ORIGINAL ARTICLE

Association of *LPIN1* gene variations with markers of metabolic syndrome in population from Bosnia and Herzegovina

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ABSTRACT

Aim To investigate association of two *LPIN1* gene variations with main traits of metabolic syndrome (MS) (waist circumference, body mass index, blood pressure, triglycerides, HDL-cholesterol and fasting glucose levels) in population from Bosnia and Herzegovina.

Methods This study included 43 patients with metabolic syndrome and 43 healthy controls from General Hospital in Tešanj, Bosnia and Herzegovina. Subjects were genotyped for two *LPIN1* gene variations (rs11693809: C>T and rs2716610: C>T) by real time PCR method.

Results In control subjects *LPIN1* polymorphism, rs2716610: C>T, was significantly associated with a lower body mass index (BMI) (p=0.008) and waist circumference (p=0.008). The second analyzed rs11693809: C>T polymorphism was associated with lower blood HbA1c levels (p=0.048) in a group of MS patients.

Conclusion Results of our study suggest that rs2716610: C>T polymorphism of *LPIN1* gene could have a protective effect against development of metabolic syndrome, while rs11693809: C>T might affect a glucose control in patients with MS.

Keywords: metabolic syndrome, *LPIN1* gene, markers, gene variations.

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INTRODUCTION

The metabolic syndrome (MS) is also known as syndrome X(1), the insulin resistance syndrome (2) and the deadly quartet (3). Metabolic syndrome is a combination of medical disorders that, when occurring together, increase the risk of developing cardiovascular disease and diabetes (4). The pathogenesis of the metabolic syndrome is thought to involve a complex interaction of multiple factors, including obesity and abnormal fat distribution, insulin resistance, hepatic, vascular, and immunologic factors, as well as lifestyle and genetic contributions (4). The available evidence indicates that between 20% and 30% of the world's adult population have MS (5). New harmonized definition and criteria for MS was accepted finally in 2009, which included measurement of waist circumference, blood pressure, triglycerides, HDL-cholesterol and serum glucose (6). Insulin resistance and abdominal obesity appear to be predominant underlying risk factors of MS. Beside this, other associated conditions for this syndrome can be genetic factors, physical inactivity, aging, and hormonal imbalance (7).

A newly discovered gene for lipin 1 (*LPIN1*) resides in the 2p25 region, and codes for phosphatidic acid phosphatase, a key enzyme in triglyceride (TG) biosynthesis (8). There is evidence that chromosome region 2p25 is in linkage disequilibrium with several obesity related phenotypes, such as body mass index (BMI), waist circumference, skin-fold thickness, and percentage of body fat (9). The lipin protein family consists of three members, lipin-1, lipin-2, and lipin-3 (10). Lipin 1 is a newly discovered multifunctional protein that participates in the metabolism of lipids in different ways (11). Lipin-1 is abundantly expressed in adipose tissue and skeletal muscle, and lipin-1 protein localizes to either the cytosol or the nucleus, which may be related to its two known functions (12). Namely, in the cytosol, lipin-1 acts as a phosphatidate phosphatase (PAP) enzyme converting phosphatidate to diacylglycerol during triglyceride biosynthesis (13-14), while in the nucleus of adipocytes and hepatocytes lipin-1 acts as a transcriptional coactivator that interacts with the nuclear receptor peroxisome proliferator-activated receptor α (PPARα) and PPARγ (PPARG) coactivator 1α (PPARGC1α) in a complex that modulates fatty acid oxidation gene expression (10, 15-16).

Null mutations in the murine *Lpin1* gene result in a severe defect in adipose tissue development, which is related to insulin resistance and fatty liver dystrophy (*fld* mice) (10). Based upon the comprehensive biological knowledge of lipin-1 role in energy metabolism, human lipin-1 has been considered as an obvious biological candidate to explain some of the inter-individual variations in the common metabolic phenotypes. In addition, variants in *LPIN1* have been associated with the fasting serum insulin levels, body mass index (BMI), waist circumference, and obesity development (17-19). Previous data from four meta-analysis studies found an association of *LPIN1* variants with increased BMI (20), while another study found an association with hypertension (21). Other studies have shown correlation of expression of *LPIN1* gene in adipose tissue with BMI and insulin resistance in humans (14, 16). Recently published German population study (n=1674), showed an interesting association of *LPIN1* gene variants with metabolic phenotype (22). They identified three associated three-marker haplotypes, one common haplotype that increased the risk for metabolic syndrome, while other two were associated with lower blood pressure levels, lower BMI, waist circumference, and HbA_1C levels (22).

