Angiotensin-converting enzyme insertion or deletion dimorphism in predisposition to cardiovascular diseases amongst United Arab Emirates nationals

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ABSTRACT

Objectives: The absence of a 287 base pairs Alu sequence in the angiotensin-converting enzyme gene (D allele) is associated with higher angiotensin converting enzyme levels than its presence (I allele). There is, however, a huge body of conflicting reports that have linked angiotensin-converting enzyme insertion/deletion to hypertension, ischaemic heart disease, myocardial infarction, left ventricular hypertrophy, as well as several other clinical entities. We carried out a retrospective, case-control study of the angiotensin-converting enzyme insertion/deletion dimorphism in relation to circulating angiotensin-converting enzyme activity, as well as to hypertension, ischemic heart disease, myocardial infarction and left ventricular hypertrophy, amongst United Arab Emirates nationals subjects (Emirati).

Methods: We investigated a sample population of 285 Emirati comprising groups of controls and of patients with clinical diagnoses of hypertension, ischaemic heart disease, myocardial infarction and left ventricular hypertrophy. Angiotensin-converting enzyme insertion/deletion genotypes were determined by assays based on polymerase chain reaction.

Results: The D allele was associated with increased circulating angiotensin-converting enzyme activity, and the angiotensin-converting enzyme insertion/deletion marker accounted for 28% of the variance of the phenomenon determining angiotensin-converting enzyme levels. We found, however, no association between angiotensin-converting enzyme insertion/deletion and clinical diagnoses of hypertension, ischaemic heart disease, myocardial infarction and left ventricular hypertrophy.

Conclusion: Although the D allele of the angiotensin converting enzyme insertion/deletion dimorphism tracks with circulating angiotensin-converting enzyme activities in United Arab Emirates nationals, it does not constitute a predictive marker for CVDs in this population.

Keywords: Angiotensin-converting enzyme, cardiovascular diseases, genetics, hypertension, myocardial infarction, polymerase chain reaction.


The aim of molecular genetics with respect to the field of cardiovascular diseases (CVDs) is to identify the quantitative trait loci (QTLs) that play a role in the onset or progression of the various disease processes. Amongst the different strategies that have been put to trial to decipher the molecular framework of complex clinical phenotypes, association (case-control) studies using candidate genes represent increasingly preferred methods. This approach indeed offers great power for
detecting QTLs of reduced or low penetrance, and as the number of available candidate genes increases rapidly, it is bound to develop even further in the future.\textsuperscript{9,10} 

In combination with environmental influences, the molecular and genetic structures of CVD may exert their effects through acute processes (such as vasoconstriction, thrombosis and plaque rupture) or through chronic processes (including hypertension, atherosclerosis, cardiac hypertrophy, endothelial and vascular changes), and intricate interactions in the various pathways of atherosclerosis and hypertension have led to the suspicion that common genetic effects underlie these disorders.\textsuperscript{1,5,6,11,12} As the angiotensin-converting enzyme (ACE) converts the inactive angiotensin I into the vasoactive and aldosterone-stimulating octapeptide angiotensin II, as well as inactivates bradykinin,\textsuperscript{11,12} the human ACE gene has been a target of choice in CVD studies.\textsuperscript{5,11-14}

An insertion (I)/deletion (D) dimorphism, due to 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 activity.\textsuperscript{15,16} This ACE/D dimorphism has no direct causative effect, but it may be in linkage disequilibrium with variants that could influence the onset and progression of CVD. Although this marker shows neither linkage nor association with essential hypertension in many different ethnic groups,\textsuperscript{6,11,13,14} the D allele has been found to be associated with such diverse clinical entities as, for example, myocardial infarction (MI) and ischaemic heart disease (IHD), cardiac hypertrophy, lacunar stroke, atheromatous renal artery stenosis, left ventricular hypertrophy (LVH), diabetic nephropathy,\textsuperscript{11,13,17,18} elevated fasting blood glucose levels,\textsuperscript{19} ischaemic stroke\textsuperscript{20} blood pressure response to physical exercise,\textsuperscript{21} arterial wall thickness\textsuperscript{22} and restenosis after coronary stenting.\textsuperscript{23} As most observations have been challenged by various investigators on populations of different origins,\textsuperscript{7,11,13,14,17} data from genetically isolated populations are of utmost importance to resolve issues of contention related to studies based on the concept of linkage disequilibrium.\textsuperscript{4}

