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EFFECTS OF THE *PPARG* GENE POLYMORPHISMS ON MARKERS OF OBESITY AND THE METABOLIC SYNDROME IN BOSNIAN SUBJECTS

EFEKTI POLIMORFIZAMA *PPARG* GENA NA POKAZATELJE GOJAZNOSTI I METABOLIČKI SINDROM KOD BOSANSKIH ISPITANIKA

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Summary

Background: Peroxisome proliferator-activated receptor gamma (PPARγ) is a key transcription factor in adipogenesis, and also regulates a number of genes associated with lipid storage and insulin sensitivity. Single nucleotide polymorphisms (SNPs) in the *PPARG* gene have been associated with obesity and diabetes. In this study, we explored the relationship of three *PPARG* gene variants with the metabolic syndrome (MetS) and related traits in a population from Bosnia and Herzegovina.
Methods: Anthropometric and biochemical parameters were measured in 43 patients with MetS and 43 healthy controls. Subjects were genotyped for Pro12Ala (rs1801282) and 1431C>T (rs3856806) SNPs by classic PCR–restriction fragment length polymorphism analysis, and for -681C>G (rs10865710) variant by real-time PCR.
Results: The genotype distributions for the three polymorphisms were not significantly different between MetS patients and controls. The Pro12Ala and 1431C>T variants were associated with lower body mass index in the control subjects ($p=0.012$ and $p=0.049$, respectively). In this group, the carriers of Pro12Ala had also lower waist circumference compared to the wild-type homozygotes ($p=0.045$).

Kratak sadržaj

Uvod: Peroksizomni proliferatorom aktivirani receptor gama (PPARγ) predstavlja ključni transkripcijski faktor u adipogenezi, i reguliše brojne gene povezane sa skladištenjem lipida i insulinskom osetljivošću. Pojedinačni nukleotidni polimorfizmi *PPARG* gena povezani su s gojaznošću i dijabetesom. U ovoj studiji, istražili smo povezanost tri varijante *PPARG* gena s metaboličkim sindromom (MetS) i njegovim karakteristikama u populaciji Bosne i Hercegovine.
Metode: Antropometrijski i biohemijski parametri su izmereni kod 43 pacijenta s MetS-om i 43 zdrava kontrolna subjekta. Polimorfizmi Pro12Ala (rs1801282) i 1431C>T (rs3856806) genotipizirani su klasičnom PCR metodom praćenom analizom polimorfizma dužine restrikcijskih fragmenata (RFLP), a -681C>G (rs10865710) varijanta je analizirana PCR metodom u stvarnom vremenu (*real-time PCR*).
Rezultati: Distribucija genotipova za sva tri polimorfizma se nije značajno razlikovala između pacijenata s MetS-om i kontrolnih subjekata. Pro12Ala i 1431C>T varijante su bile povezane s nižim indeksom telesne mase kod kontrolnih ispitanika ($p=0,012$ i $p=0,049$). U ovoj grupi, nosioci Pro12Ala varijante su takođe imali niži obim struka u odnosu na homozigote divljeg tipa ($p=0,045$).

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List of Abbreviations: BP, blood pressure; BMI, body mass index; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; HOMA-IR, homeostasis model assessment insulin resistance index; LD, linkage disequilibrium; LDL, low-density lipoprotein; MetS, metabolic syndrome; PPARγ, peroxisome proliferator-activated receptor gamma; *PPARG*, PPARγ gene; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SNP, single nucleotide polymorphism.

Conclusions: Results of our preliminary study indicate a beneficial effect of a common Pro12Ala variant on the metabolic phenotype in healthy non-obese subjects.

Keywords: insulin resistance, metabolic syndrome, obesity, peroxisome proliferator-activated receptor gamma (PPARγ), polymorphism, genetic

Zaključak: Rezultati naše preliminarne studije ukazuju da česta Pro12Ala varijanta ima povoljan efekat na metabolički fenotip zdravih negojaznih ispitanika.

