

## Original Article

# Association of APJ G212A gene polymorphisms with plasma APJ levels and coronary artery disease risk in Turkish population

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**Abstract:** Apelin and apelin receptor (APJ) are required for normal cardiovascular development. To investigate the relationship between the APJ G212A polymorphisms and plasma APJ levels and the risk of coronary artery disease (CAD), allele and genotype frequencies of the APJ G212A gene were analysed in 152 Turkish patients with CAD and 110 controls by the PCR-RFLP method. The frequencies of genotype GG (57.2% vs 18.2%,  $P = 0.000$ , OR = 0.21, 95% CI 0.1 to 0.44) and frequencies of allele G (70.1% vs 46.8%,  $P = 0.000$ , OR = 2.65, 95% CI 1.84 to 3.83) at APJ G212A gene polymorphisms were significantly increased in CAD compared with controls. Although plasma APJ level of the patients with GG genotype was lower, it was not statistically significant. CAD patients had significantly lower plasma APJ levels as compared with controls ( $P = 0.018$ ). On the basis of our results, patients with CAD seem to have lower APJ levels and higher APJ-212 GG genotype and G allele. These results suggested that the APJ G212A gene may be associated with risk for CAD and the APJ G212A gene may play a role in CAD in the Turkish population.

**Keywords:** APJ G212A gene, coronary artery disease, polymorphism, Turkish population

## Introduction

The apelin receptor (APJ) is a G protein-coupled receptor [1] which binds apelin [2, 3]. The APJ gene is located on chromosome 11 (11q12) [1] and its mRNA has been detected in vascular endothelial cells, central nervous system, myocardium, as well as other organs (lungs, kidneys, stomach, pancreatic islets, adipose tissue) [4]. The encoded protein is related to the angiotensin receptor, but is actually an APJ that inhibits adenylate cyclase activity and plays a counter-regulatory role against the pressure action of angiotensin II by exerting hypertensive effect. It functions in the cardiovascular and central nervous systems, in glucose metabolism, in embryonic and tumor angiogenesis and as a human immunodeficiency virus (HIV-1) coreceptor [5]. The apelinergic system, consisting of apelin and APJ has been described to participate in fluid homeostasis, regulation

of appetite, cardiac contractility, blood pressure and apoptosis; nowadays, studies focus on the role of apelin in glucose metabolism [6].

Coronary artery disease (CAD) is the leading cause of death worldwide. Though the symptoms and signs of CAD are noted in the advanced state of disease, a majority of CAD patients remain asymptomatic for decades prior to any overt signs of diseases [7].

The present study is the first investigation to examine the association between APJ G212A gene polymorphism and plasma APJ levels in Turkish CAD patients. APJ G212A gene polymorphism can provide benefits to improve clinical treatments and help us understand CAD pathophysiology and development. The aim of this study was to investigate the role of the plasma APJ and genotype and allele frequencies of APJ G212A gene polymorphism in Turkish patient

## APJ G212A gene polymorphism, plasma APJ levels and coronary artery disease

**Table 1.** Characteristics of the control and CAD (mean  $\pm$  SEM)

	Control (n = 110)	CAD (n = 152)	p-value
Sex (M/F)	58/52	114/38	-
Age (years)	61.4 $\pm$ 1.00	64.4 $\pm$ 0.96	0.203
Height (cm)	170.1 $\pm$ 0.65	169.3 $\pm$ 0.57	0.349
Weight (kg)	75.8 $\pm$ 0.85	77.5 $\pm$ 0.74	0.697
BMI (kg/m <sup>2</sup> )	26.2 $\pm$ 0.27	27.0 $\pm$ 0.24	0.364

Abbreviations: CAD, coronary artery disease; BMI, body mass index.

with CAD and compare them with healthy controls.

