Original Article

Population-Based Case-Control Study of Renin-Angiotensin System Genes Polymorphisms and Hypertension among Hispanics

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The effect of polymorphisms of the RAS genes on the incidence of hypertension seems to be population-dependent. We studied the effects of the angiotensinogen T174M and M235T, angiotensin converting enzyme insertion/deletion (ACE I/D), and angiotensin II receptor 1 (AT_1R) A1166C gene polymorphisms on the risk of hypertension among Hispanics. We selected all cases (n=256) and 257 age and sex group-matched controls from a random sample of free living Colombians (n=2,989). Logistic regression was used to estimate the independent effect of each polymorphism. All polymorphisms were in Hardy-Weinberg equilibrium in controls, with the exception of M235T, which showed a small excess of heterozygotes (p=0.005; disequilibrium coefficient, D=–0.0264). After adjustment for age, sex, body mass index, race, physical activity, family history of hypertension and cardiovascular disease, and other polymorphisms, subjects with the ACE DD genotype were 1.56 times (95% confidence interval [CI]: 1.05, 2.33) more likely to be hypertensive than carriers of the I allele (p=0.03). Also, adjusted systolic and diastolic blood pressure were 4.58 (95% CI: –0.39, 9.56) and 3.32 (95% CI: 0.78, 5.86) mmHg higher in DD homozygous individuals than in carriers of the I allele, respectively. Approximately 15% of the cases of hypertension in this population could be attributed to carriage of the DD genotype. None of the other polymorphisms was associated with either hypertension or blood pressure level. In conclusion, the ACE DD genotype appears to be an independent risk factor for development of hypertension and may explain a significant fraction of incident cases among Hispanics. (*Hypertens Res* 2008; 31: 401–408)

***Key Words*: hypertension, renin-angiotensin system, genetic polymorphism, angiotensin converting enzyme, Colombia**

Introduction

Essential hypertension, a major cardiovascular risk factor, is recognized as a multifactorial trait resulting from the interplay of environmental and genetic factors. The renin-angiotensin system (RAS) plays a fundamental role in blood pressure regulation, and polymorphisms of the genes encoding angiotensinogen, angiotensin II, and angiotensin II type 1 receptor (AT_1R) could influence RAS activity and increase the risk of hypertension. However, consistent associations between RAS genes polymorphisms and hypertension have been difficult to demonstrate.

Most studies of angiotensinogen have focused on two

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Table 1. Distribution of Risk Factors in a Sample Cases of Hypertension and Age and Sex Matched Controls from Colombia

Risk factor	Controls (n=257)		Cases (n=256)		p value
	Mean	95% CI	Mean	95% CI	
Age (years)	52.1	51.0, 53.2	52.6	51.4, 53.7	0.58
Male sex (%)	32.7	27.0, 38.8	32.8	27.1, 38.9	0.98
Diastolic blood pressure (mmHg)	71.6	70.6, 72.6	85.0	83.3, 86.7	<0.001
Systolic blood pressure (mmHg)	116.4	114.7, 118.0	148.0	144.9, 151.2	<0.001
Body mass index (kg/m^2)	26.6	26.0, 27.2	27.7	27.2, 28.3	0.006
Waist circumference (cm)	84.7	83.3, 86.1	87.6	86.1, 89.0	0.006
Mixed race (%)[†]	54.5	48.2, 60.7	62.1	55.9, 68.1	0.048
Diabetes (%)	14.0	10.0, 18.9	9.8	6.4, 14.1	0.089
Parental history of hypertension (%)	43.5	37.3, 49.7	59.1	53.0, 65.2	0.001

CI, confidence interval. [†]Includes 4 black subjects.

genetic variations in exon 2 of the angiotensinogen gene resulting in a methionine-to-threonine substitution at position 235 (M235T) and a threonine-to-methionine substitution at position 174 (T174M) (*1*). Some (*2*, *3*) but not all studies (*4*, *5*) have reported an elevated risk of hypertension in subjects with the angiotensinogen T235T genotype. Angiotensinogen T174M is in linkage disequilibrium with M235T (*3*) and some studies have reported that carriage of the 174M allele increases the risk of hypertension (*3*, *4*), a finding that was not replicated in a later study (*6*).

