BRAZILIAN JOURNAL
OF MEDICAL AND BIOLOGICAL RESEARCH
www.bjournal.com.br

ISSN 0100-879X
Volume 42 (8) 692-775 August 2009

CLINICAL INVESTIGATION

Braz J Med Biol Res, August 2009, Volume 42(8) 717-721

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The Brazilian Journal of Medical and Biological Research is partially financed by

CNPq Conselho Nacional de Desenvolvimento Científico e Tecnológico
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Brazilian Journal of Medical and Biological Research (2009) 42: 717-721
ISSN 0100-879X

Association of angiotensin-converting enzyme activity and polymorphism with echocardiographic measures in familial and nonfamilial hypertrophic cardiomyopathy

P.C. Buck[1], F. Fernandes[1], E. Arteaga[1], A.Y. Matsumoto[1], A.Q. Araujo[1], E.M. Oliveira[2], B.M. Ianni[1], F.J.A. Ramires[1], J.E. Krieger[1] and C. Mady[1]

[1]Unidade Clínica de Miocardiopatias, Instituto do Coração, Hospital das Clínicas, Faculdade de Medicina, [2]Escola de Educação Física e Esporte, Universidade de São Paulo, São Paulo, SP, Brasil

Correspondence to: P.C. Buck, Unidade Clínica de Miocardiopatias, InCor, HC, FMUSP, Av. Dr. Enéas de Carvalho Aguiar, 44, 05403-900 São Paulo, SP, Brasil
Fax: +55-11-3069-5346. E-mail: paula.buck@incor.usp.br

Angiotensin-converting enzyme (ACE) activity and polymorphism contribute significantly to the prognosis of patients with cardiomyopathy. The aim of this study was to determine the activity and type of ACE polymorphism in patients with familial and nonfamilial hypertrophic cardiomyopathy (HCM) and to correlate these with echocardiographic measurements (echo-Doppler). We studied 136 patients (76 males) with HCM (69 familial and 67 nonfamilial cases). Mean age was 41 ± 17 years. DNA was extracted from blood samples for the polymerase chain reaction and the determination of plasma ACE levels. Left ventricular mass, interventricular septum, and wall thickness were measured. Mean left ventricular mass index, interventricular septum and wall thickness in familial and nonfamilial forms were 154 ± 63 and 174 ± 57 g/m^2 (P = 0.008), 19 ± 5 and 21 ± 5 mm (P = 0.02), and 10 ± 2 and 12 ± 3 mm (P = 0.0001), respectively. ACE genotype frequencies were DD = 35%, ID = 52%, and II = 13%. A positive association was observed between serum ACE activity and left ventricular mass index (P = 0.04). Logistic regression showed that ACE activity was twice as high in patients with familial HCM and left ventricular mass index ≥190 g/m^2 compared with the nonfamilial form (P = 0.02). No other correlation was observed between ACE polymorphisms and the degree of myocardial hypertrophy. In conclusion, ACE activity, but not ACE polymorphisms, was associated with the degree of myocardial hypertrophy in the patients with HCM.

Key words: Echocardiography; ACE activity; ACE polymorphism; Hypertrophic cardiomyopathy; Left ventricular mass index

Received July 10, 2008. Accepted March 24, 2009

Introduction

Hypertrophic cardiomyopathy (HCM) is a complex genetic disease with broad phenotypic heterogeneity resulting in a range of outcomes and prognoses (1,2). The angiotensin-converting enzyme (ACE) gene deletion/insertion polymorphism, which has three distinct genotypes (II, ID, and DD) (1,3-5), is an important modifying factor of HCM. The ACE DD genotype is more common than the ID or II genotypes in patients with the familial form of HCM and has been associated with the severity of left ventricular hypertrophy and with interventricular septal, apical, and wall thickness (6-8).

