

## Beta1-adrenergic Receptor Polymorphisms Associated with Atrial Fibrillation in Systolic Heart Failure

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### Abstract

**Background:** The sympathetic nervous system is of great importance in the pathogenesis of atrial fibrillation in systolic heart failure. The identification of polymorphisms in the beta1-adrenergic receptor gene (ADBR1) represents an important step in understanding this pathogenesis.

**Objective:** This study assessed the association between the two functional polymorphisms of the beta1-adrenergic receptor gene (ADBR1), Ser49Gly and Arg389Gly, and the presence of atrial fibrillation in patients with systolic heart failure.

**Methods:** Case-control study with 144 patients with systolic heart failure, including 24 with atrial fibrillation (cases) and 120 without atrial fibrillation (controls). Genomic DNA was extracted from peripheral blood leukocytes and the genotypes of Ser49Gly and Arg389Gly polymorphisms were identified in all individuals by PCR/RFLP (polymerase chain reaction / restriction fragment length polymorphism).

**Results:** Mean age was  $59 \pm 13$  years, 70% of patients were males, 42% had ischemic causes and 74% had hypertension. Genotypes Ser49Ser and Arg389Arg were significantly associated with atrial fibrillation ( $p = 0.005$  and  $p = 0.01$ , respectively). After logistic regression, both adjusted for left atrial size and age, the significant association persisted (Arg389Arg - odds ratios: 2.78, 95% confidence interval = 1.02 to 7.56 and Ser49Ser - odds ratios: 8.02, 95% confidence interval = 1.02 to 63.82).

**Conclusion:** Both genotypes were associated with atrial fibrillation in patients; however, only Ser49Gly polymorphism was in Hardy-Weinberg equilibrium. (Arq Bras Cardiol 2012;98(5):384-389)

**Keywords:** Atrial fibrillation / genetics; heart failure / complications; polymorphism, genetic; genotype.

### Introduction

Atrial fibrillation (AF) and Systolic Heart Failure (SHF) frequently coexist because of common risk factors and similar pathogenic process<sup>1,2</sup>. The high incidence of AF in patients with SHF may be influenced by numerous factors, including: (i) the hemodynamic effects, especially atrial dilation and diastolic dysfunction, (ii) neurohumoral activation, and (iii) inflammation and oxidative stress. AF can cause SHF (tachycardiomyopathy) or be responsible for its deterioration. The coexistence of AF and SHF is associated with adverse prognosis, such as disease progression, more hospitalizations, cerebrovascular accidents and death<sup>3-6</sup>.

The association between FA and SHF has been extensively studied. However, the pathophysiological knowledge remains incomplete and some aspects need further investigation.

Currently, the study of genetics proposes that for a considerable number of cardiovascular diseases, susceptibility is related, in part, to genetic polymorphisms, particularly those related to neurotransmitter receptors. Considering the alterations that genetic variations of the beta1-adrenergic receptor can cause in the adrenergic system functions and the impact on predisposition to disease, these polymorphisms have been linked to propensity, evolution and outcome of several heart diseases, including HF<sup>7</sup>. The central role of the sympathetic nervous system and its receptors in HF makes the study of genetic polymorphisms of these receptors (Arg389Gly and Ser49Gly) an important step towards a better understanding of this pathogenesis<sup>8,9</sup>.

Some studies have associated the genotype Arg389Arg to higher adrenergic response in physiological states and in acute stages of cardiovascular disease. Moreover, it is associated with increased incidence of ventricular tachycardia<sup>10-15</sup>. Ser49Ser The genotype is associated with lower down-regulation of beta1-adrenergic receptors and, therefore, shows high levels of expression agonist in chronic diseases with increased adrenergic activity, such as HF. Furthermore, this genotype is associated with adverse events in patients with HF<sup>15-19</sup>.

Our study evaluated the association between ADRB1 gene polymorphisms and presence of AF in patients with SHF.

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Manuscript received April 30, 2011; revised manuscript received May 03, 2011; accepted January 20, 2012.