Ključne reči: gojaznost, insulinska rezistencija, metabolički sindrom, peroksizomni proliferatorom aktivirani receptor gama (PPARγ), polimorfizam, genetički

Introduction

The metabolic syndrome (MetS) consists of several metabolic and physiological abnormalities, including abdominal obesity, dyslipidemia, impaired glucose homeostasis and hypertension. Over the last ten years, various criteria for the diagnosis of MetS have been proposed by several different organizations, and recently unified guidelines were finally accepted (1). MetS is associated with 5-fold increased risk for type 2 diabetes and 2-fold increased risk for cardiovascular disease (2, 3). MetS prevalence, in most countries, ranges between 20 and 30% in adults (2). The most important etiological factors are low physical activity, excess calorie intake, aging and genetic factors (4). The heritability of the MetS has been estimated to be about 30% (5).

Peroxisome proliferator-activated receptor gamma (PPARγ), a nuclear hormone receptor, is a ligand-activated transcription factor that controls adipocyte differentiation and regulates a number of genes associated with energy homeostasis. PPARγ upregulates the transcription of genes involved in free fatty acids trapping and triglyceride synthesis (6). Activation of the receptor enhances insulin sensitivity by modifying the profile of adipokines secreted from adipose tissue and by favoring lipid accretion in, less hormonally sensitive, subcutaneous depots (6). Rare loss-of-function mutations in the ligand-binding domain of human *PPARG* gene lead to partial lipodystrophy with severe insulin resistance, early-onset diabetes, dyslipidemia and hypertension (7). The *PPARG* gene is located on chromosome 3p25, and is composed of nine exons (A1, A2, B and exons 1–6) and four promoters. Four different transcripts are produced by alternate promoter use and splicing (*PPARG* 1–4). *PPARG*1, *PPARG*3, and *PPARG4* produce the same protein, while *PPARG*2 produces a protein with 30 additional amino acids at the N-terminus (8). The most prevalent human *PPARG* gene variant is the Pro12Ala (p.P12A, rs1801282) polymorphism in exon B, with allelic frequency ranging between 2% and 23%, in different ethnic groups (9). *In vitro*, the Pro12Ala variant exhibits reduced binding to DNA and modest impairment of transcriptional activation (10, 11). Although early findings were inconsistent, replication studies and meta-analyses confirmed modest but significant increase in type 2 diabetes risk associated with the Pro allele (12). However, the relationship of Pro12Ala variant with obesity, MetS and related traits is less defined (13–23). Similarly, conflicting results were obtained regarding the association between metabolic phenotypes and another frequent variant, a silent mutation in exon 6 of the *PPARG* gene, 1431C>T (c.1431C>T, rs3856806) (13, 14, 16–18, 23, 24). A recently discovered polymorphism in the *PPARG*3 promoter, located 5′ of exon A2, -681C>G (c.-681C>G, rs10865710), was associated with lower promoter activity *in vitro* (25).

This is the first study conducted in subjects from Bosnia and Herzegovina in which we explored the relationship of three *PPARG* gene variants (Pro12Ala, 1431C>T, and -681C>G) with MetS and its features.

Materials and Methods

Subjects

A total of 86 subjects were enrolled in the study: 43 patients with the MetS, recruited from General Hospital Tešanj, Zenica–Doboj Canton, and 43 healthy controls. Each participant gave written informed consent. The study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Cantonal Hospital Zenica and by the Institute for Genetic Engineering and Biotechnology, University of Sarajevo. MetS was diagnosed according to the harmonized definition from 2009 that includes presence of at least three of the following five criteria: increased waist circumference (≥94 cm in men or ≥80 cm in women), triglycerides ≥1.7 mmol/L (or specific treatment for this lipid abnormality), high-density lipoprotein cholesterol (HDL-cholesterol) <1.0 mmol/L in men or <1.3 mmol/L in women (or specific treatment for this lipid abnormality), blood pressure ≥130/85 mmHg (or treatment for previously diagnosed hypertension) and fasting glucose ≥5.6 mmol/L (or previously diagnosed type 2 diabetes) (1). Patients with an acute infection and/or inflammation, endocrine disorders, and those on insulin therapy were excluded from the study. The patients received antihypertensive (74% of all patients), hypoglycemic (58%) and hypolipidemic therapy (47%). A control group was comprised of 43 healthy, non-obese subjects who had less than three MetS components, and were not taking any medication.