In this study, for the first time in population from Bosnia and Herzegovina, it was analyzed whether *LPIN1* gene polymorphisms (rs11693809: C>T and rs2716610: C>T), including haplotype analysis, were associated with the traits of metabolic syndrome. An association between biochemical parameters including, but not limited to, glucose, HbA1c, insulin levels, HDL and LDL cholesterol, triglycerides, serum proteins levels, and activity of liver enzymes and these two polymorphisms in *LPIN1* gene was analyzed in patients with metabolic syndrome and healthy controls.

PATIENTS AND METHODS

Study participants

The study included 43 patients with metabolic syndrome and 43 healthy controls from General Hospital in Tešanj, Zenica-Doboj Canton, Bosnia and Herzegovina. Investigation was done in accordance with ethical recommendations and practices of the General Hospital Tešanj, and with ethical

principles outlined in the World Medical Association Declaration of Helsinki – Ethical Principles of Medical Research Involving Human Subjects (initiated in June 1964, last amendment in October 2000). Each subject in the study signed written informed consent. Metabolic syndrome was diagnosed according to new harmonized definition and criteria for MS from 2009. According to this new definition, MS is diagnosed when any three of the following five criteria are met: increased waist circumference (recommended waist circumference thresholds for Euripides) ≥ 94 cm for men, and ≥ 80 cm for women), triglycerides ≥ 1.7 mmol/L, HDL-cholesterol < 1.0 mmol/L in males and < 1.3 mmol/L in females, blood pressure ≥130/85 mmHg and fasting glucose ≥ 5.6 mmol/L (6). Patients treated with insulin and patients with acute infection and / or inflammation and endocrine disorders were excluded from the study. All patients included in the study were using heterogeneous therapy (74% of all patients received antihypertensive therapy, 58% were treated with glucose-lowering drugs, and 47% were treated with lipid-lowering drugs). Healthy control group consisted of 43 non-obese, age-matched subjects, who had less than three features of MS. They were not taking any medication during the course of the study.

Biochemical and anthropometrical measurements

Waist circumference, height, weight, systolic and diastolic blood pressure were measured in all participants. BMI was calculated as weight (kg)/(height (m))^2. Serum levels of fasting glucose, triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol, albumin, globulin, bilirubin, creatinine, urea, urate, HbA1c and C-reactive protein (CRP), as well as activities of aspartate aminotransferase, alanine aminotransferase, and γ-glutamyltransferase were determined by using the VITROS auto analyzer 350 Chemistry System (Ortho-Clinical Diagnostics, Rochester, New York, USA). Serum insulin levels were measured by the Abbott AxSYM (Abbott Diagnostics, North Chicago, Illinois, USA) analyzer. HOMA IR index was calculated by using following formula: fasting insulin (mU/L) x fasting glucose (mmol/L)/22.5 (23).

Genotyping analysis

The Miller extraction protocol was used for the DNA extraction (24). Genotyping analysis for rs11693809 (IVS1 +3341C > T, denoted intron 1 SNP) and rs2716610 (IVS17-228C > T, denoted intron 17 SNP) polymorphisms was performed with real-time PCR allelic discrimination on ABI PRISM with C_2096848_10 and C_16280532_10 assays, respectively (Applied Biosystems, Foster City, CA, USA). We double-genotyped twenty percent of all samples with 100% concordant results.

Statistical analysis

Chi-square (χ^2) and Fisher's exact tests (in the case where frequencies were less or equal to 5) were applied to examine differences in allele frequencies and genotype distributions between healthy controls and patients with MS. Significance of difference of biochemical and anthropometrical measurements according to genotypes of analyzed polymorphisms, sex and age were estimated by linear regression Corrections for

