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Impact of β-2 Thr164Ile and combined β-adrenergic receptor polymorphisms on prognosis in a cohort of heart failure outpatients

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Abstract

Genetic polymorphisms of adrenergic receptors (ARs) have been associated with the development, progression, and prognosis of patients with heart failure (HF), with few data for the Brazilian population. We evaluated the role of the β2-AR Thr164Ile polymorphism at codon 164 on prognosis in a prospective study on 315 adult Brazilian HF patients, predominantly middle-aged Caucasian men in functional class I-II, with severe left ventricular systolic dysfunction. Genomic DNA was extracted from peripheral blood and β2-AR164 genotypes were detected by PCR followed by restriction fragment length analysis. During a median follow-up of 3 years, 95 deaths occurred and 57 (60%) were HF-related. Unexpectedly, Ile164 carriers (N = 12) had no HF-related events (log-rank P value = 0.13). Analysis using genotype combination with β1-AR polymorphisms at codons 49 and 389 identified patients with favorable genotypes (Thr164Ile of β2-AR, Gly49Gly of β1-AR and/or Gly389Gly of β1-AR), who had lower HF-related mortality (P = 0.01). In a Cox proportional hazard model adjusted for other clinical characteristics, having any of the favorable genotypes remained as independent predictor of all-cause (hazard ratio (HR): 0.41, 95%CI: 0.17-0.95) and HF-related mortality (HR: 0.12, 95%CI: 0.02-0.90). These data show that the β2-AR Thr164Ile polymorphism had an impact on prognosis in a Brazilian cohort of HF patients. When combined with common β1-AR polymorphisms, a group of patients with a combination of favorable genotypes could be identified.

Key words: Heart failure; Adrenergic receptors; Genetic polymorphisms; Prognosis

Introduction

Heart failure (HF) is an important cause of mortality and hospitalization in Brazil and worldwide and, despite recent advances in the medical treatment of HF, mortality rates and morbidity due to this entity remain elevated (1-4). Identification of prognostic factors is an important aspect of HF management that has been the focus of intense clinical and basic research (5,6). Activation of the adrenergic system underlies the genesis of HF and progression; the adrenergic receptors (ARs) are central for adrenergic system regulation and their function and distribution are dramatically altered in pathologic conditions such as HF (7-10). The clinical importance of this system has been demonstrated by the remarkably beneficial therapeutic effect of β-blockade on HF morbidity and mortality (11-14).

Genetic AR polymorphisms have been associated with the functional modulation of these receptors, as well as with the development, progression, exercise response, and prognosis of HF patients (15-18). A polymorphism of the β2-AR at position 164 (Thr164Ile) has been described, and the 164Ile allele seems to modulate cardiac contractility negatively (19,20). Liggett et al. (21) have demonstrated that the β2-AR Ile164 allele is associated with a poorer prognosis in HF patients. This finding, however, was not supported by other investigators, mostly in North American and European populations (22-25). Very few studies have addressed the impact of AR polymorphisms on HF prognosis in Brazil

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(26,27). We reported the effects of β1-AR polymorphisms at codons 49 (Ser49Gly) and 389 (Arg389Gly) on arrhythmogenesis and prognosis in patients with HF (26). In the present prospective study, we determined 1) whether the functionally relevant β2-AR Thr164Ile polymorphism is associated with HF-related and all-cause mortality in a cohort of Brazilian outpatients, and 2) the ability of a combination of favorable genotypes (β2- and β1-AR polymorphisms) to identify patients with a better prognosis.

Subjects and Methods

Subjects

HF patients were recruited from a tertiary care university hospital in Porto Alegre, Brazil. Consecutive eligible patients who agreed to participate were enrolled at the Heart Failure Clinic between October 2003 and October 2007. The eligibility criteria were age ≥18 years and left ventricular ejection fraction (LVEF) less than 45%, irrespective of functional class or etiology. Patients with HF due to obstructive or hypertrophic cardiomyopathies were excluded from the study, as well as patients with reduced life expectancy. Brazilian Amerindians were not included in this study, but no other ancestry inclusion criterion was defined *a priori*. The racial classification of all participants was self-reported.

The study protocol was approved by the Research and Ethics Committee of the Hospital de Clínicas de Porto Alegre and by the National Agency of Ethics in Research, and all subjects signed written informed consent forms. Demographic, clinical, and routine laboratory data were collected from all patients using a structured data form.

