

THE ANGIOTENSIN-CONVERTING ENZYME (ACE) GENE INSERTION/DELETION DIMORPHISM TRACKS WITH HIGHER SERUM ACE ACTIVITIES IN BOTH YOUNGER AND OLDER SUBJECTS

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Background: The absence of a 287 base pair *alu* sequence in the ACE gene (D allele) is associated with higher ACE levels than its presence (I allele) in adults. We carried out a case-control study of the ACE*I/D dimorphism in relation to circulating ACE activities to evaluate associations between the two variables in adults, compared to younger (18 years or less) individuals.

Materials and Methods: Genotypes of the ACE*I/D dimorphism were determined on DNA samples from a population of 164 random (unrelated) Emirate nationals, composed of two groups: 112 subjects above 18 years of age (range=20-77 years), and 52 subjects of 18 years or less (range=1-18), and analyzed for putative associations with serum ACE activities. ACE*I/D genotypes of the 164 individuals were determined by assays based on polymerase chain reaction. ACE activities were determined on serum samples of these subjects by colorimetric assays.

Results: The D allele was associated with increased ACE values in both adult and younger individuals. Mean ACE activity levels associated with II, ID and DD genotypes, however, were 42%-61% higher in the 18 years and under group of subjects. The ACE*I/D marker accounted for 28% of the variance of the phenomenon determining ACE levels in adults, and for 30% among youngsters.

Conclusion: The ACE*I/D dimorphism correlated strongly with circulating ACE activities in both adult and young Emirati subjects, and the corresponding mean ACE activities were significantly higher among the youngsters.

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Key words: ACE, cardiovascular diseases, genetics, hypertension, polymerase chain reaction, serum activity.

The angiotensin-converting enzyme (ACE) converts the inactive angiotensin I into the vasoactive and aldosterone-stimulating octapeptide angiotensin II, as well as inactivating bradykinin.¹⁻³ For these reasons, the human ACE gene has been a preferred target in unraveling the molecular architecture of cardiovascular diseases (CVD).¹⁻³

Circulating ACE levels (or ACE activities) show extensive interindividual variability and are highly genetically determined.⁴⁻⁷ An insertion/deletion (I/D) dimorphism, due to the presence or absence of a 287 base pair (bp) *alu*-type sequence in the 16th intron of the ACE gene, has been shown to cosegregate with serum and tissue ACE activities, and major locus inheritance explains best the findings that the D allele is associated with elevated

ACE levels.^{5,7} These findings have been recurrently confirmed by several investigators in populations of various ethnic origins.⁸⁻¹³ Thus, the ACE gene is viewed as a quantitative trait locus (QTL) that modulates circulating ACE levels, and the ACE*I/D dimorphism is a marker that is in linkage disequilibrium with functional variants located in the ACE gene.³

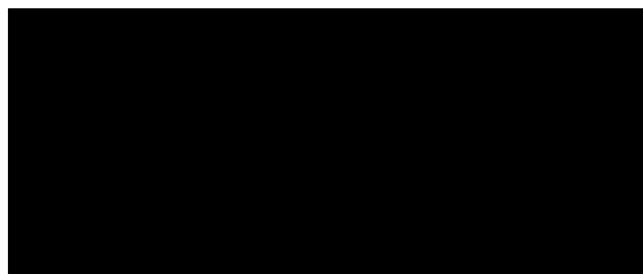


FIGURE 1. Visualization of the ACE*I/D marker on 14 DNA samples (lanes 1-6 and 8-15; lane 7 is a "negative control") by ethidium bromide staining of PCR products after electrophoretic migration through a 6.5% polyacrylamide gel. The I (insertion) allele is seen as a 490bp, and the D (deletion) allele as a 190bp fragment.

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TABLE 1. Sample sizes among three groups of Emirati subjects.

	<18 years	>18 years	Combined groups
Sample size	52	112	164
Sex (M/F)	27/25	57/55	84/80
Age (yrs) \pm SD	10 \pm 6	35 \pm 14	27 \pm 17
Age range (yrs)	1-18	20-77	1-77
Total serum cholesterol (mmol/L)	n.d.	5.7 \pm 3.5	-

TABLE 2. Distribution of mean serum ACE activities according to ACE*I/D genotypes in three groups of Emirati subjects.

