

## ASSOCIATION BETWEEN AN ANF GENE I/D DIMORPHISM AND LEFT VENTRICULAR HYPERTROPHY IN A GULF ARAB POPULATION

Enyioma N.Obineche, FRCP; Philippe M. Frossard, DSc; Awais M. Bokhari, FRCP

**Background:** An association case-controlled study was carried out on a group of 151 United Arab Emirates nationals—62 normotensives with and without left ventricular hypertrophy (LVH) and 89 hypertensives, also with and without LVH—with a view to evaluating the value of an insertion/deletion (I/D) dimorphism located in the second intron of the human atrial natriuretic factor (ANF) gene in relation to left ventricular hypertrophy.

**Subjects and Methods:** Criteria used for LVH inclusion were: demonstration of Sokolow and Lyon ECG criteria (sum of *S* wave in  $V_1$ , and tallest *R* wave in lead  $V_5$  or  $V_6$  >35 mm) and echocardiography findings (interventricular septum >1.2 cm; posterior LV wall >1.3 cm) in the long axis. ANF gene was obtained according to the usual methods by DNA extraction by means of polymerase chain reactions (PCR). The frequencies of this marker were performed according to the Hardy-Weinberg proportions.

**Results:** Our findings show that there was a significant difference in the distribution of the I and D alleles between the two groups (LVH vs non-LVH), with  $\chi^2 = 12.34$ , 2df,  $P=0.002$ , making this a significant association of the D allele with LVH.

**Conclusion:** Our results do suggest that variants of the ANF gene might be involved in the determination of left ventricular hypertrophy.

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**Key Words:** Association study, atrial natriuretic factor gene, left ventricular hypertension, I/D polymorphism polymerase chain reaction.

The aim of molecular geneticists in respect of the field of cardiovascular diseases (CVDs) is to identify the quantitative trait loci (QTLs) that play a role in the onset or progression of various disease processes.<sup>1,2</sup> Among the various strategies that have been put to trial to decipher the molecular framework of complex clinical phenotypes, association (case-control) studies using candidate genes increasingly represent preferred methods.<sup>3,4</sup> This approach offers great power for detecting QTLs of reduced low penetrance, and as the number of available candidate genes increase rapidly, it is bound to develop even further.<sup>5,6</sup>

In combination with environmental influences, the molecular and genetic structures of CVDs 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. Intricate interaction in the various pathways of atherosclerosis and hypertension have led to the suspicion that common genetic effects underlie these disorders.<sup>1</sup>

As most observations have been challenged by various investigators,<sup>7,8</sup> data from genetically isolated populations are of utmost importance in resolving issues of contention related to studies based on the concept of linkage equilibrium.<sup>4</sup> We have previously described a lack of association between the ANF gene and hypertension in the UAE population, and that the 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.<sup>9</sup>

Human atrial natriuretic factor (ANF) has potent natriuretic and vasodilatory activities, and has also been reported to inhibit synthesis and release of aldosterone as well as suppress renin activity.<sup>10</sup> Furthermore, in pathological doses, ANF lowers blood pressure and promotes salt excretion in young hypertensive rats.<sup>11</sup> Somatic delivery of human ANF has induced a sustained reduction of systemic blood pressure. The human ANF gene has, for a long time, been included in the list of candidate genes for familial susceptibility to

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From the Department of Internal Medicine, Faculty of Medicine and Health Sciences, UAE University, Al Ain, United Arab Emirates.

Address reprint requests and correspondence to Dr. Obineche: Department of Internal Medicine, Faculty of Medicine and Health Sciences, UAE University, P.O. Box 17666, Al Ain, United Arab Emirates.

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TABLE 1. Sociodemographic characteristics of subjects.

	LVH	No LVH
Hypertensives (n=89)		
Number (M/F)	76 (39/37)	13 (9/4)
Age $\pm$ SD	56 $\pm$ 11.2	53.9 $\pm$ 9.7
Mean SBP $\pm$ SD	174 $\pm$ 17.7	151.8 $\pm$ 14.1
Mean DBP $\pm$ SD	97.5 $\pm$ 14.4	94.7 $\pm$ 10.5
Normotensives (n=62)		
Number (M/F)	20 (18/2)	42 (21/21)
Age $\pm$ SD	54.1 $\pm$ 17.1	52.9 $\pm$ 14.7
Mean SBP $\pm$ SD	126 $\pm$ 14.1	118.6 $\pm$ 12.5
Mean DBP $\pm$ SD	81.7 $\pm$ 9.4	76.7 $\pm$ 7.4

hypertension.<sup>12</sup> Availability of reported genetic markers at the ANF gene locus helps to facilitate these studies, and this gene has already been the subject of investigations.<sup>11-13</sup>

