

Prevalence of the Angiotensin I Converting Enzyme Insertion/Deletion Polymorphism, Plasma Angiotensin Converting Enzyme Activity, and Left Ventricular Mass in a Normotensive Chilean Population

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The aim of this study was to estimate the prevalence of the different alleles of the angiotensin converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism and associated plasma ACE activity, as well as cardiac echocardiographic structure, in a healthy Chilean population. We selected 117 healthy normotensive subjects (aged 45 to 60 years, middle socioeconomic status, nonobese, and nondiabetic) from a population-based study concerning the prevalence of risk factors for chronic diseases (Conjunto de Acciones Para la Reducción Multifactorial de las Enfermedades no Transmisibles [CARMEN]).

The frequencies of the I and D alleles were 0.57 and 0.43, respectively. Mean plasma ACE activity was 15.3 ± 3.9 U/mL. Compared with subjects with the II genotype, plasma ACE activity was significantly higher in subjects with the ID and DD genotypes with no difference between them. No correlation was observed between blood pressure and plasma ACE activity. Among the

three different genotypes there was no difference in left ventricular (LV) dimensions or in LV mass. No correlation between plasma ACE activity and LV mass was observed for either gender or different genotypes. Multivariate linear regression analysis using LV mass and LV mass index as dependent variables showed independent effects ($P < .05$) for gender (higher LV mass in men) and diastolic blood pressure, but not for the DD genotype.

In conclusion, in this population, the presence of the D allele on the ACE gene determined higher circulating ACE activity. However, in this normotensive healthy population, male gender and diastolic blood pressure, but not the presence of the D allele, were associated with increased LV mass.

KEY WORDS: Angiotensin converting enzyme polymorphism, angiotensin II.

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The angiotensin I converting enzyme (ACE) (kininase II, EC 3.4.15.1) converts the decapeptide angiotensin I to the vasoactive octapeptide angiotensin II and also degrades bradykinin. In this way, the activity of this enzyme determines the circulating and tissue levels of angiotensin II, contributes to regulate the vascular tone, and may have some effects on cardiac mass and structure.¹

In a white population, an insertion/deletion (I/D) polymorphism of the ACE gene determined almost half the variance of circulating ACE.^{2,3} In this population, higher levels of circulating ACE were associated with the presence of the deletion allele and were possibly related to increased cardiovascular and renal morbidity.⁴⁻⁶ In humans, this polymorphism could be a marker for a closely linked but unidentified sequence variant that modulates the expression of the ACE gene in such a way that the deletion allele is associated with higher ACE activity in plasma,³ T lymphocytes,⁷ and in the heart.⁸

Even though there are numerous studies in North American, European, and Japanese subjects on the relationship between the ACE I/D polymorphism and certain cardiovascular diseases, there is only one study in Latin American people assessing the prevalence of the different ACE I/D genotypes,⁹ and no study assessing the relationship between this polymorphism and circulating levels of ACE or cardiac mass in this population.

The aim of this study was to estimate the prevalence of the different alleles of the ACE gene insertion/deletion polymorphism and associated plasma ACE activity in normotensive, middle-aged Chilean subjects. We also determined the relationship between the ACE I/D polymorphism and consequent plasma ACE activity with regard to left ventricular (LV) mass and diastolic function, eliminating by design the covariables age, body mass, and blood pressure.

MATERIALS AND METHODS

Study Design The study was approved by the Research Commission of the Medical School, Pontificia Universidad Católica de Chile. Participants were healthy, normotensive subjects (blood pressure [BP] < 140/90 mm Hg, measured twice in the seated position; not taking antihypertensive drugs) selected from a population-based study concerning the prevalence of risk factors for chronic diseases performed in Valparaíso, Chile (Conjunto de Acciones Para la Reducción Multifactorial de las Enfermedades no Transmisibles [CARMEN]). In this study, a sample of 3120 individuals was randomly selected. Given the few existing population registers in Chile, the main private water company in the area (ESVAL) gave access to their database, with full addresses of all their clients arranged geographically. There were 56,174 house-

TABLE 1. DEMOGRAPHIC CHARACTERISTICS AND LABORATORY RESULTS (n = 117; MEAN ± SD)

