

## Genetic Polymorphism, Medical Therapy and Sequential Cardiac Function in Patients with Heart Failure

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### Summary

**Background:** Functional variants of angiotensin-converting enzyme (ACE) gene may be associated with response to therapy in patients with heart failure (HF).

**Objective:** To test the hypothesis of differences in sequential echocardiographic evaluations of left ventricular ejection fraction in patients with HF on medical therapy, including ACE inhibitors in relation to insertion (I)/deletion (D) polymorphism of the ACE gene.

**Methods:** We studied 168 patients (mean age  $43.3 \pm 10.1$  years), 128 (76.2%) men, with HF and sequential echocardiograms. The I/D polymorphism was determined by polymerase chain reaction. Left ventricular ejection fraction (LVEF) was analyzed comparatively to genotypes. More than 90% of patients were on ACE inhibitors.

**Results:** There was a significantly greater increase in mean LVEF in patients with the D allele compared to patients with the II genotype ( $p=0.01$ ) after a mean follow-up of 38.9 months. The D allele was associated with an increase of 8.8% in mean LVEF over the same period. Furthermore, there was a tendency toward a D allele "copy number" effect on the increase of mean LVEF over time: a 3.5% difference in LVEF variation between patients with the II and the ID genotypes ( $p = 0.03$ ) and a 5% difference between patients with the II and DD genotypes ( $p=0.02$ ).

**Conclusion:** ACE gene deletion polymorphism may be operative in response to medical treatment that included ACE inhibitors in patients with HF. Further controlled studies may contribute to better understanding of genetic influences on response to therapy. (Arq Bras Cardiol 2008; 90(4): 252-256)

**Key words:** Angiotensin-converting enzyme inhibitors; genetics; heart failure.

### Introduction

Insertion (I)/deletion (D) polymorphism of the angiotensin-converting enzyme gene may be associated with response to medical therapy. Regression of left ventricular hypertrophy in patients with arterial hypertension treated with enalapril was greater in homozygotes for the D allele<sup>1</sup>. Poorer transplant-free survival was observed in patients with heart failure and systolic dysfunction with the D allele not treated with beta-blockers<sup>2</sup>. Furthermore, the benefits of high-dose angiotensin-converting enzyme inhibitors associated with beta-blockers appeared maximal for DD patients<sup>3</sup>. Moreover, only patients with heart failure and ID or II genotypes demonstrated improvement in left ventricular systolic function after treatment with spironolactone<sup>4</sup>. Thus, different observations in patients with heart failure suggest a significant role for the insertion/deletion polymorphism of the angiotensin-converting enzyme gene in response to therapy.

Such findings may be associated with the fact that the variability of angiotensin-converting enzyme activity is partly modulated by functional variants determined by this biallelic polymorphism located at intron 16 of the angiotensin-converting enzyme gene. The difference between the two alleles lies in the presence or absence of a 287-base pair sequence and determines the I (insertion) or D (deletion) allele. The D allele was associated with higher plasma levels and activity of the angiotensin-converting enzyme<sup>5,6</sup> and has been associated with adverse outcomes in patients with heart failure<sup>2,3,7,8</sup>. Hence, we hypothesized that there may be differences in the sequential echocardiographic evaluation of left ventricular ejection fraction in follow-up of patients with heart failure on medical therapy including angiotensin-converting enzyme inhibitors in relation to functional variants of the angiotensin-converting enzyme gene.

Thus, the objective of the present study was to evaluate the variation in left ventricular ejection fraction, estimated by sequential echocardiographic assessment, of heart failure patients on medical therapy including angiotensin-converting enzyme inhibitors in relation to the functional variants of the angiotensin-converting enzyme gene. These patients were referred from a general outpatient clinic of a referral

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## Methods

**Study design** - Patients from an ongoing inception cohort of patients with heart failure were genotyped for functional variants of angiotensin-converting enzyme gene between 1995 and 2001 and submitted to sequential echocardiographic evaluation.

**Patients** - One hundred and sixty-eight patients were studied in the General Outpatient Clinic of the Heart Institute of São Paulo University Medical School. Patients' age ranged from 19 to 64 (mean 43.3; standard deviation 10.1) years, 128 (76.2%) being men and 40 (23.8%) women. The diagnosis of heart failure was made according to previously published criteria<sup>9</sup>. The classification of the etiology of heart failure followed previous recommendations<sup>10,11</sup>.

