Angiotensin-Converting Enzyme Insertion/Deletion Gene Polymorphism and Progression of Chagas' Cardiomyopathy

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Chagas' disease is common in Latin America and is caused by *Trypanosoma cruzi*. It is usually associated with chronic cardiomyopathy, the progression of which could be related to genetic factors. As alterations in the renin-angiotensin-aldosterone system have been reported in the disease, the aim of this study was to determine whether associated genetic polymorphisms influence the development of myocardial damage. The study involved 125 patients who were divided into 2 groups according to whether they had mild or severe cardiomyopathy. The insertion/deletion polymorphism of the angiotensin-converting enzyme gene was investigated using standard techniques and results were correlated with disease stage. The genotypes were in Hardy-Weinberg equilibrium. After adjusting for demographic variables, no significant relationship was found between the polymorphism and progression of chronic Chagas' disease. Although our sample was limited, the results suggest that the progression of cardiomyopathy in chronic Chagas' disease is unrelated to the insertion/deletion polymorphism.

Key words: *Chagas' disease. Insertion/deletion polymorphism of the angiotensin-converting enzyme gene.*

Polimorfismo I/D del gen de la enzima de conversión de angiotensina y progresión de la miocardiopatía chagásica

La enfermedad de Chagas es frecuente en Latinoamérica y la causa *Trypanosoma cruzi*. Suele asociarse a miocardiopatía crónica, cuya progresión podría relacionarse con factores genéticos. Dado que se han publicado alteraciones del sistema renina-angiotensina-aldosterona en esta enfermedad, este trabajo buscó determinar si los polimorfismos genéticos relacionados afectaban a la progresión del daño miocárdico. Se seleccionó a 125 pacientes y se agrupó según presentasen miocardiopatía leve o severa. El polimorfismo de inserción/deleción del gen de la enzima de conversión de angiotensina se analizó por pruebas estandarizadas, y los resultados se correlacionaron con el estadio de la enfermedad. Los genotipos cumplieron el equilibrio de Hardy-Weinberg. Después de ajustar según variables demográficas, no hubo relación significativa entre el polimorfismo estudiado y la progresión de la enfermedad de Chagas. Pese a que la muestra fue limitada, los resultados indican que la progresión de la miocardiopatía chagásica no está relacionada con el polimorfismo de inserción/deleción.

Palabras clave: *Enfermedad de Chagas. Polimorfismo inserción/deleción del gen de la enzima de conversión de angiotensina.*

INTRODUCTION

Chagas' disease, caused by *Trypanosoma cruzi*, affects around 15 million persons, and is associated with 20 000-50 000 deaths annually.[1,2] Although it is native to America, migration has taken the disease to other regions.[1,2] The disease involves an acute phase of fever and adenopathy, followed by a chronic phase, frequently characterized by cardiac lesions (20%-30%), a long time after parasite entry.[1,3] Not all patients develop chagasic cardiomyopathy, and when it does occur it does not follow a definite pattern, even though the environment may be similar; this implies that progression cannot be explained solely by environment.[1,3] Factors contributing to disease progression should be differentiated from those of its contraction, as the latter depend on exposure and the former depend on other variables, including genetic variables.[4]

T cruzi itself presents considerable genetic variability[4] related to its pathogenic potential. Thus, its interaction with the human species varies, but it is precisely human susceptibility which is least

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understood,[4] and for this reason the genetic factors of disease progression require to be studied. Exploration of the possible link with genotypes of the renin-angiotensin system (RAS) proves interesting, with respect to which relationships to manifestations of heart disease[5-7] and therapeutic response have been published.[6]

For this reason, this study was oriented towards determining whether a human genetic variation in the RAS known to be related to heart disease (insertion/deletion polymorphism [I/D] of the angiotensin-converting enzyme [ACE] gene) is associated with the course of Chagas' disease.

METHODS

We undertook an explanatory field study, which also employed case-control methods as it began with the "absence" (controls, stages la-lb) or "presence" (cases, stages II and III) of the event of interest, to retrospectively investigate risk factors. The population included seropositive patients registered with the Molecular Cardiology Laboratory of the Central University of Venezuela. The sample was non-probabilistic. Blood samples were taken over a period of 6 months.

Procedures

Data were obtained primarily through revision of medical charts. The I/D polymorphism was confirmed, as previously described,[5] with 100 ng of genomic DNA, 50 pmol of primer (5'CTGGAGACCACTCCCATCCTTTCT3' and 5'AGACTGCTTACACTACCGGTGTAG3') and 2 U of Taq polymerase (denaturalization: 94°C, 5 min [×1]; denaturalization: 94°C, 1 min; alignment: 58°C, 1 min; extension: 72°C, 2 min [×30]; final extension: 72°C, 5 min [×1]). Two fragments of 490 base pairs for genotype II, 2 of 190 pairs for DD, and 1 of each type for ID were obtained. The products were visualized using agarose electrophoresis, stained with ethidium bromide, and compared with the respective scales.

