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Angiotensin II Type 1 Receptor Gene A1166C Polymorphism Was Not Associated With Acute Coronary Syndrome in an Iranian Population

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Abstract

Background: There are very limited data for Iranian populations on the predisposing genetic factors for acute coronary syndrome (ACS).
Objectives: The objective of the present study was to investigate the association of the angiotensin II type 1 receptor (AT1R) gene polymorphism and ACS in an Iranian population.
Patients and Methods: This cross-sectional study was conducted among 263 subjects (97 men and 166 women). Patients (n = 128) aged 30 - 80 years with chest pain were recruited from the emergency department of Ghaem Hospital (Mashhad, Iran). A 12-lead electrocardiograph plus creatine kinase MB (CK-MB) levels were used as the basis for the diagnosis of myocardial ischemia. The control group was selected from age-matched healthy subjects (n = 135). Non-enzymatic kits were used for extraction of DNA from blood samples. Polymerase chain reaction (PCR) was performed to amplify the DNA fragments. For restriction fragment length polymorphism (RFLP) determination, the DdeI enzyme was used to digest the amplified DNA fragments. Statistical analyses were performed using SPSS version 13.0.
Results: There was no statistical difference in the genotype frequency of patients and healthy subjects with regard to age and gender ($P > 0.05$).
Conclusions: The AT1R A1166C polymorphism appeared not to be associated with the presence of ACS in the population studied.

Keywords: Acute Coronary Syndrome, Angiotensin II Type 1 Receptor, Gene Polymorphisms, Genotype Frequency, AT1R A1166C

1. Background

Chest pain is the second most common cause for visits to emergency departments (1, 2), and it is a common manifestation of coronary heart disease (CHD), as either acute coronary syndrome (ACS) or exertional angina (3). In Iran, it has been reported that approximately 46% of all deaths result from CHD (4).

ACS is an important type of CHD that is highly prevalent worldwide (5, 6). This condition refers to a wide spectrum of heart problems, such as slight chest discomfort, atypical electrocardiogram changes to ST-segment elevation, myocardial infarction, and cardiogenic shock (7). There have been several previous studies on the genetic determinants of ACS (8).

The renin-angiotensin system (RAS) is a regulatory cascade of blood pressure, cardiovascular remodeling, and vascular tone. The RAS is composed of a number of hormones and enzymes, including angiotensinogen, angiotensin-converting enzyme (ACE), angiotensin II (ATII), and various receptors (9). Recent reports have highlighted the potential importance of genetic polymorphisms associated with the RAS (10). Two subtypes of receptors, ATII type 1 receptor (AT1R) and ATII type 2 receptor (AT2R), have been identified as the main receptors of the RAS. Most of the actions of ATII are mediated by AT1,

which is expressed in the myocardium (11). Although several polymorphisms of the AT1R gene have been identified, one of the most widely studied is an A/C substitution at position 1166 (A/C1166). Sugimoto et al. showed a positive association between AT1R/A1166C and various cardiovascular features, such as aortic stiffness, left ventricular hypertrophy (LVH), increased carotid intima-media thickness, or atheromatous plaque formation (10). The AT1 receptor gene (AF245699) is located on chromosome 3 at q21-q25, and extends over a 55 kb segment (3). Previous studies performed by our group indicated that AT1R/A1166C polymorphism is associated with the presence of diabetes mellitus and metabolic syndrome in patients with documented coronary artery disease (12). We have also reported the association between AT1R/A1166C gene polymorphism and metabolic syndrome in a young female Iranian population (13).

However, there is limited data for Iranian populations on the genetic basis of ACS, especially in relation to the effects of AT1R polymorphisms.

2. Objectives

Therefore, in this study, we have investigated the possible association between AT1R gene polymorphisms and ACS in an Iranian population.

