An Association Study of Five Genetic Loci and Left Ventricular Hypertrophy amongst Gulf Arabs

Enyioma N. OBINECHE, Philippe M. FROSSARD*, and Awais M. BOKHARI**

We carried out an association (case-control) study of five candidate genes — G-protein β3 subunit gene variant; methylene tetrahydrofolate reductase (MTHFR); angiotensin converting enzyme (ACE) gene; and paraoxonase 1 and 2 (PON 1 and 2) genes — in a United Arab Emirate population. The aim was to establish a possible relationship between these five candidate genes and clinical left ventricular hypertrophy (LVH) in a genetically homogenous group. DNA samples were collected from 213 unrelated Nationals who were further segregated into 98 subjects with LVH (78 hypertensives and 20 normotensives) and 115 (23 hypertensives and 92 normotensives) age- and sex-matched controls who did not present with LVH. Of the five candidate gene markers studied, no significant differences in the genotype distribution of the MTHFR, PON 1 and 2 or ACE markers were found between the LVH and non-LVH groups. However, a possible association was found between the β3 G-protein C825T marker and LVH. In conclusion, our results suggest an association between LVH and the C825T allele of the G-protein β3 subunit gene. (Hypertens Res 2001; 24: 635–639)

Key Words: alleles, association study, candidate genes, Emirates, G-proteins, hypertrophy

Introduction

One of the main concerns of molecular geneticists with interest in the field of cardiovascular diseases (CVD) is to decipher the genetic and molecular aetiologies of the various disease processes by identifying the quantitative trait loci (QTLs) that participate in the onset and progression of disease (1, 2). With respect to the aetiology of essential hypertension (EH), several mechanisms with complex interactions contribute to the regulation of blood pressure, and their effects are modulated by a variety of environmental factors (3).

Among the various strategies that have been tested to decipher the molecular framework of complex clinical phenotypes, association (case-control) studies using candidate genes are increasingly preferred (4).

This approach offers great power for detecting QTLs of reduced low penetrance, and as the number of available candidate genes increases rapidly, further development is bound to occur (4). In association with environmental influences, the molecular and genetic structures of CVD may exert their effects through acute processes (such as vasoconstriction and thrombosis) or through chronic processes (including hypertension, atherosclerosis and left ventricular hypertrophy). Clarification of the complicated interactions in the various pathways of atherosclerosis and hypertension has led to the suspicion that common genetic effects underlie these disorders (5).

The genetic basis of essential hypertension is complex. Variations in a variety of genes have shown an association with hypertension in some studies, but these associations are often not reproducible in studies of other populations. For example, an angiotensin converting enzyme (ACE) insertion/deletion (I/D) polymorphism was associated with hypertension in African Americans but not in Europeans (6, 7).
Table 1. The Characteristics of Patients Studied

<table>
<thead>
<tr>
<th></th>
<th>LVH (n=98)</th>
<th>No LVH (n=115)</th>
<th>p-value* significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertensives (n=101)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number (M/F)</td>
<td>78 (41/37)</td>
<td>23 (12/11)</td>
<td></td>
</tr>
<tr>
<td>Age±SD</td>
<td>56.0±11.2</td>
<td>53.9±9.7</td>
<td>NS</td>
</tr>
<tr>
<td>Mean SBP±SD</td>
<td>174.0±17.7</td>
<td>151.8±14.1</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Mean DBP±SD</td>
<td>97.5±14.4</td>
<td>94.7±10.5</td>
<td>NS</td>
</tr>
<tr>
<td>Normotensives (n=112)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number (M/F)</td>
<td>20 (18/2)</td>
<td>92 (46/46)</td>
<td></td>
</tr>
<tr>
<td>Age±SD</td>
<td>54.1±17.1</td>
<td>52.9±14.7</td>
<td>NS</td>
</tr>
<tr>
<td>Mean SBP±SD</td>
<td>126±14.1</td>
<td>118.6±12.5</td>
<td>p=0.02</td>
</tr>
<tr>
<td>Mean DBP±SD</td>
<td>81.7±9.4</td>
<td>76.7±7.4</td>
<td>p= 0.01</td>
</tr>
</tbody>
</table>

*Student’s t-test was used to determine the statistical significance of differences between the two group means. NS=Nonsignificant

This inconsistency is explained in part by the subsequent finding that I/D polymorphism is in linkage disequilibrium (L/D) with some other single nucleotide polymorphisms (SNPs) in the ACE gene (8).

Some of these SNPs may be of functional significance and their frequencies may vary between different ethnic groups. In another example, an angiotensinogen (AGT) coding polymorphism (M235T) showed an association with hypertension in several studies in European and Japanese but not in studies of African Americans (9, 10).

It has been suggested by Williams et al. and other workers (11, 12) that genetic interactions between multiple loci rather than variants of a single gene underlie the genetic basis of hypertension in their study subjects. They hypothesise that such interactions may account for the inconsistent findings in previous studies.

