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Association of angiotensin-converting enzyme gene variations with coronary artery disease in the Iranian population

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Original Article

Abstract

BACKGROUND: The purpose of this study was to identify the angiotensin-converting enzyme (ACE) gene (I/D) variations in CAD patients and healthy controls in an Iranian population (West Azerbaijan province of Iran).

METHOD: This cross-sectional study included 95 CAD patients and 203 healthy controls. ACE I/D polymorphisms were assessed using PCR, and their frequency was determined.

RESULTS: There were 298 people, 95 CAD patients, and 203 controls, with an average age of 50.96±3.45 and 51.14±10.20. We discovered that the frequency of the D allele was significantly higher in CAD patients than in controls (P = 0.0009). In contrast, the frequency of the I allele was significantly higher in controls than in CAD patients (P = 0.0009). The D allele carriers genotypes (DD + ID) were more frequent in the CAD patients than in the control group (P = 0.008). The ACE II genotype-state carriers were more common in the control group than in CAD patients (P = 0.008). However, in the case of the ACE ID genotype, no significant differences were not found in the tested groups (P = 0.47).

CONCLUSIONS: These findings suggest that individuals with the ACE DD genotype are predisposed to CAD, whereas individuals with the ACE II genotype state are protected.

Keywords: Coronary artery disease, Angiotensin-Converting Enzyme, Genetic, Insertion/deletion, Polymorphism

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Introduction

The most common cause of ischemic cardiac events is coronary artery disease (CAD), which can result in congestive heart failure, myocardial infarction, sudden cardiac death, and cardiac arrhythmias.[1] The Angiotensin-Converting Enzyme (ACE) is a crucial enzyme in the renin-angiotensin system that catalyzes the angiotensin I to angiotensin II conversion.[2-5] The human ACE gene is found on chromosome 17q23, consisting of 26 exons and 25 introns.[4] If the 287 bp sequence exists or not in its intron 16, it is classified as an insertion (I) or deletion (D) allele (ACE Gene I/D polymorphism).

Human populations have three genotypes (DD, II, ID). Although the pathogenesis is unknown, recent findings suggest that I/D polymorphism in ACE gene intron 16 is correlated with CAD.[5] Because the insertion/deletion (I/D) affects the level of circulating ACE, the angiotensin II plasma level changes.[6] High levels of angiotensin II caused by

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the mutation of the D allele may result in vascular tissue remodeling and amplifying the atherosclerotic process.[7] The association of ACE polymorphism with CAD has yielded conflicting results.[8] This study aimed to investigate the relationship between ACE/ID polymorphisms and CAD in Iranian Turks (West Azerbaijan province of Iran).

Materials and Methods

Subjects

The research ethics committee at the Urmia University of Medical Sciences has approved all aspects of the study [ir.umsu.rec.1394.138]. This study was conducted in the Cellular and Molecular Research Center of Urmia University of Medical Sciences and sampled in Seyed-ol Shohada University Hospital, Cardiology Poly Clinic Unit, Catheterism Ward (Angiography) (Urmia, Iran) in 2015-2016. Individuals voluntarily participated in this study.[9]

The cases and controls have been selected from all the individuals referred for coronary angiography by an expert specialist. Ninety-five CAD patients and 203 controls were included in the study.

Every participant was made aware of the study. All male and female patients were younger than 55 and 65, respectively. Clinical symptoms and non-invasive tests, such as exercise tolerance testing, nuclear myocardial perfusion imaging, exercise stress echocardiography, and CT angiography, were used to determine the need for coronary angiography.

The patients in the case group had at least 50% stenosis in one of their coronary arteries. The participants selected for the control group all had fully patent coronary lumens or luminal irregularities compared to those with diameter reductions of less than 50%. Successively referred patients were chosen, and no intervention was performed to prevent any research bias.

Exclusion criteria include patients with FMF, acute coronary syndrome, acute illness, or other inflammatory diseases. Whole blood samples ranging from three to five milliliters were collected from the patients and controls and added to tubes containing EDTA. A common salting out procedure was used to extract genomic DNA.[10]

The polymerase chain reaction was used to amplify two different ACE I and D allele types (PCR). Electrophoresis in 2% agarose gel was used to determine if ACE I and D alleles were present or absent. ACE genotyping was accomplished by PCR using the primers 5'-ctg gag acc act ccc atc ctt tct-3' and 5'-gat gtg gcc atc aca ttc gtc aga t-3'. Thirty-five cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 1 min made up the cycling schedule.[11] Next, 2% agarose gel electrophoresis with ethidium bromide was used to separate the PCR products.

UV transilluminator was used to detect the presence or absence of amplified fragments (490 and 190 bp). A single 490 bp fragment, a 190 bp band, and both 490 and 190 bp fragments indicated the I/I genotype, D/D genotype, and I/D genotypes, respectively.

Statistical analysis

Direct counting was used to determine the frequency of ACE I and D alleles and II, ID, and DD genotypes. The Hardy-Weinberg equilibrium (HWE) test was used to measure the genotype distribution's deviation in the control group. The x^2 test or Fisher's exact test was used to compare the frequencies between case and control groups. Statistical Package for the Social Sciences (SPSS) version 20.0 and Microsoft Excel 2007 was used for statistical analysis and to calculate the x^2 and *P*-value, the odds ratio (OR), and the 95% confidence interval (CI). Two-sided tests with power (1-β):90% were done, and a P-value of less than 0.05 was regarded as statistically significant.

