Non-synonymous polymorphisms in the *HRC* and *ADRB1* genes may be associated with all-cause death in patients with non-ischemic heart failure

TANISE MACHADO TELLES[1], BRUNA MIERS MAY[2], MAURICIO PIMENTEL[2],
BRUNA LETÍCIA DA SILVA PEREIRA[3], MICHAEL ANDRADES[4],
LUIS EDUARDO ROHDE[2,5] and KÁTIA GONÇALVES DOS SANTOS[1]

[1]Laboratory of Human Molecular Genetics, Lutheran University of Brazil, Canoas, Rio Grande do Sul 92425-900;
[2]Cardiology Division; [3]Cells, Tissues and Genes Laboratory; [4]Cardiovascular Research Laboratory,
Clinical Hospital of Porto Alegre, Porto Alegre, Rio Grande do Sul 90035-903; [5]Department of Internal Medicine,
Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul 90035-003, Brazil

Abstract. Sudden cardiac death (SCD) is an unpredictable and common mode of death in patients with heart failure (HF). Alterations in calcium handling may lead to malignant arrhythmias, resulting in SCD, and variants in calcium signaling-related genes have a significant association with SCD. Therefore, the aim of the present retrospective cohort study was to investigate the association of Ser96Ala [histidine-rich calcium-binding protein (*HRC*)], Ser49Gly [β1-adrenergic receptor (*ADRB1*)], Arg389Gly (*ADRB1*) and Gly1886Ser [ryanodine receptor 2 (*RYR2*)] polymorphisms with serious arrhythmic events and overall mortality in patients with HF with reduced left ventricular ejection fraction of non-ischemic etiology. In total, 136 patients with HF underwent physical examination, routine laboratory tests, non-invasive assessment of cardiac function and an invasive electrophysiological study. The primary outcome was the occurrence of serious arrhythmic events, set as either SCD or appropriate implantable cardioverter-defibrillator (ICD) therapy, and the secondary outcome was all-cause death. During a median follow-up of 37 months, arrhythmic events occurred in 26 patients (19%) and 41 patients (30%) died. Patients carrying the Ser allele of the Ser96Ala polymorphism in *HRC* had worse survival than those with the Ala/Ala genotype (log-rank P=0.043). Despite the difference in survival time, the Ala/Ala genotype was not associated with all-cause death in the regression analysis [unadjusted hazard ratio (HR)=0.17; 95% CI, 0.02-1.21]. Regarding the Ser49Gly and Arg389Gly polymorphisms in *ADRB1*, homozygosity for the major alleles at both sites (Ser49Ser and Arg389Arg) was associated with a two-fold increased risk of all-cause death compared with the other genotype combinations (unadjusted HR=1.98; 95% CI, 1.02-3.82). However, this association was lost after controlling for clinical covariates. No association was observed for the Gly1886Ser polymorphism in *RYR2*. Overall, the present findings are concurrent with the hypothesis that the Ser96Ala (*HRC*), Ser49Gly (*ADRB1*) and Arg389Gly (*ADRB1*) polymorphisms may be associated with HF prognosis. In particular, the Ser96Ala polymorphism might aid in risk stratification and patient selection for ICD implantation.

Correspondence to: Professor Kátia Gonçalves dos Santos, Laboratory of Human Molecular Genetics, Lutheran University of Brazil, 8001 Farroupilha Avenue, Building 22, 5th Floor, Canoas, Rio Grande do Sul 92425-900, Brazil
E-mail: katia.santos@ulbra.br

Introduction

Heart failure (HF) is a clinical syndrome resulting from structural and functional abnormalities of the heart, which result in elevated intracardiac pressures and inadequate cardiac output at rest and/or during exercise (1). Chronic HF is classified according to the left ventricular ejection fraction (LVEF) into HF with reduced EF (LVEF ≥40%), HF with mildly reduced EF (LVEF 41-49%) and HF with preserved EF (LVEF ≥50%) (2). HF is a major public health problem due to its high mortality rate, recurrent hospitalizations and its association with a low quality of life (1,3). In recent years, several randomized controlled trials have provided new evidence that can change the management of patients with HF with mildly reduced or preserved EF, with the introduction of new medical therapies, such as the sodium-glucose co-transporter 2

inhibitor (dapagliflozin or empagliflozin), thus reducing the risk of HF hospitalizations and cardiovascular death (2). Meanwhile, up to 40% of deaths among patients with HF are sudden and unexpected, resulting from an interplay between a vulnerable substrate (created by conditions such as aging and myocardial scars resulting from acute myocardial infarction) and multiple transient factors that trigger a fatal event (4). In this regard, implantable cardioverter-defibrillators (ICDs) are effective at correcting potentially lethal ventricular arrhythmias (VAs) and are recommended for the primary prevention of sudden cardiac death (SCD) and all-cause mortality in patients with symptomatic HF of ischemic etiology and LVEF ≤35% (1). However, the overall survival benefit of ICD therapy in patients with HF of non-ischemic etiology is unclear, as it is restricted to some subgroups of patients or even not recommended in others (5-8). Therefore, the risk stratification of SCD in patients with non-ischemic HF remains challenging. In this scenario, genetic variants have emerged as potential biomarkers of arrhythmia and SCD risk (9,10), especially those located in genes that code for calcium-signaling proteins responsible for excitation-contraction (9,11-13).

Histidine-rich calcium-binding protein (HRC) is a luminal sarcoplasmic reticulum (SR) protein that regulates calcium handling by mediating its uptake, storage and release (14). HRC protein levels are downregulated in human and experimental HF, and either the overexpression or ablation of HRC results in aberrant calcium cycling, leading to the development of HF upon aging or after pressure overload-induced stress (14). The gene encoding human HRC, located on chromosome 19q13.3 (15), is expressed in striated muscles and arteriolar smooth muscle cells (14). The Ser96Ala (286A>C; rs3745297) common variant in exon 1 of the *HRC* gene is functionally linked to protein activity in normal and failing hearts (16-19). The Ala allele was first identified as causing malignant VAs in patients with idiopathic dilated cardiomyopathy (20), which has not been fully confirmed in patients with predominantly ischemic cardiomyopathy (21,22).

β1-adrenergic receptor (β1-AR/*ADRB1*) is a G-protein-coupled receptor predominantly found in the myocardium, where it regulates cardiomyocyte contraction and relaxation, heart rate and atrioventricular conduction (23). In HF, sustained sympathetic adrenergic activation leads to desensitization and downregulation of β1-AR, ryanodine receptor 2 (RyR2) dysfunction and calcium leak, with these last two abnormalities exacerbating cardiac dysfunction, ventricular arrhythmogenesis and SCD (23). The Ser49Gly (145A>G; rs1801252) and Arg389Gly (1165C>G; rs1801253) variants in the *ADRB1* intronless gene, located on chromosome 10, exert functional effects on agonist-promoted downregulation and response to β-blockers (24). The Gly49 allele is associated with greater receptor desensitization and downregulation than the Ser49 allele (25,26), whereas the Gly389 allele is associated with decreased G-protein coupling of β1-AR and decreased cardiac contractility compared with the Arg389 allele (25-27). Both polymorphisms have been reported to be associated with HF prognosis in some studies but not in others (24,26).