Genotyping

Genomic DNA was extracted from peripheral blood samples using a commercial kit (Puregene; Gentra Systems, USA). The adrenergic receptor genotypes were detected by polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP) analysis, as previously described for the Thr164Ile polymorphism (28). Briefly, PCR were carried out in a total volume of 25 µL containing 0.2 µM of each primer, 1X PCR buffer (20 mM Tris-HCl, pH 8.4, 500 mM KCl), 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, and 1 unit *Taq* DNA polymerase (Invitrogen, USA). Genomic DNA was amplified using the primers 5'-GTGATCGCAGTGGATCGCTACT-3' (forward) and 5'-AGACGAAGACCATGATCACCAG-3' (reverse). PCR cycling conditions consisted of an initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, primer annealing at 58°C for 45 s, extension at 72°C for 1 min, and a final 7-min extension at 72°C.

The amplified products of 280 bp were digested with 5 units of *Mnl*I restriction enzyme (MBI Fermentas, USA) at 37°C, resulting in fragments of 116, 114 and 50 bp in the presence of the Thr allele, while fragments of 230 and 50 bp were yielded in the presence of the Ile allele. The digested fragments were separated by electrophoresis on a 2% agarose gel containing ethidium bromide and visualized under ultraviolet light. To improve genotyping accuracy, samples with known genotypes were used in each batch.

Drug therapy classification

Drug therapy strata were defined based on the last follow-up visit or the visit preceding clinical events. Regarding β-blocker treatment, patients were classified as high-dose users if they were at or above 50% of target doses as defined by HF treatment guidelines (50 mg/day carvedilol, 150 mg/day metoprolol tartrate or 200 mg/day metoprolol succinate) (29). Patients on lower doses or patients who did not receive β-blockers were classified as low-dose/non-users.

Outcome evaluation

Enrolled patients were followed up at the Heart Failure and Transplant outpatient clinic of our institution. Vital status was determined using the last registry assessed in the hospital's electronic database (electronic records since 2000). Telephone contact was attempted for all patients for whom no registry was found in the 4 months prior to follow-up assessment. Vital status was also verified through the State Death Certificate Database. Analyses were stratified by the presumptive cause of death, classified as 1) all-cause mortality, and 2) HF-related mortality, defined as sudden unexpected death (within 1 h of initiation of symptoms) or death caused by advanced refractory disease.

Statistical analysis

Continuous data are reported as means ± SD or median (interquartile ranges) and categorical variables are reported as absolute numbers and percentages. Groups were compared by the chi-square test, the Student *t*-test, analysis of variance, or nonparametric statistics as appropriate. Allele frequencies were determined by gene counting, and departures from Hardy-Weinberg equilibrium were verified using the chi-square test. The chi-square test was also used to evaluate the allele and genotype distributions among groups of subjects. Kaplan-Meier survival curves were constructed from the date of entry at the outpatient clinic to the last registry of follow-up or death, and compared by the log-rank statistics. Cox proportional hazard models were created and adjusted for age, left ventricular function, etiology, and functional class. A two-tailed P value <0.05 was considered to be statistically significant. All statistical analyses were performed using SPSS version 12.0 or SAS version 9.0 for Windows.

Results

Patients' characteristics

The present cohort consisted of 315 HF patients whose complete baseline clinical characteristics are shown in Table 1. The study sample consisted predominantly of middle-

Table 1. Baseline clinical characteristics of 315 heart failure (HF) patients.

