IUBMB *Life*, 48: 205–207, 1999

1521-6543/99 $12.00 + .00

Original Research Article

Association Between Angiotensin-Converting Enzyme Gene Polymorphism and Coronary Artery Disease

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Summary

An insertion/deletion (I/D) polymorphism in the gene for angiotensin-converting enzyme (ACE) is associated with myocardial infarction and other cardiac pathology. There is evidence for a role of the renin–angiotensin system in cell growth and in the repair of damaged arterial walls, so the ACE gene is postulated to be a candidate gene affecting the important clinical problem of coronary artery disease (CAD). In view of the clinical importance of the ACE as a major marker of cardiovascular diseases, we investigated the I/D polymorphism of the ACE gene in Turkish CAD patients in comparison with control subjects to evaluate a possible association between CAD and the gene encoding ACE. Polymerase chain reaction, restriction fragment length polymorphism, and agarose gel electrophoresis techniques were used to determine the ACE genotype in 58 subjects. The frequencies of ACE D and ACE I allele among the patients with CAD were 62.26% and 37.73% and in the control subjects were 49.3% and 50.76%, respectively. The greater frequency of deletion allele (D) was in the CAD group than in the control subjects was significant ($P < 0.01$).

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Keywords Angiotensin-converting enzyme gene polymorphism; coronary artery disease.

INTRODUCTION

Coronary artery disease (CAD)[3] is a multifactorial disease caused by genetic and environmental factors (*1*). Recently, polymorphisms in the genes encoding components of the renin–angiotensin system (RAS) have been proposed as an independent genetic factor for hypertension and other cardiovascular diseases (*2–6*). Several studies have shown that the DD genotype of the insertion/deletion (I/D) polymorphism in intron 16 of the angiotensin-converting enzyme (ACE) gene encoding kinase II (EC 3.4.15.1, which converts angiotensin I to the bioactive angiotensin II) is associated with a higher risk for myocardial infarction (*3*), CAD (*1*), and cardiac hypertrophy (*7*). However, other studies failed to show any correlation between the DD genotype and coronary or ischemic heart disease (*8*, *9*) or between the genotype and endothelial dysfunction (*10*). The studies reported here were made in an attempt to resolve these differences.

EXPERIMENTAL PROCEDURES

Subjects. ACE gene polymorphism was studied in 58 patients (22 women, 36 men; mean age 53.16 ± 12.02 years). There was no significant difference in age between patients with CAD, the control subjects with severe coronary vascular disease documented by angiography. Angiographic inclusion criteria were: ≥ 50% stenosis of at least one major coronary vessel because of atherosclerosis, and a vascular event, defined as myocardial infarction, percutaneous transluminal coronary angioplasty, or coronary artery bypass grafting. Patients were included irrespective of concomitant risk factors for atherosclerosis such as smoking, arterial hypertension, hyperlipidemia, increased body mass index, and diabetes mellitus. There were 36 nonsmoking patients with CAD, 4 with diabetes mellitus, and 6 with hypertension. Patients who had a cholesterol level > 200 mg/dl were also included.

Healthy persons (mean age: 51.95 ± 19.46) without any symptoms of CAD were selected for the control group. Coronary angiography was not performed on these individuals, and therefore the presence of atherosclerotic coronary arteries cannot be

Received 25 September 1998; accepted 19 January 1999.

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[3] Abbreviations: ACE, angiotensin-converting enzyme; CAD, coronary artery disease; I/D, insertion/deletion; PCR, polymerase chain reaction; RAS, renin–angiotensin system.

excluded. However, none of these individuals had any history of a vascular event.

Isolation of DNA. Blood specimens were collected in tubes containing EDTA, and DNA was prepared from the leukocyte pellets by sodium dodecyl sulfate lysis, ammonium acetate extraction, and ethanol precipitation (*11*).

Polymerase Chain Reaction (PCR) for ACE Gene Polymorphism. Template DNA (0.5–1.0 μg) was used in a PCR under stringent conditions to avoid the possibility of false positives for ACE genotyping. Reactions were performed with 10 pmol of each primer: forward primer 5′-CTG GAG ACC ACT CCC ATC CTT TCT-3′ and reverse primer 5′-GAT GTG GCC ATC TTC GTC AGA T-3′ in a final volume of 50 μl containing 3 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 8.4), 0.5 mM of each dNTP (MBI Fermentas), and 1 unit of Taq polymerase (MBI Fermentas). Amplification was carried out in a DNA Thermal Cycler (MJ Research Techne) for 30 cycles with denaturation steps at 94 °C for 1 min., annealing at 58 °C for 1 min., and extension at 72 °C for 2 min. PCR products were separated on 3% agarose gel, and DNA was visualized by ethidium bromide staining. The PCR product is a 190-bp fragment in the presence of the deletion (D) allele and a 490-bp fragment in the presence of the insertion (I) allele. Thus, each DNA sample revealed one of three possible patterns after electrophoresis: a 490-bp band (genotype II), a 190-bp band (genotype DD), or both 490- and 190-bp bands (genotype ID) (*12*).

