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RESEARCH

Association of Kruppel-like factor 2 polymorphisms with the risk of coronary artery disease

Kruppel benzeri faktör 2 polimorfizmlerinin koroner arter hastalığı riski ile ilişkisi

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Abstract

Purpose: Coronary artery disease is defined as complications that develop in proportion to the prevalence of ischemia due to occlusion of the coronary arteries and the resulting cell death. The development of atherosclerosis is significantly influenced by endothelial cell dysfunction. Kruppel-like factor 2, a transcription factor, has been shown to regulate critical biological events in endothelial biology, such as vascular tone, migration, proliferation, vasoreactivity, and angiogenesis. In our study, it was aimed to clarify the relationship between coronary artery disease and Kruppel-like factor 2 protein levels and C1239A polymorphisms.

Materials and Methods: 191 individuals who underwent coronary angiography at Mersin University Faculty of Medicine, Department of Cardiology, Health, Research and Application Center were included in the study. Measurements of serum fasting blood glucose, HDL cholesterol, total cholesterol, and triglyceride levels were performed on the AU5800 (Beckman Coulter, United States) autoanalyzer. Serum LDL levels were calculated using the Friedwald equation. Serum Kruppel-like factor 2 protein levels were measured by sandwich enzyme-linked immunoassay method on the Multiskan GO (Thermo Scientific, Finland) device. Kruppel-like factor 2 C1239A variations were detected on the Applied Biosystem VIIA™ 7 Real-Time PCR (Life Technologies Co., United States) device by TaqMan® single nucleotide polymorphism (SNP) genotyping method.

Results: Men had a 3.8-fold higher risk of CAD than women. (Odd's ratio 3.83, 95% Confidence interval 1.98-7.39; p<0.001). Kruppel-Like Factor 2 protein levels were

Öz

Amaç: Koroner arter hastalığ, koroner arterlerin tıkanmasıyla iskemi oluşması ve sonucunda gerçekleşen hücre ölümlerinin yaygınlığıyla orantılı gelişen komplikasyonlar olarak tanımlanır. Ateroskleroz oluşumunda endotel hücrelerinin disfonksiyonu önemli rol oynamaktadır. Bir transkripsiyon faktörü olan Kruppel benzeri faktör 2'nin endotel biyolojisinde vasküler tonus, migrasyon, proliferasyon, vazoreaktivite ve anjiyogenez gibi önemli biyolojik olayları düzenlediği ortaya konulmuştur. Çalışmamızda Kruppel benzeri faktör 2 protein düzeyleri ve C1239A polimorfizmlerinin koroner arter hastalığıyla ilişkisinin aydınlatılması amaçlandı.

Gereç ve Yöntem: Mersin Üniversitesi Tıp Fakültesi Hastanesi Kardiyoloji Polikliniğinde koroner anjiyografi yapılan 191 birey çalışmaya dahil edildi. Serum açlık kan şekeri, total kolesterol, HDL kolesterol ve trigliserit düzeylerinin ölçümleri AU5800 (Beckman Coulter, Amerika Birleşik Devletleri) otoanalizöründe yapıldı. Serum LDL düzeyleri Friedwald eşitliği kullanılarak hesaplandı. Serum Kruppel benzeri faktör 2 protein düzeyleri sandviç enzim bağlı immünoassay yöntemiyle Multiskan GO (Thermo Scientific, Finlandiya) cihazında çalışıldı. Kruppel benzeri faktör 2 C1239A varyasyonları TaqMan® tek nükleotid polimorfizm (SNP) genotiplendirme yöntemiyle Applied Biyosistem VIIA ™ 7 Real-Time PCR (Life Technologies Co., Amerika Birleşik Devletleri) cihazında saptandı.