Anthropometric and biochemical measurements

In all the subjects arterial blood pressure and anthropometric data (height, weight, and waist circumference) were measured. Waist circumference was measured at the midpoint between the lowest rib and the iliac crest (26). Blood samples were drawn after an overnight fast. Serum glucose, total choles-

terol, HDL-cholesterol, low-density lipoprotein cholesterol (LDL-cholesterol), triglycerides and C-reactive protein (CRP) were determined by standard procedures with an autoanalyzer VITROS 350 Chemistry System (Ortho-Clinical Diagnostic, Rochester, New York, USA). Serum insulin was measured by the immunoenzymometric assay on an AxSYM analyzer (Abbott Laboratories, Abbott Park, IL, USA). Glycohemoglobin (HbA_{1c}) was determined in the whole blood with EDTA by a NGSP-certified affinity separation method, using the NycoCard reader (Axis-Shield, Oslo, Norway). To assess insulin resistance, the homeostasis model assessment insulin resistance index (HOMA-IR) was calculated according to the formula: fasting insulin (mU/L) x fasting glucose (mmol/L) /22.5 (27).

Genotyping

Genomic DNA was extracted from the whole blood using Miller's protocol (28). Single nucleotide polymorphisms (SNPs), Pro12Ala and 1431C>T, were analyzed by classic PCR followed by restriction fragment length polymorphism (RFLP) analysis. Pro12Ala variant was genotyped using forward primer F: 5'CAAGCCCAGTCCTTTCTGTG-3', and a mismatch reverse primer R: 5'-AGCTATGACCAGTGAAGGAATCGCTTTCCG-3', which introduced a restriction site in the wild-type allele (29). For 1431C>T, F: 5'-CAGGTTTGCTGAATGTGAAGC-3' and R: 5'-TGGCTCAGGACTCTCTGCTAGT-3' primers were used. The amplified PCR products containing Pro12Ala (247 bp) and 1431C>T (259 bp) SNPs were digested with *Hpa*II and *Nla*III restriction enzymes, respectively, at 37 °C overnight. Restriction fragments were analyzed by 3% agarose gel electrophoresis.

SNP -681C>G was genotyped by hydrolysis probes real-time PCR using TaqMan® SNP Genotyping Assay (Applied Biosystems), ID C_938 4417_10. Real-time PCR was performed on the ABI PRISM™ 7700 Sequence Detection System (Applied Biosystems).

Genotyping was repeated for fifty percent of all samples (including all mutant homozygous and all heterozygous samples for Pro12Ala and 1431C>T SNPs and randomly selected other samples) with 100% reproducibility.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 19 software. Statistical significance was set as $p<0.05$. The differences in genotype frequencies between cases and controls were assessed by chi-square (χ^2) test and Fisher's exact test (in the case where frequencies were less or equal to 5). Genotype and diplotype associations with clinical and biochemical markers were tested by Mann-Whitney test and Kruskal-Wallis test, respectively. Linkage disequilibrium (LD) between polymorphisms was calculated by 2LD program (30). Haplotype analyses were performed using PHASE 2.1 software (31).

Results

The characteristics of MetS patients and control subjects are presented in *Table I*. Values of all the measured traits were significantly different between

Table I Anthropometric, clinical, and biochemical characteristics of patients with the metabolic syndrome and control subjects.

	MetS patients (n=43)	Controls (n=43)	p*
Age (years)	49 (40–56)	45 (41–51)	0.206
Males/Females	19/24	10/33	0.040
BMI (kg/m^2)	33.0 (29.2–35.5)	24.7 (22.2–27.4)	<0.001
Waist circumference (cm)	110 (96–120)	83 (78–90)	<0.001
Systolic BP (mmHg)	143 (130–158)	120 (110–125)	<0.001
Diastolic BP (mmHg)	90 (80–100)	78 (70–80)	<0.001
Fasting insulin (mU/L)	10.6 (8.0–13.9)	7.3 (6.4–10.1)	0.010
Fasting glucose (mmol/L)	8.4 (5.5–11.7)	5.0 (4.7–5.2)	<0.001
HOMA-IR	4.1 (2.7–6.1)	1.6 (1.4–2.3)	<0.001
HbA_{1c} (%)	6.1 (5.5–7.2)	5.6 (4.8–6.0)	<0.001
Total cholesterol (mmol/L)	5.6 (5.1–6.3)	5.8 (5.2–6.5)	0.385
LDL-cholesterol (mmol/L)	3.20 (2.60–4.01)	3.37 (2.87–4.19)	0.133
HDL-cholesterol (mmol/L)	1.07 (0.88–1.30)	1.67 (1.38–1.87)	<0.001
Triglycerides (mmol/L)	2.26 (1.77–3.27)	1.17 (0.76–1.45)	<0.001
hsCRP (mg/L)	5.0 (3.0–6.0)	1.3 (0.8–4.0)	<0.001

