Association Study Between the ANF Gene and Hypertension in a Gulf Arab Population

Philippe M. Frossard, Eniyoma N. Obineche, Gilles G. Lestringant, and Yassin I. Elshahat

We have studied an insertion/deletion (I/D) dimorphism located in the second intron of the human atrial natriuretic factor (ANF) gene among 232 United Arab Emirates (UAE) nationals (112 normotensives and 120 hypertensives) from the Abu Dhabi Emirate, with a view to evaluating the value of this marker in relation to hypertension. Our findings show that genotype frequencies of this I/D marker occur in Hardy-Weinberg proportions (respective genotype frequencies in the overall sample population are: II, 51%; ID, 42%; DD, 7%). No association, however, was evidenced between this dimorphic site and clinical diagnosis of essential hypertension. This suggests that: 1) this I/D dimorphism is not a useful marker to study the relationship between the ANF gene and hypertension in the UAE; and 2) variations of the ANF gene that may be in linkage disequilibrium with this marker do not play a major role in the determination of hypertension in this Arab population. Am J Hypertens 1997;10:1308–1310 © 1997 American Journal of Hypertension, Ltd.

KEY WORDS: Association study, atrial natriuretic factor gene, essential hypertension, I/D polymorphism, polymerase chain reaction, United Arab Emirates.

In the unraveling of the genetic architecture of human essential hypertension, many candidate gene loci are available for study.1–5 The human atrial natriuretic factor (ANF) has potent natriuretic and vasodilatory activities;1 it has also been reported to inhibit synthesis and release of aldosterone as well as to suppress renin activity.6 Furthermore, in pharmacological doses, ANF lowers blood pressure and promotes salt excretion;1 in young hypertensive rats, somatic delivery of human ANF induces a sustained reduction of systemic blood pressure.7 The human ANF gene8 has thus long been included in the list of candidate genes for familial susceptibility to hypertension. The availability of reported genetic markers at the ANF gene locus facilitates these studies and this gene has already been the subject of several investigations.9–13 Ramasawmy et al14 have reported the existence of an 8-base pair (bp) bi-allele, insertion/deletion (I/D) polymorphism that is located within a polyadenylate stretch in the second intron of the ANF gene. These authors also mentioned the existence of “atypical” haplotypes with other known dimorphic sites (Hpa II and Sca I) of this gene locus, indicating that rapid changes may have occurred within this region.14

We have carried out an association (case–control type) study on nationals from the Abu Dhabi Emirate with a view to evaluating the relationship of this I/D marker of the ANF gene to essential hypertension in a genetically homogeneous ethnic group.
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. Until recently, Gulf Bedouins of this area were organized into tribes that have been characterized by restricted population migrations. The incidence of essential hypertension in the UAE is similar to that in other parts of the world.15

MATERIALS AND METHODS

Subjects All individuals were nationals from the Abu Dhabi Emirate and belonged to two groups: hypertensives and normotensives.

Normotensives This group consisted of 112 unrelated subjects (58 women, 54 men) with a mean age of 48.5 ± 7.3 years. Resting systolic blood pressures were <140 mm Hg and diastolic blood pressures <90 mm Hg on at least three separate occasions. No patient had a personal or family history of hypertension and none was on antihypertensive or other therapies affecting blood pressure.

Hypertensives This group comprised 120 unrelated patients (60 women, 60 men) suffering from essential hypertension. Their mean age group was 49.0 ± 6.0 years. Patients were classified as having essential hypertension if they had systolic blood pressures >160 mm Hg and diastolic blood pressures >95 mm Hg on at least three separate occasions, and had no clinical signs, symptoms, or laboratory findings suggestive of secondary hypertension.

DNA Analysis DNA was extracted from 5 mL blood samples according to usual methods; polymerase chain reactions (PCR) were performed on 100 ng DNA samples under the following conditions: 5 pmol of each primer14 were put into a final volume of 50 μL containing 5 mmol/L MgCl₂, 50 mmol/L KCl, 10 mmol/L Tris-HCl pH 8.4, 0.1 mg/mL gelatin, 0.2 mmol/L of each dNTP (Gibco BRL, Grand Island, NY) and 0.25 unit of Taq polymerase (Gibco BRL, Grand Island, NY). Thirty amplification cycles (denaturation at 95°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 1 min) were done in a Biometra thermal cycler. Then, 10 μL of PCR products were digested with 1 U of HaeIII ( Sigma Chemicals, St. Louis, MO), size-separated by polyacrylamide gel electrophoresis (PAGE) through 16 cm long, 6.5% gels (Gibco BRL) at 100 volts for 16 h and visualized by staining with ethidium bromide. Deletion (D) alleles were visualized as 204 base pairs (bp) fragments and insertion (I) alleles as 212 bp fragments.14

RESULTS

Genotypes for the I/D dimorphism located in the second intron of the ANF gene were determined in the two populations of 120 hypertensive and 112 normotensive subjects, respectively. Table 1 displays the results of this association (case-control) study. Frequencies of the I alleles were 0.27 ± 0.03 among hypertensives and 0.30 ± 0.03 among normotensives. D and I allelic distributions were found to be in Hardy-Weinberg proportions (Table 1) in each of the two groups (normotensives, χ² = 0.18, 2 df, P = .91; hypertensives, χ² = 0.07, 2 df, P = .97).

