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Original Article

The relationship between acute coronary artery diseases with c-reactive protein +1059 G/C and angiotensin-converting enzyme I/D gene polymorphisms

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Abstract: Objective: The purpose of this study was to evaluate the presence of an association between the CRP +1059 G/C and ACE I/D gene polymorphisms and patients who were diagnosed to have acute coronary syndrome and underwent coronary angiography. Methods: A total of 126 patients (mean age: 60.0±12.9) and 144 healthy individuals (mean age: 52.1±13.0) were included to this study. The presence of CRP +1059 G/C and ACE I/D gene polymorphisms were analyzed using the RFLP method. Results: When the patient and control groups were evaluated in terms of ACE I/D gene polymorphism, no statistically significant difference was found in the frequency of ACE DD and ACE ID between the two groups (*P>0.05*), while the percentage of ACE II genotype was statistically significantly higher in the patient group compared with the control group (*P*<0.032). For the distribution of CRP G/C genotype; CRP GG, CRP GC and CRP CC genotype frequencies were similar in the patient and control groups (*P>0.05*). When the presence of the ACE I/D genotype and CRP G/C genotype was compared in patients with vessel disease (one vessel, two vessels and three vessels) among the patients with coronary artery diseases with the control group, statistically significant differences were found between the two groups (*P<0.05*). In addition, the frequency of the ACE I/D genotype in hypertensive patients with coronary artery disease was statistically significantly higher (*P<0.033*). Also, the frequency of the CRP +1059 G/C genotype was found to be statistically significantly higher in the patient group (*P<0.026*). Conclusion: This study demonstrated that CRP +1059 G/C and ACE I/D gene polymorphisms may be a genetic marker associated with coronary artery disease in patients diagnosed with ACS.

Keywords: Polymorphism, ACE I/D, CRP +1059, frequency, RFLP

Introduction

As with the rest of the world, cardiovascular diseases are the most common cause of death in Turkey. The clinical picture in Acute Coronary Syndrome (ACS) is associated with the extensiveness and severity of the myocardial ischemia that can be encountered in the form of unstable angina, myocardial infarction (MI) without ST elevation and MI with ST elevation [1]. ACS is a progressive, systemic and multifactorial (genetic, environmental factors and inflammatory) disease, in which atherosclerotic plaque plays a primary role [2]. C-reactive protein (CRP) is an inflammatory factor, extensively used in the detection of systemic inflammation related diseases and recent studies associated with genetics, and genomics have established that changes in the CRP levels may be affected by genetic factors [3-6]. Also, contradictory studies performed in this field have suggested that various CRP polymorphisms may be a risk factor for acute coronary diseases [3-6].

One of the enzymes playing a major role in coronary artery disease is angiotensin-converting

Table 1. Demographic data of the individuals in the patient and control groups

	ACS (n=126)	Controls n=144)	*P
Meanage, years	60.0±12.9	52.1±13.0	
Male sex n (%)	90 (71.4%)	79 (54.9%)	*0.006*
Female n (%)	36 (28.6%)	65 (45.1%)	
CRP, n>500	106 (99.1%)	0	*0.000*
CRP, n<500	1 (09.0%)	0	
Troponin, n>0.20	92 (76.7%)	0	*0.000*
Troponin, n<0.20	28 (23.3%)	0	
DM, n (%)	39 (31.0%)	0	*0.000*
Hypertension, N (%)	40 (31.7%)	0	*0.000*
Smoking, n (%)	69 (55.2%)	66 (45.8%)	*0.143*
HPL	10 (7.9%)	0	*0.000*
ACE I/D polymorphisms			
D/D	47 (37.3)	44 (30.6)	*0.242*
I/D	67 (53.6)	80 (55.6)	*0.748*
I/I	31 (25.0)	21 (14.6)	*0.032*
CRP +1059 G/C polymorphisms			
G/G	87 (69.0)	88 (61.1)	*0.173*
G/C	16 (12.7)	12 (8.3)	*0.241*
C/C	5 (4.0)	1 (0.7)	*0.101*

ACE I/D: Angiotensinconvertingenzyme, Insertion/Deletion. CRP: C-reactive protein. DM: Diabetes mellitus. *Chi-square test.

enzyme (ACE). ACE is synthesized by vascular endothelial cells and expressed in the plasma membrane as class 1 integral ectoenzyme [7]. There are still many uncertainties about the ACE gene expression, which has been suggested to be tissue-specific [8]. About 80 polymorphisms of the ACE gene have been detected [9], and the most known of these is the ACE insertion/deletion (I/D) polymorphism. This gene is localized on the 17th chromosome and a polymorphism is developed when a part of it, including the 287 base pair at the intron 16 of the gene, is repeated [10]. ACE genotypes and various vessel diseases have been suggested to have a significant association, while it is still debatable whether ACE gene polymorphisms affect various cardiovascular pathologies [11]. Among the studies performed, there are some reporting that ACE gene polymorphisms are related with myocardial infarcts and coronary artery diseases [12, 13], while others have demonstrated no association between ACE gene polymorphism and cardiovascular diseases [14, 15]. Thus, controversial results have been reported in the literature about the presence of a significant association between ACE genotypes and coronary artery diseases [16, 17].