Bulgular: Erkeklerin kadınlara göre KAH açısından 3,8 kat daha riskli olduğu saptandı (Odd's Oranı 3,83, %95 Güven Aralığı 1,98-7,39; p<0,001). Kruppel Benzeri Faktör 2 protein düzeylerinin gruplar arasında anlamlı

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not significantly different amongst the groups. Kruppel-like factor 2 C1239A polymorphisms were not associated with coronary artery disease.
Conclusion: More comprehensive studies with larger sample groups including other gene regions and meta-analysis studies are needed to determine the relationship between Kruppel-like factor 2 gene polymorphisms and coronary artery disease.
Keywords:. Coronary artery disease, atherosclerosis, endothel, kruppel-like factor 2, single nucleotide polymorphism.

farklılık göstermediği saptandı. Kruppel benzeri faktör 2 C1239A polimorfizmlerinin koroner arter hastalığı için risk teşkil etmediği saptandı.
Sonuç: Kruppel benzeri faktör 2 gen polimorfizmlerinin KAH için risk faktörü olup olmadığının ortaya konulması için diğer gen bölgelerini de içeren daha geniş örneklem gruplarıyla yapılacak daha kapsamlı çalışmalara ve meta analiz çalımalarına ihtiyaç duyulduğu düşünülmektedir.
Anahtar kelimeler: Koroner arter hastalığı, ateroskleroz, endotel, kruppel benzeri faktör 2, tek nükleotid polimorfizmi

INTRODUCTION

In a patient with atrial fibrillation, cardiovascular Diseases (CVD) may originate from different etiologies, such as thromboembolism resulting in ischemic stroke or rheumatic fever causing valvular heart disease. However, since atherosclerosis and atherosclerotic risk factors are the common ground of the pathophysiology of these diseases, they are particularly significant[1,2]. Coronary artery diseases are described as the complications of myocardial ischemia that may occur due to the inability to carry enough blood to supply the heart due to occlusion in the coronary arteries and the extent of necrosis in undersupplied cells[3]. Atherosclerosis is the cause of stenosis and occlusion in the coronary arteries[4]. Many risk factors that initiate or cause the progression of atherosclerosis have been identified. The etiological risk factors include hyperlipidemia, hypertension, diabetes, obesity, smoking and a sedentary lifestyle[5]. Atherosclerosis is defined as a chronic inflammatory process that is triggered by the accumulation of low-density lipoprotein (LDL) particles in the arterial wall. Elevated serum LDL levels and endothelial dysfunction may cause atherogenesis. The reaction of low-density lipoprotein with reactive oxygen species produces oxidized LDL (ox-LDL). Oxidized LDL may cause endothelial dysfunction at the onset of atherosclerosis[6]. Endothelial dysfunction results in both reduced synthesis of the endothelial nitric oxide synthase (eNOS) enzyme, causing a reduced release of nitric oxide (NO), a vasodilator agent and increased release of vasoconstrictor factors, such as endothelin-1 (ET-1). In addition, ox-LDL induces endothelial cells to synthesize a chemotactic protein called monocyte chemoattractant protein-1 (MCP-1), E-selectin and adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1). Monocyte chemoattractant protein-1 mediates the direction of free-floating monocytes to the luminal surface of endothelial cells, while VCAM-1 and E-selectin mediate these cells' rolling, adhesion and transmigration on the endothelial surface. Monocytes that penetrate into the endothelium and internal elastic membrane differentiate into macrophages and ingest ox-LDL, causing lipid deposition, hence forming atherosclerotic plaques[7]. The results of clinical, pathological and experimental observations suggest that endothelial cell dysfunction plays a central role in the formation stages of vascular disorders, such as atherosclerosis and thrombosis[8,9]. Studies in recent years have revealed that Kruppel-like factor 2 (KLF2) protein, a member of the Kruppel-like factor family and a transcriptional factor, is the key regulator of endothelial cell activity. In many inflammatory diseases, the capture of immune cells and their transfer to the relevant site by endothelial cells are critical steps. Adhesion of immune cells occurs thanks to endothelial cells that release adhesion molecules, such as VCAM-1 and E-selectin[10]. A study conducted by Sen Banerjee et al. showed that KLF2 inhibited the expressions of VCAM-1 and E-selectin[11]. One of the most significant tasks of endothelial cells is to maintain constant blood fluidity. eNOS and thrombomodulin (TM) released by these cells may have anti-inflammatory properties[12,13].

