Original Article

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

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We carried out an association (case-control) study of five candidate genes —G-protein β_3 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 Emirati 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 β_3 G-protein C825T marker and LVH. In conclusion, our results suggest an association between LVH and the C825T allele of the G-protein β_3 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).

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Table 1. The Characteristics of Patients Studied

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

*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 candi-

date 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_3 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 Sokolow 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 (interventricular 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

	MTHFR C677T			PON1 Glu 192 Arg			PON2 Cys 311 Ser			β_3 G Protein C825T			I/D ACE		
	AA	AV	VV	AA	AB	BB	CC	CS	SS	CC	CT	TT	II	ID	DD
LVH	66	30	2	12	62	16	16	26	34	40	40	18	6	58	28
(%)	67.3	30.6	4.0	13.3	68.9	17.8	21.1	34.2	44.7	40.8	40.8	20.0	6.5	63.0	30.4
no-LVH	73	30	6	20	44	10	8	18	32	28	60	25	8	62	30
(%)	67.0	27.5	5.5	23.8	52.4	23.0	13.8	31.0	55.2	24.8	53.1	22.1	8.0	62.0	30.0
<i>p</i> -value*	<i>p</i> =0.412			<i>p</i> =0.0851			<i>p</i> =0.407			<i>p</i> =0.0445			<i>p</i> =0.925		
(Significance)	(NS)			(NS)			(NS)			(NS)			(NS)		

*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 (192 Glu) and B (192 Arg) alleles (28); 3) Cys311Ser in the PON2 gene detected as PON2*C and PON2*S alleles (21, 28); 4) C825T in the β_3 -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 $\text{GN}\beta_3$, 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 $\text{GN}\beta_3$ 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 homogenous 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 homogenous. 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 $GN\beta_3$ gene, which encodes the β_3 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 β_3 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 $GN\beta_3$ 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 $GN\beta_3$ 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 β_3 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 β_3 subunit. Additional studies in other ethnic groups should be performed to confirm this association.

References

1. Soubrier F, Bonnardeaux A: Role and identification of the genes involved in human hypertension. *J Hum Hypertens* 1994; **8**: 579–585.
2. Thibonnier M, Schork NJ: The genetics of hypertension. *Curr Opin Genet Dev* 1995; **5**: 362–370.
3. Ward RH: Familial aggregation and genetic epidemiology of blood pressure. in Laragh JH, Brenner BM (eds): Hypertension; Pathophysiology, Diagnosis, and Management. New York, Raven Press, 1990, pp81–100.
4. Lander ES, Schork NJ: Genetic dissection of complex traits. *Science* 1994; **265**: 2037–2048.
5. Williams RR, Hunt SC, Hopkins PN, *et al*: Genes, hypertension and early familial coronary artery disease, in Laragh JH, Brenner BM (eds): Hypertension; Pathophysiology, Diagnosis and Management. New York, Raven Press, 1990, pp127–136.
6. Duru K, Farrow S, Wang J-M, Lockette W, Kurtz T: Frequency of a deletion polymorphism in the gene for angiotensin converting enzyme is increased in African-Americans with hypertension. *Am J Hypertens* 1994; **7**: 759–762.
7. Barley J, Blackwood A, Miller M, *et al*: Angiotensin converting enzyme gene I/D polymorphism, blood pressure and the renin-angiotensin system in Caucasian and Afro-Caribbean peoples. *J Hum Hypertens* 1996; **10**: 31–35.
8. Rieder MJ, Taylor SL, Clark AG, Nickerson DA: Sequence variation in the human angiotensin converting enzyme. *Nat Genet* 1999; **22**: 59–62.
9. Hata A, Namikawa C, Sasaki M, Sato K, Nakamura T, Tamura K: Angiotensinogen as a risk factor for essential hypertension in Japan. *J Clin Invest* 1994; **93**: 1285–1287.
10. Rotimi C, Morrison L, Cooper R, *et al*: Angiotensinogen gene in human hypertension: lack of an association of the 235T allele among African Americans. *Hypertension* 1994; **24**: 591–594.
11. Williams SM, Addy JH, Phillips III JA, *et al*: Combinations of variations in multiple genes are associated with hypertension. *Hypertension* 2000; **36**: 2–6.
12. Kainulainen K, Perola M, Terwilliger J, *et al*: Evidence for involvement of the type 1 angiotensin II receptor locus in essential hypertension. *Hypertension* 1999; **33**: 844–849.
13. Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP: Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *N Engl J Med* 1990; **322**: 1561–1566.
14. Drayer JIM, Weber MA, DeYoung JL: BP as a determinant of cardiac left ventricular muscle mass. *Arch Intern Med* 1983; **143**: 90–92.
15. Devereux RB, Roman MJ: Left ventricular hypertrophy in