## Methods

We conducted an observational, case-control study in patients with systolic HF, from the HF clinic of Universidade Federal Fluminense. The case group consisted of 24 patients with atrial fibrillation and the control group, of 120 without the arrhythmia. The aim of this study was to evaluate the association between genotypes Arg389Arg and Ser49Ser and AF in patients with systolic HF.

Patients were included when they were admitted at the HF outpatient clinic. The study involved the following steps: (i) registration of clinical and demographic data of cases and controls, (ii) blood sampling for genetic analysis of beta-adrenergic receptors, and (iii) analysis of demographic, clinical, laboratory, echocardiographic data and genotypes in relation to groups of cases and controls.

Inclusion criteria were age  $\geq 18$  years, patients with history and physical examination compatible with HF and ejection fraction (LVEF)  $\leq 50\%$  by the echocardiographic method of Simpson. Exclusion criteria were patients with indication of or undergoing cardiac resynchronization therapy, active myocarditis, episode of aborted sudden death or presence of defibrillator and indication for angioplasty or coronary artery bypass surgery.

Peripheral blood samples were obtained for DNA isolation and genotyping. Demographic data were recorded: sex, age and self-reported ethnicity. Clinical data were assessed: presence of systemic arterial hypertension (SAH), diabetes mellitus, coronary artery disease (CAD) and use of cardiovascular medication.

Patients with a history of acute myocardial infarction, coronary angioplasty, CABG, coronary artery disease confirmed by coronary angiography and positive provocative test for myocardial ischemia were considered as having CAD. All patients were functionally evaluated according to the New York Heart Association (NYHA). Echocardiographic assessment was performed at baseline by two experienced echocardiographers, according to the guidelines of the European Society of Cardiology<sup>20</sup>. The analyses performed were: (i) systolic and left ventricular end-diastolic diameter (ii) size of the left atrium (LA), and (iii) LVEF by Simpson's method (Vivid 3, GE Medical Systems Ultrasound, Wisconsin, USA). Heart rate was recorded by means of standard 12-lead electrocardiogram at patient admission to diagnose AF.

The samples were submitted to lysis with 1000  $\mu\text{L}$  of Tris-1 (Tris-HCl 10mM pH 8.0, KCl 10mM, MgCl<sub>2</sub> 10mM, EDTA 2mM pH 8.0) containing Triton X-100 at 2.5%. After centrifugation at 5,000 rpm for 5 minutes in a centrifuge Beckman® the cell nuclei were lysed with 200  $\mu\text{L}$  of Tris-2 containing SDS at 1%. Proteins were removed by saline precipitation with 100  $\mu\text{L}$  of 5M NaCl. The DNA present in the supernatant was isolated by ethanol precipitation, and finally resuspended in 100  $\mu\text{L}$  of TE (Tris-HCl 10mM and 1mM EDTA, pH 8.0) and kept at  $-20^\circ\text{C}$  until the time of use. After extraction, the integrity of the DNA samples was analyzed by an electrophoresis system (BIO RAD electrophoresis) in agarose gel at 0.8% in TBE 1X buffer (Tris-HCl 90mM, boric acid 90mM and 2mM EDTA) and stained

with ethidium bromide. ADBR1 gene polymorphisms (Arg389Gly and Ser49Gly) of the beta1-adrenergic receptor were analyzed by PCR/RFLP (polymerase chain reaction/restriction fragment length polymorphism)<sup>21,22</sup>.

The PCR reaction was performed in a total volume of 25  $\mu\text{L}$  and the following was used: 50-100 ng of genomic DNA, after concentration adjustment, 1U of Fermentas Taq DNA polymerase, reaction buffer (KCl 50mM, MgCl<sub>2</sub> 1,5mM, Tris-HCl 10mM), 200 mM of each deoxynucleotide (dATP, dCTP, dGTP, dTTP) and 15 pmol of each oligonucleotide. The amplification and digestion conditions for the identification of polymorphisms are detailed in Table 1. The wild-type allele was defined according to the frequency of the study population.

