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Rs214101 Variation of NUCB2/Nesfatin-1 Gene: Effects on Metabolic Parameters Independent of Type 2 Diabetes or Obesity in Coronary Artery Disease

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Abstract

Nesfatin-1 (NUCB2) gene, was introduced as a novel satiety factor involves in the control of energy homeostasis in the hypothalamus. Since NUCB2/nesfatin-1 has an effect on diabetes and obesity, it is a candidate gene that can be responsible for causing coronary artery disease (CAD).Therefore, we aimed to identify the association between rs214101 (C/T) SNP of NUCB2 gene and the possible amplified effects of this genetic variation together on the development risk of CAD in this study.This study was carried out in 110 patients with CAD, and 69 CAD-free controls. The NUCB2/nesfatin-1 rs214101 genotypes were analysed by PCR-RFLP technique. The CC genotype frequency of the NUCB2 rs214101 was greater in control group than CAD group (34.8% vs.52.7%; P = 0,019).The CC-genotype was associated with low serum HDL-C level compared to T allele in female CAD subgroup (P = 0.031), but, not in males. Unlike the CAD group, it was observed that the male control subjects carrying CC genotype have higher serum HDL-cholesterol and diastolic blood pressure levels than those with T allele (P = 0.043 and P = 0.031, respectively) but, not in females. Logistic regression analysis confirmed that the NUCB2/Nesfatin-1 rs214101 CC-genotype is associated with low serum HDL-cholesterol in female CAD patients. Our results suggest that the CC genotype of NUCB2 rs214101 may contribute to susceptibility CAD risk in relation to low HDL-C and that the genetic effect could differ by gender.

Keywords: NUCB2/ Nesfatin-1, Gene, Atherosclerosis, Diabetes, Obesity

1. Background

Nesfatin-1 is processed by nucleobinding-2 (NUCB2) and it is a neuropeptide which is identified as a satiety molecule in hypothalamus (1, 2). It is composed of 396 amino acid with a remarkable sequence homology (about 85%) among mouse, rat and human species. NUCB2 gene codes three types of protein as nesfatin-1, Nesfatin-2, and Nesfatin-3. Results showed that intracerebroventricular injection of nesfatin-1 in rats reduces food intake and has an appetite suppressant effect. Nesfatin-1 has a role in central pathways that control food intake, while Nesfatin-2 and Nesfatin-3 have not an effect related to appetite. Nesfatin-1 is expressed in tissues, which are in the relationship with metabolism and energy homeostasis. It was observed that the glucose injection has increased the secretion of nesfatin-1 in rodents (3). Two important findings we have observed from these results are that nesfatin-1 has an appetite-reducing effect and that the anorectic effect could be observed even if it is the leptin resistance which plays a role in the melanocortin pathway (4) and there has been demonstrated a gender-specific association between the variation rs214101 A/G of nesfatin-1 and obesity (5).

Pathology of coronary artery disease (CAD) mainly relies on the deposition of lipids in the arteries that supplying blood to the heart, which may lead to stenosis and myocardial infarction. Circulating lipids such as low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) are commonly used clinical markers for CAD (6-9). Since obesity is one of the risk factors for coronary artery disease, the variations of Nesfatin-1 gene may play a role in the formation of atherosclerosis indirectly (10, 11). The variations that affect the Nesfatin-1 protein functions could be important in affecting lipid levels and development of CAD. There are many studies regarding the association of Nesfatin-1 gene variations with the obesity, childhood adiposity, metabolic syndrome, type2

diabetes or polycystic ovary syndrome (5, 12-15).

Several studies showed that there is an association between serum nesfatin-1 concentrations and development and severity of peripheral arterial disease (16), acute myocardial infarction (17), and hypertension (18). However, the effects of functional gene variations of NUCB2/Nesfatin-1 gene on CAD risk has not been studied yet.

Therefore, in this preliminary study we questioned the effects of the NUCB2 gene rs214101 on coronary artery disease risk. We also investigated whether combined effects of the NUCB2 rs214101 gene variation and atherosclerotic risk factors increase risk of developing CAD in the present study.