In this study our aim was to evaluate the presence of an association between coronary artery disease and both CRP +1059 G/C and ACE I/D polymorphisms in patients who had presented to the emergency service with chest pain and were diagnosed to have acute coronary syndrome.

Material and methods

Collection and storage of blood samples

In this study, 125 patients who had presented to the emergency services between June 2013 and October 2013 with chest pain and were diagnosed as ACS [18] after an anamnesis, biochemical tests, ECG (electrocardiography) and ECHO (echocardiography) and had undergone coronary angiography, and a group of 144 individuals (healthy control group), randomly selected from the population living in the same geographical area and who had undergone ECG, ECHO and biochemical tests and who had no overt symptoms of coronary artery disease, were included.

The angiographic presence of ≥20% stenosis in lumen diameter in any of the epicardial coronary arteries in patients who underwent coronary angiography was accepted as coronary artery disease [19]. In addition, the disease was classified as one, two or three vessel disease according to the number of vessels involved (LAD; Left Anterior Descending, LCX; left circumflex artery and RCA; Right Coronary Artery).

Blood samples were obtained from patients and controls and were stored at -20°C until the targeted number of patients was reached. Approval from the local ethics board was obtained for this study. Demographic data of the individuals included in the study, such as age, gender, and cigarette smoking is demonstrated in **Table 1**.

Genomic DNA extraction

For genomic DNA isolation, 5 ml blood samples were collected in tubes with EDTA from the sub-

jects of both groups. Qiamp DNA Blood Midi Kit (Qiagen, France) was used for DNA isolation. Isolation was conducted by following the manufacturer's test procedure. The genomic DNA obtained by alcohol precipitation at the last step was dissolved in the 1xTE tamponade solution (10 mM Tris HCl, 0.1 mM EDTA, pH 8.0) and was stored at -20°C.

Detection of genomic DNA

A sample of 10 µl was taken from the isolated DNA samples and was subjected to gel electrophoresis in 1% agarose gel and stained with ethidium bromide. The concentration of the isolated DNA was determined by reading the absorbance at 260 wavelengths in the spectrophotometer. 20 µl was taken from the stock DNA samples and 980 µl of distilled water was added and the absorbance was measured in the spectrophotometer. Also, protein contamination was tested by measuring at 280 nm wavelength.

PCR amplification for CRP +1059 G/C gene

In this study, the following primer sequence was used and the target regions were amplified by PCR [20].

Primer sequence: CRP +1059 Forward 5'-GATCTGTGTGATCTGAGAAACCTCT-3'; CRP +1059 Reverse-5'GAGGTACCAGAGACAGAGACGTG-3'.

PCR reaction was conducted to give a total volume of 50 µl, including 5 µl genomic DNA, 0.5 U Taq polymerase (fermentas) in 10xPCR buffer, 200 µmol deoxynucleoside triphosphates (dNTP), 2 mM $MgCl_2$ and 20 pmol of each primer. PCR cycles were performed as follows: The first denaturation was performed at 94°C for five minutes, then at 95°C for 30 seconds, annealing was performed at 60°C for 45 seconds and extension was performed at 72°C for 45 seconds. PCR cycles were arranged so as to be 30 cycles in total. The cutting products obtained were projected to agarose gel electrophoresis including 3% 0.5 µg/ml ethidium bromide at gel imaging equipment (Wealtec, Dolphin-View, USA).

Determination of CRP +1059 G/C gene polymorphism

The +1059 G/C CRP polymorphism was determined, based on the method defined by Cao et al. [21]. PCR reaction was performed in a total volume of 50 µl. 200 ng genomic DNA and 25 pmol of each primer were used: 2.0 mM $MgCl_2$, 175 µM each dNTP, 1.5 U TaqDNA polymerase enzyme and PCR buffer (Roche, Germany). DNA amplification was performed in the following amplification cycles: Thirty-five cycles of denaturation at 95°C for 30 seconds, annealing at 69°C for 30 seconds and extension at 72°C for 30 seconds were performed. The first denaturation of the reaction and the last extension were arranged to be at 95°C for three minutes and at 72°C for 10 minutes, respectively. To detect the +1059 G/C gene polymorphisms, 5 µl of 744 bp PCR was taken and cut with 3 U Mae III (Roche) enzyme at 55°C. As a result of the cut, the two fragments of the rarely seen 1059 C allele developed two bands of 434 and 310 bp, while the three fragments of the more frequently seen 1059G allele were seen to form bands. The reaction products were subjected to 2% agarose gel electrophoresis and were stained with ethidium bromide and analyzed.

PCR amplification for ACE I/D gene

The genomic DNA extraction was performed according to the kit procedure (Qiagen, Germany). The isolated DNA samples were stored at -20°C until they were studied. Field specific primers were used, in order to evaluate insertion and deletion polymorphisms at the 287 bp. region localized at 16th intron.