We have previously described a lack of association between the ACEI/D dimorphism and clinical diagnosis of essential hypertension in a population of Gulf Arabs from the United Arab Emirates.\textsuperscript{24} In this paper, we report the results of a pilot association (case-control type) study on nationals from the Abu Dhabi Emirate, the aim of which was to evaluate putative correlations between the ACEI/D dimorphism and MI, IHD, LVH, hypertension as well as serum ACE levels in a genetically homogeneous ethnic group, of which members have no personal history of alcohol consumption or smoking.

**Methods. Subjects.** The United Arab Emirates (UAE) is a Federation of seven Emirates (the Abu Dhabi Emirate being the largest) with an indigenous population comprising UAE nationals, who are Gulf Arabs of Bedouin descent. In this pilot, case-control study, we investigated a sample population of 285 UAE nationals (Emirati) from the Abu Dhabi Emirate, for putative associations between the ACEI/D dimorphism and cardiovascular diseases. This project was approved by the Research Ethics Committee of the Faculty of Medicine and Health Sciences (UAE University, Al Ain, UAE).

The overall group of 285 unrelated subjects (155 males, 130 females) had a mean age (± standard deviation) of 55.5±4.8 years and was composed of (Table 1): 141 patients with hypertension (HT; mean age=53.0±12.3 yr.); 110 patients with ischaemic heart disease (IHD; mean age=57.1±12.7 yr.); 44

| Table 1 - Sample sizes by gender, mean ages (years), total cholesterol levels (mmol/L) with standard deviations, and body mass index (BMI) values amongst the different groups of Emirati subjects (patients with hypertension, IHD, MI and LVH; normal, healthy controls; and combined sample populations of this study). |
|-----------------|--------|--------|--------|--------|---------|---------|
| Sample size | 141 | 110 | 44 | 45 | 80 | 285 |
| Gender (M/F) | 77/64 | 60/50 | 30/14 | 28/15 | 40/40 | 155/130 |
| Age (years) | 55.0±12.3 | 55.1±11.3 | 55.0±11.3 | 56.9±13.3 | 55.7±15.2 | 55.5±14.8 |
| Total serum cholesterol (mmol/L) | 7.0±3.0* | 6.3±0.7* | 6.3±0.7* | 7.8±3.5* | 5.3±0.1 | 6.0±2.0* |
| BMI | 30.1±6.0 | 29.2±6.5 | 29.2±6.5 | 28.9±6.3 | 29.2±6.5 | 29.3±6.5 |

*indicates a significant difference at p<0.01 from control values

HT-Hypertension
IHD-Ishchaemic Heart Disease
MI-Myocardial Infarction
LVH-Left ventricular hypertrophy
patients who were myocardial infarction (MI) survivors (mean age = 55.0 ± 11.3 yr.); 45 patients with left ventricular hypertrophy (LVH; mean age = 56.9 ± 13.3 yr.); and 80 "controls" used as a comparison group (mean age = 55.7 ± 15.2 yr.).

Patients were classified as having essential hypertension if they had systolic blood pressures above 160 mm Hg and diastolic blood pressures above 95 mm Hg on at least three separate occasions, had no clinical signs, symptoms and laboratory findings suggestive of secondary hypertension, and had positive family histories of hypertension.

IHD, a syndrome resulting from coronary insufficiency, can manifest itself as exertional angina, unstable angina or myocardial infarction. Positive histories of IHD were considered in the case of patients who presented with any of these conditions documented either alone or in combination.

Diagnosis of myocardial infarction was applied to patients who had documented evidence of an episode of coronary thrombosis or occlusion manifested as: ECG changes of infarction (presence of pathological Q-waves in the region of infarction; presence of T wave inversions in regional leads); presence of regional wall motion abnormalities in the region of the MI on trans-thoracic echocardiography; serial enzyme elevations (TCK, CPK-MB, LDH, AST) on consecutive days following hospital admission.

