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Role of β1- and α2c-adrenergic receptor polymorphisms and their combination in heart failure: A case-control study☆

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Abstract

Background: Adrenergic activation has a central role in the development of HF. The function of the β_1- and the α_{2C}-adrenergic receptors is influenced by gene polymorphisms: the β_1Arg389 variant is associated with increased β_1-receptor sensitivity and the α_{2C}-receptor Del322-325 variant is associated with decreased α_{2C} receptor function and increased norepinephrine release. We hypothesised that these polymorphisms could influence the prevalence of heart failure.

Methods: The role of the β_1- and α_{2C}-adrenergic receptor gene polymorphisms as risk factors for heart failure (HF) was assessed in an Italian white Caucasian population using a case-control study design. Genomic DNA was analysed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RLFP).

Results: We compared 260 Caucasian patients with HF and 230 normal subjects. The β_1Arg389 allele was frequent both in the patients with HF (69%) and in the normal subjects (73%). The α_{2C}Del322-325 variant was rare in both groups (9% and 8%, respectively). Patients homozygotes for either the β_1Arg389 or the α_{2C}Del322-325 alleles had no increased risk of HF (odds ratio [OR], 0.8; 95%CI: 0.5–1.2 and OR, 0.8; 95% CI: 0.4–1.8, respectively). Patients homozygotes for both the β_1Arg389 and the α_{2C}Del322-325 alleles had no increased risk of HF as well (OR: 0.6; 95% CI: 0.2–2.1).

Conclusions: β_1-ARs and α_{2C}-ARs polymorphisms are not associated with an increased risk of HF in an Italian white Caucasian population.

Keywords: β-Adrenergic receptors; α-Adrenergic receptors; Gene polymorphisms

1. Introduction

Increased adrenergic drive has a central role in the progression of HF [1,2]. Norepinephrine (NE) release is increased 5- to 10-fold in the failing heart [3,4] and it causes ischaemia, apoptosis, pathologic hypertrophy and arrhythmias. NE has high affinity for β_1-receptors with a relative potency for $\beta_1/\beta_2/\alpha_1$-receptors of 20:1:2. Thus, despite β_1-receptors downregulation, most of the total adrenergic receptor occupancy is of the β_1-subtype [5]. Gene polymorphisms influencing the sensitivity of the β_1-receptors may influence the prevalence and progression of HF. The most common gene polymorphism for the β_1-receptors leads to the substitution of Gly for Arg at position 389 [6]. In-vitro studies have shown that the β_1-receptor Arg389 variant has enhanced G protein coupling with a 2- to 3-fold greater cyclicAMP and inotropic response to agonist stimulation, compared to the Gly389 allele [7]. This gene variant may have functional significance. Studies in targeted transgenic models have shown that the Arg389 variant overexpression may recapitulate the dilated cardiomyopathy phenotype [9].

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The Arg389 gene polymorphism was associated with a higher heart rate and blood pressure in one study [10] but not in another [11]. Case-control studies have shown that it is associated with a greater prevalence of hypertension [12] and acute myocardial infarction [13] and with a greater peak VO_2, amongst the patients with heart failure (HF) [14].

Presynaptic α_2-receptors (α_{2A} and α_{2C} subtypes) inhibit NE release from sympathetic nerve endings. Disruption of these receptors leads to chronically elevated sympathetic tone, left ventricular hypertrophy and fibrosis, development of dilated cardiomyopathy and increased mortality in animal models [15,16]. A polymorphism of the gene coding for the α_{2C}-receptor, consisting of the deletion of 4 consecutive amino acids at positions 322 to 325, has been associated with reduced α_{2C} receptor function and increased NE release [8,17]. Its relationship with the risk of HF has been assessed in African American and Caucasian subjects in a landmark case-control study [18]. Amongst African Americans, the prevalence of the α_{2C}-Del322-325 polymorphism was 10 times more common, compared to Caucasian subjects. In black patients, the homozygotes for the α_{2C}-Del322-325 variant had an increased risk of HF which further increased when it was combined with the β_1Arg389 allele [18]. However, no increase in the risk of HF was associated with these polymorphisms in the white subjects. These results may have been caused by the low number of Caucasian subjects showing the α_{2C}-Del322-325 polymorphism, so that it was insufficient to reach statistical significance. Alternatively, a specific interaction with race must be taken into account. We have therefore assessed the role of the β_1- and α_2-receptors gene polymorphisms, alone and combined, as risk factors for the development of HF in a relatively large group of Italian Caucasian subjects.

