Insertion/Deletion Polymorphism of the Angiotensin Converting Enzyme Gene in Coronary Artery Disease in Southern Turkey

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Genetic factors are important in the pathogenesis of coronary artery disease (CAD). Angiotensin converting enzyme (ACE) gene insertion(I)/deletion(D) polymorphism is one of the genetic factor found to be related with CAD. We investigated the association between I/D polymorphism of the ACE gene and the presence of CAD. Threehundred and seven patients (187 males and 120 females, aged between 35-80, mean 54.3 ± 9.8 years) who underwent diagnostic coronary angiography were included in the study. ACE I/D polymorphism was detected by polymerase chain reaction. Of the 307, 176 had CAD. The most frequently observed genotype in all subjects was ID (47.9 %). However, in patients with CAD the frequency of II genotype was lower whereas DD genotype was higher compared to the controls ($p < 0.05$). The number of D allele carrying subjects were also higher ($p < 0.05$) in CAD patients. The logistic regression analysis indicated that the ACE D allele is an independent risk factor (odds ratio = 1.48, 95% CI = 1.01-2.18, $p < 0.05$). In conclusion, the I/D polymorphism of ACE gene (carrying D allele) is an independent risk factor for CAD in the studied Turkish population.

Keywords: ACE, Allele, Coronary artery disease, Gene polymorphism, Southern Turkey

Introduction

Coronary artery disease (CAD) is a multifactorial disease for which hyperlipidemia, hypertension, smoking, diabetes mellitus (DM) and family history are important risk factors. The renin-angiotensin system has an important role in cardiovascular physiology. Angiotensin -I- converting enzyme (ACE) also involves in many pathological conditions including vasoconstriction, coronary thrombosis, heart failure and ventricular remodelling (Dzau, 1994). The ACE gene, located at chromosome17q23 contains a polymorphism in intron 16 of an insertion (I) or deletion (D) of a 287 pair Alu repeat sequence which results in three genotypes as II, DD and ID (Mattei *et al.*, 1989; Rigat *et al.*, 1992). The relation between ACE gene polymorphism (DD genotype) and CAD first has been reported by Cambien *et al.* (1992). Since then numerous studies have been done to evaluate the relationship (Wang *et al.*, 1996; Gardemann *et al.*, 1998; Ferrieres *et al.*, 1999; Eichner *et al.*, 2001). A large case-control study showed a strong association between ACE D allele with the risk of CAD (Gardemann *et al.*, 1998). In a group of patients living in Turkey an association has also been found (Akar *et al.*, 1998). However some studies have shown no such associations (Friedl *et al.*, 1995; Ferrieres *et al.*,1999; Pfohl *et al.*, 1999; Agerholm *et al.*, 2000; Canavy *et al.*, 2000). We investigated ACE genotype distribution in patients with angiographically documented CAD, living in Southern Turkey.

Methods

Patients   The study population consisted of 307 patients (187 males and 120 females, aged between 35 - 80, mean 54.3 ± 9.8 years) who were admitted for diagnostic coronary angiography for the assessment of a suspected or confirmed clinical diagnosis of CAD. As a routine procedure, an informed written consent was obtained from all patients before angiography. The study was also approved by the local Ethics Committee.

   Risk factors were considered as hypertension, hyperlipidemia, diabetes mellitus, cigarette smoking and family history for CAD. A sustained blood pressure equal or greater than 140 mmHg systolic and 90 mmHg diastolic or using an antihypertensive medication was defined as hypertension (Seventh Report of the Joint National

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Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure, 2003). Diabetes mellitus was defined as hyperglycemia, requiring antidiabetic drugs or fasting blood sugar over 126 g/dL (American Diabetes Association, the Expert Committee on the diagnosis and classification of diabetes mellitus, 1999). Patients reporting cigarette use during the year prior to examination were considered as smokers. Hyperlipidemia was defined as plasma low-density lipoprotein cholesterol (LDL-C) > 130 mg/dL or using lipid-lowering drugs at the time of investigation. Hypertriglyceridemia was defined as TG level > 150 mg/dL and low high-density lipoprotein cholesterol (HDL-C) was considered present if the concentration of HDL-C was < 40 mg/dL according to The Third Report of the National Cholesterol Education Program guidelines. (Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Cholesterol in Adults , 2001).

