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ORIGINAL ARTICLE

Association of *ace* polymorphisms with left ventricular hypertrophy

Mohammad Saeed[1], Sammer Siddiqui[1], Aisha Khan[1], Zahid A. Butt[1], S. Hasan Parvez[2] & Philippe M. Frossard[1]

[1] Department of Biological and Biomedical Sciences, Aga Khan University, Karachi, Pakistan;
[2] Neuroendocrinology and Neuropharmacology Unit, CNRS, Gif-sur-Yvette, France.

Correspondence to: Dr. Philippe M. Frossard
Department of Biological and Biomedical Sciences
The Aga Khan University
Stadium Road, P. O. Box 3500,
Karachi – 74800, PAKISTAN
TEL: +92 (21) 4859 4540
FAX: +92 (21) 493 4294
EMAIL: philippe.frossard@aku.edu

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Abstract

The angiotensin converting enzyme gene (*ACE*) is a candidate gene for an individual's genetic susceptibility to left ventricular hypertrophy (LVH). LVH has long been thought to be an end point of essential hypertension (EH), rather than a separate entity, though it is influenced by a unique set of hormonal, vascular and genetic factors.

In this study, we attempted to determine whether two representative polymorphisms of the *ACE* gene, *ACE* I/D and 2350 G>A, known to be associated with EH and to influence plasma ACE levels most significantly, could implicate *ACE* as a quantitative trait locus (QTL) for LVH. We carried out a retrospective, case–control study of the two *ACE* polymorphisms amongst 180 nationals (50 LVH patients and 130 controls) from the United Arab Emirates – an ethnic group characterized by no alcohol intake and no cigarette smoking – for correlations with LVH. Clinical diagnosis of LVH was based on echocardiographic and ECG criteria. *ACE* I/D and 2350 G>A genotypes were determined by PCR and restriction digestion.

Univariate and multivariate logistic regression analyses revealed an association between *ACE* polymorphisms and LVH. Haplotype analysis further supported this finding. *ACE* I/D and *ACE* 2350 G>A polymorphisms are in strong linkage disequilibrium and are associated with LVH, suggesting that *ACE* is likely to be a QTL for LVH.

Introduction

Left ventricular hypertrophy (LVH), a major independent cardiovascular risk factor for mortality, is widely believed to be a consequence of essential hypertension (EH) [Cambien *et al.*, 1992]. There is now some evidence that LVH is an independent clinical entity, with its own unique genetic and environmental influences, rather than solely an end point of EH [Ganau *et al.*, 1990; Drayer *et al.*, 1983]. Neuroendocrine factors such as angiotensin II have also been implicated in the etiopathogen-

esis of LVH Lindpaintner and Ganten [1991]. Thus, of the candidate genes thought to be involved in conferring an individual's genetic susceptibility to LVH, the angiotensin-converting enzyme gene (*ACE*) is of much interest.

In this study, we attempted to determine whether two representative polymorphisms of the *ACE* gene, *ACE* I/D [Cambien *et al*., 1992] and 2350 G>A [Zhu *et al.,* 2001], known to influence plasma ACE levels most significantly, could implicate *ACE* as a quantitative trait locus (QTL) for LVH. The influence of the I/D polymorphism on LVH has been studied previously and the results are conflicting [Lindpaintner *et al.,* 1996; Schunkert *et al.,* 1994]. We recently found that the *ACE* 2350 G>A polymorphism, known to have the most significant influence on systolic blood pressure, is associated with EH [Mahmood *et al.,* 2003]. The effects of this polymorphism on LVH have not been studied.

We investigated a population of United Arab Emirates (UAE) nationals (Emiratis) that has been described before [Obineche *et al.,* 2001]. This population is marked by high levels of consanguinity, which raise homozygosity levels and minimize the influence of selection bias and population stratification. Moreover, this population is characterized by absence of smoking and alcohol intake, which provided the added advantage of elimination of these usual environmental confounders in the study [Obineche *et al.,* 2001].