ACE*I/D	<18 years	>18 years	Combined groups
II	53 \pm 18 (14)	33 \pm 8 (9)	45 \pm 16 (23)
ID	67 \pm 25 (24)	43 \pm 17 (48)	50 \pm 22 (72)
DD	85 \pm 11 (14)	60 \pm 18 (55)	66 \pm 18 (69)

Values are expressed in units of activity as defined by the manufacturer (see text). Number of cases in each cell is indicated between parentheses. Differences of mean ACE activities according to genotypes between adults and youngsters are significant at $P < 0.01$.

As transient increase of ACE levels has been reported to occur during adolescence,⁴ we sought to determine: 1) whether the ACE*I/D dimorphism tracked with ACE levels in younger individuals as well; 2) how these associations compared to those observed in adults; and 3) what the variance of the phenomenon among both adult and younger subjects was. For this, we studied a homogeneous ethnic group of nationals from the Abu Dhabi Emirate.

Materials and Methods

Subjects

We investigated a random sample population of 164 unrelated UAE nationals (Emirati) from Abu Dhabi. The sample population consisted of the two following sub-groups: 112 subjects above 18 years of age, and 52 subjects of age 18 or less (Table 1). We ensured that individuals taking part in the study were not on any medication that could have modified circulating ACE levels, and that they had no history of diabetes, renal or liver disease. Therefore, at the time of selection for the study, all the subjects were considered as representative of a random and "healthy" Emirati population from the Abu Dhabi Emirate. This project was approved by the Research Ethics Committee of the Faculty of Medicine and Health Sciences (UAE University, Al Ain, UAE).

Off-medication, total serum cholesterol values were gathered from the medical charts of the subjects. These were available for adult individuals only. The mean cholesterol level of the adult group was 5.7 \pm 3.5 mmol/L, which put it in the "borderline hypercholesterolemic" category (Table 1). There was no history of smoking in these subjects, and no member of the sample populations admitted to alcohol intake. Controlling for these two

confounding external influences is an added value of this study, as smoking (through a direct effect of nicotine), as well as (although to a lesser extent) alcohol, tend to raise circulating ACE levels.

Determinations of ACE Activities

Serum activities of angiotensin-converting enzyme (ACE) were determined on all 164 subjects of this study. ACE activity determinations were done by colorimetric assays, using assay kits (Bühlman Laboratories AG, Allschwil, Switzerland). The assay is based on the ability of ACE to cleave a synthetic substrate, N-hippuryl-L-histidyl-L-leucine, into the dipeptide histidyl-leucine and hippuric acid. One unit of ACE activity is defined as the amount of enzyme required to release 1 μ mol of hippuric acid per minute and per liter of serum at 37 °C. Normal ACE serum activity ranges with this assay have been determined as 18-55 units in a population of normal Swiss adults aged 18-60 years (Bühlman Laboratories AG).

DNA Analysis

DNA was extracted from 5 mL blood samples according to usual methods.¹⁴ The I/D dimorphism of the ACE gene was visualized by polymerase chain reaction (PCR) according to protocol conditions and primer sequences that have been published previously.¹⁵ D alleles were observed as 190 base pair (bp) fragments and insertion (I) alleles as 490 bp fragments upon ethidium bromide staining of electrophoresis products (Figure 1). Individual DNA samples on which DD genotypes were observed were subsequently analyzed by complementary procedures described elsewhere¹⁶ to avoid DD versus ID typing mistakes.

Data Analyses

Bivariate correlations between ACE*I/D dimorphism and serum ACE activities were studied by Spearman correlation coefficients (R_s) with an SPSS[®] version 6.1 for Windows[®] software package (Gorinchem, The Netherlands). Comparisons of means in both younger and older subjects were evaluated by two-way ANOVA procedures, and Hardy-Weinberg proportions of allele distributions were investigated by chi-squared analyses. For all analyses, statistical significance was considered when significance level (P) values were < 0.01 .

Results

Figure 1 shows the visualisation of D and I alleles of the ACE*I/D dimorphism according to conditions described earlier. Frequencies of D and I alleles were 0.5 and 0.5 in youngsters, and 0.7 and 0.3 among adults (see Table 2 for corresponding numbers in each cell).

