The association between apelin gene polymorphism and coronary artery disease in young patients with acute obstructive coronary syndrome

Genç akut koroner sendromlu hastalarda apelin gen polimorfizminin tıkıçıcı koroner arter hastalığı ile ilişkisi

Ramazan Güven, M.D.,¹ Kamil Can Akyol, M.D.,² Nermin Bayar, M.D.,³ Mustafa Keşaplı, M.D.,² Muhammed İkbal Şasmaz, M.D.,⁴ Asım Ari, M.D.,⁵ Şakir Arslan, M.D.³

¹Department of Emergency Medicine, Bitlis State Hospital, Bitlis, Turkey
²Department of Emergency Medicine, Antalya Training and Research Hospital, Antalya, Turkey
³Department of Cardiology, Antalya Training and Research Hospital, Antalya, Turkey
⁴Department of Emergency Medicine, Servergazi State Hospital, Denizli, Turkey
⁵Department of Emergency Medicine, Dr. Ersin Arslan State Hospital, Gaziantep, Turkey

Objective: The purpose of this study was to evaluate the association between V103V, 6140AG, TGA-Stop-TAA Stop, and 6016CA polymorphisms of the apelin (APLN) gene detected for the first time among young patients with acute coronary syndrome (ACS) and coronary artery disease (CAD).

Methods: This was a prospective cross-sectional study. The study population was divided into 2 groups. The first group included 132 patients who were found to have critical lesions in their coronary arteries, while the control group consisted of 41 patients who were found to have normal coronary arteries or non-critical atherosclerotic lesions.

Results: Among the gene polymorphisms, V103V was found to be more common in the critical CAD patients with the GG genotype compared with the control group (67.4% vs. 46.3%). On the other hand, the GT genotype was more common in the control group (53.7% vs. 32.6%). Univariate and multivariate logistic regression analysis revealed that the GG genotype of V103V was an independent predictor for the presence of critical CAD (odds ratio: 2.397; 95% confidence interval, 1.174–4.892; p=0.016).

Conclusion: In cases of V103V polymorphism of the APLN gene, patients with the GG genotype were at a greater risk for the presence of atherosclerotic critical lesions compared with the control group.
Coronary artery disease (CAD) is defined as the most important cause of morbidity and mortality globally.\(^1,2\) Although coronary atherosclerosis develops early in life, acute coronary syndrome (ACS) is rarely found in young adults.\(^3\) Therefore, a majority of the studies conducted on ACS focus on middle-aged and elderly patients.\(^4,5\) The prevalence of CAD is low among young patients, whereas there is increased mortality among middle-aged and elderly patients.\(^6\) Furthermore, the traditional risk factors are known to be less common among young ACS patients. Therefore, it is imperative to explore new risk factors for this group of patients. Many clinicians have studied the traditional risk factors associated with ACS. Meanwhile, cardiovascular genetic studies have provided significant contributions explaining the mechanisms of premature coronary atherosclerosis.\(^7\)

Apelin (APLN)/APJ is prevalent in the vascular endothelial cells. The apelinergic system plays a significant role in the proliferation of endothelial cells in the system; hence, it is proposed as a prognostic factor in ischemic events.\(^8\) The APLN gene is one of the major genes debated in recent cardiovascular genetic studies. The APLN gene, coded on the Xq25–26.1 chromosome, comprises 3 exons and 1726 base pairs.\(^9\) As ongoing research supports the important role of the APLN gene in the cardiovascular system, there is an increasing number of studies exploring the association between APLN/APJ gene polymorphism and hypertension (HT), diabetes mellitus (DM) and CAD.

The aim of this study was to evaluate the association between the presence of critical CAD and APLN gene polymorphisms (V103V, 6140AG, TGA-Stop-TAA Stop, 6016CA polymorphisms) in patients aged ≤45 years who were admitted with ACS.

### METHODS

This single-center, prospective, cross-sectional study included the 173 young patients (18–45 years of age), who were admitted to the emergency department from 2011 to 2015 who were considered to have ACS and who underwent coronary angiography. Approval for the study was obtained from the ethics committee of Antalya Education and Research Hospital. Written, informed consent was obtained from all participants. The genetic analysis of the study population was performed in the laboratory of the medical biology and genetics department. All patients underwent coronary angiography and all records were re-evaluated by a blinded physician who was unaware of the genetic analysis.