The following primers were selected for the PCR procedure ACE I/D Forward 5' CTG GAG ACCACT CCCATC CTT TCT 3'; ACE I/D Reverse 5' GAT GTG GCC ATC ACATTC GTC AGAT 3'.

The total volume of PCR reaction was performed as 50 µl, which was composed of 10 pmol of each primer, 3 mM $MgCl_2$, 50 mM KCl, 10 m MTris-HCl, pH 8.4, 0.1 mg/ml gelatin, 0.5 mM of each dNTP, 2.5 U Taq DNA polymerase (fermentas) and 2 µl of genomic DNA. The PCR cycle was performed at the following temperatures: Denaturation was performed for one minute at 94°C, annealing for one minute at 60°C and extension for one minute at 72°C for a total 30 cycles. In addition, the first denaturation was performed at 94°C for four minutes and the last extension at 72°C for seven minutes, using a thermal cycler.

Determination of CRP +1059 G/C gene polymorphism

RFLP analysis was performed, following the method described by Sundarrajan et al., in

Table 2. Distributions of genotypes according to genders and smoking

Characteristics	Genotype D/D, n (%)	Genotype I/D, n (%)	Genotype I/I, n (%)	Genotype G/G, n (%)	Genotype G/C, n (%)	Genotype C/C, n (%)
Male, n (%)	60 (35.5%)	87 (51.8%)	36 (21.6%)	107 (63.3%)	19 (11.2%)	5 (3.0%)
Female, n (%)	31 (30.7%)	60 (59.4%)	16 (15.8%)	68 (67.3%)	9 (8.9%)	1 (1.0%)
Male, meanage ± SD	56.4±12.6	56.0±13.1	56.4±11.9	55.8±12.9	58.5±14.0	59.0±16.7
Female, meanage ± SD	59.1±15.7	58.7±14.5	54.4±14.1	56.2±15.6	53.7±15.6	52.0
**P values*	0.418	0.224	0.252	0.504	0.543	0.416
Smoking, n (%)	49 (36.3%)	76 (56.3%)	26 (19.4%)	83 (61.5%)	17 (12.6%)	3 (2.2%)
No-smoking, n (%)	42 (31.3%)	71 (53.4%)	25 (18.8%)	91 (67.9%)	11 (80.2%)	3 (2.2%)
**P values*	0.391	0.632	0.900	0.270	0.239	0.993

*Chi-square test.

Table 3. Distribution of ACE I/D and CRP G/C polymorphisms in the patient and control groups

	ACE I/D polymorphisms			CRP G/C polymorphisms		
	Genotype D/D	Genotype I/D	Genotype I/I	Genotype G/G	Genotype G/C	Genotype C/C
Patients, n (%)	47 (37.3%)	67 (53.6%)	31 (25.0%)	87 (69.0%)	16 (12.7%)	5 (4.0%)
Control, n (%)	44 (30.6%)	80 (55.6%)	21 (14.6%)	88 (61.1%)	12 (8.3%)	1 (0.7%)
**P values*	*0.242*	*0.748*	*0.032*	*0.173*	*0.241*	*0.101*

ACE I/D: Angiotensin converting enzyme, Insertion/Deletion. CRP G/C: C-Reactive protein Guanine/Cytosine.

order to demonstrate the presence of polymorphism. The presence of 190 bp PCR was evaluated as the presence of D allele, and 477 bp PCR product was evaluated as presence of I allele. Both band profiles (477 and 190 bp) were encountered in the heterozygote samples [22].

Imaging amplification products

Approximately 8 µl was taken from the amplification product and mixed with 2 µl loading buffer (50% glycerol, 0.1 M EDTA, 0.1% bromophenol blue and Xylene cyanol). The 1.5% agarose gel was prepared in the TBE tamponade (0.089 M Tris, 0,089 M Boric Acid and 0.011 M EDTA, pH: 8.3): Agarose gel was prepared using 10 X TBE. The 0.5 µg/ml ethidium bromide, included in this solution, was prepared.

Statistical analysis

The statistical analysis was carried out using Statistical Package for Social Sciences (*SPSS*, ver. 17.5, Chicago, USA) software. *P* value less than 0.05 was considered statistically significant. Chi-square and Fisher's exact tests were used to compare categorical variables between the groups, while numerical variables were compared with Student's t-test after checking for normal distribution.

Results

The number of male and female patients included in the study was 90 (71.4%) (57.0±11.8) and 36 (28.6%) (67.6±12.1), respectively (*P<0.001*). Thirty-nine patients (31.0%) had DM (*Diabetes mellitus*), 40 (31.7%) had HT, and 10 (7.9%) had HPL. Sixty-nine patients (54.8%) were cigarette smokers. Hs-CRP (high sensitivity CRP) levels were >0.500 mg/L in 106 (84.1%) patients, and was less than this level in one patient. The troponin level was <0.02 mg/L in 28 (22.2%) patients and was >0.02 mg/L in 92 (73.0%) patients. Among the 126 patients who underwent coronary angiography, eight (6.3%) of them had no coronary artery disease. One vessel, two vessels and three vessels disease were present in 36 (28.6%), 45 (35.7%) and 37 (29.4%) patients, respectively. In the control group, 79 of the volunteers were male (54.9%) (52.3±12.6) and 65 (45.1%) were female (51.8±13.4) (*P=0.808*). None of the individuals in the healthy control group had DM, HT or HPL. Troponin and CRP levels were within the normal ranges. The number of cigarette smokers was 66 (52.4%).