Statistical analysis was performed using the SPSS statistical software, release 17.0 (SPSS Inc., Chicago, Illinois). Continuous data were expressed as mean  $\pm$  standard deviation (SD) and categorical variables as absolute numbers and percentage. The chi-square test was performed to analyze the deviation from Hardy-Weinberg equilibrium regarding the distribution of genotypes of beta-1-adrenergic receptor polymorphisms and to investigate their association with the presence of AF. The Hardy-Weinberg Law describes the principle of genetic equilibrium in a population. This equilibrium will occur if in a given population, over the generations, both allele frequencies and genotype frequencies remain at the same proportion. For that to occur there must be forces that will favor the formation of another gamete, such as migration, mutation or natural selection.

To verify whether there is a significant association between clinical, laboratory, and echocardiographic variables and those of polymorphisms (Arg389Arg and Ser49Ser) with AF, the following methods were applied: (i) the chi-square test ( $\chi^2$ ) or Fisher's exact test was applied for comparisons of categorical data; and (ii) Student's *t* test for independent samples or the Mann-Whitney test (nonparametric) were used to compare numerical data. The homogeneity of variance was tested using Levene test.

The logistic regression analysis was performed to identify the significant independent variables that are associated with AF. The criterion for determining significance was set at 5%.

This study was performed in accordance with the principles of the Declaration of Helsinki. The protocol was approved by a research ethics committee and all patients signed a free and informed consent form before enrolling in the study, for which there was no funding source.

## Results

Clinical characteristics of patients are shown in Table 2. One hundred and forty-four patients were included in the study. The group of cases, patients with AF, consisted of 24 patients (17%). The mean age was  $59 \pm 13$  years, 70% were males and 50% were non-Blacks. Regarding comorbidities, CAD occurred in 42% of patients, hypertension in 74% and diabetes mellitus in 34%. All patients were symptomatic at baseline, 25% of them presenting in functional class III or IV

**Table 1 – Amplification and digestion conditions for the identification of beta1-adrenergic receptor polymorphisms**

	ADBR1	
	Arg389Gly	Ser49Gly
Technique	PCR-RFLP	PCR-RFLP
Amplified Product	530bp	564bp
Cycling	Initial denaturing	94 °C-5min
	Denaturing*	94 °C-30s
	Annealing*	58 °C-30s
	Extension*	72 °C-1min
	Final extension	72 °C-7min
Restriction Enzyme	BCG1	Eco0109I
Effect of SNP on DNA sequence	ABS	ABS
Fragments of wild allele	342,154,34pb	345,219pb
Fragments of mutant allele	530bp	564bp
Temperature of digestion	37 °C	37 °C
Time of digestion	20h	20h

PCR - polymerase chain reaction; RFLP - restriction fragment length polymorphism; bp - base pairs; min-minutes; s-seconds; h-hours; SNP - single nucleotide polymorphism; ABS - Abolishes binding site; CBS - Creates binding site; (\*)30 cycles.

according to NYHA. Ninety-four percent were treated with angiotensin-converting enzyme inhibitor or an angiotensin-receptor blocker; 58%, with an aldosterone antagonist, 72%, with a beta-blocker and 3%, with amiodarone. The echocardiograms included in the study showed a prevalence of patients with severe systolic LV dysfunction (mean LVEF = 35.3 ± 9.3%) and enlarged left atrium (4.7 ± 1.2 cm).

The data on allelic and genotype frequencies are listed in Table 3. The study population was in Hardy-Weinberg equilibrium in relation to the polymorphism Ser49Gly. However, in relation to the polymorphism Arg389Gly, the population was not in equilibrium, which may be due to the fact that the patients already had a disease (HF) that may have generated it.

Only the genotypes Ser49Ser and Arg389Arg, age and left atrial size were significantly associated with the group of cases (AF) compared to controls (without AF). There was no significant difference, at 5% level, in the proportion of the remaining variables between the two groups.