The angiotensin converting enzyme (ACE) gene is characterized by the insertion (I) or deletion (D) of a 287-bp repeated Alu sequence in intron 16, which is associated with 20–50% of the variability in ACE levels (*7*). However, the role of the I/D polymorphism in the incidence of hypertension remains quite controversial (*1*). A non-functional substitution of a cytosine for an adenosine at position 1166 of the 3′ untranslated region of the AT_1R gene has also been associated with hypertension. A higher prevalence of the C allele has been found in Caucasians with severe hypertension in some (*8*) but not all studies (*9*).

Although the role of RAS genes polymorphisms on the incidence of hypertension seems to be population-dependent (*1*), only a few studies on these polymorphisms have been conducted among Hispanics (*2*, *5*, *10*, *11*). In the present study we assessed whether the angiotensinogen M235T, angiotensinogen T174M, ACE I/D and AT_1R A1166C polymorphisms are associated with blood pressure and essential hypertension in a Colombian population.

Methods

We conducted a population-based case-control study in a random sample of 2,989 individuals from Bucaramanga, Colombia (*12*). The study was approved by the Research Ethics Committee of the Universidad Industrial de Santander and written informed consent was obtained from each participant. Blood pressure was measured in the seated position, after a 5-min resting period, with a mercury sphygmomanometer, following standard recommendations (*13*). Two trained observers independently measured the blood pressure in each participant three times with a 1-min resting period between measurements. Room temperature ranged from 21–26°C in 97% of the measurements. The average of the last two blood pressure measurements was used in the analysis. Subjects with systolic blood pressure (SBP) ≥140 mmHg or diastolic blood pressure (DBP) ≥90 mmHg and those with a physician diagnosis of hypertension who were taking antihypertensive medication were considered as cases of hypertension. People who participated in the original survey and who had a stored blood sample were eligible for the study. All cases (n=256) and up to 4 sex- and age group–matched controls per case (n=257), randomly selected from the corresponding age-by-gender strata, were included in this analysis. All controls had an average SBP <140 mmHg and average DBP <90 mmHg and were not taking antihypertensive medication.

Fasting blood samples were drawn from each participant and white cells were collected and stored at −70°C until extraction of DNA. DNA was amplified a first time by polymerase chain reaction (PCR) using primers that flank the polymorphic region Alu in intron 16 on chromosome 17q23 (5′-CTGGAGACCACTCCCATCATTTCT-3′ and 5′-GAT GTGGTCGCCATCACATTGGTCAGAT-3′). The first primer pair produced a ≈490-bp fragment corresponding to an insertion (I) and/or a ≈190-bp fragment corresponding to a deletion (D) (*14*). Because the D allele in heterozygous samples is preferentially amplified, each DD sample was subjected to a second, independent amplification with a primer pair that recognizes an insertion-specific sequence (5′-TGG GACCACAGCGCCCGCCACTAC-3′; and 5′-TCGCCA GCCCTCCCATGCCCATAA-3′), under the same PCR conditions except for an annealing temperature of 67°C (*15*). This reaction yields a 335-bp fragment in the presence of the I allele. The angiotensinogen M235T polymorphism was determined by PCR amplification of a 303-bp fragment in exon 2 of the gene, using the primers 5′-GATGCGCACAAG GTCCTG-3′ and 5′-CAGGGTGCTGTCCACACTGGCTCG C-3′. Digestion with the restriction-endonuclease *SfaNI*

Table 2. Distribution of Polymorphisms of the Renin-Angiotensin System Genes in a Sample of Cases of Hypertension and Age and Sex Matched Controls from Colombia

