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The association of ACE gene D/I polymorphism with cardiovascular risk factors in a population from Rio de Janeiro

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Our aim was to determine the frequencies of the angiotensin-converting enzyme (ACE) gene alleles D and I and any associations to cardiovascular risk factors in a population sample from Rio de Janeiro, Brazil. Eighty-four adults were selected consecutively during a 6-month period from a cohort subgroup of a previous large cross-sectional survey in Rio de Janeiro. Anthropometric data and blood pressure measurements, echocardiogram, albuminuria, glycemia, lipid profile, and ACE genotype and serum enzyme activity were determined. The frequency of the ACE*D and I alleles in the population under study, determined by PCR, was 0.59 and 0.41, respectively, and the frequencies of the DD, DI, and II genotypes were 0.33, 0.51, and 0.16, respectively. No association between hypertension and genotype was detected using the Kruskal-Wallis method. Mean plasma ACE activity (U/mL) in the DD (N = 28), DI (N = 45) and II (N = 13) groups was 43 (in males) and 52 (in females), 37 and 39, and 22 and 27, respectively; mean microalbuminuria (mg/dL) was 1.41 and 1.6, 0.85 and 0.9, and 0.6 and 0.63, respectively; mean HDL cholesterol (mg/dL) was 40 and 43, 37 and 45, and 41 and 49, respectively, and mean glucose (mg/dL) was 93 and 108, 107 and 98, and 85 and 124, respectively. A high level of ACE activity and albuminuria, and a low level of HDL cholesterol and glucose, were found to be associated with the DD genotype. Finally, the II genotype was found to be associated with variables related to glucose intolerance.

Key words: Angiotensin-converting enzyme gene polymorphism; Left ventricular hypertrophy; Angiotensin-converting enzyme activity; Albuminuria; Hypertension

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Introduction

The variability in the prevalence of cardiovascular risk factors and their association with stroke or ischemic heart disease in different populations is certainly due to a complex interaction between environmental and genetic factors. Among the multiple genetic polymorphisms described, and possibly playing an active role in the pathogenesis of hypertension and cardiovascular disease (1), is the angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism (2). The physiologic and pathophysiologic role of this polymorphism in the function of the renin-angiotensin-aldosterone system and in the clinical manifestations of the vascular atherothrombotic process, as well as its association with the classical cardiovascular risk factors (CVRF), is not completely understood.

Angiotensin II is a vasoconstrictor agent that also promotes cell proliferation and myocardial hypertrophy. Left

ventricle hypertrophy (LVH) increases by 1.5 times the cardiovascular morbidity and mortality (3), and the presence of LVH in hypertension cannot be totally explained by pressure overload (4). Therefore, it is reasonable to assume that LVH or left ventricular mass index (LVMI) might be associated with the DD genotype since plasma ACE activity is higher in DD patients (5).

Proteinuria is a common finding in hypertension and diabetic nephropathy and a well-established risk of death (6). In hypertensive patients, the ACE*D allele poses a higher risk for microalbuminuria and treatment with ACE inhibitors produces a greater reduction in microalbuminuria in hypertensive patients homozygous for the ACE*I allele (7). In addition, coronary artery disease may be associated with the DD genotype in a low risk group, as shown by the ECTIM case-control study (8). The ACE*I allele has also been shown to be associated with glucose intolerance and with insulin resistance in women with body mass index (BMI) >25 kg/m^2 (9). Finally, triglyceride levels have been found to be higher in patients with the DD genotype (10).

Much remains to be studied in order to better understand the complex mechanisms of blood pressure (BP) regulation and the associations between CVRF and the genetic background of individuals at risk. In the Brazilian population, little is known about the frequency of the I/D polymorphism of the ACE gene and its associations with CVRF. In the present study, we investigated the association of the ACE gene I/D polymorphism with arterial hypertension, LVMI, BMI, microalbuminuria, blood lipid profile, and blood glucose in a population sample from Rio de Janeiro, Brazil.

