HF (ms ²)	1272 (\pm 232)	1611 (\pm 145)	1632 (\pm 176)	0.31
LF (%)	50 (\pm 4)	50 (\pm 5)	49 (\pm 4)	0.98
HF (%)	50 (\pm 4)	50 (\pm 5)	51 (\pm 4)	0.98
LF/HF	1.01 (\pm 0.12)	0.99 (\pm 0.11)	0.95 (\pm 0.11)	0.95
Var SAP	51 (\pm 10)	57 (\pm 8)	55 (\pm 11)	0.53
LF (mmHg ²)	5 (\pm 1.2)	7 (\pm 1)	7 (\pm 1)	0.33
α index (ms/mmHg)	16 (\pm 0.8)	18 (\pm 0.9)	18 (\pm 1.6)	0.30

Values are mean and standard deviation (\pm SD). P-values=comparison among groups.

In humans, the functional importance of the Arg16Gly and Gln27Glu β -2 AR polymorphisms has been studied in cardiac and vasodilator responses. The β -2 AR is responsible for vasodilatation in the vasculature via the cAMP pathway in smooth muscle cells or through the release of nitric oxide (NO) from vascular endothelium [27]. The effect of the β -2 AR genotype has been investigated for responsiveness to local infusions and systemic infusions of agonists. Evidence has been collected supporting the concept that Gly16, and possibly Glu27, is associated with greater β -2 AR-agonist-mediated vasodilatation than Arg16 and Gln27 [8].

The difference between the responses of local infusion studies, which reveal that Gly16 and Glu27 homozygotes presents greater vasodilatation, and systemic infusion studies, which show greater vasodilatation in Arg16 or Arg16+Gln27 homozygotes, is probably due to the impact of counter-regulatory baroreflex activation and the compensation for this reflex in reason of augmented vasodilatation in Gly16 or Glu27 carriers. Once baroreflex inhibition was established, raising blood pressure to baseline levels, systemic vascular resistance tended to come under the influence of haplotype, and it was significant at position 16, since Gly16 homozygotes had a lower systemic vascular resistance response to terbutaline than Arg16 homozygotes [8].

In the heart, β -2 is located in the presynaptic terminal sympathetic nerve and in the myocardium. Presynaptic β -2 AR activation stimulates cardiac norepinephrine release, and postsyn-

aptically enhances cardiac frequency and contractility [2].

The β -2 AR genotype may be linked to ventricular function. In normotensive humans, echocardiographic evaluation showed Gly16 homozygotes have greater fractional shortening, ejection fraction, midwall shortening, and stress-corrected midwall shortening than either heterozygotes or Arg16 homozygotes [28]. These differences were independent of various confounders, such as age, sex,

ethnicity, and hemodynamic parameter. Moreover, Gly16 homozygotes had greater cardiac output and stroke volume at rest than Arg16 homozygotes [29], as well as during low- and high-intensity exercises [23].

These studies of cardiovascular function in healthy young subjects demonstrated that Arg16 allele carriers have attenuated resting cardiovascular function. Our results add to this information, because we demonstrate a reduced baseline heart rate and increased baroreflex sensitivity and vagal modulation in homozygous carriers of the Arg16 allele. However, since blood pressure is a phenotype controlled by several other mechanisms, it is necessary to consider the link of Arg16Gly polymorphism to other systems and environmental factors, such as sodium intake and renal sodium excretion, and its impact on blood pressure levels [30].

In conclusion, the variability of β -2-AR function resulting from genetic polymorphisms may account for the cardiac autonomic modulation, in special heart rate variability and baroreflex sensitivity in Brazilian health subjects, seen in this study. Our results strongly support the hypothesis that the Arg16 allele has a protective effect showed by increased parasympathetic modulation in studied individuals.

Acknowledgements

B.R., M.C.I, and F.M.C.C. had grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and from Fundação de

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Amparo à Pesquisa do Estado de São Paulo (FAPESP).

Disclosure of conflict of interest

None to disclose.