### Materials and methods

In this study, we screened 152 consecutive patients with stable angina pectoris who had at least 70% stenosis in any coronary artery diagnosed by coronary angiography (CAD Group) and 110 subjects with normal coronary anatomy that shown by coronary angiography (Control Group). CAD was diagnosed on the basis of the patients' clinical history, a physical examination and coronary angiography, according to the World Health Organization criteria [8]. The study was conducted with the collaboration of Department of Cardiovascular Surgery of Namik Kemal University in Tekirdağ, Turkey and the Department of Physiology of Dumlupınar University in Kütahya, Turkey. The study was approved by the ethical review board of Uludağ University (Bursa, Turkey). All patients were informed about the study, and their written consent forms were obtained.

### Biochemical investigation

Blood samples were taken after a 10-h overnight fast before angiography and centrifuged at 1000 g for 10 min, then plasma specimens were stored at -80°C until analysis. Plasma APJ levels were analyzed with human ELISA assay kit using the chemiluminescence method (Cusabio, China) according to the instruction of manufacturer by an ELISA microplate reader (Spectro Star Nano, Bmg Labtech, Germany).

### Analysis of APJ G212A gene polymorphism

Isolation of DNA from peripheral blood sample was performed with a commercial kit (GeneJET, Thermo, Cat No: # K0722) and DNA was stored in a deep freezer (-80°C) until the genetic anal-

ysis. By applying cycles of polymerase chain reaction (PCR) the genomic DNA containing the APJ gene was amplified using 5'-GGA GGT GGG AGG AGG AG-3' as forward primer and 5'-CCG TTG CCC GTG GTG CCC-3' as reverse primer [9, 10]. The PCR protocol was as follows: initial 2 min at 94°C followed by 35 cycles, consisting of denaturation for 30 s at 94°C, annealing for 30 s at 60°C, and extension for 30 s at 72°C. The PCR product (450 bp in size) was digested at 37°C for 16 h with Ddel restriction enzyme (New England Biolabs, Whitby, Ontario, Canada). Digestion products were examined by gel electrophoresis in 2% agarose gel. The detection of a single DNA band sized 152 bp indicated the GG genotype, the detection of a single 198 bp band suggested AA and the detection of two zones (152 bp, 198 bp) indicated the presence of heterozygosity GA.

The study was conducted in two steps. Firstly, we compared allele and genotype distributions of the CAD patients and controls. Secondly, we studied the correlation between plasma APJ levels and APJ G212A gene polymorphism.

### Statistical analysis

Statistical analyses were done by SPSS (Statistical Package for Social Sciences, Chicago, IL, USA) 16.0 package program. All data are given as mean  $\pm$  standard error of the mean (SEM). Independent sample t test were used to analyze differences in continuous variables between control and CAD patients. Both parametric and nonparametric tests were used for evaluation of the data. The difference in allele and genotype frequencies were compared between control and CAD using Pearson's Chi-Square test, from which the odds ratio (OR) and the 95% confidence interval (95% CI) were calculated. Statistical significance of the observed genotype frequencies was evaluated according to the Hardy-Weinberg rule and compared to the expected genotype frequencies. All *p* values  $\leq$  0.05 were accepted as statistically significant.

### Results

#### Characterization of study population

The general characteristics of the study groups are shown in **Table 1**. Characteristics of CAD patients and controls were similar (**Table 1**). The population control group consisted of 58

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**Table 2.** Allele/Genotype frequencies and test of Hardy-Weinberg (HW) equilibrium

	Controls		CAD	
f (G)	0.468		0.703	
f (A)	0.532		0.297	
	O	E	O	E
GG	20	24.1	87	75.3
GA	63	54.7	40	63.3
AA	27	31.1	25	13.3

$$\chi^2 = 2.49, df = 2, P = 0.286 \quad \chi^2 = 20.6, df = 2, P \leq 0.05$$

f = observed frequency of each allele (G or A); O = observed genotype numbers; E = expected genotype numbers under a Hardy-Weinberg (HW) equilibrium assumption;  $\chi^2$  = Chi-square values; P = probability of difference.