RyR2 is a calcium-release channel protein located in the SR membrane that is essential for excitation-contraction coupling in cardiac muscle (28). Post-translational modifications in RyR2 increase its activity (and hence calcium leak)

in failing hearts in humans and experimental HF (29). These alterations in RyR2 can induce VAs and may also contribute to supraventricular defects, such as sinus node dysfunction and atrial fibrillation (29). Human RyR2 is encoded by a large gene on chromosome 1q43 (28), and the Gly1886Ser (5656G>A; rs3766871) polymorphism in exon 37 is located in the functional domain of the tetrameric channel (30), which is involved in excitation-contraction coupling and the binding of physiological ligands (31). The Ser allele has been reported to be associated with VAs (32) and SCD (33) in patients with HF.

The aim of the present study was to investigate whether Ser96Ala (*HRC*), Ser49Gly (*ADRB1*), Arg389Gly (*ADRB1*) and Gly1886Ser (*RYR2*) polymorphisms were associated with serious arrhythmic events and all-cause death in a cohort of patients with non-ischemic HF with reduced LVEF in Southern Brazil.

Materials and methods

Study design and population. A retrospective cohort study of 136 adults with HF and reduced LVEF (≤40%) of non-ischemic etiology was performed. Patients were enrolled between March 2011 and November 2016 at the Heart Failure and Cardiac Transplant Clinic, Clinical Hospital of Porto Alegre (HCPA) (Porto Alegre, Brazil), a tertiary care university hospital. The ejection fraction was assessed by two-dimensional transthoracic echocardiography, and non-ischemic cardiomyopathy was defined as the absence of atherosclerotic coronary lesions >75% on coronary arteriography, or absence of necrotic or ischemic areas on single-photon emission computed tomography or cardiac magnetic resonance. Patients were not included in the study if they had a history of SCD with resuscitation, cardiogenic syncope, sustained ventricular tachycardia (VT), advanced cerebrovascular disease or a life expectancy of <1 year due to non-cardiovascular diseases.

At baseline, the patients underwent a comprehensive clinical and laboratory evaluation consisting of physical examination, routine laboratory tests, non-invasive assessment of cardiac function (two-dimensional echocardiography, 12-lead resting electrocardiogram, 24-h Holter monitoring and cardiopulmonary exercise test) and an invasive electrophysiological study (EPS), as previously described (34,35). The present study was approved by the Research Ethics Committee of HCPA (Institutional Review Board no. 0000921) under CAAE no. 93278918.9.0000.5327 and consolidated review no. 2.824.527. All subjects provided written informed consent before data collection, blood collection and clinical evaluation.

Follow-up and outcomes. Patients were followed up on regular outpatient visits at 3 and 6 months, and every 6 months thereafter. Patients who failed to return were contacted by telephone, received home visits or were followed up indirectly through their relatives. The primary outcome of the present study was the occurrence of serious arrhythmic events, defined as SCD or appropriate ICD therapy, whereas the secondary outcome was all-cause death. ICD shocks were considered appropriate if they were caused by VT or ventricular fibrillation (VF). The outcomes were adjudicated independently by two investigators who were blinded to the baseline assessment and genotype data. The adjudication of outcomes was based on clinical history,

statements from family members, review of ICD or pacemaker electrograms, hospital charts and death certificates. The outcome data were updated in November 2020.

DNA isolation and genotyping. At the beginning of the EPS, a 20-ml sample of peripheral venous blood was collected from each subject in EDTA-containing tubes for the analysis of biomarkers potentially associated with HF outcomes. Samples were then centrifuged at 1,258 x g for 15 min at 4˚C at The Cardiovascular Research Laboratory of HCPA. Plasma was aspirated with a micropipette, aliquoted and stored in microtubes at -70˚C, while the blood cells were kept at -20˚C until DNA isolation. Genomic DNA was isolated using a salting out procedure (36), quantified by spectrophotometry (NanoDrop 1000; NanoDrop; Thermo Fisher Scientific, Inc.), diluted to a concentration of 10 ng/μl and stored at -20˚C until genotyping.

Genotyping of *HRC*, *ADRB1* and *RYR2* polymorphisms was performed by quantitative polymerase chain reaction (qPCR) using pre-designed commercial assays containing specific primers and hydrolysis probes (TaqMan® Genotyping Assay, ID numbers C__11506744_1_, C__8898508_10, C__8898494_10 and C__27513673_20 for the Ser96Ala, Ser49Gly, Arg389Gly and Gly1886Ser polymorphisms, respectively; cat. no. 4351379; Thermo Fisher Scientific, Inc.). Amplification reactions were carried out in a final volume of 10 μl containing 20 ng genomic DNA, 1X TaqMan Genotyping Master Mix (cat. no. 4371355; Thermo Fisher Scientific, Inc.) and 1X TaqMan SNP Genotyping Assay (cat. no. 4351379; Thermo Fisher Scientific, Inc.). Reaction plates were loaded into a qPCR thermal cycler (StepOnePlus Real-Time PCR System; Thermo Fisher Scientific, Inc.) and heated for 10 min at 95˚C, followed by 40 cycles of denaturation at 95˚C for 15 sec and annealing/extension at 60˚C for 1 min. The fluorescence data and amplification plots were analyzed using automated allele-calling software (SDS 2.1; Thermo Fisher Scientific, Inc.).

To improve genotyping accuracy, a DNA sample of each genotype (also collected from the present study patients) was used in all PCR runs as a positive control. The investigators who performed the genotyping were blinded to the clinical and laboratory data, and the amplification plots were visually checked by two investigators. The genotyping success rate ranged from 89% (Arg389Gly) to 97% (Ser49Gly and Gly1886Ser), and 116 (85%) samples were successfully genotyped for all four polymorphisms. Approximately half of the samples were re-genotyped for each polymorphism and the concordance rate was 100%. Genotyping data generated during the present study and a minimal clinical dataset are available in a public repository (https://doi.org/10.6084/m9.figshare.22929857).

Statistical analysis. Data are presented as the mean ± standard deviation, median (25-75th percentiles), absolute frequency (%) or relative frequency. After checking for normal distribution using the Shapiro-Wilk test, continuous data were compared between groups using the unpaired Student's t-test or Mann-Whitney U test. Categorical data, including genotype and allele frequencies, were compared between groups using the Pearson χ^2 test, likelihood-ratio χ^2 test (used for contingency tables larger than 2x2 with >20% of the cells having expected counts <5) or Fisher's exact tests, as appropriate.

Analysis of adjusted residuals was performed where appropriate to examine how different cells contributed to the χ^2 values. Allele frequencies were determined by gene counting, and departures from the Hardy-Weinberg equilibrium were evaluated using the goodness-of-fit χ^2 test (37).

The association of the four polymorphisms in the *HRC*, *ADRB1* and *RYR2* genes with serious arrhythmic events and all-cause death was also evaluated using Kaplan-Meier survival analysis. Survival curves obtained for different genotypes were compared using the log-rank test, and hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated using Cox regression with the backward stepwise (Wald) procedure in the multivariable analysis. Statistical analyses were performed using SPSS (version 18; SPSS, Inc.) and WinPEPI (version 11.50) (38) statistical software. Two-tailed P<0.05 was considered to indicate a statistically significant difference.

Results

Baseline characteristics of the study population. The majority of the 136 subjects included in the present study were middle-aged or elderly (≥40 years old; 84%), male (58%) and white (75%). Furthermore, the majority were in New York Heart Association functional classes (3) I and II (81%), had a conduction disorder (65%) and had severe ventricular dysfunction caused by idiopathic, hypertensive or alcoholic cardiomyopathy (75%). Regarding the pharmacological treatment of HF, most patients were using β-blockers, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, spironolactone and/or digoxin (Table I).