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References

- [1] Insel PA. Seminars in medicine of the Beth Israel Hospital, Boston. Adrenergic receptors-evolving concepts and clinical implications. *N Engl J Med* 1996; 334: 580-5.
- [2] Brodde OE, Michel MC. Adrenergic and muscarinic receptors in the human heart. *Pharmacol Rev* 1999; 51: 651-690.
- [3] Ge D, Huang J, He J, Li B, Duan X, Chen R, Gu D. Beta2-Adrenergic receptor gene variations associated with stage-2 hypertension in northern Han Chinese. *Ann Hum Genet* 2005; 69: 36-44.
- [4] Eriksson P, Dahlman I, Rydén M, Hoffstedt J, Arner P. Relationship between beta-2 adrenoceptor gene haplotypes and adipocyte lipolysis in women. *Int J Obes Relat Metab Disord* 2004; 28: 185-190.
- [5] 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: 519-526.
- [6] Gjesing AP, Andersen G, Burgdorf KS, Borch-Johnsen K, Jørgensen T, Hansen T, Pedersen O. Studies of the associations between functional beta2-adrenergic receptor variants and obesity, hypertension and type 2 diabetes in 7,808 white subjects. *Diabetologia* 2007; 50: 563-568.
- [7] Gavin MC, Newton-Cheh C, Gaziano JM, Cook NR, VanDenburgh M, Albert CM. A common variant in the β 2-adrenergic receptor and risk of sudden cardiac death. *Heart Rhythm* 2011; 8: 704-710.
- [8] Dorn GW. Adrenergic signaling polymorphisms and their impact on cardiovascular disease. *Physiol Rev* 2010; 90: 1013-1062.
- [9] Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation* 1996; 93: 1043-1065.
- [10] Rodefeld MD, Beau SL, Schuessler RB, Boineau JP, Saffitz JE. Beta-adrenergic and muscarinic cholinergic receptor densities in the human sinoatrial node: identification of a high beta 2-adrenergic receptor density. *J Cardiovasc Electrophysiol* 1996; 7: 1039-1049.
- [11] Brodde OE. Beta-1 and beta-2 adrenoceptor polymorphisms: functional importance, impact on cardiovascular diseases and drug responses. *Pharmacol Ther* 2008; 117: 1-29.
- [12] Verriar RL, Tan A. Heart Rate, autonomic markers, and cardiac mortality. *Heart Rhythm* 2009; 6 Suppl 11: S68-S75.
- [13] Matsunaga T, Yasuda K, Adachi T, Gu N, Yamamura T, Moritani T, Tsujimoto G, Tsuda K. Association of beta-adrenoceptor polymorphisms with cardiac autonomic modulation in Japanese males. *Am Heart J* 2007; 154:759-766.
- [14] Miller SA, Dykes DD, Polesk HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research* 1988; 16: 1215.
- [15] Mansur AJ, Fontes RS, Canzi RA, Nishimura R, Alencar AP, de Lima AC, Krieger JE, Pereira AC. Beta-2 adrenergic receptor gene polymorphisms Gln27Glu, Arg16Gly in patients with heart failure. *BMC Cardiovascular Disorders* 2009; 9: 50.
- [16] Frattola A, Parati G, Gamba P, Paleari F, Mauri G, Di Rienzo M, Castiglioni P, Mancia G. Time and frequency domain estimates of spontaneous baroreflex sensitivity provide early detection of autonomic dysfunction in diabetes mellitus. *Diabetologia* 1997; 40: 1470-1475.
- [17] Davies CL, Molyneux SG. Routine determination of plasma catecholamines using reversed-phase, ion-pair high-performance liquid chromatography with electrochemical detection. *J Chromatogr* 1982; 231: 41-45.
- [18] Eisenach JH, Wittwer ED. {beta}-Adrenoceptor gene variation and intermediate physiological traits: prediction of distant phenotype. *Exp Physiol* 2010; 95: 757-764.
- [19] Palatini P. Heart rate as an independent risk factor for cardiovascular disease: current evidence and basic mechanisms. *Drugs* 2007; 67 Suppl 2: 3-13.
- [20] Hsia J, Larson JC, Ockene JK, Sarto GE, Allison MA, Hendrix SL, Robinson JG, LaCroix AZ, Manson JE; Women's Health Initiative Research. Resting heart rate as a low tech predictor of coronary events in women: prospective cohort study. *BMJ* 2009; 338: b219.
- [21] Masuo K, Katsuya T, Fu Y, Rakugi H, Ogihara T, Tuck ML. Beta2-adrenoceptor polymorphisms relate to insulin resistance and sympathetic overactivity as early markers of metabolic disease in nonobese, normotensive individuals. *Am J Hypertens* 2005; 18: 1009-1014.
- [22] Masuo K, Katsuya T, Fu Y, Rakugi H, Ogihara T, Tuck ML. Beta2- and beta3-adrenergic recep-

Arg16Gly and Gln27Glu β 2 adrenergic polymorphisms in young Brazilians

- tor polymorphisms are related to the onset of weight gain and blood pressure elevation over 5 years. *Circulation* 2005; 111: 3429-3434.
- [23] Snyder EM, Beck KC, Dietz NM, Eisenach JH, Joyner MJ, Turner ST, Johnson BD. Arg16Gly polymorphism of the beta2-adrenergic receptor is associated with differences in cardiovascular function at rest and during exercise in humans. *J Physiol* 2006; 571: 121-130.
- [24] Green SA, Turki J, Innis M, Liggett SB. Amino-terminal polymorphisms of the human beta 2-adrenergic receptor imparts distinct agonist-promoted regulatory properties. *Biochemistry* 1994; 33: 9414-9419.
- [25] Brodde OE, Leineweber K. Beta2-adrenoceptor gene polymorphisms. *Pharmacogenet Genomics* 2005; 15: 267-275.
- [26] Green SA, Turki J, Bejarano P, Hall IP, Liggett SB. Influence of beta 2-adrenergic receptor genotyping on signal transduction in human airway smooth muscle cells. *Am J Resp Cell Mol Biol* 1995; 13: 25-33.
- [27] Eisenach JH, Clark ES, Charkoudian N, Dinunno FA, Atkinson JL, Fealey RD, Dietz NM, Joyner MJ. Effects of chronic sympathectomy on vascular function in the human forearm. *J Appl Physiol* 2002; 92: 2019-2025.
- [28] Tang W, Devereux RB, Kitzman DW, Province MA, Leppert M, Oberman A, Hopkins PN, Arnett DK. The Arg16Gly polymorphism of the beta2-adrenergic receptor and left ventricular systolic function. *Am J Hypertens* 2003; 16: 945-951.
- [29] Snyder EM, Hulsebus ML, Turner ST, Joyner MJ, Johnson BD. Genotype related differences in beta2 adrenergic receptor density and cardiac function. *Med Sci Sports Exerc* 2006; 38: 882-886.
- [30] Eisenach JH, Schroeder DR, Pike TL, Johnson CP, Schrage WG, Snyder EM, Johnson BD, Garovic VD, Turner ST, Joyner MJ. Dietary sodium restriction and beta2-adrenergic receptor polymorphism modulate cardiovascular function in humans. *J Physiol* 2006; 574: 955-965.