Age (years)	51.9 ± 4.9
Men/women (n)	40/77
Body mass index (kg/m ²)	24.1 ± 2.6
Systolic blood pressure (mm Hg)	123.9 ± 9.4
Diastolic blood pressure (mm Hg)	74.7 ± 7.1
Serum creatinine (μmol/L)	67.1 ± 17.7
Hematocrit (%)	43.2 ± 3.9
Potassium (plasma, mmol/L)	4.3 ± 0.5
n (%) genotype II	42 (36)
n (%) genotype ID	56 (48)
n (%) genotype DD	19 (16)
Plasma ACE activity (U/mL)	15.3 ± 3.9
End diastolic dimension (mm)	47 ± 4
End systolic dimension (mm)	27 ± 5
Left atrial dimension (mm)	36 ± 4
E wave velocity (m/sec)	63.1 ± 12.7
A wave velocity (m/sec)	60 ± 12.2
E/A ratio	1.1 ± 0.3
LV mass (g)	121.3 ± 35.8
LV mass index (g/m ²)	72.4 ± 17.7

holds in the database, covering 78.4% of the Valparaíso population. Only one individual, aged 25 to 64 years, was interviewed in each house. To select the participant at each house a census of its inhabitants was performed. The subject was chosen from among eligible individuals by applying a Kihss table for individual random selection. People from this original sample fulfilling the inclusion criteria for this study were contacted by mail, phone, or personal visits and invited to participate. There were 264 people who fulfilled the inclusion criteria in the database, 55 of whom were initially excluded because of foreign surnames or absence of one surname; 157 were examined and 117 participated (40 were excluded after examination because of foreign surnames or treated hypertension).

Blood pressure was determined twice in the sitting position and later at the time of echocardiographic examination (three times in the seated position). For the analysis, the last blood pressure determinations were averaged and considered.

Inclusion Criteria We enrolled healthy, nonhypertensive people, aged 45 to 60 years, born in Chile, with their surnames and parental surnames all Spanish, who were middle socioeconomic status (Graffar scale 2 to 4), nonobese (body mass index [BMI] < 28 kg/m²), and nondiabetic. These criteria were selected to control the sample for genetic background, sociogenetic influences,¹⁰ and the effects of body mass and diabetes on LV mass. The subjects' demographic characteristics and laboratory results are depicted in Table 1.

DNA Extraction and ACE Polymorphism Determination The ACE polymorphism was determined in DNA extracted from leukocytes and amplified by polymerase chain reaction (PCR). After subjects signed the informed consent, one blood sample was obtained in a tube with EDTA. The blood was then centrifuged at room temperature, the supernatant was eliminated, and the cells were resuspended in sterile NaCl 0.9% and centrifuged. The pellet was washed in lysis buffer, and resuspended in 1 mL DNazol (Gibco BRL, Grand Island, NY). Absolute ethanol was added to precipitate the DNA. The ethanol was removed, the DNA was dried, resuspended in NaOH 8 mmol/L, and incubated at 50°C for 20 min.

DNA Amplification and Gel Electrophoresis DNA was amplified by PCR according to Rigat et al¹¹ in a Techne (Cambridge, England) thermal cycler. The reaction mixture contained PCR buffer 1×, MgCl₂ 1.5 mmol/L, deoxynucleotide triphosphates (dNTPs) 200 μmol/L, Taq polymerase 1UI (Gibco BRL), the sense oligonucleotide primer: 5' CTG GAG ACC ACT CCC ATC CTT TCT 3' (ACE1, Eurogentec, Seraing, Belgium), the antisense primer: 5' GAT GTG GCC ATC ACA TCC GTC AGAT 3' (ACE2, Eurogentec), and DNA (50 to 200 ng). The DNA amplification cycles were one cycle at 94°C for 5 min, then 30 cycles each (1 min at 94°C, for denaturation), 1 min at 60°C (for annealing), and 1 min at 72°C (for extension).

Amplification products were mixed with bromophenol blue, incubated at 65°C, and transferred to agarose electrophoresis gel. The gel was stained with ethidium bromide, visualized, and photographed in an ultraviolet transilluminator. The PCR products corresponded to a 190-base pair fragment in the presence of the deletion and to a 490-base pair fragment in the presence of the insertion.