Methods

The clinical characteristics of these two groups of subjects have been previously described Obineche, *et al.,* [2001]. Briefly, LVH was defined based on echocardiographic criteria and Sokolow-Lyon index Sokolow and Lyon [1949]. "Control" (comparison) subjects were individuals who had no documented evidence of LVH, EH or ischemic heart disease as confirmed by a clinical cardiologist [Obineche *et al.,* 2001]. DNA was extracted from peripheral blood lymphocytes according to standard procedures Sambrook *et al.* [1989]. PCR and restriction assays were carried out as described elsewhere [Cambien *et al.,* 1992; Zhu *et al.,* 2001; Mahmood *et al.,* 2003].

Statistical analyses was carried out using the SPSS® (Statistical Package for Social Sciences) Software Version 10.0 for Windows® (Gorinchem, The Netherlands). Distribution differences of genotypes in the patients (LVH) as compared to distribution in the control group were assessed by chi-squared analyses on 3X2 tables followed by univariate and multivariate regression analyses. Estimations of departures from Hardy-Weinberg (HW) equilibria (D_A) and the tests for statistical significance were calculated as reported by Haviland *et al.* [1991]. Haplotype frequencies for the sites were estimated by maximum likelihood using an expectation maximization algorithm. The amount of linkage disequilibrium (LD) was calculated using the D-statistic [Thompson *et al.,* 1988]. Haplotype analysis was obtained using EHPLUS [Zhao *et al.,* 2000] with the T5 statistic is distributed as a χ^2 with 3 degrees of freedom [Zhao *et al.,* 2000]. For all analyses, statistical significance was considered when significance level (*P*) values were lower than 0.05. This project was approved by the Research Ethics Committee of the Faculty of Medicine and Health Sciences (UAE University, Al Ain, UAE), and informed consent was obtained from all subjects recruited for this study.

Results

In this pilot, retrospective case-control study, we investigated a sample population of 180 Emiratis, composed of 50 LVH patients and 130 controls, to identify putative associations of the polymorphisms *ACE* I/D and 2350 G>A with LVH. The study population included 94 males and 86 females, 43 to 72 years of age. There was no statistical difference in the ages of cases (56.5 ± 13 years) and controls (57.1 ± 15.5 years) nor in their gender distributions.

Table 1 shows the genotype frequencies for *ACE* I/D and 2350 G>A polymorphisms. *ACE* I/D genotype frequencies occurred in HW proportions in cases but not in controls, while *ACE* 2350 G>A genotype frequencies occurred in HW proportions in controls only. Deviations from HW equilibrium may not necessarily reflect sample population recruitment bias, but may indicate an underlying gene effect on the phenotype under consideration, as has been extensively discussed in the literature [Mahmood *et al.,* 2003].

An association was found between *ACE* I/D as well as *ACE* 2350 G>A genotypes and LVH (respectively, χ^2=10.9; 2df; P=0.004 and χ^2=6.2; 2df; P=0.04). Multivariate logistic regression analysis for *ACE* I/D and 2350 G>A genotypes after adjustment for the effect of age and gender, showed that neither of the polymorphisms was associated with LVH independently (*Table 2*). The association reached statistical significance, however, when the effect of the other marker was taken into consideration (*Table 2*).

We then evaluated the association of the combination of the two polymorphisms with LVH by constructing haplotypes. This increased the heterozygosity index of the marker system from 0.34 and 0.47 for *ACE* I/D and 2350 G>A polymorphisms respectively, to 0.65 for the haplotypes. *Table 3* shows the distribution of *ACE* I/D and 2350 G>A haplotypes in cases and controls. For the *ACE* I/D and 2350 G>A polymorphisms we found D=–0.08 (D'=0.67; χ^2=43.2; 1df; *P*<0.01), which signifies that the D allele in intron 16 is in strong LD with the A allele in exon 17. Haplotype association analysis carried out using EHPLUS revealed a strong association of the haplotypic markers with LVH (T5 statistic= 10.51; 3df; *P*=0.02). Further analysis indicated that I/G haplotype was associated with LVH (χ^2=24.8; OR=4.96; CI_{95}=2.48–10.11; *P*<0.001).