Values represent medians (lower-upper quartile). BMI – body mass index; BP – blood pressure; HOMA-IR – homeostasis model assessment insulin resistance index; LDL – low-density lipoprotein; HDL – high-density lipoprotein; hsCRP – high-sensitivity C-reactive protein. *Significance of difference in Mann-Whitney test.

Table II Genotype and variant allele frequencies for Pro12Ala, 1431C>T, and -681C>G polymorphisms.

Polymorphism	Genotype	MetS patients		Controls		p*
		No. of subjects (%)	Mutated allele frequency	No. of subjects (%)	Mutated allele frequency	
Pro12Ala	Pro/Pro	31 (73.8%)	0.18	31 (72.1%)	0.17	0.876
	Pro/Ala	7 (16.7%)		9 (20.9%)		
	Ala/Ala	4 (9.5%)		3 (7.0%)		
	Total	42		43		
1431C>T	C/C	32 (78.0%)	0.16	32 (74.4%)	0.14	0.189
	C/T	5 (12.2%)		10 (23.3%)		
	T/T	4 (9.8%)		1 (2.3%)		
	Total	41		43		
-681C>G	C/C	25 (61.0%)	0.27	24 (57.1%)	0.25	0.377
	C/G	10 (24.4%)		15 (35.7%)		
	G/G	6 (14.6%)		3 (7.1%)		
	Total	41		42		

*Significance of χ^2 / Fisher's exact test for comparison of genotype frequencies between MetS patients and controls.

Table III Anthropometric, clinical and biochemical characteristics according to the genotypes of Pro12Ala, 1431C>T, and -681C>G polymorphisms in the group of patients with metabolic syndrome.

	Pro12Ala		p*	1431C>T		p	-681C>G		p
	Pro/Pro (n=31)	Pro/Ala+Ala/Ala (n=11)		C/C (n=32)	C/T+T/T (n=9)		C/C (n=25)	C/G+G/G (n=16)	
Age (years)	48 (39–54)	53 (43–57)	0.169	48 (39–54)	54 (52–57)	0.035	49 (39–54)	52 (43–57)	0.556
BMI (kg/m^2)	31.0 (29.2–35.4)	35.0 (27.0–38.0)	0.647	33.0 (29.6–35.5)	29.4 (26.5–39.8)	0.581	30.8 (28.6–34.7)	34.7 (27.6–37.6)	0.370
Waist circumference (cm)	110 (96–119)	115 (96–125)	0.629	110 (97–120)	111 (96–125)	0.985	110 (96–116)	115 (97–125)	0.506
Systolic BP (mm Hg)	143 (130–153)	140 (135–160)	0.849	143 (130–153)	145 (140–160)	0.607	140 (130–150)	140 (130–160)	0.858
Diastolic BP (mm Hg)	90 (80–101)	90 (80–98)	0.702	90 (80–100)	90 (83–99)	0.918	90 (80–103)	90 (80–98)	0.615
Fasting insulin (mU/L)	10.6 (8.4–14.1)	10.9 (8.1–13.8)	1.000	10.6 (8.1–13.9)	10.9 (7.4–14.0)	0.956	10.6 (8.8–14.2)	10.5 (8.0–13.9)	0.908
Fasting glucose (mmol/L)	7.6 (5.3–11.8)	10.0 (5.8–11.7)	0.453	7.6 (5.2–11.5)	9.4 (6.1–11.8)	0.361	7.6 (5.2–12.0)	8.9 (5.4–11.5)	0.761
HOMA-IR	4.6 (2.5–6.9)	3.6 (3.4–5.3)	0.959	4.6 (2.5–7.3)	3.6 (3.3–4.9)	0.784	4.8 (2.5–6.5)	3.9 (3.2–5.1)	0.842
HbA1c (%)	5.9 (5.3–6.7)	7.0 (6.1–8.0)	0.016	5.9 (5.3–6.9)	6.6 (6.0–7.5)	0.102	5.9 (5.2–6.6)	6.6 (5.8–7.7)	0.077
Total cholesterol (mmol/L)	5.7 (5.1–6.4)	5.6 (5.3–6.0)	0.808	5.6 (5.0–6.3)	5.7 (5.4–6.3)	0.570	5.7 (5.1–6.3)	5.7 (5.3–6.3)	0.633
LDL-cholesterol (mmol/L)	3.16 (2.75–4.07)	3.20 (2.48–4.03)	0.804	3.07 (2.19–3.93)	3.72 (3.11–4.06)	0.157	3.21 (2.75–4.07)	3.11 (2.58–4.05)	0.987
HDL-cholesterol (mmol/L)	1.04 (0.86–1.29)	1.15 (0.92–1.49)	0.398	1.04 (0.85–1.26)	1.20 (0.94–1.45)	0.223	1.12 (0.89–1.36)	1.00 (0.85–1.34)	0.656
Triglycerides (mmol/L)	2.33 (1.85–3.93)	2.19 (1.69–2.43)	0.317	2.33 (1.82–3.82)	2.19 (1.68–2.84)	0.373	2.32 (1.75–3.27)	2.19 (1.72–3.25)	0.762
hsCRP (mg/L)	4.0 (3.0–5.8)	6.0 (4.5–8.3)	0.053	4.0 (3.0–6.0)	6.0 (2.5–8.6)	0.324	4.0 (3.0–5.8)	6.0 (3.0–7.0)	0.218