Figure 1. Genotyping for the rs214101 polymorphism in the NUCB2/Nesfatin-1 gene. DNA bands were visualized by using 3% agarose gel electrophoresis after digested by the SfaNI restriction enzyme (Fermentas). Lane 1: 50 bp DNA size marker (MBI Fermentas); lanes 2, 3: CT genotype (266, 187 and 79 bp); lanes 4 - 7: CC genotype (266 bp).

2. Methods

2.1. Subjects and Clinical Investigation

Sixty-nine Turkish healthy control subjects (48 men and 21 women; mean age, 58.77 $\pm$ 6.20 years) and one hundred and ten Turkish patients with CAD (70 men and 40 women; mean age 58.97 $\pm$ 11.09 years) were recruited in the study. Control group consists of healthy individuals who have not any symptoms of coronary artery disease, metabolic disorder (diabetes mellitus, kidney failure, liver failure etc.), lipid metabolism disorder and history of ischemic heart disease in the family. CAD group consists of subjects diagnosed with coronary artery disease who were in the follow-up of department of cardiology in Istanbul faculty of Medicine and all blood samples were classified and sent to the department of molecular Medicine by this unit. The patients with the severe coronary vascular disease were documented by angiography. Angiographic inclusion criteria were 50% stenosis of at least one major coronary vessel because of atherosclerosis, and a vascular event, defined as myocardial infarction, percutaneous transluminal coronary angioplasty, or coronary artery bypass grafting. All subjects were subjected to full history taking with the special emphasis on coronary risk factors including smoking, family history of coronary artery disease, hypertension, diabetes mellitus, and hyperlipidemia. CAD group includes subjects who has 40.90% hypertension, 44.54% diabetes and 34.54% left ventricular hypertrophy.

The study protocol was approved by both the local ethical committee and the scientific research projects coordination unit of our University (approval number: 2013/693, date: 18th June 2013). The protocol followed was consistent with the world Medical association declaration of Helsinki "ethical principles for Medical research involving human subjects". All participants received medical approval from their personal physicians and gave written, informed consent prior to giving their blood sample.

2.2. Genotyping

DNA samples for each subject were obtained from blood samples with DNA isolation kit (Roche) and were checked with ND-1000 spectrophotometer. DNA samples added to the reaction mixture (Fermentas) that was prepared according to the PCR protocol and amplifications were performed by using a thermal cycler (Applied Biosystems). The PCR-amplified fragments, which was 266 base pair, were digested by the SfaNI restriction enzyme (Fermentas) which cuts CC genotype. DNA bands were visualized and were checked by using agarose gel electrophoresis. DNA bands for the NUCB2/Nesfatin-1 polymorphism rs214101 (C/T) TT genotype are 187 and 79 bp, CC genotype is 266 bp and CT genotype is 266, 187 and 79 bp (Figure 1).

2.3. Statistical Analysis

We used SPSS software package (version 20.0 SPSS Inc., Chicago, IL, U.S.A.) for Windows to conduct all statistical analyses. Student's t-test was used to compare descriptive statistical methods (mean, standard deviation) such as blood pressures, lipid levels, and body mass index. Differences in the distributions of genotypes according to clinical phenotypes were assessed by using chi-square test in 2 $\times$ 2 tables to determine the relative risks, odds ratios and 95% confidence intervals. Parametric analyses were performed between patients and control subjects by the unpaired Student's t-test and ANOVA test.

Multivariate analysis was carried out by the linear logistic regression model. Estimation of the relative risk was determined by calculating odds ratios (OR) and confidence intervals (CI). For this analysis, we used to define

Table 1. Linear Logistic Regression Analysis for the Association Between Nesfatin-1 rs214101 CC Genotype, HDL-Cholesterol Level, Smoking, Blood Pressures and CAD Risk in Study Group (Level of Significance: P < 0.05)