Table 1. Characteristics of the study participants

Parameter*	MS patients (n=43)	Controls (n=43)	p†
Age (years)	49 (40-56)	45 (41-51)	0.206
BMI (kg/m2)	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 (mm Hg)	143 (130-158)	120 (110-125)	<0.001
Diastolic BP (mm Hg)	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
Blood HbA1c (%)	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
CRP (mg/L)	5.0 (3.0-6.0)	1.3 (0.8-4.0)	<0.001
Creatinine (mmol/L)	82.5 (72.5-94.5)	96.0 (59.0-78.0)	<0.001
Urea (mmol/L)	5.05 (4.15-6.12)	4.30 (3.70-5.40)	0.036
Urate (mmol/L)	297.0 (239.0-333.0)	272.0 (229.0-323.0)	0.018
Albumin (g/L)	49.4 (43.0-52.7)	44.0 (42.0-48.1)	0.012
Globulin (g/L)	24.6 (21.4-30.7)	32.0 (28.0-33.0)	<0.001
Bilirubin (mmol/L)	12.9 (10.7-14.7)	11.5 (9.7-14.6)	0.302
AST (IU/L)	26.0 (18.2-31.0)	26.0 (21.0-31.0)	0.593
ALT (IU/L)	27.0 (22.2-42.2)	23.0 (16.0-30.0)	0.017
GGT (IU/L)	23.5 (17.7-38.0)	17.5 (14.0-26.5)	0.298

*Values represent medians (lower-upper quartile); †significance of difference in Mann-Whitney test.
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; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT – γ, glutamyl transferase γ

multiple testing were performed by using Bonferroni correction for a total of two SNPs. A p value ≤ 0.05 was considered statistically significant. Linkage disequilibrium was calculated by using the 2LD program (25). Haplotype reconstruction was done with the PHASE program (26).

RESULTS

All analyzed parameters were significantly different between patients and control groups except total cholesterol, LDL cholesterol, and bilirubin levels, as well as aspartate aminotransferase (AST) and γ-glutamyltransferase activity (GGT) (Table 1).

Table 2. Allele and genotype frequencies for *LPIN1* gene polymorphisms*

Polymorphism		MS	T-allele frequency	Controls	T-allele frequency	p†
Intron 1SNP (rs11693809)	CC	16 (40.0%)		14 (33.3%)		
	CT	19 (47.5%)	0.36	23 (54.8%)	0,39	0.792
	TT	5 (12.5%)		5 (11.9%)		
	Total	40		42		
	p	0.985		0.631		
Intron 17 SNP (rs2716610)	CC	26 (65.0%)		32 (76.2%)		
	CT	12 (30.0%)	0.20	9 (21.4%)	0.13	0.513
	TT	2 (5.0%)		1 (2.4%)		
	Total	40		42		
	p‡	0.925		0.931		

*rs11693809: C>T and rs2716610: C>T; †Significance of $\chi 2$ / Fisher's exact test for comparison of genotype frequencies between healthy controls and MS patients; ‡p value for Hardy-Weinberg equilibrium.

Table 3. Effects of *LPIN1* SNPs on biochemical and anthropometrical parameters in controls