In addition, the tissue factor (TF) has procoagulant properties and the plasminogen activator inhibitor-1 (PAI-1) has antifibrinolytic properties[14-16]. Lin et al. showed that KLF2 induces the expressions of TM and eNOS, whereas it inhibits the expressions of PAI-1 and TF[17]. Vascular endothelium-derived growth factor (VEGF) induces the proliferation and migration of endothelial cells and promotes angiogenesis. VEGF exerts this effect by binding to certain receptors, such as vascular endothelium-derived growth factor receptor-1 (VEGFR1) and vascular endothelium-derived growth factor receptor-2 (VEGFR2)[18-20]. Semaphorin 3F (SEMA3F) is known to inhibit migration[21]. Dekker et

al. showed that KLF2 inhibits the expression of VEGFR2, one of the VEGF receptors and induces the expression of SEMA3F[22].

Unveiling genetic risk factors for coronary artery diseases can help determine novel diagnostic and therapeutic approaches. Therefore, we aimed to establish a link between coronary artery disorders and C1239A variations in the KLF2 gene area.

MATERIALS AND METHODS

Study population

The study comprised 191 individuals who had coronary angiography at the Cardiology Department, Health, Research and Application Center of Mersin University Faculty of Medicine. Coronary angiography was performed with femoral intervention using the standard Judkin technique by Cardiology Department faculty members. CAD was defined as ≥70% of luminal stenosis in at least one major coronary vessel based on the result of coronary angiography. Individuals with stenosis in any of the coronary arteries constituted the case group, and those stenosis were the control group. Each participant provided their written informed consent, which the Mersin University Clinical Research Ethics Committee authorized.

The cohort participants were divided into a CAD group and a control group. Participants with malignant or autoimmunological disease, severe kidney or liver disease and cardiomyopathy were excluded from the study. 115 people were reached in the case group. While 8 people were excluded from the study due to exclusion criteria, 15 people refused to participate in the study. 111 people were reached in the control group, but 12 people refused to participate in the study. As a result, the study completed with a total of 191 individuals, 92 individuals in case and 99 individuals in control groups.

Demographic data included age, sex, smoking status (smokers were defined as having smoked at least one cigarette per day for more than one year), diabetes mellitus (type 2), hypertension and blood lipid profile were retrospectively extracted from registered documents or questionnaires. The study was approved with the decision of Mersin University Clinical Research Ethics Committee dated 08.03.2018 and numbered 2018/122.

SNP selection

According to the criteria of minor allele frequencies (MAFs) ≥0.05, three SNPs were targeted for genetic analysis. These were rs15336 (3 prime UTR variant), rs3745318 (missense variant) and rs12459387 (2 KB upstream variant). As a result of our examination, we saw that the gene region contains many circular structures and that the Guanine-Cytosine bases are dominant. Therefore, we chose one SNP (rs15336) for further analysis.

DNA extraction and genotyping of KLF2

Peripheral blood was obtained from all individuals and collected in EDTA-containing tubes. A highly purified PCR template preparation kit (Cat No. K182002, PureLink® Genomic DNA Mini Kit, Thermo Fischer Scientific, United States) was used to extract DNA from the leukocytes. Kruppel-like factor 2 C1239A (rs15336) variations were detected on the ViiA 7 Real-Time PCR instrument (ABI) using the TaqMan® single nucleotide polymorphism (SNP) genotyping kit (Cat No: 4351379, Thermo Fischer Scientific, United States).

