POLYMORPHISMS RS562556 AND RS2479409 OF THE *PCSK9* GENE ASSOCIATED WITH OBESITY AND CARDIOVASCULAR DISEASE

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SUMMARY

Objective: The primary objective was to comprehensively assess the association between single nucleotide polymorphisms (rs562556 and rs2479409) in the *PCSK9* gene with biochemical parameters – C-reactive protein (CRP), glucose (GLU), triglyceride (TAG), low-density lipoprotein cholesterol (LDL CHOL), non-high-density lipoprotein cholesterol (non HDL CHOL), high-density lipoprotein cholesterol (HDL CHOL), cholesterol (CHOL), and anthropometric parameters (visceral fat), overweight/obesity and cardiovascular risk.

Methods: A total of 71 women aged 23–64 years were divided into three groups based on body mass index (BMI). BMI ≥ 25/≥ 30 kg/m^2 was the criterion for assessment of overweight/obesity. Anthropometric, biochemical and genetic examinations were performed on the probands. Changes in markers in each group and their association with cardiovascular risk were monitored.

Results: We can conclude that in our study population we observed differences between the BMI categories for biochemical markers (CRP, LDL CHOL, non HDL CHOL, HDL CHOL, LDL CHOL) and anthropometric marker (visceral fat). Atherogenic index of plasma (AIP), Castelli's Risk Index I (CRI-I) and atherogenic coefficient (AC) confirmed high cardiovascular risk for the obese women category (0.045); (< 0.013); (< 0.010). Genotype and allele frequencies for the *PCSK9* gene in the overweight and obese groups showed higher allele frequencies of allele A for both polymorphisms of the gene.

Conclusions: PCSK9 gene expression is associated with biological processes such as lipid metabolism and inflammation. Cholesterol-lowering therapies are the gold standard for reducing the risk of cardiovascular mortality and morbidity. Administration of monoclonal antibodies (mAbs) against *PCSK9* is a novel lipid-lowering therapeutic approach in adults to reduce the risk of cardiovascular disease.

Key words: PCSK9, BMI, obesity, women, cardiometabolic risk

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INTRODUCTION

Nowadays, obesity is a major health problem, leading to the development of metabolic comorbidities in the form of cardiovascular complications. Obesity is defined by the enlargement of adipocytes and adipose tissue and the secretion of inflammatory cytokines that induce pathological complications of the cardiovascular system (1). Cardiovascular disease (CVD) is the leading cause of death worldwide, claiming 17.9 million lives each year. CVDs are a group of disorders of the heart and blood vessels and include ischaemic heart disease, cerebrovascular disease, myocardial infarction, and other conditions (2). Pro-protein convertase subtilisin/kexin type 9 (*PCSK9*) is a soluble protease that, since its discovery, has been studied in the field of cholesterol homeostasis and in cardiovascular biology (3). The best-known function of *PCSK9* is its effect on the low-density lipoprotein receptor (LDLR). *PCSK9* has been the focus of several studies in various clinical contexts as a marker of cardiovascular risk (4, 5). *PCSK9* functions as an inhibitor of the LDLR pathway by targeting receptors in the lysosomal degradation pathway (1). Circulating *PCSK9* levels are associated with low-density lipoprotein (LDL) cholesterol (6). Obesity upregulates *PCSK9* expression, and high levels of *PCSK9* are associated with disease progression. Adipose tissue *PCSK9* expression levels are positively correlated with body mass index, suggesting that obesity and adiposity induce *PCSK9* expression (5). Adipose tissue secretes and expresses *PCSK9*, which, through LDLR-independent mechanisms, may contribute to obesity and therefore to the development of cardiovascular disease. Keeping optimal blood lipid levels is essential for cardiovascular health (7). The increasing prevalence of obesity and cardiovascular disease suggest that increased attention should be paid to preventive measures that correctly and in a timely manner identify at-risk individuals in the premorbid stage of the disease. Research into the genetic and biochemical basis of cardiovascular

disease and obesity holds the potential for reducing the prevalence of these diseases in the population. The objective of this study was to investigate the relationship of overweight and obesity with the values of the studied parameters, i.e., the association of alleles of *PCSK9* gene polymorphisms with higher biochemical values and atherogenic indices.

