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Angiotensin-Converting Enzyme Gene Polymorphism in Turkish Hypertensive Patients and its Association with Left Ventricular Hypertrophy

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TÜRK HİPERTANSİF HASTALARDA ANJİYOTENSİN-KONVERTİNG ENZİM GEN POLİMORFİZMİ VE SOL VENTRİKÜL HİPERTROFİSİ İLİŞKİSİ

ÖZET

Anjiyotensin-konverting enzim (AKE) gen polimorfizmi ile sol ventrikül hipertrofisi (SVH) arasındaki ilişki farklı populasyonlarda çalışılmış ve çelişkili sonuçlar alınmıştır. Bu çalışmanın amacı esansiyel hipertansiyonu olan Türk hastalarda AKE genotipi ve allel dağılımını araştırmak ve bunun sol ventrikül hipertrofisiyle ilişkisini ortaya koymaktır.

Çalışmaya benzer yaş ve cinsiyette 117 hipertansif hasta ve 75 sağlıklı kontrol alınmıştır. Sol ventrikül kütle indeksi (SVKİ) heriki grupta da iki boyutlu ekokardiyografiyle hesaplandı. AKE geninin 16. intronundaki insersiyon(I)/delesyon (D) polimorfizmini araştırmak üzere polimeraz zincir reaksiyonu kullanıldı.

DD, ID ve II genotipi dağılımı hastalar (%42, %49 ve %9) ve kontrol grubu (%35, %53 ve %12) arasında farklı bulunmadı. Her iki gruptaki allel dağılımı da farklı bulunmadı; hasta ve kontrol grupları için, D alleli %66'ya karşı 62, I alleli %34'e karşı %38 olarak saptandı. Hipertansif grupta SVH sıklığı %35 idi. AKE genotip dağılımı (DD, ID, II) sol ventrikül hipertrofisi olan (%41, %57, %2) ve olmayan hastalarda (%42, %45, %13) farklı bulunmadı. SVKİ heriç genotipte faklı saptanmadı, ortalama değerler DD için 113±37 g/m²; ID için 110±36 g/m²; ve II için 96±11 g/m² (p=0.5) idi.

Sonuç olarak esansiyel hipertansiyonu olan Türk hastalarda AKE geni I/D polimorfizminin genotipik dağılımı ve allel sıklığı sağlıklı kontrollere göre farklı bulunmadı. Ayrıca AKE geni polimorfizminin sol ventrikül hipertrofisi ve sol ventrikül kütlesi ile anlamlı bir ilişkisi olmadığı ortaya kondu. ***Türk Kardiyol Dern Arş 2002; 30: 743-748***

Anahtar kelimeler: *ACE gen polimorfizmi, hipertansiyon, sol ventrikül hipertrofisi*

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Left ventricular hypertrophy (LVH) is an independent risk factor for cardiovascular morbidity and mortality [1-4]. The pathogenetic background leading to LVH is multifactorial and both hemodynamic (increased peripheral vascular resistance, volume oveload) and nonhemodynamic factors (age, sex,obesity, genetics) seem to be involved in its development. The fact that many subjects with LVH are actually normotensive individuals further emphasizes the possible role of nonhemodynamic factors in its pathogenesis [4]. The evidence that left ventricular mass is a familial trait further supports the genetic theory in etiology [5,6].

Renin angiotensin system (RAS) is also implicated in the development of LVH. Angiotensin II (AII) facilitates LVH through enhancement of protein synthesis in cardiac and vascular smooth muscle cells [7-9]. There is increased expression of AII in the hypertrophied ventricle suggesting its intracardiac formation, independent of circulating RAS [10]. The role of RAS is additionally supported by experimental and clinical studies which have shown that angiotensin converting enzyme inhibitors (ACEI) can prevent or lead to the regression of hypertensive LVH [11,12].

The human ACE gene is located on chromosome 17 at position 23 (17q23). A deletion polymorphism of a 287-base pair fragment of intron 16 of the ACE gene was detected [13,14], resulting in allele D if deletion is present and in allele I (insertion) if absent and the three resulting genotypes are: DD, ID, II. Homozygous presence of the deletion polymorphism (DD) was shown to be associated with higher plasma ACE activity [15,16] which in turn may theoreti-

cally result in increased AII formation in cardiac and vascular tissues. Furthermore, the presence of allele D has been associated with target organ damage including hypertensive retinopathy, microalbuminuria and LVH in some studies [17,18]. The ACE gene polymorphism has therefore been investigated for its possible association with essential hypertension or ischemic heart disease and the findings seem to vary between populations of different genetic and environmental backgrounds [19-21].

These intriguing findings led to the speculation that the DD genotype may be the genetic factor involved in the development of LVH in essential hypertension through its influence on the local formation of AII within the cardiac tissue.