3. Patients and Methods

3.1. Study Population and Sample Collection

This cross-sectional study was conducted among 263 subjects (97 men and 166 women), comprising 128 patients with ACS and 135 control subjects without symptoms of heart disease, recruited from Ghaem hospital (Mashhad, Iran). ACS was confirmed by a cardiologist based on changes in troponin and CK-MB levels, as well as an electrocardiogram (ECG) analysis. The changes in ECG used to diagnose ACS included ST-segment elevation, ST-segment depression, or inverted T. The troponin and CK-MB levels were analyzed as previously described (14, 15). The age range for the population was between 30 and 80 years, and the control group was age-matched to the case group. The study was approved by the Medical Ethics Committee of Mashhad University of Medical Sciences, and written consent was obtained from all subjects for their participation in experimental procedures. Demographic data were collected via questionnaires. Patients taking angiotensin-converting enzyme inhibitors (ACEIs), angiotensin II receptor blockers (ARBs), or beta-1 blockers, as well as pregnant patients and those with a past diagnosis of ischemic heart-disease (IHD) or acute or chronic renal failure, were excluded from our study. Blood samples were collected from the patients and controls (blood donors from the same hospital) either in tubes containing citrate for hematological examinations, or in citrate-free tubes for biochemical evaluations. Serum and plasma samples were immediately separated by centrifugation at 3,000 rpm for 15 minutes at 4°C.

3.2. Anthropometric and Other Measurements

Anthropometric parameters, including weight, height, and body mass index (BMI) were measured, as previously described (16).

3.3. Biochemical Analysis

The patients fasted for 12 hours before lipid profile and fasting blood sugar measurements. Concentrations of low-density lipoprotein cholesterol (LDL-C), triglycerides, high-density lipoprotein cholesterol (HDL-C), and blood glucose were measured by enzymatic methods using a semi-automatic analyzer (TP Analyzer Plus®, Thermo-plate, China). Biochemical markers were measured with the standard methods.

3.4. Genetic Analysis

A commercial kit (Biogen, Iran) was used to extract DNA from the blood samples (13). Whole blood was collected from the study subjects, and genomic DNA was isolated from peripheral blood leukocytes. The A1166C genotypes of the AT1R gene were identified by PCR, followed by restriction enzyme digestion of the amplified product, as previously described (10, 12-14). The oligonucleotide primers used in the PCR reaction were 5′-GCACCATGTTTTGAGTTG-3′ as the forward and 5′-GACTACTGCTTAGCATA-3′ as the reverse, under conditions described elsewhere (17, 18). The PCR products were digested with the DdeI restriction enzyme (MBI Fermentas, USA). The digested products were separated by electrophoresis on a 1% (w/v) agarose gel and visualized directly under UV light (230 nm) after staining with ethidium bromide.

3.5. Statistical Analysis

Statistical analysis was performed using SPSS for Windows™ version 13.0 (SPSS Inc., Chicago, IL, USA). The data were expressed as mean $\pm$ SD for normally distributed variables, and as median and interquartile range for non-normally distributed variables. The statistical differences in polymorphic genotype frequencies and existence were assessed using Hardy–Weinberg equilibrium to perform the χ^2 test. To identify whether or not the distribution of data was normal, a one-sample Kolmogorov-Smirnov

analysis was performed. The demographic characteristics were compared using the independent sample t-test or the Mann-Whitney U test. To identify independent findings of ACS, a binary logistic regression analysis in the forward conditional method was performed. A two-sided P value of < 0.05 was considered statistically significant. The model included the presence of ACS as a dependent variable, while the presence or absence of polymorphic (AC/CC) genotypes, as well as HDL-C, LDL-C, triglycerides, gender, age, and BMI, were inserted as covariates. In addition, the interactions between each of the aforementioned parameters and the presence or absence of polymorphic (AA/CC) genotypes were inserted as covariates.

4. Results

4.1. Demographic Characteristics

Overall, the values for anthropometric indices, including weight, height, BMI, waist circumference, waist/hip ratio, and fat mass were higher in patients with ACS compared to the control group. There was no significant age difference between the patients (59.73 $\pm$ 9.8 years) and the controls (59.49 $\pm$ 13.4 years) (P = 0.18). There were also no significant differences between the ACS subjects and the control group with regard to LDL-C and HDL-C concentrations. Although the mean triglyceride level in the ACS subjects was higher than in the control group, this difference was not significant (P $>$ 0.05). Also, the BMI mean values were not significantly different between the case and control groups (P $>$ 0.05). The demographic characteristics of the study subjects are summarized in Table 1.