Subjects and Methods

Population under study

A cross-sectional population survey stratified by socioeconomic class was performed during 1991 and 1992 to determine the prevalence of arterial hypertension and other CVRF in Rio de Janeiro, Ilha do Governador (11). A total of 1272 individuals were interviewed and referred to the Clementino Fraga Filho University Hospital for clinical evaluation and follow-up. In the first semester of 1998, 85 consecutive subjects attending their regular outpatient appointments were invited to sign an informed consent form to participate in the present study, which had been approved by the National Research Ethics Committee. After exclusion of one patient due to the use of immunosuppressive drugs, which alter ACE levels, data were collected and analyzed from the remaining 84 subjects. Upon entry into the present study, from 1998 to 2001, all 84 subjects were submitted to a thorough clinical examination and their hospital records were reviewed since their initial visit in 1991/1992. Blood samples were used to determine ACE gene I/D polymorphism and serum ACE enzyme activity. Twenty-four-hour urine samples were collected to measure proteinuria and microalbuminuria. The following data were collected at the initial (1991/1992) and last (current) examinations (1998/2001): age, height, weight, BMI, BP, use of anti-hypertensive drugs, blood glucose and use of hypoglycemic drugs, total cholesterol, HDL cholesterol, triglycerides and use of hypolipemic drugs. Patients with systolic BP $\geq$140 mmHg or diastolic BP $\geq$90 mmHg, or taking anti-hypertensive drugs were diagnosed as hypertensive. One specialist blind to the clinical data reviewed the first transthoracic echocardiogram. Echocardiograph data included the thickness of the posterior wall and septum and the diameter of the left ventricle at the end of diastole and systole. Left ventricular mass (LVM) was derived from the Devereux equation (12). LVMI was calculated by dividing LVM by body surface area in square meters.

Genotyping and determination of plasma ACE activity, proteinuria and microalbuminuria

Genomic DNA was extracted from white blood cells and the ACE gene polymorphism was determined by PCR as described in the literature (8). Briefly, primers flanking the polymorphic region were used to PCR-amplify a portion of the ACE gene, and the amplified product was analyzed by UV light after gel electrophoresis and ethidium bromide staining to determine the I/D pattern. Allele-specific I primers were used in 10% of the DD individuals to validate the genotype assigned with the flanking primers. Plasma ACE activity was determined in blood samples by kinetic spectrophotometry by Sigma Diagnostics (USA). In patients using ACE inhibitors, plasma ACE activity was determined after a drug washout period of 15 days. Proteinuria and microalbuminuria were determined in 24-h urine samples by immunoturbidimetry/nephelometry (Dade Behring Marburg, Germany).

Statistical analysis

The software packages STATA 7.0 and SPSS 10.0 were used for statistical analysis. The chi-square method was used to determine the presence of Hardy-Weinberg equilibrium and to compare genotype frequencies in patients with and without hypertension. Genotype and hypertension data were used each in turn as the dependent variable in analysis of variance by the non-parametric Kruskal-Wallis test. The independent variables were: gender, age, plasma ACE activit y, BMI, blood glucose, total

cholesterol, HDL cholesterol, triglycerides, microalbuminuria, and LVMI. Sex interaction with total and HDL cholesterol, blood glucose, LVMI, and BMI was also used in the logistic model since these variables were found to present sex differences. The multinomial logistic regression method was used to determine the final model of association among independent variables with genotype as the dependent variable (13).

Results

All 84 subjects were genotyped for ACE gene polymorphism, while plasma ACE activity was determined in 63 subjects and microalbuminuria in 55 subjects. The mean follow-up period since the initial visit was 6.4 ± 2 years. Table 1 shows the clinical characteristics of the participants in the present study at the initial visit to the hospital (1991/1992) in the survey study at Ilha do Governador (11) and at the last visit (1998/2001). The female sex predominated (65.5%), with females being 6.6 years younger on average than males. Mean systolic and diastolic BP reduction during follow-up was 11 mmHg (146 to 135 mmHg) and 6 mmHg (88 to 82 mmHg), respectively. At the beginning of the follow-up period (1991/1992), 55.2% of men and 45.5% of women were hypertensive, with 50% of men and 68% of women taking anti-hypertensive drugs. At the last visit (1998/2001), all hypertensive subjects were taking anti-hypertensive medication. Of these, 41 had been previously classified as hypertensive and 4 became hypertensive during follow-up. Eleven subjects were diabetic on the occasion of the first visit, and only 4 of them were taking hypoglycemic drugs. One subject became diabetic during follow-up. No subject was using hypolipemic drugs at the initial visit.

