

ORIGINAL ARTICLE

Influence of ACE Polymorphism on Echocardiographic Data of Patients with Heart Failure

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Abstract

Background: Angiotensin converting enzyme (ACE) polymorphism has been associated with different clinical and echocardiographic parameters in patients with heart failure (HF). However, no studies have been investigated such association with HF caused by Chagas disease.

Objectives: To perform a genetic study to evaluate the frequency of ACE polymorphism in patients with HF caused by Chagas disease attending a university hospital in the central-west region and its association with echocardiographic findings.

Methods: Descriptive study of ACE polymorphism (I/D) and echocardiographic data of 103 patients with HF caused by Chagas disease. Echocardiographic parameters were compared between the genotypes using the ANOVA test.

Results: Genotypic distribution of the ACE polymorphism was 16.5% DD, 57.3% DI and 26.2% II. There was no statistically significant difference in the distribution of genotypes between men and women. The echocardiographic findings were: left ventricular ejection fraction: 43.8 ± 14.8 (DD) vs. 42.3 ± 11.6 (ID) vs. 44.9 ± 13.0 (II), $p = 0.664$; left ventricular diastolic diameter: 59.2 ± 9.7 (DD) vs. 60.3 ± 7.6 (ID) vs. 59.7 ± 7.8 (II), $p = 0.879$; left ventricular systolic diameter: 48.6 ± 12.8 (DD) vs. 50.6 ± 9.7 (ID) vs. 49.3 ± 11.9 (II), $p = 0.753$; and left atrial volume: 44.9 ± 10.1 (DD) vs. 40.9 ± 9.6 (ID) vs. 38.2 ± 7.8 (II), $p = 0.068$. Significant correlation coefficients were found for gender, age, ethnicity, heart rate and dyslipidemia.

Conclusion: ACE polymorphism was not associated with echocardiographic findings in patients with HF caused by Chagas disease. (Int J Cardiovasc Sci. 2019;32(1)55-60)

Keywords: Heart Failure; Angiotensins; Polymorphism, Genetic; Chagas Disease; Echocardiography / methods.

Introduction

Chagas disease, described more than 100 years ago by Carlos Chagas, is considered one of the most neglected diseases in the world by the World Health Organization (WHO).¹ It is an important cause of heart failure (HF) in low socioeconomic regions, leading to high morbidity and mortality.² In the central-west region of Brazil, Chagas disease has been considered the main cause of HF.³⁻⁵

Most health problems, including HF, have a multifactorial etiology, that involves environmental, lifestyle, and genetic factors.⁶ Multifactorial disorders

are characterized by genotypic contributions from many genes that interact with each other and with environmental factors.⁶ Genetic composition, associated with environmental factors, can predispose an individual to diseases and response to pharmacological interventions.⁷

Doppler echocardiography is a useful method for diagnostic confirmation, evaluation of etiology, pathophysiological and hemodynamic model, prognosis and indication of therapeutic alternatives for patients with HF.⁸ Due to the importance and magnitude of this condition, clinical therapy of HF patients require a multidisciplinary approach, and search for new techniques.

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In this context, analysis of genetic polymorphisms is a non-invasive technique that may open new avenues and indicate potential preventive therapies for these patients.

Angiotensin converting enzyme (ACE) gene is located on chromosome 17q23 and consists of 24 introns.⁹ Several approaches on ACE polymorphism in HF have been reported in the literature, including three clinical trials that did not find an association between ACE polymorphism and HF.^{4,10,11} Other studies, however, have suggested the DD phenotype as a risk factor for cardiac hypertrophy and HF¹² and associated this phenotype with worse survival.¹³ Figure 1 shows the localization of the gene that encodes ACE.¹⁴

In the last years, ACE polymorphism has drawn much attention due to conflicting results of the studies on HF. The present study aimed to identify ACE gene D/I (deletion/insertion) in patients with HF caused by Chagas disease and compare it with echocardiographic results.

Methods

This was an observational cohort study. Clinical data were collected from the medical records of patients seen at the Cardiology Unit of the Federal University of Goias, Brazil. Genetic analysis was performed at the Laboratory of Genetics and Molecular Cardiology (Incor/University of Sao Paulo, Brazil).

One hundred and three patients were evaluated from February 2014 to October 2015. All patients were on optimized drug therapy according to current guidelines.⁸

All patients included in the study underwent complete genotyping process for I/D alleles. Alleles were determined after the patients were included in the study. All clinical data were extracted from medical records by the first author of this article, before patients' visits, and ten patients were invited to participate in the study. Inclusion criteria were age older than 18 years, diagnosis of HF as established by the Framingham criteria, and doppler echocardiography using the Teichholz method (more recent and available tests were considered for analysis). Exclusion criteria were: unavailable or inadequate medical records, and HF caused by other than Chagas disease.

The study was analyzed and approved by the Ethics Committee of the General Hospital of the Federal University of Goias (approval number 908.870). All patients signed an informed consent form, and the study was conducted according to current ethical issues.

