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β1-adrenergic receptor gene polymorphisms in Mexican patients with idiopathic dilated cardiomyopathy

José Manuel Fragoso [a], José Manuel Rodríguez-Pérez [a], Jaime González [b], David Cruz [c], Oscar Pérez-Méndez [a], José de Jesus García [d], Aurora de la Peña [e], Minerva Arce [f], Pedro A. Reyes [g], Gilberto Vargas-Alarcón [a,*]

[a] *Department of Physiology, Cardiovascular Disease's Genomic and Proteomic Study Group, Instituto Nacional de Cardiología Ignacio Chávez, Juan Badiano No. 1, Tlalpan 14080, Mexico City, Mexico*
[b] *Department of Echocardiography, Instituto Nacional de Cardiología Ignacio Chávez, México City, México*
[c] *Department of Pathology, Cardiovascular Disease's Genomic and Proteomic Study Group, Instituto Nacional de Cardiología Ignacio Chávez, México City, México*
[d] *Department of Biochemistry, Cardiovascular Disease's Genomic and Proteomic Study Group, Instituto Nacional de Cardiología Ignacio Chávez, México City, México*
[e] *Department of Pharmacology, Cardiovascular Disease's Genomic and Proteomic Study Group, Instituto Nacional de Cardiología Ignacio Chávez, México City, México*
[f] *Department of Immunoparasitology, Cardiovascular Disease's Genomic and Proteomic Study Group, Instituto Nacional de Cardiología Ignacio Chávez, México City, México*
[g] *Department of Research Direction, Instituto Nacional de Cardiología Ignacio Chávez, México City, México*

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Abstract

The objective of the study was to evaluate the role of β1-adrenergic receptor gene polymorphisms (Ser49Gly and Arg389Gly) as susceptibility markers for idiopathic dilated cardiomyopathy (IDC) in Mexican patients. The polymorphisms were analyzed in 47 patients with IDC and 93 ethnically matched healthy controls by polymerase chain reaction-restriction fragment length polymorphism. The Ser49Gly allele and genotype frequencies were similar in patients and healthy controls. On the other hand, the analysis of the Arg389Gly polymorphism showed an increased frequencies of the *Gly allele (pC = 0.022, OR = 2.16) and *Arg/*Gly genotype (pC = 0.027, OR = 2.70) in the group of IDC patients when compared to healthy controls. The data suggest that Arg389Gly polymorphism could be involved in the genetic susceptibility to develop IDC in Mexicans.

Keywords: Alleles; Genetic markers; Genotypes; Idiopathic dilated cardiomyopathy; Polymerase chain reaction; Polymorphism; Populations

Introduction

Idiopathic dilated cardiomyopathy (IDC) is a relatively homogeneous form of dilated cardiomyopathy, which constitutes approximately 60% of heart failure cases. It is characterized by ventricular dilatation and depressed myocardial contractility, producing progressive refractory congestive heart failure and sudden death from ventricular arrhythmias (principally ventricular tachycardia (VT) and subsequent ventricular fibrillation). IDC is considered a multifactorial disease with a strong genetic component including the β-adrenergic receptor genes (Forleo et al., 2004); the adrenergic nervous system, especially through β-adrenergic receptor (β-AR) activation, appears to be of major importance not only in the genesis or progression of cardiac remodeling but also in induction of VT (Forleo et al., 2004; Iwai et al., 2002; Satwani et al., 2004). The β-adrenoceptors (β-AR) belong to a large family of G-protein-coupled receptors. Three subtypes of β-ARs (β1, β2 and β3) have been characterized by pharmacological, biochemical and molecular biological

* Corresponding author. Fax: +525 5573 09 26.
E-mail address: gvargas63@yahoo.com (G. Vargas-Alarcón).

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cloning approaches (Nagatomo et al., 2001; Strosberg, 1997). The β1-adrenergic receptor (β1AR) is expressed on the heart, mediating the physiological and pathophysiological effect of noradrenaline (White et al., 2002; Borjesson et al., 2000; Brodde et al., 2001). Thus, in normal physiological conditions, heart rate and contractility are under the control of β1AR. The gene coding the β1AR has been cloned and sequenced and comprising a single exon located on chromosome 10q24–26 and consists of 447 amino acids (Frielle et al., 1987; Yang-Feng et al., 1990). Two polymorphic sites located on the gene (positions A145G and C1165G) have been associated with the genetic susceptibility to several types of cardiovascular diseases and cardiovascular risk factors, including obesity, hypertension, diabetes, congestive heart failure and dilated cardiomyopathy (Feldman, 2001; Iwai et al., 2002). The first polymorphism is located at nucleotide position 145, resulting in an amino acid substitution of serine by glycine at amino acid position 49 (Ser49Gly). The second polymorphism is located at nucleotide position 1165, resulting in an amino acid substitution of arginine by glycine at amino acid position 389 (Arg389Gly). Several reports demonstrated large ethnic differences in the allele frequencies of both polymorphisms. The aim of this study was to analyze the relevance of the polymorphisms in the β1AR gene in the susceptibility to develop IDC in a well clinically characterized cohort of Mexican Mestizo patients.