Measurement of Plasma ACE Activity Another blood sample was obtained in a chilled heparinized tube (after one overnight fasting). The sample was then centrifuged within 3 h at 4°C. Plasma was stored in liquid nitrogen and processed within 4 weeks. The method used was based on spectrofluorimetric determination of histidyl-L-leucine (HL) using Z-phenyl-histidyl-leucine (Bachem Bioscience Inc., Torrance, CA) as an ACE substrate.¹²⁻¹⁴ Fifty microliters of plasma were incubated for 20 min at 37°C. Then, 100 μL of cold trichloroacetic acid (10%) was added to stop the reaction. The samples were then centrifuged at 4°C and the supernatant was neutralized adding NaOH, followed by the addition of *o*-phthaldialdehyde solution. Samples were again incubated at 37°C for 10 min and the reaction was stopped by adding 2N HCl. Fluorescence was measured within 60 min in an Aminco-Bowman spectrofluorimeter (λ excitation = 365 nm, λ emission = 500 nm). Readings were inter-

polated in a calibration curve and the amount of HL formed during the incubation time was calculated. ACE activity was expressed as U/mL (1 U = nmol HL produced in 20 min in 0.05 mL). All determinations were performed in duplicate. Intraassay and interassay coefficients of variation were both 1%.

Echocardiographic Measurements These were obtained with a 3.5-Mhz transducer at the same time of blood sampling by one echocardiographer using an Aloka SSD 875 device. All measurements were performed according to the recommendations of the American Association of Echocardiography (ASE),¹⁵ measuring three to five consecutive cardiac cycles. The following variables in the parasternal short axis were measured: interventricular septal thickness (IVSpTh) and posterior wall thickness (PWTh), end-diastolic (EDD) and end-systolic dimension (ESD). With these variables LV mass and LV mass index were calculated according to the formula developed by Devereux et al and modified by the ASE.¹⁶ Diastolic function was assessed by pulsed Doppler with the sample volume at the mitral valve level in the apical four-chamber view. The following variables were measured: E and A wave velocities and E/A ratio.

Statistical Analysis The results are presented as means ± SD. Unpaired *t* test, one-factor ANOVA, and χ^2 tests were used. When one-factor ANOVA was statistically significant ($P < .05$), subsequent comparisons with the Student-Newmann-Keuls test were performed. Linear regression and multivariate linear regression analyses (using LV mass and LV mass index as dependent variables) were also used.

RESULTS

One hundred-seventeen subjects were consecutively evaluated. Their demographic characteristics and laboratory results are shown in Table 1. Both genders were of similar age, body mass index, and had similar systolic blood pressures. Diastolic blood pressure, serum creatinine, hematocrit, and potassium were slightly higher in men ($P < .05$, Table 2).

ACE I/D Genotypes The D allele was present in 64% of the subjects (55% in men and 69% in women) (Table 2). The I allele was present in 84% of the subjects (80% in men and 81% in women). The frequencies of the I and D alleles were 0.57 and 0.43, respectively. The allele frequencies did not deviate statistically from the Hardy-Weinberg equilibrium.

No significant difference in the distribution of the three genotypes by gender was observed (Table 2). In the three genotypes, age, BMI, blood pressure, hematocrit, plasma sodium, and potassium were comparable, but plasma creatinine was slightly higher in subjects with the II genotype (Table 3).

TABLE 2. CHARACTERISTICS BY GENDER (MEAN \pm SD)