ACE converts angiotensin I to angiotensin II (5), an important mediator in secondary myocardial hypertrophy, postmyocardial infarction remodeling, and heart failure. Although blood levels of angiotensin II are not increased in HCM patients, a relation seems to exist between HCM phenotype and ACE genetic polymorphism (9). In addition, angiotensin II has well-established profibrotic effects, and studies have suggested myocardial fibrosis as another important cause of diastolic dysfunction in HCM (10,11). We have also shown that losartan, a renin-angiotensin-aldosterone system (RAAS) blocker, leads to clinical im-

provement evaluated by functional class (NYHA), to a decrease in plasma levels of B-type natriuretic peptide, and to improved diastolic dysfunction in HCM patients (12). Nevertheless, there are no reports in the literature correlating ACE activity with myocardial hypertrophy in patients with familial and nonfamilial forms of HCM.

The aim of this study was to determine ACE polymorphism and its plasma activity in patients with the familial and nonfamilial forms of HCM and to correlate it with the echocardiographic measures.

Material and Methods

We selected 136 consecutive patients with HCM from the Cardiomyopathy Unit, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo/Heart Institute (InCor). Sixty-nine patients presented the familial form and 67 the nonfamilial form of HCM. Mean age was 41 ± 17 years, and 76 patients were males. All patients signed an informed consent form. The study was approved by the Ethics Committee of Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo (#360/98).

The inclusion criteria were diastolic septum thickness >15 mm in HCM patients and >13 mm in their relatives (on echocardiography), and absence of left ventricular dilation (13). The exclusion criteria were use of ACE inhibitors and/or angiotensin receptor blockers, hypertension (blood pressure ≥139/89 mmHg) (14) and other comorbidities known to influence septum thickness. The familial form was characterized by a history of sudden death and a diagnosis of HCM among third-degree relatives younger than 40 years of age. Patients who met none of these criteria were classified as presenting nonfamilial HCM (15).

Table 1. Clinical data of patients with familial and nonfamilial hypertrophic cardiomyopathy (HCM).

Variables	Familial HCM (N = 69)	Nonfamilial HCM (N = 67)
Age (years)	38 ± 18	43 ± 7
Gender		
Male	35 (51%)	41 (61%)
Female	34 (49%)	26 (39%)
Functional class (NYHA)		
I	35 (51%)	38 (57%)
II	29 (42%)	20 (30%)
III	4 (6%)	8 (12%)
IV	1 (1%)	1 (1%)

Data are reported as means ± SD for age and number of patients with percent of group in parentheses for all other variables. NYHA: New York Heart Association functional classification of heart failure. There were no statistical differences between groups for all parameters (age: nonpaired *t*-test; proportions: chi-square test).

Echocardiographic studies were performed using M-mode echocardiography according to the recommendations of the American Society of Echocardiography. The following parameters were determined: left ventricular mass, interventricular septum, and wall thickness (Acuson Sequoia 512 unit, USA, and a 2-3.5-MHz multifrequency transducer) (16). Left ventricular mass index was obtained using the formula "mass/BMI (g/m^2)", where mass (g) = 0.8 $\{1.04[(Dd + PW + IVS)^3 - (Dd)^3]\}$ + 0.6 (Dd = left ventricular diastolic diameter, PW = posterior wall thickness, IVS = interventricular septal thickness, in cm), and body mass index (m^2) = $weight^{0.425}$ x $height^{0.725}$ x 0.00718 (17,18). The echocardiographer was blind to the objective of the study.

Venous blood was collected into tubes containing EDTA (disodium salt) for the isolation of peripheral blood leukocytes. Genomic DNA was extracted from these cells by a standard method and amplified by the polymerase chain reaction (19).

Serum ACE activity was determined by the method described by Alves et al. (20), which is based on the use of a fluorescent peptide (Abz-FRK (Dnp) P-OH) hydrolyzed with high affinity by ACE (Kcat/Km = 45.4/s). ACE activity is reported as fluorescence units (FU)/mg of protein, or FU/mL of serum (1 m FU = nmol of Abz-FRK (Dnp) P-OH hydrolyzed per min).