**Inclusion criteria** - Patients with symptomatic heart failure of different etiologies and left ventricular ejection fraction  $\leq 45\%$  on two-dimensional transthoracic Doppler echocardiography were eligible for the study. Patients were also evaluated for surgical treatment of heart failure, including heart transplantation. Specifically, patients with valvular cardiomyopathy enrolled in the study were those with severe left ventricular dysfunction to the point that they would not be eligible for valve repair or replacement, but rather candidates for heart transplantation. We have only included in this study patients with at least two echocardiographic studies (baseline and follow-up) during the follow-up period.

**Exclusion criteria** - Patients with valvular heart disease that would be candidates for conventional surgical treatment, such as valve repair or replacement; patients with hypertrophic cardiomyopathy, chronic obstructive pulmonary disease, recent myocardial infarction and unstable angina were excluded. In addition, patients with severe renal or hepatic dysfunction, severe peripheral arterial disease, cerebrovascular disease, active infection, coexisting neoplasm and active peptic ulcer disease were also excluded<sup>12</sup>.

**Etiology of heart failure** - Heart failure was ascribed to idiopathic dilated cardiomyopathy in 61 (36.3%) of the patients. The etiology of heart failure was ischemic cardiomyopathy in 36 (21.4%) patients, hypertensive cardiomyopathy in 25 (14.9%), Chagas' heart disease in 21 (12.5%), alcoholic cardiomyopathy in 10 (6%), valvular heart disease in 10 (6%), and peripartum cardiomyopathy in 5 (2.9%) patients.

**Left ventricular function assessment** - Sequential left ventricular ejection fraction was determined by M-mode echocardiography. In some patients two-dimensional method was employed, using Simpson. The evaluation of the left ventricular function was performed by the echocardiography staff in a blinded way in relation to the genotypes and was conducted according to previously published recommendations<sup>13</sup>.

**Genotype determination** - Extraction of genomic DNA was performed from leukocytes separated from whole blood using a standard method<sup>14</sup>. The I/D polymorphism was detected by a polymerase chain reaction (PCR) technique using

oligonucleotide primers flanking the respective fragments D and I from the intron 16 of the human angiotensin-converting enzyme gene<sup>6</sup>. Genotyping was undertaken in a blinded way after the samples had been separated with electrophoresis on a 1% agarose gel and stained with ethidium bromide (1 $\mu$ g/ml). Because the preferential amplification of the D allele in heterozygous samples, each sample found to have the DD genotype was subjected to a second, independent PCR amplification with a primer that recognizes an insertion-specific sequence<sup>15</sup>.

**Statistical analysis** - The  $\chi^2$ -square test was used to evaluate associations between the I/D polymorphism and discrete variables. The Student t-test was used for the comparison of means of two groups, and ANOVA was used for comparison of more than two groups of continuous variables.

The relation between the I/D polymorphism and evolution of patients with heart failure was analyzed in the light of the pattern of genetic inheritance conferred to the D allele<sup>16</sup>. In this way, assuming a codominant model for the D allele, the three genotypes were analyzed separately (DD versus ID versus II), whereas assuming a recessive model of the D allele, patients with the DD genotype were compared with patients with the I allele (DD versus ID + II). Finally, assuming a dominant model of the D allele, patients with the D allele were analyzed in relation to homozygosity for the I allele (DD + ID versus II).

Statistical analyses were performed with SAS software<sup>17</sup>. A p value  $< 0.05$  was considered significant.

**Ethics** - The study protocol was approved by the Ethics Committee for Medical Research on Human Beings of the Hospital das Clínicas from University of São Paulo Medical School. Signed informed consent was obtained from all participants.

## Results

The frequency of the angiotensin-converting enzyme gene genotypes was 32.5% for the DD, 49.7% for the ID and 17.8% for the II genotype. Genotype frequencies were in accordance with the Hardy-Weinberg equilibrium. It was not possible to determine the genotype of only one of the patients due to technical problems.

The comparison of baseline demographic and clinical characteristics did not reveal a statistically significant difference between the study groups (Table 1).

In relation to the etiology of heart failure there was no statistically significant difference among patients with the DD, ID or II genotypes.