KLF2 protein assay

Peripheral blood was drawn and placed in tubes containing coagulant from all induviduals and were centrifuged at 3000 rpm for 10 minutes. Sufficient amount of serum to measure KLF2 protein levels were stored at -80 °C until the study day. According to the manufacturer's recommendations, the Multiskan GO (Thermo Scientific, Finland) device was used to measure the levels of serum KLF2 protein using commercially available human KLF2 ELISA kits (Cat No: SED367Hu, Enzyme-linked Immunosorbent Assay Kit for Kruppel Like Factor 2, Uscn Life Science Inc., Wuhan, China).

Statistical analysis

In the study, power analysis was used to determine the number of samples. According to analysis results, it was planned to include at least 90 individuals in the present study by taking the opinion of the Department of Biostatistics and Medical Informatics. SPSS for Windows, Version 25.0 (SPSS Inc., Chicago, IL, USA) was used to analyse the data. Descriptive statistics of number and percentage were calculated for the categorical variables, and mean, SD, minimum and maximum for numerical variables. Shapiro-Wilk test was used for testing normality.

Comparisons of numerical variables in independent groups were performed using the Mann- Whitney U test for 2 groups and the Kruskal-Wallis test for more than 2 groups without normal distribution. Besides, ANOVA and independent t-test were used for the variables with normal distribution. Chi-square test were applied for analysing the categorical variables. The level of statistical significance applied was $p<0.05$.

RESULTS

The distribution of risk factors and the descriptive data of the control and CAD groups are presented in Table 1. The findings obtained in this study showed that the number of male patients in the CAD group was greater than that in the control group, and men had a 3.8 times (Odd's ratio 3.83, 95% Confidence interval 1.98-7.39; $p<0.001$) greater risk of having CAD. Among risk factors of coronary artery disease, diabetes, hypertension and smoking were not associated with higher CAD risk ($p>0.05$).

There was no significant difference between the control and CAD groups regarding total cholesterol (TC), LDL cholesterol, VLDL cholesterol, triglyceride, FBG and KLF2 levels ($p>0.05$). However, the median cholesterol level of the control group was greater than the CAD group, although this difference did not reach statistical significance ($p>0.05$). Table 2 shows the serum lipid profile, FBG and KLF2 level of the subjects in the control and CAD groups

Table 1. The distributions of the KLF2 C1239A genotype and alleles

KLF2 Genotype	Control n (%)	CAD n (%)	p-value
AA+AC	28 (28.3)	24 (26.1)	0.748
CC	71 (71.7)	68 (73.9)	
Allele frequency			
C	157 (51.6)	147 (48.4)	0.900
A	41 (52.6)	37 (47.4)	

n: number in sample, p: degree of significance between groups.

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Table 2. Age, FBG and lipid profiles of the CC and AC+CC genotypes in the CAD group

KLF2 C1239A	CC	AC+AA	p-value
Age	60.60±9.69	62.54±10.55	0.599
TC	192.35±49.23	198.63±52.33	0.413
HDL-C	[35.92;46.52] 40.55	[36.70;50.20] 41.50	0.566
LDL-C	[97.62;142.40] 113.65	[93.55;144.65] 121.80	0.929
VLDL-C	[20.55;36.50] 24.60	[21.40;35.25] 29.80	0.309
TG	[102.75;182.50] 123.00	[107.00;176.25] 149.00	0.309
FBG	[97.17;122.90] 104.25	[94.25;129.12] 101.50	0.866

p: degree of significance between groups.

The subjects with coronary artery disease were divided into three subgroups as 1-vessel, 2-vessel, and 3 or more vessel based on the number of occluded vessels (left main coronary artery, left anterior descending artery, circumflex artery and right coronary artery). There was no significant difference between the serum KL2 levels of the 1-vessel, 2-vessel and 3 vessel or more (multiple-vessel) groups (p=0.439). The relationship between serum KLF2 levels and the number of occluded vessels is shown in Table 3.

Table 3. The relationship between serum KLF2 levels and the number of occluded vessels

Variables	n	KLF2 (Mean rank)	Chi square value	p-value
1-Vessel	53	43.44	1.645	0.439
2-Vessel	20	50.33		
Multiple-vessel	19	51.00		

n: number in sample, p: degree of significance between groups.