	Odds Ratio	95% CI for OR	P Value
Nesfatin-1 rs214101 CC genotype	0.171	0.002 - 0.299	0.047
Serum HDL-cholesterol level	-0.149	-0.505 - 0.024	0.074
Smoking	0.216	0.042 - 0.334	0.012
SBP	0.183	-0.007 - 0.002	0.256
DBP	0.41	0.002- 0.019	0.012

Abbreviations: CI: Confidence Interval; DBP, Diastolic Blood Pressure; OR: Odds Ratio; SBP, Systolic Blood Pressure.

an association of NUCB2/ Nesfatin-1 rs214101 SNP among several independent factors. Coronary artery disease was used as dependent variable and NUCB2/Nesfatin-1 rs214101 CC genotype, serum HDL-cholesterol level, smoking and blood pressures were used as independent variables (Table 1).

Second multivariate logistic regression analysis was performed to assess the effects of rs214101 SNP on decreased serum HDL-cholesterol (serum HDL- cholesterol level classified as above or below 0.90 mmol/L) and smoking in patients with CAD including gender subgroups (Table 2). The analysis was used to identify an association of NUCB2/ Nesfatin-1 rs214101 SNP among several independent factors. In this regression analysis, NUCB2/ Nesfatin-1 rs214101 CC genotype and smoking status were used as independent variables. The model included decreased serum HDL-cholesterol (below 0.90 mmol/L) as the dependent variable was used.

Table 2. Binary Logistic Regression Analysis for the Association Between Nesfatin-1 rs214101 CC Genotype, Smoking and Low HDL-Cholesterol Level Risk in the CAD Group (Level of Significance: P < 0.05)

Variables	Odds Ratio (Exp(B))	95% CI for OR	P Value
Women			
Nesfatin-1 rs214101 CC genotype	7.558	1.045 - 54.654	0.045
Smoking	0.177	0.115 - 2.087	0.169
Men			
Nesfatin-1 rs214101 CC genotype	1.019	0.352 - 2.949	0.972
Smoking	0.978	0.308 - 3.105	0.970

Abbreviations: CI, Confidence Interval; OR, Odds Ratio.

3. Results

The baseline characteristics of the study population are given in Table 4. It was not found significant differences between the patients with coronary artery disease and controls, when age and sex distributions were investigated (P > 0.05).

Systolic blood pressure (SBP) (P < 0.01) and diastolic blood pressure (DBP) (P < 0.001), fasting blood glucose (P < 0.001) and smoking (P < 0.001) was found to be higher in CAD group than the controls, while HDL-cholesterol level (P < 0.01) was found to be lower in CAD group than the controls. There is no statistical difference observed in the parameters of Total-cholesterol, VLDL-cholesterol, LDL-cholesterol, body mass index (BMI), alcohol consumption and CAD family history between the groups (Table 4).

The frequencies of NUCB2/Nesfatin-1 rs214101 TT, CC and CT genotypes among the diabetic CAD patients were 5.5%, 52.7% and 41.8%, respectively; among the healthy control subjects were 4.3%, 34.8% and 60.9%, respectively. Our results showed that CC genotype frequency is higher in CAD group than control group (34.8% vs. 52.7%; P = 0.019, OR: 1.380, 95% CI: 1.062 - 1.793) (Table 5).

We didn't observe any significant association when comparing effects of NUCB2/Nesfatin-1 genotypes on serum lipid profiles, blood pressure and BMI in both of study groups (without sex sub-grouping). It was only found that CAD patients carrying CC genotype were prone to decreased HDL level (P = 0.071). This association indicated the NUCB2/Nesfatin-1 rs214101 on plasma HDL-C levels as ascending levels in the order of CC < CT< TT in the CAD group. To evaluate whether this polymorphism had any effect on lipid and metabolic parameters, the serum lipid levels and blood pressure values among the NUCB2/Nesfatin-1 genotypes in the control and CAD groups in different sexes were compared using Student's t-test (Table 6). In the CAD group, it was observed that serum HDL cholesterol levels in CC genotype are lower than G allele in the subgroup female subjects (CC: 0.93 $\pm$ 0.21, T allele: 1.12 $\pm$ 0.18, P = 0.010). Whereas, it was observed that the male subjects carrying CC genotype have higher serum HDL-cholesterol and diastolic blood pressure levels than those with T allele (P = 0.043 and P = 0.031, respectively) but, not in females in the control group. Since there were only 6 CAD patients and 3 control subjects carrying the rare TT homozygote genotype, ANOVA test was not valid for the statistical analysis of these three groups. Therefore, we used Student's t-test for the comparison of NUCB2/Nesfatin-1 rs214101genotypes with the values of serum lipid profiles, blood pressure, and BMI.