There was a statistically significant difference in the variation of left ventricular ejection fraction over time. There was a significant increase in the mean value of left ventricular ejection fraction in patients with the DD genotype comparatively to patients with the ID and II genotypes (Figure 1).

Considering the D allele in a dominant mode, we observed that there was a statistically significant increase in the mean value of left ventricular ejection fraction with time in patients with the D allele when compared to patients homozygous for the I allele ( $p = 0.01$ ). The presence of at least one D allele

**Table 1 - Baseline demographic and clinical characteristics relative to angiotensin-converting enzyme genotypes**

	DD N = 54	ID N = 83	II N = 30	p
Age (years) mean (SD)	4.1 (11.2)	43.9 (9.9)	45.6 (8.2)	NS
Gender (%)				NS
Male	82.4	68	85.7	
Female	17.6	32	14.3	
Ethnicity (%)				
White	79.2	73.7	82.1	NS
Mulatto	14.6	15.8	14.3	
Black	6.2	10.5	3.6	
Functional Class NYHA (%)				NS
I	-	1.4	-	
II	66.7	53.6	60	
III	26.7	40.6	32	
IV	6.7	4.3	8	
Hypertension (%)	49	42	50	NS
Diabetes mellitus (%)	12	10.5	0	NS
Smoking history (%)	51.2	47	37.5	NS
Left ventricular diastolic diameter (mm) mean (SD)	72.9 (6.8)	73.4 (7)	71 (6.1)	NS
Ejection fraction, mean (SD)	35.8 (5)	35.8 (5.9)	38.4 (6.1)	NS
Months elapsed between echocardiograms, mean (SD)	41.5 (27.1)	37.7 (27.7)	43 (26.6)	NS
ACE inhibitors (%)	97.6	93.8	95.5	NS
Digoxin (%)	95.2	89.2	90.9	NS
β-blockers (%)	21.4	15.4	18.2	NS
Diuretics (%)	97.6	100	95.5	NS

NS - not significant.

was associated with an ejection fraction increase of 8.8% after a mean follow-up of 38.9 (standard deviation 24) months compared to 1.73% decrease in patients with the II genotype, mean follow-up 43 (standard deviation 26.6) months.

There was a tendency, although not significant, toward a D allele “copy number” effect on ejection fraction progression: a 3.5% difference between ejection fraction progression of patients with the II versus ID genotypes ( $p = 0.03$ ) and a 5% difference between patients with the II versus DD genotypes ( $p = 0.02$ ) (Figure 2).

## Discussion

We observed in the present series that patients with heart failure and the D allele treated with angiotensin-

converting enzyme inhibitors had a significant improvement in left ventricular ejection fraction assessed by sequential echocardiograms compared to patients with the II genotype.

This finding may add further information in the hypothesis of the influence of functional variants of the angiotensin-converting enzyme gene on the response to pharmacological treatment of heart failure. Our study showed the same trend of previously published studies. For instance, a greater enalapril-induced regression of left ventricular hypertrophy and improvement in left ventricular impaired diastolic filling was observed in hypertensive patients with the DD genotype compared to patients with the I allele<sup>1</sup>. In another study, there was a significantly poorer transplant-free survival mainly in patients with the D allele and left ventricular systolic dysfunction not treated with beta-blockers<sup>2</sup>. More recently, a study analyzing patients with left ventricular systolic dysfunction showed that the benefits of beta-blockers and high doses of angiotensin-converting enzyme inhibitors appeared maximal for the DD patients<sup>3</sup>. These data suggest that the D allele may be a marker of pharmacological response to angiotensin-converting enzyme inhibitors and beta-blockers. Hence, determination of the insertion/deletion polymorphism might help target therapy for patients with heart failure.

It is noteworthy that a study with patients with heart failure randomly assigned to treatment with spironolactone showed a significant improvement in left ventricular function only in patients with a non-DD genotype<sup>4</sup>. The different results observed in these studies must be due in part to the different genetic backgrounds of the populations analyzed, the number of patients enrolled and the different classes of drug studied, with their distinct sites and mechanisms of action.