MATERIALS AND METHODTS

Study Population

The study comprised 71 women aged 23–64 years. The mean age of the probands was 42.63 ± 9.99 years. The inclusion criteria for the study was female sex and age ranging from 20–65 years, with no major health problems. The probands who had cardiovascular disease or were under treatment for cardiovascular disease at the time of the study were excluded from the study. Other exclusion criteria were metabolic disease, cancer, liver and kidney disease. The probands were informed about the purpose and process of testing and signed an informed consent form.

Anthropometric, Biochemical, Genetic Measurement with Indices for Cardiovascular Risk

Each proband underwent baseline anthropometric measurements (body height, body weight, waist and hip circumference). Total body fat percentage was determined using bioelectrical impedance of a commercially available multifrequency Bodystat QuadScan 4000 (Bodystat Body Composition Technology, GB). On the basis of anthropometric parameters and bioelectrical impedance, probands were divided into three groups according to BMI (Table 1).

A buccal swab of the oral cavity was taken from the probands. DNA isolation was performed from buccal swabs using the NucleoSpin®00000 Tissue Macherey-Nagel kit (GmbH & Co. KG).

Genetic analysis was performed by real-time polymerase chain reaction (real-time PCR) using TaqMan technology (the Custom TaqMan® SNP Genotyping Assay, Applied Biosystems, Foster City, CA, USA). Genotyping was performed on an Applied BiosystemsTM7500 Fast Real-Time PCR System.

The biochemical profile of each parameter was determined from the medical documentation of the participating individuals.

The atherogenic index and lipid ratios were calculated using the following established formulas:

AIP = (TAG/HDL – CHOL); CRI-I = CHOL/HDL CHOL; CRI-II = LDL CHOL/HDL CHOL; AC = CHOL – HDL CHOL/ HDL CHOL

AIP – atherogenic index of plasma; TAG – triglyceride; HDL CHOL – high-density lipoprotein cholesterol; CRI-I – Castelli's risk index I; CHOL – cholesterol; CRI-II – Castelli's risk index II; LDL CHOL – low-density lipoprotein cholesterol; AC – atherogenic coefficient

The following are the abnormal values of atherogenic index of plasma and lipid ratios for cardiovascular risk: AIP > 0.1 low risk, AIP 0.11–0.21 medium risk, AIP > 0.21 high risk; CRI-I > 3.5 in males and > 3.0 in females, CRI-II > 3.3, AC > 3.0 (8, 9).

Table 1. *Selected markers of the cohort divided by BMI values with associated cardiovascular risk indices*

	Visceral fat	CRP mg/L	GLU mmol/L	TAG mmol/L	LDL CHOL mmol/L	non-HDL CHOL mmol/L	HDL CHOL mmol/L	CHOL mmol/L	CRI-I	CRI-II	AIP	AC
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Optimal body weight (n = 11) 18–24.9 kg.m^{-2}	3.73 (1.67)	3.11 (2.57)	5.33 (0.82)	1.07 (0.40)	2.85 (0.45)	2.73 (1.02)	1.69 (0.55)	4.74 (0.54)	3.05 (0.90)	1.90 (0.81)	0.15 (0.28)	1.71 (0.91)
Overweight (n =30) 25–29.9 kg.m^{-2}	6.23 (1.45)	2.95 (2.88)	5.24 (0.95)	1.19 (0.55)	2,94 (0.73)	3.27 (0.83)	1.53 (0.33)	4.77 (0.79)	3.22 (0.84)	2.00 (0.69)	0.209 (0.263)	2.18 (0.92)
Obesity (n = 30) 30–55.2 kg.m^{-2}	10.17 (2.86)	5.44 (4.55)	5.53 (0.85)	1.37 (0.55)	3.12 (0.74)	3.474 (1.047)	1.41 (0.32)	5.07 (0.78)	3.7 (0.96)	2.33 (0.82)	0.319 (0.241)	2.56 (0.99)
p-values	<0.001	<0.020*	<0.344	<0.085	<0.209	<0.030*	<0.034*	<0.132	<0.013*	<0.063	<0.045*	<0.010*