Results

We studied 298 individuals, including 95 CAD patients and 203 healthy controls. The average age of the cases and controls was 50.96±3.45 and 51.14±10.20, respectively. Significant differences in demographic records, including high-quality household history,

dyslipidemia, diabetes mellitus, blood stress, and cigarette smoking, were not discovered between examined corporations (P-value >0.05).

Table 1 provides the ACE Genotype/allele, observed F(%F), observed allele frequency, and expected frequency in the tested control group. The statistical analysis revealed that controls (χ^2 = 2.162 < 3.84, P-value with 2 degrees of freedom = 0.339 > 0.05) agreed with Hardy-Weinberg equilibrium. The I and D allele frequencies were 0.408 and 0.591 in the controls, respectively.

Table 2 lists the frequency of the examined groups (case and controls) for the ACE I/D alleles and genotypes (I, D, II, ID, and DD), P-values, and OR (95% CI). The ACE D and I allele frequencies were 73.16% and 26.84% in the case group and 59.11% and 40.69% in the control group. The ACE D/D, D/I, and I/I genotypes were found in 51 (53.68%), 37 (38.95%), and 7 (7.37%) of tested cases, and 76 (37.44%), 88 (43.35%), and 39 (19.21%) of tested controls. Statistical analysis revealed significant differences between the CAD patients and the controls regarding ACE D and I alleles and DD and II genotypes (Table 2). The CAD patients and control group had a higher prevalence of ACE DD and II genotype-state carriers. In this regard, ACE DD genotype-state carriers may be predisposed to CAD, and ACE II genotype- and I allele-state carriers are protected against CAD.

Table 1. ACE Genotype, observed F(%F), observed allele frequency and expected frequency in control group*

ACE Genotype	Observed genotype frequencies F(%F)	Expected genotype frequencies F	Observed genotypic frequency
II	39(19.2)	33.9	0.19
ID	88(43.3)	98.2	0.43
DD	76(37.4)	70.9	0.37

*Values in parentheses are percentages. ACE indicates angiotensin-converting enzyme; I, insertion; D, deletion; χ^2 = 2.162 < 3.84, P-value with 2 degrees of freedom = 0.339 > 0.05

Table 2. Angiotensin-Converting Enzyme Genotypes and Alleles among CAD and Controls*

ACE	Cases (n=95)	Controls (n=203)	OR (95% CI)	*P*-value
II	7(7.37)	39(19.2)	0.33 (0.14, 0.78)	0.01
ID	37(38.9)	88(43.3)	0.83 (0.51, 1.37)	0.47
DD	51(53.7)	76(37.4)	1.94 (1.18, 3.17)	0.01
I	51(26.8)	166(40.9)	0.53 (0.36, 0.77)	0.01
D	139(73.2)	240(59.1)	1.89 (1.29, 2.75)	0.01

*Values in parentheses are percentages. ACE indicates angiotensin-converting enzyme; I, insertion; D, deletion; OR, odds ratio; and CI, confidence interval. Microsoft Excel 2007 was used for statistical analysis to calculate the P-value, the odds ratio (OR), and the 95% confidence interval (CI)

Discussion

The underlying pathophysiologic mechanisms for CAD begin with atherosclerosis. The renin-angiotensin system (RAS), along with endothelial dysfunction, inflammation, and plaque stabilization, is a critical mediator of the atherosclerotic process.[12] The findings of our study revealed that D allele carrier status and DD genotype in the ACE gene were associated with an increased risk of CAD. This finding is consistent with previous research that found a

link between CAD and the DD genotype.[13]

Niemiec et al. [13] in Poland reported that the DD genotype/D allele increases the risk of CAD in the presence of traditional risk factors. The D allele has been linked to an increased risk of severe coronary stenosis and acute coronary events. Zintzaras et al. (2008) also reported a modestly positive relationship between ACE I/D polymorphism and CAD in their meta-analysis.[14]

However, no correlation was found between the frequency of ACE I/D genotype in controls and CAD patients in this study. In our research, we discovered that the ACE I and II genotypes are predominant in the control group, implying that the ACE II and I alleles may have a protective effect on the coronary arteries. Some studies have found no link between ACE polymorphisms and the severity of CAD.[15]

According to the findings of this study, there is a link between D allele carrier status and DD genotype in the ACE gene and the incidence of CAD among Iranian Turks. Further research is needed to determine the role of the other genes in the pathogenesis of CAD.

Conclusion

These findings suggested that people with the ACE DD genotype are predisposed to CAD, whereas people with the ACE II and I alleles are protected.

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Conflict of Interest

The authors state that they do not have any competing interests.

Authors' Contribution

AG, MN, and MB contributed to the study's concept and design. MH, KK, and SB helped with data collection and DNA extraction. MB and KK monitored the sample collection and DNA extraction procedures. MB and MN did the statistical analysis. All authors contributed to and approved the manuscript.

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