Polymorphism	Controls (*n* (%))	Cases (*n* (%))	HWE *p* value*	Odds ratio†	95% CI†
Angiotensin converting enzyme I/D					
II	38 (16.4)	37 (14.5)	0.689		
ID	116 (50.2)	113 (44.3)			
DD	77 (33.3)	105(41.2)		1.38	0.95, 2.00
Angiotensin II type 1 receptor A1166C					
AA	207 (89.6)	222 (87.1)	0.496		
AC	23 (10.0)	33 (12.9)			
CC	1 (0.4)	0 (0.0)		1.30	0.74, 2.27
Angiotensinogen T174M					
MM	2 (0.9)	0 (0.0)	0.544		
MT	52 (22.5)	76 (29.8)			
TT	177 (76.6)	179 (70.2)		1.41	0.93, 2.08
Angiotensinogen M235T					
MM	1 (0.4)	5 (2.0)	0.005		
MT	79 (34.2)	90 (35.3)			
TT	151 (65.4)	160 (62.7)		0.88	0.61, 1.28

*Hardy-Weinberg equilibrium exact test in the group of controls. †Age- and sex-adjusted odds ratio and 95% CI: ACE DD *vs*. I-carriers; AGT A1166A *vs*. C-carriers; AGT M-carriers *vs*. T174T; and AGT T235T *vs*. M-carriers. CI, confidence interval; ACE, angiotensin converting enzyme; AGT, angiotensinogen.

results in a 266-bp fragment corresponding to the M allele and a 303-bp undigested fragment corresponding to the T allele. The T174M genotype was determined by digestion of the same 303-bp amplified product with the endonuclease NcoI, which results in a 211-bp fragment corresponding to the T allele and a 92-bp fragment corresponding to the M allele (*16*). Primers 5′-ATAATGTAAGCTCATCCACCAAGA AG-3′ (downstream) and 5′-TCTCCTTCAATTCTGAAA AGTACTTAA-3′ (upstream) were used for genotyping AT_1R A1166C (*17*). The 166-bp PCR amplicon was digested with the restriction enzyme *Afl II*, which produces 139-bp and 27-bp products in the presence of the C allele, but fails to cleave the amplicon containing the A allele. Amplification products were separated on 2.5% agarose gel and visualized by ultraviolet transillumination after ethidium bromide staining. All genotyping was conducted with blinding to case-control status.

Data Analysis

The distribution of risk factors was described using means and proportions with their corresponding 95% confidence intervals (CI). Allele frequencies in the control group were compared to that predicted by the principle of Hardy-Weinberg using an exact test. DNA amplification was not achieved in blood samples from 1 case and 26 controls. To avoid selection bias due to exclusion of participants without genotype data, we assumed that genotype data were missing at random and used multivariate imputation by chained equations to fill out missing values and generate 20 imputed data sets (*18*). Each completed data set was analyzed independently and the parameters of interest were averaged across the 20 copies to give a single estimate. Standard errors were calculated using Rubin's formula (*19*). Multiple logistic regression was used to estimate the independent effect of each genetic variant on the risk of hypertension. Age and sex were included in all regression models to account for the matched design. Variables other than the exposures of interest were retained in the final model if they were significantly associated with the disease or confounded the exposure-disease association (*20*). Interactions between the DD genotype and age, gender, obesity, race and other RAS gene polymorphisms were evaluated in the multivariate models. All *p* values were two-sided. For purposes of interpretation we also estimated the power of the study to detect a doubling of the effect of the DD polymorphism in people with other risk factors (*i.e.*, an interaction relative risk of 2).

Dominant and recessive models were used to evaluate the effect of the ACE I/D polymorphism. Due to the reduced number of C homozygous subjects, carriers of the AT_1R 1166C allele were compared to subjects homozygous for the A allele (A1166A). Similarly, angiotensinogen T174T homozygous subjects were compared with carriers of the 174M allele and angiotensinogen T235T homozygous subjects were compared to carriers of the 235M allele.