### Study group

The study included 173 patients aged 18 to 45 years who were diagnosed with ACS (ST-elevated myocardial infarction, non-ST-elevated myocardial infarction, unstable angina). The demographic data of the patients were recorded. Electrocardiogram and angiography records of all the patients included in the study were evaluated by the same cardiologist. In our study, those patients who had a minimum of 50% stenosis in the left main coronary artery and a minimum of 70% stenosis in the other vessels according to the coronary angiography were categorized as the “critical CAD group,” while those patients with normal or non-critical atherosclerotic lesions were assigned to the control group. A flow chart of the patient selection procedure is presented in Figure 1.

### DNA extraction and sequencing

From patients in each group, 3 cc of whole blood was taken in ethylenediaminetetraacetic acid tubes, and stored at 80°C. The DNA extraction was performed using the QIAamp DNA Mini Kit (Qiagen, N.V., Hilden, Germany). Polymerase chain reaction amplifications were performed with Qiagen HotStarTaq DNA Polymerase Kit (Qiagen, N.V., Hilden, Germany) using a standard application protocol described in the manufacturer’s instructions. In a total volume of 12 mL for 40 cycles, each cycle consists of denaturation for 10 minutes at 95°C and 40 cycles of 15 seconds at 95°C, primer annealing for 30 seconds at the specific Tm for each primer pair, extension at 72°C for 1 minute, and at the end of the cycles, a last extension for 7 minutes at 72°C in a thermal cycler (Applied Biosystems, Inc., Foster City, CA, USA). Shrimp alkaline phosphatase and exonuclease 1 purification protocol was applied for PCR purification. The purified PCR products were submitted for sequencing using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA, USA). The sequenced products were run on the Applied Biosystems AB3100 (Ap-
plied Biosystems, Inc., Foster City, CA, USA). The primers and sequences of the polymorphisms are provided in Table 1.

### Statistical analysis

The sequence analysis was performed using Mutation Surveyor, Version 2.09 software (Softgenetics, LLC, State College, PA, USA). The data collected from the patients during the study were uploaded to IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA) and subjected to percentage and statistical analysis. The Shapiro-Wilk goodness of fit test was used to check if the assumptions of the parametric test statistic were achieved. The Kolmogorov-Smirnov goodness of fit test was used to check if the assumptions of the parametric test statistic were realized. Continuous variables without normal distribution were analyzed using the Mann-Whitney U test and the obtained values were presented as median (50th) values and interquartile ranges (25th and 75th), while the categorical variables were presented as percentages. The chi-square test was used, since the expected frequency was lower than 25% for the statistical distribution of the APLN V103V and 6140AG genotypes and HT (blood pressure ≥140/90 mmHg or under anti-HT treatment), hyperlipidemia (total cholesterol ≥200 mg/dL, low-density lipoprotein cholesterol ≥130 mg/dL), family history (CAD before the age of 55 in males and 65 in females among first-degree relatives), DM (fasting blood glucose >126 mg/dL, second hour value in oral glucose tolerance test

### Table 1. Location of the designed primers on the APLN gene

<table>
<thead>
<tr>
<th>Name of primer</th>
<th>5’→ 3’ sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apelin-Exon1-F</td>
<td>CCGATGACCACATGACCAAG</td>
</tr>
<tr>
<td>Apelin-Exon1-R</td>
<td>GAATAGGGCGGAGGGAAAG</td>
</tr>
<tr>
<td>Apelin-Exon2-F</td>
<td>GTGGTCTGAGGCCCTTG</td>
</tr>
<tr>
<td>Apelin-Exon2-R</td>
<td>ATCACCCGGCTTCTAGGC</td>
</tr>
</tbody>
</table>
>200 mg/dL) and smoking, whereas Fischer’s exact test was used for the other categorical variables. Those variables that were statistically significant (p<0.25) in the univariate analysis were entered into multivariate logistic regression analysis using the backward logistic regression method in order to determine the independent prognostic factors (HT, hyperlipidemia, DM, family history, smoking) in the critical CAD group. P <0.05 was considered to be statistically significant.

**RESULTS**

The demographic data of the patients are provided in Table 2. The mean age of the patients was 40.13±4.8 years. There were significant differences between the groups with respect to gender, smoking, and hyperlipidemia, which are the conventional risk factors for ACS.