Follow-up and outcomes. During a median follow-up of 37 months (25-75th percentiles, 25-68 months), 11 patients (8.1%) died of cardiovascular causes, 8 patients (5.9%) underwent heart transplantation and 3 patients (2.2%) had cardiac syncope. Furthermore, 1 patient died during transplant surgery. The primary outcome occurred in 26 patients (19.1%; 17 had SCD and 9 had appropriate ICD therapy), whereas the secondary outcome (all-cause death) occurred in 41 patients (30.1%). A total of 86 patients (63.2%) experienced neither primary nor secondary outcomes.

Table I summarizes the baseline clinical and demographic characteristics of the population of the present study stratified by primary and secondary outcomes. Patients with serious arrhythmic events had lower levels of lymphocytes, higher levels of uric acid, larger left ventricular diameters, longer QRS duration and HV interval, and a higher frequency of conduction disorder, non-sustained VT and periodic breathing during exercise than those without the primary outcome. Patients who died from any cause were older, had lower body mass index, lower levels of lymphocytes, higher levels of uric acid and creatinine, lower LVEF, larger left ventricular diameters, longer QRS duration and HV interval, lower peak oxygen uptake (VO$_2$), higher VE/VCO$_2$ slope, and had a higher frequency of ventricular pacing, conduction disorder, and periodic breathing during exercise compared with those who were alive. Tables SI and SII provide a more detailed description of the baseline profile of the present study population stratified by the primary and secondary clinical outcomes, respectively.

Table I. Clinical characteristics of the study population stratified by the primary and secondary clinical outcomes.

Characteristic	All patients (n=136)	Serious arrhythmic events			All-cause death		
		Without (n=110)	With (n=26)	P-value	Alive (n=95)	Deceased (n=41)	P-value
Age, years	55.0±13.0	54.9±12.7	55.6±14.3	0.794	53.2±12.8	59.2±12.7	0.005
Male	79 (58.1)	64 (58.2)	15 (57.7)	>0.999	50 (52.6)	29 (70.7)	0.076
White[a]	98 (74.8)	79 (75.2)	19 (73.1)	>0.999	68 (74.7)	30 (75.0)	>0.999
New York Heart Association class							
I	57 (41.9)	44 (40.0)	13 (50.0)	0.640	38 (40.0)	19 (46.4)	0.730
II	53 (39.0)	44 (40.0)	9 (34.6)		39 (41.1)	14 (34.1)	
III	26 (19.1)	22 (20.0)	4 (15.4)		18 (18.9)	8 (19.5)	
Heart failure etiology							
Idiopathic	60 (44.1)	50 (45.5)	10 (38.5)	0.353	48 (50.5)	12 (29.3)	0.108
Hypertensive	25 (18.4)	22 (20.0)	3 (11.5)		16 (16.8)	9 (21.9)	
Alcoholic	17 (12.5)	14 (12.7)	3 (11.5)		9 (9.5)	8 (19.5)	
Others	34 (25.0)	24 (21.8)	10 (38.5)		22 (23.2)	12 (29.3)	
Physical examination							
Systolic blood pressure, mmHg	119.4±21.9	119.8±21.7	117.9±23.0	0.701	120.0±21.5	118.0±22.9	0.557
Diastolic blood pressure, mmHg	74.5±12.8	74.6±12.5	74.0±14.3	0.511	75.4±13.0	72.4±12.3	0.143
Body mass index, kg/m^{2b}	28.2±6.0	28.4±6.0	27.6±6.1	0.465	29.0±6.2	26.4±5.1	0.019
Routine laboratory tests							
Hemoglobin, g/dl	13.3±1.6	13.4±1.6	13.2±1.7	0.565	13.4±1.5	13.2±1.9	0.468
Lymphocytes, mm^3	1,975 [1,542-2,465]	2,015 [1,652-2,520]	1,530 [905-2,335]	0.008	2,010 [1,660-2,520]	1,780 [1,190-2,330]	0.043
Creatinine, mg/dl	0.98 [0.79-1.34]	0.96 [0.75-1.17]	1.05 [0.88-1.53]	0.070	0.93 [0.72-1.15]	1.09 [0.93-1.54]	0.001
Sodium, mEq/l[c]	140.1±2.7	140.0±2.9	140.4±2.1	0.544	140.4±2.7	139.4±2.6	0.054
Potassium, mEq/l	4.6±0.4	4.6±0.4	4.5±0.4	0.521	4.6±0.4	4.6±0.4	0.288
Uric acid, mg/dl[d]	7.2 [5.9-9.0]	6.9 [5.7-8.7]	8.7 [7.1-9.9]	0.004	7.0 [5.7-8.7]	8.0 [6.6-9.8]	0.028
LDL cholesterol, mg/dl[d]	106±36	106±38	105±28	0.830	107±36	104±37	0.650
Echocardiography							
LV ejection fraction, %[e]	27.5±8.0	28.0±8.1	25.4±7.2	0.131	28.6±8.1	25.1±7.3	0.023
LV end-diastolic diameter, mm[f]	67.9±8.8	66.6±8.1	73.1±9.7	0.001	66.6±8.1	71.0±9.6	0.007
LV end-systolic diameter, mm[e]	59.1±9.1	58.1±8.5	63.6±10.3	0.010	57.7±8.2	62.5±10.4	0.006
Left atrium diameter, mm[e]	47.4±6.6	47.3±6.8	47.9±5.9	0.641	46.2±6.1	50.1±7.0	0.001
Electrocardiography							
Rhythm							
Normal sinus rhythm	108 (79.5)	89 (81.0)	19 (73.1)	0.666	79 (83.1)	29 (70.8)	0.007
Atrial fibrillation	21 (15.4)	16 (14.5)	5 (19.2)		15 (15.8)	6 (14.6)	
Ventricular pacing	7 (5.1)	5 (4.5)	2 (7.7)		1 (1.1)	6 (14.6)	

Table I. Continued.

Characteristic	All patients (n=136)	Serious arrhythmic events			All-cause death		
		Without (n=110)	With (n=26)	P-value	Alive (n=95)	Deceased (n=41)	P-value
QRS duration, msec	120 [102-157]	118 [100-153]	143 [112-174]	0.025	114 [100-150]	140 [115-173]	0.001
Conduction disorder	88 (64.7)	64 (58.2)	24 (92.3)	0.002	50 (52.6)	38 (92.7)	<0.001
24-h Holter monitoring							
Non-sustained VT[e]	47 (34.8)	32 (29.4)	15 (57.7)	0.013	32 (34.0)	15 (36.6)	0.929
Cardiopulmonary exercise testing							
VO$_2$ peak, ml/kg/min	17.9±4.9	18.1±4.8	17.0±5.3	0.287	18.6±5.0	16.2±4.5	0.009
VE/VCO$_2$ slope[g]	41.3±12.0	40.2±10.8	46.6±15.8	0.107	38.9±9.9	47.2±14.7	0.005
Periodic breathing during exercise, %	24 (17.6)	15 (13.6)	9 (34.6)	0.020	12 (12.6)	12 (29.3)	0.037
Electrophysiological study							
Induced VT or VF	16 (11.8)	12 (10.9)	4 (15.4)	0.508	11 (11.6)	5 (12.2)	>0.999
HV interval, msec[h]	52.6±10.5	51.4±9.7	58.0±12.3	0.020	51.5±10.3	55.4±10.7	0.042
Heart failure medication							
β-blocker	132 (97.1)	106 (96.4)	26 (100.0)	>0.999	92 (96.8)	40 (97.6)	>0.999
ACE inhibitor or ARB	132 (97.1)	108 (98.2)	24 (92.3)	0.165	92 (96.8)	40 (97.6)	>0.999
Spironolactone	93 (68.4)	76 (69.1)	17 (65.4)	0.896	68 (71.6)	25 (61.0)	0.308
Digoxin	111 (81.6)	88 (80.0)	23 (88.5)	0.407	75 (78.9)	36 (87.8)	0.326
Amiodarone	8 (5.9)	7 (6.4)	1 (3.8)	>0.999	7 (7.4)	1 (2.4)	0.435