	Men (n = 40)	Women (n = 77)	P
Age (years)	51.7 \pm 5.3	52.1 \pm 4.7	ns
Body mass index (kg/m ²)	24.3 \pm 3	24.1 \pm 2.3	ns
Systolic blood pressure (mm Hg)	122.9 \pm 9.7	124.4 \pm 9.2	ns
Diastolic blood pressure (mm Hg)	76.7 \pm 6.3	73.7 \pm 7.3	<.04
Serum creatinine (μ mol/L)	76 \pm 17.7	62.8 \pm 8.8	<.01
Hematocrit (%)	46.7 \pm 3.3	41.3 \pm 2.7	<.01
Sodium (plasma, mmol/L)	140.7 \pm 2	140 \pm 3	<.04
Potassium (plasma, mmol/L)	4.4 \pm 0.4	4.2 \pm 0.5	<.02
n (%) genotype II	18 (45)	24 (31)	ns
n (%) genotype ID	14 (35)	42 (55)	ns
n (%) genotype DD	8 (20)	11 (14)	ns
Plasma ACE activity (U/mL)	15.2 \pm 3.6	15.3 \pm 4.1	ns
End diastolic dimension (mm)	49 \pm 5	46 \pm 4	<.01
End Systolic dimension (mm)	30 \pm 5	26 \pm 4	<.01
Left atrial dimension (mm)	37.3 \pm 4	35.2 \pm 4	<.01
E wave velocity (m/sec)	61 \pm 12	64 \pm 13	ns
A wave velocity (m/sec)	57.7 \pm 13.6	61.2 \pm 11.4	ns
E/A ratio	1.1 \pm 0.3	1.1 \pm 0.3	ns
LV mass (g)	144 \pm 42.6	109 \pm 24.2	<.01
LV mass Index (g/m ²)	81 \pm 21	68 \pm 14	<.01

Plasma ACE Activity The mean value was 15.3 \pm 3.9 U/mL and was identical in both genders (Table 2). Compared with subjects with the II genotype, plasma ACE activity was significantly higher in subjects with the ID and DD genotypes, with no difference between them (Table 3).

There was no correlation between plasma ACE activity and BMI, blood pressure, potassium, or hematocrit. No correlation was observed between blood pressure and plasma ACE activity in any of the three different genotypes.

The distribution of the classical blood groups among the three genotypes was similar, as shown in Table 4.

LV Mass and Function LV systolic and diastolic diameters, and interventricular and posterior wall thickness were higher in men, leading to an increased LV mass and therefore increased LV mass index in this group (Table 2). Among the three different genotypes there was no difference in either LV dimensions or in

TABLE 3. GENERAL CHARACTERISTICS AND PLASMA ACE BY GENOTYPE (MEAN \pm SD)

	II (n = 42)	ID (n = 56)	DD (n = 19)	F	P
Men/women (n)	18/24	14/42	8/11		
Age (years)	53.3 \pm 5.1	51.5 \pm 4.9	50.4 \pm 4.1	2.8	ns
Body mass index (kg/ m ²)	24 \pm 3	24.5 \pm 2.2	23.3 \pm 2.8	1.7	ns
Systolic blood pressure (mm Hg)	123.4 \pm 9.8	123.6 \pm 9.6	126.1 \pm 8.2	0.6	ns
Diastolic blood pressure (mm Hg)	74.3 \pm 7.2	74.6 \pm 7.3	76.2 \pm 6.3	0.5	ns
Serum creatinine (μ mol/L)	71.6 \pm 17.7	62.8 \pm 17.7	68.9 \pm 8.8	4.9	<.01
Hematocrit (%)	44.2 \pm 3.9	42.4 \pm 3.3	41.2 \pm 5	2.4	ns
Sodium (plasma, mmol/L)	139.5 \pm 2.8	140.4 \pm 1.7	140.1 \pm 2.9	1	ns
Potassium (plasma, mmol/L)	4.3 \pm 0.5	4.3 \pm 0.4	4.4 \pm 0.5	0.9	ns
Plasma ACE activity (U/mL)	12.6 \pm 2.6	16.5 \pm 3.6*	17.6 \pm 4*	21.7	<.001

*P < .05 v II genotype (after ANOVA).

LV mass (Table 5). There was no difference in LV mass between subjects with the II genotype and those with I/D + DD genotypes.

In the whole group, LV mass and LV mass index were slightly correlated with diastolic blood pressure ($r = 0.31$ and 0.25 , respectively; $P < .01$), but not with plasma ACE activity or systolic blood pressure. No correlation between plasma ACE activity and LV mass was found in either gender or in different genotypes.

Multivariate linear regression analysis (stepwise) using LV mass and LV mass index as dependent vari-

TABLE 4. BLOOD GROUP FREQUENCY IN EACH GENOTYPE

Blood Group	II (n = 42)	ID (n = 56)	DD (n = 19)
O	22	33	7
A	14	18	10
B	4	4	0
AB	2	1	2

$\chi^2 = 4.763$, $P = .57$.

