

# Association between 11 $\beta$ -hydroxysteroid dehydrogenase type 1 gene polymorphisms and metabolic syndrome in Bosnian population

Tanja Dujic<sup>1\*</sup>, Tamer Bego<sup>1</sup>, Barbara Mlinar<sup>2</sup>, Sabina Semiz<sup>1</sup>, Maja Malenica<sup>1</sup>, Besim Prnjavorac<sup>3</sup>, Barbara Ostanek<sup>2</sup>, Janja Marc<sup>2</sup>, Adlija Causevic<sup>1</sup>

<sup>1</sup>Department of Biochemistry and Clinical Analysis, Faculty of Pharmacy, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

<sup>2</sup>Department of Clinical Biochemistry, Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia

<sup>3</sup>General Hospital of Tesanj, Tesanj, Bosnia and Herzegovina

\*Corresponding author: tanja.dujic@gmail.com

### Abstract

**Introduction:** The enzyme 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD1) catalyzes the conversion of the hormonally inactive cortisone to active cortisol, thus facilitating glucocorticoid receptor activation in target tissues. Increased expression of 11 $\beta$ -HSD1 in adipose tissue has been associated with obesity and insulin resistance. In this study, we investigated the association of two 11 $\beta$ -HSD1 gene (*HSD11B1*) polymorphisms with the metabolic syndrome (MetS) and its characteristics in the Bosnian population.

**Materials and methods:** The study included 86 participants: 43 patients diagnosed with MetS and 43 healthy controls. Subjects were genotyped for two *HSD11B1* gene polymorphisms: rs846910: G>A and rs45487298: insA, by the high resolution melting curve analysis. Genotype distribution and an influence of genotypes on clinical and biochemical parameters were assessed.

**Results:** There was no significant difference in the mutated allele frequencies for the two *HSD11B1* gene polymorphisms between MetS patients and controls. In MetS patients, no significant associations between disease-associated traits and rs45487298: insA were found. Regarding rs846910: G>A variant, heterozygous patients (G/A) had significantly lower systolic ( $P = 0.017$ ) and diastolic blood pressure ( $P = 0.015$ ), lower HOMA-IR index ( $P = 0.011$ ) and higher LDL-cholesterol levels ( $P = 0.049$ ), compared to the wild-type homozygotes. In the control group, rs45487298: insA polymorphism was associated with lower fasting plasma insulin levels ( $P = 0.041$ ), lower homeostasis model assessment insulin resistance (HOMA-IR) index ( $P = 0.041$ ) and lower diastolic blood pressure ( $P = 0.048$ ). Significant differences between rs846910: G>A genotypes in controls were not detected. Haplotype analysis confirmed the association of rs45487298: insA with markers of insulin resistance in the control subjects.

**Conclusions:** Our results indicate that a common rs45487298: insA polymorphism in *HSD11B1* gene may have a protective effect against insulin resistance.

**Key words:** metabolic syndrome; insulin resistance; obesity; 11-beta-hydroxysteroid dehydrogenase type 1; polymorphism, genetic; high resolution melting curve analysis

Received: July 26, 2011

Accepted: November 29, 2011

### Introduction

The metabolic syndrome (MetS) represents a cluster of metabolic disorders including abdominal obesity, dyslipidemia (elevated triglycerides and lowered high-density lipoprotein cholesterol (HDL-cholesterol)), hyperglycemia and hypertension (1). These metabolic abnormalities are associated with

increased risk for cardiovascular disease and type 2 diabetes mellitus (2,3). It is estimated that about 20-30% of the world's adult population has MetS (3). Diagnostic criteria for MetS have changed during the last decade with new insights into the pathogenesis of disease. Different health organi-

zations had proposed their own criteria and finally in 2009, a new harmonized definition was accepted (1). MetS is diagnosed when any three of the following five criteria are met: increased waist circumference (with population- and country-specific cut points), triglycerides of at least 150 mg/dL ( $\geq 1.7$  mmol/L), HDL-cholesterol less than 40 mg/dL ( $< 1.0$  mmol/L) in males and less than 50 mg/dL ( $< 1.3$  mmol/L) in females, blood pressure of at least 130/85 mmHg and fasting glucose of at least 100 mg/dL ( $\geq 5.6$  mmol/L) (1). Recommended waist circumference thresholds for Europids are  $\geq 94$  cm for men and  $\geq 80$  cm for women (1). Abdominal obesity and physical inactivity are considered as major causes for MetS. Beside this, genetic factors, aging and endocrine disorders, all contribute to the susceptibility for disease (2).