Criteria for LVH inclusion were: demonstration of Sokoloe and Lyon ECG criteria (sum of S wave in V1 and the tallest R wave in lead V5 or V6 > 35 mm) and echocardiography findings (inter-ventricular septum > 1.2 cm; posterior LV wall > 1.3 cm).

Several patients presented with more than one of the described clinical entities. For example, MI patients were a sub-group of IHD patients; most patients with LVH were hypertensive; some patients had both IHD and hypertension. This is why the sum of cases from each sub-group is more than the total number of 285 subjects. During data analysis, however, dissociation of confounding, common clinical entities was made possible by using partial correlations procedures.

"Control" (comparison) subjects were individuals who had no personal history of hypertension, LVH, IHD or MI (as documented by individual medical records).

Total serum cholesterol was taken as off lipid-lowering medication values from subject's medical charts. So were height and weight measurements, which were used to calculate body mass index (BMI) as [weight (in kg)]/[height (in m)]^2 (Table 1). No member of the sample populations admitted to alcohol intake. There was also no history of smoking in most subjects.

**ACE level determinations.** Serum activities of angiotensin-converting enzyme (ACE) were determined on 55 subjects who were picked at random in the group of controls, as individuals in this group were off any medication that could have modified ACE levels. ACE activity determinations were done by colorimetric assays using assay kits (Bühlman Laboratories AG, Allschwil, Switzerland) according to the supplier's recommendations. Normal serum ranges of this assay have been determined as 18-55 Units in a population of normal Swiss adults (Bühlman Laboratories AG).

**DNA analysis.** DNA was extracted from 5 mL blood samples according to usual methods. The I/D dimorphism of the ACE gene was evidenced by polymerase chain reaction (PCR) according to protocol conditions and primer sequences that have been published previously. PCR products were size-separated by polyacrylamide gel electrophoresis (PAGE) through 10 cm long, 6.5% gels (Hoeffer) at 75 Volts for one hour and visualized by staining with ethidium bromide. D alleles were visualized as 190 base pairs (bp) fragments and insertion (I) alleles as 490 bp fragments. Individual DNA samples on which DD genotypes were observed were subsequently analyzed by complementary procedures described elsewhere so as to avoid DD versus ID typing mistakes.

**Data analyses.** Bivariate and partial correlation determinations were carried out with the help of an SPSS® version 6.1 for Windows® software package (Gorinchem, The Netherlands). Correlations between dichotomous variables (presence of I and D alleles versus presence of hypertension, IHD, MI and LVH) were assessed by Pearson correlations coefficients;
Table 4 - Distribution of ACE I/D genotypes and alleles frequencies in the different populations of Emirati subjects (patients with hypertension, IHD, MI and LVH; normal, healthy controls).

<table>
<thead>
<tr>
<th>ACE Genotypes</th>
<th>HT</th>
<th>IHD</th>
<th>MI</th>
<th>LVH</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Size</td>
<td>141</td>
<td>110</td>
<td>44</td>
<td>45</td>
<td>80</td>
</tr>
<tr>
<td>DD</td>
<td>0.33</td>
<td>0.30</td>
<td>0.28</td>
<td>0.29</td>
<td>0.34</td>
</tr>
<tr>
<td>ID</td>
<td>0.57</td>
<td>0.61</td>
<td>0.63</td>
<td>0.64</td>
<td>0.56</td>
</tr>
<tr>
<td>II</td>
<td>0.10</td>
<td>0.09</td>
<td>0.09</td>
<td>0.07</td>
<td>0.10</td>
</tr>
<tr>
<td>ACE Alleles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>0.37</td>
<td>0.40</td>
<td>0.39</td>
<td>0.39</td>
<td>0.38</td>
</tr>
<tr>
<td>D</td>
<td>0.63</td>
<td>0.60</td>
<td>0.61</td>
<td>0.61</td>
<td>0.62</td>
</tr>
</tbody>
</table>

Correlations between ACE I/D dimorphism and serum ACE levels were studied with Spearman correlation coefficients. 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. This study included 285 UAE national (Emirati) subjects (155 men, 130 women) with complete phenotypic and genotypic data. Table 1 summarizes the demographic and clinical characteristics of this sample population. Total serum cholesterol levels were significantly higher in all disease groups when compared to controls, as were individual ages. There was, however, no difference in BMI values amongst all groups (including controls).

