Angiotensin-1–Converting Enzyme (ACE) Gene Polymorphism, Plasma ACE Levels, and Their Association With the Metabolic Syndrome and Electrocardiographic Coronary Artery Disease in Pima Indians

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In Caucasian subjects, an insertion/deletion (I/D) polymorphism of the angiotensin-converting enzyme (ACE) gene is associated with coronary artery disease (CAD) and fatal myocardial infarction. The underlying mechanism(s) of this association is not fully understood. Pima Indians have a low incidence of nonfatal and fatal CAD despite a high prevalence of diabetes. In Pima Indians, circulating ACE levels are related to ACE genotype, but the frequency of the D allele is significantly lower than in Caucasians. A lower frequency of the D allele may underlie a low risk of CAD in this population. We examined the relationship of the ACE genotype and plasma ACE level with electrocardiographic evidence of CAD (Tecumseh criteria), hypertension, and metabolic variables associated with insulin resistance in 305 (146 men and 159 women aged 47 ± 9.0 years) Pima Indians characterized for the ACE I/D genotype. The distribution of ACE genotypes was unrelated to diabetes and obesity. Fasting plasma insulin, plasminogen activator inhibitor-1 (PAI-1) activity, plasma triglyceride concentrations, and systolic (SBP) and diastolic (DBP) blood pressure were not significantly different between the three ACE genotypes among nondiabetic and diabetic subjects. There was no significant association of ACE genotype with electrocardiographic evidence of CAD or with hypertension. Plasma ACE concentrations were not significantly different between nondiabetic and diabetic subjects (median, 77 [range, 21 to 169] *v* 83 [7 to 238] IU/mL, P = NS). In all subjects, plasma ACE levels were associated weakly with plasma triglyceride (partial $r = .20$, $P < .01$) and total cholesterol (partial $r = .13$, $P < .03$) concentrations, but not with fasting plasma insulin or PAI-1 activity. In diabetic subjects, ACE levels were related to fasting plasma glucose concentrations (partial $r = .15$, $P = .07$). These findings would suggest that ACE gene I/D polymorphism is unlikely to be a major determinant of susceptibility to CAD in Pima Indians. Plasma ACE levels, but not ACE genotype, correlated with lipids, plasma glucose, and blood pressure, suggesting that elevated plasma ACE levels may contribute to the link between insulin resistance and CAD disease or may be a consequence of it.

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ANGIOTENSIN-CONVERTING ENZYME (ACE) regulates systemic and renal vascular tone by its key role in the activation of angiotensin II and inactivation of bradykinin.[1] In Caucasians, plasma levels of ACE relate to the insertion/deletion (I/D) genotype, with the highest levels in DD and the lowest levels in II homozygotes.[2] A recent study showed a significant relationship between cardiac ACE activity and ACE I/D gene polymorphism, with subjects of DD genotype having the highest levels.[3] In Caucasian populations from France and Northern Ireland, ACE genotype is related to the development of myocardial infarction, and the association was stronger in subjects at low risk of coronary artery disease (CAD) as defined by the apolipoprotein B level and body mass index (BMI).[4] However, a recent prospective study of US male physicians found no association between the ACE genotype and risk of ischemic heart disease.[5] ACE genotype is also associated with CAD in Caucasian subjects with non–insulin-dependent diabetes (type II diabetes).[6,7] Even if there is an association between ACE genotype and CAD, it is not clear whether it is mediated through high plasma ACE levels or through yet other unidentified mechanisms. In some studies, ACE genotype is associated with hypertension in subjects with type II diabetes.[8,9]

Hyperinsulinemia, obesity, hypertension, glucose intolerance, and dyslipidemia often congregate, with insulin resistance as the underlying metabolic abnormality,[10] also known as the insulin resistance syndrome or syndrome X. This syndrome is strongly associated with macrovascular disease, although the underlying mechanism of this association is unclear.[11] Pima Indians of Arizona are hyperinsulinemic and insulin-resistant and have a high prevalence of type II diabetes.[12] Despite these features, Pima Indians have a low prevalence of electrocardiographic evidence of CAD and a low incidence of fatal myocardial infarction.[13-14] Plasma ACE levels in Pima Indians are associated with ACE I/D gene polymorphism, but the frequency of the D allele is significantly lower than in Caucasian subjects.[15]

The aims of this study therefore were to assess if ACE gene I/D polymorphism and plasma ACE levels correlate with metabolic variables associated with the insulin resistance syndrome, and to determine if the lower frequency of the ACE D allele may underlie the low rate of CAD in this population.