Data are presented as the mean ± standard deviation, median [25-75th percentiles] or absolute number (%). [a]Missing data for 5 patients without serious arrhythmic events (4 who were alive and 1 who died). [b]Missing data for 1 patient with serious arrhythmic events who died. [c]Missing data for 1 patient without serious arrhythmic events who died. [d]Missing data for 5 patients without serious arrhythmic events who died, 1 patient with serious arrhythmic events who died and 1 patient with serious arrhythmic events who were alive. [e]Missing data for 2 patients without serious arrhythmic events who were alive. [f]Missing data for 1 patient without serious arrhythmic events; 7 were alive and 6 have died). [g]Missing data for 13 patients (8 without serious arrhythmic events, 5 with serious arrhythmic events; 7 were alive and 6 have died). [h]Missing data for 7 patients (5 without serious arrhythmic events who were alive; 2 were alive and 5 have died). LDL, low-density lipoprotein; LV, left ventricular; VT, ventricular tachycardia; VO$_2$, oxygen consumption; VE/VCO$_2$, arrhythmic events, 2 with serious arrhythmic events; 2 were alive and 5 have died). LDL, low-density lipoprotein; LV, left ventricular; VT, ventricular tachycardia; VO$_2$, oxygen consumption; VE/VCO$_2$, ventilatory equivalent for carbon dioxide; VF, ventricular fibrillation; ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker.

Table II. Genotype and allele frequencies of *HRC*, *ADRB1* and *RYR2* polymorphisms stratified by the primary and secondary clinical outcomes.

Polymorphism	All patients	Serious arrhythmic events			All-cause death		
		Without	With	P-value	Alive	Deceased	P-value
Ser96Ala, *HRC*	128	106	22		90	38	
Genotype							
Ser/Ser, AA	58 (45.3)	51 (48.1)	7 (31.8)	0.364	40 (44.4)	18 (47.4)	0.060
Ser/Ala, AC	53 (41.4)	42 (39.6)	11 (50.0)		34 (37.8)	19 (50.0)	
Ala/Ala, CC	17 (13.3)	13 (12.3)	4 (18.2)		16 (17.8)[a]	1 (2.6)[a]	
Allele							
Ser, A	0.660	0.679	0.568	0.215	0.633	0.724	0.211
Ala, C	0.340	0.321	0.432		0.367	0.276	
Ser49Gly, *ADRB1*	132	106	26		91	41	
Genotype							
Ser/Ser, AA	89 (67.4)	69 (65.1)	20 (76.9)	0.312	58 (63.7)	31 (75.6)	0.172
Ser/Gly, AG	40 (30.3)	34 (32.1)	6 (23.1)		30 (33.0)	10 (24.4)	
Gly/Gly, GG	3 (2.3)	3 (2.8)	0 (0.0)		3 (3.3)	0 (0.0)	
Allele							
Ser, A	0.826	0.811	0.885	0.296	0.802	0.878	0.184
Gly, G	0.174	0.189	0.115		0.198	0.122	
Arg389Gly, *ADRB1*	121	99	22		84	37	
Genotype							
Arg/Arg, CC	64 (52.9)	53 (53.5)	11 (50.0)	0.471	42 (50.0)	22 (59.5)	0.557
Arg/Gly, CG	48 (39.7)	40 (40.4)	8 (36.4)		36 (42.9)	12 (32.4)	
Gly/Gly, GG	9 (7.4)	6 (6.1)	3 (13.6)		6 (7.1)	3 (8.1)	
Allele							
Arg, C	0.727	0.737	0.682	0.575	0.714	0.757	0.598
Gly, G	0.273	0.263	0.318		0.286	0.243	
Gly1886Ser, *RYR2*	132	106	26		91	41	
Genotype							
Gly/Gly, GG	119 (90.1)	94 (88.7)	25 (96.2)	0.415	81 (89.0)	38 (92.7)	0.606
Gly/Ser, GA	12 (9.1)	11 (10.4)	1 (3.8)		9 (9.9)	3 (7.3)	
Ser/Ser, AA	1 (0.8)	1 (0.9)	0 (0.0)		1 (1.1)	0 (0.0)	
Allele							
Ser, G	0.947	0.939	0.981	0.385	0.940	0.963	0.615
Gly, A	0.053	0.061	0.019		0.060	0.037	

Data are expressed as absolute number (%) or relative frequency. [a]Frequencies that deviate significantly from expected in the analysis of adjusted residuals. *ADRB1*, β1-adrenergic receptor; *HRC*, histidine-rich calcium-binding protein; *RYR2*, ryanodine receptor 2.

Association between polymorphisms and outcomes. The frequency distributions of *HRC*, *ADRB1* and *RYR2* polymorphisms are shown in Tables II and SIII. The genotype frequencies of the four studied polymorphisms were in accordance with the Hardy-Weinberg equation in the overall population and in the subgroups stratified by primary and secondary clinical outcomes. The differences observed in the genotype and allele frequencies between the groups with and without serious arrhythmic events and between the subjects who died and those who were alive did not reach statistical significance (Table II). Moreover, the genotype and allele frequencies of all polymorphisms did not differ between white and non-white patients with HF (Table SIII), and the four polymorphisms were not associated with the primary and secondary outcomes in the analyses stratified by self-declared ethnicity (data not shown).

However, the analysis of adjusted residuals indicated that the Ala/Ala genotype of the Ser96Ala polymorphism in *HRC* was more frequent than expected under the null hypothesis (that is, assuming that the genotypes are not associated with the outcome) among the patients who were alive (Table II). Notably, carriers of the major allele (Ser/Ser or Ser/Ala) had worse survival rates than those homozygous for the Ala allele during follow-up (Fig. 1). Despite this difference, the Ala/Ala
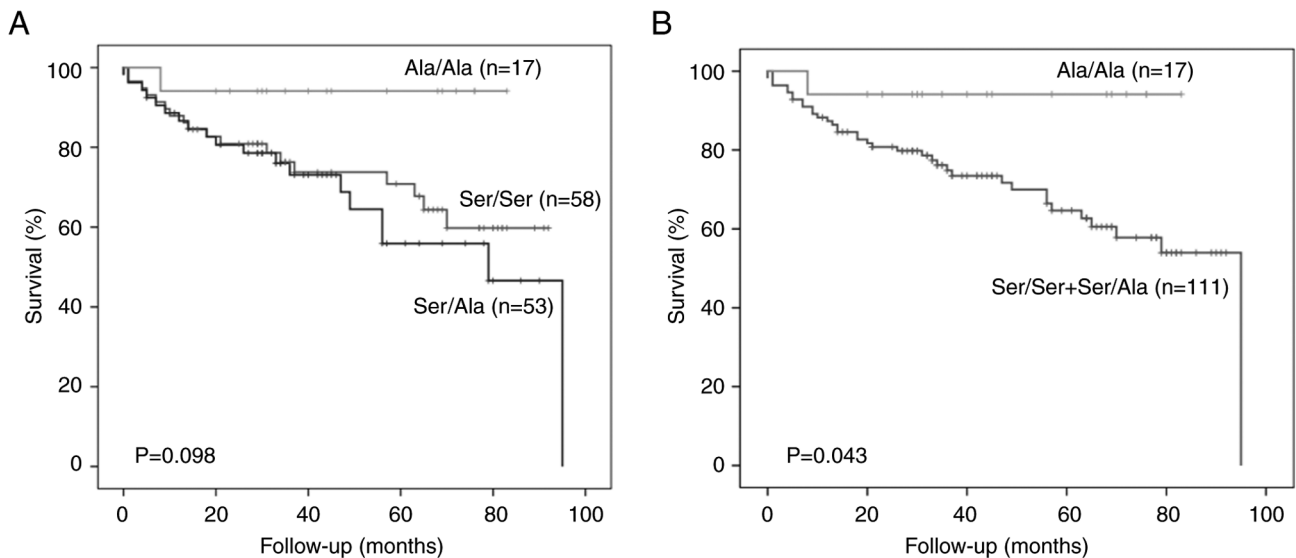
Figure 1. Kaplan-Meier survival estimates of all-cause death according to the (A) genotypes of the Ser96Ala polymorphism and the (B) carriers of the Ser96 allele in the histidine-rich calcium-binding protein gene.