Correlation analyses between the ACE I/D dimorphism and clinical variables indicated a positive trend with serum ACE activities (R=0.53; p<0.001; n=55). All 55 subjects who were included in this analysis (these were randomly picked) belonged to the “control” group; they were neither on ACE inhibitors nor on any other drug. Mean ACE levels associated with II, ID and DD genotypes are shown in Table 2 and reveal a marked association between the D allele and increased mean serum ACE values. Mean plasma ACE activity was lowest amongst II homozygotes (33±9 Units; n=4), intermediate in ID heterozygotes (43±18 Units; n=24) and highest amongst DD homozygotes (60±18 Units).

When the ACE I/D marker was investigated for putative associations with HT, IHD, LVH or MI, no correlation was observed with any of these four clinical variables (Table 3). Partial correlation analyses, whereby correlations between ACEI/D and a given clinical entity was corrected for possible confounding effects of any of the other three variables, failed to identify any trend towards significant correlations, as respective correlation coefficients remained unchanged from values determined upon bivariate correlations (data not shown).

Table 4 shows the distributions of I and D alleles as well as II, ID and DD genotypes of the ACE I/D dimorphism, in the five following categories of subjects: HT, IHD, MI, LVH, and control groups. Frequencies of the D alleles ranged from 0.59 to 0.63 in the five different clinical categories of subjects (table 4). I and D genotypic distributions were found to be in Hardy-Weinberg proportions in all groups.

Table 4 illustrates the fact that there was no significant difference in the distribution of the I and D alleles of the ACE I/D marker between any of the five groups. Therefore, this data supports the conclusion that there is lack of association between ACE I/D and HT, IHD, MI and LVH in the Emirati sample population studied here.

Discussion. Association (case-control) studies are influenced by the effects of selection bias, population stratification, confounding by other variables, and clinical criteria used to define patient groups. As for the first two, it is of utmost importance to explore the nature of reported associations in various ethnic groups that may be more genetically homogeneous. The Emirati population that was the target of this investigation offered another advantage - absence of alcohol intake and of smoking, which are usual, confounding environmental factors in these types of studies. BMI values (Table 1) indicate that all individuals were overweight (26<bMI<30) and that hypertensives were even slightly obese (BMI>30).

"Controls" individuals that were included in this investigation constituted a "comparison" rather than a "control" group. There were indeed free of disease and chosen because both their age and their BMIs matched those of the patients (Table 1). Their cholesterol levels, however, were significantly lower (5.3±0.1 versus 7.2±3.0 mmol/L for all combined patients). As these control individuals had neither personal nor family history of CVDs, and as genetic markers remain stable over the life span of an individual, these subjects nevertheless represented a valid comparison group for these association studies.

Some cases had multiple phenotypes (combinations of HT, LVH, IHD or MI), but the admixture of phenotypes, which could confound the results in association studies, was resolved with the use of partial correlation procedures.

ACE I/D and ACE levels. Numerous investigations have demonstrated that circulating ACE levels (or ACE activities) show both extensive inter-individual variability and are highly genetically
The ACE I/D marker itself correlates strongly with plasma ACE concentration, and major locus inheritance explains best the findings that the D allele is associated with elevated ACE levels. 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. McKenzie et al. 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.

Amongst 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). The strength of LD is lower in Jamaican families, where the ACE I/D marker accounts for 9% of the total variance, or in Pima Indians, where it accounts for only 6.5%. In the Emirati population of the UAE that was part of this study, the ACE I/D marker correlates strongly with circulating plasma ACE activities ($R = 0.53; p < 0.001$), and the D allele also associates with higher values (Table 2). Therefore, in the sample group of 55 random Emirati individuals screened here, we observed a similar relationship between ACE I/D and ACE activities as has been found in other ethnicities. When the Spearman correlation coefficient is squared to make up an estimate of the variance of the observed phenomenon, then our data indicates that the ACE I/D marker accounts for 28% of total variance, which is within the broad range of values (6.5-49%) reported by various groups.

ACE I/D and hypertension. Based on the hypothesis that circulating ACE levels constitute informative intermediate phenotypes of the molecular mechanisms underlying blood pressure regulation and CVDs, the ACE I/D marker has been the subject of extensive investigations in many different ethnic groups.