Materials and methods

Subjects

Between January 2003 and February 2004, a total of 47 patients with heart failure were referred to our institution. Idiopathic dilated cardiomyopathy was diagnosed on the basis of clinical history, physical examination with electrocardiography, chest radiography, echocardiography, left ventriculography and coronary angiography. The diagnosis of IDC was made according to the World Health Organization and the National Heart, Lung, and Blood Institute (Richardson et al., 1996; Manolio et al., 1992). A group of 93 healthy unrelated individuals with neither symptoms nor previous diagnosis of cardiovascular problem and systemic disease was studied as control group. All included subjects (patients and controls) were ethnically matched, and we considered as Mexican Mestizos only those individuals who for three generations, including their own, had been born in Mexico. The Institutional Ethics and Research Committees approved the study, and all subjects signed informed consent.

DNA extraction

Genomic DNA was isolated from peripheral blood by a rapid non-enzymatic method (Lahiri and Nurnberger, 1991).

β1 receptor polymorphisms detection

Both polymorphisms were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. For the Ser49Gly polymorphism, the primers used were 5′-CCGGGCTTCTGGGGTGTTCC-3′ (sense) and 5′-GGCGAGGTGATGGCGAGGTAGC-3′ (antisense) previously described (Maqbool et al., 1999). The amplification of the 564 bp specific fragment was followed by restriction enzyme digestion by *Eco*0109I, as described previously (Maqbool et al., 1999). In the same way, a fragment of 530 bp that contains the Arg389Gly polymorphic site was amplified using the 5′-CGCTCTGCTGGCTGCCCTTCTTCC-3′ (sense) and 5′-TGGGCTTCGAGTTCACCTGCTATC-3′ (antisense) primers and was followed by restriction enzyme digestion by *Bcg*I (Molenaar et al., 2002). Cleavage of amplified fragments was detected by electrophoresis in 1.8% agarose gel and visualized by ethidium bromide staining.

Statistical analysis

Allele and genotype frequencies of the β1AR gene polymorphisms were obtained by direct counting. Furthermore, Hardy–Weinberg equilibrium was evaluated by chi-square test. The differences between IDC patients and healthy controls were evaluated by the Mantel–Haenszel chi-square test that combined the 2 × 2 contingency tables using the EPIINFO statistical program (EPIINFO Version 5.0: USD Incorporated 1990, Stone Mountain, Georgia). If the number in any cell was <5, Fisher's Exact Test was used. *P* values were corrected by multiplying by the number of comparisons made. *P* values equal or less than 0.05 were considered statistically significant. Relative risks with 95% confidence interval (95% CI) were estimated as the odds ratios (OR) by Woolf's method (Woolf, 1955).

Results

Allele and genotype frequencies of β1-adrenergic receptor gene polymorphisms at positions A145G (Ser49Gly) and C1165G (Arg389Gly) in IDC patients and healthy controls are shown in Table 1. Observed and expected frequencies in both polymorphic sites were in Hardy–Weinberg equilibrium. A145G (Ser49Gly) allele and genotype frequencies were equally distributed between IDC patients and healthy controls. Analysis of C1165G (Arg389Gly) polymorphism showed increased frequencies of G (*Gly) allele (pC = 0.022, OR = 2.16, 95% CI = 1.13–4.12) and C/G (*Arg/*Gly)

Table 1
Allele (af) and genotype (gf) frequencies (%) of β1-adrenergic receptor gene polymorphisms (A145G and C1165G) in IDC patients and healthy controls

	Patients		Controls		pC	OR	95% CI
A145G (Ser49Gly)	(*n* = 47)		(*n* = 93)				
Allele	*n*	af	*n*	af			
*A (*Ser)	69	73.4	150	80.6	NS	–	—
*G (*Gly)	25	26.6	36	19.4	NS	–	—
Genotype	*n*	gf	*n*	gf			
*A/*A (*Ser/*Ser)	25	53.2	61	65.6	NS	–	—
*A/*G (*Ser/*Gly)	19	40.4	28	30.1	NS	–	—
*G/*G (*Gly/*Gly)	3	6.4	4	4.3	NS	–	—
C1165G (Arg389Gly)							
Allele	*n*	af	*n*	af			
*C (*Arg)	68	72.3	158	84.9	0.022	0.46	0.24–0.89
*G (*Gly)	26	27.7	28	15.1	0.022	2.16	1.13–4.12
Genotype	*n*	gf	*n*	gf			
*C/*C (*Arg/*Arg)	24	51.0	69	74.2	0.018	0.36	0.16–0.81
*C/*G (*Arg/*Gly)	20	42.6	20	21.5	0.027	2.70	1.18–6.21
*G/*G (*Gly/*Gly)	3	6.4	4	4.3	NS	–	—

All populations were in Hardy–Weinberg equilibrium. Abbreviations: IDC = idiopathic dilated cardiomyopathy; OR = odds ratio; NS = not significant. pC: *P* corrected.