Echocardiographic variables

All patients had echocardiography results available in the medical records. The following echocardiographic parameters were evaluated (Table 1) – LAV: left atrial volume; EF: ejection fraction; LVDD: left ventricular diastolic diameter; LVSD: left ventricular systolic diameter (Teichholz method).

Echocardiographic measures of cavity diameter and muscle thickness were obtained following the American Echocardiography Society recommendations.¹⁵

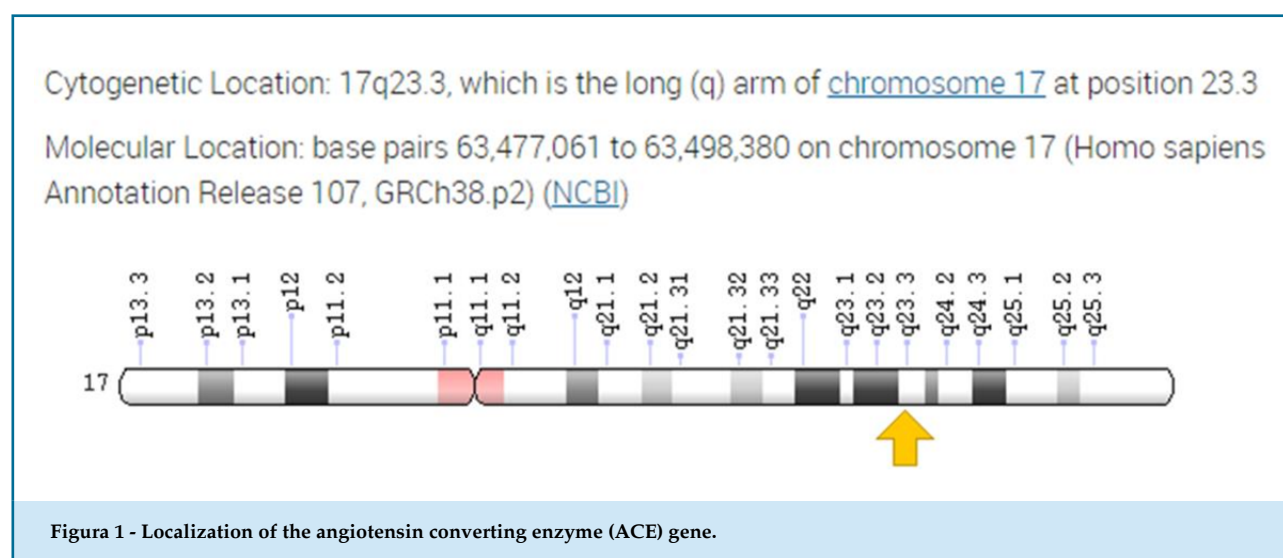


Table 1 - Echocardiographic parameters according to angiotensin-converting enzyme (ACE) (Deletion/Insertion, D/I)

	DD n (%)	DI n (%)	II n (%)	
N	17	59	27	
EF (%)				0.664
Mean	43.8	42.3	44.9	
Standard deviation	14.8	11.6	13.0	
LVDD				0.879
Mean	59.2	60.3	59.7	
Standard deviation	9.7	7.6	8.1	
LVSD				0.753
Mean	48.6	50.6	49.3	
Standard deviation	12.8	9.7	11.0	
LAV				0.068
Mean	44.9	40.9	38.2	
Standard deviation	10.1	9.6	7.8	

* ANOVA test. LAV: left atrial volume; DD: deletion/deletion; DI: deletion/insertion; LVDD: left ventricular diastolic diameter; LVSD: left ventricular systolic diameter; EF: ejection fraction; I/I: insertion/insertion.

Genetic analysis

Blood (8 mL) was collected in tubes containing EDTA, and submitted to DNA extraction. Subsequently, polymorphism genotyping was performed by polymerase chain reaction (PCR) and classified as DD, DI or II. One pair of primers was used to amplify the D and I alleles, resulting in 319-bp (base pairs) and 597 bp amplicons.

Hace 3,5'GCCCTGCAGGTGTCTGCAGCATGT3'

Hace 3,5'GGATGGCTCTCCCCGCCTTGCTCTC3'

The protocol of PCR using a thermal cycle consisted of a cycle of denaturation at 94°C for 30 seconds, annealing at 56°C for 45 minutes and extension at 72°C for 7 minutes. Amplification products of the D and I alleles were identified using an ultraviolet transilluminator. Since the D allele in heterozygous samples is preferentially amplified, each sample initially found to have the DD genotype was equally subjected to a second, independent amplification with a primer pair that recognizes an insertion-specific sequence with identical PCR conditions except for an annealing temperature of 67°C.¹⁶

Hace 5',5'TGGGACCACAGCGCCCGCCACTAC3'

Hace 5',5' TCGCCAGCCCTCCCATGCCATAA3'

Statistical analysis

The Shapiro-Wilk test was used for testing normality of data. Mean and standard deviation of EF, LVDD, LVSD and LAV were calculated for patients grouped according to ACE polymorphism (DD, DI and II). Differences in echocardiographic parameters between these groups were compared by analysis of variance (ANOVA, F test). P-values lower than 0.05 were considered statistically significant. Analysis was performed using the SPSS software version 18.0.