The enzyme 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD1) catalyzes the conversion of the hormonally inactive cortisone to active cortisol, thus facilitating glucocorticoid receptor activation in target tissues. It is a microsomal, nicotinamide adenine dinucleotide-dependent dehydrogenase, identified in many tissues, with highest levels of expression in the liver, gonads, adipose tissue, and brain (4). The similarities between Cushing's syndrome and MetS lead to the hypothesis that increased cortisol concentrations in adipose tissue are implicated in the pathogenesis of visceral obesity and MetS. A transgenic mouse overexpressing 11 $\beta$ -HSD1 in adipose tissue, became a model for MetS with increased visceral fat mass, glucose intolerance, insulin resistance, dyslipidemia and hypertension (5). In contrast, 11 $\beta$ -HSD1 knockout mice, showed improved insulin sensitivity and lipid profile (6). Various studies (7-16), although not all (17), have reported increased 11 $\beta$ -HSD1 expression in subcutaneous adipose tissue in obese subjects. Some of them confirmed this association also in visceral adipose depots (7,11,16), while others did not (9,18,19). Several researches found positive correlations of 11 $\beta$ -HSD1 subcutaneous fat expression with markers of insulin resistance (10,12,14,15,18,19). Investigations in animal models and early results of human studies suggest that inhibitors of 11 $\beta$ -HSD1 are promising therapeutic drugs for obesity and diabetes (20), further

supporting the role of 11 $\beta$ -HSD1 in the pathogenesis of MetS (21).

The gene encoding 11 $\beta$ -HSD1 (*HSD11B1*) is located on chromosome 1q32-q41. Several studies investigated associations of single-nucleotide polymorphisms (SNPs) of *HSD11B1* with metabolic phenotype. Two SNPs within *HSD11B1* gene (rs846910: G>A and rs12086634: G>T) have been associated with type 2 diabetes in Pima Indians (15). SNP rs846910: G>A was also associated with hypertension (22). Another polymorphism, insertion of adenine in intron 3 (rs45487298: insA), was associated with obesity and insulin resistance in children (23). On the contrary, Robitaille *et al.* found no association of three *HSD11B1* gene variants with components of the MetS (24). Four other *HSD11B1* polymorphisms were not associated with type 2 diabetes or metabolic parameters in Koreans (25).

This is the first study performed in population from Bosnia and Herzegovina, in which we examined a significance of genetic variations in the *HSD11B1* gene (rs846910: G>A and rs45487298: insA), in the pathogenesis of MetS. Influence of genotypes on clinical and metabolic parameters was also assessed.

## Materials and methods

### Subjects

The study included a total of 86 participants from Bosnia and Herzegovina: 43 patients of General Hospital Tescanj, Zenica-Doboj Canton, diagnosed with MetS, and 43 healthy subjects. All research involving human subjects was done in accordance with the ethical recommendations and practices of the General Hospital Tescanj, and compiled with ethical principles of the Declaration of Helsinki. The study was approved by Ethics Committee of Cantonal Hospital Zenica and by the Institute for Genetic Engineering and Biotechnology, University of Sarajevo. Written informed consents were obtained for each participant involved in the study. MetS was diagnosed according to harmonized criteria from 2009 that include three or more of the following abnormalities: waist circumference  $\geq 94$  cm in men or  $\geq 80$  cm in women, triglycerides  $\geq$

1.7 mmol/L (or specific treatment for this lipid abnormality), HDL-cholesterol < 1.0 mmol/L in men or < 1.3 mmol/L in women (or specific treatment for this lipid abnormality), blood pressure  $\geq$  130/85 mmHg (or treatment for previously diagnosed hypertension) and fasting glucose  $\geq$  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. Only 9 patients did not receive any therapy; 47% of all patients were treated with lipid-lowering drugs, 58% were treated with glucose-lowering drugs, and 74% received antihypertensive therapy. Control group consisted of 43 age-matched, healthy, non-obese subjects who had less than three features of MetS, and were not taking any medication during the course of the study.