Linear logistic regression analysis shows that Nesfatin-1 rs214101 CC genotype, smoking, and diastolic blood pres-

Table 3. Effects NUCB2/Nesfatin-1 rs214101 Genotype on Serum Lipid Levels, Blood Pressure Body Mass Index

Groups	NUCB2/Nesfatin-1 rs214101				
	Genoypes			Alleles	
	TT	CT	CC	TT/CT	CC/CT
Control					
Total-C, mmol/L	3.61 ± 2.39	4.91 ± 1.18	4.88 ± 1.34	4.85 ± 1.25	4.92 ± 1.23
TG, mmol/L	1.34 ± 0.31	1.77 ± 1.11	1.51 ± 0.81	1.75 ± 1.09	1.70 ± 1.03
HDL-C, mmol/L	1.16 ± 0.36	1.09 ± 0.35	1.16 ± 0.28	1.10 ± 0.34	1.12 ± 0.33
LDL -C, mmol/L	1.83 ± 2.37	3.08 ± 0.99	3.14 ± 1.21	3.02 ± 1.08	3.11 ± 1.07
VLDL -C, mmol/L	0.61 ± 0.14	0.73 ± 0.26	0.61 ± 0.22	0.73 ± 0.26	0.69 ± 0.26
BMI, kg/m^2	23.10 ± 4.10	25.68 ± 3.25	24.75 ± 2.45	25.47 ± 3.27	25.26 ± 3.00
SBP, mmHg	120.00 ± 0.0	122.97 ± 15.62	119.18 ± 4.29	122.89 ± 15.40	121.75 ± 13.48
DBP, mmHg	75.00 ± 7.07	73.62 ± 14.42	75.37 ± 6.20	73.81 ± 14.26	74.24 ± 12.64
CAD					
Total-C, mmol/L	5.55 ± 1.31	5.24 ± 1.40	5.16 ± 1.36	5.27 ± 1.38	5.20 ± 1.37
TG, mmol/L	1.19 ± 0.44	1.79 ± 1.14	1.89 ± 2.15	1.73 ± 1.11	1.84 ± 1.74
HDL-C, mmol/L	1.13 ± 0.18	1.03 ± 0.21	0.97 ± 0.18	1.04 ± 0.21	1.00 ± 0.20
LDL -C, mmol/Lf	3.18 ± 1.11	3.31 ± 1.27	3.19 ± 0.99	3.36 ± 1.25	3.25 ± 1.13
VLDL -C, mmol/L	0.54 ± 0.20	0.76 ± 0.28	0.75 ± 0.36	0.74 ± 0.28	0.76 ± 0.32
BMI, kg/m^2	23.38 ± 3.07	25.77 ± 3.17	25.45 ± 3.58	25.54 ± 3.21	25.60 ± 3.37
SBP, mmHg	128.33 ± 28.57	134.76 ± 31.52	137.60 ± 38.02	133.97 ± 30.97	136.26 ± 34.94
DBP, mmHg	80.00 ± 10.95	82.32 ± 16.63	84.68 ± 19.74	82.04 ± 15.97	83.57 ± 18.27

Abbreviations: BMI, Body Mass Index; DBP, Diastolic Blood Pressure; HDL-C, High Density Lipoprotein-Cholesterol; LDL-C, Low Density Lipoprotein -Cholesterol; LVH, Left Ventricular Hypertrophy; N, Number of Individuals; SBP, Systolic Blood Pressure; Total-C, Total Cholesterol; VLDL-C, Very Low Density Lipoprotein -Cholesterol.
[a]Values are given as mean value ± standard error of mean (X ± SE) in the Table.