SD – standard deviation; BMI – body mass index; CRP – C-reactive protein; GLU – glucose; TAG – triglyceride; LDL CHOL – low-density lipoprotein cholesterol; non HDL CHOL – non-high-density lipoprotein cholesterol; HDL CHOL – high-density lipoprotein cholesterol; CHOL – cholesterol; CRI-I – Castelli's risk index I; CRI-II – Castelli's risk index II; AIP – atherogenic index of plasma; AC – atherogenic coefficient; * < 0.05 – statistically significant

Statistical Analysis

Data were converted into Microsoft Office Excel 2013 and statistically processed in SPSS for Windows version 19.0. The Levene's test of homogeneity was used to verify the normal distribution of the data. The Student's unpaired t-test and Mann-Whitney U test were used to compare the data. For each parameter, the mean value, standard deviation (SD) and p value were determined. The chi-square test of independence was used with the use of a contingency table. ANOVA test (GraphPad 2022) was used to compare the individual parameters with respect to the genotypes of each polymorphism. Statistical significance of the performed tests was evaluated at the 5% level ($p < 0.05$). The Pearson correlation coefficient was used to assess the dependence between parameters. Values higher than 0.7 indicate a strong positive dependence between the parameters. Values greater than −0.7 indicate a strong negative dependence between the parameters.

RESULTS

The study comprised 71 women aged 23–64 years. The mean age of the probands was 42.63 ± 9.99 years. The probands were divided on the basis of BMI into three categories. The first category included women (n = 11) with normal body weight according to BMI of 18–24.9 $kg.m^{-2}$ with a mean value of 22.74 ± 1.53 $kg.m^{-2}$. The second category included women (n = 30) who were overweight with BMI 25–29.9 $kg.m^{-2}$ with a mean value of 27.31 ± 1.46 $kg.m^{-2}$. The third category included women (n = 30) with obesity BMI 30–55.2 $kg.m^{-2}$ with a mean value of 35.04 ± 6.06 $kg.m^{-2}$.

The Pearson correlation coefficient was used to assess the dependence between the parameters. A strong positive dependence was demonstrated between the anthropometric parameters body fat in kg and with visceral fat (0.88); muscle mass in kg and with body fat in kg (0.73); active body mass in kg and with body fat in kg (0.73); total body water in kg and with body fat in kg (0.71); total body water in kg with muscle mass in kg (0.95). A strong positive correlation was shown between biochemical parameters of non HDL cholesterol with total cholesterol (0.89); LDL cholesterol with total cholesterol (0.87); LDL cholesterol with non HDL cholesterol (0.90). A strong negative correlation was demonstrated between anthropometric parameters of muscle mass (%) with total body fat (%) (−0.99) and with visceral fat (−0.88). A strong negative dependence was demonstrated between anthropometric parameters total body water (%) with body fat (%) (−0.96) and with visceral fat (−0.87).

Table 1 reports the mean values of visceral fat, biochemical parameters, and indices for cardiovascular risk for each category by BMI. Statistically significant values were for visceral fat ($p < 0.001$), CRP ($p < 0.020$), non HDL cholesterol ($p < 0.030$), HDL cholesterol ($p < 0.034$), CRI-I ($p < 0.013$), AIP ($p < 0.045$), and AC ($p < 0.010$). According to the atherogenic index of plasma, obese women have a high risk (0.319) and overweight women have an intermediate risk (0.20). According to Castelli's Risk Index I, obese women have the highest risk (3.740) in the third category.