TABLE 5. CARDIAC DIMENSIONS, DIASTOLIC FUNCTION, AND LV MASS BY GENOTYPE (MEAN \pm SD)

	II (n = 42)	ID (n = 56)	DD (n = 19)	F	P
End-diastolic dimension (mm)	47 \pm 4	48 \pm 5	46 \pm 6	1.3	ns
End-systolic dimension (mm)	26 \pm 3	28 \pm 4	28 \pm 7	0.1	ns
Left atrial dimension (mm)	36.1 \pm 4	35.8 \pm 4	36 \pm 4	1.7	ns
E wave velocity (m/sec)	62.3 \pm 11.5	62.1 \pm 12.3	68.1 \pm 16	3.9	ns
A wave velocity (m/sec)	61.7 \pm 11.3	58.4 \pm 12.6	60.8 \pm 13.1	0.9	ns
E/A ratio	1.03 \pm 0.2	1.11 \pm 0.3	1.17 \pm 0.4	1.7	ns
LV mass (g)	124.1 \pm 43.6	119.7 \pm 27	119.3 \pm 39.9	0.2	ns
LV mass Index (g/m ²)	74 \pm 21	71 \pm 14	73 \pm 20	0.3	ns

LV, left ventricular.

ables showed independent effects ($P < .05$) for gender (higher LV mass in men) and diastolic blood pressure, but not for the DD genotype.

DISCUSSION

One of the factors influencing plasma ACE activity is age.¹⁷ In whites, circulating ACE activity is higher in children than in adults, and decreases before puberty.¹⁸⁻²¹ To control for this variable, our study was performed in middle-aged adults. Furthermore, circulating ACE activity is not associated with other environmental factors.²²

On the other hand, plasma ACE concentrations are very stable in each individual, with large differences between subjects,²³ suggesting a significant genetic influence on its activity. The I/D ACE polymorphism is correlated with differences in plasma levels of ACE in healthy white subjects, accounting for 47% of the total phenotypic variance of plasma ACE.² A similar genetic determinism of ACE activity has been observed in rats.²⁴ In addition, in the human heart ACE activity is higher in subjects with the DD genotype than in subjects with the ID and the II genotypes.⁸

Based on comparative responses to acetylcholine and nitroprusside in the vessels of hypertensive subjects, it has been suggested that homozygosity for the D allele is characterized by a significant reduction in the endothelium-dependent vasorelaxation, compared with the ID genotype.²⁵ In subjects without hyperten-

sion, ischemic heart disease, or diabetes, higher plasma ACE activity has been associated with increased carotid wall thickening.²⁶ Thus, this polymorphism could also affect the vascular structure and function.

In a subgroup of patients from the CATS study, plasma ACE activity determined after the onset of a myocardial infarction²⁷ and the presence of the DD genotype²⁸ were significant predictors of a subsequent increase in the left ventricular volume, suggesting a role for this genotype in promoting deleterious LV remodeling in this condition.

These associations underscore the significance of estimating the distribution of these genotypes in the general population and in subgroups of patients with cardiovascular disease. A recent metaanalysis on this polymorphism and cardiovascular disease concluded that the D allele behaves as a marker of atherosclerotic cardiovascular complications and diabetic nephropathy.²⁹

In our normotensive healthy population sample, the prevalences of the II, ID, and DD genotypes were 0.36, 0.48, and 0.16, respectively. These prevalences can be extrapolated to the general population because a random sampling from the population was taken. This is one of the main strengths of this study, differing from most of the observational studies concerning this polymorphism in which sampling from the general population has not been performed.

In a metaanalysis of 15 studies in patients with acute myocardial infarction from white and Japanese populations the prevalences of the II, ID, and DD genotypes in the control subjects were 0.23, 0.49, and 0.28, respectively, with significant differences in the distribution of the genotypes in the three Japanese control populations, compared with the white controls.³⁰ In the Japanese studies the D allele frequency was significantly lower (0.39) than in the white studies (0.54)²⁸ and the DD genotype was associated, in the Japanese, with a higher odds ratio for myocardial infarction, compared with the ID/II genotypes. The observed prevalence of the D allele and the distribution of the three genotypes in our normotensive, nondiabetic sample was closer to the Japanese control population than to the white controls. Interestingly, plasma ACE activity in our normotensive healthy subjects was the same in the heterozygous ID subjects as in the homozygous DD subjects, suggesting dominance of the D allele. This result is different from what has been reported in French² and German³¹ studies, where a gradient of plasma ACE activity has been observed within the three genotypes (DD > ID > II), but is similar to that reported in Japanese subjects, where plasma ACE levels were the same in both DD and ID genotypes and lower in the II subjects.³² These two similarities between Chilean normotensives and Japa-