The median age of the patients included in the study was 42.0 years (25th and 75th percentile: 39.0–44.0 years). Four new APLN gene polymorphisms that had not been defined previously were identified through genetic analysis. The first polymorphism was V103V, which caused valine 103 valine-change (conversion of G base into T base at 103rd amino acid). This polymorphism led to the base conversion, while it did not change the protein structure. The V103V polymorphism has 2 genotypes: GG and GT. A total of 67.4% of the ACS patients with critical CAD (n=89) had the GG genotype, and 32.6% (n=43) had the GT genotype, while 46.3% of the cases in the control group (n=19) had the GG genotype, and 53.7% (n=22) had the sGT genotype. Critical lesions were found to be 2.397 times more common in the patients with the GG genotype of the V103V polymorphism, when compared with those with the GT genotype (Odds ratio [OR]: 2.397; 95% confidence interval [CI], 1.174–4.892; p=0.016); however, the GT geno-

<table>
<thead>
<tr>
<th>Table 2. Distribution of the groups according to CAD risk factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Critical CAD (+) (n=132)</td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>Male, n (%)</td>
</tr>
<tr>
<td>Age, years</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
</tr>
<tr>
<td>Hyperlipidemia, n (%)</td>
</tr>
<tr>
<td>Family history, n (%)</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
</tr>
</tbody>
</table>

Continuous data are presented as median and interquartile range (25th and 75th); categorical data are presented as number and percentage. CAD: Coronary artery disease; CI: Confidence interval; OR: Odds ratio.

<table>
<thead>
<tr>
<th>Table 3. Distribution of GG-GT genotypes of V103V, TT-TC genotypes of 6140AG, CC-CA genotypes of 6016CA, CC-CT genotypes of Stop-Stop polymorphism in the groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Critical CAD (+) (n=132)</td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>GG genotype, n (%) (V103V polymorphism)</td>
</tr>
<tr>
<td>GT genotype, n (%) (V103V polymorphism)</td>
</tr>
<tr>
<td>TT genotype, n (%) (6140AG polymorphism)</td>
</tr>
<tr>
<td>TC genotype, n (%) (6140AG polymorphism)</td>
</tr>
<tr>
<td>CC genotype, n (%) (6016CA polymorphism)</td>
</tr>
<tr>
<td>CA genotype, n (%) (6016CA polymorphism)</td>
</tr>
<tr>
<td>CC genotype, n (%) (Stop-Stop polymorphism)</td>
</tr>
<tr>
<td>CT genotype, n (%) (Stop-Stop polymorphism)</td>
</tr>
</tbody>
</table>

CAD: Coronary artery disease; CI: Confidence interval; OR: Odds ratio.
The prevalence of the GG-GT genotypes of the V103V polymorphism in the groups is shown in Table 3. According to Hardy-Weinberg equilibrium, the distribution and significance of the genotype and allele frequencies of the V103V polymorphism across the groups are shown in Table 4. A significant difference was found between the 2 groups in terms of the G and T allele frequencies of the V103V polymorphism; the T allele was observed to be protective against critical CAD compared with the G allele (OR: 0.531; 95% CI, 0.295–0.955; p=0.033).

The comparison between the groups with regard to the other gene polymorphisms of APLN gene, which were 6140AG, TGA-Stop-TAA Stop, and 6016CA, did not reveal any significant differences (p=0.522; p=0.665; p=0.694, respectively).

The results of the univariate and multivariate logistic regression analysis, performed to identify the independent risk factors for the development of ACS (age, gender, V103V polymorphism, DM, HT, smoking, hyperlipidemia, and family history), are presented in Table 5. Univariate regression analysis revealed that there was a significant association between the presence of ACS and age, gender, V103V (GG/GT) polymorphism, smoking, and hyperlipidemia (p=0.047; p=0.001; p=0.016; p=0.007, respectively). However, no significant association was found between the presence of ACS and DM, HT, or family history (p=0.112; p=0.166; p=0.109, respectively). Multivariate logistic regression analysis was performed for the variables that were found to be significant according to p<0.1. Accordingly, gender (OR: 0.194; 95% CI, 0.052–0.725; p=0.015), GG genotype of the V103V polymorphism (OR: 3.587; 95% CI, 1.301–9.888; p=0.014), and hyperlipidemia (OR: 0.115; 95% CI, 0.021–0.628; p=0.013) were found to be independent risk factors for ACS.

**DISCUSSION**

Environmental and genetic factors play a role in the development of ACS. Studies show that the conventional risk factors are less common, especially among young ACS patients. It is important to identify new risk factors, which includes genetic polymorphism,
in these patients. In our study, the GG/GT polymorphisms of the APLN gene were independently associated with the presence of critical CAD. This finding may help identify individuals who are at greater risk of CAD.