The genotype and allele frequencies of the *PCSK9* gene and its polymorphisms (rs562556; rs2479409) for each parameter are shown in Table 2. The mean values of visceral fat, TAG, CHOL, non-HDL CHOL, HDL CHOL, LDL CHOL, GLU, and CRP were above normal in our study set of women and thus can be considered pathological. For the rs562556 polymorphism of *PCSK9* gene, we can conclude that allele A has the highest frequency for the following markers: visceral fat (81.81%), TAG (75%), CHOL (79.69%), non HDL CHOL (80%), HDL CHOL (71.87%), LDL CHOL (79.41%), GLU (77.5%), and CRP (85.71%). These observations suggest that allele A for rs562556 polymorphism of *PCSK9* gene can be considered a risk allele. For the rs2479409 polymorphism of the *PCSK9* gene, we can conclude that allele A has the highest frequency for the following markers: visceral fat (59.09%), non HDL CHOL (62.5%), LDL CHOL (58.82%), and GLU (60%). For the other markers, the A and G alleles of the rs2479409 polymorphism of the *PCSK9* gene are at approximate frequencies, given that the AG genotype is the most frequent.

Allele and genotype frequencies for the rs562556 polymorphism of the *PCSK9* gene for each category according to BMI are shown in Table 3. In each group, allele A had the highest frequency, but it was highest in the obese women category (86.67%). In the normal weight category, genotype AG was the most represented (54.55%), and in the overweight and obese category, genotype AA was the most represented (70%, 76.67%, respectively). The values were not statistically significant ($p < 0.336$). Table 4 shows the allele and genotype frequency for the rs2479409 polymorphism of the *PCSK9* gene for each category according to BMI. Allele A had the highest frequency in each group but was highest in the overweight category (60%). In all three categories, the AG genotype was the most frequent (63.64%, 46.67%, 60%). The values were not statistically significant ($p < 0.924$).

DISCUSSION

Pro-protein convertase subtilisin/kexin type 9 (*PCSK9*) was originally called neural apoptosis regulated convertase 1 (NARC1) and is part of a family of secretory serine proteinases called protein concentrates (PCs). The human *PCSK9* gene is 22 kb in length and is located on chromosome 1p32. *PCSK9* consists of 12 exons and 11 introns (10). The primary source of *PCSK9* is hepatocytes, but it is produced and secreted by cells in the intestine, pancreas, adipose tissue, kidney, and brain (11, 12). The binding of *PCSK9* and LDLR, which leads to its degradation, plays an important role in cholesterol homeostasis (13). *PCSK9* inhibits cholesterol efflux and thereby disrupts cholesterol homeostasis (14). Destruction of LDLR by *PCSK9* leads to hyperlipidaemia, which is associated with many cardiovascular complications. In particular, lipid uptake and accumulation by macrophages in the vascular wall and foam macrophage formation lead to the development of atheromas (13). Atherosclerosis is characterized by chronic inflammation of the vasculature accompanied by lipid retention in the arteries and plaque formation. *PCSK9* is expressed on aortic endothelial cells, smooth muscle cells, macrophages, dendritic cells, and epithelial cells (15). This fact suggests that *PCSK9* regulates atherosclerosis through LDL cholesterol levels, but also by interacting with and influencing cellular processes in the vascular wall (16). The severity of atherosclerosis is positively correlated with circulating *PCSK9* levels (17). Serum *PCSK9* levels are also elevated in another cardiovascular disease, acute myocardial infarction. Acute myocardial infarction leads to increased expression of SREBP-2, hepatocyte nuclear factor 1α NLRP3, which leads to increased

Table 2. *Frequency of genotypes and alleles for the PCSK9 gene within exceeding reference values of biochemical and anthropometric parameters*