**Table 3.** Distribution of APJ G212A gene polymorphism in control and CAD

APJ G212A Genotypes	Control (n = 110) (%)	CAD (n = 152) (%)	OR (95% CI)	p
GG	20 (18.2%)	87 (57.2%)	0.21 (0.1-0.44)	0.000
GA	63 (57.3%)	40 (26.3%)	1.46 (0.74-2.86)	0.271
AA	27 (24.5%)	25 (16.4%)	Reference	-
$\chi^2 = 41.5 df = 2 P = 0.000$				
Allele				
G	103 (46.8%)	199 (70.1%)	2.65 (1.84-3.83)	0.000
A	117 (53.2%)	85 (29.9%)	Reference	
$\chi^2 = 27.9 df = 1 P = 0.000$				

OR-odds ratio.

males/52 females and also 114 males/38 females in the CAD groups. The median age was 62 years (interquartile range: 50-90 years).

### Allele/Genotype frequencies and test of Hardy-Weinberg (HW) equilibrium

The frequency of APJ G212A gene polymorphism genotypes in controls did not show a significant deviation from Hardy-Weinberg equilibrium ( $P = 0.286$ ), but it was showed a significant in the CAD group ( $P \leq 0.05$ ) (**Table 2**).

### Effect of the APJ G212A gene polymorphisms on the risk of CAD

Three different genotypes and allele frequencies of the APJ G212A polymorphism were GG, GA and AA genotypes and G and A alleles, respectively. Allele frequencies and genotypes of the APJ G212A polymorphism in CAD patients and healthy controls are shown in **Table 3**. Significant differences were observed in the genotype and allele frequencies of APJ G212A

polymorphism between CAD patients and healthy controls ( $P = 0.000$  and  $P = 0.000$ , respectively). The frequencies for each of the APJ G212A genotype were found as 57.2% for GG (87), 26.3% for GA (40) and 16.4% for AA (25) in the CAD group; 18.2% for GG (20), 57.3% for GA (63) and 24.5% for AA (27) in the control group. The distribution of APJ genotypes was found significantly different between groups ( $\chi^2 = 41.5$ ;  $df = 2$ ;  $P = 0.000$ ) (**Table 3**). For G212A variants in the APJ gene, the risk genotypes GG were associated with an increased risk of CAD (odds ratio for GG = 0.21; 95% CI = 0.1-0.44;  $P = 0.000$ ). Furthermore, The G allele was encountered in 70.1 (199) of the CAD and 46.8% (103) of the controls. The A allele was seen in 29.9% (85) of the CAD and 53.2% (117) of the controls. Distribution of the allele was found significantly different between groups ( $\chi^2 = 27.9$ ;  $df = 1$ ;  $P = 0.000$ ). The G allele in APJ gene increased the risk of CAD 2.65 times (95% CI 1.84-3.83,  $P = 0.000$ ) as compared to controls ( $P = 0.000$ ) (**Table 3**).

### Effect of plasma APJ levels on the risk of CAD

The plasma APJ levels were significantly lower in CAD group than control group ( $211.4 \pm 5.66$  and  $190.6 \pm 4.12$  pg/mL, respectively,  $P = 0.018$ ) (**Figure 1**).

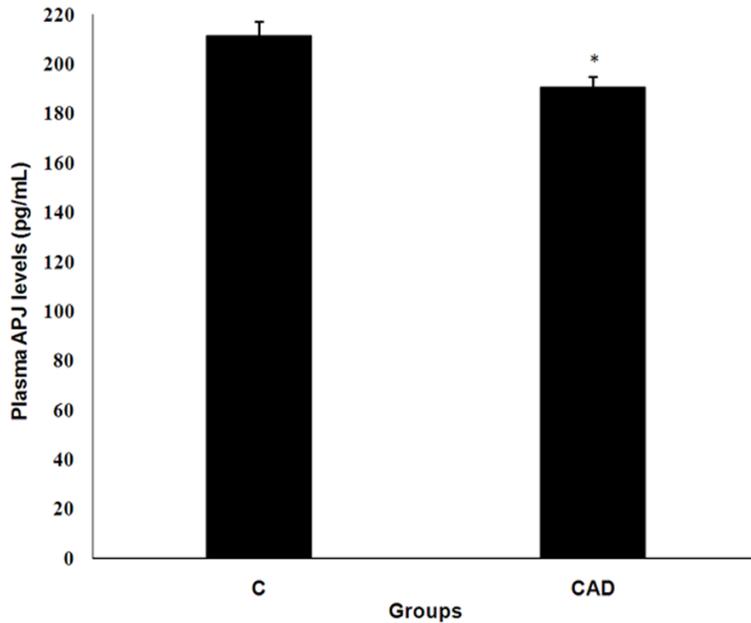
### Effect of APJ G212A gene polymorphisms on plasma APJ levels in the CAD