Values represent medians (lower–upper quartile). BMI – body mass index; BP – blood pressure; HOMA–IR – homeostasis model assessment insulin resistance index; LDL – low-density lipoprotein; HDL – high-density lipoprotein; hsCRP – high-sensitivity C-reactive protein. *Significance of difference in Mann–Whitney test.

Table IV Anthropometric, clinical and biochemical characteristics according to the genotypes of Pro12Ala, 1431C>T, and -681C>G polymorphisms in the control group.

	Pro12Ala		p^*	1431C>T		p	-681C>G		p
	Pro/Pro (n=31)	Pro/Ala+Ala/Ala (n=12)		C/C (n=32)	C/T+T/T (n=11)		C/C (n=24)	C/G+G/G (n=18)	
Age (years)	46 (42–52)	45 (40–49)	0.605	45 (41–52)	45 (40–50)	0.974	45 (42–52)	47 (41–51)	0.646
BMI (kg/m^2)	25.2 (22.7–28.7)	22.2 (20.8–24.5)	0.012	24.8 (22.5–28.7)	23.1 (20.7–25.4)	0.049	25.0 (22.6–28.8)	23.8 (21.3–25.9)	0.178
Waist circumference (cm)	86 (79–93)	80 (74–86)	0.045	86 (78–91)	80 (75–88)	0.082	86 (78–90)	82 (79–93)	0.345
Systolic BP (mmHg)	120 (110–125)	121 (114–135)	0.256	120 (118–125)	118 (109–131)	0.375	120 (120–125)	120 (110–125)	0.216
Diastolic BP (mm Hg)	80 (70–80)	77 (75–85)	0.366	80 (70–80)	77 (70–85)	0.868	80 (70–80)	78 (70–80)	0.815
Fasting insulin (mU/L)	7.3 (6.5–9.9)	7.9 (6.2–10.6)	0.499	7.3 (6.6–12.4)	6.9 (6.1–9.4)	0.296	7.1 (6.3–8.2)	8.1 (6.3–11.4)	0.564
Fasting glucose (mmol/L)	5.0 (4.8–5.2)	5.0 (4.7–5.4)	0.416	5.0 (4.8–5.3)	4.9 (4.5–5.1)	0.433	5.0 (4.8–5.2)	5.0 (4.7–5.4)	0.596
HOMA–IR	1.6 (1.3–2.2)	1.8 (1.4–2.7)	0.647	1.7 (1.4–2.7)	1.4 (1.3–2.1)	0.190	1.6 (1.4–1.8)	1.7 (1.3–2.8)	0.811
HbA_{1c} (%)	5.6 (4.7–6.0)	5.6 (5.0–6.0)	0.820	5.6 (4.7–6.0)	5.6 (5.1–6.1)	0.684	5.6 (4.7–6.0)	5.6 (5.1–6.0)	0.538
Total cholesterol (mmol/L)	6.1 (5.2–6.7)	5.4 (5.1–5.8)	0.060	5.9 (5.1–6.7)	5.8 (5.2–6.3)	0.766	6.0 (5.1–6.7)	5.8 (5.2–6.3)	0.457
LDL-cholesterol (mmol/L)	3.56 (3.03–4.74)	3.16 (2.74–3.50)	0.070	3.41 (2.86–4.43)	3.34 (2.87–4.18)	0.719	3.48 (3.03–4.68)	3.30 (2.86–4.18)	0.432
HDL-cholesterol (mmol/L)	1.61 (1.37–1.87)	1.77 (1.41–1.89)	0.400	1.68 (1.37–1.90)	1.62 (1.39–1.81)	0.975	1.70 (1.39–1.94)	1.58 (1.35–1.83)	0.424
Triglycerides (mmol/L)	1.17 (0.89–1.63)	0.93 (0.62–1.37)	0.201	1.17 (0.78–1.43)	1.08 (0.68–1.46)	0.791	1.15 (0.78–1.45)	1.13 (0.68–1.45)	0.578
hsCRP (mg/L)	1.3 (0.8–4.3)	1.3 (0.7–3.4)	0.570	1.4 (0.8–5.0)	1.2 (0.6–1.7)	0.342	1.1 (0.7–6.0)	1.4 (1.0-2.2)	0.790