Parameters	Mean (SD)	Genotype and allele frequency	Polymorphism rs 562556 of the *PCSK9* gene n (%)	Polymorphism rs 2479409 of the *PCSK9* gene n (%)
Visceral fat	13.09 (2.165)	AA	8 (72.72)	3 (27.27)
		AG	2 (18.19)	7 (63.64)
		GG	1 (9.09)	1 (9.09)
		A	18 (81.81)	13 (59.09)
		G	4 (18.18)	9 (40.91)
TAG mmol/L	2.072 (0.297)	AA	8 (57.14)	3 (21.43)
		AG	5 (35.72)	10 (71.43)
		GG	1 (7.14)	1 (7.14)
		A	21 (75.0)	16 (57.14)
		G	7 (25.0)	12 (42.86)
Total cholesterol mmol/L	5.584 (0.475)	AA	20 (62.50)	8 (25.0)
		AG	11 (34.38)	16 (50.0)
		GG	1 (3.12)	8 (25.0)
		A	51 (79.69)	32 (50.0)
		G	13 (20.31)	32 (50.0)
Non-HDL cholesterol mmol/L	4.388 (0.531)	AA	13 (65.0)	6 (30.0)
		AG	6 (30.0)	13 (65.0)
		GG	1 (5.0)	1 (5.0)
		A	32 (80.0)	25 (62.50)
		G	8 (20.0)	15 (37.50)
HDL cholesterol mmol/L	1.07 (0.086)	AA	8 (50.0)	5 (31.25)
		AG	7 (43.75)	10 (62.50)
		GG	1 (6.25)	1 (6.25)
		A	23 (71.87)	20 (62.50)
		G	9 (28.13)	12 (37.50)
LDL cholesterol mmol/L	3.567 (0.493)	AA	21 (61.77)	10 (29.41)
		AG	12 (35.29)	20 (58.83)
		GG	1 (2.94)	4 (11.76)
		A	54 (79.41)	40 (58.82)
		G	14 (20.59)	28 (41.18)
GLU mmol/L	6.39 (1.056)	AA	11 (55.0)	6 (30.0)
		AG	9 (45.0)	12 (60.0)
		GG	0 (0.0)	2 (10.0)
		A	31 (77.50)	24 (60.0)
		G	9 (22.50)	16 (40.0)
CRP mg/L	8.633 (3.779)	AA	16 (76.19)	4 (19.05)
		AG	4 (19.05)	14 (66.67)
		GG	1 (4.76)	3 (14.28)
		A	36 (85.71)	22 (52.38)
		G	6 (14.29)	20 (47.62)

SD – standard deviation; CRP – C-reactive protein; GLU – glucose; TAG – triglyceride; LDL CHOL – low-density lipoprotein cholesterol; non HDL CHOL – non-high-density lipoprotein cholesterol; HDL CHOL – high-density lipoprotein cholesterol; CHOL – cholesterol

PCSK9 expression (18). *PCSK9* has a major role in targeting other receptors for degradation, thereby regulating a variety of processes including hypercholesterolemia and associated atherosclerosis, vascular inflammation, viral infections, and immune checkpoint regulation in cancer (19). Total levels of circulating *PCSK9* are influenced by gender, as women have higher levels compared to

Table 3. *Allele and genotype frequency of the PCSK9 gene rs562556 polymorphism for BMI categories*

Polymorphism rs 562556 of the *PCSK9* gene	Optimal body weight n = 11 n (%)	Overweight n = 30 n (%)	Obesity n = 30 n (%)
AA	5 (45.45)	21 (70.0)	23 (76.67)
AG	6 (54.55)	8 (26.67)	6 (20.0)
GG	0 (0.0)	1 (3.33)	1 (3.33)
A	16 (72.73)	30 (83.83)	52 (86.67)
G	6 (27.27)	10 (16.62)	8 (13.33)
ANOVA F-ratio value	1.106		
p-value	0.337		

Table 4. *Allele and genotype frequency of the PCSK9 gene rs2479409 polymorphism for BMI categories*