2. Methods

2.1. Patients

This study is part of a case-control study carried out to investigate the role of genetic polymorphisms in HF [19]. A total of 260 consecutive subjects admitted to our Institute with a diagnosis of HF were enrolled as cases between January and December 2002. Only cases born in Italy, Caucasian and with HF due to coronary heart disease or idiopathic dilated cardiomyopathy were included. The diagnosis of HF was based on the presence of the typical clinical signs and symptoms of HF with a left ventricular ejection fraction (LVEF) $\leq$ 40% at two-dimensional echocardiography [20].

A random sample of 230 subjects from the general population living in the same area, all Caucasian, of the same age range as the cases (30–80 years), with no clinical signs or symptoms of HF and with a normal echocardiogram, were selected as controls.

A 20-ml blood sample was taken by venipuncture from all the subjects and the serum was stored at −80 °C until further analysis. A local ethics committee approved the project and written informed consent was obtained from all subjects.

2.2. Genotyping

Genomic DNA was extracted from 200 μl of EDTA anti-coagulated blood using a QIAamp DNA Blood Mini Kit (QIAGEN S.p.A., Milano, Italy). Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RLFP). Analysis of β_1Arg389Gly gene polymorphism has been previously described in detail [21]. For the analysis of the α_{2c}Del322-325 polymorphism, the amplification reaction was carried out in a final volume of 25 μl containing 12.5 μl of PCR Master Mix 2X (Promega Corporation, Madison, WI USA), nuclease-free water and 300–500 ng of DNA template and primers. The primers were 5′-AGCCCGACGAGAGCAGCGCA-3′ and 5′-AGGCCTCGCGGCAGATGCCGTACA-3′ [17]. The DNA was amplified for 35 cycles with denaturation at 94 °C, annealed at 62 °C, with extension at 72 °C for 1 min at each step using a PCR Express thermal cycler (Celbio S.r.l., Milan, Italy). PCR products were analysed by 2% agarose gel electrophoresis (NuSieve 3:1, BMA) and visualized by ethidium bromide staining. For detection polymorphism the amplified products of PCR was digested at 37 °C for 1 h with 2 U of NciI (Promega Corporation, Madison, WI, USA). The fragments were resolved on a 2.5% ultra-pure DNA agarose gel electrophoresis (NuSieve 3:1, BMA) and visualized under ultraviolet illumination by ethidium bromide staining. This digestion produced fragments of the following sizes: 150, 82, 62 and 42 bp in wild-type homozygotes; 150, 111, 82, 62 and 42 bp in heterozygotes; 150, 111, 62 and 42 bp in Del322-325 homozygotes.

2.3. Statistical analysis

Results are shown as mean ± standard deviation unless otherwise specified. Differences in clinical variables and in the distribution of different genotypes studied among cases and controls were assessed using the unpaired *t*-test, chi-square and exact test analysis. To test for Hardy-Weinberg equilibrium for each polymorphism, the expected genotype numbers were calculated from the allele frequencies, and deviation from the observed genotype numbers was determined using the chi-square test. The odd ratios (OR) and their 95% confidence intervals (CI) were computed by unconditional logistic regression analyses using the maximum likelihood method. Sex and age were included in the logistic regression model as possible

confounders. All the statistical tests were performed with an alpha value of <0.05 to reject the null hypothesis. All the analyses were computed using the STATA statistical package (Stata Statistical Software: Release 7.0, College Station, TX: Stata Corporation).

As the frequency of the β_1Arg389 allele is approximately of 50%, we calculated that a study group including >160 cases and >160 controls yields a power >90% to detect an odds ratio of 2, with an alpha error of 0.05, for this single gene polymorphism. In contrast, as the frequency of the α_{2C}Del322-325 allele is of approximately 5%, it would be necessary to have a study group of 400 cases and 400 controls to have a power of 80% to obtain an odds ratio of 2 with an alpha error of 0.05.

3. Results

The clinical characteristics of the study group are shown in Table 1. There were no differences between cases and controls with regard to all the variables except for a higher prevalence of diabetes among the patients with HF.

There were no deviations from Hardy-Weinberg equilibrium for the polymorphisms considered when comparing expected and actual genotypes frequencies for both cases and controls. The frequencies of the β_1Arg389 and of the α_{2C}Del322-325 alleles were similar between the patients with HF and the normal subjects (69% vs. 73% and 9% vs. 8%, respectively; both n.s.). The frequency of homozygotes for the Arg389 allele was 46.2% in the patients with HF and 53.1% in normal subjects (n.s.). The frequency of homozygotes for the α_{2C}-Del322-325 polymorphism was 6.2% in cases and 6.5% in controls (n.s.). Neither the patients homozygotes for the β_1Arg389 allele nor those homozygotes for the α_{2C}Del322-325 allele had an increased risk of HF (OR: 0.8; 95%CI: 0.5–1.2 and OR: 0.8; 95% CI: 0.4–1.8, respectively). Patients who were homozygotes for both the β_1Arg389 and α_{2C}Del322-325 alleles had no increased risk of HF as well (OR: 0.6; 95% CI: 0.2–2.1; Table 2). Similar results were found when male patients, patients with idiopathic dilated cardiomyopathy and patients with coronary artery disease were analyzed separately (Table 3).

