Association between angiotensin converting enzyme (ACE) gene I/D polymorphism frequency and plasma ACE concentration in patients with idiopathic dilated cardiomyopathy

İdiyopatik dilate kardiyomiyopatili hastalarda anjiyotensin dönüştürücü enzim (ADE) geni I/D polimorfizm sıklığı ile plazma ADE konsantrasyonu arasında ilişki

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Dilated cardiomyopathy (DCM) is a syndrome characterized by cardiac enlargement and impaired systolic function of one or both ventricles. The etiology of DCM is idiopathic in about half the patients. These patients present unknown cause. However, familial studies revealed that 20% to 25% of idiopathic DCM had a familial origin. This finding raised the hypothesis that gene defects might play an important role in the disease pathogenesis. Angiotensin converting enzyme (ACE) gene (Gene Bank accession number: NM 000789.2) is one of these genes affecting idiopathic DCM (1). This gene contains a polymorphism based on the presence (insertion, I) or absence (deletion, D) within intron 16, of a 287 base pair ALU repeat sequence; resulting in 3 genotypes: DD and II homozygotes and ID heterozygote. DD genotype is associated with higher concentrations of circulating ACE (2).

Plasma ACE concentration is important in regulating blood pressure and proliferation of muscle cells. As a result, ACE gene I/D polymorphism may be a genetic marker for idiopathic DCM (2-5).

We evaluated 29 (11 female and 18 male) unrelated, patients with idiopathic DCM and 20 (11 female and 9 male) healthy subjects as controls, admitted to the Research Hospital of the Osmangazi University between 2003 and 2004.

DNA was extracted from venous blood by salt method and polymerase chain reaction (PCR) of amplification of DNA was performed with thermo stable taq polymerase (Sigma diagnostics, St. Louis, US) according to Gunes et al (6).

The PCR products were separated by electrophoresis on 2 % agarose gel containing 4 Il of ethidium bromide and were visualized by using CCD camera. Results were evaluated with the gel analysis Software (Lab Works, Cambridge UK)

Plasma ACE concentrations were determined by using ELISA kit (Temecula, CA, Chemicon International Inc.).

Echocardiograms were obtained by using Acuson-Siemens Sequoia-C256, (Acuson Corporation, Mountain View, CA, USA) with a 3.5 MHz transducer. Subjects were examined in the left lateral decubitus position according to the standardization of the American Society of Echocardiography (7). Standard views for M-mode and cross-sectional studies were obtained. Left ventricular (LV) end-diastolic dimension, LV end-systolic dimension, % fractional shortening, diastolic thickness of the ventricular septum and the posterior LV wall and LV mass were determined, The LV ejection fraction was calculated from the conventional apical 4-chamber images using the biplane Simpson's method (8). One investigator without knowledge of the clinical characteristics analyzed all echocardiographic measurements. All examinations were recorded on videotape.

Case and control genotype frequencies were compared by χ^2 exact testing and plasma ACE concentrations and echocardiographic results were compared by unpaired t test. The ACE concentrations according to ACE genotypes were compared with the use of Kruskal Wallis non-parametric ANOVA test. A p-value of <0.05 was considered significant.

In this study, we have demonstrated that there was no statistically significant difference between ACE genotypes and D and I allele frequencies in idiopathic DCM patients as compared with healthy subjects (Table 1). D allele frequency was 43% in controls versus 52% in patients and I allele - 57% versus 48%, respectively. Consistently with our results Candy et al. (2), Montgomery et al. (4) and Vancura et al. (5) have demonstrated that ACE gene I/D polymorphism is not a risk factor for idiopathic DCM and there was no significant difference between genotypes and alleles compared with idiopathic DCM patients and controls. In contrast, Raynolds et al. (3) have suggested that ACE DD genotype is a risk factor for the development of end stage heart failure due to cardiomyopathy.

When we have compared the echocardiographic values between patients and controls; LV end-diastolic dimension, LV end-systolic dimension, posterior wall thickness were significantly increased and ejection fraction and fraction shortening were sig-

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Table 1. Frequencies of genotypes, ACE concentrations, and association of ACE genotypes with ACE concentrations in idiopathic DCM patients and controls

	ACE Genotypes			p*	ACE, ng/ml	p**	ACE concentrations according to ACE genotypes, ng/ml			p***
	DD, n (%)	ID, n (%)	II, n(%)				DD	ID	II	
Controls (n=20)	4 (20)	9 (45)	7 (35)	0.396	269.65±93.92	0.01	264.55 (120.63-481.58) (n=4)	269.65 (116.06-426.75) (n=9)	239.43 (207.44-349.08) (n=7)	NS
Patients (n=29)	6 (20)	18 (62)	5 (18)		371.92±138.5		371.92 (180.03-554.68) (n=6)	371.92 (193.74-764.85) (n=18)	371.92 (161.75-390.20) (n=5)	NS

Values are presented as proportions, Mean+SD and Median (Minimum - Maksimum)
* Chi-square test
** - Unpaired t test for independent variables
*** - Kruskal Wallis non-parametric ANOVA test
ACE- angiotensin converting enzyme, DCM- dilated cardiomyopathy, NS- non-significant

Table 2. Echocardiographic variables in patients with DCM and controls

Parameters	Controls (n=20)	Patients (n=29)	p
LVEDD, mm	44.57±4.49	62.4±7.08	0.001
LVESD, mm	28.03±3.56	52.08±7.63	0.001
IVST, mm	9.19±0.86	9.95±1.58	NS
PWT, mm	8.75±0.79	9.46±1.38	<0.05
LV EF, %	66.55±4.92	33.82±8.96	0.001
LV FS, %	36.62±3.85	16.55±4.25	0.001
Stroke volume, mL	63.65±17	66.93±19.49	NS
LV mass, g	162.45±35.88	328.06±106.58	0.001
Left atrial dimension, mm	33.65±3.93	43.02±7.07	0.001

Values gives as means ± SD.
Unpaired t test for independent samples
DCM- dilated cardiomyopathy, EF- ejection fraction, FS- fractional shortening, LV- left ventricular, LVEDD- left ventricular end-diastolic dimension, LVESD- left ventricular end-systolic dimension, IVST- interventricular septal wall thickness, NS- non-significant, PWT- posterior wall thickness

nificantly decreased in patients versus to controls (Table 2). We have also found that there is no any relationship between ACE genotypes and echocardiographic values. Our findings are consistent with Yamada et al. (9). But in a study performed on the same population by Gunes et al. (6), it is suggested that left atrial diameter was dilated in homozygote II groups as compared to homozygote DD groups. On the other hand, Candy et al. (2) have suggested that patients with DD genotype had a significantly decreased ejection fraction than patients with other genotypes. These results show that homozygote DD group patients have a large dimension in echocardiographic parameters. So, echocardiographic parameters should be evaluated in DD homozygote patients.

Plasma ACE concentrations were significantly increased in patients. However, when we have compared plasma ACE concentrations with genotypes there were no significant differences between the groups. We could not find any findings about the association between plasma ACE concentrations and ACE gene I/D polymorphism genotypes in patients with DCM to compare with our results (Table 1). However, when we have compared our results with the results studied in various diseases; Cambien et al. (10) have found that there were no significant differences between patients and controls according to both plasma ACE concentrations and, ACE concentration and ACE gene polymorphism. Nevertheless, Gunes et al. (6) have found that plasma ACE concentrations were higher in hypertensive patients with DD genotypes.

As a conclusion, we suggest that there was no association between ACE gene I/D polymorphism and idiopathic DCM, although ACE concentration was high in this category of patients.

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