In this paper, we report a pilot association study (case-control type) on nationals of the Abu Dhabi Emirate, with a view to establishing the relationship of this I/D marker of the ANF gene to left ventricular hypertrophy (LVH) in a genetically homogenous group, as this, to our knowledge, has not been previously reported. 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 were characterised by restricted population migrations. The incidence of essential hypertension in the UAE is similar to that in the other parts of the world,<sup>14</sup> but the relationship between the ANF gene and left ventricular hypertrophy (LVH) has yet to be determined.

### Subjects and Methods

All 151 subjects were nationals from the Abu Dhabi Emirate and belonged to two main groups: hypertensives with and without LVH and normotensives also with and without LVH. No member of the sampling population admitted to alcohol intake, and there was no history of smoking in any of the subjects. Exclusion criteria questionnaire was used to exclude subjects who smoked. The project was approved by the Research Ethics Committee of the Faculty of Medicine and Health Sciences (UAE University, Al Ain, UAE) and all subjects gave their informed consent.

Patients were classified as suffering from essential hypertension if they had systolic blood pressures >140 mm Hg and diastolic blood pressures >90 mm Hg on at least three separate occasions and had no clinical signs, symptoms or laboratory findings suggestive of secondary hypertension, and had a positive family history as assessed by direct questioning of relatives, of occurrence of hypertension in any close relative.

Criteria for LVH inclusion were: demonstration of Sokolow and Lyon ECG criteria (sum of S wave and V1 and

tallest R wave in lead V5 or V6 >35 mm) and echocardiographic findings (interventricular septum >1.2 cm; posterior LV wall >1.3 cm) in the long axis. Both criteria were used in assessment of LVH. The subjects were all known hypertensive patients who were recruited for this study during ambulatory visits to the Hypertension Unit for routine check-ups. Care was taken to ensure that no subject of this study (whether in the hypertensive or normotensive groups) was affected by other confounding clinical phenotypes, including non-insulin dependent diabetes mellitus (that is otherwise quite prevalent in this population).

### DNA Analysis

DNA was extracted from 5 mL blood samples according to usual methods, and polymerase chain reactions (PCR) were performed on 100 ng DNA samples under the following conditions: 5 pmoles of each primer (15) were put into a final volume of 50 L containing 5 mM MgCl<sub>2</sub>, 50 mM KCl, 10 mM Tris-HCl pH 8.4, 0.1 mg/mL gelatin, 0.2 mM of each dNTP (Gibco BRL), and 0.25 unit of Taq polymerase (Gibco BRL). 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. Ten microliters of PCR products were digested with one unit of Hae III (Sigma), size separated by polyacrylamide gel electrophoresis (PAGE) through 16 cm long, 6.5% gels (Gibco BRL) at 100 volts for 16 hours, 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.<sup>15</sup>

### Data Analyses

Raw data was coded, entered and processed in an IBM-PC compatible computer using the Statistical Packages for Social Sciences (SPSS<sup>®</sup>) version 7.5 for Windows<sup>®</sup> software package. Clinical and laboratory values were expressed as mean and standard deviation (SD) unless otherwise stated. Chi-squared analyses were performed to test for differences in proportions of categorical variables between two or more groups. Differences in the distributions of I/D ANF genotypes according to clinical phenotypes (LVH vs. no LVH) were assessed using 3x2 tables of association and chi-squared procedures. Hardy-Weinberg proportions of allele distributions were investigated by chi-squared analyses. For all analyses, statistical significance was considered at significance level (P) values of <0.05.

### Results

Table 1 shows sociodemographic characteristics of subjects (hypertensive and normotensive). None of the normotensive patients had a family history of hypertension and none was on any antihypertensive or other therapies

TABLE 2. Distribution of the I/D alleles of the ANF gene among normotensives with and without LVH and hypertensives with and without LVH in UAE nationals, with corresponding frequencies and associated standard errors.