sure remained significant after adjustment for Nesfatin-1 rs214101 CC genotype, serum HDL-cholesterol level, smoking and blood pressures (Table 1). The linear logistic regression analysis confirmed that the Nesfatin-1 rs214101 CC genotype is associated with an increased risk of developing CAD. In the second multivariate logistic regression model (Binary), low serum HDL-cholesterol (below 0.90 mmol/L) used as the dependent variable and significantly associated with Nesfatin-1 rs214101 CC genotype in female CAD subgroup (OR = 7.558; 95% CI = 1.045 - 54.654, P = 0.045), and no association was observed in male subjects (P > 0.05) (Table 2). The logistic regression analysis revealed that the NUCB2/Nesfatin-1 rs214101 CC genotype could predict decreased serum HDL-cholesterol in female CAD patient.

4. Discussion

Coronary artery diseases (CAD) have a remarkable role in morbidity and mortality rates worldwide. According to World Health Organization CAD has the highest rate for the cause of death (19). Obesity, diabetes, and levels of lipids are major risk factors in the development of CAD (6, 20, 21). It is known that the regulation of energy and the melanocortin pathway, which has a special role in appetite, is effective in the development of obesity. The novel satiety factor nesfatin-1, which is a newly identified gene in this pathway; reduces food intake. Nesfatin-1 immunoreactivity was detected in the paraventricular nucleus of the hypothalamus which can alter food intake also affect cardiovascular function (22). Ding et al. showed that there is an association of serum nesfatin-1 concentrations and the development and severity of peripheral arterial disease in type 2 diabetes mellitus patients (16). Dai et al. showed that lower nesfatin-1 concentration may have an important role in the development of acute myocardial infarction (17). Sahin et al. reported that Nesfatin-1 levels play an important role in the pathogenesis of hypertension in patients with polycystic ovary syndrome (18). Angelone et al. demonstrated that Nesfatin-1 elicits an incisive influence on the heart by depressing contractility and relaxation (23). Based on mentioned above findings NUCB2/Nesfatin-1 involved in energy homeostasis and atherosclerosis patho-

Table 4. Characteristics of Study Groups[a]

Characteristics	Groups		P Value
	Control (N = 69)	CAD (N = 110)	
Age, y	58.77 ± 6.20	58.97 ± 11.09	0.496
Sex (women/man), n	21/48	40/70	0.415
BMI, kg/m2	25.27 ± 3.02	25.49 ± 3.37	0.676
Total-C, mmol/L	4.85 ± 1.27	5.21 ± 1.37	0.101
TG, mmol/L	1.67 ± 1.00	1.81 ± 1.70	0.580
HDL-C, mmol/L	1.12 ± 0.32	1.01 ± 0.20	0.007
LDL-C, mmol/L	3.05 ± 1.11	3.28 ± 1.13	0.237
VLDL-C, mmol/L	0.68 ± 0.25	0.75 ± 0.32	0.238
SBB, mmHg	121.71 ± 12.96	135.77 ± 34.50	0.005
DBB, mmHg	74.20 ± 12.19	83.35 ± 17.89	0.001
Fasting blood glucose, mg/dL	87.62 ± 36.78	170.31 ± 117.58	0.000
Smoking, %	26.7%	0.55	0.001
Family history of CAD, %	53.3%	36.3%	0.213

Abbreviations: BMI: body Mass Index; DBP: Diastolic Blood Pressure; HDL-C, high density lipoprotein-Cholesterol; LDL-C, Low Density Lipoprotein -Cholesterol; N, Number of Individuals; SBP: Systolic Blood Pressure; Total-C, Total Cholesterol; VLDL-C, Very Low Density Lipoprotein -Cholesterol.
[a]Age, serum lipid, BMI, and the blood pressure values are given as mean value ± standard error of mean (X + SE), remaining values are given as % in the Table.

genesis, it can be a candidate gene affecting the development of CAD.