genotype was not associated with all-cause death in regression analysis (unadjusted HR=0.17; 95% CI, 0.02-1.21; P=0.076). To further examine the potential association of the Ser96Ala polymorphism in *HRC* with cardiac dysfunction, an exploratory analysis of the association between this gene variant and individual clinical outcomes (SCD, cardiovascular-related death, heart transplantation, cardiac syncope and appropriate ICD therapy) was explored. Notably, homozygotes for the Ala allele had more appropriate ICD shocks than carriers of the Ser allele [3/17 (17.6%) vs. 3/111 (2.7%), respectively; P=0.031], whereas 7/8 patients who underwent heart transplantation were homozygous for the Ser allele (data not shown).

Regarding the Ser49Gly and Arg389Gly polymorphisms in the *ADRB1* gene, neither polymorphism was individually associated with arrhythmic events or all-cause death (Table II). However, patients homozygous for the major alleles at both sites (Ser49Ser and Arg389Arg) had a higher rate of all-cause death than those with other genotype combinations (Ser49Ser + Arg389Gly, Ser49Ser + Gly389Gly, Ser49Gly + Arg389Arg, Ser49Gly + Arg389Gly, or Gly49Gly + Arg389Arg; Fig. 2). Carrying the four major alleles was associated with a two-fold increased risk of all-cause death, but this association was lost in the multivariate analysis after controlling for covariates that were also associated with this outcome in the univariate analysis (Table III).

Discussion

In the present study, evidence of an association of Ser96Ala (*HRC*), Ser49Gly (*ADRB1*), Arg389Gly (*ADRB1*) and Gly1886Ser (*RYR2*) polymorphisms with the combined outcome of SCD or appropriate ICD therapy was not found in outpatients with non-ischemic HF from Southern Brazil. However, the Ser96Ala polymorphism appeared to be associated with all-cause death and appropriate ICD therapy when the outcomes were analyzed individually. Moreover, the Ser49Gly and Arg389Gly polymorphisms were found to be jointly associated with all-cause death in the univariate analysis.
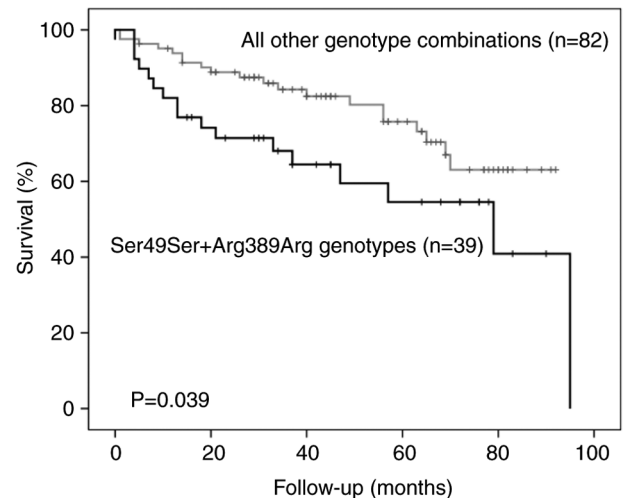

Figure 2. Kaplan-Meier survival estimates of all-cause death according to the genotype combinations of the Ser49Gly and Arg389Gly polymorphisms in the β1-adrenergic receptor gene.

Regarding the number of appropriate ICD shocks and the Ser96Ala polymorphism, the findings of the present study are in line with those reported in a large cohort of predominantly white patients with ischemic cardiomyopathy from the Genetic Risk Assessment of Defibrillator Events multicenter study. In this previous study, carriers of the Ala allele experienced more appropriate shocks than those homozygous for the Ser allele. However, in contrast to the findings of the present study, the secondary endpoint of death, heart transplant, or ventricular assist device use was not associated with the Ser96Ala polymorphism (21). Similarly, in a study of patients with idiopathic dilated cardiomyopathy from Greece, carriers of the Ala allele were more likely to need ICD due to a history of sustained VT or VF prior to study entry than those homozygous for the Ser allele. During follow-up, homozygotes for the Ala allele were more susceptible to life-threatening ventricular arrhythmic events (including SCD and episodes

Table III. Cox regression analysis for all-cause death (secondary outcome).

Variable[a]	Univariate analysis			Multivariate analysis		
	HR	95% CI	P-value	HR	95% CI	P-value
Ser49Ser + Arg389Arg genotypes	1.98	1.02-3.82	0.043	1.62	0.80-3.27	0.181
Body mass index, kg/m^2	0.92	0.87-0.98	0.012	0.91	0.85-0.97	0.004
LVEDD, mm	1.06	1.02-1.09	0.004	1.07	1.02-1.12	0.003
VO$_2$ peak, ml/kg/min	0.92	0.86-0.98	0.012	0.87	0.81-0.94	<0.001

[a]Age at study entry (years), QRS duration (msec), ventilatory equivalent for carbon dioxide, periodic breathing during exercise (%) and HV interval (msec) were also entered in the initial model and were removed during the backward stepwise (Wald) procedure. HR, hazard ratio; CI, confidence interval; LVEDD, left ventricular end-diastolic diameter; VO$_2$, oxygen consumption.

of unstable VT or VF) and cardiac death from any cause than Ser allele carriers (20). However, another study observed no association between the Ser96Ala polymorphism and VA in a cohort of Caucasian patients with predominantly ischemic cardiomyopathy from Southern Italy (22). In addition to the Ser96Ala polymorphism being poorly studied in the clinical setting in humans, studies are heterogeneous in terms of population, HF etiology and outcomes evaluated (20-22), which may explain the partially discrepant findings regarding the existence and direction of the association between this polymorphism and HF prognosis. Nevertheless, two of the three previous studies (20,21) and the present study have found evidence suggesting that the Ser96Ala polymorphism in *HRC* is associated with life-threatening arrhythmias and/or death.