We chose the initial laboratory results (1991/1992) for data analysis to improve the odds of observing an association between the ACE I/D polymorphism and the variables studied by minimizing the effects of therapy. The I/D allele frequencies were 0.41 and 0.59, respectively, and were in Hardy-Weinberg equilibrium (P = 0.96). The heterozygous DI genotype was the most frequent (0.51) and the homozygous II genotype was the least frequent (0.16). Genotype frequencies were DD = 0.28, DI = 0.62, and II = 0.10 among men and DD = 0.36, DI = 0.46, and II = 0.18 among women. Thus, genotypes II and DD were relatively more frequent among women when compared to men, and heterozygous DI was present at a higher percentage among men. Table 2 shows analysis of variance using the non-parametric Kruskal-Wallis method with genotype as the dependent variable and possible intermediate phenotypes as independent variables. The only variable significantly associated with genotype was plasma ACE activity (P = 0.02 for men and 0.003 for women). Women showed higher levels of plasma ACE activity than men for any genotype group, and the difference between genders was higher in the DD genotype group.

Women with the DD genotype had the highest mean values of triglyceride levels, microalbuminuria, plasma ACE activity and BMI, among all genotype groups, while women with the II genotype had the highest mean values of glycemia and HDL cholesterol. Men with the DD genotype had the highest mean values of LVMI and cholesterol. Women with the DD genotype had the lowest mean values of LVMI which, however, were similar to those of women with the II genotype. BMI was always higher in women than in men for any genotype group. DD women had the highest mean BMI, but the highest difference in mean BMI by gender was noted between men with the II genotype (24 kg/m^2) and women with the II genotype (28 kg/m^2). Microalbuminuria increased from the lowest values in the II genotype to the highest values in the DD genotype, both in men and in women.

The prevalence of hypertension was also analyzed by genotype and gender. We found an increasing prevalence of hypertension from DD to II genotype in women. Con-

Table 1. Clinical characteristics of the subjects under study at the first and last hospital visits.

Clinical characteristics	Total (N = 84)		Male (N = 29; 34.5%)		Female (N = 55; 65.5%)	
	First	Last	First	Last	First	Last
Age (years)	51.3 ± 16.3	57.7 ± 17.2	55.6 ± 14	62 ± 15	49 ± 17	55.4 ± 18
SBP (mmHg)	146 ± 27	135 ± 20	147.6 ± 23.7	134.5 ± 16.8	144.6 ± 29	135.5 ± 22.3
DBP (mmHg)	88 ± 15.3	82 ± 10.8	90.9 ± 16.4	83 ± 9.8	86.5 ± 14.6	81 ± 11.3
Hypertension (N)	41 (48.8%)	45 (53.5%)	16 (55.2%)	18 (62.1%)	25 (45.5%)	27 (49.1%)
Diabetes mellitus (N)	11 (13.0%)	12 (14.0%)	4 (14.0%)	5 (17.0%)	7 (13.0%)	7 (13.0%)

Dara are reported as mean ± SD unless otherwise indicated. SBP = systolic blood pressure; DBP = diastolic blood pressure; First = initial clinical examination (1991/1992); Last = last clinical examination (1998/2001). N = number of individuals.

versely, in men there was an increasing prevalence of hypertension with the ACE*D allele. The relationship of intermediate phenotypes with hypertension and normotension was also analyzed by the non-parametric Kruskal-Wallis method (Table 3). Association between arterial hypertension and glycemia, total cholesterol, triglycerides as well as LVMI was observed in the population under study. However, when we analyzed the intermediate phenotypes by gender we noted that the LVMI was associated exclusively with the female hypertensive group.

Finally, we performed multinomial logistic regression, with the genotype being considered to be the dependent variable. The independent variables were the intermediate phenotypes previously shown in Table 2 along with gender and age. The final model is shown in Table 4. The DD genotype showed a positive association with plasma ACE activity and microalbuminuria while HDL cholesterol and glucose were negatively associated, meaning that HDL cholesterol was lower in subjects with the DD genotype while glycemia was higher in subjects with the II genotype.

Discussion

In 1991/92, a survey to study the prevalence of arterial

Table 2. Mean values of intermediate phenotypes according to genotype of ACE gene I/D polymorphism and gender.