Parameter*	rs11693809 C>T					rs2716610 C>T				
	C/C (n=14)	C/T + T/T (n=24)	B (95% CI) †	p†	pB	C/C (n=29)	C/T + T/T (n=10)	B (95% CI)†	p†	pB
BMI (kg/m2)	23.3 (20.5-25.4)	25.1 (22.2-28.7)	2.156 (-0.758, 5.071)	0.141	0.282	25.4 (22.9-28/.7)	21.0 (20.2-24.0)	-4.272 (-7.104, -1.441)	0.004	0.008
Waist circumference (cm)	81 (72-85)	88 (78-93)	5.168 (-1.405, 11.740)	0.119	0.238	86 (80-93)	78 (72-81)	-9.637 (-16-032, -3.243)	0.004	0.008
Systolic BP (mm Hg)	120 (101-137)	120 (120-125)	3.803 (-8.272, 15.878)	0.524	1.000	120 (120-130)	120 (102-122)	-5.752 (-18.509, 7.004)	0.364	0.728
Diastolic BP (mm Hg)	75 (66-87)	80 (75-80)	2.255 (-5.21, -9.728)	0.541	1.000	80 (70-80)	75 (67-82)	-0.974 (-8.974, 7.026)	0.805	1.000
Fasting insulin (mU/L)	7.3 (6.2-14.7)	6.9 (5.8-7.7)	-3.646 (-7.582, 0.290)	0.068	0.136	6.9 (6.1-9.9)	7.3 (6.0-9.6)	-1.695 (-6.112, 2.722)	0.438	0.876
Fasting glucose (mmol/L)	5.1 (4.5-5.6)	4.9 (4.7-5.1)	-0.089 (-0.392, 0.213)	0.553	1.000	5.0 (4.8-5.2)	4.6 (4.3-5.3)	-0.298 (-0.606, 0.010)	0.057	0.114
HOMA-IR	1.6 (1.3-3.1)	1.5 (1.3-1.7)	-0.880 (-1.905, 0.144)	0.089	0.178	1.5 (1.4-2.3)	1.5 (1.2-2.2)	-0.566 (-1.697, 0.564)	0.313	0.626
Blood HbA1c (%)	5.6 (5.0-6.0)	5.6 (4.4-5.9)	0.161 (-0.289, 0.611)	0.472	0.944	5.6 (4.5-6.0)	5.6 (5.0-5.8)	0.065 (-0.420, 0.549)	0.788	1.000
Total cholesterol (mmol/L)	6.2 (5.3-7.0)	5.7 (5.1-6.4)	-0.334 (-1-059, 0.392)	0.357	0.714	5.9 (5.1-6.4)	6.0 (5.5-6.8)	0.199 (-0.584, 0.981)	0.609	1.000
LDL-cholesterol (mmol/L)	3.81 (2.97-4.85)	3.35 (2.93-4.18)	-0.154 (-0.904, 0.596)	0.679	1.000	3.34 (2.88-4.46)	3.69 (2.98-4.54)	0.123 (-0.679, 0.926)	0.757	1.000
HDL-cholesterol (mmol/L)	1.72 (1.48-2.03)	1.66 (1.37-1.86)	-0.205 (-0.474, 0.064)	0.130	0.260	1.69 (1.37-1.87)	1.68 (1.48-1.92)	0.014 (-0.283, 0.312)	0.922	1.000
Triglycerides (mmol/L)	1.15 (0.92-1.67)	1.12 (0.67-1.44)	-0.073 (-0.439, 0.294)	0.690	1.000	1.13 (0.77-1.47)	1.29 (0.63-1.49)	-0.008 (-0.401, 0.385)	0.968	1.000
hsCRP (mg/L)	1.0 (0.5-1.4)	1.7 (0.9-4.7)	1.154 (-0.582, 2.889	0.185	0.370	1.5 (0.8-3.8)	1.1 (0.5-2.0)	-1.018 (-2.890, 0.853)	0.277	0.554
Creatinine (mmol/L)	63.0 (56.0-71.0)	70.0 (59.0-80.5)	4.348 (-3.075, 11.771)	0.242	0.484	70.0 (60.5-80.0)	56.0 (51.5-70.0)	-8.440 (-15.985, -0.894)	0.029	0.058
Urea (mmol/L)	4.5 (3.6-5.5)	4.4 (3.8-5.4)	0.188 (-0.70, -1.082)	0.671	1.000	4.7 (4.0-5.4)	3.8 (3.3-4.8)	-0.809 (-1.724, 0.106)	0.081	0.162
Urate (mmol/L)	251.5 (223-320)	289.5 (228-326)	3.283 (-38.087, 44,634)	0.873	1.000	285 (229-323)	244 (213-330)	-2.706 (-46.896, 41.485)	0.902	1.000
Albumin (g/L)	44.5 (42.7-49.1)	44.0 (42.2-47.4)	-1.690 (-4.365-0.985)	0.208	0.416	44.0 (42.0-47.8)	44.5 (42.7-47.4)	0.619 (-2.302, 3.540)	0.669	1.000
Globulin (g/L)	30.0 (25.4-33.0)	32.0 (28.7-34.5)	2.900 (-0.689-6.488)	0.110	0.220	32.0 (28.8-34.0)	31.5 (27.4-34.0)	-0.075 (-4.062, 3.913)	0.970	1.000
Bilirubin (mmol/L)	9.6 (6.9-11.4)	12.5 (10.5-16.2)	2.488 (-0.389-5.365)	0.088	0.176	11.7 (9.6-15.6)	10.6 (7.7-11.9)	-1.401 (-4.578, 1.777)	0.377	0.754
AST (IU/L)	22.0 (20.5-30.0)	26.0 (21.5-30.7)	1.358 (-2.611-5.326)	0.491	0.982	26.0 (21.5-30.5)	23.5 (19.5-30.0)	-1.147 (-5.399, 3.105)	0.587	1.000
ALT (IU/L)	21.0 (11.7-29.0)	24.0 (16.0-29.7)	0.343 (-7.313-8.000)	0.928	1.000	23.0 (14.0-28.5)	22.0 (16.7-35.2)	4.157 (-3.894, 12.208)	0.301	0.602
GGT (IU/L)	16.0 (13.7-28.7)	18.0 (15.0-26.0)	-5.483 (-22.380-11.414)	0.514	1.000	17.0 (15.0-25.5)	18.5 (13.7-35.5)	15.814 (-1.500, 33.127)	0.072	0.144