Left ventricular hypertrophy (LVH) is a major independent risk factor for morbidity and mortality from cardiovascular disease (13). Blood pressure is an important determinant of LVH (14, 15), and a large percentage of patients with essential hypertension develop this complication. However, the degree of such complication varies greatly from patient to patient (15, 16). Moreover, epidemiological studies have demonstrated that subjects with LVH may have near-normal blood pressure (16), suggesting that other factors may be important in the development of this hypertrophy. Family and twin studies have indicated that left ventricular mass is a familial trait influenced by both genetic and environmental factors (17).

The United Arab Emirates (UAE) is a federation of seven emirates, with the Abu Dhabi Emirate being the largest. The indigenous population of the Abu Dhabi Emirate comprises UAE nationals, who are Gulf Arabs of Bedouin descent. Until recently, these Gulf Bedouin were organized into tribes that were characterized by restricted population migrations. Within these populations, the usual confounding environmental factors, i.e., alcohol consumption and smoking, are absent.

Several studies have been carried out to correlate candidate genes with various cardiovascular diseases and essential hypertension (18–26). The ACE gene was studied in relation to LVH, but the results were conflicting (20, 22, 23). For this reason, in order to investigate the relationship between five candidate genes, (G-protein β subunit variant, methylene tetrahydrofolate reductase (MTHFR), ACE gene and paroxonase (PON) 1 and 2 genes) and LVH, we carried out a study in a genetically homogenous Emirati population.

Materials and Methods

Subjects

All subjects recruited for this study were UAE nationals from the Abu Dhabi Emirate. The overall study group was composed of 213 subjects who were further segregated into 98 subjects with LVH (78 hypertensives and 20 normotensives) and 115 (23 hypertensives and 92 normotensives) age- and sex-matched controls who did not present with LVH. Details of the subject group are presented in Table 1.

Criteria for inclusion in the group affected by LVH were as follows: demonstration of Sokoloe and Lyon ECG criteria (sum of the S wave in V1 and the tallest R wave in leads V5 or V6 > 35 mm) and echocardiography findings (intraventricular septum > 1.2 cm; posterior LV wall > 1.3 cm) in the long axis view.

The project was approved by the Research Ethics Committee of the Faculty of Medicine and Health Sciences (UAE University, Al Ain, United Arab Emirates) and all subjects gave their informed written consent for genetic analysis as per the study protocol.

DNA Analysis

We studied the distributions of genotypes of intragenic markers in five candidate genes. All five bi-allele polymorphisms have been described previously and we used experimental conditions and nomenclatures that have been published by the corresponding authors. The five markers were:
Table 2. Comparison in the Distribution of Genotypes of the Dimorphic Polymorphisms in the Five Candidate Genes, between LVH Patients and Non-LVH Emirati Subjects

<table>
<thead>
<tr>
<th></th>
<th>MTHFR C677T</th>
<th>PON1 Glu192 Arg</th>
<th>PON2 Cys311 Ser</th>
<th>β-G Protein C825T</th>
<th>I/D ACE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>AV</td>
<td>VV</td>
<td>AA</td>
<td>AB</td>
</tr>
<tr>
<td>LVH (%)</td>
<td>66</td>
<td>30</td>
<td>2</td>
<td>12</td>
<td>62</td>
</tr>
<tr>
<td>LVH (%)</td>
<td>67.3</td>
<td>30.6</td>
<td>4.0</td>
<td>13.3</td>
<td>68.9</td>
</tr>
<tr>
<td>LVH (%)</td>
<td>73</td>
<td>30</td>
<td>6</td>
<td>20</td>
<td>44</td>
</tr>
<tr>
<td>LVH (%)</td>
<td>67.0</td>
<td>27.5</td>
<td>5.5</td>
<td>23.8</td>
<td>52.4</td>
</tr>
<tr>
<td>p-value*</td>
<td>p=0.412</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Significance</td>
<td>(NS)</td>
<td></td>
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</tbody>
</table>

*Chi-square test was performed to determine significant differences among the three proportions. NS = Nonsignificant.

1) C677T, leading to an Ala-to-Val substitution in the MTHFR gene and to visualization of A (677Ala) and V (677Val) alleles (27); 2) Glu192Arg in the PON1 gene leading to visualization of A (192Glu) and B (192Arg) alleles (28); 3) Cys311Ser in the PON2 gene detected as PON2*C and PON2*S alleles (21, 28); 4) C825T in the β-G protein gene, detected as C (825C) and T (825T) alleles (18); 5) and an I/D polymorphism in the ACE gene (23).

Data Processing, Statistical Methods and Analysis

The data were coded, entered into a computer and processed on an IBM-PC compatible computer using the Statistical Packages for Social Sciences (SPSS) according to Norusis (29). Data are expressed as the mean plus SD unless otherwise stated. The Student’s t-test was used to ascertain the significance of differences between mean values of two continuous variables, and the Mann-Whitney test was used for non-parametric distribution. Chi-square analysis was performed to test for differences in proportions of categorical variables between two or more groups. Values of p < 0.05 were considered to indicate statistical significance.

