KLİNİK ÇALIŞMA / ORIGINAL ARTICLE

Association of *APOA5-1131T>C* polymorphism with obesity in coronary artery disease

APOA5-1131T>C polimorfizminin koroner arter hastalarında obezite ile ilişkisi

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ABSTRACT

Objective: Genetic risk factors that cause coronary artery disease (CAD) demonstrate variations in different populations. In this study, a single nucleotide polymorphism in the *APOA5* gene was targeted to determine genetic contributors to atherosclerotic CAD. The effects of this polymorphism on the development of CAD and known risk factors of the disease were examined.

Methods: A total of 448 patients with angina or acute myocardial infarction who underwent coronary angiography were grouped as individuals with normal coronary arteries (≤30% stenosis) and critical disease (≥50% stenosis). The angiographic severity and the extent of atherosclerotic CAD were assessed using the Gensini and SYNTAX scores. Individuals were genotyped for the *APOA5*–1131T>C polymorphism using hydrolysis probes and the results were evaluated.

Results: The *APOA5*–1131T>C polymorphism was found associated with the serum lipid levels in the non-CAD group ($p<0.05$). In addition, the effect of *APOA5* gene polymorphism on clinical status and other parameters was determined to vary depending on gender. A borderline association was found between *APOA5* –1131T>C and type 2 diabetes mellitus ($p=0.055$). This polymorphism was found to be associated with obesity and it was observed that the *APOA5* -1131C allele carriers had a reduced risk for obesity ($p<0.05$). Logistic regression analysis adjusted for age and gender indicated that *APOA5* -1131C allele carriage had a protective effect against obesity in the study group (odds ratio: 0.48, 95% confidence interval: 0.29-0.78; $p=0.003$).

Conclusion: In this study, the *APOA5* gene polymorphism, one of the genetic factors that may lead to atherosclerotic CAD, was found to be associated with obesity. The *APOA5* –1131T>C polymorphism was associated with important risk factors for CAD, obesity, and serum lipid levels.

ÖZET

Amaç: Koroner arter hastalığına (KAH) yol açan genetik risk faktörleri toplumlar arasında farklılıklar göstermektedir. Aterosklerotik KAH oluşumuna neden olan genetik faktörlerin belirlenmesi amacıyla yapılan bu çalışmada, *APOA5* aday genindeki bir tek nükleotid polimorfizminin (SNP) KAH ve KAH risk faktörleri üzerindeki etkisi araştırıldı.

Yöntemler: Anjina veya akut miyokart enfarktüsü nedeniyle koroner anjiyografi yapılan 448 birey normal koroner arter (koroner lezyon ≤30 darlık) ve anlamlı KAH (≥1 koroner lezyon ≥50 darlık) taşımalarına göre iki gruba ayrıldı. Gensini ve SYNTAX skorları ile KAH'ın ciddiyeti ve yaygınlığı değerlendirildi. Bireyler *APOA5* –1131T>C polimorfizmi için hidroliz probları kullanılarak genotiplendi ve sonuçlar incelendi.

Bulgular: Çalışmamızda, anlamlı KAH olmayan grupta, seçilen *APOA5* gen varyantı serum lipit düzeyleri ile ilişkili bulundu ($p<0.05$). Ayrıca, *APOA5* gen polimorfizminin incelenen klinik durum ve diğer parametreler üzerine olan etkisinin cinsiyete bağlı olarak değişkenlik gösterdiği belirlendi. *APOA5* genindeki bu polimorfizm ve tip 2 diabetes mellitus arasında sınırda bir ilişki saptandı ($p=0.055$). Bu polimorfizmle obezitenin ilişkili olduğu ve *APOA5* –1131C allelini taşıyanlarda obezite riskinin azaldığı saptandı ($p<0.05$). Lojistik regresyon analizinde yaş ve cinsiyete göre ayarlama yapıldığında, nadir allel taşıyıcılığının tüm grupta obeziteye karşı koruyucu etkiye sahip olduğu gözlendi (odds oranı: 0.48, %95 güven aralığı: 0.29-0.78, $p=0.003$).

Sonuç: Çalışmamızda, aterosklerotik KAH gelişimine yol açabilecek önemli genetik faktörlerden biri olarak gösterilen *APOA5* gen polimorfizminin çalışma grubunda obezite ile ilişkisi saptanmıştır. *APOA5* –1131T>C polimorfizmi obezite ve lipit düzeyleri gibi KAH'ın önemli risk faktörleri ile ilişkilendirilmiştir.