Discussion

Our data suggests that the *ACE* polymorphisms are associated with LVH, after the confounding effects of EH and other variables are controlled for through case selection and regression analysis. This is in accordance with some of the earlier findings that the *ACE* gene is likely a QTL underlying individuals' genetic susceptibility to LVH [Schunkert *et al*., 1994] but in contradiction to other studies [Lindpaintner *et al.,* 1996; Shlyakhto *et al.,* 2001]. Lindpaintner *et al*. [1996] and Schunkert *et al*. [1994] showed absence of an association of the *ACE* I/D polymorphism with LVH. A significant proportion of their sample population, however, suffered from EH as well. We have recently described a positive association of *ACE* 2350 G>A with EH in the same population (Emiratis) [Mahmood *et al.,* 2003]. Since ACE is known to influence cardiac hypertrophy [Mathew *et al.,* 2001] and *ACE* I/D and G>A polymorphisms are associated with increased plasma ACE levels [Zhu *et al.,* 2001] and EH [Mahmood *et al.,* 2003], we specifically selected LVH patients who did not suffer from EH to exclude the confounding effects of EH. The patients, however, did suffer from ischemic heart disease (IHD).

Although the *ACE* I/D and G>A polymorphisms were found to be independently associated with LVH, the association was lost on adjustment for age and gender. The association was found to be significant only when the mutual effect of both polymorphisms was considered. This demonstrates that the two polymorphisms play a combined role in increasing the genetic susceptibility to LVH.

Constructing haplotypes allowed an increase in heterozygosity index of the marker system, thus increasing the power of the study to compensate for the small sample size. The I/G haplotype was found to be strongly associated with LVH. Zhu *et al.* [2001] demonstrated that the G allele of the *ACE* G>A 2350 polymorphism is most significantly associated with increased plasma ACE levels as well as elevated systolic blood pressure values. We hypothesize here that the G allele is involved in increasing susceptibility to LVH in the Emirati population.

The 2350A allele does not seem to increase an individual's risk of developing LVH and it is inherited predominantly in the "control", non-LVH population. Judging solely from the allele frequencies, it seems that the 2350G allele was first mutated into 2350A, and that the I occurred later on a 2350G allele. Therefore I/G haplotypes occur more frequently amongst LVH subjects and the haplotype provides a better marker for LVH than G alleles only (mean OR = 4.96, $P<0.001$).

This is the first study demonstrating the association of the *ACE* G>A 2350 polymorphism with LVH. The sample size of our study remained small due to the strict selection criteria designed to exclude LVH patients with EH. One way to overcome this limitation is to study more informative markers such as haplotypes, which enhanced the visibility of the association of the *ACE* genetic markers with LVH.

Table 1: Distribution of ACE I/D and 2350 G>A genotypes and allele frequencies (± standard errors) as well as D_A statistics for Hardy-Weinberg equilibrium in the LVH and normal control Emirati subjects.

	LVH	Controls
ACE I/D		
II	3 (6)	8 (6.2)
ID	33 (66)	51 (39.2)
DD	14 (28)	71 (54.6)
p (I)	0.39 ± 0.06*	0.25 ± 0.04
q (D)	0.61 ± 0.06	0.75 ± 0.04
D_A (I/D)	–0.09	–0.01
χ^2 (I/D)	0.08	10.16
***P* (I/D)**	>0.2	<0.01
ACE 2350 G>A		
GG	13 (26)	59 (45.4)
GA	25 (50)	43 (33)
AA	12 (24)	28 (21.5)
p(G)	0.51 ± 0.07	0.62 ± 0.04
q(A)	0.49 ± 0.07*	0.38 ± 0.04
D_A (G>A)	0.01	0.07
χ^2 (G>A)	7.16	0.16
***P* (G>A)**	<0.01	>0.2

*Indicates a genotype / allele distribution statistically different from the one in the control group ($P<0.05$). (Numbers in parentheses indicate percentages).

Table 2: Multivariable Conditional Logistic Regression Analysis for Association of ACE I/D and 2350 G>A polymorphisms with LVH.

	Wald Statistic	P	Adjusted for
ACE I/D	2.82	0.24	Gender
	3.73	0.15	Gender, Age
	8.59	0.01*	Gender, Age, *ACE* G>A
ACE G>A	5.29	0.07	Gender
	2.26	0.32	Gender, Age
	5.96	0.05*	Gender, Age, *ACE* I/D

*Significant *P*-values

Table 3: Distribution of haplotypes of the ACE I/D and 2350 G>A polymorphisms in LVH and control Emirati subjects.