Characteristic	All patients (N = 315)	Alive N = 220 (70%)	All-cause death N = 95 (30%)	HF-related death N = 57 (18%)
Age (years)	54 ± 13	53 ± 13	57 ± 12*	55 ± 12**
Males	218 (69)	148 (67)	70 (73)	38 (66)
Self-reported race				
Caucasian	221 (70)	157 (71)	64 (68)	40 (71)
Mixed	45 (14)	31 (14)	14 (15)	6 (10)
Black	49 (16)	32 (15)	17 (17)	11 (19)
HF etiology				
Ischemic	118 (37)	76 (35)	42 (44)	20 (35)
Idiopathic	92 (29)	62 (28)	30 (31)	23 (40)
Hypertensive	75 (24)	53 (24)	22 (23)	12 (21)
Chagas disease	9 (3)	8 (4)	1 (1)	1 (2)
Functional class (SAS)				
I and II	247 (78)	180 (82)	67 (70)*	35 (62)**
III and IV	68 (22)	40 (18)	28 (30)	22 (38)
Comorbidities				
COPD	43 (14)	28 (13)	15 (16)	8 (14)
Stroke	26 (8)	11 (5)	15 (16)*	7 (12)
Liver disease	12 (4)	9 (4)	3 (3)	3 (5)
Diabetes mellitus	95 (30)	59 (27)	36 (37)	17 (29)
Current smoking	38 (12)	23 (10)	15 (16)	8 (14)
Echocardiography				
LV ejection fraction (%)	31 ± 8	33 ± 8	30 ± 8*	29 ± 9**
LV diastolic diameter (mm)	66 ± 9	65 ± 9	69 ± 10*	70 ± 14**
Left atrium diameter (mm)	48 ± 8	47 ± 8	49 ± 8*	50 ± 8**
PASP (mmHg)	49 ± 13	47 ± 13	52 ± 14*	53 ± 15**
Electrocardiogram				
Sinus rhythm	235 (76)	164 (76)	71 (74)	41 (71)**
Atrial fibrillation	57 (18)	42 (19)	15 (16)	9 (16)
Pacemaker rhythm	18 (6)	9 (4)	9 (9)	8 (14)
LBBB	92 (30)	56 (26)	36 (38)*	25 (44)**
QRS duration (ms)	129 ± 36	123 ± 33	141 ± 40*	153 ± 39**
Laboratory variables				
Creatinine (mg/dL)	1.3 ± 0.5	1.2 ± 0.4	1.4 ± 0.5*	1.4 ± 0.6**
Urea (mg/dL)	57 ± 30	53 ± 26	67 ± 36*	68 ± 39**
Sodium (mEq/L)	140 ± 3.4	141 ± 3.4	139 ± 3.4*	139 ± 3.6**
Potassium (mEq/L)	4.5 ± 0.6	4.4 ± 0.5	4.5 ± 0.6	4.5 ± 0.5
Hemoglobin (g/dL)	13.1 ± 1.7	13.3 ± 1.7	12.7 ± 1.8*	12.7 ± 1.7**
Initial drugs				
ß-blockers	277 (88)	198 (90)	79 (82)*	49 (85)
ACEi	277 (88)	192 (87)	85 (88)	51 (88)
Digoxin	247 (79)	171 (78)	76 (80)	48 (83)
Spironolactone	121 (75)	73 (66)	48 (94)*	29 (91)**
Hydralazine	62 (49)	36 (39)	26 (77)*	17 (74)**
Isosorbide	75 (60)	41 (47)	34 (90)*	20 (91)**
Anticoagulation	63 (20)	46 (22)	17 (18)	14 (24)

Data are reported as means ± SD or N (%). SAS = Specific Activity Scale; COPD = chronic obstructive pulmonary disease; LV = left ventricular; PASP = pulmonary artery systolic pressure; LBBB = left bundle branch block; ACEi = angiotensin-converting enzyme inhibitor. *P ≤ 0.05 for the comparison between total dead and living patients (Student *t*-test or chi-square test); **P ≤ 0.05 for the comparison between HF-related death and living patients (Student *t*-test or chi-square test).

aged Caucasian men, in functional class I and II and with a mixed etiology profile. Overall, HF patients had severe LV systolic dysfunction and mild renal failure, and 30% had left bundle branch block. Most patients were using angiotensin-converting enzyme inhibitors and β-blockers.

Table 2. Baseline clinical characteristics as a function of the Thr164Ile genotype.

Characteristics	Thr/Thr (N = 303)	Thr/Ile (N = 12)
Age (years)	55 ± 13	53 ± 14
Males	209 (69)	8 (67)
Caucasian	212 (70)	9 (75)
HF etiology		
Ischemic	116 (38)	2 (17)
Idiopathic	89 (29)	3 (25)
Hypertensive	72 (24)	2 (17)
Functional class (SAS)		
Class I and II	230 (77)	11 (92)
LVEF (%)	31 ± 8	32 ± 9
Electrocardiogram		
Sinus rhythm	217 (76)	8 (67)
Atrial fibrillation	53 (18)	4 (33)
LBBB	91 (31)	1 (8)
QRS duration (ms)	131 ± 36*	105 ± 22
Laboratory variables		
Creatinine (mg/dL)	1.3 ± 0.5	1.2 ± 0.2
Sodium (mEq/L)	140 ± 3	141 ± 5
Hemoglobin (g/dL)	13.1 ± 1.7	12.6 ± 0.9
Initial drugs		
ß-blockers	268 (88)*	8 (67)
ACEi	264 (87)	12 (100)

Data are reported as means ± SD or N (%). SAS = Specific Activity Scale; LVEF = left ventricular ejection fraction; LBBB = left bundle branch block; ACEi = angiotensin-converting enzyme inhibitor. *P ≤ 0.05 compared to Thr164Ile (Student *t*-test or chi-square test).