Since it was discovered that APLN/APJ was intensively present in the vascular endothelial cells, there has been an increasing number of studies focusing on the effect of the APLN gene on cardiovascular diseases.[10,11] Jin et al. explored the effect of 5 polymorphisms in the APLN/APJ pathway (rs3761581, rs56204867, rs7119375, rs10501367, rs9943582) in hypertensive patients on the presence of CAD.[12] In their study, age was not a criterion for enrollment, all CAD patients and control cases included in the study were selected from among those who underwent coronary angiography. Their results revealed that none of the 5 polymorphisms analyzed either in the CAD patients or in the control group had a significant difference in terms of genotype/allele frequencies. However, the role of haplotypes with low penetrance in the male CAD patients was underlined in the haplotype analysis.

Falcone et al.[13] analyzed 664 CAD patients, including 378 hypertensive cases and 143 healthy controls, in their study and investigated the differences between the genotype and allele frequencies of the G212A and A445C APJ polymorphisms. Although HT was found to be highly significant among the CAD patients, no difference was observed in the genotype and allele frequencies between the CAD and the control groups. Like these 2 studies, all reports exploring the effect of APLN gene polymorphism on HT focus on the effect of the APLN-APJ pathway on blood pressure.[14] In another study, which comprised 200 patients, the effect of another genetic variation of APLN (rs 2235306) on type 2 DM was investigated[15] and it was found that the APLN polymorphism had a significant impact on female patients with poor glycemic control (p=0.03).

Zhang et al. investigated the effect of APLN gene polymorphisms (rs2235306, rs2235307, and rs3115759) on hypertensive and diabetic Chinese patients.[16] A total of 1757 non-diabetic patients were added to the study through validation, and as a result 3156 diabetic patients and 3736 non-diabetic cases were investigated, according to which, none of the 3 gene polymorphisms was found to be associated with HT. However, only the rs2235306 polymorphism was associated with male non-diabetic hypertensive patients (p=0.039).

Pakizeh et al. published a study in 2014, in which they explored the association between the rs3115758 and rs3115759 gene polymorphisms with CAD.[17] They included 244 individuals in the study, 134 of whom were diagnosed with CAD through coronary angiography and 110 healthy controls with normal coronary angiograms. Their results concluded that the TT genotype increased the CAD risk by 6.36 times in the rs311578 polymorphism, while the T allele increased the risk by 2.86 times. In the same study, the AA genotype of the rs3115759 polymorphism was observed to increase the CAD risk by 6.36 times while the A allele increased the risk by 2.86 times.

In our study, we investigated the association between the genotype and allele frequencies of 4 newly identified polymorphisms of the APLN gene and the presence of critical CAD. We found that the V103V polymorphism had a significant association, while the 6140AG (TT/sTC), 6016CA (CC/CA), Stop-Stop (CC/CT) polymorphisms did not have any significant associations. The GG genotype of the V103V polymorphism increased the risk of critical CAD, whereas the T allele was protective against the development of critical CAD. In the study of Pakizeh et al., multivariate regression was performed for only 2 variables (DM, HT) apart from the polymorphisms. In our study, however, the other conventional risk factors known for ACS (age, gender, DM, hypertension, hyperlipidemia, smoking, family history) were also taken into consideration.

Possible limitations of the present study include that it was a single-center experience, and that it comprised a small number of patients and the control group cannot be followed up for a long time (until they are 45 years of age). Additional limitations of the study are that the control group did not comprise only healthy individuals, and family screening could not be performed for family members of the patients who were subjected to genetic analysis.

Critical CAD is more prevalent among young ACS patients who have the GG genotype of the V103V polymorphism of the APLN gene. This finding suggests that the V103V (GG/GT) polymorphism of the APLN gene may be a predictor for the presence of
critical CAD in young ACS patients. Moreover, genetic analysis of the relatives of these young ACS patients who are found to have this polymorphism may help with the early detection of critical CAD before it develops. Furthermore, there is a need for additional large-scale studies that explore different gene polymorphisms simultaneously and APLN level.

Funding
The authors received no financial support for the research and/or authorship of this article.

Conflict-of-interest: None declared

REFERENCES


Key words: Apelin; genetic analysis; premature atherosclerosis
Anahtar sözcükler: Apelin; genetik analiz; erken ateroskleroz.