Data Analysis

The dependent variable was the stage of heart disease, according to Hagar et al.[3] Firstly, Hardy-Weinberg equilibrium was confirmed by trinomial agreement (allele frequency D)^2 + 2 × (allele frequency D) × (allele frequency I) + (allele frequency I)^2. The χ^2 test was used to determine the cited equilibrium. Logistic regression analysis was used to determine the risk of advanced chagasic cardiopathy, including age and sex as intervening variables.

TABLE 1. **Clinical Characteristics of the Study Patients**

Age, mean (SD), y	60.17 (9.19)
Time since diagnosis, y	11.80 (4.09)
Cardiac rhythm	
Heart rate, beats/min	71.44 (16.39)
Sinus node involvement	33.6
Atrioventricular block (varying degrees)	66.4
Other conduction problems	52
Other arrhythmia manifestations	9.6
Patients with pacemaker	16.8
Chamber characteristics	
Ventricular dilatation	48.8
Atrial dilatation	10.4
Ejection fraction, %	55.36 (11.84)
Medication usage	
Digoxin	72.8
Diuretics	54.4
Angiotensin converting enzyme inhibitors	44.8
Beta-blockers	28
New York Heart Association classification	
I	27.2
II	31.2
III	24.8
IV	16.8

Data are expressed as mean (standard deviation) or percentage of patients.

Three types of genetic expression were used[7]:

– DD codominance: DD genotype =1; II genotype =0; ID genotype =0.5
– DD dominance: DD and ID genotypes =1; II genotype =0
– DD recessivity: DD genotype =1; II and ID genotypes =0

The final result is shown as an odds ratio with the respective 95% confidence interval (CI). Data analysis was performed using the programs Microsoft Excel 2000 and Statistix, version 1.0.

RESULTS

The sample included 34 patients with genotype II (27.2%), 41 DD (32.8%), and 50 ID (40%), whose clinical characteristics are shown in Table 1. The trend towards linkage disequilibrium was not significant (P=.09). The mean age of the patients was DD, 59.32 (8.88) years; II, 61.86 (9.17) years; and ID, 59.76 (9.45) years. The percentage of male patients with DD, II, and ID genotypes was 46.34%, 58.82%, and 50%, respectively.

Table 2 shows the results of the logistic regression analysis, with age and sex always exhibiting a similar profile. The D allele was associated with an advanced degree of chagasic cardiomyopathy, although the results were not significant.

TABLE 2. **Effect of the Deletion Allele in 3 Genetic Models in the Progression of Chagasic Cardiomyopathy**

Model	OR	95% CI	*P*
DD dominant			
Age	1.07	1.02-1.13	.0067
Sex	0.51	0.22-1.18	.1160
Presence of D allele	2.46	0.98-6.19	.0651
DD codominant			
Age	1.07	1.02-1.13	.0066
Sex	0.51	0.22-1.19	.1182
Presence of D allele	3.09	0.84-11.34	.0685
DD recessive			
Age	1.07	1.02-1.12	.0094
Sex	0.49	0.21-1.14	.0969
Presence of D allele	1.98	0.78-5.03	.1535

OR indicates odds ratio.
Multiple logistic regression results are shown for 3 forms of genetic expression.

DISCUSSION

In this study the progression of Chagas' disease was correlated with a genetic characteristic of the RAS, given that characteristics such as these can modify the progression of parasitic diseases.[6,7] For Chagas' disease, however, information is scarce, but its relationship with polymorphic residuals of the human histocompatibility complex has been published[8,9]; in our case, an important limitation was the small sample size, in addition to the fact that only 2 intervening variables were considered, sex and age, although the clinical characteristics of the patients were more or less typical.[1,3]

A possible association of the D allele of the ACE gene I/D polymorphism with advanced degrees of cardiomyopathy has been implied. Should this be the case, there would be 2 conflicting explanations: that the D allele is, in fact, associated with progression, such that it would tend to be rapid, or that the D allele is associated with protection against death in advanced stages, which would last longer ("survival bias"). The first appears to be the most likely option and, in fact, a study by Nakahara et al[10] demonstrated higher heart weight in DD individuals, in correlation with the study by Montgomery et al,[11] who observed an association between higher ventricular hypertrophic response and exercise; this coincides with other studies in which hypertrophy is associated with the DD genotype.[11] The second possibility, however, cannot be excluded. For example, Sanderson et al[12] found no association between the I/D polymorphism and heart failure, and van Suylen et al[13] reported a protective relationship between the DD genotype and ventricular hypertrophy.

The logical explanation of the association between RAS genotypes and heart disease involves changes in plasma concentrations of the mediators,[10,13] but this association might not exist if the local formation of angiotensin II and/or the responses of different organs are altered.[6] The genotype profile could predict the response of the patient to treatment and make its optimization possible; Kuznetsova et al[5] established that the deleterious effect of the DD genotype increases in the absence of pharmacotherapy and is mitigated with it.

In conclusion, the I/D polymorphism of the ACE gene is not related to progression of chagasic cardiomyopathy, although this must be confirmed in further studies.

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