nese control populations could reflect a shared genetic background.³³

Ethnicity has an important effect on the distribution of this polymorphism. In this regard, in a comparative study with populations of different origins, the ratio of the frequencies for the II, ID, and DD genotypes was 1:2:1 in Europeans, with a tendency to higher frequency of the D allele in Nigerians. In the same study, indigenes from Samoa and Yanomani from the Amazon basin had a much higher frequency of the I allele than the D allele.³⁴ In one study in normal Chinese subjects, the frequencies of the D and I alleles were 0.3 and 0.7, respectively.³⁵ There is only one study in Latin Americans concerning the ACE gene polymorphism—from Brazil, whose population is extremely heterogeneous—performed in 200 subjects not sampled from the general population. They found frequencies of 0.659 for allele D and 0.341 for allele I.⁹ Our results on the distribution of this polymorphism and plasma ACE activity in a Chilean population are closer to those reported in Asian populations than to those in whites.

In general, no relation has been found between blood pressure and the ACE I/D polymorphism,^{22,36} and our data in normotensive subjects are consistent with these observations. However, one recent study in a large American population-based sample, with percentages of hypertensive subjects close to 50%, found that the D allele was associated with diastolic blood pressure and hypertension in men but not in women.³⁷ Furthermore, in hypertensive Italian subjects, the ACE-DD genotype was associated with higher ambulatory systolic and pulse pressures.³⁸ Both studies take into account hypertensive subjects, not included in our study.

In the general white population (from the Framingham Study), there was no relation between the ACE genotype and echocardiographically determined LV mass ($n = 2439$, 12% to 16% taking hypertensive therapy), with similar prevalences of left ventricular hypertrophy (LVH) among the three genotypes (range, 13.6% to 15.6%).³⁹ In normotensive Germans, increased posterior wall thickness has been observed in subjects with the DD genotype.⁴⁰ However, in a hypertensive white population, the DD and ID genotypes were associated with significantly higher LV mass index than was the II genotype after adjustment for other covariables.⁴¹ Similar findings were observed in untreated hypertensive patients from Italy.⁴² There are no data relating this polymorphism to LV mass in Latin American patients, but our data in middle-aged normotensive and nonobese Chileans do not show that ACE polymorphism is associated with LV mass. One explanation for this finding could be that despite its association with a major difference in plasma ACE levels, the ACE I/D polymorphism has not been

found to modify angiotensin II, aldosterone, renin, or diastolic BP increase induced by exogenous angiotensin I infusion in normotensive subjects.³⁶ It could then be hypothesized that if angiotensin II and aldosterone participate by inducing cardiac growth and they do not vary among the three genotypes in response to stress, no different effects on LV mass or structure would be observed. A second explanation for our findings could be that, because no hypertensive subjects were incorporated, no relation of the I/D polymorphism with LV mass exists at all, as LV mass might be the result of an interaction of this polymorphism with hypertension. This hypothesis should be explored further.

Probably, the ACE I/D polymorphism is not a direct determinant of cardiovascular risk, but it might induce differential responses or risk incidences after physiologic or pathologic stimuli. This concept has been suggested experimentally by observations in rats with genetically determined high levels of ACE developing higher neointimal proliferation after carotid injury, compared with those with low ACE levels.⁴³ In humans, some observations also support this notion, such as higher restenosis rate after coronary stenting in patients with the D allele⁴⁴ or increased LV mass in response to exercise in subjects with the D allele.⁴⁵

In conclusion, in a healthy, middle-aged normotensive Chilean population, the presence of the D allele on the ACE gene determined higher circulating ACE activity, which might be associated with increased cardiovascular morbidity. In this normotensive healthy population, the male gender and diastolic blood pressure, but not the presence of the D allele, were associated with increased LV mass.

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