Coronary angiography Coronary angiography was performed by Judkins technique and images of coronary tree were obtained in routine, standardized projections. The angiograms were assessed by at least two cardiologists. CAD was diagnosed as 50% reduction of internal diameter of left anterior descending, right coronary, circumflex artery or their primary branches. Patients without angiographic lesions were considered as the patients without CAD.

Blood chemistry Venous blood samples were collected after 12-h fasting before angiography. Plasma was separated within 4 h and stored at –20°C. Total cholesterol (TC), LDL-C, TG and HDL-C were measured by enzymatic methods by an Olympus 5200 autoanalyzer (Olympus Diagnostica GmbH, Hamburg, Germany).

Determination of ACE I/D polymorphism Genomic DNA from leukocytes was purified according to the method of Miller (Miller *et al.*, 1988). ACE I/D polymorphism was detected by polymerase chain reaction (PCR) by using the primer sequences derived from Zee *et al.* (1992). The sequences of the primers were chosen such that they flank the targeted region of the genome on the intron 16 of ACE gene (17q23). The template DNA (0.4 mg) was amplified by following primers: (forward) 5'-CTG GAG ACC ACT CCC ATC CTT TCT 3', and (reverse) 5'-GAT GTG GCC ATC ACA TTC GTC AGA T 3'. These primers (10 pmol of each) were added to a mixture containing 5 ml of 10X Cetus buffer (pH 8.3), 0.5 mM dNTP (dATP, dCTP, dGTP, dTTP) and 1.0 units of Taq DNA polymerase (Perkin Elmer Cetus). PCR p Program (Perkin Elmer 9600 Thermal Cycler) was initiated in a final total of 50 ml volume with thirty cycles of denaturation for 1 minute at 94°C, annealing for 1 minute at 58°C and primer extension for 1 minute at 72°C was applied for amplification. PCR products of ACE gene locus were examined by agarose gel electrophoresis (3% agarose) at 150V for 60 minutes and visualized at room temperature under UV after ethidium bromide staining.

Statistical analysis Statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS), version 9.0, and all values were expressed as mean ± standard deviation for both genders. Student's t-test and chi-square test were used to evaluate the mean age, plasma lipid profile and other risk factors. A p value

Table 1. Demographic characteristics of the patients with and without coronary artery disease[x]

	CAD (-) (n = 131)	CAD (+) (n = 176)	p
Male/female	61/70	126/50	0.000
Hypertension	48 (36.6%)	85 (48.3%)	0.04
Diabetes mellitus	11 (8%)	35 (20%)	0.005
Smoking	36 (28%)	83 (47%)	0.000
Family history	42 (32%)	81 (46%)	0.8
Age	53 ± 9	56 ± 10	0.01
Total C (mg/dL)	197 ± 38	212 ± 48	0.004
HDL-C (mg/dL)	44 ± 10	43 ± 8	0.4
LDL-C (mg/dL)	120 ± 31	132 ± 39	0.003
TG (mg/dL)	168 ± 84	177 ± 106	0.4
Total C/HDL-C	5.7 ± 1.1	5.1 ± 1.4	0.004

[x]Values are expressed as mean ± standard deviation.
(+), present; (-), absent; CAD, coronary artery disease; HT, hypertension; DM, diabetes mellitus; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride.

of < 0.05 was considered significant. Allelic frequencies were calculated by gene-counting method and the genotype distribution with Hardy- Weinberg expectations by a x^2 test. The differences in subjects with and without CAD were analyzed by a x^2 and Fisher's exact tests. The stepwise logistic regression analysis was performed for the determination of the independent predictors for CAD and the odds ratios as estimators of relative risk together with their 95% approximate confidence intervals were calculated to assess the association with and without CAD.