When we compared the plasma APJ levels and APJ G212A gene polymorphism of the CAD patients with GG genotype was  $188.5 \pm 4.75$  pg/mL. It was  $189.3 \pm 9.69$  pg/mL and  $194.8 \pm 9.23$  pg/mL in the patients with GA and AA genotypes, respectively. Although plasma APJ level of the patients with GG genotype was lower, it was not statistically significant. There was no significant difference between the plasma APJ levels of the patients with different genotypes ( $P = 0.932$ ) (**Figure 2**).

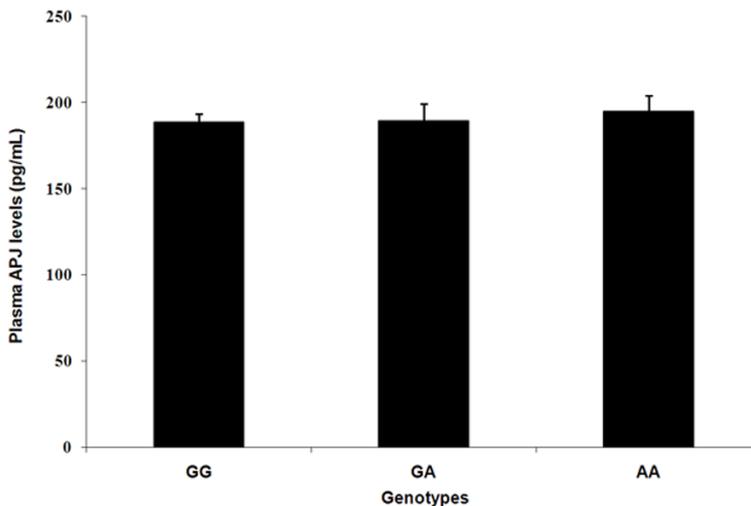
### Discussion

The pathogenesis of Coronary artery disease (CAD) is very complicated, to which multiple

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**Figure 1.** Plasma APJ levels of the CAD patients. Data were expressed as mean  $\pm$  standard error. \* $P \leq 0.05$  vs control group.



**Figure 2.** Plasma APJ levels of the CAD patients with three genotypes.

genetic and environmental factors contribute. CAD genesis is related to oxidative stress, which might be induced by CAD risk factors such as hypertension, dyslipidemia, diabetes, high hyperhomocysteinemia and obesity [11, 12]. The apelin-APJ receptor signaling pathway has emerged as an important novel mediator of cardiovascular control and blood pressure homeostasis [13]. This was a pilot study investigating, for the first time, the role of APJ G212A

gene polymorphism as both susceptibility and modifier candidate gene in Turkish population with CAD. In this study, it was aimed to analyze the distribution of the APJ G212A gene polymorphisms and plasma APJ levels in patients with CAD.

Differences in allele frequencies were apparent in various pathophysiologic conditions, including idiopathic dilated cardiomyopathy (IDC), hypertension or obesity for APJ G212A polymorphism. In a clinical study, the 212A allele was proposed as beneficial to human physiology in a study conducted in patients with CAD, since it was associated with lower risk for hypertension in these patients [9]. Conversely, a high frequency of G212 allele was found in the subgroup of hypertensive CAD patients as well [9]. Additionally, Falcone et al. [9] showed that in a subpopulation of patients with hypertension there was a significant increase in the frequency of the G allele than in patients with normal blood pressure. Moreover, a significant increase in the frequency of the homozygous AA genotype was noted in CAD patients without hypertension, when compared with patients with hypertension [9]. Hinohara et al. [14] also have shown that although the prevalence of

genotypes did not differ significantly between IDC and controls, the 212A allele was associated with a diminished risk for heart failure in the IDC group [14].