Haplotypes*	LVH	Controls
I/G	74 (0.33)	12 (0.09)
I/A	15 (0.07)	1 (0.01)
D/G	49 (0.22)	77 (0.59)
D/A	84 (0.38)	41 (0.31)

Parentheses indicate frequencies of haplotypes
* T5 statistic = 10.51, 3df, $P=0.02$
+ χ^2 (Yates corrected) = 24.8; 2df; odds ratio = 4.96; confidence interval (95%) = 2.48 – 10.11; $P<0.001$.

REFERENCES

1 Cambien F, Poirier O, Lecerf L, et al. Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. Nature 1992; **359**:641–644.
2 Drayer, JIM, Weber MA, DeYoung JL. BP as a determinant of cardiac left ventricular muscle mass. Arch Intern Med 1983; **143**:90–92.
3 Ganau, A, Devereux RB, Pickering TG, et al. Relation of left ventricular hemodynamic load and contractile performance to left ventricular mass in hypertension. Circulation 1990; **81**:25–36.
4 Haviland MB, Kessling AM, Davignon J, Sing CF. Estimation of Hardy-Weinberg pairwise disequilibrium in apolipoprotein AI-CIII-AIV gene cluster. Am J Hum Genet 1991; **49**:350–365.
5 Lindpaintner K, Ganten D. The cardiac renin-angiotensin system: an appraisal of present experimental and clinical evidence. Circ Res 1991; **68**:905–921.
6 Lindpaintner K, Lee M, Larson MG, et al. Absence of association or genetic linkage between the angiotensin-converting-enzyme gene and left ventricular mass. N Engl J Med 1996; **334:**1023–8.
7 Mahmood MS, Saboohi K, Ali SO, Bokhari AM, Frossard PM. Association of the angiotensin-converting enzyme (ACE) gene G2350A dimorphism with essential hypertension. J Hum Hypertension 2003; **17**:719–723.
8 Mathew J, Sleight P, Lonn E, Johnstone D. Heart Outcomes Prevention Evaluation (HOPE) Investigators. Reduction of cardiovascular risk by regression of electrocardiographic markers of left ventricular hypertrophy by the angiotensin-converting enzyme inhibitor ramipril. Circulation. 2001; **104**:1615–21.
9 Obineche EN, Frossard PM, Bokhari AM. An association study of five genetic loci and left ventricular hypertrophy amongst Gulf Arabs. Hypertens Res 2001; **24**:635–639.
10 Sambrook J, Fritsch EF, Maniatis T. Molecular cloning, a laboratory manual, 2nded. Cold Spring: Cold Spring Harbour Laboratory Press, New York. 1989.
11 Schunkert H, Hense HW, Holmer SR, et al. Association between a deletion polymorphism of the angiotensin-converting-enzyme gene and left ventricular hypertrophy. N Engl J Med 1994; **330**:1634–8.
12 Shlyakhto EV, Shwartz EI, Nefedovam YB, Zukovam AV, Vinnic TA, Conrady AO. Lack of association of the renin-angiotensin system genes polymorphisms and left ventricular hypertrophy in hypertension. Blood Press 2001; **10**:135–41.
13 Sokolow M, Lyon TP. The ventricular complex in left ventricular hypertrophy as obtained by unipolar precordial and limb leads. Am Heart J 1949; **37**:161–186.
14 Thompson EA, Deeb S, Walker D, Motulsky AG. The detection of linkage disequilibrium between closely linked markers: RFLPs at the AI-CIII apolipoprotein genes. Am J Hum Genet 1988; **42**:113–124.
15 Zhao JH, Curtis D, Sham PC. Model-free analysis and permutation tests for allelic associations. Hum Hered 2000; **50:**133–9.
16 Zhu X, Bouzekri N, Southam L, et al. Linkage and association analysis of angiotensin I-converting enzyme (ACE)-gene polymorphisms with ACE concentration and blood pressure. Am J Hum Genet 2001; **68**:1139–48.