*Values represent medians (lower-upper quartile); †Effects (unstandardized coefficients B) and p values were assessed using multiple linear regression adjusted for age and gender, under dominant genetic model; pB, adjusted by using Bonferroni correction for two SNPs;
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; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT-γ; glutamyltransferase;

Table 4. Effects of *LPIN1* SNPs on biochemical and anthropometrical parameters in the patients

Parameter*	rs11693809 C>T					rs2716610 C>T				
	C/C (n=16)	C/T + T/T (n=24)	B (95% CI)†	p†	pB	C/C (n=26)	C/T + T/T (n=14)	B (95% CI)†	p†	pB
BMI (kg/m2)	33.5 (29.3-36.4)	33.0 (26.9-35.3)	0.280 (-3.845, 4.404)	0.891	1.000	33.5 (28.9-36.9)	33.0 (25.7-35.1)	-1.825 (-5.904, 2.253)	0.370	0.740
Waist circumference (cm)	111 (103-117)	111 (96-123)	-5.482 (-17.623, 6.659)	0.365	0.730	112 (106-120)	110 (87-122)	-6.339 (-18.423, 5.746)	0.293	0.586
Systolic BP (mm Hg)	145 (140-160)	140 (130-155)	-6.002 (-20.321, 8.317)	0.398	0.796	145 (140-160)	140 (130-150)	-7.799 (-21.990, 6.392)	0.270	0.540
Diastolic BP (mm Hg)	95 (81-100)	90 (80-97)	-4.814 (-14.276, 4.647)	0.307	0.614	92 (81-100)	85 (70-97)	-6.985 (.16.248, 2.277)	0.134	0.268
Fasting insulin (mU/L)	10.3 (7.3-12.6)	11.5 (8.9-16.4)	0.381 (-4.951, 5.712)	0.885	1.000	10.4 (7.4-12.9)	11.3 (9.0-16.0)	-0.869 (-6.191, 4.454)	0.741	1.000
Fasting glucose (mmol/L)	11.0 (7.2-14.0)	6.2 (5.4-9.8)	-2.315 (-5.305, 0.675)	0.125	0.250	9.0 (5.8-11.9)	6.1 (5.5-10.3)	-2.118 (-5.124, 0.888)	0.162	0.324
HOMA-IR	4.3 (3.6-5.6)	4.3 (2.6-7.7)	0.088 (-1.909, 2.084)	0.929	1.000	4.3 (3.3-8.0)	3.6 (2.3-5.6)	-1.047 (-3.006, 0.912)	0.284	0.568
Blood HbA1c (%)	6.9 (5.9-7.7)	5.9 (5.2-6.6)	-1.082 (-2.010, -0.154)	0.024	0.048	6.4 (5.5-7.4)	5.9 (5.5-6.5)	-0.223 (-1.222, 0.775)	0.653	1.000
Total cholesterol (mmol/L)	5.6 (5.1-6.0)	5.6 (5.0-6.4)	0.142 (-0.629, 0.913)	0.711	1.000	5.7 (5.1-6.2)	5.5 (4.5-6.3)	-0.228 (-0.996, 0.549)	0.551	1.000
LDL-cholesterol (mmol/L)	3.02 (2.56-3.28)	3.31 (2.46-4.13)	0.309 (-0.515, 1.134)	0.451	0.902	3.16 (2.77-3.90)	3.05 (2.01-4.18)	-0.016 (-0.848, 0.815)	0.969	1.000
HDL-cholesterol (mmol/L)	1.11 (0.83-1.42)	1.00 (0.88-1.32)	0.043 (-0.181, 0.266)	0.701	1.000	1.20 (0.93-1.46)	0.91 (0.83-1.13)	-0.228 (-0.438, -0.019)	0.033	0.066
Triglycerides (mmol/L)	2.38 (1.69-3.21)	2.24 (1.88-3.69)	-0.595 (-1.473, 0.283)	0.177	0.354	2.22 (1.85-3.15)	2.37 (1.69-4.03)	-0.112 (-1.013, 0.788)	0.801	1.000
hsCRP (mg/L)	5.0 (3.2-6.0)	5.0 (2.2-6.7)	-0.103 (-1.946, 1.740)	0.910	1.000	5.0 (3.0-6.0)	5.0 (2.0-7.2)	0.233 (-1.608, 2.074)	0.798	1.000
Creatinine (mmol/L)	83.0 (78.7-88.5)	83.0 (70.0-110.0)	2.708 (-12.677, 18.093)	0.723	1.000	82.0 (74.2-89.5)	89.0 (67.5-105.5)	-0.377 (-15.788, 15.034)	0.961	1.000
Urea (mmol/L)	4.9 (3.9-5.8)	5.3 (4.66.2)	0.444 (-0.703, 1.590)	0.437	0.874	5.0 (4.3-6.0)	5.0 (3.9-6.5)	-0.110 (-1.266, 1.047)	0.848	1.000
Urate (mmol/L)	275 (232-336)	325 (294-338)	38.578 (-19.443, 96.600)	0.185	0.370	295 (246-337)	316 (278-337)	-0.178 (-59.775, 59.419)	0.995	1.000
Albumin (g/L)	49.9 (45.1-51.8)	48.0 (43.0-53.3)	-0.799 (-4.794, 3.195)	0.686	1.000	50.5 (43.7-52.9)	46.4 (41.7-51.3)	-2.595 (-6.488, 1.299)	0.184	0.368
Globulin (g/L)	26.0 (21.6-30.3)	23.5 (21.4-32.0)	-1.053 (-4.852, 2.746)	0.576	1.000	24.6 (21.5-30.2)	24.6 (19.7-33.2)	-0.464 (-4.277, 3.350)	0.806	1.000
Bilirubin (mmol/L)	13.3 (12.4-15.6)	12.0 (9.1-14.0)	-2.408 (-5.738, 0.921)	0.151	0.302	13.3 (10.9-15-9)	12.0 (10.0-13.6)	-2.690 (-5.993, 0.613)	0.107	0.214
AST (IU/L)	22.0 (17.0-28.0)	26.0 (19.2-31.7)	3.968 (-4.941, 12.876)	0.372	0.744	26.0 (19.5-31.5)	22.5 (18.0-30.2)	-6.080 (-14.845, 2.686)	0.168	0.336
ALT (IU/L)	25.0 (22.0-35.0)	27.0 (22.2-48.0)	-0.843 (-12.511, 10.826)	0.884	1.000	33.0 (23.0-50.5)	23.5 (20.0-33.0)	-15.079 (-25.539, -4.619)	0.006	0.012
GGT (IU/L)	15.0 (8.75-27.5)	25.0 (16.0-38.0)	-1.335 (-18.571, 15.900)	0.876	1.000	23.0 (12.0-38.0)	22.5 (9.5-31.2)	-11.987 (-28.694, 4.721)	0.154	0.308