Polymorphism rs 2479409 of the *PCSK9* gene	Optimal body weight n = 11 n (%)	Overweight n = 30 n (%)	Obesity n = 30 n (%)
AA	3 (27.27)	11 (36.67)	8 (26.67)
AG	7 (63.64)	14 (46.67)	18 (60.0)
GG	1 (9.09)	5 (46.65)	4 (13.33)
A	13 (59.09)	36 (60.0)	34 (56.67)
G	9 (40.11)	24 (40.0)	26 (43.33)
ANOVA F-ratio value	0.078		
p-value	0.925		

men, suggesting that hormones such as estrogens are involved in the expression and secretion of *PCSK9* (20). Other factors that influence total circulating *PCSK9* levels include age, body mass index (BMI), plasma cholesterol and triacylglycerol levels, and blood pressure (21). Adipose tissue is involved in energy balance and energy storage. It has endocrine functions and plays an essential role in the metabolism of triglyceride-rich lipoproteins. Brown adipose tissue uptakes triglycerides and is also actively involved in the metabolic flux of high-density lipoprotein cholesterol to the liver (22). Bordicchia et al. (5) in their study reported that *PCSK9* is expressed in human adipose tissue. They subsequently report that *PCSK9* expression levels are significantly and positively correlated with BMI, even after adjusting for sex and age. We observed significant difference between categories for parameters such as visceral fat ($< 2.45.10^{-12}$), non HDL cholesterol (< 0.0302), HDL cholesterol (< 0.0345). These claims are supported by the fact that CRI-I and AIP in the group of obese women showed high risk (3.740, 0.319).

Ma et al. (23) reported that circulating *PCSK9* was positively correlated with LDL cholesterol, total cholesterol and triglycerides ($p < 0.001$). Positive correlations between *PCSK9* protein levels and waist circumference, fasting glycaemia, insulin resistance, systolic blood pressure, and C-reactive protein ($p < 0.001$) were observed only in women. Circulating *PCSK9* was positively associated with the incidence of high LDL cholesterol in women (OR = 1.36, 95% CI = 1.12–1.69, $p < 0.001$). In addition, *PCSK9* was significantly associated with the incidence of high triglycerides (OR = 1.31, 95% CI = 1.13–1.72, $p < 0.001$), hypertension (OR = 1.28, 95% CI = 1.08–1.53, $p < 0.011$), type 2 diabetes mellitus (OR = 1.34, 95% CI = 1.09–1.76, $p < 0.005$) and metabolic syndrome (OR = 1.30, 95% CI = 1.11–1.65, $p < 0.009$) in women only. Taken together, the results suggest that subjects with higher circulating *PCSK9* levels had progressively worse cardiometabolic risk profiles in women, an important factor in the development of atherosclerotic cardiovascular disease in women. Gago-Dominguez et al. (24) found that rs2479409 of the *PCSK9* gene was associated with stroke risk in obese subjects (OR = 0.54, 95% CI: 0.35–0.84, p = 0.006). An association with stroke risk was found in obese subjects, with more pronounced results in women. The rs2479409 values obtained were for obese men (OR = 0.66, 95% CI: 0.34–1.27, p = 0.21) and for obese women (OR = 0.49, 95% CI: 0.24–0.99, p = 0.04). The risk allele was allele A. We provided the allele and genotype frequency of the rs2479409 polymorphism in the *PCSK9* gene among the BMI categories. The results of our study were not significant, however, the genotype AG was the most frequent in the overweight and obese categories, and the A allele had the highest frequencies in these categories.