Results

Table 1 shows the clinical and laboratory characteristics of the patients according to the presence and absence of CAD. Of the 307 subjects, 176 had CAD. Patients with CAD were older and had a significantly greater incidence of hypertension, DM, smoking and also significantly higher concentrations of TC and LDL-C. The most frequently observed genotype in all subjects was ID (47.88%). ACE genotype distribution was in agreement with the Hardy-Weinberg expectations. The distribution of ACE genotypes in CAD (+) and CAD (-) patients and D allele carrying subjects are shown in Table 2. II genotype was significantly higher in CAD (-) subjects whereas DD genotype was found to be significantly higher in CAD (+) patients. The number of D allele carrying subjects were also significantly higher in CAD (+) cases. The stepwise logistic regression analysis indicated that carrying D allele of ACE gene was an independent predictor of CAD. Table 3 shows the odds ratios for CAD risk factors. We found the ACE D allele as an independent risk factor (odds ratio = 1.48, 95% CI = 1.01-2.18, *p* < 0.05) in our study population.

Table 2. Angiotensin converting enzyme gene distribution in patients with and without coronary artery disease

	CAD (-) n (%)	CAD (+) n (%)	OR (CI 95%)	p
II genotype	14 (10.7)	6 (3.4)	Ref.	
ID genotype	66 (50.4)	81 (46.0)	3.3 (1.2-9.0)	0.01*
DD genotype	51 (38.9)	89 (50.6)	1.6 (1.0-2.5)	0.04[f]
I allele	94 (35.9)	93 (26.4)	Ref.	
D allele	168 (64.1)	259 (73.6)	1.5 (1.1-2.2)	0.01

*ID+DD versus II
¶ DD versus II+ID
(+), present; (-), absent; CAD, coronary artery disease.
OR, odds ratio; CI, confidence interval

Table 3. Multivariate logistic regression analysis of coronary artery disease risk factors

	Coefficient	S.E.	X^2	p	Odds ratio	95% Cl
ACE D allele	0.394	0.197	3.987	0.046	1.483	1.01-2.18
Age	0.058	0.010	30.637	0.0000	1.060	1.04-1.08
Gender (male)	1.275	0.206	38.201	0.0000	3.580	2.39-5.37
Hypertension	0.685	0.201	11.628	0.0006	1.983	1.34-2.94
Diabetes mellitus	1.088	0.289	14.192	0.0002	2.968	1.69-5.23
Smoking	0.978	0.215	20.777	0.0000	2.659	1.75-4.05
LDL-C	0.009	0.003	10.259	0.0014	1.009	1.00-1.02
Constant	-5.763	0.755	58.202	0.0000	0.003	

For the total model : R^2: 0.270 and likelihood : 694.428
ACE, angiotensin converting enzyme; LDL-C, low density lipoprotein cholesterol.
CI, confidence interval; SE, standard error

Discussion

Besides very well known risk factors, genetic factors also play a role in the development of coronary artery disease. Genetic factors differ in various populations. Among these, ACE gene polymorphism has most frequently been studied and proposed as a coronary artery disease risk factor. ACE gene polymorphism determines the serum and tissue ACE activity which is high in subjects with DD genotype (Malik et al., 1997). ACE by causing high angiotensin II (Ang II) and low bradykinin levels may increase the risk of CAD (Cambien et al., 1992). Angiotensin II increases the macrophage derived growth factor and platelet derived growth factor which have a role in the genesis of atherosclerosis (Keidal et al., 1993). Furthermore, Ang II leads to LDL- C oxidation and stimulates neutrophil, macrophage and T-lymphocytes (Farber et al., 1990; Keidal et al., 1993). ACE decreases nitric oxide release via the bradykinin-kallikrein system and causes endothelial dysfunction which has also an important role in the genesis of atherosclerosis. Homozygous deletion subset of the ACE I/D polymorphism is associated with deteriorated endothelial function (Mulder et al., 2003).