- hypertension: stimuli, patterns, and consequences. *Hypertens Res* 1999; **22**: 1–9.
16. Levy D, Anderson KM, Savage DD, Kannel WB, Christiansen JC, Castelli WP: Echocardiographically detected left ventricular hypertrophy: prevalence and risk factors: the Framingham Heart Study. *Ann Intern Med* 1988; **108**: 7–13.
 17. Post WS, Larson MG, Myers RH, Galderisi M, Levy D: Heritability of left ventricular mass: the Framingham Heart Study. *Hypertension* 1977; **30**: 1025–1028.
 18. Siffert W, Roszkopf, Siffert G, et al: Association of a human G-protein β_3 subunit variant with hypertension. *Nat Genet* 1998; **18**: 45–48.
 19. Robinson K, Mayer EL, Miller DP, et al: Hyperhomocysteinemia and low pyridoxal phosphate: common and independent reversible risk factors for coronary artery disease. *Circulation* 1995; **92**: 2825–2830.
 20. Gharavi AG, Lipkowitz MS, Diamond JA, Jhang JS, Phillips RA: Deletion polymorphism of the ACE gene is independently associated with left ventricular mass and geometric remodelling in systemic hypertension. *Am J Cardiol* 1996; **77**: 1315–1319.
 21. Serrato M, Marian AJ: A variant of human paraoxonase/arylesterase (HUMPONA) gene is a risk factor for coronary artery disease. *J Clin Invest* 1995; **96**: 3005–3008.
 22. Schunkert H, Hense H, Holmer S, et al: Association between a deletion polymorphism of the angiotensin-converting-enzyme gene and left ventricular hypertrophy. *N Engl J Med* 1994; **330**: 1634–1638.
 23. Lindpaintner K, Lee M, Larson MG, et al: Absence of association of genetic linkage between the ACE gene and left ventricular mass. *N Engl J Med* 1996; **334**: 1023–1028.
 24. Pontremoli R, Sofia A, Tirotta A, et al: The deletion of polymorphism of the angiotensin I-converting enzyme gene is associated with target organ damage in essential hypertension. *J Am Soc Nephrol* 1996; **7**: 2550–2558.
 25. Beige J, Hohenbleicher H, Distler A, Sharma AM: G-protein β_3 subunit C825T variant and ambulatory blood pressure in essential hypertension. *Hypertension* 1999; **33**: 1049–1051.
 26. West MJ, Summers KM, Burstow DJ, Wong KK, Huggard PR: Renin and angiotensin-converting enzyme genotypes in patients with essential hypertension and left ventricular hypertrophy. *Clin Exp Pharmacol Physiol* 1994; **21**: 207–210.
 27. Frosst P, Blom HJ, Milos R, et al: A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet* 1995; **10**: 111–113.
 28. Sanghera DK, Aston CE, Saha N, Kamboh I: DNA polymorphisms in two paraoxonase genes (PON1 and PON2) are associated with the risk of coronary heart disease. *Am J Hum Genet* 1998; **62**: 36–44.
 29. Norusis MJ: SPSS/PC+ for Windows, Base System and Advanced Statistic User's Guide, Window version 10.0. Chicago, SPSS Inc., 1996.
 30. Hegele RA, Harris SB, Hanley AJG, Cao H, Zinman B: G protein β_3 subunit gene variant and blood pressure variation in Canadian Oji-Cree. *Hypertension* 1998; **32**: 688–692.
 31. Kato N, Sugiyama T, Morita H, Kurihara H, Yamori Y, Yazaki Y: G protein β_3 subunit variant and essential hypertension in Japanese. *Hypertension* 1998; **32**: 935–938.
 32. Poch E, González D, Gómez-Angelats E, et al: G-protein β_3 subunit gene variant and left ventricular hypertrophy in essential hypertension. *Hypertension* 2000; **35** (part2): 214–218.
 33. De la Sierra A, Coca A, Pare JC, Sanchez M, Valls V, Urbano-Marquez A: Erythrocyte ion fluxes in essential hypertensive patients with left ventricular hypertrophy. *Circulation* 1993; **88**: 1628–1633.
 34. Canessa M, Falkner B, Hulman S: Red blood cell sodium-proton exchange in hypertensive blacks with insulin-resistant glucose disposal. *Hypertension* 1993; **22**: 204–213.
 35. Dfez J, Alonso A, Garciandia A, et al: Association of increased erythrocyte Na^+/H^+ exchanger with Na^+ retention in patients with essential hypertension. *Am J Hypertens* 1995; **8**: 124–132.
 36. Re RN, Frolich ED: Controversies in the genetic analysis of hypertensive diseases. *Hypertension* 1996; **28**: 880 (Editorial).