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Abbreviations:

ApoA-V	*Apolipoprotein A-V*
APOA5	*Apolipoprotein A5 gene*
BMI	*Body Mass Index*
HDL	*High-density lipoprotein*
HDL-C	*HDL cholesterol*
CAD	*Coronary artery disease*
LDL-C	*Low-density lipoprotein cholesterol*
LPL	*Lipoprotein lipase*
OR	*Odds ratio*
TG	*Trigliserid*
VLDL	*Very low-density lipoprotein*

The apolipoprotein A5 gene (*APOA5*) is a member of the *APOA1-C3-A4* gene cluster located in 11q23.[1] Apolipoprotein AV (ApoA-V), an important regulator of serum triglyceride (TG) levels, is encoded by the *APOA5* gene and is expressed mostly in hepatocytes.[1–4] ApoA-V protein is an important component of lipoproteins such as high-density lipoprotein (HDL), and very-low-density lipoprotein (VLDL). [5] The lipoprotein lipase (LPL) enzyme involved in TG metabolism regulates the supply of free fatty acids to peripheral tissues. LPL is an enzyme responsible for determining plasma TG and HDL cholesterol (HDL-C) levels and providing hydrolysis of TG-rich particles that are effective in the atherosclerotic process. The LPL enzyme activated by ApoA-V decreases plasma TG levels by increasing the degradation of TG-rich lipoproteins.[5–7] High plasma TG levels have been observed to be an independent risk factor for vascular occlusion in many studies.[8]

Frequently analyzed and found in the promoter region of the *APOA5* gene, -1131T>C (rs662799) polymorphism was found associated with plasma TG, HDL-C, and low-density lipoprotein cholesterol (LDL-C) levels in various populations.[2,9] Turkish Adult Risk Factor (TARF) study conducted in the Turkish population, the non-carriage of *APOA5*-1131T>C and c.56C>G common alleles were shown to be a risk factor for dyslipidemia and metabolic syndrome. In the same study, when the combined effect of -1131T>C and c.56C>G mutations in the gene was analyzed, it was reported that women with the TC haplotype had higher plasma HDL-C and lower TG levels than noncarriers.[10] In another study, it was shown that the *APOA5* -1131T>C polymorphism is a risk factor for obesity. Besides, it was reported that the TC haplotype of -1131T>C and c.56C>G polymorphisms has a protective effect against obesity.[11] In functional studies, it was observed that overexpression of *APOA5* in transgenic mice results in lower TG levels. Moreover, APOA-V knockout mice had shown higher plasma TG concentrations.[2] Also, *APOA5*-1131T> C polymorphism was found associated with TG levels in different ethnic populations.[12–16] These findings indicate that the *APOA5* gene plays an important role in the regulation of plasma TG levels. In addition, it has been reported that the *APOA5*-1131T>C polymorphism is associated with the risk of coronary artery disease (CAD) in many ethnic populations due to its association with hypertriglyceridemia.[17–20]

Based on these results, in this study, we aimed to investigate the association between *APOA5*–1131T>C polymorphism, which has a very remarkable role in TG metabolism, with obesity and other risk factors in patients with angiographically defined CAD.

METHODS

Study population

The study group comprised 448 consecutive individuals who underwent invasive coronary angiography at Ufuk University Cardiology Department between 2015 and 2017.

Gensini and SYNTAX scores of individuals that undergone coronary angiography were calculated. The Gensini score was calculated as previously described by assigning a severity score to each lesion according to the degree of stenosis in the epicardial coronary arteries and its localization.[21] In addition, the SYNTAX score was calculated using a score calculator (version 2.28, www.syntaxscore.com) to evaluate the severity and prevalence of CAD for the individuals in the patient group. A high Gensini score indicates that the disease is severe and prevalent, while a high SYNTAX score indicates more complex CAD.[22,23]

As a result of coronary angiography which carried out according to the findings of clinical and non-invasive ischemia tests, the participants included in the study were grouped into individuals with significant CAD (coronary lesion with 50-100% stenosis, n=249) and non-CAD (with angiographically normal coronary arteries or coronary lesions with <30% stenosis n=199).