Results

Table 1 shows the characteristics of patients studied.

Genotypes for the five genetic markers in the two groups (LVH and non-LVH) were as depicted in Table 2.

In our study we found no association between the ACE gene I/D polymorphism and LVH in the Emirati population. Our present results are in good agreement with those of Lindpaintner et al. (23); both studies fail to support a role for ACE gene mutations in determining left ventricular mass (LVM) and indicate that ACE/insertion/deletion (I/D) polymorphism is not a useful marker to predict the risk of left ventricular hypertrophy.

With regard to other candidate genes, the C677T transition in the MTHFR gene provided no support for the idea that this polymorphism might be a risk factor for LVH (p = 0.412).

As regards the distribution of the biallelic PON polymorphism at Codon 192 (A and B alleles), even though a common polymorphism in this gene has been reported as an independent risk factor for Coronary Heart Disease (CHD) in populations of white ancestry, these mutations do not appear to be useful markers of the increased risks of LVH. However, in the case of C825T substitution in GNβ1, there was a difference between the two patient groups (LVH and non-LVH) which was statistically significant in the C/C genotype with p = 0.044, suggesting an association between LVH and the GNβ1 C825T polymorphism in the present cohort. Furthermore, our data would seem to indicate a general pattern of recessive transmission for the observed association.

There was thus no significant difference in the genotype distributions between the two individual groups (LVH and non-LVH) in the case of the MTHFR, PON 1 and 2, or ACE markers.

Discussion

In this investigation the correlation of LVH to five candidate genes was studied in a homogeneous Emirati population.

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. The influence of the first two variables can be minimized by exploring putative associations in various ethnic groups that may be more genetically homogeneous. The Emirati population that was the subject of this investigation offered another advantage absence of alcohol intake and of smoking, which are usual confounding environmental factors in these types of studies.

LVH occurs as a physiological adaptation to high blood pressure (HBP) in EH. However, the correlation between the severity of HBP and LVM is poor, suggesting that in addition to haemodynamic overload, other factors may contribute to the development of LVH in essential hypertension.

Contradictory findings have been reported in regard to ACE gene polymorphism and LVH in both normal (22, 23) and hypertensive populations (24, 26). In line with the find-
ings of Lindpainter et al. (23), our results showed no correlation between the ACE gene and left ventricular hypertrophy.

A possible association between the C825T dimorphism and essential hypertension has been reported in a recent case-control study (18). Results have, however, been conflicting (19, 30), and no data have been reported regarding the possible relation of C825T to organ damage. A recent case control study in Germany initially showed that the T allele was significantly associated with essential hypertension (18). However, studies carried out in other ethnic populations failed to show association of the T allele with blood pressure (30) or hypertension (31). In a Japanese study (31), however, the frequency of T alleles was much higher (49%) than that reported for the white population (30%).

Other results demonstrate that the C825T allele of the GNβ3 gene, which encodes the β3 subunit of heterotrimeric G proteins, is associated with LVH. These results are in line with those of Poch et al. (32), who recently reported that the C825T allele in the G-protein β3 subunit gene was associated with higher diastolic blood pressure, left ventricular mass and LVH in patients with essential hypertension.

The pathological significance of this association is contingent on the fact that previous studies have demonstrated that the C825T allele of GNβ3 is related to increased stimulated binding of labelled GTP in cell lines from hypertensive patients (18) in concordance with the observation of enhanced stimulated G protein activation, Na+-H+ exchanger activity, and cell growth and proliferation (33). Earlier investigators have shown that the enhanced Na+-H+ exchanger activity in hypertensive individuals is associated with several phenotypes, such as left ventricular hypertrophy (33), insulin resistance (34) and renal sodium retention (35).

Taken together, our results and those of Poch et al. (32) suggest an association between the C825T allele of the GNβ3 gene and LVH.

We are aware, however, that the design of this study could have been matched to better account for hypertension and its confounding influences on LVH. This could not be done owing to certain constraints. To further explore these influences, we compared the prevalence of the genes under study between hypertensives and normotensives and found no difference between the two groups, leading us to suggest that the observed association between the C825T allele and LVH may not be due to hypertension.

Another constraint in our study merits attention: the existence of multiple statistical testing. In association studies such as ours there is always the possibility of false positivity when multiple genetic loci are investigated and tests are performed. It has been suggested that this could be addressed by complementing with linkage studies (36). Nonetheless, our present finding of significance at the level of p = 0.0445 for the association between LVH and the 825T allele in the G-protein β3 subunit, the first such finding in a homogenous Emirati population, would seem to strongly indicate a correlation between LVH and this polymorphism.

In conclusion, no significant correlation was found in the genotype distributions between the LVH and non-LVH groups for the MTHFR, PON 1 and 2 or ACE markers. However, our data on this homogenous cohort of Emirati provided the first suggestion of a correlation between LVH and the C825T allele in the G-protein β3 subunit. Additional studies in other ethnic groups should be performed to confirm this association.

References

15. Devereux RB, Roman MJ: Left ventricular hypertrophy in