A number of studies addressing this issue in different populations had conflicting results [17,18,22-25]. The aim of the present study was to determine the allelic frequency and the genotype distribution for ACE gene polymorphism in Turkish patients with essential hypertension and to correlate the genetic findings with the presence of LVH.

MATERIAL AND METHODS

Subjects

In 1998-1999, 117 patients (85 female, 32 male) with essential hypertension older than 18 years were randomly selected in the outpatient clinic of internal medicine to participate in this study, which was a part of a larger clinical trial. All subjects were white adults of caucasion Turkish descent. Causes of secondary hypertension were excluded in all patients. Subjects with coronary artery disease, congestive heart failure, any form of cardiomyopathy, valvular heart disease, arrhythmias and diabetes were excluded. Essential hypertension was defined according to the Sixth Report of the Joint National Committee, as systolic blood pressure (SBP) of 140-170 mmHg and/or diastolic blood pressure (DBP) of 90-109 mmHg on at least three different occasions with absence of evidence for secondary hypertension. Seventy five normotensive (BP<130/85 mmHg) individuals with similar age and gender comprised the control group. None of the patients were on drug treatment at the time of the study. They had never been treated for hypertension or had been off therapy at least 4 weeks before the study. Informed consent was taken from all subjects and the study was approved by the local ethics committee.

Echocardiographic Methods

Both the study and the control groups underwent 2D- and M-mode echocardiographic evaluation for LV mass index (LVMI) calculations. ATL UM9 (Bothel, Ca., USA) device was used in all echocardiographic studies. Echocardiographic examination was undertaken at rest with subjects in supine left lateral position using standard parasternal and apical views. M-mode measurements were obtained according to the recommendations of American Society of Echocardiography [26]. Echocardiographic examination and mass calculations were performed by the same cardiologist. The intraobserver variability for echocardiographic measurements and calculations was below 5%. Left ventricular mass (LVM) was derived using the formula described by Devereux et al.[27]. Left ventricular mass was corrected for body surface area (LVMI) and expressed in units of grams/meter squared (g/m^2). Left ventricular hypertrophy was defined as LVMI $\geq$ 134 g/m^2 in males and $\geq$ 110 g/m^2 in females [28].

Determination of ACE Genotypes:

Genomic DNA was extracted from peripheral mononuclear cells with sodium dodecyl sulfate lysis, ammonium acetate extraction, and ethanol precipitation [29]. A polymerase chain reaction (PCR) was used for amplification and accurate genotyping of the ACE gene as previously defined [30,31].

Statistical Analysis

All data are expressed as mean ± SD. Chi - square was used to compare the allele and genotype frequencies between patients with essential hypertension versus controls and between patients with versus without LVH. Analysis of variance (ANOVA) was performed to test the influence of ACE genotypes on left ventricular mass. A p value of less than 0.05 was assumed to indicate statistical significance.

RESULTS

Characteristics of the study population are summarized in Table 1. The patients and the control group did not differ significantly with regard to age and gender. Mean SBP and DBP in hypertensive group were significantly higher than the control group ($p<0.05$). The echocardiographic evaluation of the study population revealed the presence of LVH in 35% of the patients while there were no cases of LVH in the control group.

The allele frequencies and distribution of ACE genotypes in patients with hypertension compared to controls are given in Table 2. The respective frequencies of DD, ID and II genotypes were 42%, 49% and 9% in the hypertensive group versus 35%, 53% and 12% in the control group. The overall frequencies of D and I alleles were 67% and 33% versus 62% and 38% in hypertensive and control groups respectively. There was no significant difference in either allele or genotype distribution between Turkish pati-

Table 1. Characteristics of the study population

	Patients	Controls
Number	117	75
Male / Female*	32/85	24/51
Age (years)*	50±10	48±9
SBP (mmHg)	151±21	110±12
DBP (mmHg)	97±14	75±5
Duration of HT (years)	7.5±1	-
LVH	42 (35%)	-

*Data presented are mean value ± SD, SBP=systolic blood pressure, DBP=diastolic blood pressure, HT= hypertension, LVH= left ventricular hypertrophy. * p>0.05 for patients vs. controls.*

ents with essential hypertension and healthy local controls.

Although baseline characteristics (duration of hypertension, stage of hypertension, number of drugs used) of patients with or without LVH were similar (Table 3), no significant difference was found for the presence or absence of LVH in patients with essential hypertension according to their ACE genotypes or alleles as summarized in Table 4. The LVMI valus were 113 ± 37 g/m^2 in DD, 110 ± 36g/m^2 in ID and 96±11 g/m^2 in II genotypes (Table 5). Although the LVMI value is lower in II genotype, the correlation of genotype data with LVMI also proved statistically non-significant (p>0.05, by ANOVA).