Initially, the chance of the genotype Ser49Ser being associated with AF was greater than the chance of Ser49Gly and Gly49Gly genotypes (odds ratio = 11.07, confidence interval = 1.44 to 85.02). After logistic regression analysis taking into account age and LA size, the chance remained high (odds ratio = 8.02, confidence interval = 1.02 to 63.82). The chance of the genotype Arg389Arg being associated with AF was also greater than the chance of Gly389Gly and Arg389Gly genotypes (odds ratio = 3.21, confidence interval = 1.29 to 8.04). After logistic regression analysis, taking into account age and LA size, the chance remained high (odds ratio = 2.79, confidence interval = 1.02 to 7.56). Table 4 shows simplified data with odds ratio (OR) and confidence interval (CI) values.

## Discussion

The main finding of this study was the association between genotypes Ser49Ser and Arg389Arg, which are involved in states of adrenergic exacerbation<sup>10-19</sup> and AF in patients with SHF. The presence of AF in patients with SHF is multifactorial. The sympathetic nervous system and genetic factors related to it, such as beta1-adrenergic receptor polymorphisms, present as an additional variable to be considered in order to explain the association between AF and SHF.

In our study, as well as in other series, the LVEF was not associated with AF in patients with SHF, which did not occur in relation to NYHA functional class, either<sup>23-25</sup>.

The parameters that were significantly associated with AF were age, LA size, genotype Ser49Ser and genotype Arg389Arg.

The LA increase is classically associated with cardiac remodeling and the physiopathology of AF in patients with SHF, so that it directly contributes to the onset of AF in SHF. Moreover, sympathetic activation that is significantly elevated in patients with SHF also contributes to the pathogenesis of electrical and mechanical remodeling that culminates with the onset of AF<sup>26</sup>. Another fact to be considered is that the time of permanence of the AF can also, by itself, generate atrial remodeling and cause the enlargement of this cavity. As the start time of the arrhythmia onset could not be obtained, it may have contributed to the increase in the LA.

Age has been described as a factor related to AF<sup>23</sup>. In this study, the older the age, the greater the chance of its association with AF; however, the statistical significance of this variable with the AF was borderline (p = 0.06).

Regarding the beta1-adrenergic receptor polymorphisms, the Arg389Arg and Ser49Ser genotypes were significantly associated with AF. Physiopathologically, AF and HF are related to greater

Table 2 – Characteristics of patients

Clinical Characteristics	Total Group	Cases	Controls	p Value
	(n = 144)	(n = 24)	(n = 120)	
Age	59 ± 13	63 ± 13	58 ± 13	0.06
M/F	102(70%)/42(30%)	19(79%)/5(21%)	83(69%)/37(29%)	0.33
Ethnicity (Black)	73 (50%)	10 (42%)	62 (52%)	0.37
NYHA (class III/IV)	35 (25%)	8 (33%)	27 (22%)	0.46
SAH	108 (75%)	19 (79%)	89 (74%)	0.61
Diabetes mellitus	50 (35%)	9 (37.5%)	41 (34.2%)	0.75
CAD	62 (43%)	7 (29%)	55 (46%)	0.13
LA	4.6 ± 0.6	5.1 ± 0.8	4.5 ± 0.6	0.0002
LVEF	35.4 ± 9.3	35 ± 9.9	35 ± 9.2	0.98
LVEDD	6.8 ± 1.0	6.7 ± 0.9	6.8 ± 1.0	0.52
LVESD	5.5 ± 1.0	5.5 ± 1.0	5.5 ± 1.1	0.80
Beta-blocker	104 (72%)	20 (83%)	84 (70%)	0.18
ACEI or ARB	135 (94%)	22 (92%)	113 (94%)	0.64
Aldosterone antagonist	83 (58%)	16 (67%)	67 (56%)	0.33
Amiodarone	5 (3.5%)	2 (8.3%)	3 (2.5%)	0.19
Hemoglobin	13.5 ± 1.8	13.8 ± 1.6	13.4 ± 1.8	0.41
Creatinine	1.1 ± 0.7	1.1 ± 0.4	1.4 ± 1.1	0.20
Sodium	139 ± 3	140 ± 3.6	139 ± 3.6	0.43
Ser49Ser	104 (72.2%)	23 (96%)	81 (67%)	0.005
Arg389Arg	36 (25%)	11 (46%)	25 (21%)	0.01

M/F - male/female; NYHA - New York Heart Association; SAH – systemic arterial hypertension; CAD – coronary artery disease; FA - atrial fibrillation; LA – left atrium; LVEF – left ventricular ejection fraction; LVEDD – left ventricular-end diastolic diameter; LVESD – left ventricular-end systolic diameter; ACEI – angiotensin-converting enzyme inhibitor; ARB – angiotensin-receptor blocker.