Table 1
Clinical characteristics

	Patients ($n=260$)	Controls ($n=230$)	P value
Sex, males, n (%)	235 (90)	145 (63)	n.s.
Age, years	61.5±11.2	62.4±7.8	n.s.
Risk factors for heart failure			
Hypertension, n (%)	101 (39)	94 (41)	n.s.
Diabetes, n (%)	62 (24)	18 (8)	<0.05
Hypercholesterolemia, n (%)	114 (44)	79 (34)	n.s.
Cause of heart failure			
Coronary artery disease	126 (48.5)		
Idiopathic dilated cardiomyopathy	134 (51.5)		

Table 2
Frequency and odd ratios for heart failure adjusted for age and sex according to β_1- and α_{2C}-receptor genotypes

Genotypes	Patients ($n=260$)	Controls ($n=230$)	Adjusted OR (95% CI)
β_1Arg389			
Gly389Gly+Arg389Gly, n (%)	140 (53.8)	108 (46.9)	reference
Arg389Arg, n (%)	120 (46.2)	122 (53.1)	0.8 (0.5–1.2)
α_{2C}Del322-325			
Wt/wt+wt/Del322-325	244 (93.8)	215 (93.5)	reference
Del322-325/Del322-325	16 (6.2)	15 (6.5)	0.8 (0.4–1.8)
β_1Arg389+ α_{2C}Del322-325			
Gly389* wt*+ Arg389Arg wt*+ Gly389* Del322-325/Del322-325	255 (98.1)	222 (96.6)	reference
Arg389ArgDel322-325/Del322-325	5 (1.9)	8 (3.4)	0.6 (0.2–2.1)

OR indicates odds ratio; CI indicates confidence interval.

4. Discussion

We investigated the role of the β_1- and the α_{2C}-receptor polymorphisms as risk factors for the development of HF using a case-control study design in an Italian Caucasian study population. The main finding of our study was that neither the common β_1-receptor Arg389Gly polymorphism nor the rare α_{2C}-Del322-325 polymorphism nor their combinations are associated with an increased risk of HF in Caucasian subjects.

4.1. β_1-receptor Arg389Gly polymorphism

The untoward effects of sympathetic stimulation are mainly mediated by β_1-receptors. Thus, gene polymorphisms influencing the sensitivity of β_1-receptors to adrenergic stimulation may be important for the development of HF. The β_1-receptor Arg389 variant has a two- to three-fold increase in agonist-stimulated activity compared with the Gly389 receptor variant [7,9]. It may therefore be hypothesized that it is associated with an increased risk of HF. Studies in transgenic animals have shown that overexpression of the β_1Arg389-receptor is associated with development of the dilated cardiomyopathy phenotype with reduced contractility, fetal patterns of expression of the α- and β-myosin heavy chain genes, reduced SERCA and phospholamban activities, myocyte loss, replacement fibrosis and increased mortality [9]. However, clinical studies did not find differences either in the prevalence of HF or in its severity related to the β_1-receptor Arg389Gly polymorphism [22,23]. Our results confirm these studies.

Table 3
Frequency and age and sex-adjusted odds ratios for the patients with heart failure caused by coronary artery disease (CAD) and idiopathic dilated cardiomyopathy (IDC) considered separately

Genotypes	CHD	Controls	OR (95% CI)	IDC	Controls	OR (95% CI)
	N (%)	*N* (%)		*N* (%)	*N* (%)	
β1-AR						
Gly389Gly+Arg389Gly	66 (52.4)	108 (46.9)	Reference	71 (54.6)	108 (46.9)	Reference
Arg389Arg	60 (47.6)	122 (53.1)	0.8 (0.5–1.2)	59 (45.4)	122 (53.1)	0.8 (0.6–1.3)
α2C-AR						
Wt/wt+wt/Del322-325	115 (91.3)	215 (93.5)	Reference	125 (96.1)	215 (93.5)	Reference
Del322-325/Del322-325	11 (8.7)	15 (6.5)	1.0 (0.4–2.5)	5 (3.9)	15 (6.5)	0.6 (0.2–1.7)

4.2. α_{2C}-Del322-325 polymorphism

Presynaptic α_{2A} and α_{-2C}-receptors inhibit NE release from sympathetic nerve endings. A gene polymorphism leading to the deletion of the 4 amino acids in positions 322 to 325 of the α_{2C}-receptor is associated with decreased receptor function and increased NE release [8,17]. It may, therefore, be related to the incidence and severity of HF. However, both its functional significance and its role as a risk factor for HF are still controversial.