We used a multiple linear regression model with the imputed data to assess the independent effect of each polymorphism on SBP and DBP. The Buckley-James method for analyzing censored data was used to adjust the level of blood pressure observed in people taking antihypertensive medica-

Table 3. Multivariate Adjusted Odds Ratios of Hypertension for Renin-Angiotensin System Genes Polymorphisms in a Sample of Colombians

Polymorphism and genetic model	Odds ratio*	95% CI	*p* value
ACE I/D			
DD *vs*. (II+ID)	1.56	1.05, 2.33	0.03
Number of D alleles	1.33	1.00, 1.76	0.05
(DD+ID) *vs*. II	1.24	0.73, 2.11	0.42
Angiotensin II type 1 receptor A1166C			
AA *vs*. (AC+CC)	1.18	0.66, 2.10	0.58
Angiotensinogen M235T			
TT *vs*. (MT+MM)	0.91	0.61, 1.34	0.63
Angiotensinogen T174M			
(TM+MM) *vs*. TT	1.38	0.90, 2.12	0.14

*Adjusted for age, sex, body mass index, race, physical activity, family history of hypertension, family history of myocardial infarction, family history of cerebrovascular disease, and the other genetic polymorphisms. CI, confidence interval.

Table 4. Relative Risks and Study Power for Interactions between the Angiotensin-Converting Enzyme DD Genotype and Different Risk Factors

Risk factor	Interaction relative risk	95% CI	*p* value	Power* (%)
Age (≥50 years)	1.30	0.71, 2.38	0.39	56
Sex†	0.87	0.49, 1.53	0.62	78†
Obesity‡	0.95	0.52, 1.74	0.86	76
Race	0.87	0.51, 1.49	0.62	72
Angiotensinogen M235T	0.84	0.49, 1.42	0.51	76
Angiotensinogen T174M	1.55	0.87, 2.75	0.13	73
Angiotensin II type 1 receptor A1166C	1.15	0.54, 2.48	0.71	36

*Power to detect an interaction relative risk ≥2.0 as statistically significant. †Assuming that the risk of hypertension in men is 2.0 times that in women. ‡Body mass index ≥30 kg/m^2. CI, confidence interval.

tion to the level of blood pressure expected without treatment (*21*).

A priori calculations based on the available number of cases, an equal number of controls, and the expected prevalence of each polymorphism showed that our study had a ≥86% power to detect as statistically significant a relative risk of 1.86.

Results

We studied 256 cases and 257 sex- and age group–matched controls. The age in controls was within ±2 years of that in the matched cases. Group matching ratios varied from 0.33 to 4.0 controls per case. One-third of all subjects were men and the mean age was 52 years (range: 17 to 64 years; Table 1). About 21%, 48%, and 20% of all cases were in the 40–49, 50–59, and 60–64 year-old age groups, respectively. Almost half of all cases were using antihypertensive drugs (48.8%; 95% CI: 42.6, 55.1). DNA amplification was not achieved in blood samples from 1 case and 26 controls (10.1%). Among controls, genotyping failure was significantly more likely in participants ≥50 years old (13.1% *vs*. 3.7%) and those who exercised regularly (13.6% *vs*. 6.0%). As expected, cases had higher SBP and DBP, higher body mass index, larger waist circumference, and higher frequency of close relatives with hypertension than controls.

The distributions of the ACE I/D, AT_1R A1166C, and angiotensinogen T174M polymorphisms among controls were consistent with those expected from the Hardy-Weinberg law (*p* values of 0.69, 0.50, and 0.54, respectively; Table 2). In contrast, the M235T polymorphism departed from Hardy-Weinberg equilibrium, with a small excess of heterozygotes among controls ($p=0.005$; estimated disequilibrium coefficient, $D=-0.0264$). Angiotensinogen T174M and M235T were in linkage disequilibrium ($r=0.20$; $p<0.001$).