Kruppel-like factor 2 gene possesses two alleles as C and A for the 171st position in the 3' UTR region. The C allele is the common allele in the general population. The findings showed that 71 subjects (71.7%) in the control group had CC genotype, 15 (15.15%) had AC genotype and 13 (13.13%) had AA genotype, while 68 subjects (73.9%) in the CAD group had CC genotype, 11 (11.11%) had AC genotype and 13 (14.13%) had AA genotype. In addition, 157 subjects (51.6%) in the control group had a C allele, and 41 (52.6%) had an A allele, while 147 subjects (48.4%) in the CAD group had a C allele, and 37 (47.4%) had A allele. Whether KLF2 C1239A genotype and alleles were related to CAD risk was examined by binary logistic regression analysis. In the risk analysis, the CC genotype was determined as the reference genotype. The distributions of the KLF2 C1239A genotype and alleles are shown in Table 4.

Table 4. The distributions of the KLF2 C1239A genotype and alleles

KLF2 Genotype	Control n (%)	CAD n (%)	p-value
AA+AC	28 (28.3)	24 (26.1)	0.748
CC	71 (71.7)	68 (73.9)	
Allele frequency			
C	157 (51.6)	147 (48.4)	0.900
A	41 (52.6)	37 (47.4)	

n: number in sample, p: degree of significance between groups.

Table 5. Age, FBG and lipid profiles of the CC and AC+CC genotypes in the CAD group

KLF2 C1239A	CC	AC+AA	p-value
Age	60.60±9.69	62.54±10.55	0.599
TC	192.35±49.23	198.63±52.33	0.413
HDL-C	[35.92;46.52] 40.55	[36.70;50.20] 41.50	0.566
LDL-C	[97.62;142.40] 113.65	[93.55;144.65] 121.80	0.929
VLDL-C	[20.55;36.50] 24.60	[21.40;35.25] 29.80	0.309
TG	[102.75;182.50] 123.00	[107.00;176.25] 149.00	0.309
FBG	[97.17;122.90] 104.25	[94.25;129.12] 101.50	0.866

p: degree of significance between groups.

The rate of the CC genotype in the CAD group (73.9%) was higher than that in the control group (71.7%), but the difference did not reach statistical significance (p=0.748).The rate of the C allele in the control group (51.6%) was higher than that in the CAD group (48.4%) although the difference between the two groups did not reach statistical significance (p=0.900). Two subgroups as homozygous normal (CC) and KLF2 variant (AC+CC) were formed in the CAD group. These groups were not significantly different concerning age, lipid profile and FBG levels (p>0.005). Age, FBG and lipid profiles of the CC and AC+CC genotypes in the CAD group are shown in Table 5. Table 6 shows the data on comparing the CC and AC+CC genotypes regarding the risk factors of sex, type 2 diabetes, hypertension and cigarette consumption in the CAD group.

Table 6. Genotypes regarding the risk factors of sex, type 2 diabetes, hypertension and cigarette consumption in the CAD group.

Variable		KLF2 Genotypes n (%)		p-value
		CC	AA+AC	
Gender	Male	56 (82.4)	19 (79.2)	0.764
	Female	12 (17.6)	5 (20.8)	
Diabetes (Type 2)	Normal	43 (63.2)	17 (70.8)	0.621
	Diabetes	25 (36.8)	7 (29.2)	
Hypertension	Normal	26 (38.2)	14 (58.3)	0.100
	Hypertensive	42 (61.8)	10 (41.7)	
Cigarette consumption	Non smoking	53 (77.9)	16 (66.7)	0.285

n: number in sample, p: degree of significance between groups.