Clinical outcomes

During follow-up (median: 3 years, interquartile range: 1.4 to 5.1 years), 95 (30%) deaths occurred and 57 (60%) were HF-related. Most clinical characteristics did not differ between patients who remained alive and those who died (Table 1). However, higher Specific Activity Scale functional class (P = 0.02 and P = 0.001), lower LVEF (P = 0.05 and P = 0.01), greater LV diastolic diameter (P = 0.002 and P = 0.005) and QRS duration (both P < 0.001), hyponatremia (P = 0.002 and P = 0.003), renal dysfunction (P = 0.001 and P = 0.01), and anemia (both P = 0.02) were more common in HF patients with worst clinical prognosis (P values for total mortality and HF-related deaths, respectively). Also, as demonstrated in Table 1, patients with HF-related deaths were more often receiving drugs such as spironolactone, hydralazine and isosorbide (all P values <0.01).

β2-AR genotype, clinical characteristics and outcomes

The genotype frequencies for Thr164Ile polymorphism were in agreement with those predicted by the Hardy-Weinberg equilibrium. The frequency of the Ile164 allele was 0.02. Analysis of genotypes and clinical characteristics are presented in Table 2. Overall, there were no major significant differences in baseline characteristics among different AR genotypes, except for QRS duration and β-blockers use. As reported in other studies (19,21), we did not identify Ile164 homozygosis in our sample.

Figure 1 presents the Kaplan-Meier survival curves as a function of the Thr164Ile genotype for all-cause mortality and HF-related mortality. Unexpectedly, Ile164 carriers (N = 12) had no HF-related events in our cohort (log-rank P value = 0.13).

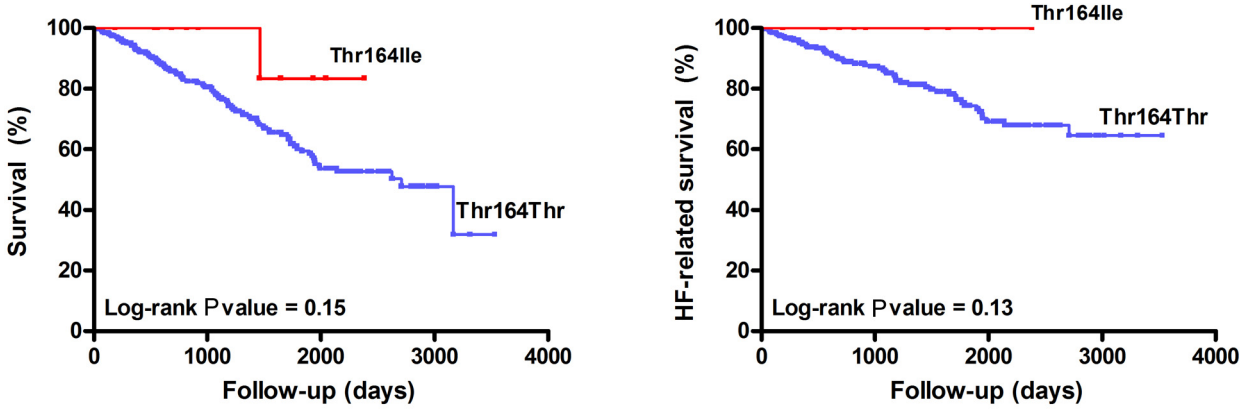

Figure 1. Kaplan-Meier survival curves as a function of the Thr164Ile genotype for all-cause mortality (N = 315, left) and heart failure (HF)-related mortality (N = 315, right).