*Values represent medians (lower-upper quartile);

†Effects (unstandardized coefficients B) and p values were assessed using multiple linear regression adjusted for age and gender, under dominant genetic model; pB adjusted by using Bonferroni correction for two SNPs;

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; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT-γ; glutamyltransferase;

Table 5. Haplotype frequencies and diplotypes of *LPIN1* gene in MS patients and healthy controls

Haplotype (intron 1 SNP-intron 17 SNP)*	Frequency		Diplotype	Diplotype No	No. of subjects	
	MS patients	Controls			MS patients	Controls
CC	0.548	0.505	CC/CC	1	14	8
CT	0.102	0.116	CC/CT	2	3	4
TC	0.252	0.344	CC/TC	3	9	12
TT	0.098	0.035	CC/TT	4	8	-
			CT/CT	5	-	1
			CT/TC	6	-	3
			CT/TT	7	2	-
			TC/TC	8	3	4
			TC/TT	9	2	1

*corresponding to the rs11693809: C>T (intron SNP1) and rs2716610: C>T (intron SNP17) polymorphism

Allele frequencies were in Hardy-Weinberg equilibrium for both, patients and control subjects (p>0.05). However, no significant differences in analyzed genotype frequencies were found between patients and healthy controls (Table 2).

In a control group, after Bonferroni correction, rs11693809: C>T polymorphism did not show significant association with any biochemical or anthropometrical measurements. However, the carriers of T allele (CT + TT) of another analyzed polymorphism of *LPIN1* gene, rs2716610: C>T, had significantly lower BMI (p=0,008), waist circumference (p=0.008), and tendency of association with lower

creatinine levels (p=0.058), as compared to the carriers of a wild type allele (CC) (Table 3).