The causal relationship between the Ser96Ala polymorphism and calcium handling in cardiomyocytes has already been demonstrated in cell and animal models (14,16-19). Substitution of Ala for Ser at position 96 has been reported to result in altered interactions of HRC with the major SR calcium cycling proteins (16), aberrant calcium transients and increased calcium leak, thus leading to more frequent calcium sparks and calcium waves (14), which in turn trigger aftercontractions and delayed afterdepolarizations (14,16). These electrical phenomena may be the underlying mechanisms of the increased propensity to VAs (17-19), decreased cardiomyocyte contractility (18,19), increased ventricular automaticity under stress conditions (19) and increased mortality (18,19), even in the absence of structural remodeling (18) or concomitant cardiac disease (19). Taken together, these studies show that Ser96 is critical for the cardioprotective effect of HRC (16-19).

In general, the present results regarding the two polymorphisms in *ADRB1* are in accordance with those obtained from previous studies by our research group in other cohorts of patients with HF with ischemic and non-ischemic etiology (39-41). In the first study by Biolo *et al* (39), the Ser49Gly polymorphism was reported to not be associated with non-sustained VT on Holter monitoring or death. By contrast, homozygous patients for the Gly389 allele had less non-sustained VT and a higher HF-related survival rate than the carriers of the Arg389 allele. In another cohort of patients with HF using ICDs for primary and secondary prevention, the Arg389Gly polymorphism was not associated

with the risk of appropriate shocks (41). A meta-analysis of studies published between 2003 and 2011, including patients with HF who were predominantly white and of non-ischemic etiology from Sweden, Denmark, Germany, Italy, the Netherlands, the United Kingdom, the USA and China, revealed that neither of the two polymorphisms of the *ADRB1* gene were associated with all-cause mortality or combined endpoints comprising death, heart transplantation and hospitalization (42). Such a lack of association of the Ser49Gly and Arg389Gly polymorphisms with adverse outcomes in patients with HF has also been reported in other studies carried out in France (43), the USA (44,45) and Japan (46), regardless of the use of ICD (45). In addition, the Arg389Gly polymorphism has been reported to not be associated with the incidence of malignant tachyarrhythmias (fast VT/VF), appropriate ICD shocks or cardiac death in Caucasian patients with HF 12 months after undergoing cardiac resynchronization therapy device implantation (47). In another study of Caucasian patients with HF, the Gly49 allele carriers were shown to have a higher risk of having appropriate ICD shocks than the homozygotes for the Ser49 allele, while the Arg389Gly polymorphism was not associated with arrhythmic events (48).

A potential explanation for the discrepancies across the studies may lie in the interaction between the Ser49Gly and Arg389Gly polymorphisms, ethnicity and the use (and dose) of β-blockers, as reported by several authors (39,49-53). For example, in a large cohort of African American and Caucasian patients with HF with both ischemic and non-ischemic etiologies, the Gly allele of the Arg389Gly polymorphism was associated with decreased survival in Caucasians only among those not taking β-blockers (51). In the present study, 132 of 136 patients (97%) were taking β-blockers. If the Ser49Gly or Arg389Gly polymorphism has a small effect on survival in patients with HF, it may have been overcome by treatment with β-blockers. Apart from the interaction between *ADRB1* polymorphisms and β-blockers, a joint effect of adrenergic receptor gene variants on event-free survival has been recurrently reported (40,41,44,46,52). For example, in one of our previous studies, a combination of the Thr164Ile (β2-adrenergic receptor), Gly49Gly and/or Gly389Gly genotypes was independently associated with lower all-cause and HF-related mortality than other genotypes (40). In line with these studies, the present study also found that patients carrying both the

Ser49Ser and Arg389Arg genotypes had a lower survival rate than those with other genotype combinations, indicating the synergistic effect of multiple common gene variants with small effect sizes on HF prognosis.

As for the Gly1886Ser polymorphism in the *RYR2* gene, the findings of the present study were partially in agreement with those obtained in a large study conducted in Han Chinese patients with chronic HF of ischemic or idiopathic etiology, in which the Gly1886Ser polymorphism was not associated with VAs among patients with HF or with HF severity, as defined by LVEF. However, patients carrying the Ser allele had an increased risk of heart transplantation and SCD (33). The results of the present study also partially differed from those reported in an Italian study of Caucasian patients with HF with both ischemic and non-ischemic etiologies who underwent primary or secondary prevention ICD implantation, in which the Ser allele was associated with an increased risk of VT/VF. However, during follow-up, 30 patients died, and only one patient was heterozygous for the Ser allele (32). In addition to having only two previous reports investigating the association of the Gly1886Ser polymorphism with HF prognosis (32,33), these studies and the present study have included patients with different inclusion criteria, HF etiologies, degrees of severity and composite endpoints, which makes the results not directly comparable.

In summary, the present study supported previous evidence that genetic variants in calcium handling and signaling genes are associated with HF prognosis. Beyond the understanding of the molecular basis of arrhythmic events and death in patients with HF with non-ischemic etiology, the findings of the present study support further research aimed at identifying new non-invasive (or minimally invasive) predictive biomarkers of arrhythmia and death that could be useful to improve the risk stratification and selection criteria for ICD implantation. For example, the Ala96 allele of the Ser96Ala polymorphism in *HRC* is a common variant in the general population and seems to have no deleterious effects in normal cardiomyocytes. However, it becomes arrhythmogenic under stress conditions such as in the failing heart (17). In the present study, almost all patients homozygous for the Ala96 allele were alive after a follow-up of 80 months, although they had more appropriate ICD shocks than the carriers of the Ser allele. This reinforces the idea that patients carrying certain alleles or genotypes are likely to benefit the most from ICD implantation. However, the main limitation of the present study was the small sample size. Although no patient was lost during follow-up, not all were using ICD, and not all DNA samples could be genotyped for the four polymorphisms. This reduced the power to detect associations of low magnitude, while the positive associations detected in the crude analyses should be considered suggestive rather than conclusive. Therefore, the findings of the present study need to be replicated in future studies.

In conclusion, the present retrospective cohort study of patients with non-ischemic HF provided suggestive evidence that the Ser96Ala polymorphism in *HRC* may be associated with all-cause death and appropriate ICD therapy, whereas the Ser49Gly and Arg389Gly polymorphisms in *ADRB1* may be jointly associated with all-cause death. This search for an improved prediction of HF prognosis is important for clinical practice, as the overall survival benefit of ICD therapy in patients with HF of non-ischemic etiology is still uncertain, and the conventional criteria used to indicate the implantation of this type of device are not sufficient to identify patients who are likely to benefit from this intervention. Randomized clinical trials, including patients undergoing contemporary treatment for non-ischemic HF and the presence of high-risk genetic polymorphisms, may indicate a subgroup of patients with an improved cost-benefit ratio for ICD implantation.

Acknowledgements

Not applicable.

Funding

Availability of data and materials

The genotyping datasets generated and/or analyzed during the current study are available in the Figshare repository (https://doi.org/10.6084/m9.figshare.22929857). The data are available from the corresponding author upon reasonable request.

Authors' contributions

BMM, MP, MA, LER and KGS conceived the present study. BMM and MP enrolled the patients, acquired clinical data and updated the database. LER supervised the study clinical stages. MA supervised DNA isolation. TMT and BLSP performed genotyping. KGS supervised genotyping. TMT updated the database. LER and KGS confirm the authenticity of all the raw data. KGS performed the statistical analyses. LER and KGS contributed to funding resources. KGS wrote and edited the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by The Research Ethics Committee of HCPA (Institutional Review Board-IRB: 0000921; CAAE no. 93278918.9.0000.5327 and consolidated review no. 2.824.527; Porto Alegre, Brazil). Written informed consent was obtained from all the participants.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Böhm M, Burri H, Butler J, Čelutkienė J, Chioncel O, *et al*: 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: Developed by the Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). With the special contribution of the Heart Failure Association (HFA) of the ESC. Eur J Heart Fail 24: 4-131, 2022.

2. McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Böhm M, Burri H, Butler J, Čelutkienė J, Chioncel O, *et al*: 2023 Focused update of the 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur Heart J 44: 3627-3639, 2023.

3. Roger VL: Epidemiology of heart failure: A contemporary perspective. Circ Res 128: 1421-1434, 2021.

4. Mulder BA, van Veldhuisen DJ and Rienstra M: Sudden cardiac death in heart failure: More than meets the eye. Eur J Heart Fail 23: 1361-1363, 2021.

5. Elming MB, Hammer-Hansen S, Voges I, Nyktari E, Raja AA, Svendsen JH, Pehrson S, Signorovitch J, Køber L, Prasad SK, *et al*: Myocardial fibrosis and the effect of primary prophylactic defibrillator implantation in patients with non-ischemic systolic heart failure-DANISH-MRI. Am Heart J 221: 165-176, 2020.

6. Jilek C, Lewalter T, Pauschinger M, von Scheidt W, Frankenstein L, Pfister O, Hambrecht R, Bruder O, Brachmann J, Hartmann A, *et al*: Cardioverter-defibrillator does not improve short-term survival among patients with non-ischemic cardiomyopathy and reduced left ventricular ejection fraction. Clin Res Cardiol 109: 115-123, 2020.

7. Merkel ED, Schwertner WR, Behon A, Kuthi L, Veres B, Osztheimer I, Papp R, Molnár L, Zima E, Gellér L, *et al*: Predicting the survival benefit of cardiac resynchronization therapy with defibrillator function for non-ischemic heart failure-Role of the Goldenberg risk score. Front Cardiovasc Med 9: 1062094, 2023.

8. Wasiak M, Tajstra M, Kosior D and Gąsior M: An implantable cardioverter-defibrillator for primary prevention in non-ischemic cardiomyopathy: A systematic review and meta-analysis. Cardiol J 30: 117-124, 2023.

9. Halliday BP, Cleland JGF, Goldberger JJ and Prasad SK: Personalizing risk stratification for sudden death in dilated cardiomyopathy: The past, present, and future. Circulation 136: 215-231, 2017.

10. Escobar-Lopez L, Ochoa JP, Mirelis JG, Espinosa MÁ, Navarro M, Gallego-Delgado M, Barriales-Villa R, Robles-Mezcua A, Basurte-Elorz MT, Gutiérrez García-Moreno L, *et al*: Association of genetic variants with outcomes in patients with nonischemic dilated cardiomyopathy. J Am Coll Cardiol 78: 1682-1699, 2021.

11. Rai V and Agrawal DK: Role of risk stratification and genetics in sudden cardiac death. Can J Physiol Pharmacol 95: 225-238, 2017.

12. Tamariz L, Balda J, Pareja D, Palacio A, Myerburg RJ, Conway D, Davis L and Goldberger JJ: Usefulness of single nucleotide polymorphisms as predictors of sudden cardiac death. Am J Cardiol 123: 1900-1905, 2019.

13. Li S, Jia Z, Zhang Z, Li Y, Yan M and Yu T: Association study of genetic variants in calcium signaling-related genes with cardiovascular diseases. Front Cell Dev Biol 9: 642141, 2021.

14. Arvanitis DA, Vafiadaki E, Johnson DM, Kranias EG and Sanoudou D: The histidine-rich calcium binding protein in regulation of cardiac rhythmicity. Front Physiol 9: 1379, 2018.

15. Hofmann SL, Topham M, Hsieh CL and Francke U: cDNA and genomic cloning of HRC, a human sarcoplasmic reticulum protein, and localization of the gene to human chromosome 19 and mouse chromosome 7. Genomics 9: 656-669, 1991.

16. Pollak AJ, Haghighi K, Kunduri S, Arvanitis DA, Bidwell PA, Liu GS, Singh VP, Gonzalez DJ, Sanoudou D, Wiley SE, *et al*: Phosphorylation of serine96 of histidine-rich calcium-binding protein by the Fam20C kinase functions to prevent cardiac arrhythmia. Proc Natl Acad Sci USA 114: 9098-9103, 2017.

17. Han P, Cai W, Wang Y, Lam CK, Arvanitis DA, Singh VP, Chen S, Zhang H, Zhang R, Cheng H, *et al*: Catecholaminergic-induced arrhythmias in failing cardiomyocytes associated with human HRCS96A variant overexpression. Am J Physiol Heart Circ Physiol 301: H1588-H1595, 2011.

18. Singh VP, Rubinstein J, Arvanitis DA, Ren X, Gao X, Haghighi K, Gilbert M, Iyer VR, Kim DH, Cho C, *et al*: Abnormal calcium cycling and cardiac arrhythmias associated with the human Ser96Ala genetic variant of histidine-rich calcium-binding protein. J Am Heart Assoc 2: e000460, 2013.

19. Tzimas C, Johnson DM, Santiago DJ, Vafiadaki E, Arvanitis DA, Davos CH, Varela A, Athanasiadis NC, Dimitriou C, Katsimpoulas M, *et al*: Impaired calcium homeostasis is associated with sudden cardiac death and arrhythmias in a genetic equivalent mouse model of the human HRC-Ser96Ala variant. Cardiovasc Res 113: 1403-1417, 2017.

20. Arvanitis DA, Sanoudou D, Kolokathis F, Vafiadaki E, Papalouka V, Kontrogianni-Konstantopoulos A, Theodorakis GN, Paraskevaidis IA, Adamopoulos S, Dorn GW II, *et al*: The Ser96Ala variant in histidine-rich calcium-binding protein is associated with life-threatening ventricular arrhythmias in idiopathic dilated cardiomyopathy. Eur Heart J 29: 2514-2525, 2008.

21. Klappa AJ, Aleong R, Vargas-Estrada A, Morgan G and London B: Abstract 20362: The Ser96Ala variant of the histidine-rich calcium-binding protein is associated with appropriate ICD shocks in cardiomyopathy patients. Circulation 130: A20362, 2014.

22. Galati F, Galati A and Massari S: RyR2 QQ2958 genotype and risk of malignant ventricular arrhythmias. Cardiol Res Pract 2016: 2868604, 2016.

23. Mahmood A, Ahmed K and Zhang Y: β-Adrenergic receptor desensitization/down-regulation in heart failure: A friend or foe? Front Cardiovasc Med 9: 925692, 2022.

24. Ahles A and Engelhardt S: Polymorphic variants of adrenoceptors: Pharmacology, physiology, and role in disease. Pharmacol Rev 66: 598-637, 2014.

25. Bencivenga L, Liccardo D, Napolitano C, Visaggi L, Rengo G and Leosco D: β-adrenergic receptor signaling and heart failure: From bench to bedside. Heart Fail Clin 15: 409-419, 2019.

26. Thomas CD and Johnson JA: Pharmacogenetic factors affecting β-blocker metabolism and response. Expert Opin Drug Metab Toxicol 16: 953-964, 2020.

27. McCrink KA and Lymperopoulos A: β1-adrenoceptor Arg389Gly polymorphism and heart disease: Marching toward clinical practice integration. Pharmacogenomics 16: 1035-1038, 2015.