	ACE polymorphism genotypes							
	DD (0.34)		DI (0.51)		II (0.15)		P value	
	M (N = 8)	F (N = 20)	M (N = 18)	F (N = 25)	M (N = 3)	F (N = 10)	M (N = 29)	F (N = 55)
Genotype frequencies	0.28	0.36	0.62	0.46	0.10	0.18	NA	NA
Glucose, mg/dL (N = 83)	93	108	107	98	85	124	0.24	0.19
Total cholesterol, mg/dL (N = 84)	244	219	196	222	189	239	0.26	0.68
HDL cholesterol, mg/dL (N = 83)	40	43	37	45	41	49	0.62	0.14
Triglycerides, mg/dL (N = 84)	165	192	184	142	90	143	0.22	0.08
Microalbuminuria, mg/dL (N = 55)	1.41	1.60	0.85	0.90	0.60	0.63	0.36	0.24
Plasma ACE activity, U/mL (N = 63)	43	52	37	39	22	27	0.02	0.003
BMI, kg/m^2 (N = 83)	28	29	26	27	24	28	0.14	0.42
LVMI, g/m^2 (N = 79)	102	79	95	82	94	80	0.56	0.63
AH prevalence (%)	62.5	40	66.7	52	33.3	60	NA	NA

N = number of individuals; M = male; F = female; HDL cholesterol = high-density lipoprotein cholesterol; ACE = angiotensin-converting enzyme; BMI = body mass index; LVMI = left ventricular mass index; AH = arterial hypertension; NA = not applicable. ACE activity: 1 U = 1 nmol of substrate converted/min. The Kruskal-Wallis test was used for statistical analysis.

Table 3. Comparison of intermediate phenotype averages by gender and clinical diagnosis of hypertension.

Intermediate phenotypes	Hypertensive (N = 45)			Normotensive (N = 39)			P value		
	M	F	T	M	F	T	M	F	T
Glucose (mg/dL)	109	122	117	88	91	90	0.01	0.12	0.01
Cholesterol (mg/dL)	221	233	228	189	215	208	0.08	0.14	0.04
HDL cholesterol (mg/dL)	38	45	42	40	45	44	0.34	0.64	0.26
Triglycerides (mg/dL)	188	184	185	138	138	138	0.06	0.02	0.003
Microalbuminuria (mg/dL)	1.12	0.72	0.88	0.72	1.32	1.18	0.06	0.22	0.81
Plasma ACE activity (U/mL)	38	43	41	36	40	39	0.89	0.57	0.61
BMI (g/m^2)	27	28	28	26	27	27	0.32	0.20	0.14
LVMI (g/m^2)	101	92	96	91	71	76	0.48	0.0001	0.0001

N = number of individuals; M = male; F = female; T = values for the total number of subjects according to clinical diagnosis of hypertension; HDL cholesterol = high-density lipoprotein cholesterol; ACE = angiotensin-converting enzyme; BMI = body mass index; LVMI = left ventricular mass index. ACE activity: 1 U = 1 nmol of substrate converted/min. The Kruskal-Wallis test was used for statistical analysis.

hypertension and other CVRF in Rio de Janeiro, Brazil (11) found the prevalence of hypertension to be 38% (14), an unexpectedly high prevalence in view of previous surveys (15). Obesity also showed a high prevalence, especially among women of lower socioeconomic level and men of higher socioeconomic level (16). All participants recruited from our cohort study at Ilha do Governador live in the same geographic area of a Brazilian city with diverse socioeconomic and cultural backgrounds. The prevalence of hypertension found later in 1998 (48.8%) was higher compared to the initial survey (38%), probably due to the fact that the subjects are now older. Furthermore, younger subjects have less health problems and tend to attend follow-up consultations less frequently than older ones.

Table 4. Multinomial logistic regression analysis of intermediate phenotypes and ACE genotype (comparison group = II genotype).

	B	P value	95% confidence interval for odds ratios
DD genotype			
HDL-C	–8.617E-02	0.091	0.830 to 1.029
Microalbuminuria	17.597	0.000	5020619.075 to 383582305.700
ACE	0.188	0.002	1.073 to 1.357
Glucose	–5.421E-03	0.758	0.961 to 1.029
DI genotype			
HDL-C	–3.099E-02	0.373	0.906 to 1.038
Microalbuminuria	15.979	–	8699884.856 to 8703622.791
ACE	0.125	0.020	1.020 to 1.259
Glucose	–3.298E-02	0.112	0.929 to 1.008

HDL-C = high-density lipoprotein cholesterol; ACE = plasma angiotensin-converting enzyme activity.