SUBJECTS AND METHODS

Three hundred five (146 men and 159 women) Pima Indians aged between 35 and 70 years were recruited from an ongoing study of diabetes and its complications in the Gila River Indian Community in Arizona. They were recruited between November 1991 and October 1992 to study ethnic differences in cardiovascular risk factors.

Subjects presented in a fasting state between 8 and 10 AM and underwent a limited physical examination, and venous blood was withdrawn. Blood pressure was measured with a random-zero sphygmomanometer, and hypertension was diagnosed as systolic (SBP) or diastolic (DBP) blood pressure above 160 or 90 mm Hg, respectively, or treatment with antihypertensive medication. A 12-lead resting electrocardiogram was performed and interpreted for CAD by the Tecumseh criteria.[16] Serum triglyceride and total serum cholesterol levels were measured by enzymatic methods using a Hitachi 717 analyzer (Boeh-

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ringer Mannheim, Indianapolis, IN). In all samples, the high-density lipoprotein (HDL) cholesterol level was measured after precipitation of very–low-density lipoprotein and low-density lipoprotein (LDL) and using the method used to measure total cholesterol. LDL cholesterol was calculated using the Friedewald equation. Blood for plasminogen activator inhibitor-1 (PAI-1) activity was collected without venous stasis, with a wide-bore needle to avoid platelet aggregation. Samples were collected into prechilled tubes containing 3.8% sodium citrate solution. Samples were cold-centrifuged immediately and separated and frozen at −70°C. PAI-1 activity was measured by a chromogenic substrate method[17] using a kit from Kabi Vitrum (Uxbridge, Middlesex, UK) modified to a microplate method. The intraassay coefficient of variation (CV) is 4.5%, and interassay CV 8.5%. Results are expressed in arbitrary units per milliliter. Plasma glucose was analyzed by the hexokinase method (EXPRESS 550 analyzer; Ciba-Corning Diagnostic, Norwood, MA), and glycated hemoglobin (HbA_{1c}) by high-performance liquid chromatography. The plasma insulin level was measured by a modification of a new monoclonal antibody–based, highly specific, two-site immunoradiometric assay modified to a microplate enzyme-linked immunosorbent assay immunoenzymometric assay[18] and was sensitive to 2.0 pmol/L. Assays for intact and des 31,32 proinsulin were performed using immunoradiometric assays with iodinated (^{125}I) antibody.[19] Diabetes was diagnosed by World Health Organization criteria.[20]

Plasma ACE levels were determined by a spectrophotometric method.[15] For ACE genotyping, the region of I/D polymorphism in intron 16 of the ACE gene was amplified using standard primers.[21] Polymerase chain reaction products were separated by electrophoresis using 3% agarose gel and visualized by ethidium bromide staining under UV light.[15] Since the ACE deletion allele can be preferentially amplified by this method, all subjects who were homozygotes for this allele underwent confirmation of genotype status with a different set of insertion-specific primers.[22]

Statistical Methods

The data are shown as the mean ± SD or median and range. The chi-square test was used to study the associations of ACE genotype and allele frequency between various groups. Other variables were compared using unpaired *t* tests for normally distributed variables and Mann-Whitney *U* tests for others. ANOVA was used to compare the group means. Correlation analyses were performed using Pearson's correlation coefficients after logarithmically transforming variables that were not normally distributed.

RESULTS

ACE Genotype and Plasma ACE Levels

The frequency of the D allele for the ACE genotype among Pima Indians was significantly lower than in healthy Caucasian subjects, and plasma ACE levels were significantly associated with ACE I/D genotype, as shown elsewhere.[15] The prevalence of ACE genotypes and plasma ACE levels were similar among nondiabetic and diabetic subjects (Table 1). The ACE genotype distribution was not significantly different among non-obese and obese Pima Indians (Table 2). Plasma levels of ACE were higher in non-obese subjects (Table 2), but only among those with diabetes. In a multiple regression analysis controlling for age, sex, and SBP and DBP, plasma ACE levels showed no significant association with the BMI.