28. Keefe JA, Moore OM, Ho KS and Wehrens XHT: Role of Ca2+ in healthy and pathologic cardiac function: From normal excitation-contraction coupling to mutations that cause inherited arrhythmia. Arch Toxicol 97: 73-92, 2023.

29. Benitah JP, Perrier R, Mercadier JJ, Pereira L and Gómez AM: RyR2 and calcium release in heart failure. Front Physiol 12: 734210, 2021.

30. Koop A, Goldmann P, Chen SR, Thieleczek R and Varsányi M: ARVC-related mutations in divergent region 3 alter functional properties of the cardiac ryanodine receptor. Biophys J 94: 4668-4677, 2008.

31. Zhang J, Liu Z, Masumiya H, Wang R, Jiang D, Li F, Wagenknecht T and Chen SR: Three-dimensional localization of divergent region 3 of the ryanodine receptor to the clamp-shaped structures adjacent to the FKBP binding sites. J Biol Chem 278: 14211-14218, 2003.

32. Francia P, Adduci C, Semprini L, Stanzione R, Serdoz A, Caprinozzi M, Santini D, Cotugno M, Palano F, Musumeci MB, *et al*: RyR2 common gene variant G1886S and the risk of ventricular arrhythmias in ICD patients with heart failure. J Cardiovasc Electrophysiol 26: 656-661, 2015.

33. Ran Y, Chen J, Li N, Zhang W, Feng L, Wang R, Hui R, Zhang S and Pu J: Common RyR2 variants associate with ventricular arrhythmias and sudden cardiac death in chronic heart failure. Clin Sci 119: 215-223, 2010.

34. Pimentel M, Zimerman A, Chemello D, Giaretta V, Andrades M, Silvello D, Zimerman L and Rohde LE: Predictors of serious arrhythmic events in patients with nonischemic heart failure. J Interv Card Electrophysiol 48: 131-139, 2017.

35. Kochi AN, Pimentel M, Andrades M, Zimerman T, Zimerman LI and Rohde LE: Predictors of total mortality and serious arrhythmic events in non-ischemic heart failure patients: The role of galectin-3. Arq Bras Cardiol 117: 531-541, 2021.

36. Lahiri DK and Nurnberger JI Jr: A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Res 19: 5444, 1991.

37. Griffiths A, Doebley J, Peichel C and Wassarman DA: Introduction to genetic analysis. Macmillan, New York, 12th ed., 2020.

38. Abramson JH: WINPEPI updated: Computer programs for epidemiologists, and their teaching potential. Epidemiol Perspect Innov 8: 1, 2011.
39. Biolo A, Clausell N, Santos KG, Salvaro R, Ashton-Prolla P, Borges A and Rohde LE: Impact of beta1-adrenergic receptor polymorphisms on susceptibility to heart failure, arrhythmogenesis, prognosis, and response to beta-blocker therapy. Am J Cardiol 102: 726-732, 2008.
40. Biolo A, Salvaro R, Clausell N, Silvello D, Santos KG and Rohde LE: Impact of beta-2 Thr164Ile and combined beta-adrenergic receptor polymorphisms on prognosis in a cohort of heart failure outpatients. Braz J Med Biol Res 43: 565-571, 2010.
41. Chemello D, Rohde LE, Santos KG, Silvello D, Goldraich L, Pimentel M, Rosa PR, Zimerman L and Clausell N: Genetic polymorphisms of the adrenergic system and implantable cardioverter-defibrillator therapies in patients with heart failure. Europace 12: 686-691, 2010.
42. Liu WN, Fu KL, Gao HY, Shang YY, Wang ZH, Jiang GH, Zhang Y, Zhang W and Zhong M: β1 adrenergic receptor polymorphisms and heart failure: A meta-analysis on susceptibility, response to β-blocker therapy and prognosis. PLoS One 7: e37659, 2012.
43. de Groote P, Lamblin N, Helbecque N, Mouquet F, Mc Fadden E, Hermant X, Amouyel P, Dallongeville J and Bauters C: The impact of beta-adrenoreceptor gene polymorphisms on survival in patients with congestive heart failure. Eur J Heart Fail 7: 966-973, 2005.
44. Kardia SL, Kelly RJ, Keddache MA, Aronow BJ, Grabowski GA, Hahn HS, Case KL, Wagoner LE, Dorn GW II and Liggett SB: Multiple interactions between the alpha 2C- and beta1-adrenergic receptors influence heart failure survival. BMC Med Genet 9: 93, 2008.
45. Sehnert AJ, Daniels SE, Elashoff M, Wingrove JA, Burrow CR, Horne B, Muhlestein JB, Donahue M, Liggett SB, Anderson JL, et al: Lack of association between adrenergic receptor genotypes and survival in heart failure patients treated with carvedilol or metoprolol. J Am Coll Cardiol 52: 644-651, 2008.
46. Ogimoto A, Okayama H, Nagai T, Suzuki J, Inoue K, Nishimura K, Shigematsu Y, Tabara Y, Miki T and Higaki J: Impact of synergistic polymorphisms in adrenergic receptor-related genes and cardiovascular events in patients with dilated cardiomyopathy. Circ J 76: 2003-2008, 2012.
47. Pezzali N, Curnis A, Specchia C, Carubelli V, Covolo L, Donato F, Auricchio A, Regoli F and Metra M: Adrenergic receptor gene polymorphism and left ventricular reverse remodelling after cardiac resynchronization therapy: Preliminary results. Europace 15: 1475-1481, 2013.
48. Zanolla L, Guarise P, Tomasi L, Vassanelli C, Cicorella N, Zanini R, Guarrera S, Fiorito G and Matullo G: Association between beta1-adrenergic receptor polymorphism and risk of ICD shock in heart failure patients. Pacing Clin Electrophysiol 39: 557-564, 2016.
49. Pereira SB, Velloso MW, Chermont S, Quintão MM, Nunes Abdhala R, Giro C, Oliveira E Alves T, Camacho V, De Fátima Maia Contarato L, Pena FM, et al: β-adrenergic receptor polymorphisms in susceptibility, response to treatment and prognosis in heart failure: Implication of ethnicity. Mol Med Rep 7: 259-265, 2013.
50. Parikh KS, Fiuzat M, Davis G, Neely M, Blain-Nelson P, Whellan DJ, Abraham WT, Adams KF Jr, Felker GM, Liggett SB, et al: Dose response of β-blockers in adrenergic receptor polymorphism genotypes. Circ Genom Precis Med 11: e002210, 2018.
51. Cresci S, Kelly RJ, Cappola TP, Diwan A, Dries D, Kardia SL and Dorn GW II: Clinical and genetic modifiers of long-term survival in heart failure. J Am Coll Cardiol 54: 432-444, 2009.
52. Petersen M, Andersen JT, Hjelvang BR, Broedbaek K, Afzal S, Nyegaard M, Børglum AD, Stender S, Køber L, Torp-Pedersen C, et al: Association of beta-adrenergic receptor polymorphisms and mortality in carvedilol-treated chronic heart-failure patients. Br J Clin Pharmacol 71: 556-565, 2011.
53. Guerra LA, Lteif C, Arwood MJ, McDonough CW, Dumeny L, Desai AA, Cavallari LH and Duarte JD: Genetic polymorphisms in ADRB2 and ADRB1 are associated with differential survival in heart failure patients taking β-blockers. Pharmacogenomics J 22: 62-68, 2022.