Table 1. Demographic Characteristics of Study Subjects[a,b]

Variable	ACS Group	Control Group	P-Value
Subjects (n)	128	135	
Age, y	59.7 $\pm$ 9.8	59.4 $\pm$ 13.4	0.18
Sex			
Female	43 (33.6)	54 (40)	0.17
Male	85 (66.4)	81 (60)	
Body mass index, kg/m^2	25 $\pm$ 8.2	24.8 $\pm$ 9.3	0.80
Fasting plasma glucose (mg/dL	106.22 $\pm$ 49.01	106.23 $\pm$ 48.69	0.9
LDL-C, mg/dL)	127.64 $\pm$ 43.77	109.11 $\pm$ 45.53	0.85
HDL-C, mg/dL	40.9 $\pm$ 14.8	48.67 $\pm$ 14.25	0.13
Triglycerides, mg/dL	134 $\pm$ 49	132 $\pm$ 50	0.88

Abbreviations: ACS, acute coronary syndrome; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.
[a]Values are expressed as mean $\pm$ SD.
[b]Comparisons between ACS and control groups were performed using independent sample t-test, Mann-Whitney test, or χ^2 test.

4.2. Association Between AT1R/A1166C Polymorphism and the Presence of ACS

DNA amplification by PCR resulted in a 540 bp DNA fragment. The PCR product was digested by the DdeI restriction endonuclease enzyme. Digestion fragments resulted in the production of 540 (AA), 430 (CC), or 540 and 430 (AC) bp fragments with identifiable patterns on agarose gel electrophoresis. Each of the samples revealed one of the three different electrophoretic patterns. The frequencies of the polymorphic genotypes (AC/CC) and the AA genotype, respectively, were 25 and 103 in the ACS subjects and 24 and 111 in the control group. The genotype frequencies are shown in Table 2.

The genotype distributions were consistent with Hardy-Weinberg equilibrium, both in the ACS subjects ($\chi^2 = 7.67$) and in the control subjects ($\chi^2 = 2.49$). The respective frequencies of genotypes, without being divided into polymorphic (AC/CC) and non-polymorphic (AA) groups, were 103, 20, and 5 for AA, AC, and CC in the ACS subjects and 111, 21, and 3 for AA, AC, and CC in the control subjects. These differences in frequencies were not significant (P = 0.85). On separate analyses for the two genders, the genotype frequencies were not significantly different (P $>$ 0.1 for women and P = 0.56 for men). Analyses were performed for two alleles (A and C) in both genders, and the results showed that no significant differences between the ACS and control group frequencies (P $>$ 0.1 for men and P $>$ 0.1 for women). The frequencies of genotypes and alleles are shown in Table 2.

According to the results of a binary logistic analysis, HDL-C (P $<$ 0.001), LDL-C (P $<$ 0.001), and triglycerides (P $<$ 0.001) were independent contributors to ACS.

5. Discussion

ATII is a vasoconstrictive peptide that can cause the growth and proliferation of fibroblastocytes. It is the most potent component of the RAS in vasoconstriction and sodium retention. The major activity of ATII is mediated by the AT1 receptor, and these effects are seen in the myocardium more than in any other tissue. The AT1 receptor is a peptide containing 360 amino acids and belonging to the G-protein-coupled receptor superfamily. When ATII binds to AT1 receptors in vascular smooth muscle cells, inositol 1, 4, 5-trisphosphate (IP3) and diacylglycerol are generated. IP3 releases calcium from the endoplasmic reticulum, which leads to the activation of certain enzymes, such as protein kinase C and calcium/calmodulin-activated kinases. These mechanisms finally result in

Table 2. Genotypes and Allele Frequencies in the ACS and Control Groups[a,b]

	ACS			Control			P Value
	AA	AC	CC	AA	AC	CC	
Genotype	103 (80.5)	20 (15.6)	5 (3.9)	111 (82.2)	21 (15.6)	3 (2.2)	0.85
Women	28 (65)	11 (25.5)	4 (9.5)	43 (79.5)	9 (16.5)	2 (4)	0.16
Men	75 (88)	9 (10.5)	1 (1.5)	68 (84)	12 (15)	1 (1)	0.56
			ACS		Control		P Value
Alleles	A		226 (88)		243 (90)		0.64
	C		30 (12)		27 (10)		
Women	A		67 (80)		95 (88)		0.16
	C		19 (20)		13 (12)		
Men	A		159 (93)		148 (91)		0.58
	C		11 (7)		14 (9)		

Abbreviation: ACS, acute coronary syndrome.
[a]Values are expressed as No. (%).
[b]Comparisons are performed using the χ^2 test. P-values refer to the comparisons between AA, AC, and CC genotypes, and between alleles.