CAD, Hypertension, and ACE Genotype

The frequency of ischemic electrocardiographic abnormalities of CAD was not significantly different between nondiabetic and diabetic subjects (3% in each group). There was no significant association of ACE genotype with electrocardiographic evidence of CAD, and the frequency of CAD in subjects with II, ID, and DD genotypes was 4.4%, 2.2%, and 0% in men and 3.1%, 1.4%, and 5% in women, respectively. We also analyzed the association of CAD and ACE genotype using the Whitehall and Pooling criteria and found similar results. Similarly, there was no significant association of ACE genotype with hypertension ($\chi^2 = 0.814$, $df 2$, P = NS).

Table 1. ACE I/D Genotype in Nondiabetic and Diabetic Pima Indians

Parameter	Diabetic (n = 169)	Nondiabetic (n = 132)
Genotype		
II	83 (49%)	72 (55%)
ID	69 (41%)	51 (39%)
DD	17 (10%)	9 (7%)
Allele frequency		
I	0.695	0.738
D	0.305	0.262
Plasma ACE level (IU · mL^{-1})	83 (7-238)	77 (21-169)*

NOTE. $\chi^2 = 1.41$, $df = 2$, P = NS for genotype distribution.
*P = NS.

Insulin Resistance, ACE Genotype, and Plasma ACE Levels

Plasma insulin and triglyceride concentrations and PAI-1 activity were unrelated to the ACE genotype among nondiabetic and diabetic subjects (Table 3). Plasma ACE levels were not significantly related to age, sex, BMI, or waist to hip ratio. When adjusted for these variables, plasma ACE levels showed a significant but weak association with plasma triglyceride (partial $r = .20$, $P < .01$) and total cholesterol (partial $r = .13$, $P < .03$), but not with the plasma insulin concentration or PAI-1 activity. In nondiabetic subjects, plasma ACE levels were related to SBP (partial $r = .19$, $P < .05$), and in diabetic subjects, to fasting plasma glucose (partial $r = .15$, $P = .07$). The relationship of ACE levels with cardiovascular risk factors was generally similar between the three ACE genotypes. In a multivariate analysis that included age, sex, BMI, SBP, DBP, triglycerides, cholesterol, insulin, intact and des 31,32 proinsulin, PAI-1, diabetes, and CAD, plasma ACE levels were significantly related to plasma triglycerides ($P < .02$) and ACE genotype ($P < .001$), and not to diabetes or electrocardiographic evidence of CAD.

Table 2. ACE I/D Genotype in Non-Obese and Obese Pima Indians

Parameter	Non-Obese (n = 112)	Obese (n = 189)
Genotype		
II	57 (51%)	98 (52%)
ID	46 (41%)	74 (39%)
DD	9 (8%)	17 (9%)
Allele frequency		
I	0.714	0.719
D	0.286	0.281
Plasma ACE level (IU · mL^{-1})	91 (7-238)	75 (7-202)*

NOTE. $\chi^2 = 0.15$, $df = 2$, P = NS for genotype distribution. Non-obese, BMI < 31; obese, BMI ≥ 31.
*$P < .001$.

Table 3. Biochemical Features in Nondiabetic and Diabetic Subjects by ACE Genotype