Studies regarding of this gene variation with metabolic disorders are scarce. Zegers et al. showed that NUCB2/Nesfatin-1 rs214101 variation has no effect on obesity in CAD and control groups but they found an association between rs214101 and obesity, body mass index, weight and fat-free mass in men population when analyzing the gender subgroups separately. Relying on these findings it is suggested that NUCB2 gene variations may have a role with gender-specific effect in developing of obesity (5).

Table 5. NUCB2/Nesfatin-1 Polymorphism rs214101 Variations in the Study Groups[a]

NUCB2/Nesfatin1 Gene Polymorphisms	Groups	
	Control (N = 69)	CAD (N = 110)
Rs214101 genotypes		
CC	24 (34.8)	58 (52.7)[b]
TT	3 (4.3)	6 (5.5)
CT	42 (60.9)	46 (41.8)
HWE	P = 0.004 (P < 0.05)	P = 0.419 (P > 0.05)
Rs214101 alleles		
C	90 (65.21)	162 (73.64)
T	48 (34.79)	58 (26.36)

Abbreviations: HWE: Hardy-Weinberg Equilibrium; N, Number of Individuals.
[a]Values are expressed as No. (%).
[b]P = 0.019 (Chi-square = 5.500, OR: 1.380 95% CI: 1.062 - 1.793).

The present study is the first one conducted which shows the association among NUCB2/Nesfatin-1 rs214101 gene variation, serum lipid levels and the risk of CAD development in a Turkish population. Our results indicated that rs214101 CC homozygotes had significantly increased the risk of CAD when compared with the T allele carriers (GG + GA) (P < 0.019). The frequencies of NUCB2/Nesfatin-1 alleles C and T among the CAD patients were 73.6% and 29.3% respectively and among the control subjects, alleles were 65.2% and 34.8% respectively. The allele frequencies of the NUCB2 rs214101 were found to be similar to the European populations (C allele: 63% and T allele: 37%) (24).

We did not find any association between NUCB2/Nesfatin-1 rs214101 and serum lipid levels in control and CAD groups. However, in the subgroup analysis, it was demonstrated that CC genotype has an association with low HDL-C levels (hypoalphalipoproteinemia) in female CAD subgroup (P = 0.031). Linear logistic regression analysis indicates low serum HDL-C level is associated with nesfatin-1 rs214101 genotype CC in the female population of CAD group. The genotypes in the CAD group show an increase as CC < CT < TT. Conversely, in the control group, the rs214101 CC genotype has the association with higher HDL-C levels in a male subgroup, while it has no effect on serum lipids in females. This conflict results in gender subgroups may result from protein's gender-specific effect. Our data supports Zegers et al. (5) and Bergmann et al. findings which demonstrate the gender difference effect on the association between NUCB2 levels and obesity (25). Also, Hofmann et al. results corroborate NUCB2/Nesfatin-1 is involved in the regulation of mood and stress in a gender specific manner (26).

HDL-C is being used as a clinical biomarker for CAD and high serum levels of HDL is protective against heart diseases and low levels of HDL-C is a major risk factor for developing cardiovascular diseases. Our data indicate that the NUCB2 rs214101 CC genotype is associated with both coronary artery disease risk and low HDL levels in a gender-specific manner. Although it is unclear that how NUCB2/Nesfatin-1 rs214101 gene variation causes decreased serum HDL-cholesterol level, nesfatin-1 is an insulinotropic peptide and NUCB2/ Nesfatin-1 is considered to be a susceptibility gene for T2DM (27). It was shown that Nesfatin-1 enhanced glucose-induced insulin secretion by promoting Ca2+ influx through L-type channels in mouse islet β-cells in vitro (28). Furthermore, Li et al. (29) reported a positive correlation in patients with HDL and fasting plasma

Table 6. NUCB2/Nesfatin-1 rs214101 Genotype Effects on Subjects About Serum Lipid Levels, Blood Pressure Body Mass Index in Comparison with Gender[a]