DISCUSSION

Left ventricular mass is a complex phenotype that is influenced by interacting genetic and environmental factors. The RAS has been postulated to be involved in the pathogenesis of LVH. A number of experimental and clinical observations showed an association between the ACE gene polymorphism and LV mass. Individuals with DD genotype were shown to have higher plasma ACE levels [15,16] and activated RAS may exert trophic influences on cardiomyocytes [7,8]. There is little information regarding the expression levels of ACE in cardiac and vascular tissues in the carriers of different ACE polymorphism genotypes. Higher in-situ A II formation may also facilitate cellular hypertrophy and extracellular matrix formation [22].

Data from various populations on the relationship between ACE gene phenotype and LVH are controversial. D allele of ACE gene was found to be an independent risk factor for target organ damage in essential hypertension. In 106 caucasian Europeans with essential hypertension Pontremoli et al. showed that DD and ID genotypes were significantly associated with the presence of retinopathy, microalbuminuria and LVH [17]. In 140 untreated southern Italian patients Perticone et al. demonstrated that LVMI was significantly enhanced in patients with the DD genotype [25]. In a Japanese study the genotype of the ACE gene was found to be a significant predictor of left ventricular hypertrophy [22]. However on a large group of subjects from the Framingham Heart Study, no role of the ACE gene influencing left ventricular mass was shown [24]. Similarly Kupari et al. failed to show a major influence of ACE gene variation on LV mass and function [23]. Both studies were done in samples of the general population and included small percentages of subjects with hypertension. Findings of another population-based study from Germany conducted by Schunkert et al.[18] suggest that LVH is partially determined by genetic disposition and DD genotype is a potential genetic marker for LVH in only middle-aged men.

In our study the ACE genotype and allelic distribution was found to be similar in Turkish patients with

Table 2. Genotypic and allelic distribution of ACE gene in hypertensive patients and controls

Groups	Genotypes*			Alleles*	
	DD n(%)	ID n(%)	II n(%)	D n(%)	I n(%)
Patients n=117	48(42)	58(49)	11(9)	154(66)	80(34)
Controls n=75	26(35)	40(53)	9(12)	92(62)	58(38)

**p>0.05 for patients vs. controls, by chi square*

Table 3. Characteristics of patients with and without LVH

	Patients with LVH n=41	Patients without LVH n=76	p value
Duration of HT (years)	6.5±7	9.5±8	0.06
Stage of HT* (n/%)			0.19**
Stage 1	15 (%37)	36 (%47)	
Stage 2	14 (%34)	31 (%41)	
Stage 3	12 (%29)	9 (%12)	
Number of drugs used			0.08**
0	4 (%10)	14 (%18)	
1	28 (%68)	50 (%66)	
2	7 (%17)	12 (%16)	
3	2 (%5)	-	

Data presented are mean value ± SD, HT= hypertension, LVH= left ventricular hypertrophy. Stage of hypertension is determined according to Sixth Report of the Joint National Committee (JNC VI). **p>0.05 by chi-square.*

Table 4. Genotypic and allelic distribution of ACE gene in hypertensive patients with and without LVH

Groups	Genotypes*			Alleles*	
	DD n(%)	ID n(%)	II n(%)	D n(%)	I n(%)
LVH n=41	16(41)	24(57)	1(2)	56(68)	26(32)
No LVH n=76	32(42)	34(45)	10(13)	98(64)	54(36)

** p>0.05 by chi square, for patients with LVH vs no LVH, LVH= left ventricular hypertrophy*

essential hypertension and normotensive healthy controls. The lack of association between I/D polymorphism of the ACE gene and the presence of essential hypertension in our study is in accordance with two other studies performed in the same population. Topaloğlu et al.[32] studied the same issue in Turkish pediatric patients with essential hypertension. They made genetic analysis in both children and their parents and found no significant difference between either hypertensive and normotensive children or between their parents. Araz M et al. also found no correlation between gene polymorphism and the development of hypertension in Turkish type 2 diabetic patients [33].

Table 5. LVMI values for the three ACE genotypes

Genotypes	LVMI (g/m²)*
DD	113 ± 37
ID	110 ± 36
II	96 ± 11

Data presented are mean value ± SD ,
*LVMI = left ventricular mass index, * p>0.05 by ANOVA*

The second major finding of our study was that the distribution for ACE genotypes and alleles were similar in hypertensive patients with and without LVH. The difference in LVMI values among the carriers of the three genotypes was also statistically non-significant.

Although our study has a relatively small sample size and may not be representative of the general population, the findings are consistent with the results of the well-conducted large scale study based on the subjects from the Framingham Heart Study. Other studies supporting the presence of an association can be criticized for small sample size, selection bias and suboptimal characterization of phenotypes.

Conflicting results may also be explained by nonuniform genetic background of different study populati-

ons. Besides, in some of the studies the confounding effect of antihypertensive treatment should also be taken into consideration.

In conclusion, our data suggests the absence of an association between the I/D polymorphism of the ACE gene and essential hypertension. The genotypic variation showed no influence on left ventricular mass. We believe that current evidence fails to support the role of the ACE gene polymorphism as a determinant and marker of left ventricular hypertrophy.

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