Mean overall ACE values were 50 \pm 20 (median=47, range=15-99) units among adults (n=112), 68 \pm 23 units (median=69, range=15-105 units) in subjects younger than

18 (the mean is 36% higher than in adults and the median is 47% higher), and 56 ± 22 (median=56, range=15-105) units in the combined groups.

There were strong correlations between the ACE*I/D dimorphism and circulating serum ACE activities in all three groups (adults, $R_s=0.53$, $P<0.0001$, $n=112$; younger subjects of 18 or less, $R_s=0.55$, $P<0.0001$, $n=52$; combined groups, $R_s=0.41$, $P<0.0001$, $n=164$). Mean ACE levels were lowest among II, intermediate in ID heterozygotes, and highest among DD homozygotes (Table 2), and two-way ANOVA showed that interclass differences were statistically significant (Table 2).

Serum ACE activity values associated with each of the three genotypes were 61%, 56% and 42%, higher, respectively, among the young, compared to the adult group (see Table 2), and the difference was statistically significant.

Discussion

Data from these investigations shows that ranges of ACE activities are similar in both young and adult groups, but mean ACE values are significantly higher among subjects who are 18 years old and younger. Also, the normal range of ACE activity in the adult Emirati population (15-99 units) is wider than in a population of normal Swiss adults (18-55 units, Bühlman Laboratories AG).

Although the frequency of D alleles in adults is slightly higher ($pD=0.7$) than what we have previously reported in another group of Emirati nationals—where $p(D)$ was 0.64 in hypertensives, and 0.67 in normotensives¹⁷—its frequency is significantly lower among younger individuals ($pD=0.5$). The fact that D allele frequencies increase with age in the two studied age groups of the Emirati population is in line with a report which showed evidence that $p(D)$ was higher in French centenarians,¹⁸ but contrasts with results which showed a marked $p(D)$ decrease with increasing age among hypertensive Australians.¹³ In both groups of the present investigation, genotype distributions occurred in Hardy-Weinberg proportions (data not shown; see Table 2), which we had already observed in another group of Emirati nationals.¹⁷ This indicates that the high level of consanguinity practiced in Gulf populations, such as the Emirati, has little effect on heterozygosity levels, at least not at the ACE gene locus.

Recent combined segregation/linkage data has shown that two ACE-linked QTLs explain 38% of the ACE variance in parents, and 49% in offspring of French subjects, and that one of these QTLs might be the ACE*I/D dimorphism itself.¹⁹ McKenzie et al.²⁰ have also shown by combined segregation/linkage analysis in a series of African Caribbean families from Jamaica that two QTLs jointly influence serum ACE levels; one QTL, located within or close to the ACE locus, explains 27% of the total variability, and a second QTL, unlinked to the

ACE locus, could explain 52% of the variability. Among French subjects, the ACE*I/D dimorphism is in almost complete linkage disequilibrium (LD), with functional mutations accounting for 15%-47% of the total variance of serum ACE levels (or activity).^{5,7,19} The strength of LD is lower in Jamaican families, where the ACE*I/D marker accounts for 9% of the total variance,²⁰ or in Pima Indians, where it accounts for only 6.5%.¹⁰

Squared values of Spearman correlation coefficients were used to estimate the variance of the phenomena determining ACE levels among Emiratis.²¹ Our data indicates that the ACE*I/D marker accounts for 28% of the total variance in adults, and for 30% in the group of younger individuals. These estimates fall within the broad range of values (6.5%-49%) reported elsewhere.^{4-10,19,20} In contrast to what has been reported on French subjects, where the variance in offspring was greater than in parents,¹⁹ there was no significant difference of variance between adult and younger Emiratis.

Our main finding is that the ACE*I/D marker tracks directly with circulating ACE activities in Emirati individuals. Such an observation has been made in adult populations from several parts of the world,⁴⁻¹³ but we show here that it holds true in younger subjects as well. In this latter group, however, mean activity levels associated with II, ID and DD genotypes are, respectively, 61%, 56% and 42% higher. These results agree with those of one other study which also correlated the ACE*I/D marker with ACE activity in Japanese children.²²

It would thus be of interest to analyze the changes in ACE activity with age as a trend, rather than as a dichotomous variable. A much larger sample size, however, would be required to probe the extent of age-related trends by genotype. This might provide some direct relevance of the reported observation to laboratory medicine.

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