Values represent medians (lower-upper quartile). BMI – body mass index; BP – blood pressure; HOMA-IR – homeostasis model assessment insulin resistance index; LDL – low-density lipoprotein; HDL – high-density lipoprotein; hsCRP – high-sensitivity C-reactive protein. *Significance of difference in Mann–Whitney test.

cases and controls, except for total and LDL-cholesterol concentrations.

Genotype and mutant allele frequencies for Pro12Ala, 1431C>T, and -681C>G polymorphisms are shown in *Table II*. Genotype frequencies for all three SNPs were in line with the Hardy-Weinberg equilibrium in the control group ($p>0.05$). There were no significant differences in the distribution of genotypes for the three *PPARG* gene variants between MetS patients and controls (*Table II*).

We also explored the relationship between genotypes and anthropometric, clinical, and biochemical features. In MetS patients group, the carriers of Pro12Ala polymorphism had higher HbA_{1c} levels compared to the wild-type homozygotes ($p=0.016$) (*Table III*). Significant associations with the measures of obesity (BMI and waist circumference) were not detected in the patients (*Table III*).

In the control subjects, the Pro12Ala variant was significantly associated with lower BMI ($p=0.012$) and lower waist circumference ($p=0.045$) (*Table IV*). It also showed a trend of association with lower total and LDL-cholesterol levels ($p=0.060$ and $p=0.070$, respectively). Likewise, the carriers of 1431C>T polymorphism had lower BMI compared to the wild-type homozygotes ($p=0.049$). Significant associations with the other parameters were not found (*Table IV*).

Two *PPARG* gene polymorphisms, 1431C>T and -681C>G, were in strong linkage disequilibrium (D'=0.94). The Pro12Ala variant was in LD at 75% with these two SNPs. We also performed haplotype analysis. *Table V* shows haplotype frequencies and diplotypes, with the following order of the three polymorphisms: -681C>G, Pro12Ala, and 1431C>T. There was no significant difference in the distribution of haplotypes between MetS patients and controls ($p=0.64$). Further, we explored the association between diplotypes and MetS-related quantitative traits. Since other diplotypes were rare, we tested the differences only between the complete wild-type diplotype, CProC/CProC, and two groups of combined diplotypes: a group of diplotypes with mutated -681C>G locus (CProC/GProC and GProC/GProC) and a group of diplotypes containing the haplotype with all three loci mutated, X/GAlaT (CProC/GAlaT,

Table V Haplotype frequencies and diplotypes of Pro12Ala, 1431C>T, and -681C>G variants in the group of patients with metabolic syndrome and control group.