Statistical Methods. Statistical analyses, using SPSS version 5.1, included the χ^2 test for genotype and allele frequencies comparison. The significance between patients and control subjects was evaluated with the unpaired Student's *t* test. ACE allele frequencies were estimated by gene counting methods.

RESULTS

The ACE genotype and allele frequencies for CAD and control subjects are presented Table 1. The respective frequencies of DD, ID, and II genotypes among the patients with CAD were 39.6% (n = 21), 45.3% (n = 24), and 15.1% (n = 8); among the control subjects, these were 30.8% (n = 20), 36.9% (n = 24), and 32.3% (n = 21) (differences nonsignificant). The frequencies of ACE D and ACE I allele among the patients with CAD were 62.26% and 37.73% and among the control subjects were 49.3% and 50.76%, respectively. The frequency of deletion allele (D) was higher in the CAD group than control subjects. Statistical analysis (Table 2) was showed that values for the CAD group differed significantly from those for the control group ($\chi^2 = 5.41$, $P < 0.01$).

Table 1
Distribution of ACE genotypes and allele frequencies in CAD and control subjects

	Control (n = 65)	CAD (n = 53)
ACE genotype		
DD	30.8% (20)	39.6% (21)
II	32.3% (21)	15.1% (8)
ID	36.9% (24)	45.3% (24)
Alleles		
I	50.76% (66)	37.73% (40)
D	49.23% (64)	62.26% (66)

The number of individuals is shown in parentheses.

Table 2
Comparison of presence of ACE D allele in CAD subjects

	Controls		CAD	
	Frequency, %	n	Frequency, %	n
ACE D allele				
Absent	33.8	22	15.11	8
Present	66.2	43	84.9	45

DISCUSSION

CAD is a multifactorial disease in which genetic and environmental factors have important roles. These factors may differ in each race or ethnic group. Thus, we investigated the association between I/D polymorphism of the ACE gene in Turkish CAD patients.

ACE is one of the constituents of the RAS and has attracted attention in the development of cardiovascular diseases. In practice, an ACE inhibitor is known to significantly reduce mortality or the incidence of myocardial infarction in patients who have hypertension or ischemic cardiovascular diseases (*6*, *13*, *14*). Genetic variants in the constituents of the RAS, which include renin, angiotensinogen, and ACE, may contribute to cardiovascular disease (*13*), as genetically determined differences in the expression of any of these factors may adversely affect angiotensin II concentrations in the heart (*6*). A polymorphic marker correlating with circulating concentrations of ACE was recently found in intron 16 of the ACE gene. The marker, which is in strong linkage disequilibrium with the true variant, consists of the presence (I) or absence (D) of a 287-bp alu repeat sequence. People with the ACE II genotype have the lowest circulating ACE concentrations, whereas those with the ACE DD genotype have the highest (*15*). Furthermore, the ACE DD genotype has been associated with the incidence of myocardial infarction in French and Irish men otherwise considered to be at low risk of CAD. In this study, the frequency of the ACE deletion allele (D) has been found to be higher in the CAD group than in the control subjects ($P < 0.01$).

Association studies between ACE gene polymorphism and cardiovascular diseases have reported varied results (*1*, *3*, *10*, *16*). Therefore, in an association study we compared the allele frequencies of the ACE gene polymorphism in CAD subjects.

In summary, a significant association between the I/D polymorphism of the ACE gene and Turkish CAD patients was noted.

The frequency of the ACE D allele was higher in the CAD group than control subjects ($P < 0.01$). The results of the study support the hypothesis that the DD genotype of the ACE gene is a linkage marker for an underlying etiological mutation that confers the risk for the development of CAD and is detectable in subjects previously unidentifiable by a history of classic risk factors. The numbers are relatively small and thus may be open to dispute, but a clear interrelation between this allele and CAD is established and should be noted by cardiac physicians.

ACKNOWLEDGEMENTS

This study was supported by a grant from the University of Istanbul, Research Foundation (project Ö-335/270697).

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