In the selection of the individuals included in the study, in addition to the coronary artery stenosis score, the presence of family history in participants with significant CAD, smoking, diabetes, the levels of biochemical parameters such as glucose hemoglobin (HbA1c) and fasting blood glucose was also taken into consideration. In the non-CAD group, age (≥55 years), not having a family history of CAD, and

cerebrovascular/peripheral artery disease were taken into consideration, as well as the rate of stenosis in the coronary artery. The definitions of cerebrovascular/peripheral artery disease were made according to the criteria of the European Society of Cardiology.[24]

For the other classification of the study, 448 individuals were grouped as obese (n=172) and non-obese (n=266) regarding their obesity status. In addition, as subgroups of the study, both significant CAD and non-CAD groups were classified according to the obesity status. Prior to the study, approval was obtained from the Istanbul University Istanbul Faculty of Medicine Clinical Research Ethics Committee, and the individuals included in the study signed an informed voluntary patient consent form.

Exclusion Criteria

Individuals with a history of previous coronary bypass graft surgery, ongoing decompensated heart failure (New York Heart Association Functional Class IV), advanced liver or kidney failure, a life expectancy of less than one year, and a known malignancy, and infectious, inflammatory, or rheumatologic disease were excluded from the study. Also, individuals with moderate coronary artery stenosis (stenosis >30% and <50%) were excluded in terms of better comparison between significant CAD and non-CAD groups. To better evaluate genetic factors other than family history, individuals with a positive family history of CAD in the non-CAD group were excluded from the study.

Measuring the risk factors

Participants were evaluated in terms of their first and second-degree relatives by the family history criteria. Presence of first and second-degree relatives (males <55 years, females <65 years) that are diagnosed with CAD was determined as a positivity criterion for the family history. Hypertension, one of the risk factors for CAD, was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg obtained in two different measurements in accordance with the criteria of the European Society of Cardiology. Smokers were classified as positive regardless of how many cigarettes consumed per day whereas individuals who had never smoked, or not smoked for at least one year before sampling were classified as negative for smoking. Bodyweight was measured with underwear and without shoes. Body mass index (BMI) was calculated by dividing body weight in kilograms by the square meter of height (kg/m^2). In the obesity classification, individuals with a body mass index of ≥30kg/m^2 were considered obese.

Biochemical analysis

Blood samples were collected before coronary angiography and stored at -80°C until analyzed. Any lipemic, icterus, and hemolysis specimens were excluded from the study. Analyzes of biochemical parameters were performed in two central laboratories. Total cholesterol, fasting TG, HDL-C, and LDL-C concentrations were determined using the UniCell DxC 800 (Beckman Coulter, USA) instrument. Fasting glucose levels were measured by the spectrophotometric method using the Hexokinase/G-6-PDH reaction principle with the Abbott c8000 Architect autoanalyzer (Abbott Lifesciences, USA). HbA1c levels were analyzed in the High-Performance Liquid Chromatography (HPLC) system, the results were given as the percentage of glycosylated hemoglobin to total hemoglobin (Hb%).

Genotyping by RT-PCR

DNA samples of 448 individuals included in the study were used for the determination of the effect of *APOA5* gene polymorphism. For the sample collection process, 10 ml of peripheral blood samples were taken from the individuals into tubes containing EDTA. Quality quantitation of DNA isolated from peripheral blood via the inorganic method was carried out using Nanodrop™ 2000 (Thermo Scientific™, USA).[25] Individuals in significant CAD and non-CAD groups were genotyped via LightCycler® 480 (Roche, Germany) device using labeled hydrolysis probes by the Real-Time PCR method.

Genotyping was performed using 1 μl DNA sample (50 ng), 5 μl LightCycler® 480 Probes Master Mix (Roche, Germany), 0.2 μl forward and reverse primers, 1 μl FAM and Yakima Yellow labeled specific hydrolysis probes, and 1.6 μl dH_2O under the following conditions: 2 minutes at 50°C (pre-incubation), 10 minutes at 95°C (denaturation) and 45 cycles of 10 seconds at 95°C, 30 seconds at 56°C and 1 second at 72°C (amplification).

Statistical Analysis

Prior to the statistical analysis, each variable was examined in terms of the normal distribution, the logarithmic transformation was applied to the TG and HbA1c

variables that are not normally distributed. Following the transformation, the distributions of these two variables were re-analyzed and it was determined that they are normally distributed. Categorical variables in groups were compared with the chi-square test, while quantitative variables were compared with Student's t-test, and the general linear model was used to adjust the covariants. Quantitative values were expressed as mean±standard deviation (SD), and nominal values as in percent (%). Logistic regression analysis was performed adjusted according to age, sex, and genotype distribution, in order to determine the odds ratio (OR) and corresponding 95% confidence intervals (CI) for models. Hardy–Weinberg equilibrium (HWE) was computed to the expected genotype distribution. A *p-value* <0.05 was considered statistically significant. All statistical calculations were made using Windows SPSS 21.0 (IBM, USA) program.