Haplotype*	Frequency		Diplotype	No. of subjects	
	MetS patients	Controls		MetS patients	Controls
CProC	0.705	0.690	CProC/CProC	25	22
CProT	0.013	0.004	CProC/CProT	1	0
CAlaC	0.017	0.056	CProC/CAlaC	0	2
GProC	0.091	0.098	CProC/GProC	4	6
GProT	0.012	0.033	CProC/GProT	0	3
GAlaC	0.032	0.016	CProC/GAlaC	2	1
GAlaT	0.129	0.102	CProC/GAlaT	3	4
			CAlaC/CAlaC	0	1
			CAlaC/GAlaT	1	1
			GProC/GProC	1	0
			GProC/GAlaC	1	0
			GProC/GAlaT	0	2
			GProT/GAlaT	1	0
			GAlaT/GAlaT	3	1

* Order of the SNPs in haplotypes/diplotypes: -681C>G, Pro12Ala, and 1431C>T.

CAlaC/GAlaT, GProC/GAlaT, GProT/GAlaT and GAlaT/GAlaT). No significant associations in MetS group were found. In controls, individuals with X/GAlaT diplotype, compared to the CProC/CProC carriers, had significantly lower BMI (data presented as median (interquartile range): 21.5 (20.7–24.5) kg/m^2 *vs*. 25.3 (22.7–29.0) kg/m^2; $p=0.023$). However, the BMI difference was not significant after applying Bonferroni's correction for multiple comparisons (corrected level of significance $\alpha=0.017$).

Discussion

The *PPARG* gene is implicated in adipocyte differentiation, obesity, dyslipidemia and insulin resistance, thus representing a good candidate gene for the MetS. This was the first study to examine the effect of *PPARG* gene variations on the MetS and its components in a population from Bosnia and Herzegovina. We found no relationship between the Pro12Ala, 1431C>T, and -681C>G polymorphisms and the MetS. Thus far, few studies have found an association of *PPARG* variants with an increased risk for MetS (21, 32), while others, in line with our results, revealed no significant associations (15–17, 33). Nevertheless, a large cohort study among non-diabetic Danish subjects found a decreased risk of insulin resistance syndrome associated with the Ala/Ala genotype (34). Our preliminary study, however, had limited power to detect possibly small effects of the investigated SNPs on the MetS, due to the insufficient sample size. In general, inconsistent findings between genetic association studies might be attributed to inadequate statistical power. According to a recent meta-analysis, the combined overall odds ratio for an association between the Pro allele of Pro12Ala variant and type 2 diabetes was 1.16 (12). A sample size of nearly 4000 patients, and the same number of controls, would be needed to detect this small effect size with 80% power. In addition, the MetS represents a very complex and heterogeneous disease, which could further compromise the results of genetic association studies (35).

In the analysis of MetS-related quantitative traits according to different genotypes, the only significant association in the MetS patient group was found between the Pro12Ala variant and higher HbA_{1c} levels. However, the majority of patients in our study received oral hypoglycemic therapy, and this result has to be taken very cautiously. In the control group, Pro12Ala and 1431C>T SNPs demonstrated significant associations with lower BMI. In controls, carriers of Pro12Ala had also lower waist circumference compared to the wild-type homozygotes. A similar beneficial effect of the Pro12Ala variant on BMI was shown in a previous study of our group, done on Slovene patients with the polycystic ovary syndrome (23). Associations of the 681C>G variant with MetS-related traits were not found in either group of subjects.