There was no significant difference in the distribution of the I and D alleles of the ANF gene between the two groups (χ² = 0.86, 2 df, P = .65).

DISCUSSION

Molecular genetic investigations of the human ANF gene are facilitated by the various polymorphisms that have been identified at that locus. Combined results reported by Barley et al9 on white Europeans and black Afro-Caribbeans, by Zee et al10 on Australians, and by Berge and Berg11 on a Norwegian population, tend to show that the ANF gene is not significantly involved in either hypertension determination or blood pressure regulation among normotensive individuals; these effects appear to be independent of ethnicity.9–11 Rutledge et al, however, showed that a HpAI1 dimorphism located in the second intron of the human ANF gene is associated with hypertension in a population of African Americans—a typically salt-sensitive group.13 In the same population, an Scal dimorphic site in exon 3 of the ANF gene was found in perfect linkage equilibrium with the disease state. It is therefore worthwhile, at this stage, to investigate the potential value of different ANF markers with respect to hypertension in different populations.

Ramasawmy et al, who reported the presence of the I/D dimorphism in the second intron of the ANF gene,14 found a wide variability in allele frequencies depending on ethnic origins. Thus, frequencies of I

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Normotensives</th>
<th>Hypertensives</th>
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</thead>
<tbody>
<tr>
<td>DD</td>
<td>53 (55)</td>
<td>64 (64)</td>
</tr>
<tr>
<td>ID</td>
<td>50 (47)</td>
<td>48 (47)</td>
</tr>
<tr>
<td>II</td>
<td>9 (10)</td>
<td>8 (9)</td>
</tr>
<tr>
<td>p(D)</td>
<td>0.70 ± 0.03</td>
<td>0.73 ± 0.03</td>
</tr>
<tr>
<td>q(I)</td>
<td>0.30 ± 0.03</td>
<td>0.27 ± 0.03</td>
</tr>
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SE = {V(p)}^{1/2}, where V(p) = p(1 - p)/n, n being the number of chromosomes screened.

P represents the frequency of D alleles and q the frequency of I alleles. Expected numbers of individuals assuming Hardy-Weinberg equilibrium are indicated in parentheses. There was no significant difference in allelic distributions between the two groups: χ² = 0.56, 2 df, P = .75.
alleles were 0.068 in Mauritian Indians, 0.541 in black Africans, and 0.225 in French whites. In the population of Emirati studied here, the overall I allele frequency (combining data from normotensives and hypertensives) is 0.28 ± 0.03. This value stands in between those obtained by Ramasawmy et al.\textsuperscript{14} on black Africans and French whites.

Genotype frequency distribution within each of the two groups (hypertensives and normotensives) occurred in Hardy-Weinberg proportions, which indicates that the extensive level of consanguinity frequently advocated in the Gulf populations does not affect genotypic heterozygositites—at least not at the ANF gene locus.

Our results also indicate lack of association between the I/D dimorphism of the ANF gene and clinical diagnosis of human essential hypertension in the population under study. Our data does suggest that putative ANF variants whose DNA variations might be in linkage disequilibrium with the I/D dimorphism, do not play a significant role in the determination of hypertension among Emiratis.

It could be argued, however, that the relatively small number of subjects (232 in total) could give a low probability of detecting a small effect of the I/D polymorphism (a small gene effect could be expected in the case of a disease as complex as hypertension). These types of study design (association studies of the case–control type) are prone to type II errors (that is, failing to reject the null hypothesis—that there is no effect be likely to be detectable through linkage disequilibrium studies alone. Only if the deleterious gene effect were confined to a single variant would this effect be likely to be detectable through linkage disequilibrium with a nearby marker. Interestingly, this seems to hold true for a HpaII marker\textsuperscript{13} (at least in black Americans) but not for the I/D dimorphism investigated here, although both markers are located in the same (second) intron of the ANF gene.

The clearest evidence for the involvement of specific genes in the onset of hypertension comes from transgenic animals. As for the involvement of the ANF gene in the onset of hypertension, John et al.\textsuperscript{12} have described generations of transgenic mice with disrupted proANF genes. Heterozygous mutants became hypertensive only when on high salt diets. Homozygous mice, however, who had no circulating or atrial ANF, developed elevated blood pressures no matter what diet they were on. This finding leads to the hypothesis that genetically reduced ANF production can induce a salt-sensitive form of hypertension.\textsuperscript{12}

The next step in the human molecular genetics of the ANF gene as it relates to elevated blood pressure will be to look directly for ANF variants involved in the genetic etiology of salt-sensitive hypertension.

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REFERENCES