RESULTS

In the study, 448 individuals (mean age; 60.5±11.7, 56.9% male) were genotyped for –1131T>C polymorphism in the promoter region of the *APOA5* gene. The clinical features and biochemical parameters of individuals that are grouped according to the significant CAD status were summarized in Table 1.

Association of *APOA5* –1131T>C polymorphism with CAD

In the significant CAD group, the genotype distributions of the *APOA5* –1131T > C polymorphism were 79.1% (n=197), 16.9% (n=42) and 4.0% (n=10) for TT, TC and CC, respectively. The frequency of the C allele was determined as 12.5% in the significant CAD group. Comparing significant CAD and non-CAD groups, no statistically significant difference was observed in genotype distributions and allele frequencies (Table 2). Furthermore, logistic regression analysis adjusted by age and sex revealed that C allele carriage had no effect on the significant CAD development (OR: 0.69, CI 95%: 0.43–1.1, *p=0.11*). For further testing, subclassifications were made according to type 2 diabetes mellitus (T2DM) status in significant CAD and non-CAD groups. In the analyses, the CC genotype was observed less frequently in T2DM patients in the non-CAD group, although this was not statistically significant (Table 3, *p=0.055*).

Table 1. Distribution of biochemical parameters and clinical features in the significant CAD and non-CAD groups

Characteristics	Significant CAD (n=249)	Non-CAD (n=199)	*p-value*
	Mean±SD; % (n)	Mean±SD; % (n)	
Age (year)	63.4±10.6	57.6±11.7	*<0.001*
Body mass index (kg/m^2)	29.0±4.2	28.4±3.9	*<0.001*
Total Cholesterol (mg/dL)	194.3±46.3	203.6±49.2	*0.033*
HDL-Cholesterol (mg/dL)	40.1±11.1	42.1±10.0	*0.039*
LDL-Cholesterol (mg/dL)	112.7±36.4	119.6±36.9	*0.042*
Triglyceride (mg/dL)[¤]	144.4±1.71	141.8±1.66	*0.707*
Fasting glucose (mg/dL)	124.1±45.5	111.1±39.0	*0.001*
HbA1c (%)[¤]	6.33±1.22	5.8±1.19	*<0.001*
SYNTAX Score	16.6±11.8	0.01±0.15	*<0.001*
Gensini Score	57.1±48.8	2.0±3.2	*<0.001*
Stenosis (%)	80.0±16.6	9.4±10.2	*<0.001*
Hipertension*	50.9 (139)	43.1 (91)	*0.089*
Obesity*	40.7 (111)	35.7 (75)	*0.268*
Smoking*	36.1 (99)	43.4 (92)	*0.104*
Lipid-lowering drug usage*	45.2 (123)	29.7 (63)	*0.001*
Antidiabetic drug usage*	39.1 (107)	23.1 (50)	*<0.001*

[¤]Logarithmic values were transformed into geometric values. *Shown as % (n). CAD: Coronary artery disease; HbA1c: Hemoglobin A1c; HDL-cholesterol: High-density lipoprotein cholesterol; LDL-Cholesterol: Low-density lipoprotein cholesterol; n: number of individuals; SD: Standard deviation.

Table 2. ***APOA5*** **-1131T>C genotype distributions and allele frequencies according to CAD and obesity status**

	Total						Non-CAD			Significant CAD		
	CAD (n=249)	Non-CAD (n=199)	*p-value*	Obese (n=172)	Non-obese (n=266)	*p-value*	Obese (n=69)	Non-obese (n=123)	*p-value*	Obese (n=103)	Non-obese (n=143)	*p-value*
TT	79.1	72.4	*0.236*	83.7	71.8	***0.013***	85.5	65.9	***0.010***	82.5	76.9	*0.119*
TC	16.9	21.6		12.2	22.9		13.0	26.0		11.7	20.3	
CC	4.0	6.0		4.1	5.3		1.5	8.1		5.8	2.8	
TC + CC	20.9	27.6	*0.960*	16.3	28.2	***0.004***	14.5	34.1	***0.045***	17.5	23.1	*0.600*
C allele frequency	12.5	16.8	*0.060*	10.2	16.8	***0.001***	7.5	21.1	***0.043***	5.9	13	*0.335*

Genotype distributions and allele frequencies are shown as percentage. n: number of individuals; CAD: Coronary artery disease.