In analyses adjusted for gender and age, carriage of the DD genotype resulted in a statistically non-significant increase in the risk of hypertension (age- and sex-adjusted odds ratio [OR_m] = 1.38; $p=0.09$; Table 2). Similarly, subjects with the AT_1R A1166A genotype had a non-significant increase in risk as compared to carriers of the C allele ($OR_m = 1.30$; $p=0.36$). The risk of hypertension was also higher in carriers

Table 5. Multivariate Adjusted Effects of Renin-Angiotensin Genes Polymorphisms on Systolic and Diastolic Blood Pressure in a Sample of Colombian Subjects

Polymorphism	Systolic BP			Diastolic BP		
	Difference in BP*	95% CI	*p* value	Difference in BP*	95% CI	*p* value
ACE I/D						
DD *vs*. (II+ID)	4.58	−0.39, 9.56	0.071	3.32	0.78, 5.86	0.011
Number of D alleles	1.94	−1.47, 5.34	0.264	1.64	−0.10, 3.38	0.065
ID *vs*. II	−2.93	−9.46, 3.60	0.377	−1.20	−4.56, 2.15	0.481
DD *vs*. II	1.93	−4.95, 8.81	0.582	2.43	−1.17, 6.03	0.185
Angiotensinogen M235T						
TT *vs*. (MT+MM)	1.68	−3.17, 6.54	0.495	0.59	−1.93, 3.11	0.646
Angiotensinogen T174M						
(MT+MM) *vs*. TT	1.73	−3.73, 7.19	0.534	1.34	−1.51, 4.20	0.355
Angiotensin II receptor 1 A1166C						
AA *vs*. (AC+CC)	−2.22	−9.55, 5.12	0.553	−0.35	−4.18, 3.49	0.859

*Adjusted for age, sex, body mass index, race, physical activity, family history of hypertension, family history of myocardial infarction, family history of cerebrovascular disease, and the other genetic polymorphisms. BP, blood pressure; CI, confidence interval.

of the angiotensinogen 174M allele as compare to TT homozygous subjects, but not significantly so (OR_m =1.41; p=0.11). In contrast, subjects with the angiotensinogen T235T genotype were less likely to be hypertensive than carriers of the M allele (OR_m =0.88; p=0.52).

After adjustment for other risk factors only the I/D polymorphism was significantly associated with hypertension (Table 3). Hypertension was 1.56 times more likely in DD homozygous subjects as compared to carriers of the I allele (p=0.03). Results from models with different degrees of adjustment were consistent with those from our final model. In a model without adjustment for previous history of hypertension, myocardial infarction or stroke, the odds ratio (OR) for the DD was 1.50 (p=0.04). Similarly, a model without adjustment for other polymorphisms resulted in an OR of 1.52 (p=0.04). The risk of hypertension also increased progressively with the number of D alleles, although the trend was of borderline statistical significance (p=0.05). The OR for the DD variant was 1.30 times higher in participants ≥50 than in those <50 years old, but this interaction was not statistically significant (p=0.39; Table 4). Similarly, no significant interactions with gender, obesity, and other polymorphisms were identified. However, the power of our study to detect a doubling of the effect of the DD polymorphism was very low for subjects aged ≥50 years and for those with the angiotensinogen M235T polymorphism, and was about 70–80% for other potentially interacting factors (Table 4). Carriage of the DD genotype accounted for 15.6% (95% CI: 2.8, 26.8) of the cases of hypertension in this population. After adjustment for other risk factors, SBP and DBP were 4.58 mmHg (p=0.07) and 3.32 mmHg (p=0.01) higher in DD homozygous subjects than in carriers of the I allele (Table 5).