	LVH			No LVH		
	HT (%)	NT (%)	Total (%)	HT (%)	NT (%)	Total (%)
Genotype						
II	2 (2.6)	2 (10)	4 (4.2)	0 (0)	12 (28.6)	12 (21.8)
ID	32 (42.1)	8 (40)	40 (41.7)	5 (38.5)	10 (23.8)	15 (27.3)
DD	42 (55.3)	10 (50)	52 (54.2)	8 (61.5)	20 (47.6)	28 (50.9)
Allele frequencies						
p(I)	0.24±0.04	0.30±0.05	0.25±0.05	0.19±0.06	0.04±0.05	0.35±0.05
q(D)	0.76±0.04	0.70±0.05	0.75±0.05	0.89±0.06	0.60±0.05	0.65±0.06

HT=hypertensives; NT=normotensives; p=frequency of I alleles; q=frequency of alleles.

that would affect blood pressure. They were recruited as normotensive individuals whose age, sex and body mass index (BMI) matched those of the hypertensive group.

Genotypes for the I/D dimorphism located in the second intron of the ANF gene were determined in the two main groups (LVH and no LVH) of the 151 subjects as shown in Table 2. On studying the allele frequencies and comparing the genotype distributions of the hypertensives and normotensives in the two main groups (LVH and no LVH), certain interesting features emerge. In Table 2, examination of the frequencies and distribution of the alleles I and D genotype distributions were provided in Hardy-Weinberg proportions in each of the two groups (hypertensives and normotensives). In this table, *p* represents the frequency of I alleles and *q*, the frequency of D alleles. There was no significant difference in allelic distributions between normotensives and hypertensives of the two groups (LVH and no LVH:  $P=0.80$  and  $P=0.19$ , respectively). However, on pooling the sum total in the LVH vs. non LVH groups, we found significant differences between the two groups ( $P<0.002$ ). Therefore, the association of D alleles with clinical diagnosis of LVH was statistically significant, and our results do suggest a significant association between the I/D dimorphism of the ANF gene and clinical diagnosis of left ventricular hypertrophy.

## Discussion

In this study, we demonstrate for the first time a statistically significant association between the I/D dimorphism of the ANF gene and clinical diagnosis of left ventricular hypertrophy in a genetically homo genous population in the United Arab Emirates. Investigations related to the molecular genetics of human ANF gene are made possible by the various polymorphisms which have been identified at that locus. Ramaswamy et al.<sup>15</sup> who reported the presence of the I/D dimorphism in the second intron of the ANF gene found a wide variability in allele frequencies depending on ethnic origins. Thus, frequencies of I alleles were 0.068 in Mauritian Indians, 0.541 in black Africans and 0.225 in French whites. In our earlier study of the Emirati population,<sup>9</sup> the overall I allele frequency

(combining data from normotensives and hypertensives) was  $0.28\pm0.03$ . This value ranks inbetween those in black Africans and French whites obtained by Ramaswamy et al.

Data suggesting that left ventricular hypertrophy (LVH) predicts the incidence of hypertension and that the converse is true (hypertension is associated with the development of LVH) may suggest that both hypertension and LVH share common antecedents.<sup>16</sup> Literature examining the risk factors for echocardiographic LVH is extensive, and comes from a variety of settings including epidemiological investigations and hypertension.<sup>17-19</sup> Some previous studies have reported a possible association between LVH and hypertension,<sup>18-20</sup> however, to our knowledge no study has been reported relating ANF to LVH. In this study, for the first time, we demonstrate an association between the ANF gene and clinical diagnosis of LVH in our Emirati population.

In our previous association study between the ANF gene and hypertension,<sup>9</sup> no association was evidenced between this dimorphic site and clinical diagnosis of essential hypertension. This suggested that, first, this I/D polymorphism was not a useful marker to study the relationship between the ANF gene and hypertension in the UAE, and second, 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. It could be argued, however, that the relatively small number of subjects in this study (232 in total) could give a low probability in 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 (i.e., failing to reject the null hypothesis—that there is no difference in allelic distribution between the two groups—when it is false).

Although the number of our present study population was smaller than the number used in our association study between the ANF gene and hypertension, significant association was observed between the ANF gene and clinical diagnosis of LVH. Ongoing investigations are examining the influence of genetic factors on LVH.<sup>21,22</sup> Twin studies and epidemiological investigations have

provided suggestive evidence for a genetic contribution to LVH.<sup>22,23</sup> However, the genetic basis of LVH remains unknown.

In conclusion, we have shown that in our homogenous Emirati population recruited for this study, the I/D dimorphic marker (the D allele) located in the second intron of the human ANF gene is associated with clinical diagnosis of LVH. It is therefore proposed that ANF variants (or variants in a nearby gene, that may be in linkage disequilibrium with ANF) could be implicated in the underlying mechanisms leading to LVH. However, association does not imply causation and such results should be interpreted with care.<sup>24</sup> Nonetheless, conclusions reached in this report warrant testing in other populations.

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