No statistically significant difference was found in terms of gene polymorphisms, depending on cigarette smoking and gender, in the whole series. No statistically significant difference

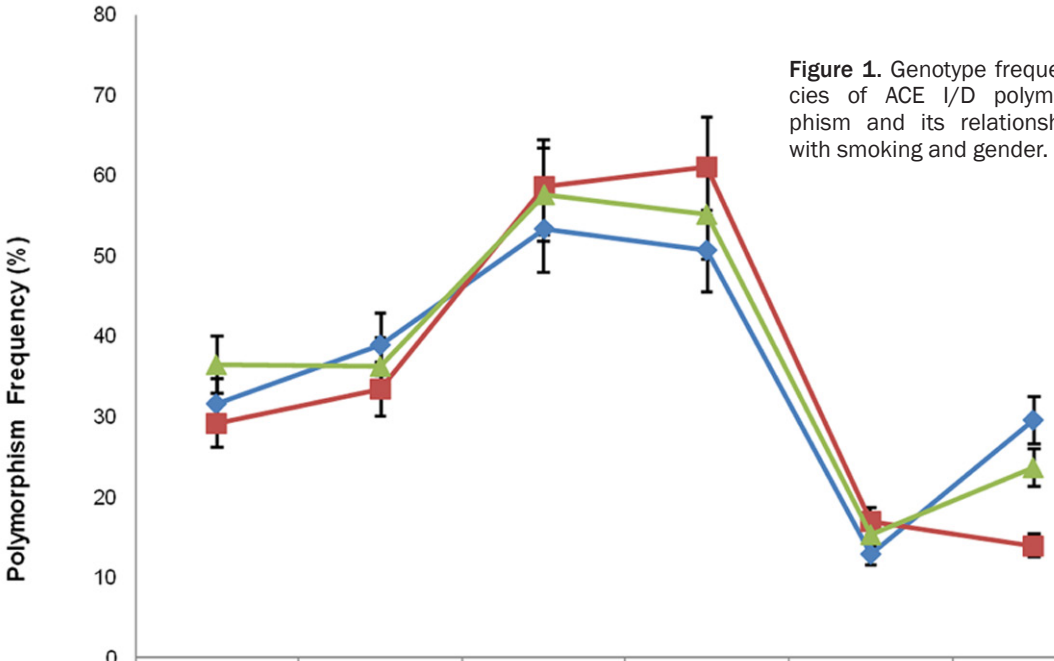

Figure 1. Genotype frequencies of ACE I/D polymorphism and its relationship with smoking and gender.

	Control group	Patient group	Control group	Patient group	Control group	Patient group
	D/D Genotype		I/D Genotype		I/I Genotype	
Men	31,6	38,9	53,2	50,6	12,7	29,5
Women	29	33,3	58,5	61,1	16,9	13,9
Smoking	36,4	36,2	57,6	55,1	15,2	23,5

	Control-Patient Genotype D/D		Control-Patient Genotype I/D		Control-Patient Genotype I/I	
Male	25/79 (31.6%)	35/90 (38.9%)	42/79 (53.2%)	45/89 (50.6%)	10/79 (12.7%)	26/90 (28.9%)
Female	19/65 (29.%)	12/36 (33.3%)	38/65 (58.5%)	22/36 (61.1%)	11/65 (16.9%)	5/36 (13.9%)
P values	0.754	0.560	0.524	0.284	0.471	0.068
Smoking	24/66 (36.4%)	25/69 (36.2%)	38/66 (57.6%)	38/69 (55.1%)	10/66 (15.2%)	16/68 (23.5%)
P values	0.164	0.726	0.654	0.795	0.859	0.805

was found in the genotype distribution for both genes (**Table 2**).

Genotype distribution of the ACE I/D and CRP +1059 G/C polymorphisms in the control and patient groups are demonstrated in **Table 3**. The occurrence rates of ACE DD, ID and II genotypes in the patient group were 47 (37.3%), 67 (53.6%) and 31 (25.0%), respectively. In the control group, on the other hand, ACE DD, ID and II genotypes were detected in 44 (30.6%), 80 (55.6%) and 21 (14.6%) cases, respectively.

CRP +1059 GG, GC and CC genotypes in the patient group were detected in 87 (69.0%), 16 (12.7%) and five (4.0%) cases, while the respective genotypes were seen in 88 (61.1%), 12 (8.3%) and one (0.7%) cases, respectively.