Table 3 – Genotypic distribution and allele frequency of polymorphisms Ser49Gly and Arg389Gly

Variable	Ser49Gly			p Value	Allele frequency
	SerSer	SerGly	GlyGly		Ser / Gly
Expected	105.6%	35.88%	3.06%		
Found	105%	36%	3%	> 0.05	0.85 / 0.15
Variable	Arg389Gly			p Value	Allele frequency
	ArgArg	ArgGly	GlyGly		Arg / Gly
Expected	25%	70%	49%		
Found	36%	48%	60%	< 0.001	0.42 / 0.58

adrenergic activity. Some aforementioned studies associated these genotypes with states of adrenergic exacerbation<sup>10-19</sup>, supporting the idea that these genotypes are actually involved in the physiopathology of HF and AF.

The present study showed there is a greater chance of genotype Arg389Arg being related to AF than Arg389Gly and Gly389Gly genotypes. However, the sample lacked Hardy-Weinberg equilibrium, which may have been due to the fact

that the patients already had a disease (HF) that may have generated it. This fact weakens the value of the association found in this study.

The finding involving genotype Ser49Ser, however, was more significant than the first one. The chance of this genotype to be associated with atrial fibrillation was eight times higher than the other two genotypes, even after multivariate analysis, and additionally, the sample was in Hardy-Weinberg equilibrium.

**Table 4 – Multivariate analysis for age, size of LA and genotypes Arg389Arg and Ser49Ser**

Variables	Univariate analysis		Multivariate Analysis	
	OR	95% CI	OR	95% CI
Ser49Ser	11.07	1.44 – 85.02	8.02	1.02 – 63.82
Arg389Arg	3.21	1.29 – 8.04	2.78	1.02 – 7.56

The association of AF with heart failure is associated with adverse prognosis. Recently, a meta-analysis involving more than 50,000 patients showed that the presence of AF in patients with HF is independently associated with increased mortality<sup>27</sup>. The identification of a high risk of developing AF in patients with HF is crucial. The right time to use antithrombotic agents or anticoagulants, and thereby reduce thromboembolic events, and even the use of new antiarrhythmic agents to prevent the exacerbation of HF symptoms caused by the presence of AF are examples of this importance<sup>28</sup>. The acceleration of gene technology has provided greater understanding of the consequences of genetic variations.

The discovery of genetic polymorphisms of adrenergic receptors and their interactions represent a significant part of this development, contributing to greater understanding of the AF-HF association, concerning the diagnosis, prevention and treatment<sup>29</sup>. The genesis of these heart diseases is involved in a complex mechanism that may also be related to genetic factors. Thus, the polymorphisms studied here, especially Ser49Gly, which was associated with AF in patients with systolic HF, may contribute to a better understanding of these diseases.

There are some limitations in our study: first, the studied patients are from a single center, in a specific geographical area; second, the number of patients studied could have been higher; third, the study does not allow establishing cause and effect between variables and four, as the diagnosis of AF was carried

out only by ECG during the admission consultation, it was not possible to identify the paroxysmal attacks.

## Conclusion

Our data add to knowledge about the association between AF in patients with SHF and genotypes of the beta 1-adrenergic receptors. Although the association does not prove causality, there is biological plausibility in the mechanism between the AF and beta 1-adrenergic receptor polymorphisms. If this finding is confirmed in larger studies, these data should contribute to better risk stratification for the development of AF in the context of SHF, and thus, prevent it.

## Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

## Sources of Funding

There were no external funding sources for this study.

## Study Association

This article is part of the thesis of master submitted by Bruno Costa do Nascimento, from Universidade Federal Fluminense.

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