GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Traditional risk factors and angiotensin-converting enzyme insertion/deletion gene polymorphism in coronary artery disease

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Genet. Mol. Res. 14 (1): 2063-2068 (2015)
Received March 24, 2014
Accepted October 23, 2014
Published March 20, 2015
DOI http://dx.doi.org/10.4238/2015.March.20.16

ABSTRACT. We investigated whether the insertion/deletion (I/D) polymorphism in the angiotensin-converting enzyme (ACE) gene and serum ACE levels are associated with traditional risk factors of coronary artery disease (CAD). We enrolled 250 individuals without CAD and 750 individuals suffering from CAD who were angiographically diagnosed. Biochemical risk factors, the *ACE* (I/D) gene polymorphism, and ACE serum levels were compared. *ACE* genotypes were determined using real-time polymerase chain reaction. ACE serum levels were determined using an enzyme-linked immunosorbent assay. Lipid parameters were determined spectrophotometrically using an autoanalyzer. Compared to the control group, the CAD group showed significantly higher serum

ACE levels ($P < 0.001$). The highest ACE levels were found in those with the DD genotype. Other genotypes also presented statistically significant differences. We observed a significant difference between the control and coronary patient groups regarding the levels of total cholesterol, triglyceride, high-density lipoprotein-cholesterol, and low-density lipoprotein-cholesterol ($P < 0.05$). *ACE* (I/D) genotypes and serum ACE levels may be associated with risk factors and the development of CAD.

Key words: Angiotensin-converting enzyme; Coronary artery disease; Polymorphism

INTRODUCTION

The relationships between genetic determinants and environmental risk factors of disease have been widely studied in recent years. Coronary artery disease (CAD) begins in the early stages of life and ends with death. It is the leading cause of death in developing countries; Turkey has one of the highest incidence rates among European countries (Zhang et al., 2011). The disease is defined as atherosclerotic blockage of the arteries supplying the oxygen-rich blood to artery muscle and may be prevented through the control of risk factors. Prevention methods can lower mortality rates (Levy et al., 1990; Kones, 2011). In addition to well-known risk factors such as hypertension, hyperlipidemia, smoking, and diabetes mellitus, recent attention has been focused on emerging factors (Makino and Kawano, 1998).

The angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism is a genetic determinant that plays an important role in hypertension development and is a significant, modifiable, and strong risk factor for CAD (Johnson et al., 2009; Hamelin et al., 2011). ACE, a key element of the renin-angiotensin and kallikrein-kinin systems, is expressed on the surface of cells in the lungs, endothelial cells, and plasma (Cambien et al., 1992). ACE hydrolyzes angiotensin I to convert it into angiotensin II, a potent vasoconstrictor that regulates blood pressure. It also inactivates the vasodilator bradykinin. The human *ACE* gene is located at 17q23 and contains 26 exons and 25 introns. The I/D of a 287-base pair element in intron 16 is the most thoroughly studied and clinically important polymorphism in the *ACE* gene (Mattu et al., 1995). The deletion polymorphism has been found to be associated with elevated levels of circulating ACE and myocardial infarction (MI) (Arbustini et al., 1995). Hypertension, which is associated with high systolic and diastolic blood pressure values, is a potent risk factor for CAD. It causes endothelial dysfunction and prevents vasodilatation. Lipid parameters affect the pathogenesis of atherosclerosis. Elevated plasma lipid concentrations lead to thrombus in the coronary arteries. A relationship between the DD genotype of the *ACE* gene and hyperlipidemia has been demonstrated previously (Niemiec et al., 2007).

In this study, we investigated the relationship between the *ACE* I/D polymorphism and CAD. We examined lipid parameters that are defined risk factors for CAD in Turkish subjects in Tokat, who have a specific food consumption style and are at high risk for MI. We examined whether these parameters are related to the polymorphism or ACE levels.

MATERIAL AND METHODS

Study population

The study included 1000 people admitted to the Cardiology Department, Medical Faculty of Gaziosmanpasa University, Tokat. A total of 285 female and 465 male patients with CAD confirmed by coronary angiography (presence or history of at least 1 stenosis >50%) were considered to be the patient group. In addition, 138 female and 112 male subjects without CAD were included in the control group. Before catheterization, clinical examination was performed to determine demographics, cardiac history, cardiovascular risk factors, features of extra coronary vascular disease, and related comorbidities.

Laboratory methods

Venous blood samples were drawn into tubes with ethylenediaminetetraacetic acid and centrifuged within 10 min at 1000 *g*. We measured serum ACE levels using a human enzyme-linked immunosorbent assay kit (QUANTIKINE; R&D Systems, Inc., Minneapolis, MN, USA) and serum lipid parameters, total cholesterol, triglyceride, high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein-cholesterol (HDL-C) were assayed spectrophotometrically using an autoanalyzer.

Genotyping

Genomic DNA was extracted from whole blood using the MagaZorb® DNA Mini-Prep Kit (BioFlux, Tokyo, Japan). Genotyping was performed using the real-time polymerase chain reaction (PCR) method with LightCycler Fast Start DNA Master SYBR Green I on a LightCycler 480 II system (Roche Diagnostics GmbH, Basel, Switzerland).

Statistical analyses

Conventional methods were used to calculate means and standard deviations (SD). All results are reported as means ± SD. Baseline characteristics of study patients are summarized in terms of frequencies and percentages for categorical variables and by means and SD for continuous variables. Categorical variables were compared using the χ^2 test and continuous variables were compared using the Student *t*-test. Comparison of continuous variables between all 3 genotypes was conducted using one-way analysis of variance. P values equal to or less than 0.05 were considered to be statistically significant. All statistical computations were performed using SPSS 13.0 for Windows (SPSS, Inc., Chicago, IL, USA).