Our study revealed that there was statistically significant difference between CAD patients and healthy control group for APJ G212A genotypes and alleles in Turkish patients ( $\chi^2 = 41.5$ ,  $df = 2$ ,  $P = 0.000$  and  $\chi^2 = 27.9$ ,  $df = 1$ ,  $P =$

0.000, respectively). We obtained GG genotype in eighty seven cases, GA genotype in forty cases, and AA genotype was found only in twenty five cases of CAD. GG genotype frequency of CAD patients was found higher than controls (odds ratio for GG = 0.21; 95% CI = 0.1-0.44; P = 0.000). When allele frequencies of control subjects (46.8% G, 53.2% A) were compared with those of the patient group, we observed a significant disequilibrium with respect to a higher G prevalence in patients (70.1% G, 29.9% A; P = 0.000). An A allele carrier of control subjects was higher than subjects from the CAD patient group. According to our data we may predict that the A allele has protective effects against CAD. In addition, allele G (OR = 2.65, 95% CI 1.84 to 3.83) may increase the risk of the appearance of the CAD disease. This study's results are compatible with the aforementioned study. The G212A allele of the APJ seems to be a key polymorphism in various pathophysiologic conditions. Our findings suggest that APJ G212A gene polymorphism might be one of the genetic factors involved in CAD and the enhanced G allele has a role in the development of CAD in Turkish population.

Studies have shown that In the process of heart failure, the expression levels of apelin and the APJ underwent down-regulation in end-stage failing human hearts. In vivo animal modeling or in vitro studies showed that expression of endogenous apelin and the APJ receptor was increased immediately after the myocardium was under hypoxia stress, and this up-regulation was confirmed to have a protective effect on the cardiomyocytes from apoptosis or injury [15-17]. Kadoglou et al. [18] and Ye et al. [19] also showed that plasma apelin levels in patients with acute MI were lower than those in controls, and were even lower than those in patients with stable coronary artery disease (CAD).

In this study, we found that plasma APJ concentration in CAD patients was lower than that control group (P = 0.018) (**Figure 1**). In addition, although plasma APJ level of the CAD patients with GG genotype was lower, it was not statistically significant. There was no significant difference between the plasma APJ levels of the patients with different genotypes (P = 0.932) (**Figure 2**). There is a lack of information about the relationship between variants in the APJ

G212A gene, plasma APJ levels and CAD in the literature that can be compared with the results of this study. In a recent study with obesity patients the investigators demonstrated that, although G212A genotype frequencies did not differ between obese and other Caucasians, apelin levels were found to be higher in obese youngsters homozygous for the A allele; obese participants homozygous for the G allele exhibited lower apelin levels as compared with the rest of the study population [10]. Our findings suggest us that plasma APJ level can be independent from APJ G212A polymorphism, which should be confirmed with further studies performed in larger populations.

### Conclusions

This study is the first investigation of the association between apelin receptor (APJ) G212A gene polymorphism and plasma APJ levels and the risk of coronary artery disease (CAD). The results of the present study demonstrated that CAD patients differ from healthy subjects for APJ G212A gene polymorphism in the Turkish population. According to our data, we can say that G allele carriers may have more risk than A allele individuals for development of CAD. In addition, we think it is also could be a sign that there is a relationship between the GG genotype and development of CAD. Because, we observed that, the GG genotype is significantly higher in CAD, compared to the control group. Plasma APJ level of the CAD patients with GG genotype was lower. Patients with CAD who have GG genotype and G allele may be evaluated from the population because of decreased plasma APJ level, which may have deleterious effects on CAD pathophysiology. Further investigation into this signaling pathway may further enhance the understanding of the pathophysiology and therapy of CAD.

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### Disclosure of conflict of interest

None.

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