smooth muscle contraction and cell proliferation in the artery (19-21). Other processes, including vascular hypertrophy, sodium retention, cardiac remodeling, hypertension, and fibrinogenesis, are mediated by activation of AT1 receptors (22-25). The A/C1166 polymorphism of AT1R is the most-studied polymorphism among all AT1R polymorphisms (26). Cardiovascular disorders, especially myocardial infarction and coronary artery disease, have shown inheritable features (27). Many studies have confirmed that the A/C1166 polymorphism of AT1R is a risk factor for cardiovascular disorders (28-30). However, at least one study involving 10,000 cases rejected this association (8). This controversy may be due to differences in study populations, reporting bias, and publishing bias (8, 31). Moreover, several studies have evaluated disease associations of the A/C1166 polymorphism of AT1R, but none evaluated the association between this polymorphism and ACS.

In a study of 1,254 patients with coronary catheterizations, different polymorphisms of angiotensinogen (G217A, G152A, A20C, G6A, M235T, and T174M) and the A/C1166 polymorphism of AT1R were studied with regard to CHD. The association of these polymorphisms with the incidence of CHD in the patient population was confirmed (32). The effects of ACEIs in relation to the AT1R A1166C polymorphism were evaluated, and the results showed that the polymorphism of AT1R A1166C does not predict the response to antihypertensive treatment with ACEIs (33). The association of the A1166C polymorphism with the AT1 receptor gene polymorphism in myocardial infarction patients was also investigated. It was found that the CC genotype of the receptor was not a risk factor for myocardial infarction in a South Indian population (19). However, to the best of our knowledge, no work similar to our study has been conducted in an Iranian population.

The present study was initiated to evaluate whether the AT1R A1166C gene polymorphism is associated with ACS in an Iranian population. The study was carried out with a total of 263 subjects, comprising 128 patients and 135 controls.

Our results showed that the frequencies of genotypes AA, AC, and CC were not significantly different in the ACS and control groups even when determined for separate genders. HDL-C, LDL-C, and triglyceride concentrations were independent contributors to ACS. In addition, the frequencies of alleles were not different in ACS subjects and the control group.

There have been no studies investigating the association between the AT1R A1166C polymorphism and the presence of ACS. We found no relationship between the AT1R polymorphism and ACS in our population. The participants in this study were patients with documented ACS, in whom the co-existence of other complications, such as renal failure, could have led to changes in results. In addition, other factors, such as triglycerides, LDL-C, and HDL-C, which might serve as contributors to the development of ACS, were evaluated with a binary logistic regression analysis. One of the limitations of our study was the fact that we looked specifically at patients with ACS, and therefore it may not be possible to generalize these results to the whole population. Another limitation of our study was that all ACS patients (with different stages of disease, such as ST-segment elevation myocardial infarction, non-

ST-segment elevation myocardial infarction, and inverted-T myocardial infarction) were considered as one group. Division of ACS subjects into more subgroups, with greater numbers of patients, could increase the accuracy of the results and the value of the study. Finally, since ACS is a polygenic disease with many predisposing factors, several polymorphisms and several SNPs are probably necessary to be assessed by linkage studies and haplotype analyses, in order to identify genes might play a significant role in the development of ACS.

5.1. Conclusion

To our knowledge, the present study is the first to research the association between a single-nucleotide polymorphism in the AT1R gene and ACS in an Iranian population. The AT1R A/C1166 single nucleotide polymorphism does not appear to be associated with the presence of ACS in the population studied. Further studies with a larger number of patients and controls would shed more light on this issue. There were some limitations to this study. This cross-sectional study was conducted among 263 subjects in two groups that did not differ significantly for gender and age, but the gender ratio was not representative of the population as a whole. Further research is needed to confirm our findings in larger multicenter and multiethnic trials, and to investigate the diagnostic utility of the AT1R A/C1166 allele as a conservation factor for recognizing patients who are more resistant to the expansion of ACS.

Footnotes

Authors' Contribution: Navid Delshad and Majid Ghayour-Mobarhan equally contributed in this study. Navid Delshad, Majid Ghayour-Mobarhan, Kamal Razavi-Azarkhiavi, Javad Behravan, design of the study, data collection, and literature review; Mohsen Moohebati, Hamed Mirzaei, Mitra Hassany, Jamal Kasaian, Mohammad Reza Etemadzadeh, Maryam Sadat Alavi, Javad Behravan, review of the manuscript and editing.

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