Variable	ACE Genotype					
	Nondiabetic			Diabetic		
	II (n = 72)	ID (n = 52)	DD (n = 8)	II (n = 83)	ID (n = 69)	DD (n = 17)
Age (yr)	44.2 ± 8.1	45.8 ± 7.6	39.6 ± 4.5	49.9 ± 8.3	49.2 ± 8.5	48.0 ± 10.1
Sex (M/F)	45/27	17/35	2/6	45/38	30/39	3/14
BMI ($kg \cdot m^{-2}$)	35.1 ± 7.3	36.4 ± 9.5	37.0 ± 4.0	32.2 ± 6.6	33.5 ± 7.0	31.9 ± 4.3
SBP (mm Hg)	128 ± 20	124 ± 16	118 ± 13	128 ± 20	123 ± 15	129 ± 12
DBP (mm Hg)	77 ± 10	76 ± 10	80 ± 12	77 ± 10	78 ± 10	76 ± 6
Plasma triglycerides ($mmol \cdot L^{-1}$)	1.2 (0.5-1.8)	1.3 (0.5-4.2)	1.4 (0.6-5.4)	1.4 (0.4-9.2)	1.4 (0.6-8.0)	1.4 (0.4-3.3)
Total cholesterol ($mmol \cdot L^{-1}$)	4.45 ± 0.75	4.33 ± 0.90	4.52 ± 0.50	4.63 ± 0.94	4.65 ± 1.11	4.78 ± 1.02
HDL cholesterol ($mmol \cdot L^{-1}$)	1.08 ± 0.24	1.05 ± 0.21	1.13 ± 0.36	1.00 ± 0.22	1.01 ± 0.25	1.23 ± 0.27
LDL cholesterol ($mmol \cdot L^{-1}$)	2.67 ± 0.61	2.61 ± 0.73	2.56 ± 0.55	2.72 ± 0.83	2.66 ± 0.90	2.87 ± 0.74
Fasting plasma glucose ($mmol \cdot L^{-1}$)	5.5 ± 0.6	5.4 ± 0.6	5.3 ± 0.7	10.7 ± 4.5	10.8 ± 3.8	9.5 ± 3.2
HbA_{1c} (%)	5.5 ± 0.4	5.6 ± 0.5	5.4 ± 0.3	9.0 ± 2.2	9.2 ± 2.3	8.2 ± 2.0
PAI-1 activity ($AU \cdot mL^{-1}$)	19.1 ± 8.1	17.2 ± 9.5	20.3 ± 8.1	18.3 ± 9.5	19.2 ± 9.2	18.4 ± 9.1
Fasting plasma insulin ($pmol \cdot L^{-1}$)	108 (26-796)	102 (30-547)	155 (35-287)	124 (15-3,225)	132 (23-511)	124 (24-360)
Intact proinsulin ($pmol \cdot L^{-1}$)	6.2 (1.5-46.8)	4.9 (1.3-15.1)	5.3 (3.0-26.9)	15.1 (1.5-53.7)	12.7 (2.1-63.1)	11.0 (3.3-36.3)
Des 32,33 proinsulin ($pmol \cdot L^{-1}$)	3.7 (0.7-31.8)	3.2 (0.6-15.0)	3.3 (2.0-12.3)	6.0 (0.9-28.9)	6.5 (0.9-15.5)	5.8 (1.9-15.9)
Plasma ACE ($IU \cdot mL^{-1}$)	70 (22-155)	81 (29-169)	107 (57-138)*	75 (15-202)	93 (7-238)	92 (55-215)*

NOTE. Data are the mean ± SD or median (range).

*$P < .02$ for comparison by genotype among nondiabetic and diabetic subjects.

DISCUSSION

The ACE DD genotype was not significantly associated with electrocardiographic evidence of CAD and therefore is unlikely to be a useful marker for susceptibility to CAD in Pima Indians. However, as reported previously, the frequency of the DD genotype in this ethnic group is significantly lower than in healthy Caucasians,[15] and the number of subjects with the DD genotype was small in the present investigation. Furthermore, CAD was assessed by electrocardiographic evidence alone and therefore may have been underestimated. Therefore, these results need to be interpreted with caution. However, our findings do not support previous observations of an association between this genotype and a higher risk of CAD.[4,6,7] A number of previous case-control studies have explored the association of ACE genotype with macrovascular and microvascular disease with variable results. A previous study has shown an association of ACE gene polymorphism with macrovascular disease in type II diabetes.[6] A cross-sectional study by Ruiz et al[6] showed that the DD genotype in subjects with type II diabetes was an independent risk factor for CAD when controlling for other risk factors. In the UK Prospective Diabetes Study, the DD genotype was associated with a higher risk of acute myocardial infarction, and the results were stronger when cases and controls were matched for hypertension. Furthermore, this risk was substantially higher in subjects at low risk of CAD, defined as subjects with LDL cholesterol less than 3.6 mmol/L and triglycerides less than 1.58 mmol/L.[7] The results of these two studies were supportive of previous findings in nondiabetic subjects,[4] which showed that the DD ACE genotype conferred a higher risk of acute myocardial infarction in a subgroup at low risk of CAD. A possible explanation for this paradoxical relationship could be that subjects with the DD genotype in the higher-risk group may have already died and therefore were not included in cross-sectional studies. In a prospective study on US male physicians, ACE genotype was not demonstrated to be a useful risk marker for CAD.[5] We also did not find any significant association of ACE genotype with hypertension in this population in nondiabetic and diabetic subjects, results that are opposite to those reported in some previous studies of subjects with type II diabetes.[8,9]