Echocardiographic parameters (EF, LVDD, LVSD and LAV) were treated as continuous variables with normal distribution, and the DD, DI and II genotypes treated as categorical variables.

Results

A total of 103 patients with HF caused by Chagas disease, mean age of 62.4 years (36 – 95 years), 63% men, were included in the study. All patients were on optimized drug therapy according to current guidelines.

Genotypic distribution of the ACE polymorphism was 16.5% DD, 57.3% DI and 26.2% II. No statistically

significant difference was found in the distribution of genotypes between men and women, with a predominance of men with DI genotype (59.3%).

Functional class varied from II to IV; most patients were in NYHA functional class IV. Analysis of repeated measures for categorical data showed that there was no significant variation in functional class and D/I genotype ($p = 0.472$).

No difference was found in mean values of echocardiographic variables or genotypes. Patients with the DI genotype showed higher LVDD (60.3) as compared with the other genotypes.

Significant correlations were found for the variables sex, age, smoking, heart rate and dyslipidemia; male sex was associated with increased risk of HF ($p = 0.023$). Smoking was considered a risk factor for HF. Most patients did not consume alcohol, and thereby alcohol consumption was considered a protective factor ($p = 0.008$).

Dyslipidemia was associated with a five times higher risk for HF. DD, DI and II genotypes were not considered a risk factor or HF.

Discussion

Many researches involving ACE polymorphisms have been conducted to study the pathophysiology of HF and, so far, it is still controversial whether ACE polymorphism is associated with HF. Although ACE polymorphism has been associated with several pathophysiological events and with morbidity and mortality of cardiovascular diseases, in this study, we aimed to determine a relationship of this polymorphism with HF caused exclusively by Chagas disease.

Most of our patients were men (63%), similarly to other studies.^{4,17,18} HF is an increasing epidemic that affects mostly older men, and its increasing incidence has been associated with higher survival.⁴ No statistically significant difference in genotype distribution was found between men and women, which is in agreement with a previous study conducted in 2014.¹⁹

To evaluate an association of ACE polymorphism with HF severity, we investigated a possible association of D/I genotype with echocardiographic parameters, but no association was found. In contrast, a previous national study²⁰ reported an association of DD and ID genotype with worse and better echocardiographic profile, respectively.²⁰ We also did not find differences in EF values between the two genotypes ($p = 0.664$).

HF is a common clinical condition with high morbidity and mortality, affecting 1.5% - 2.0% of the general population. Its prevalence increases with age and affects approximately 10% of individuals older than 65 years.²¹ Our findings corroborate this finding, since we found a statistically significant association of age with HF in the study population.

We did not find any difference in the frequency of genotypes after adjustment for gender, which agrees with the results of a study conducted in a Chinese population.²²

A national study performed in 2005²³ reported augmented LVSD in patients with DD genotype, which was associated with higher morbidity and mortality in patients with different causes of HF. Our results differ from these findings, since we did not find any relationship between D/I genotypes and LVSD.

Similar occurrence of D/I genotypes was found in the study population, contradicting the hypothesis that D/I genotype is a risk factor for HF.^{4,24}

We did not find any association of D/I genotypes with HF severity, differently from previous studies^{25,26} reporting an association of the D allele with HF progression and higher mortality as compared with the I allele. Our study corroborates another Brazilian, clinical comparative study on 193 patients, that did not find any difference in the frequency of D/I genotype in patients with HF caused by Chagas disease without systolic dysfunction.²⁷

Hospitalization for HF is an important public health problem.²⁸ Clinical treatment of this patients involves multidisciplinary approach, since they have many comorbidities that may have an impact on the clinical course of the disease. There is evidence that the risk for HF in the general population depends on a genetic predisposition characterized by a very complex genetic architecture. Predisposition to individual conditions and genetic variations modulate the pathophysiological responses.²⁹

Possible limitations of this study include a small number of patients; however, there are available in the literature studies involving larger number of patients,^{10,23} but also others with smaller samples.^{27,30} Due to the lack of patients' follow-up in the study, we could not evaluate the natural course of the disease. Socioeconomical aspects can interact with genetic factors and influence HF outcomes. Our study included only patients attending public clinics and therefore our findings may not be reproduced to other populations.

Conclusions

This study suggests that ACE (D/I) polymorphism is not related with echocardiographic parameters of patients with HF caused by Chagas disease.

Further larger, prospective studies are needed to determine which factors may be associated with HF caused by Chagas disease and investigate the best intervention with minimal adverse effects in this population.

Author contributions

Conception and design of the research: Silva SJ, Rassi S. Acquisition of data: Silva SJ. Analysis and interpretation of the data: Silva SJ, Rassi S, Pereira AC. Statistical analysis: Silva SJ. Obtaining financing: Pereira AC. Writing of the manuscript: Silva SJ. Critical revision of the manuscript for intellectual content: Rassi S, Pereira AC.

Potential Conflict of Interest

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

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Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Hospital das Clínicas (UFG) under the protocol number 908.870. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

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