Statistical analysis

Left ventricular mass was stratified into quintiles. Qualitative variables are reported as absolute frequencies (n) and percentages (%). The association of these variables with left ventricular mass index ≥190 g/m^2 was determined by the χ^2 test. The association between echocardiographic measures and ACE polymorphism and activity was determined by the Spearman test. Quantitative variables are reported as means ± SD or as minimum and maximum values. Mean values were compared by the Student *t*-test. Variables that were statistically significant in univariate analysis were used to adjust the logistic regression model.

A P value of ≤0.05 was considered to be significant (21).

Results

The distribution of ACE gene polymorphisms was as follows: DD was observed in 47 patients (35%), ID in 71 (52%) patients, and II in 18 (13%) patients. Table 1 describes the main clinical data for the patients studied.

Table 2 shows the mean left ventricular mass indexes (P = 0.008), interventricular septal thickness (P = 0.02) and wall thickness (P = 0.0001) in familial and nonfamilial HCM.

Mean ACE activity in the familial and nonfamilial forms was 56,414 ± 19,236 and 55,085 ± 22,634 FU/mL, respectively (P = 0.71). No correlation was found between ACE polymorphism and degree of myocardial hypertrophy. ACE activity was associated with left ventricular mass index (P = 0.04).

A logistic regression curve showed that ACE activity was twice as high in familial HCM patients with a left ventricular mass index ≥190 g/m^2 compared to patients with the nonfamilial form (Figure 1; r = 0.766; P = 0.02). ACE activity was not correlated with either interventricular septal thickness (P = 0.08) or wall thickness (P = 0.18).

Discussion

The present results indicate that there are no differences between the familial and nonfamilial forms of HCM regarding ACE insertion/deletion polymorphism.

Marian et al. (22) observed that the ACE D allele was more frequent in affected relatives of HCM patients than in unaffected relatives. They also observed that the DD genotype was more frequent in HCM patients, especially in those with a family history of sudden death. Yoneya et al. (7) concluded that the ACE D allele is a genetic factor for cardiac hypertrophy and that the ACE DD genotype is a potential genetic marker. They demonstrated that the frequency of the D allele in nonfamilial forms was higher than that in the familial form and controls. However, we showed that the D allele and DD genotype had no influence on hypertrophy.

As also observed here, Yamada et al. (23) reported that ACE polymorphism was not related to HCM in Japanese patients, and that variants of this polymorphism did not contribute to the genesis or progression of the disease. When compared with previous studies, Yamada et al. (23) found a different distribution of ACE genotypes: in 71 patients with the nonfamilial form of HCM, they identified 11% with DD, 45% with ID, and 44% with II, a distribution similar to that observed in control subjects (14% with DD, 45% with ID, and 41% with II).

Lopez-Haldon et al. (24) studied the effect of ACE polymorphism in patients with the familial and nonfamilial forms of HCM and concluded that the D allele and DD genotype were associated with a predisposition to hypertrophy. Doolan et al. (25) investigated the progression of left ventricular hypertrophy and the ACE polymorphism in HCM patients and demonstrated that progression of left ventricular hypertrophy is higher in the presence of the DD genotype than in other genotypes. Thus, the DD genotype could be an important predictor of disease progression in HCM. In the present study, however, we did not find a significant difference between genotypes and alleles in the familial and nonfamilial forms of HCM and between patients with different degrees of myocardial hypertrophy determined by echocardiographic parameters, in agreement with the observations made by Rai et al. (26).

Table 2. Mean left ventricular mass index (LVMI), interventricular septum (IVS), posterior wall (PW), and left atrium (LA) in patients with familial and nonfamilial hypertrophic cardiomyopathy (HCM).

Variables	Familial HCM (N = 67)	Nonfamilial HCM (N = 67)
LVMI (g/m^2)	154 ± 63	174 ± 57*
IVS (mm)	19 ± 5	21 ± 5*
PW (mm)	10 ± 2	12 ± 3*
LA (mm)	42 ± 8	44 ± 9

Data are reported as means ± SD. *P < 0.05 compared to familial HCM (nonpaired *t*-test).