In our study, more than 90% of the patients were on angiotensin-converting enzyme inhibitors but only those with the D allele showed a significant improvement in left ventricular ejection fraction over time. This finding may be related to a better response to angiotensin-converting enzyme inhibitors in individuals with higher neurohormonal activity. In fact, individuals with the DD genotype have higher plasmatic and tissue angiotensin-converting enzyme activity<sup>18</sup> and may present higher angiotensin II levels. In accordance with this observation, a previously published study showed an increased pressure response to infusion of angiotensin I in normotensive men with the DD genotype, probably as a consequence of the generation of increased angiotensin II levels in subjects homozygous for the D allele<sup>19</sup>. Interestingly, the same authors later showed that the effect of intravenous enalaprilat was significantly greater and lasted longer in normotensive men with the II genotype<sup>20</sup>.

The trend toward a D allele copy number effect on left ventricular ejection fraction increase over time could be analyzed as the genetic and molecular expression of improvement in left ventricular function observed in patients with heart failure on angiotensin-converting enzyme inhibitors. In fact, as demonstrated in previous studies, the benefits from angiotensin-converting enzyme inhibition were more pronounced in patients with elevated plasma neurohormonal concentrations<sup>21-23</sup>. Furthermore, it seems that the maximal survival benefit is obtained in patients with the DD genotype with increasing levels of neurohormonal

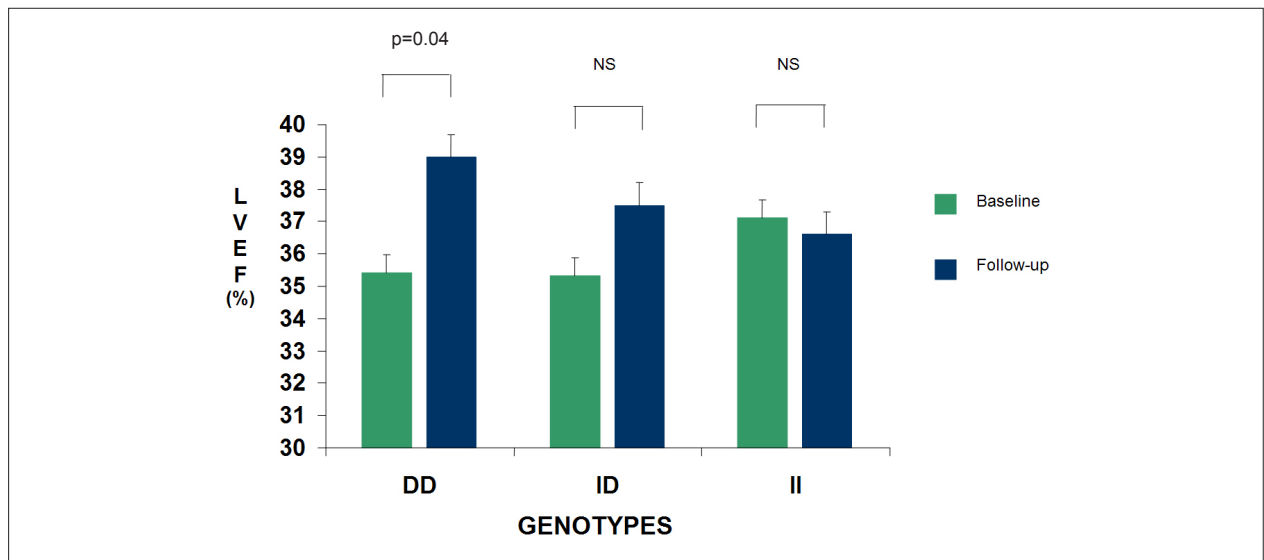


Figure 1 - Sequential left ventricular ejection fraction relative to the DD, ID and II genotypes. LVEF - Left Ventricular Ejection Fraction.