ACE gene polymorphism has been studied in patients with hypertension, dilated cardiomyopathy and restenosis (Zee et al., 1992; Dzau et al., 1994). Association of the DD genotype

of the ACE gene and myocardial infarction (MI) has also been reported in numerous studies (Cambien et al., 1992, Gardemann et al., 1998; Yoshida et al., 1999; Eichner et al., 2001). Cambien et al. (1992) has proposed the ACE I/D polymorphism as a marker of susceptibility to MI. However, the relation between ACE gene polymorphism and CAD has been challenged in different studies. Gardemann et al. have shown a relation between the presence of CAD and ACE D allele in a large case-controlled study (Gardemann et al., 1998). Furthermore, DD genotype of the ACE gene has been reported as a risk factor for the development of early atherosclerosis also in carotid arteries in a Chinese population (Jeng, 2000). There are some other studies in which ACE I/D gene polymorphism has not been found to be associated with the prevalence of CAD (Pfohl et al., 1999; Lindpainter et al., 1995). In a sample in European population with the lowest risk ACE I/D polymorphism has been reported not to be associated with an increased risk of CAD or MI (Ferrieres et al., 1999). It has been suggested that the different results from the studies may be due to either the population-specific effect in relation to the ACE gene polymorphism or the lack of the documentation of CAD by means of coronary angiography (Wang et al., 1996; Pfohl et al., 1999).

There are several studies regarding ACE gene polymorphism and CAD in Turkey. Akar and colleagues (Akar et al., 1998)

have shown an association between D allele and CAD in patients living in the middle part of Turkey. Similar findings have been found by Isbir and colleagues (Isbir *et al.*, 1999) in patients living in Western Turkey. Whereas a study from South-eastern Turkey and another study from middle part of Turkey have not shown such an association (Tokgozoglu *et al.*, 1997; Nacak *et al.*, 2004). Another study from Western part of Turkey pointed out that ACE gene polymorphism did not play a role in the development of congestive heart failure in 209 CAD patients proven by angiography (Akbulut *et al.*, 2003). However, there were no data on controls in that study. Gurlek *et al.* (2000) showed that 35% of 158 patients with CAD who underwent coronary-stenting had ACE I/D polymorphism. The authors also found that ACE I/D polymorphism was significantly associated with the risk of restenosis. However, routine determination of the ACE genotype may not identify patients who are at a higher risk of thrombotic and restenotic events after coronary stenting (Koch *et al.*, 2000).

Our study shows that the DD genotype is a significant predictor of CAD in population living in Southern Turkey. In the present study the frequency of II genotype was significantly low whereas DD genotype was significantly high in patients with CAD compared to the patients with normal coronary arteries proven by angiography. We found the ACE D allele as an independent risk factor (odds ratio = 1.48, 95% CI = 1.01-2.18, $p < 0.05$). ACE polymorphism analyzed were in Hardy-Weinberg equilibrium which indicates that they were normally distributed between the patients with and without CAD.

An association of the ACE DD genotype with the presence of CAD has also been found in Australian population (Wang *et al.*, 1996). On the other hand van Bockxmeer reported that ACE I/D polymorphism did not play a role in the development of CAD or MI in Western Australian Caucasian population (van Bockxmeer *et al.*, 2000). Another study by Jeunemaitre *et al.* (1997) showed that no association between ACE I/D polymorphism and CAD in 463 Caucasians. Gardemann *et al.* (1998) found a relation of the ACE I/D gene polymorphism to CAD and MI in 2267 male Caucasians and found an increased familial risk of MI in those carrying the D allele.

Our findings are not consistent with some other studies coming from Turkey and other countries. This might be due to the genetic heterogenity of the population living in Southern Turkey.

In conclusion, the insertion/deletion polymorphism of ACE gene (carrying D allele) is an independent risk factor for coronary artery disease in the studied Turkish population.

Limitations of the study　The nature itself of cross-sectional studies is one of the limitations of this and other case-control studies assessing the influence of single polymorphism in CAD. Another limitations are the sample size and the lack of follow-up.

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