We found no association of ACE genotype with type II diabetes in this population-based study. Plasma ACE levels were not significantly higher in subjects with diabetes than in nondiabetic subjects, in contradiction to some prior reports of elevated ACE levels in Caucasian subjects with diabetes.[23] The variables commonly associated with insulin resistance were not significantly different among the three genotypes in either nondiabetic or diabetic subjects, as would be expected if the excess risk of CAD seen in subjects with the DD genotype was mediated through insulin resistance. Our findings are similar to the results seen in other populations.[24] Furthermore, in the latter study, subjects with the DD genotype were more insulin-sensitive than those with the ID or II genotype.[24] The results of our study and studies by Katsuya et al[24] and Panahaloo et al[25] suggest that the previously reported excess risk of CAD in subjects with the DD ACE genotype is unlikely to be mediated through insulin resistance. We also did not find any association of intact proinsulin and des 31,32 proinsulin concentrations with ACE genotype.

It is possible that such an association of ACE genotype and CAD may be mediated locally through elevated levels of ACE in the myocardium, as a recent study showed that subjects with the DD genotype had higher ACE levels in the myocardium.[3] There is evidence that tissue ACE concentrations have multiple effects that may be closely linked to the pathogenesis of CAD. The effects of ACE inhibitors on endothelial function[26] and other antiatherogenic effects[27] are of particular interest in subjects with type II diabetes, a group at extremely high risk of CAD and acute myocardial infarction. Clinical trials have shown that ACE inhibitors can modify the clinical course of CAD in selected patients.[28] The ACE genotype may determine the response of patients with nephropathy to ACE inhibition,

with subjects with the DD genotype responding less favorably,[29-30] and such studies may be of interest in subjects with CAD with left ventricular dysfunction treated with ACE inhibitors, to determine if their response is dependent on the ACE genotype.

A recent study in a population from Trinidad showed a weak relationship of plasma ACE levels with obesity.[31] In that study, this association persisted after controlling for other variables. However, the obese group had a higher proportion of subjects with the D allele, which may partly explain the finding of higher plasma ACE levels in obese subjects. Subjects in our study were generally obese, and we found no relationship of plasma ACE with the BMI or waist to hip ratio in all subjects or in men and women separately. The distribution of ACE genotype was similar among obese and non-obese subjects. It is now well known that adipose tissue has renin angiotensin system (RAS) activity with a capacity for de novo renin synthesis.[32,33] The adipose tissue RAS may have multiple functions including prostaglandin production and adipose tissue lipolysis.[34,35] These observations raise the possibility that the adipose tissue RAS may play a role in adipocyte metabolic function and hence total-body energy balance. Indeed, studies in hypertensive subjects treated with ACE inhibitors have shown significant weight loss.[36,37] We found a significant relationship of ACE levels with blood pressure, and the strength of this association was similar to that observed by Cooper et al.[31] Whether the adipose tissue RAS plays a role in regulating blood pressure in obese individuals remains to be seen.

Plasma ACE levels, on the other hand, were significantly associated with variables associated with insulin resistance, and this relationship was independent of obesity. However, these relationships were weak and inconsistent among nondiabetic and diabetic subjects, and may be chance findings. However, a significant relationship between blood pressure and plasma ACE levels similar to that observed in our study has previously been reported in Caucasian men.[38] A relationship of plasma ACE levels (but not ACE genotype) with insulin resistance variables suggests that circulating ACE levels may modify variables associated with the insulin resistance syndrome or that ACE levels may be elevated as a consequence of CAD risk factors and the disease process itself.

In summary, we found no association of the ACE genotype with electrocardiographic evidence of CAD or hypertension in a Pima Indians, a population with a high prevalence of diabetes and obesity but a low risk of CAD. The association of plasma ACE levels with triglycerides, cholesterol, and blood pressure, although weak, is suggestive that plasma ACE levels may modify variables associated with the insulin resistance syndrome or that these may be elevated as a consequence. Since the ACE genotype is not a major determinant of circulating plasma ACE levels in Pima Indians, the ACE genotype or plasma ACE levels are unlikely to play a major role in determining the variables associated with insulin resistance syndrome.

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