Table 3. ***APOA5*** **-1131T>C genotype distributions and allele frequencies according to CAD and T2DM status**

	Non-CAD			Significant CAD		
	T2DM (n=62)	Non-T2DM (n=137)	*p-value*	T2DM (n=124)	Non-T2DM (n=125)	*p-value*
TT	77.4	70.1	*0.055*	78.2	80.0	*0.802*
TC	22.6	21.2		16.9	16.8	
CC	0.0	8.8		4.8	3.2	
TC + CC	22.6	29.9	*0.283*	21.8	20.0	*0.731*

Genotype distributions and allele frequencies are shown as percentage. n: number of individuals; CAD: Coronary artery disease; T2DM: Type 2 diabetes mellitus.

Association of *APOA5* –1131T>C polymorphism with cardiometabolic risk

It was observed that total cholesterol, LDL-C, and TG levels were higher and BMI values were lower in C allele carrier non-CAD individuals (Table 4, $p<0.05$), compared to significant CAD patients. For further testing, univariate analyses were performed adjusted to BMI, age, lipid-lowering, and antidiabetic drug usage. In these analyses, it was observed that the total cholesterol and TG levels were significantly higher in -1131C allele carrier non-CAD individuals whereas the BMI was lower (Table 5, $p<0.05$).

In sex-specific analyses, a statistically significant association was observed between plasma lipid levels, BMI, and the *APOA5* –1131T>C polymorphism. BMI was found lower in both male and female minor allele carriers in the non-CAD group (Table 6, $p<0.05$). In addition, in minor allele carrier non-CAD individuals, total cholesterol was found significantly higher in males and females, while LDL-C and TG were significantly higher only in females (Table 6, $p<0.05$). In the significant CAD group, there was no significant association between these parameters and the *APOA5* –1131T>C polymorphism.

Association of *APOA5* –1131T>C polymorphism with obesity

By classifying the whole study group into obese and non-obese, the genotype distributions of *APOA5* –1131T>C polymorphism were examined. TT and TC+CC genotypes in the non-obese group were 71.8% (n=191) and 28.2% (n=75), respectively. The *APOA5* –1131C allele carriage was found in higher frequency in the non-obese group (Table 2, $p=0.004$). In addition to this finding, the frequency of the C allele was 16.8% in the non-obese group. When the obese and non-obese groups were compared, it was observed that the frequency of the C allele was higher in the non-obese group (Table 2, $p=0.001$).

Genotype distributions and allele frequencies of *APOA5* –1131T>C polymorphism were examined in

Table 4. Association of *APOA5* -1131T>C polymorphism with cardiometabolic risk factors in CAD study group

	Non-CAD			Significant CAD		
	TT	TC+CC		TT	TC+CC	
	Mean±SD	Mean±SD	*p-value*	Mean±SD	Mean±SD	*p-value*
Stenosis	10.1±10.8	8.7±9.0	*0.414*	79.6±16.5	83.8±16.2	*0.111*
Genesini	2.3±3.5	1.5±2.0	*0.132*	57.6±49.1	52.3±46.1	*0.482*
SYNTAX	0.0±0.2	0.0±0.0	*0.407*	16.3±11.5	17.5±12.3	*0.543*
T-Cholesterol (mg/dL)	196.2±47.0	219.2±52.2	***0.004***	192.3±42.2	203.5±58.9	*0.126*
LDL-C (mg/dL)	115.1±35.6	127.6±52.2	***0.036***	111.7±33.4	119.0±43.4	*0.195*
HDL-C(mg/dL)	42.0±10.4	42.2±10.0	*0.874*	39.8±11.2	39.9±10.2	*0.944*
Triglyceride (mg/dL)[¤]	133.4±1.6	161.5±1.7	***0.016***	145.1±1.7	154.3±1.7	*0.460*
HbA1c (%)[¤]	5.8±1.2	5.7±1.2	*0.579*	6.4±1.2	6.2±1.2	*0.328*
Glucose (mg/dL)	113.4±43.6	107.4±30.7	*0.358*	125.9±49.9	126.5±40.4	*0.945*
BMI (kg/m^2)	29.0±4.0	27.0±3.5	***0.001***	29.1±3.9	29.3±5.5	*0.719*

[¤]Logarithmic values were transformed into geometric values. BMI: Body mass index; HbA1c: Hemoglobin A1c; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; T-Cholesterol: Total cholesterol; SD: Standart deviation.