Combined β1- and β2-AR genotypes and multivariable analysis

Prognostic analyses of β1-AR polymorphisms have been previously published by our laboratory (26). In the present study, we analyzed the effect of combined "favorable" AR genotypes on HF-related and all-cause mortality, by grouping all patients with any of the polymorphisms associated with better prognosis (β2-AR Thr164Ile and/or β1-AR Gly49Gly and/or β1-AR Gly389Gly). We then compared this group of patients to those patients without any of the favorable genotypes. Figure 2 shows the effect of favorable genotypes for all-cause mortality (P = 0.06) and HF-related mortality (P = 0.01). Interestingly, when analyzing only β1-AR polymorphisms in our previous study (26), we detected a significant effect on HF-related mortality but not on all-cause mortality. However, by combining β1-AR and β2-AR polymorphisms, we observed a non-significant statistical trend (P = 0.06, Figure 2) for all-cause mortality.

In a Cox proportional hazard model adjusted for other clinical characteristics (Table 3), favorable genotypes remained as independent predictors of all-cause and HF-related deaths (for all cause mortality, hazard ratio (HR): 0.41, 95%CI: 0.17-0.95, for HF-related mortality, HR: 0.12, 95%CI: 0.02-0.90).

Discussion

We evaluated the role of β2-AR Thr164Ile polymorphism in a cohort of 315 Brazilian HF patients with systolic dysfunction who regularly attend a tertiary care university hospital outpatient clinic. In our cohort, HF patients carrying the β2-164Ile allele had no HF-related events. Furthermore, we showed that a combination of favorable genotypes (Thr164Ile of β2-AR, Gly49Gly of β1-AR and/or Gly389Gly of β1-AR) was an independent predictor of better prognosis (lower HF-related and all-cause mortality).

There is no consensus about the effects of β2-AR Thr164Ile polymorphism on HF prognosis. Liggett et al.

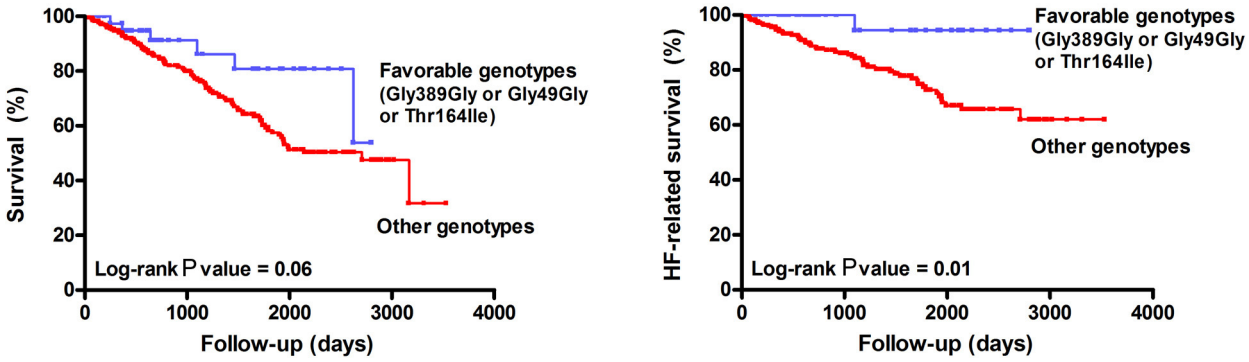

Figure 2. Kaplan-Meier survival curves for all-cause mortality (N = 315, left) and heart failure (HF)-related mortality (N = 315, right) according to the combination of "favorable" adrenergic receptor genotypes. Favorable genotypes include Thr164Ile and/or Gly49Gly and/or Gly389Gly patients.

Table 3. Cox proportional hazard model for all-cause and heart failure (HF)-related mortality.

	All-cause mortality			HF-related mortality		
	Hazard ratio	95%CI	P*	Hazard ratio	95%CI	P*
LVEF (increments of 1%)	0.99	0.96-1.01	0.26	0.98	0.95-1.02	0.28
Sodium (increments of 1 mEq/L)	0.89	0.83-0.94	<0.001	0.86	0.80-0.93	<0.001
SAS functional class (increments of 1 class)	1.23	0.99-1.52	0.06	1.42	1.10-1.94	0.008
QRS duration (increments of 1 ms)	1.007	1.001-1.012	0.02	1.01	1.005-1.02	0.001
Spironolactone use	2.24	1.48-3.39	<0.001	2.22	1.29-3.81	0.004
High-dose β-blockade	0.64	0.42-0.95	0.04	0.69	0.40-1.18	0.17
Favorable genotypes	0.41	0.17-0.95	0.04	0.12	0.02-0.90	0.04

CI = confidence interval; LVEF = left ventricular ejection fraction; SAS = Specific Activity Scale. *P value for association with all-cause or HF-related mortality in the multivariate model.