Zamarrón-Licona et al. (25) in the study reports that the rs2479409 polymorphism of the *PCSK9* gene was associated with an increased risk of subclinical atherosclerosis (OR = 1.53, p recessive = 0.041). The rs2479409 polymorphism of the *PCSK9* gene was significantly associated with several cardiometabolic parameters. The rs2479409 polymorphism could be considered as a risk marker for subclinical atherosclerosis. The A allele of the *PCSK9* gene rs2479409 was associated with a high risk of subclinical atherosclerosis (SA) (OR = 1.539, 95% CI: 1.018–2.328, p = 0.041). Different TAG concentrations were observed for rs2479409 genotypes in the control group (p = 0.016). In our study, we provided the parameters that are above the reference values and can be considered cardiometabolic risk parameters. For the rs2479409 polymorphism of the *PCSK9* gene, we observed the highest frequencies (%) for allele A in the following parameters: visceral fat (59.09%), non HDL cholesterol (62.5%), HDL cholesterol (62.5%), LDL cholesterol (58.82%), and glucose (60%). Gai et al. 2021 (26) report that the G allele for the *PCSK9* gene of the rs562556 polymorphism (G > A) is associated with lower LDL cholesterol and blood glucose levels and BMI. The dominant model (AA vs. GG+AG) of rs562556 showed a significant association with cardiovascular disease. The GG genotype of rs562556 of the *PCSK9* gene showed beneficial effects on health (OR = 0.63, 95% CI: 0.45–0.90, p = 0.011). The G rs562556 allele may reduce the risk of cardiovascular disease by lowering circulating levels of *PCSK9* to lower LDL cholesterol. These findings support our hypothesis that the A allele is risk and the G allele has beneficial effects on cardiovascular health because of the rs562556 polymorphism of the *PCSK9* gene. We observed the highest frequency of A allele (%) at risk reference levels for the following parameters: visceral fat (81.81%), TAG (75%), total cholesterol (79.69%), non HDL cholesterol (80%), HDL cholesterol (71.87%), LDL cholesterol (79.41%), glucose (77.5%), and CRP (85.71%).

Chuan et al. (27) in their meta-study revealed that carriers of the G allele in the *PCSK9* gene rs562556 polymorphism had lower total cholesterol (95% CI: 0.06–0.23, p = 0.001) and LDL

cholesterol (95% CI: −0.55–0.22, p=0.002) levels than A allele carriers. The allele and genotype frequency for the rs562556 polymorphism of the *PCSK9* gene in our study set was highest in the overweight (83.83%) and obese (86.67%) groups, respectively. The AA genotype was the most abundant genotype in the entire study set, but showed the highest frequency in the overweight and obese group of women. There were no statistically significant differences in this cohort. The plasma atherogenic index (AIP), has been proposed as a powerful marker in the prediction of atherogenicity and cardiovascular events. Compared with simple lipid parameters, AIP has stronger associations and better predictive ability for cardiovascular disease (28). AIP and other cholesterol-derived indices, the atherogenic coefficient, Castelli risk index I, and Castelli risk index II are all associated with CVD risk; in this study, we evaluated the relationship between AIP (<0.045), AC (<0.010), CRI-I (<0.063), and CRI-II (<0.013) levels and obesity, which is closely related to the development of cardiovascular disease. We found a significant difference between the groups. According to the atherogenic index of plasma, obese women have a high risk (0.31) and overweight women have an intermediate risk (0.20). According to Castelli's risk index I, obese women have the highest risk (3.74) in the third category.

CONCLUSIONS

Complete absence of circulating *PCSK9* with LDL cholesterol of 15–20 mg/dl, full cardiovascular health and normal reproductive, physical and mental abilities were observed in some subjects. *PCSK9* inhibitors are a major innovation in lipid management and are undoubtedly effective in reducing cardiovascular events and LDL-C by 50%. Based on this knowledge, it is important to continue to monitor these risk factors such as BMI, visceral fat, lipid profile of individuals, CRI-I, CRI-II, AIP and AC which are major predictors of cardiovascular disease and through which we can reduce prevalence and mortality. In our study, we found statistically significant differences between groups in the parameters of visceral fat, non HDL cholesterol and HDL cholesterol. Significant difference between groups was also confirmed for CRI-I, AIP and AC. No significant difference was confirmed for the two *PCSK9* gene polymorphisms studied, but considering previous studies and the results of our study, a possible risk allele for both polymorphisms is allele A. These findings provide a basis for further studies. On the basis of these findings, we can support that primary prevention based on biochemical and genetic observation has a validity in the context of cardiovascular health.

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Conflicts of Interest

None declared

Adherence to Ethical Standards

Ethical approval for the study was obtained from the University Ethics Committee of the University of Prešov, Prešov, Slovakia, No: ECU-PO42022PO-1/7.

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