Table 5. Association of the *APOA5* -1131T>C polymorphism with cardiometabolic risk factors in covariance analysis

	Non-CAD			Significant CAD		
	TT	TC+CC		TT	TC+CC	
	Mean±SD	Mean±SD	*p-value*	Mean±SD	Mean±SD	*p-value*
T-Cholesterol (mg/dL)	196.1±4.1	218.0±6.9	***0.009***	192.5±3.1	203.8±6.2	*0.104*
LDL-C (mg/dL)	115.3±3.2	126.1±5.3	*0.089*	112.0±2.5	119.3±4.8	*0.182*
Triglyceride (mg/dL)[¤]	133.4±1.0	164.8±1.9	***0.008***	145.2±1.0	153.1±1.1	*0.510*
BMI (kg/m^2)	29.0±0.32	27.0±0.55	***0.002***	29.1±0.31	29.3±0.59	*0.757*

[¤]Logarithmic values were transformed into geometric values. Values were shown as mean±standard deviation. Adjusted by BMI, Age, lipid-lowering, and anti-diabetic drugs. BMI: Body mass index; LDL-C: Low-density lipoprotein cholesterol; T-Cholesterol: Total Cholesterol; SD: Standard deviation.

groups where CAD and obesity were evaluated together. Minor allele carriage was found in a higher frequency in non-obese individuals in the non-CAD group (Table 2, *p=0.045*).

Moreover, in logistic regression analysis adjusted for age and sex, the minor allele carriage was observed to have a protective effect against obesity in the whole group, (OR: 0.48, CI 95%: 0.29–0.78, *p=0.003*) (Table 7). When logistic regression analyses adjusted according to age was performed in the whole group classified by sex, it was determined that *APOA5* -1131T>C allele carriage had a protective effect against obesity in males (OR: 0.43, CI 95%: 0.21–0.87, *p=0.019*) (Table 7).

DISCUSSION

In this study, the effect of *APOA5* -1131T>C polymorphism on CAD development in patients who underwent coronary angiography was investigated along with other cardiovascular risk parameters. When the obese and non-obese groups were compared, the C allele carriage was found in lower frequency in the obese group. According to the results of our study, it is thought that the *APOA5* -1131T>C polymorphism in the significant CAD group may affect the risk of CAD through its protective impact on obesity.

The frequencies of the *APOA5* -1131T>C polymorphism vary among populations. *APOA5* –1131T>C

Table 6. Association of *APOA5* -1131T>C polymorphism with cardiometabolic risk factors in CAD study group stratified according to sex

	Non-CAD					
	Male			Female		
	TT	TC+CC		TT	TC+CC	
	Mean±SD	Mean±SD	*p-value*	Mean±SD	Mean±SD	*p-value*
Stenosis	11.5±11.4	8.3±9.2	*0.218*	8.9±10.3	9.0±9.1	*0.935*
Genesini	3.0±4.4	1.3±1.8	*0.061*	1.7±2.5	1.7±2.1	*0.917*
SYNTAX	0.1±0.3	0.0±0.0	*0.416*	0.0	0.0	–
T-Cholesterol (mg/dL)	189.2±39.2	212.3±57.6	***0.038***	201.8±51.9	224.4±47.9	***0.040***
LDL-C (mg/dL)	113.6±29.1	120.4±42.1	*0.405*	116.3±40.2	133.2±37.7	***0.049***
HDL-C (mg/dL)	38.0±8.7	38.3±11.0	*0.915*	45.1±10.6	45.3±8.0	*0.943*
Triglyceride (mg/dL)¤	142.3±1.6	164.3±1.9	*0.264*	126.7±1.6	159.4±1.5	***0.022***
HbA1c (%)¤	5.8±1.2	5.5±1.2	*0.176*	5.8±1.2	5.9±1.2	*0.746*
Glucose (mg/dL)	109.2±26.2	105.8±32.6	*0.618*	116.8±53.6	108.8±29.6	*0.438*
BMI (kg/m^2)	28.6±4.2	26.3±3.5	***0.025***	29.4±3.8	27.5±3.5	***0.019***
	Significant CAD					
Stenosis	80.7±15.9	83.2±16.9	*0.428*	77.4±17.7	84.6±15.4	*0.106*
Genesini	60.7±50.0	56.9±47.1	*0.700*	51.2±47.0	45.0±44.7	*0.602*
SYNTAX	17.2±11.3	18.1±12.5	*0.677*	14.5±11.8	16.4±12.3	*0.554*
T-Cholesterol (mg/dL)	185.5±43.2	194.9±59.0	*0.310*	206.6±36.3	217.9±57.4	*0.308*
LDL-C (mg/dL)	106.7±33.1	114.3±44.7	*0.279*	122.2±31.8	126.9±41.2	*0.601*
HDL-C (mg/dL)	37.6±10.8	39.4±11.4	*0.408*	44.4±10.6	40.8±8.1	*0.370*
Triglyceride (mg/dL)¤	145.5±1.7	158.6±1.8	*0.436*	144.2±1.6	147.2±1.5	*0.839*
HbA1c (%)¤	6.3±1.2	5.8±1.1	*0.061*	6.7±1.3	6.9±1.2	*0.864*
Glucose (mg/dL)	121.4±44.4	115.5±31.3	*0.487*	135.5±59.0	143.5±47.3	*0.585*
BMI (kg/m^2)	28.9±4.0	28.9±5.1	*0.918*	29.6±3.7	29.9±6.3	*0.751*