Studies in animal models have shown that disruption of α_{2A} and α_{2C} receptors leads to chronically elevated sympathetic tone, left ventricular hypertrophy and fibrosis, development of dilated cardiomyopathy and increased mortality [15,16]. In patients with HF, the α_{2C}-Del322-325 polymorphism has been associated with an increased severity of symptoms [16] and with an increased uptake of [125I] MIBG, a radiolabeled NE analog and an indirect index of NE release [24]. However, in a more recent study in patients with HF, cardiac NE release, directly assessed by the radiotracer NE spillover method, was tightly related to the pulmonary wedge pressure without any influence of the α_{2C}-Del322-325 polymorphism [25]. This result was explained by the importance of hemodynamic factors, namely left ventricular filling pressure, as causes of the increased cardiac sympathetic drive, and by the concomitant downregulation of the pre-synaptic α_{2C}-receptors in the failing hearts [26].

The role of the α_{2C}-Del322-325 polymorphism as a risk factor for HF is controversial as well. Small et al. assessed the frequency of the β_1- and the α_{2C}-adrenergic receptor polymorphisms in 348 subjects, 162 African Americans and 186 Caucasians, 159 with HF and 189 normals [18]. Among the African Americans, the α_{2C}-Del322-325 gene polymorphism was 10-fold more common than in the white subjects and it was associated with an increased risk of HF (OR, 5.65; 95%CI, 2.67–11.95). These results were not confirmed among the Caucasian subjects. This could be explained by the low frequency of the Del322-325 polymorphisms with only 6 of 81 patients with HF and 2/105 controls showing it [18]. Similar results were, however, obtained in another study in a Caucasian population of 91 patients with HF and 105 control subjects. The frequency of the α_{2C}-Del322-325 variant was low and not different in the patients with HF (11%) compared to the controls (11.4%) [16]. In our study, we assessed a larger group of Caucasian subjects than in the previous trials (260 patients and 230 controls). Our results confirm the low frequency of the α_{2C}-Del322-325 variant (11% of the patients with HF and 9% of the normal subjects) in the Caucasian population with no significant difference between the two study groups. All these three studies are therefore consistent in showing a lack of difference in the frequency of the α_{2C}-Del322-325 polymorphism between the patients with HF and the normal subjects in the Caucasian subjects. In contrast, this polymorphism was associated with an increased risk of HF in the African American patients [18].

Our data should be interpreted in the light of more recent findings regarding the characteristics of the α_{2C} gene polymorphisms. It has been shown that the α_{2C} polymorphisms are organized into 24 different haplotypes and that their frequencies have substantial ethnic variations [27]. The association between the α_{2C}-Del322-325 polymorphism, black race and the increased risk of HF may therefore be secondary to a specific haplotype, particularly deleterious in the African American population. The α_{2C}-Del322-325 polymorphism may therefore be a marker of an associated haplotype, deleterious in the African American population, rather than having untoward effects by itself. In agreement with this hypothesis, and contrary to what was found by Small et al. in the African Americans [18], we did not find any increase in risk of HF in our Caucasian subjects who were homozygotes for both the β_1-receptor Arg389 and the α_{2C}-receptor Del322-25 polymorphisms. This last analysis must, however, be taken with some caution. In fact, the size of our study group was powered to detect meaningful differences between single nucleotide polymorphisms. The combination of the two homozygotic alleles had a lower frequency.

4.3. Limitation of the study

Although our study had a large statistical power to detect a difference between cases and controls with regards to the β_1Arg389 gene polymorphism, it was underpowered to detect a difference with regard to the α_{2C} Del 322-325

polymorphism. As the frequency of this allele is approximately 5%, it would be necessary to have a study population of at least 400 patients and 400 normal subjects to have the statistical power to detect an odds ratio of 2 between these two study groups. However, despite this limitation, our study remains the largest in which the frequency of such a polymorphism has been assessed in a Caucasian population. Our findings agree with and extend those of previous studies [16,18] showing both the low frequency of the α_{2C} Del 322-325 polymorphism and the lack of differences in its frequency between normal subjects and HF patients in the Caucasian population.

4.4. Conclusion

In conclusion, we did not find any association between the α_{2C} and the β_1-receptors gene polymorphisms and their combination and the risk of HF in a relatively large Italian Caucasian population.

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