When the subgroups of homozygous normal (CC) and KLF2 variant (AC+AA) genotype of the CAD group were compared, no significant difference was found between the rates of the risk factors sex, type 2 diabetes, hypertension and smoking (p>0.005). The comparison of the groups regarding KLF2 levels was performed using the Mann-Whitney U test instead of the independent t-test since the KLF2 variable had a non-normal distribution (Shapiro-Wilk p>0.05). Table 7 shows serum KLF2 levels of the control and CAD groups by KLF2 C1239A genotypes.

Table 7. Serum KLF2 levels of the control and CAD groups by KLF2 C1239A genotypes

KLF2	Control (mg/dL)	CAD (mg/dL)	p-value
CC	[58.70;73.10] 64.50	[61.00;71.15] 64.65	0.850
AC	[63.50;96.50] 68.50	[56.90;87.50] 64.00	0.938
AA	[59.60;69.85] 65.40	[57.70;77.70] 64.50	0.880

p: degree of significance between groups.

In the control group, a comparison of the CC, AC and AA genotypes (CC-AC, CC-AA, AC-AA) regarding serum KLF2 levels revealed no statistically significant difference (p>0.005). Similarly, a comparison of the control and CAD groups by genotype showed no significant difference (p>0.005). Two subgroups as homozygous normal (CC) and KLF2 variant (AC+CC) were formed in the CAD group. No statistically significant difference was found between these groups (p>0.005). Table 8 shows the relationship between the KLF2 C1239A genotypes and the number of occluded vessels in the CAD group.

Table 8. Relationship between the KLF2 C1239A genotypes and the number of occluded vessels in the CAD group

Occluded vessels	KLF2 Genotype n (%)		p-value
	CC	AC+AA	
1-Vessel	41 (60.3)	12 (50.0)	0.562
2-Vessel	13 (19.1)	7 (29.2)	
Multiple-vessel	14 (20.6)	5 (20.8)	

DISCUSSION

The first study investigating the relationship between cardiovascular diseases and lifestyle was published by Keys et al. in the United States of America in 1963. That study established that hypertension, elevated serum cholesterol level and smoking as the CVD risk factors[23]. The data obtained in the 1970s from the Framingham heart study, which have continued to this date, have helped account for the relationship between the development of atherosclerosis and a high LDL cholesterol level, a low HDL cholesterol level, sedentary lifestyle and obesity[24]. Information about our subjects; age, sex, type 2 diabetes, hypertension and smoking status was obtained by questioning their past medical history. In addition, the duration of exposure to other CAD risk factors and atherosclerotic causal agents increases as people age. Our study found that the mean age of the CAD group was higher than the mean age of the control group, this difference did not reach statistical significance. Since CAD occurs approximately 10 years earlier in men than women and it more commonly affects men, male sex is considered an independent risk factor. A study conducted in Finland, which enrolled 14786 middle-aged men and women, found that the risk of CAD was 3.3 times greater in men than women[25]. In our study, the percentage of male subjects in the CAD and control groups were 81.5% and 53.5%, respectively; the risk of CAD was 3.8 times greater in men compared with women. A large body of epidemiological and pathological studies indicates that diabetes is an independent CAD risk factor for both women and men. Diabetic women lose their natural protection against CVD, which increases their tendency to acquire CVD. Furthermore, the cause of death of 65% of the individuals with diabetics is CVD[26]. Our study revealed that type 2 diabetes was present in 34.8% and 33.3% of the subjects in the CAD and control groups, respectively. Although the percentage of diabetics was greater in the CAD group, the inter-group difference in the rate of diabetes did not reach a statistical significance. Hypertension is one of the independent CAD risk factors, which makes the most common and strong contribution to the development of CAD, which is the leading cause of death in the United States of America. Hypertension increases the risk of atherosclerotic cardiovascular diseases by 2-3 folds[27]. Our study showed no significant difference between the study groups concerning the rate of hypertension. We compared the TC, LDL-C, VLDL-C and FBG levels in the control and CAD groups but found no significant difference regarding these parameters. The HDL-C level was significantly higher in the control group. Among the subjects in the control group, 32.3% were using antidiabetic drugs, 59.6% antihypertensives, and 26.3% statins; among those in the CAD group, 30.4% were using antidiabetic drugs, 72.8% antihypertensives and 48.9% statins (Statistical data on medication use was presented as a poster at the 4th Çukurova International Scientific Research Congress, Adana, 2020). We believe that the findings indicating that diabetes, hypertension and lipid parameters did not statistically increase the CAD risk stem from a high prevalence of medication use. When we compared serum KLF2 levels by the number of occluded vessels, we found a KLF2 level of 43.4 pg/mL in subjects with one occluded vessel, 50.3 pg/mL in those with two occluded vessels and 51 pg/mL in those with three or more occluded vessels. Although the KLF2 level increased in proportion to the number of occluded vessels, the inter-group differences were statistically non-significant.