In group of MS patients, the carriers of T allele (CT + TT) of rs11693809: C>T polymorphism had significantly lower blood HbA_{1c} (%) (p=0.048), as compared to the carriers of a wild type allele (CC). The carriers of T allele (CT + TT) of rs2716610: C>T polymorphism had significant lower ALT activity (p=0.012) and tendency of association with lower HDL cholesterol levels (p=0.066), as compared to the carriers of a wild type allele (CC). As shown in Table 4, no association was found for both analyzed *LPIN1* gene polymorphisms with anthropometrical measurements (body mass index (BMI) and waist circumference).

The selected *LPIN1* variants, rs11693809: C>T and rs2716610: C>T were in a weak linkage disequilibrium (D'=0.261). No significant differences in distribution of haplotype frequencies between patients with MS and control subjects were demonstrated. Furthermore, an association of *LPIN1* haplotypes with biochemical and anthropometrical parameters was also tested. In control group, the carriers of CC haplotype had significantly higher plasma insulin (p=0.024), higher glucose levels (p=0.036) and higher HOMA IR index (p=0.024) as compared to the carriers of CT haplotype. No significant associations of *LPIN1* haplotypes with traits of MS were found in patients group (Table 5).

DISCUSSION

Members of the lipin protein family have a newly discovered enzymatic role in triglyceride and phospholipid biosynthesis as a phosphatidate phosphatase, and act also as inducible transcriptional coactivators in conjunction with peroxisome proliferator-activated receptor c (PPARc) coactivator-1a and PPARa (13). Through these activities, the founding member of the family, lipin-1, influences lipid metabolism and glucose homeostasis (13).

Results of our study showed significant association of rs2716610: C>T genetic variant with body mass index (BMI) and waist circumference. No significant associations between disease-associated traits and rs11693809: C>T were found.

Since the majority of patients participating in this study were using medications (antihypertensive, glucose-lowering or lipid-lowering drugs), the influence of *LPIN1* gene polymorphisms on the most of selected biochemical parameters in MS patients should be interpreted cautiously. However, it was reasonable to analyze the effects of *LPIN1* gene variations on the BMI and waist circumference in these patients.

The selected genotype-phenotype analysis showed that, the mutant T allele of rs11693809: C>T polymorphism was associated with lower blood HbA_1 in a group of MS patients. T allele of another analyzed polymorphism in our study, rs2716610: C>T**,** was associated with lower BMI and waist circumference, and creatinine levels in controls, and lower HDL cholesterol levels and lower ALT activity in a group of MS patients.

Only a few studies analyzed association of LPIN1 gene polymorphisms (rs11693809: C>T and rs2716610: C>T) with metabolic syndrome (17,22,27). Suviolahti et al. analyzed seven polymorphisms in the *LPIN1* gene. They found an association of rs11693809: C>T polymorphism with the insulin levels. In addition, rs11693809: C>T and rs2716610: C>T polymorphism were associated with BMI in lean males. Since an association of *LPIN1* variants with BMI in the lean or obese females was not found, the association of *LPIN1* alleles with BMI appeared to be sex specific (17). Mlinar et al., in their study tested an association of *LPIN1* polymorphisms (rs11693809: C>T and rs2716610: C>T) with polycystic ovary syndrome (PCOS) (28). Their results showed that mutated T allele of rs11693809: C>T polymorphism was associated with lower plasma LDL-cholesterol levels in controls, with lower glucose levels after OGTT in the PCOS patients, and with lower insulin levels and HOMA-IR in nonobese PCOS patients. These results suggest a protective role of mutated T allele of rs11693809: C>T polymorphism against development of IR and dyslipidemia. Mutated allele T of another analyzed polymorphism in this study (28), rs2716610: C>T, showed an association with higher triglyceride levels in control subjects, suggesting a negative effect of this polymorphism on the metabolic phenotype. Wiedmann at al. in their study analyzed an association of 15 genetic variants of *LPIN1* gene, including rs2716610: C>T, with metabolic phenotype. Their results showed the borderline significant