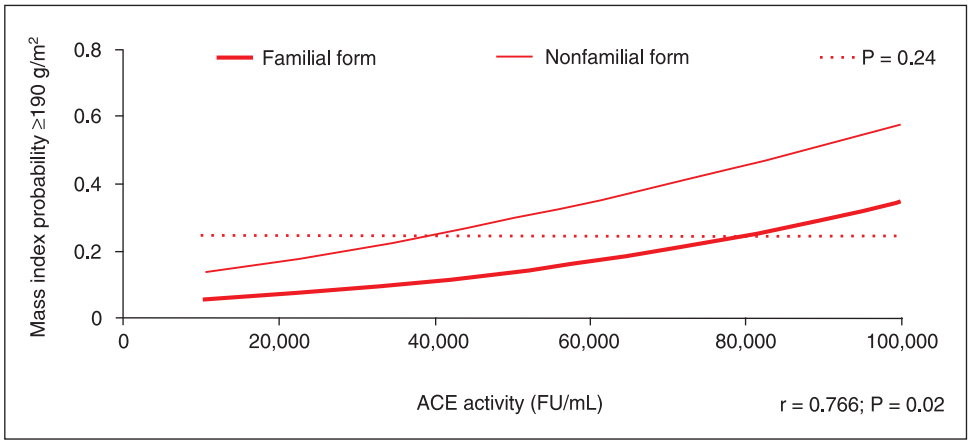

Figure 1. Logistic regression analysis of ventricular mass index ≥190 g/m^2 *vs* angiotensin-converting enzyme (ACE) activity in patients with familial and nonfamilial hypertrophic cardiomyopathy. FU = fluorescence units.

Perkins et al. (15) evaluated ACE polymorphism according to the genesis and progression of the disease in patients with the nonfamilial form of HCM. They demonstrated that patients with the DD genotype had a higher level of ACE and angiotensin II and, consequently, an increase in hypertrophy and fibrosis, and concluded that the DD genotype influences the degree of left ventricular hypertrophy and the severity of the disease.

Other findings indicate that HCM can be considered a polygenic disease with different degrees of penetrance and mutations, leading to different genotypes and phenotypic expression. In Brazil, this fact could be explained by the high incidence of miscegenation throughout our history (27). Tesson et al. (28) showed that the ACE genotype can influence the phenotypic expression of hypertrophy, and that this influence depends on mutation, which supports the concept of multiple modifier genes in patients with the familial form of HCM. Likewise, Lechin et al. (4) showed that the ACE gene modifies the phenotypic expression of hypertrophy in HCM patients. In addition, Ortlepp et al. (29), who studied the genetic polymorphism of the RAAS associated with the expression of left ventricular hypertrophy in HCM, observed several polymorphisms that influenced both the prevalence and the degree of left ventricular hypertrophy caused by mutation in the cardiac myosin-binding protein C gene.

Our study showed higher left ventricular mass index and increased interventricular septum and wall thickness hypertrophy in patients with the nonfamilial form of HCM compared with patients with the familial form. In addition, we observed that patients with higher levels of ACE activity had a large left ventricular mass index. To our knowledge, this is the first report of ACE activity in patients with HCM. Angiotensin I is converted by ACE into angiotensin II, which is an important mediator in myocardial hypertrophy and fibrosis. Masutomo et al. (30), in an experimental study of HCM using losartan, demonstrated a decrease in left ventricular collagen concentration. Lim et al. (31) observed similar results in an experimental study using the troponin T gene in a transgenic mouse model of human HCM. These investigators observed that losartan treatment decreased myocardial fibrosis and the expression of collagen type I.

The present study allowed us to individualize and select HCM patients with higher levels of ACE and consequently a large left ventricular mass index for treatment using RAAS blockers. A logistic regression curve showed that in patients with familial HCM and left ventricular mass index $\geq$190 g/m^2 ACE activity was twice that of nonfamilial form patients ($P = 0.02$). For this reason, we believe that ACE antagonism could be different in the familial and nonfamilial forms of HCM. More studies are necessary to evaluate this hypothesis.

We observed an association between ACE activity and left ventricular mass index. Otherwise, there was no correlation between the ACE polymorphism and the degree of myocardial hypertrophy.