¤Logarithmic values were transformed into geometric values. BMI: Body Mass Index; HbA1c: Hemoglobin A1c; HDL-C: High-Density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; T-cholesterol: Total cholesterol; SD: Standart deviation.

Table 7. Effects of the *APOA5* -1131T>C genotype for obesity on logistic regression classified by sex

	Total		Male		Female	
Model	OR (CI 95%)	*p-value*	OR (CI 95%)	*p-value*	OR (CI 95%)	*p-value*
Gender (female)	0.69 (0.47–0.103)	*0.069*				
Age (year)	1.01 (0.99–1.028)	*0.200*	1.01 (0.99–1.03)	*0.383*	1.01 (0.98–1.04)	*0.321*
APOA5 TC+CC[1]	0.48 (0.29–0.78)	***0.003***	0.43 (0.21–0.87)	***0.019***	0.53 (0.27–1.01)	*0.072*

Regarding obesity, the genotype interaction of APOA5 -1131T>C polymorphism by gender was found significant (p<0.001). [1]For TC + CC genotypes, the TT genotype is considered as a reference. In the analyses, 438 individuals (172 obese) in the whole group, 249 male (89 obese), and 189 female (83 obese) were examined. OR: Odds ratio; CI: Confidence interval.

polymorphism frequency was found to be 26-30% in East Asians,[26,27] 6–9%[18,27] in Caucasians and 12.8% in Turkish population.[28] A comprehensive meta-analysis study revealed that CC and TC genotypes are observed in a higher frequency in Asian populations compared to other populations.[29]

Many studies showed that the *APOA5* -1131T>C polymorphism is significantly associated with CAD risk in terms of TG levels. These studies showed that minor allele carriers have higher levels of fasting and nonfasting TG levels.[1,30] The percentage rate of the effect of rare allele carriers on TG levels varies in Caucasians (30%), Chinese (36%), and Turks (60%).[28,31,32] In the present study, it was determined that TG levels were higher in carriers of the -1131C allele in the non-CAD group when compared to non-carriers.

In a meta-analysis study, the minor allele of the *APOA5* -1131T>C polymorphism was found to be a risk factor for CAD, especially in the Chinese population. It was reported that large-scale studies carried out in different populations are needed to better assess the effect of this polymorphism on gene-gene and gene-environmental interactions and CAD risk.[1] A study conducted in the Japanese population investigating the effect of *APOA5* -1131T>C polymorphism on lipid levels and CAD risk showed that it was strongly associated with TG levels and CAD risk.[33] The low ApoA-V protein level observed in *APOA5* -1131C allele carriers is thought to increase the risk of CAD through the mechanism responsible for the regulation of serum TG level.[34,35] In the study of Jang et al.[35] it was observed that plasma ApoA-V levels were lower for the individuals carrying the CC genotype in both control and CAD patients. Also, it was shown that TG levels were higher for the -1131C allele carriers in both groups. In our study, comparing the individuals with and without significant CAD in terms of their rare allele carriage, no significant difference was observed between the two groups.