Only a few studies have examined the effect of –681C>G polymorphism on the metabolic phenotype. Results from these studies have demonstrated an association of –681C>G with higher LDL-cholesterol concentrations and body weight in a French population (25, 36). The lack of association in our study might be attributed to the small sample size. On the other hand, a number of studies have explored the relationship of Pro12Ala and 1431C>T with obesity and MetS parameters, with conflicting results. Deeb et al. initially reported an association of Pro12Ala with lower BMI and improved insulin sensitivity in normal weight and slightly overweight Finns (10). In the later study, Pro12Ala and 1431C>T SNPs were associated with increased BMI in obese Finn women (37). In a cohort of Danish men, the Ala/Ala genotype was associated with a higher BMI in obese, but lower BMI in lean subjects (38). A large meta-analysis detected a significant association of the Pro12Ala variant with higher BMI only in subjects with a BMI above 27 kg/m^2 (39). Another meta-analysis of data from non-diabetic subjects found an association of the Ala allele with greater BMI and greater insulin sensitivity only in selected subgroups, such as Caucasians and obese subjects (40). Our results were partially in accordance with the mentioned studies, since we found an association of the two *PPARG* variants with lower BMI only in the group of healthy control non-obese subjects (median BMI 24.7 kg/m^2). Disparate effects of the Pro12Ala variant on BMI in obese and lean subjects may be explained by the gene–nutrient interaction. Luan et al. showed that a low dietary polyunsaturated/saturated fatty acids ratio is associated with a greater BMI in Ala12 carriers, but when this ratio is high, the opposite is found (41). These observations are in line with the results from a large prospective study in a French population (42) and a study in Italian diabetic patients (43). Two other studies reported opposite findings: total dietary fat intake was associated with higher BMI in Pro/Pro individuals, but not among Ala12 carriers in a French Canadian population (44) and healthy American women (45). Similar effects of diet on waist circumference (44) and plasma lipid concentrations (45) were observed. The discrepancy between the mentioned studies may be due to the difficulty of accurately assessing intake of specific types of dietary fat (45). However, such evidence of the gene–environment interaction could, at least partially, explain the controversial effects of Pro12Ala and 1431C>T SNPs on BMI.

The Pro12Ala polymorphism modulates transcriptional activity (10, 11) and leads to the less efficient stimulation of *PPARG* target genes. Protective effect of the Ala allele in type 2 diabetes is associated with improved insulin sensitivity (10, 12, 40, 46). It has been shown that moderate reduction of PPARγ activity in heterozygous PPARγ-deficient mice decreases triglyceride content of white adipose tissue, skeletal muscle, and liver due to increased leptin expression and increase in fatty acid combustion and decrease in lipogenesis, thereby ameliorating high fat diet-induced obesity (47). Decreased lipogenesis in white adipose tissue prevents adipocyte hypertrophy, which is associated with alleviation of insulin resistance, presumably due to decreases in free fatty acids, tumor necrosis factor α, and upregulation of adiponectin (47). In our preliminary study, associations with markers of insulin resistance were not detected. However, we observed a trend of association of the Pro12Ala variant with lower total and LDL-cholesterol levels in the control subjects. Previous studies that analyzed the association of Pro12Ala with blood lipids have reported opposing results. Only a few studies detected an association of the Ala allele with lower total cholesterol (18, 48), and lower LDL-cholesterol levels (48). However, it has been more frequently associated with lower triglyceride (10, 34, 49–51) and higher HDL-cholesterol levels (10, 18, 51), which has been confirmed by a recent meta-analysis (52). As decreased HDL-cholesterol and increased triglyceride levels represent two components of the MetS, these findings support the hypothesis that the Pro allele may be one of the genetic factors that contribute to the MetS.

In the present study, we also showed an association of the 1431C>T variant with lower BMI in controls. This could be a consequence of the linkage disequilibrium between 1431C>T and Pro12Ala, or another polymorphism. Nevertheless, positive effects of this SNP on the metabolic phenotype have been demonstrated earlier. It has been associated with a favorable lipid profile (24, 33, 53), and reduced risk of coronary artery disease (54) and severe atherogenesis (24).

Our data showed that three *PPARG* gene SNPs were in high linkage disequilibrium, similarly as in a previous study (32). Haplotype analysis confirmed the beneficial effect of mutated GAlaT haplotype on BMI in controls, although the significance was lost after multiple testing correction.

In conclusion, we found a significant association of Pro12Ala and 1431C>T polymorphisms with lower BMI in the control subjects. The Pro12Ala variant was also associated with lower waist circumference in controls. Associations of the investigated polymorphisms with markers of insulin resistance were not detected. Findings of our preliminary study indicate a beneficial effect of the common Pro12Ala variant on the metabolic phenotype in healthy non-obese subjects. Future, larger population-based studies are needed to clarify the role of this and other *PPARG* gene variants in MetS risk.

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Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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