There were more women (65.5%) than men and men were older (mean age: 55.6 years) than women (mean age: 49 years). Retired men and housewives tend to come more often for consultation, a fact that might explain this difference. The prevalence of the ACE genotype was in Hardy-Weinberg equilibrium and in agreement with that expected for the Brazilian population (17,18). Therefore, no major selection bias appears to have been present regarding the genetic frequencies of the subjects studied. Our results showed no relationship between ACE gene polymorphism and hypertension, in agreement with the results of Cambien et al. (8). Staessen et al. (19), in a meta-analysis of 23 studies, found no association of ACE gene polymorphism with arterial hypertension. We found a higher frequency of hypertension among women with the II genotype, while in men the higher frequency of hypertension was found in the DD and DI genotypes, as also previously shown in the Framingham study (20). Also, women had higher plasma ACE activity than men in all genotype groups. High plasma ACE activity has been reported to be associated with the development of diabetic nephropathy in type 1 diabetes (21) and may play a role in obesity. Thus, the different association patterns of the II genotype in men and women may reflect gender differences in the role of factors predisposing to the development of hypertension, diabetes and obesity.

LVMI increase or cardiac hypertrophy is known to occur with elevated BP, as confirmed in the present study. Other factors causing myocardial hypertrophy or cardiac interstitial matrix growth certainly influence this association (22). In the present study, hypertensive versus normotensive patients showed statistically significant differences in LVMI ($P < 0.0001$), mainly due to the subgroup of women. LVMI was also higher in hypertensive men but the difference was not statistically significant, possibly due to the small number of men in our sample. Among men, BMI, glycemia and BP were lower in the II genotype group, and men had higher LVMI than women, especially among subjects with the DD genotype. Several studies have described higher LVMI in men with the DD genotype (23,24). This difference was even more prominent when hypertensive and untreated men were compared (24). Men with the DD genotype were shown to be more prone to LVH associated with physical exercise than men with the DI or II genotype (25). These data also suggest that gender may affect the influence of several risk factors such as hypertension, BMI and glycemia.

Women had higher microalbuminuria levels than men in all genotype groups. Microalbuminuria values in the DD genotype group were more than twice those shown by the II genotype groups in both sexes. Microalbuminuria was higher in hypertensive than in normotensive men, exactly the opposite found in equivalent groups of women. Kruskal-Wallis tests for microalbuminuria and genotype showed higher values for the DD genotype than for the DI or II genotypes, in agreement with the literature (21,26). It has also been reported that hypertensives with the DD genotype are less likely to recover from microalbuminuria and more likely to develop microalbuminuria irrespective of the anti-hypertensive drug in use (27).

Analysis of variance with hypertensive and normotensive patients showed significantly higher cholesterol, triglyceride and glycemia levels in hypertensive than in normotensive subjects. When analyzed by gender, the increase in glycemia remained statistically significant in

hypertensive men (P = 0.01). Cholesterol levels were higher in men than in women (P = 0.08). Triglyceride levels were higher in hypertensive men and women than in normotensive subjects (P = 0.06 and 0.02, respectively), while HDL cholesterol did not vary significantly among groups. We detected a tendency for glucose levels to be higher in women with the II genotype, BMI to be lower in men with the II genotype and triglycerides to be higher in women with the DD genotype, although with no statistical significance. Viitanen et al. (28) also found higher triglyceride levels in DD participants, but the number of subjects studied was small. Our results agree with those of Ryan et al. (9), who showed that the ACE*I allele was associated with insulin resistance in overweight women. Since hyperinsulinism may influence the development of hypertension, the different prevalence of hypertension in men and women with the II genotype could be explained by gender variation in the predisposition to insulin resistance.

In our analysis, the II genotype was associated with higher HDL cholesterol levels, especially in women. In contrast, the only report in the literature (29) investigating HDL cholesterol levels and ACE gene polymorphism found no associations even when adjusted for age and gender.

Our results show that the DD genotype is associated with higher plasma ACE activity and microalbuminuria, and with lower glucose and HDL cholesterol levels. We hypothesize that the ACE*D allele may increase the risk of cardiovascular disease by facilitating the development of LVH, microalbuminuria and low HDL cholesterol, especially among men. On the other hand, the ACE*I allele may protect men from obesity and hypertension and may facilitate the development of diabetes in women. The latter hypothesis is supported by the observation that the Pima Indians from Arizona, USA, have a high prevalence of the ACE*I allele and of diabetes mellitus (2). In addition, the Yanomami Indians of the Amazon, who have no hypertension, obesity, diabetes, or hypercholesterolemia, have a predominance of the ACE*I (30).

Finally, gender differences in the role of factors predisposing to the development of diseases must always be considered in clinical studies in which association analyses of genetic polymorphisms are performed. Finding the more common patterns of association will help to better define prognosis and treatment, with more benefit to the patients.

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