The presence of genotypes, CRP +1059 GG, GC and CC and ACE, DD and ID were statistically similar in both patient and control groups, while the presence of the ACE II genotype was statistically significantly higher in the patient group compared with the control group (*P=0.032*).

No statistically significant differences were observed between the groups and in-group comparisons of gene polymorphisms of ACE D/I and CRP G/C distribution by gender and smoking (**Figures 1** and **2**).

In the in-group comparisons, male or female gender, smoking, troponin level >0.02, hypertension, diabetes and presence of HPL were found to be statistically not significant for ACE D/I and CRP G/C gene polymorphisms. Distribution of ACE DD, ID and II genotypes in

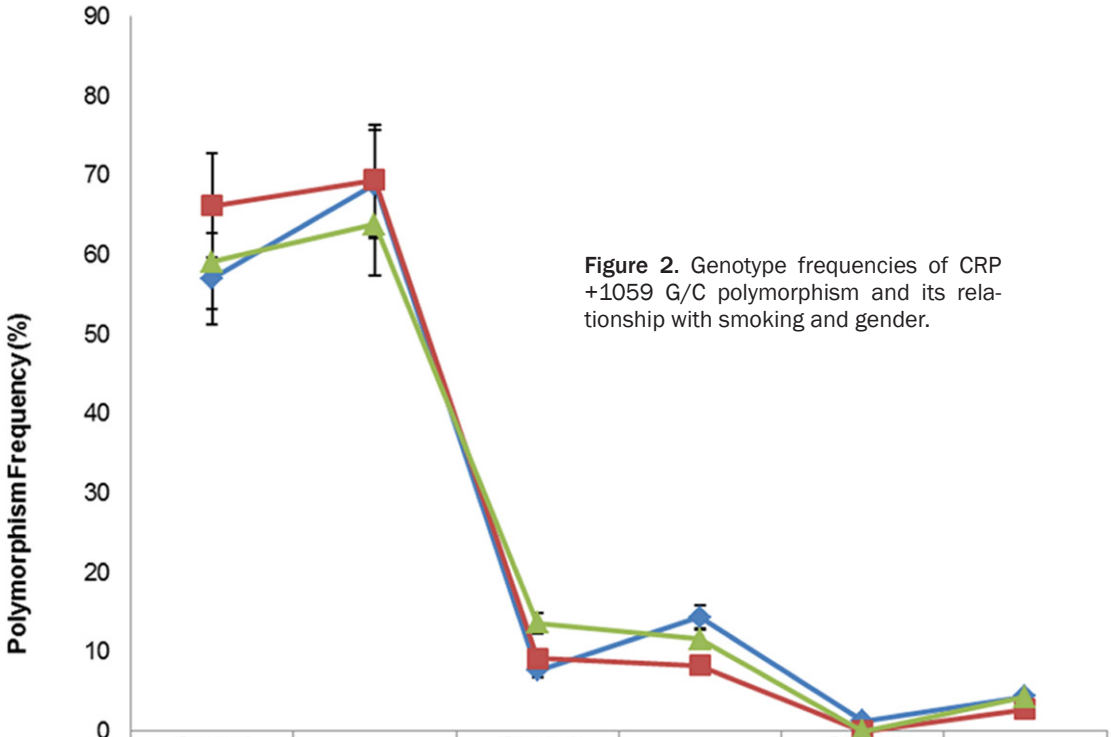

Figure 2. Genotype frequencies of CRP +1059 G/C polymorphism and its relationship with smoking and gender.

	Control - Patient G/G genotype		Control - Patient G/C genotype		Control - Patient C/C genotype	
Male	45/79 (57.0%)	62/90 (68.9%)	6/79 (7.6%)	13/90 (14.4%)	1/79 (1.3%)	4/90 (4.4%)
Female	43/65 (66.2%)	25/36 (69.4%)	6/65 (9.2%)	3/36 (8.3%)	0/65 (0.0%)	1/36 (2.8%)
P values	0.260	0.951	0.724	0.352	0.363	0.665
Smoking	39/66 (59.1%)	44/69 (63.8%)	9/66 (13.6%)	8/69 (11.6%)	0/66 (0.0%)	3/69 (4.3%)
P values	0.647	0.178	0.66/0,34	0.654	0.356	0.826

patients with hypertension was found to be 42.5%, 67.5% and 23.1%, respectively, while distribution of CRP +1059 GG, GC and CC were 82.5%, 17.5% and 2.5%, respectively. The frequency of ACE DD and II genotypes and CRP +1059 GC and CC genotypes were similar in patients who had or did not have hypertension, while the percentage of ACE ID (*P<0.033*) and CRP +1059 GC (*P<0.026*) genotypes was statistically significantly higher in patients who had hypertension (**Table 4**).

In-group comparisons in the control group demonstrated that gender, age and smoking had no significant influence on genotype distribution (**Table 5**).

ACE D/I and CRP +1059 G/C gene polymorphisms were statistically significantly higher in patients with vessel coronary artery disease (**Figures 3** and **4**).