The adjusted analysis showed a small, nonsignificant increase in risk among subjects homozygous for the AT_1R 1166A allele, as compared to carriers of the C allele (OR=1.18; p=0.58; Table 3). In contrast, subjects with the angiotensinogen T235T genotype were slightly less likely to be hypertensive than carriers of the 235M allele, but not significantly so (p=0.63). Finally, the risk of hypertension was 38% higher among carriers of the angiotensinogen 174M allele, as compared to subjects with the T174T genotype, but this association was not statistically significant (p=0.14).

Discussion

We found that carriage of the ACE D allele was independently associated with hypertension in Colombians, with a 56% increase of risk in DD homozygous subjects. DBP was also significantly higher among DD homozygous individuals. If this association is indeed causal, a considerable proportion of cases of hypertension in this population (about 15%) could be attributed to the DD genotype. Conversely, the angiotensinogen T174M, angiotensinogen M235T, and AT_1R A1166C polymorphisms were not significantly associated with hypertension and blood pressure values.

The prevalence of the D allele in our study was very close to that reported in African Americans (61.0% *vs*. 60.3%), but higher than in Caucasians (56%), Asians (39%) (*22*) and Latinos living in Los Angeles (48.6%) (*11*). Overall, studies in populations of African and Asian origin show an increased risk of hypertension among DD homozygous subjects, but studies among Caucasians do not (*22*). In the only study among Hispanics published to date, Henderson *et al*. found no association between self-reported hypertension and the DD polymorphism in Latinos living in Los Angeles (*11*). However, a potential effect of the DD genotype in Henderson's study may have been missed, since up to 30% of hypertensive people are unaware of their condition (*23*). The fact that, in

our previous study in the same Colombian population (*24*), the DD genotype was also associated with the risk of acute myocardial infarction adds confidence to our present results on hypertension.

Although the I/D polymorphism is an intronic marker, it may be in linkage disequilibrium with another functional mutation within the ACE gene. In fact, the I/D polymorphism is known to be associated with circulating levels of ACE in Europeans (*7*). Whether this is also the case among Colombians is currently unknown. However, Kammerer *et al*. (*25*) have shown that the levels of ACE activity increase progressively with the number of D alleles in Mexican-Americans, a population that shares some ancestry with Colombians.

An important additional finding in our study was the lack of interaction of the DD genotype with selected risk factors and other polymorphisms of the RAS genes. Our power analysis suggests that in this population, sex, obesity, self-reported race, and the angiotensinogen M235T and T174M polymorphisms are unlikely to double the effect of the DD genotype, although weaker interactions cannot be ruled out.

Our study provides little evidence of an effect of the angiotensinogen T174M and angiotensinogen M235T polymorphisms on the risk of hypertension. However, for these two polymorphisms the power of our study was adequate (≥80%) to detect only OR≥1.73. An increased risk of hypertension among angiotensinogen T235T subjects has been reported in Brazilians (OR=1.33, 95% CI: 1.04, 1.70) (*2*) and Chileans (OR=1.75; 95% CI: 0.75, 4.19) (*5*). A combined estimate obtained from those studies and our study results in a borderline statistically significant increase of 22% in the risk of hypertension among T homozygous subjects (OR=1.22; 95% CI: 1.00, 1.50; $p=0.05$). Therefore, an association between angiotensinogen M235T and hypertension cannot be ruled out, based on the available data. In fact, large and well-conducted cohort studies suggest that M235T is significantly associated with the incidence of hypertension among whites, but not in African Americans (*26*). In spite of the potential effect of angiotensinogen M235T on the risk of hypertension, it is worth noticing that this polymorphism is at some distance from the angiotensinogen cleavage sites and the promoter region and seems to be only weakly associated with plasma angiotensinogen levels (*27*).

The angiotensinogen T174M polymorphism was not associated with hypertension in our study, a finding consistent with other studies of this polymorphism (*16*). In fact, in studies suggesting an association, the relationship was restricted to women (*28*), men (*29*), and non-obese individuals (*30*). Moreover, it is unclear how angiotensinogen T174M might functionally influence the activity of the RAS, since it is far from functional regions of the gene and is weakly associated with angiotensinogen levels (*3*, *27*).