Contrary to the abovementioned studies, it is also stated that -1131T>C polymorphism may have a protective role against CAD. In the study of Furuya et al.,[36] the relationship between -1131T>C polymorphism and lipid changes and CAD was investigated. As a result of the study, the C allele carriers were found to have lower LDL-C levels. Since elevated LDL-C levels are strongly associated with the risk of CAD, it is also thought that this polymorphism may have a protective effect against CAD.[36,37]

In the TARF study conducted in our country, it was stated that the non-carriage of TC haplotype of *APOA5* -1131T>C and c.56C>G polymorphisms is risk factors for dyslipidemia and metabolic syndrome in Turkish women. Women carrying the TC haplotype for these polymorphisms were found to exhibit higher HDL-C and lower TG levels. Supporting this result, lower HDL-C and higher TG levels were observed in women with the *APOA5* -1131C allele.[10] In our study, in minor allele carrier females, the higher TG levels observed in the non-CAD group supports the findings of the TARF study.

In another study, the effect of *APOA5* -1131T>C polymorphism on plasma lipid properties and BMI value was investigated in hyperlipidemic and overweight male individuals who followed a low-fat diet. It was observed that minor allele carrier individuals had higher TG levels before and after the diet than individuals with TT genotype. After the diet, minor allele carriers had a 20.6% decrease in TG levels and were reported to have lost more weight compared to individuals with TT genotype.[38] Similar to this study, as a result of the grouping by the significant CAD status in our study, it was observed that minor allele carriage was protective in terms of BMI value in the non-CAD group.

To date, several studies have shown that variations in the *APOA5* gene play a potential role in the development of obesity.[39,40] However, it is known that the results vary between different populations. The -1131T>C polymorphism in the *APOA5* gene is strongly associated with an increased risk of obesity in the Moroccan population. It was also stated in the same study that having common allele haplotypes in terms of two APOA5 polymorphisms had a protective effect against obesity.[11] On the contrary, in another study that investigated the effect of the -1131T>C polymorphism on obesity risk and total fat intake, a gene-diet interaction was found. It was observed that the C allele carriers who followed a high-fat diet had lower weight gain compared to those who did not.[40] In another study, it was found that the *APOA5* -1131C allele has a protective effect against obesity and higher TG levels, two important CAD risk factors in individuals following a high-fat diet. This supports the hypothesis that gene-diet interaction is one of the underlying causes of the association between the *APOA5* gene and cardiovascular disease risk.[41] Our study showed similar results to the abovementioned study due to the high frequency of the C allele in non-obese individuals observed both in the study population and non-CAD group. Our results suggest that this polymorphism may reduce the risk of CAD because

of its protective effect on obesity. However, these results show that the varied results between CAD and the *APOA5* gene seen across populations may be due to environmental factors, diet, ethnicity, and other genetic factors.

One of the most important limitations of our study is the small sample size of the significant CAD and non-CAD groups. In addition, even if no significant coronary stenosis was observed in individuals who were determined as a control group via coronary angiography, the control group does not fully represent healthy individuals and it can be assumed that non-CAD individuals have more additional risk factors compared to the general population. However, since invasive coronary angiography is not possible to be carried out in healthy individuals, those who did not undergo angiography cannot be assumed to be healthy. Moreover, the administration of the study from a single center prevents the results from reflecting the precise population of our country. By increasing the sample size and increasing the number of centers involved in the study to reflect the population of Turkey, the findings may reflect the characteristics of the Turkish population allowing more detailed analysis for the results of this polymorphism. It is known that the interactions of the polymorphism that we examined with other genetic factors may increase the risk of the disease or have a protective effect against the disease.[40] Another limitation of our study is that only a single polymorphism in the *APOA5* gene was examined. The precise determination of the effect of this variant will be possible by examination of other polymorphisms other than this one in the same gene or polymorphisms in the associated genes together.

As a result of this study, it was found that the *APOA5* gene variant was associated with serum lipid levels in the group without significant CAD. In addition, it was determined that the association between the *APOA5* -1131C allele and clinical outcomes along with other biochemical parameters varies by sex. *APOA5* -1131C allele carriage was found to reduce the risk of obesity. Moreover, in the logistic regression analysis, it was observed that minor allele carriage had a protective effect against obesity in the whole group. These results suggest that *APOA5* -1131T>C polymorphism has a protective effect against CAD by reducing the risk of obesity in our study group.

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