ACE D/I genotype distribution is demonstrated in vessel disease in **Figure 3**. In the study, ACE DD, ID and II genotypes in 36 patients with one vessel disease was found to be present in 10.6%, 25.4% and 3.2%, respectively. The rate of ACE DD, ID and II genotypes in 45 patients with two vessel disease was 44.7%, 35.8% and 41.9%, respectively. The respective rates of the genotypes in 37 patients with three vessel disease were 40.4%, 35.8% and 51.6%, respectively (**Figure 3**).

Table 4. Comparison of the distributions of CRP +1059 G/C and ACE I/D genotypes in the patient group

Parameters	D/D Genotype	I/D Genotype	I/I Genotype	G/G Genotype	G/C Genotype	C/C Genotype
Male, n (%)	35 (38.9%)	45 (50.6%)	26 (29.5%)	62 (68.9%)	13 (14.4%)	4 (4.4%)
Female, n (%)	12 (33.3%)	22 (61.1%)	5 (13.9%)	25 (69.4%)	3 (8.3%)	1 (2.8%)
Mean age of males Mean ± SD	59.6±10.3	60.1±11.7	59.1±10.3	57.9±12.6	61.2±11.8	62.8±16.6
Mean age of females Mean ± SD	71.5±10.7	69.3±11.3	66.4±12.5	68.4±12.8	70.7±6.3	52.0±0
**P value*	0.560	0.284	0.068	0.951	0.352	0.665
Troponin >0.20, n (%)	33 (35.9%)	54 (58.7%)	19 (21.1%)	67 (72.8%)	13 (14.1%)	5 (5.4%)
Troponin <0.20, n (%)	10 (35.7%)	12 (44.4%)	7 (25.0%)	18 (64.3%)	2 (7.1%)	0
**P value*	0.988	0.190	0.665	0.384	0.516	0.589
DM n (%)	15 (38.5%)	23 (59.0%)	8 (21.1%)	28 (71.8%)	4 (10.3%)	0
P value	0.857	0.417	0.500	0.655	0.581	0.127
HT, n (%)	17 (42.5%)	27 (67.5%)	9 (23.1%)	33 (82.5%)	7 (17.5%)	1 (2.5%)
**P value*	0.411	0.033	0.738	0.026	0.270	0.565
Smoking, n (%)	25 (36.2%)	38 (55.1%)	16 (23.5%)	44 (63.8%)	8 (11.6%)	3 (4.3%)
P value	0.726	0.795	0.805	0.178	0.654	0.826
HPL, n (%)	4 (40.0%)	4 (44.4%)	2 (20.0%)	9 (90.0%)	2 (20.0%)	0
**P value*	0.854	0.568	0.703	0.135	0.470	0.503

DM: Diabetes mellitus. HT: Hypertension. HPL: Hyperlipidemia. *Chi-square test.

When the association of vessel disease and CRP +1059 and G/C genotype distribution was evaluated in the study, CRP +1059 GG and GC genotype distribution was 23.0% and 12.5%, respectively in 36 patients with one vessel disease, while no CC genotype was encountered. The rate of CRP +1059 GG, GC and CC genotypes were 36.8%, 18.8% and 20.0%, respectively in 45 patients who had two vessels disease. The respective rates of the genotypes were 36.8%, 68.8% and 80.0%, respectively in 37 patients who had three vessels disease (**Figure 4**).

Discussion

Angiotensin converting enzyme is an important enzyme in the renin-angiotensin-aldosterone system. Currently, ACE inhibitors are quite commonly used in the treatment [8-10]. It has been suggested that ACE gene polymorphisms could be associated with various pathological conditions in many diseases [23-25]. There are many studies reporting the effect of insertion/deletion (I/D) gene polymorphism (II, ID, DD) of ACE on the risk of occurrence, severity, course and response to treatment in hypertension, heart failure, myocardial infarct, diabetes, diabetic nephropathy and cancer [15]. In this study, we evaluated the association of ACE gene polymorphisms, and specifically one or more vessel diseases, in individuals who have been diagnosed as ACS and undergone coronary angiography. A possible association between coronary artery disease and D allele has been reported in previous studies conducted in Turkey [15]. In another study, a significant difference was reported in the mortality of individuals with DD genotype, in decreased left ventricle systolic performance, progressive left ventricle dilation and heart failure, compared with individuals with ACE II genotype [26, 27]. In this present study, no statistically significant difference was found in the rates of gene polymorphisms of ACE DD and DI, between the patient group with the diagnosis of ACS and healthy controls, while rate of ACE II polymorphism was found to be significantly high in the patient group (*P<0.032*).