RESULTS

Serum ACE levels were higher in the CAD group than in the control group ($P < 0.001$) (Table 1) and higher in subjects with the DD genotype compared to other genotypes ($P < 0.001$). HDL-C levels were the highest in subjects with the II genotype ($P < 0.05$). Total cholesterol, triglyceride, HDL-C, and LDL-C were higher in CAD patients ($P < 0.05$) (Tables

2 and 3). No significant differences were observed in the allele frequencies between the groups (D allele: CAD group 0.58, control group 0.59; I allele: CAD group 0.42, control group 0.41) (P = 0.95) (Table 4).

Table 1. Levels of risk factors in different groups.

	Control	CAD	P value
ACE (ng/mL)	167.2 ± 35.2	177.4 ± 33.9	<0.001
Total cholesterol (mg/dL)	209.8 ± 42.8	200.4 ± 47.9	<0.05
Triglyceride (mg/dL)	154.4 ± 82.2	170.2 ± 94.4	<0.05
LDL-C (mg/dL)	133.3 ± 38.2	126.6 ± 40.6	<0.05
HDL-C (mg/dL)	45.4 ± 12.1	40.7 ± 11.4	<0.05

Table 2. Comparison of polymorphisms analyzed and risk factors in the control group.

N (250)	DD	ID	II	P value
ACE (ng/mL)	185.4 ± 37.6	164.7 ± 27.5	138.8 ± 24.1	<0.001
Total cholesterol (mg/dL)	212.0 ± 41.5	207.2 ± 46.6	211.7 ± 36.7	
Triglyceride (mg/dL)	165.4 ± 89.8	145.2 ± 77.6	154.0 ± 76.0	
LDL-C (mg/dL)	134.7 ± 38.7	131.7 ± 38.8	134.0 ± 36.6	
HDL-C (mg/dL)	44.1 ± 9.6	46.4 ± 13.3	46.9 ± 13.2	

Table 3. Comparison of polymorphisms analyzed and risk factors in CAD group.

N (750)	DD	ID	II	P value
ACE (ng/mL)	190.3 ± 35.1	175.5 ± 32.0	156.9 ± 24.6	<0.001
Total cholesterol (mg/dL)	203.3 ± 47.9	197.3 ± 46.6	204.0 ± 51.4	
Triglyceride (mg/dL)	166.8 ± 84.8	171.7 ± 103.0	173.0 ± 86.3	
LDL-C (mg/dL)	129.6 ± 39.6	123.6 ± 39.6	129.2 ± 43.4	
HDL-C (mg/dL)	40.6 ± 11.3	40.0 ± 10.4	42.91 ± 14.0	<0.05

Table 4. Comparison of allele frequencies in the control and CAD groups.

	N	D allele	I allele	P value
Control	250	0.59	0.41	0.95
CAD	750	0.58	0.42	

DISCUSSION

Several studies have examined the genetic determinants of CAD in recent years. *ACE* gene polymorphisms play a role in the disease (Dzielińska et al., 2011; Pandey et al., 2011). Further, the *ACE* I/D polymorphism has been examined as a risk factor for CAD, MI, cardiomyopathies, and sudden cardiac death, among others (Marian et al., 1993; Alvarez et al., 2000). The frequency of the D allele of *ACE* (I/D) differs among populations (Tokgözoğlu et al., 1997; Oren et al., 1999). Previous studies revealed a relationship between CAD and the D allele (Akar et al., 1998). However, some studies reported no such association (Arca et al., 1998; Araz et al., 2002). Differences in genotypes in the same population according to ethnic-

ity have also been described (Foy et al., 1997; Akbulut et al., 2004; Zhang et al., 2010). ACE activity is higher in subjects with the DD genotype (Alvarez et al., 2000; Elshamaa et al., 2011). The ACE genotype also affects the pathologies of the vascular endothelial tissue (Johnston, 1994; Morishita et al., 1994). Serum ACE activity, MI, and CAD are related (Marian et al., 1993; Cambien et al., 1994; Payne et al., 1994). The D allele and serum ACE activity are considered to be risk factors for the development of CAD (Turgut, 2005). Induced ACE activity worsens endothelial functions, similar to other risk factors (Evrengül et al., 2008).

In this study, serum ACE levels were higher in the CAD group than in the control group. ACE levels were the highest in subjects with the DD genotype. HDL-C levels were the highest in those with the II genotype. Total cholesterol, triglyceride, HDL-C, and LDL-C levels were higher in CAD patients. There was no significant difference in allele frequencies between groups.

Ethnicity is an important factor in gene polymorphisms. We investigated the frequency distribution of the *ACE* (I/D) polymorphism in Turkish subjects in Tokat. The results showed that the *ACE* (I/D) polymorphism and ACE levels are associated with total cholesterol, triglyceride, HDL-C, and LDL-C, which are important factors of CAD.

Although there was no significant relationship between the D allele and CAD, lipid parameters were higher in subjects with the DD genotype; thus, the D allele can be considered a risk factor for CAD. ACE activity was associated with both CAD and the genotypes. The prevalence of the DD genotype was related to elevated ACE levels, supporting the results of previous studies. Furthermore, our results suggest a relationship between the activity of ACE and the *ACE* (I/D) polymorphism. This may have an important clinical implication for the definition of different reference ranges based on genotypes.

The present study revealed an association between the *ACE* (I/D) polymorphism and the pathogenesis of CAD. Our results can be used in the planning of preventive strategies for patients with risk factors for CAD. Further and larger studies are required to confirm these results and determine whether the activity of ACE and the *ACE* D allele can be used to predict CAD risk.

ACKNOWLEDGMENTS

Research supported by the Gaziosmanpasa University Scientific Research Projects Committee.

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