References

1. Marian AJ, Roberts R. Recent advances in the molecular genetics of hypertrophic cardiomyopathy. *Circulation* 1995; 92: 1336-1347.
2. Arteaga E, Ianni BM, Fernandes F, Mady C. Benign outcome in a long-term follow-up of patients with hypertrophic cardiomyopathy in Brazil. *Am Heart J* 2005; 149: 1099-1105.
3. Danser AH, Schalekamp MA, Bax WA, van den Brink AM, Saxena PR, Riegger GA, et al. Angiotensin-converting enzyme in the human heart. Effect of the deletion/insertion polymorphism. *Circulation* 1995; 92: 1387-1388.
4. Lechin M, Quinones MA, Omran A, Hill R, Yu QT, Rakowski H, et al. Angiotensin-I converting enzyme genotypes and left ventricular hypertrophy in patients with hypertrophic cardiomyopathy. *Circulation* 1995; 92: 1808-1812.
5. Pfeufer A, Osterziel KJ, Urata H, Borck G, Schuster H, Wienker T, et al. Angiotensin-converting enzyme and heart chymase gene polymorphisms in hypertrophic cardiomyopathy. *Am J Cardiol* 1996; 78: 362-364.
6. Iwai N, Ohmichi N, Nakamura Y, Kinoshita M. DD genotype of the angiotensin-converting enzyme gene is a risk factor for left ventricular hypertrophy. *Circulation* 1994; 90: 2622-2628.
7. Yoneya K, Okamoto H, Machida M, Onozuka H, Noguchi M, Mikami T, et al. Angiotensin-converting enzyme gene polymorphism in Japanese patients with hypertrophic cardiomyopathy. *Am Heart J* 1995; 130: 1089-1093.
8. Ishanov A, Okamoto H, Yoneya K, Watanabe M, Nakagawa I, Machida M, et al. Angiotensinogen gene polymorphism in Japanese patients with hypertrophic cardiomyopathy. *Am Heart J* 1997; 133: 184-189.
9. Kawano H, Do YS, Kawano Y, Starnes V, Barr M, Law RE, et al. Angiotensin II has multiple profibrotic effects in human cardiac fibroblasts. *Circulation* 2000; 101: 1130-1137.
10. Olivotto I, Cecchi F, Casey SA, Dolara A, Traverse JH, Maron BJ. Impact of atrial fibrillation on the clinical course of hypertrophic cardiomyopathy. *Circulation* 2001; 104: 2517-2524.
11. Yamaji K, Fujimoto S, Yutani C, Ikeda Y, Mizuno R, Hashimoto T, et al. Does the progression of myocardial fibrosis lead to atrial fibrillation in patients with hypertrophic cardiomyopathy? *Cardiovasc Pathol* 2001; 10: 297-303.