(21) found that HF patients carrying the Ile164 allele had a poorer prognosis, with rapid progression to death or heart transplant compared to Thr164 allele homozygotes. Other studies have found no impact of the Ile164 allele on HF prognosis (24,25), although multivariable analysis suggested that β-blocker treatment may negatively impact survival in the heterozygote group (24). Our data suggest that the Ile164 allele may positively impact prognosis. Several considerations may help to explain the contradictory findings from different studies evaluating AR polymorphisms in HF. Ethnic heterogeneity might be one of these factors, suggesting that genetically based information cannot be totally applicable to patients of different geographic and/or genetic origin. Moreover, specific gene-gene or gene-environmental interactions might result in different consequences of altered receptor function. It is also reasonable to suggest that the analysis of different clinical outcomes, population-specific interactions, and the inherent difficulty in defining the onset of HF in an individual patient may be responsible, in part, for the absence of consensus regarding the role of this polymorphism in HF prognosis. Therefore, studies on different populations should be conducted and published in an attempt to build a more consistent data set that could answer this question. Our study contributes to this goal with information on a Brazilian population living in the South of Brazil.

Another issue that has been overlooked is the specific combination of polymorphisms (haplotypes) in different populations. Small et al. (30) have elegantly demonstrated the impact of haplotypes on the expression of cardiac adrenergic receptors in a model of whole-gene transfections. Firstly, they identified at least 15 polymorphisms with allele frequencies of 0.05 or more in the β1-AR. These polymorphisms were organized into six common haplotypes, leading to as much as 2-fold differences in receptor expression. These investigators concluded that the β1-AR is highly polymorphic, with different clusters of variation, providing the molecular basis to explain why individual polymorphisms may not have the same clinical meaning in distinct populations (30).

We have observed better HF-related prognosis in patients homozygous for the Gly389 allele of the β1-AR, as well as a clear pharmacogenetic interaction between Arg389 allele carriers and β-blocker use and doses (26). In the present study, we further determined the effect of combined "favorable" AR genotypes (patients carrying β2-AR Thr164Ile, β1-AR Gly49Gly and/or β1-AR Gly389Gly genotypes) on HF-related and all-cause mortality. The best outcome was observed in HF patients with "favorable" genotypes and in those with high-dose β-blockade. The "favorable" genotypes remained as independent predictors of HF-related and all-cause mortality after adjustment for other clinical characteristics. In the current analysis, higher Specific Activity Scale functional class, lower LVEF, greater LV diastolic diameter and QRS duration, hyponatremia, renal dysfunction, and anemia were more common in HF patients with a worse clinical prognosis. This is consistent with that demonstrated in other studies and would be expected from predictive models such as the Seattle HF score (5,6). Our findings also agree with previous studies, which observed a beneficial effect of β-blockers on HF (12-14). Regarding the effects of "favorable" genotypes, it seems reasonable to propose that aggregated information from several polymorphisms may permit us to understand the net impact of genetic variations in the adrenergic system on HF.

Some aspects of our study design deserve special comment. Our study population was heterogeneous compared with other studies that selected their patients on the basis of etiology and LVEF. Because the purpose of our study was to identify the genetic polymorphisms that can affect the prognosis of HF patients receiving contemporary pharmacotherapy, the exclusion of one etiology might have prevented us from achieving this goal. In the current study, we did not perform a comprehensive evaluation of β1-AR and β2-AR haplotypes. Such analysis could have optimized prognostic power of genetic variables in this sample of Brazilian HF patients. Our findings should be interpreted with caution given that our analysis was based only on 12 heterozygous patients. However, this limitation is inherent to any analysis of a rare polymorphism.

In summary, the β2-AR Thr164Ile polymorphism had a significant impact on prognosis in a Brazilian cohort of HF patients. When β2-AR164 polymorphism was aggregated to common β1-AR polymorphisms, a group of patients with a combination of favorable genotypes and prognosis was identified. These findings add to those reported in other populations (18,21,24,25), mostly from Europe and North America, although contrasting with some previous data. Finally, in order to identify the complex factors that affect the progression of HF, one must take into account genetic factors and expect future prospective studies to analyze the impact of multiple genes and pathways on the HF syndrome.

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