CRP is an acute phase reactant, released in both acute and chronic inflammation, and it rises rapidly with infection, tissue damage or tissue inflammation. A concentration of C-reactive protein is a hereditary condition and predictive in cardiovascular diseases [27]. Chronic inflammation has been reported to play a role in the development of cardiovascular diseases and atherosclerosis [28]. Although high sensitivity CRP levels have been reported to play a role in many diseases, studies reporting its

Table 5. Comparison of CRP +1059 G/C and ACE I/D genotypes distribution in the control group group

Parameter	D/D Genotype	I/D Genotype	I/I Genotype	G/G Genotype	G/C Genotype	C/C Genotype
Male, n (%)	25 (31.6%)	42 (53.2%)	10 (12.7%)	45 (57.0%)	6 (7.6%)	1 (1.3%)
Female, n (%)	19 (29.2%)	38 (58.5%)	11 (16.9%)	43 (66.2%)	6 (9.2%)	0
Mean age of males Mean ± SD	52.0±11.3	51.6±13.1	49.5±13.3	52.9±12.7	52.7±17.6	44.0
Mean age of females Mean ± SD	51.3±13.2	52.5±12.4	49.0±12.0	49.1±12.4	45.2±10.5	
**P value*	0.754	0.524	0.471	0.260	0.724	0.363
Smoking, n (%)	24 (36.4%)	38 (57.6%)	10 (15.2%)	39 (59.1%)	9 (13.6%)	0
**P value*	0.164	0.654	0.859	0.647	0.34	0.356

*Chi-square test.

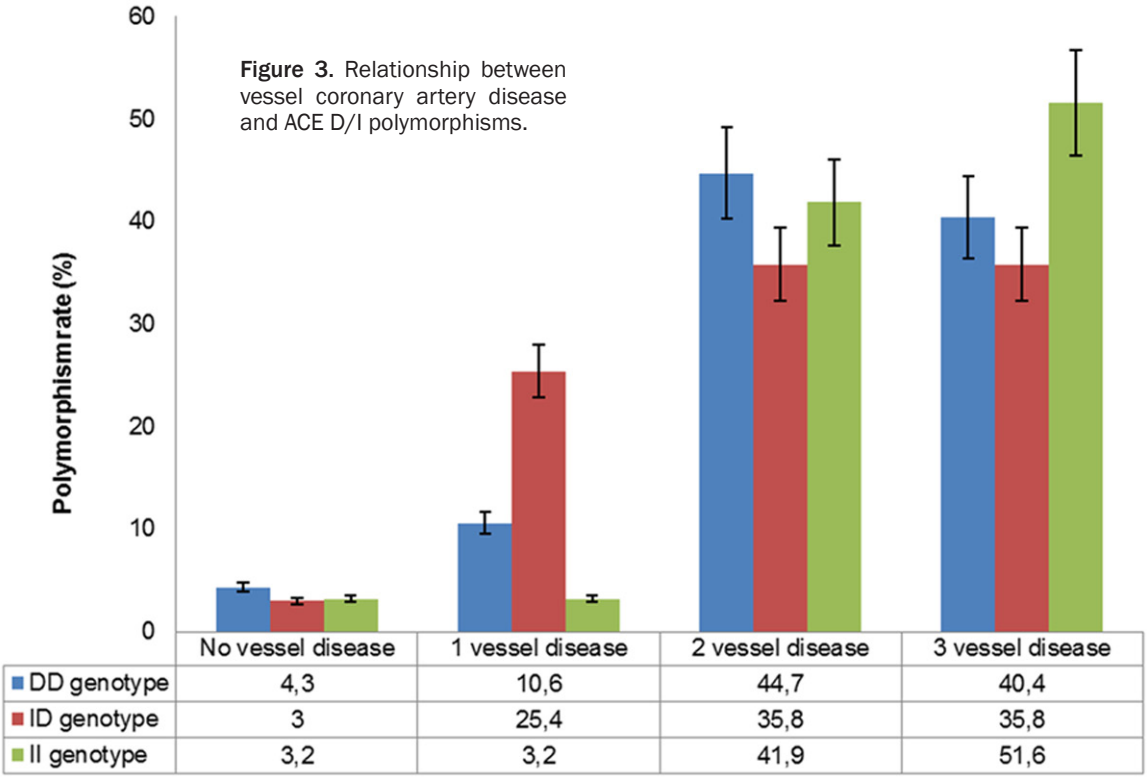

Figure 3. Relationship between vessel coronary artery disease and ACE D/I polymorphisms.

	0 Vessel n=8	1 Vessel n=36	2 Vessels n=45	3 Vessels n=37	*P values*	Total (126)
Genotype DD	2 (4.3%)	5 (10.6%)	21 (44.7%)	19 (40.4%)	0.002	47 (100.0%)
Genotype ID	2 (3.0%)	17 (25.4%)	24 (35.8%)	24 (35.8%)	0.022	67 (100.0%)
Genotype II	1 (3.2%)	1 (3.2%)	13 (41.9%)	16 (51.6%)	<0.001	31 (100.0%)

association with cardiovascular diseases are controversial, and such studies undertaken in Turkey are in a very limited number [29-31].