It is estimated that the genetic contribution to the development of CAD is 20-60%, with genetic factors having a greater contribution to premature CAD [28]. Studies to date have established the significance of many loci and genes for CAD. Based on the hypothesis that the gene encoding the KLF2 protein affects CAD development due to its regulatory functions in endothelial biology, we compared the

genotype and allele frequency distributions of the C1239A variants. The rate of CC genotype (73.9%) was higher in the CAD group compared with that in the control group (71.7%), but the difference was statistically non-significant. Additionally, it was demonstrated that the rate of the C allele was more significant in the control group (51.6% vs. 48.4%) than in the CAD group, but the difference was not significant. Two subgroups as homozygous normal (CC) and KLF2 variant (AC+CC) were formed in the CAD group. The subgroups were then compared regarding age, lipid profile and FBG levels and no significant difference was found for any parameter. A comparison of the homozygous normal (CC) and the KFL2 variant (AC+CC) subgroups in the CAD group concerning sex, diabetes, hypertension and smoking revealed no significant difference. A comparison of the CC, AC and AA genotypes in the CAD group concerning serum KFL2 levels failed to show any significant difference, either. The CAD group formed two subgroups of homozygous normal (CC) and KFL2 variant (AC+CC) to examine the relationship between KFL2 C1239A genotypes and the number of occluded vessels. No significant difference was found between the subgroups.

Our literature review found no study to date that examined KFL2 gene variations in CAD. However, there are studies related to obesity, diabetes, and hypertension, which are the independent CAD risk factors. Among these studies, Meirhaeghe et al. showed that KFL2 gene variations were not related to obesity[29]. Gutierrez-Aguilar et al. reported that KFL2 gene variations were not in a relationship with type 2 diabetes[30]. A study conducted by Eichsteadt et al. on hereditary pulmonary arterial hypertension reported that, apart from the known disease-related gene variations, a mutation that is located in the KFL2 gene region and causes an amino acid change in the zinc finger motive was related to the disease[31].

Limitation of our study is that it can not represent the profile of the entire country, as data from only one center was used.

In conclusion, unveiling genetic risk factors for coronary artery diseases can help determine novel diagnostic and therapeutic approaches. Thus, it is believed that further studies examining the KFL2 gene region with larger study groups and the series analysis method will contribute to revealing the genetic background of CAD.

Author Contributions: Concept/Design : UD, SY; Data acquisition: UD, DÇY, MA; Data analysis and interpretation: ROÖ, BT; Drafting manuscript: UD, MY, SY; Critical revision of manuscript: MY, SY; Final approval and accountability: UD, MY, MA, AEY, DÇY, ROÖ, BT, SY; Technical or material support: DÇY; Supervision: SY; Securing funding (if available): n/a.
Ethical Approval: This study was approved with the decision of Mersin University Clinical Research Ethics Committee dated 08.03.2018 and numbered 2018/122.
191 individuals who underwent coronary angiography at Mersin University Faculty of Medicine, Department of Cardiology, Health, Research and Application Center were included in the study. Written informed consent was obtained from each participant, which was approved by the Mersin University Clinical Research Ethics Comittee.
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