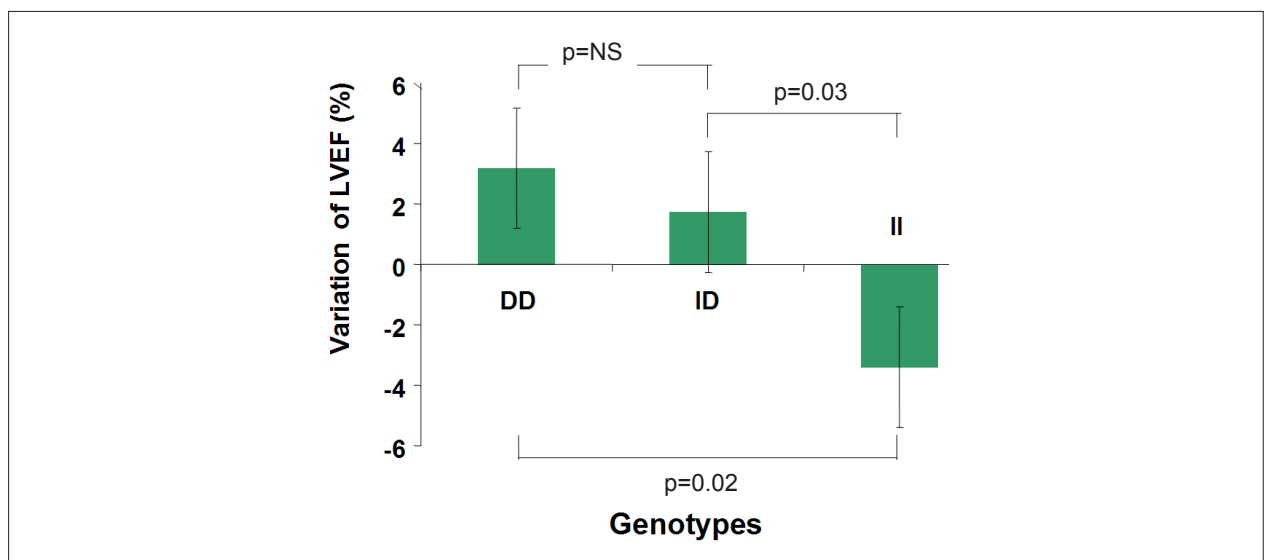


Figure 2 - D allele "copy number" effect on left ventricular ejection fraction variation. LVEF - Left Ventricular Ejection Fraction

inhibition<sup>3</sup>. In our study, there was a tendency to a linear progression of left ventricular function in patients with the II genotype and probably lower angiotensin-converting enzyme activity towards patients homozygous for the D allele and probably higher angiotensin-converting enzyme activity who might show a better response to angiotensin-converting enzyme inhibitors.

*Limitations of the study* - Our study has limitations. There was a relatively small number of patients analyzed in light of the heterogeneity of etiologies. Nevertheless, if we consider heart failure as the final phase of a series of different pathological conditions, such an etiologic heterogeneity enriches our observation since it allows us to assess heart failure and genetic background in the light of different pathogenetic characteristics. Another potential

limitation may be related to the small number of patients on beta-blocker therapy at the beginning of the study. This fact hinders further analysis on the interaction of this therapy and the angiotensin-converting enzyme genetic variants as previously demonstrated<sup>2,3</sup>.

In our study the angiotensin-converting enzyme plasma levels were not determined because more than 90% of the patients were on therapy with angiotensin-converting enzyme inhibitors. However, as previously outlined, plasma hormone levels do not by themselves describe the activity of neurohormonal activation and it is thus possible that tissue effect and receptor sensitivity might be playing a role<sup>23</sup>.

Our study is a non-randomized analysis and only patients with sequential echocardiographic studies were evaluated. These characteristics may imply a potential selection bias.

It is possible that patients with more advanced disease and an increased mortality incidence do not show the same pharmacogenetic association here described.

Another potential limitation lies in the fact that the angiotensin-converting enzyme inhibitor dose was not determined in the present study. Patients with the DD genotype and heart failure show the maximal benefits in terms of outcome on high-dose angiotensin-converting enzyme inhibitors, as recently published<sup>3</sup>.

Finally, the assessment of left ventricular function by echocardiography however practical may be also less than optimal and thus further studies that evaluate left ventricular ejection fraction by radionuclide ventriculography might be performed.

*Clinical implications* - The findings of our study add further information to the understanding of the angiotensin-converting enzyme gene polymorphism modulation of clinical response of patients with heart failure to treatment with angiotensin converting enzyme inhibitors. Further studies are necessary

to demonstrate whether genetic variants of the angiotensin-converting enzyme might be of help to individualize those patients with heart failure more prone to show a better response to angiotensin-converting enzyme inhibitors and consequently a better outcome.

#### Potential Conflict of Interest

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

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#### Study Association

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