CRP +1059 gene polymorphism is a silent polymorphism that has been reported to play a possible role in the development of coronary artery disease, by affecting the amino acid levels and thus CRP protein levels [32]. Unlike the other studies in the literature, we performed this study to demonstrate the presence of an association between CRP +1059 GC gene polymorphisms and coronary artery disease in patients who were diagnosed with ACS and had undergone coronary angiography. No statistically significant difference was found in the CRP +1059 GG, GC and CC gene polymorphism in patients who were diagnosed as having ACS and had

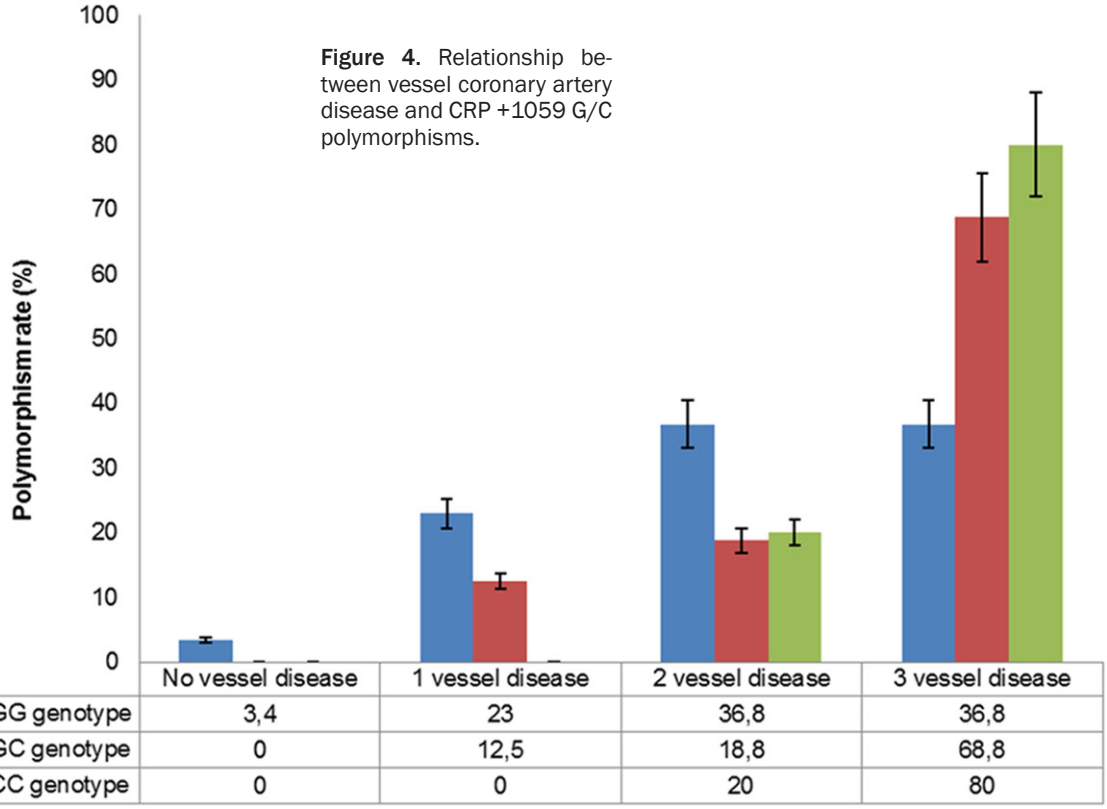

Figure 4. Relationship between vessel coronary artery disease and CRP +1059 G/C polymorphisms.

	0 Vessel n=8	1 Vessel n=36	2 Vessels n=45	3 Vessels n=37	*P values*	Total (126)
Genotype GG	3 (3.4%)	20 (23.0%)	32 (36.8%)	32 (36.8%)	0.001	87 (100.0%)
Genotype GC	0 (0.0%)	2 (12.5%)	3 (18.8%)	11 (68.8%)	0.001	16 (100.0%)
Genotype CC	0 (0.0%)	0 (0.0%)	1 (20.0%)	4 (80.0%)	0.021	5 (100.0%)

undergone coronary angiography, compared with healthy controls; however, the CRP +1059 GG genotype was found to be significantly high in hypertensive patients (82.5%) (*P<0.026*). In addition, ACE DI gene polymorphism was also found to be significantly high in the same patient group (67.5%) (*P<0.033*), (**Table 4**).

Also, the rates of the ACE I/D genotype and CRP +1059 G/C genotype was found to be statistically significantly higher in patients with vessel disease and coronary artery disease (individuals with one vessel, two vessels and three vessels disease) compared with individuals with no vessel disease (*P<0.05*), (**Figures 3** and **4**).

Study limitations

There were some shortcomings in our work. The number of patients was low, due to economic difficulties. Also, the number of the patients with vessel disease and coronary artery disease (individuals with one vessel, two vessels and three vessels disease) was low.

Conclusion

In conclusion, the findings of this study suggest that CRP +1059 GC and ACE DI gene polymorphisms might be a genetic marker associated with vessel disease. CRP +1059 GC and ACE DI gene polymorphisms might also be considered as a risk factor in cardiovascular diseases.

Disclosure of conflict of interest

None.

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