Groups	Man		Woman	
	TT + CT	CC	TT + CT	CC
Control				
Total-C, mmol/L	4.66 ± 1.16	4.91 ± 1.40	5.20 ± 1.38	4.67 ± 1.21
TG, mmol/L	1.90 ± 1.26	1.60 ± 0.84	1.42 ± 0.45	1.07 ± 0.48
HDL-C, mmol/L	0.97 ± 0.23	1.11 ± 0.96[b]	1.36 ± 0.38	1.42 ± 0.56
LDL -C, mmol/L	2.96 ± 0.90	3.22 ± 1.29	3.10 ± 1.39	2.76 ± 0.63
VLDL -C, mmol/L	0.74 ± 0.28	0.64 ± 0.22	0.69 ± 0.21	0.49 ± 0.20
SBP, mmHg	120.16 ± 10.01	118.33 ± 4.43	128.33 ± 22.08	121.75 ± 2.87
DBP, mmHg	70.20 ± 8.96	76.66 ± 6.15[c]	81.00 ± 19.66	71.50 ± 5.19
BMI, kg/m^2	25.21 ± 3.09	24.57 ± 2.31	26.16 ± 3.62	25.41 ± 3.09
CAD				
Total-C, mmol/L	4.89 ± 0.91	5.30 ± 1.34	5.91 ± 1.80	4.80 ± 1.40
TG, mmol/L	1.51 ± 0.55	1.96 ± 2.50	2.10 ± 1.63	1.71 ± 0.89
HDL-C, mmol/L	0.99 ± 0.21	0.98 ± 0.18	1.13 ± 0.17	0.94 ± 0.19[d]
LDL -C, mmol/L	3.14 ± 080	3.23 ± 0.97	3.72 ± 1.73	3.11 ± 1.08
VLDL -C, mmol/L	0.69 ± 0.25	0.74 ± 0.33	0.83 ± 0.31	0.78 ± 0.42
SBP, mmHg	132.41 ± 31.00	132.50 ± 26.87	136.66 ± 31.62	152.91 ± 59.48
DBP, mmHg	80.32 ± 17.07	83.19 ± 17.65	85.00 ± 13.82	89.16 ± 25.39
BMI, kg/m^2	25.34 ± 2.48	24.70 ± 3.48	25.81 ± 4.05	27.67 ± 3.04

Abbreviations: BMI, Body Mass Index; DBP, Diastolic Blood Pressure; HDL-C, High Density Lipoprotein-Cholesterol; LDL-C, Low Density Lipoprotein -Cholesterol; N, Number of Individuals; SBP, Systolic Blood Pressure; Total-C, Total Cholesterol; VLDL-C, Very Low Density Lipoprotein -Cholesterol.
[a]Values are given as mean value ± standard error of mean (X ± SE) in the Table.
[b]P = 0.043.
[c]P = 0.031.
[d]P = 0.031.

nesfain-1 level between type 2 diabetes mellitus. In the present study, we didn't observe any association NUCB2/Nesfatin-1 rs214101 gene variation and presence of type 2 diabetes mellitus in patients with CAD (data not shown). However, there was a significant association between this variation, risk of CAD and low serum HDL-cholesterol levels. The main limitation in this report is relatively small study population. Therefore, future studies are needed to be conducted with larger sample-size including expression levels of Nesfatin-1 and evaluation of other key proteins, which can affect serum lipid levels, in lipoprotein metabolism to get more reliable results.

4.1. Conclusion

Our data indicate that the NUCB2 rs214101 CC genotype is may be one of the genetic risk factors for the hypoalphalipoproteinemia and coronary artery disease in a gender-specific manner. We presume that more information can be obtained that can support protein's effect on lipid levels by increasing number of subjects included in the study and investigate other polymorphisms related with Nesfatin-1.

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Footnotes

Authors' Contribution: The planning and conduct of this work has been made by Hulya Yilmaz-Aydogan and Fatih Yanar. Clinic examinations have been made by Zehra Bugra. The DNA isolations and gene analysis have been made by Fatih Yanar and Bengu Tokat. Oguz Ozturk and Hulya Yilmaz-Aydogan have contributed to the statistical analysis and interpretation of data.

Conflict of Interest: Authors declare that they have no conflict of interests.

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