As ACE is a key player in the renin-angiotensin-aldosterone system, the involvement of ACE I/D was first looked for in the molecular mechanisms underlying blood pressure regulation and hypertension. It soon became evident that both association and linkage analyses of the ACE gene in hypertension were negative. Since then, a few scattered reports have hinted at weak associations with blood pressure variations. Zee et al. for example, observed greater frequencies of I alleles in hypertension amongst Australians, although this early data was later explained as caused by increased death rate; Duru et al. found higher frequencies of D alleles in African-Americans hypertensives, as did Morise et al. amongst Japanese; significant associations between D alleles and diastolic blood pressure were reported on a German population during exercise and post-stress; and recently, a “modest” (although non-significant) association was reported between ACE I/D and hypertension in Chinese.

Most investigations, however, have pointed to negative associations of ACE I/D with hypertension. Such negative findings have recently been reported on Greeks and Chinese. We have previously found a lack of association between ACE I/D and hypertension in an Emirati population. We have now repeated this finding in another, independent group of Emirati subjects who were part of the present investigation. In our first report, frequencies of D alleles were found to be 0.64 in hypertensives and 0.67 in normotensives; they are, respectively, 0.63 and 0.62 in this study (Table 4), which represents a good agreement between both independent case-control studies.

As several clinical entities that all participate in the onset of CVDs were available on all subjects, the added value of this investigation was the possibility to perform partial correlation analyses. Yet, correcting the association between ACE I/D and HT for possible confounding effects of MI, IHD or LVH did not modify the values of correlations coefficients. Therefore, this data definitely rules out an involvement of mutations that would be in linkage disequilibrium (LD) with ACE I/D in hypertension amongst Emirati.

ACE I/D and coronary heart disease. Hypotheses have been put forth as for the patho-physiology of ACE in CVDs. A reasonable consensus seems to be that a mechanism, underlined by variants that are in LD with ACE I/D, acts at the vascular level of the heart. The D allele correlates with elevated local ACE levels and must be associated with increased production of angiotensin II and increased bradykinin degradation. The net results would thus be increased neointimal proliferation, cellular matrix formation, and vasoconstriction, all of which contribute to progression towards MI.

Hence the numerous, recurrent reports that have linked ACE I/D to MI, CAD and IHD, although conflicting results have come to cloud this hypothesis as well. More recently, lack of association between ACE I/D has been found with premature myocardial infarction in Hawaiian Indians and with coronary artery disease (CAD) in Koreans. and Italian women.

Our data indicates lack of association of ACE I/D with either IHD or MI (the group of MI survivors constituted a subset of the IHD group) amongst Emirati subjects. Here again, these results remained unchanged when we performed partial correlation.
analyses. All of the subjects were overweight, which by itself may reduce the chance of seeing a relationship with MI, according to an initial report by Cambien in which “low-risk" MI patients were the ones who showed significance.

**ACE I/D and LVH.** Several conflicting results have been published regarding the value of ACE I/D in evaluating an individual’s genetic susceptibility to cardiac hypertrophy. With yet another recent compelling evidence of lack of correlation between LVH and ACE genotypes, one is led to wonder if ACE gene polymorphisms do contribute to molecular mechanisms leading to LVH, although it has also been shown that exercise-induced LV growth is strongly associated with the ACE I/D dimorphism in young and healthy males. Our results on the Emirati population show an absence of correlation between ACE I/D and LVH (Tables 3 and 4) amongst cardiology patients of this ethnic group, and this conclusion was backed up by our data using partial correlations.

In conclusion, our data on the investigation of the role of ACE I/D in a group of Emirati subjects indicates that the D allele of the dimorphism correlates directly with circulating ACE activity, and that it explains 28% of the variance of this phenomenon. Our data, however, failed to demonstrate any association with HT, IHD, MI and LVH. Similar results were obtained when men and women were analyzed independently (data not shown). These findings are strengthened by the absence in these subjects of alcohol intake and smoking, which constitute usual confounding environmental factors elsewhere. As great care was insured to avoid I/D mistyping (see “Methods" section), we are confident that ACE I/D is not a marker for CVDs amongst Emiratis.

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**References.**