12. Araujo AQ, Arteaga E, Ianni BM, Buck PC, Rabello R, Mady C. Effect of Losartan on left ventricular diastolic function in patients with nonobstructive hypertrophic cardiomyopathy. *Am J Cardiol* 2005; 96: 1563-1567.
13. Spirito P, Bellone P, Harris KM, Bernabo P, Bruzzi P, Maron BJ. Magnitude of left ventricular hypertrophy and risk of sudden death in hypertrophic cardiomyopathy. *N Engl J Med* 2000; 342: 1778-1785.
14. IV Brazilian Guidelines in Arterial Hypertension Work Groups. IV Brazilian Guidelines on Hypertension. *Arq Bras Cardiol* 2004; 82 (Suppl 4): 7-22.
15. Perkins MJ, Van Driest SL, Ellsworth EG, Will ML, Gersh BJ, Ommen SR, et al. Gene-specific modifying effects of pro-LVH polymorphisms involving the renin-angiotensin-aldosterone system among 389 unrelated patients with hypertrophic cardiomyopathy. *Eur Heart J* 2005; 26: 2457-2462.
16. Henry WL, DeMaria A, Gramiak R, King DL, Kisslo JA, Popp RL, et al. Report of the American Society of Echocardiography Committee on Nomenclature and Standards in Two-dimensional Echocardiography. *Circulation* 1980; 62: 212-217.
17. Gottdiener JS, Bednarz J, Devereux R, Gardin J, Klein A, Manning WJ, et al. American Society of Echocardiography recommendations for use of echocardiography in clinical trials. *J Am Soc Echocardiogr* 2004; 17: 1086-1119.
18. DuBois D, Dubois EF. A formula to estimate the approximate surface area if height and weight be known. *Arch Intern Med* 1961; 17: 863.
19. Mullis KB. The unusual origin of the polymerase chain reaction. *Sci Am* 1990; 262: 56-61-64-65.
20. Alves MF, Araujo MC, Juliano MA, Oliveira EM, Krieger JE, Casarini DE, et al. A continuous fluorescent assay for the determination of plasma and tissue angiotensin I-converting enzyme activity. *Braz J Med Biol Res* 2005; 38: 861-868.
21. Rosner B. *Fundamentals of biostatistics*. 4th edn. New York: Duxbury Press; 1994.
22. Marian AJ, Yu QT, Workman R, Greve G, Roberts R. Angiotensin-converting enzyme polymorphism in hypertrophic cardiomyopathy and sudden cardiac death. *Lancet* 1993; 342: 1085-1086.
23. Yamada Y, Ichihara S, Fujimura T, Yokota M. Lack of association of polymorphisms of the angiotensin converting enzyme and angiotensinogen genes with nonfamilial hypertrophic or dilated cardiomyopathy. *Am J Hypertens* 1997; 10: 921-928.
24. Lopez-Haldon J, Garcia-Lozano JR, Martinez MA, Nunez-Roldan A, Burgos CJ. [The effect of polymorphisms of the angiotensin-converting enzyme and angiotensinogen genes on the phenotypic expression of Spanish patients with hypertrophic cardiomyopathy]. *Med Clin* 1999; 113: 161-163.
25. Doolan G, Nguyen L, Chung J, Ingles J, Semsarian C. Progression of left ventricular hypertrophy and the angiotensin-converting enzyme gene polymorphism in hypertrophic cardiomyopathy. *Int J Cardiol* 2004; 96: 157-163.
26. Rai TS, Dhandapany PS, Ahluwalia TS, Bhardwaj M, Bahl A, Talwar KK, et al. ACE I/D polymorphism in Indian patients with hypertrophic cardiomyopathy and dilated cardiomyopathy. *Mol Cell Biochem* 2008; 311: 67-72.
27. Tirone AP, Arteaga E, Pereira AC, Krieger JE, Buck PC, Ianni BM, et al. [Research of markers for the genes of the heavy chain of cardiac beta-myosin and myosin binding protein C in relatives of patients with hypertrophic cardiomyopathy]. *Arq Bras Cardiol* 2005; 84: 467-472.
28. Tesson F, Dufour C, Moolman JC, Carrier L, al-Mahdawi S, Chojnowska L, et al. The influence of the angiotensin I converting enzyme genotype in familial hypertrophic cardiomyopathy varies with the disease gene mutation. *J Mol Cell Cardiol* 1997; 29: 831-838.
29. Ortlepp JR, Vosberg HP, Reith S, Ohme F, Mahon NG, Schroder D, et al. Genetic polymorphisms in the renin-angiotensin-aldosterone system associated with expression of left ventricular hypertrophy in hypertrophic cardiomyopathy: a study of five polymorphic genes in a family with a disease causing mutation in the myosin binding protein C gene. *Heart* 2002; 87: 270-275.
30. Masutomo K, Makino N, Fushiki MS. Effects of losartan on the collagen degradative enzymes in hypertrophic and congestive types of cardiomyopathic hamsters. *Mol Cell Biochem* 2001; 224: 19-27.
31. Lim DS, Lutucuta S, Bachireddy P, Youker K, Evans A, Entman M, et al. Angiotensin II blockade reverses myocardial fibrosis in a transgenic mouse model of human hypertrophic cardiomyopathy. *Circulation* 2001; 103: 789-791.