association of this polymorphism (rs2716610: C>T) with higher plasma triglyceride levels (22). In our recent study we have also tested an association of *LPIN1* and *PPARG* variants with biochemical and anthropometrical parameters in patients with MS and type 2 diabetes. Interestingly, results of this study showed that mutated T allele of rs11693809: C>T polymorphism was associated with higher insulin levels in patients with MS and type 2 diabetes (27). Neither of these studies analyzed an association of two *LPIN1* variants (rs11693809: C>T and rs2716610: C>T) with the biochemical parameters, including albumin, globulin, creatinine, urea and uric acid levels, as well as ALT, AST and GGT activity that we tested in the current study. The mechanism of an association of *LPIN1* polymorphisms with above biochemical parameters is not completely understood. However, many previous studies demonstrated an association of selected biochemical parameters, which are known as possible markers, with increased risk of development Type 2 diabetes (T2D) (29-33). Recently published studies found a positive correlation between higher creatinine and urea levels with increased risk for T2D development (32-33). Furthermore, other studies found a correlation of higher activity of liver enzymes (AST, ALT or GGT) with increased risk for MS and T2D development, or their association with obesity and insulin resistance (29-31).

Effects of two polymorphisms of *LPIN1* gene (rs11693809: C>T and rs2716610: C>T) on metabolic phenotype was further tested by the haplotype analyses. An association of rs2716610: C>T polymorphism with the lower BMI and waist circumference, and protective role of this polymorphism were confirmed by this analysis. Another analyzed polymorphism, rs11693809: C>T, did not show any association disease-associated traits, that is also confirmed by haplotype analysis. In control subjects, carriers of CT haplotype had significant lower insulin levels, glucose levels and HOMA IR levels as compared to the carriers of CC haplotype. Thus, these findings suggested that non-mutated first locus, and mutated second locus (CT haplotype) decreased metabolic risk, while non-mutated first and second locus (CC haplotype) increased metabolic risk. These positive findings have to be replicated in a larger cohort of subjects.

In conclusion, the reason why we have chosen these two SNPs of LPIN1 gene is because previous studies have shown association with metabolic phenotype, but with opposite results, prompting us to examine impact of these polymorphisms of LPIN1 gene in development of metabolic syndrome in the population of Bosnia and Herzegovina. These two polymorphisms did not cover the role of all variation of LPIN1 gene, which is a limitation of our study.

Results of our study showed that rs2716610: C>T decrease the risk of MS, while rs11693809: C>T did not show any association with risk factors of MS. An association of rs2716610: C>T polymorphism with lower BMI and waist circumference suggest that this genetic variant of *LPIN1* gene could have a protective role against development of metabolic syndrome. Future investigations including larger cohorts of subjects, genes and larger number of SNPs, as well as studies on other populations, are needed to substantiate these findings. Illuminating the mechanism and pathogenesis of this complex disorder, might lead to effective treatment, and also to predict individual's risk of developing of metabolic syndrome.

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Povezanost varijacija *LPIN1* gena s markerima metaboličkog sindroma u populaciji Bosne i Hercegovine

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SAŽETAK

Cilj Istražiti povezanost dvije varijante *LPIN1* gena s glavnim karakteristikama metaboličkog sindroma (MS) (opseg struka, indeks tjelesne mase, krvni pritisak, trigliceridi, HDL holesterol i glukoza) u populaciji Bosne i Hercegovine.

Metode Studija je uključila 43 pacijenta s metaboličkim sindromom i 43 zdrava ispitanika (kontrole) iz Opće bolnice u Tešnju. Varijante *LPN1* gena (rs11693809: C>T i rs2716610: C>T) analizirane su PCR metodom (*real time* PCR).

Rezultati Kod kontrolnih ispitanika, polimorfizam *LPIN1* gena, rs2716610: C>T, sa statistički značajnom razlikom bio je povezan s nižim vrijednostima indeksa tjelesne mase (ITM) (p=0.008) i opsega struka (p=0.008). Drugi analizirani genski polimorfizam, rs11693809: C>T, pokazao je povezanost s nižim vrijednostima HbA1c (p=0.048) u skupini pacijenata s MS-om.

Zaključak Rezultati studije sugerišu da bi rs2716610: C>T polimorfizam *LPIN1* gena mogao imati zaštitnu ulogu u razvoju metaboličkog sindroma, dok bi polimorfizam rs11693809: C>T mogao imati ulogu u kontroli glukoze kod pacijenata s MS-om.

Ključne riječi: metabolički sindrom, *LPIN1* gen, marker, varijacije gena