Our study provides little support for a strong association between AT_1R A1166C and hypertension, a finding consistent with the results from a study in adolescents from Argentina (*10*). However, only a handful of subjects were carriers of the C allele and the power of our study was adequate (≥80%) only to detect an OR≥2.78. The results of epidemiologic studies of the A1166C-hypertension association have been rather controversial (*1*). Results from many positive studies are questionable because cases with hypertensive parents were compared to controls without hypertensive parents, cases and controls were selected from different sources, and known confounding factors were not accounted for (*8*). On the other hand, AT_1R was not associated with the incidence of hypertension in a well conducted large prospective cohort study (*9*). Finally, the A1166C is a non-functional mutation occurring in the 3′ untranslated region of the AT_1R gene and does not seem to be in linkage disequilibrium with multiple single nucleotide polymorphisms identified in the promoter region of the gene (*31*).

Results from models with different degrees of adjustment were consistent with those from our final model. Selection bias is an unlikely explanation of our findings. Comparability of cases and controls was achieved by studying all cases and a random sample of controls from the same population and by matching on gender and age. Although we studied prevalent cases, survival bias is unlikely because survival in hypertensive patients is likely independent of the studied polymorphisms. Finally, we avoided selection bias from "non-participation" by using multiple imputation and including in our analysis all eligible subjects instead of only those with genotype data (*18*). Exclusion of participants without genotype data would have resulted in a slight overestimation of the effect of the DD genotype (OR=1.60; 95% CI: 1.07, 2.40, based on 472 cases and controls with complete genotype data).

Our results should not have been substantially affected by confounding due to genetic background (population stratification). Wacholder *et al*. have shown that the bias from population stratification should be small in well-designed epidemiologic studies (*32*). Moreover, our results were adjusted for self-reported race and studies of population structure have shown that self-reported population ancestry likely provides a suitable proxy for genetic background in epidemiologic studies (*33*). On the other hand, we were unable to adjust for levels of inflammatory markers, which have been shown to increase the risk of hypertension (*34*) and may be modulated by the ACE I/D genotype (*35*). However, since the levels of C-reactive protein seem to be lower in DD homozygous subjects, at least in this population (*35*), adjustment for this marker would have resulted in a larger effect of this polymorphism.

Genotyping errors in our study should be non-differential and should weaken the estimated associations, since all of the genotyping was performed with blinding to case-control status. We used an expected maximization algorithm to obtain unbiased estimates of the ORs (*36*) under the assumption of a mistyping error rate of 5%, which is larger than the 1–3% reported in series that have been retyped (*37*). Under these conditions, the corrected ORs for ACE DD, angiotensinogen

M235T, angiotensinogen T174M, and AT_1R A1166C were 5%, 1%, 7% and 15% higher than the observed ones, respectively. Therefore, genotyping errors are an unlikely explanation for the lack of association observed for polymorphisms other than the ACE I/D.

Our data provide evidence that the ACE DD genotype is an independent risk factor for development of hypertension among Hispanics. The increase in the risk of hypertension among ACE DD subjects in this population does not seem to be greatly affected by gender, race or obesity. Based on the combined data from our study and previous studies among Hispanics (*2*, *5*), a small increase in the risk of hypertension among angiotensinogen T235T homozygous subjects cannot be ruled out. On the other hand, our study provides little support for a large effect of angiotensinogen T174M and AT_1R A1166C, but larger studies are needed to make conclusive inferences about the role of these polymorphisms. Also, we cannot rule out a role of these polymorphisms on salt-sensitive hypertension, such as has been shown in other populations (*38*). Our findings suggest that the role of RAS genetic polymorphisms on the incidence of hypertension may be different among ethnic groups and that studies of specific genetic risk factors within individual ethnic populations are warranted.

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