### Anthropometric measurements and biochemical markers determination

Height, weight, waist circumference, systolic and diastolic blood pressure were measured in all subjects. Waist circumference was measured at the midpoint between the lowest rib and the iliac crest (26). After overnight fast, blood samples were collected for the determination of following biochemical markers: insulin, glucose, total cholesterol, HDL-cholesterol, low-density lipoprotein cholesterol (LDL-cholesterol), triglycerides, glycohemoglobin (HbA<sub>1c</sub>) and C-reactive protein (CRP). Whole blood with EDTA was used for HbA<sub>1c</sub> determination and serum was used for all other measurements. Fasting insulin levels were measured by immunoassay on an AxSYM analyzer (Abbott Laboratories, Abbott Park, IL, USA). HbA<sub>1c</sub> was determined by NGSP certified method based on affinity separation, using the NycoCard reader (Axis-Shield, Oslo, Norway). Other markers were determined by standard methods with the use of autoanalyzer VITROS 350 Chemistry System (Ortho-Clinical Diagnostic, Rochester, New York, USA). The homeostasis model assessment insulin resistance index (HOMA-IR) was calculated using the formula: fasting insulin (mU/L) x fasting glucose (mmol/L) / 22.5 (27).

### Genetic analyses

Genomic DNA was isolated from peripheral blood using Miller's protocol (28). Two *HSD11B1* gene polymorphisms, rs846910: G>A and rs45487298: insA, were genotyped by previously described high-resolution melting (HRM) method (29). HRM analysis was performed on the LightCycler<sup>®</sup>480 Real-Time PCR System (Roche Diagnostics GmbH, Mannheim, Germany). Briefly, DNA fragments containing the analyzed polymorphisms were amplified using the following primers and probes: F: 5'- CTC TGT TGC TTG TGC TTG ATT C-3', R: 5'-AGA GCA GGC TTT CAG CAG ATA C-3' and unlabeled probe: 5'-CTGGT-GGGAATGATCAAATCAGAGAGA-3' for rs846910: G>A, and F: 5'-GGT GAG GCT GTT TCT CTT ACC T-3', R: 5'-GGT TGA TAC CTT TCC CAT TCT-3' and unlabeled probe: 5'-GGT CAC CAA GAG CTT TTG GGA GG-3' for rs45487298: insA. The amplification protocol consisted of initial denaturation at 95 °C for 10 min and 60 cycles with denaturation at 95 °C for 10 s, then 10 s annealing with a touchdown protocol covering a range of annealing temperatures from 68 to 58 °C (decreasing 1 °C per cycle) and elongation at 72 °C for 10 s. After amplification, PCR products were denatured at 95 °C for 1 min and cooled to 40 °C. High resolution melting was performed by heating from 55 °C to 90 °C with 25 acquisitions per 1 °C.

During each run, two positive control samples for each genotype and one negative control were used for quality control. Positive controls were previously confirmed by PCR-RFLP method (29). Twenty percent of all samples were double-genotyped with 100% concordant results.

### Statistical analysis

Statistical analysis was performed using SPSS Statistics 17.0 software. A P value  $\leq$  0.05 was considered statistically significant. The differences in genotype frequencies between study groups were analyzed by chi-square ( $\chi^2$ ) test and Fisher's exact test (in the case where expected frequencies were less or equal to 5). Genotype association with clinical and biochemical markers was tested by Mann-Whitney test (data did not follow normal distribution). Linkage disequilibrium between two polymorphisms was calculated by 2LD program (30).

Haplotype analyses were performed using PHASE 2.1 software (31).

Power analysis was performed using the Quanto program version 1.2.4 (32) and G\*Power 3 software available at <http://www.psych.uni-duesseldorf.de/abteilungen/aap/gpower3>.

## Results

Clinical and biochemical variables of MetS patients and controls are presented in Table 1. Most of the measured anthropometric, hemodynamic and metabolic parameters were significantly different between the two groups except for total and LDL-cholesterol concentration (Table 1).

Allele and genotype frequencies for rs846910: G>A and rs45487298: insA variants are presented in Table 2. As the rs846910: G>A polymorphism is rare, there were no homozygous mutant genotypes (A/A). There was no significant deviation from the Hardy-Weinberg equilibrium in two groups of subjects studied, with respect to both polymorphisms

Distribution of genotypes did not differ significantly between controls and MetS patients (Table 2). However, the study had limited power to detect potentially small effects of these variants on MetS, which is multifactorial and very complex disease. The power of the study to detect odds ratios (ORs) between 1.2 and 2.0 at  $\alpha = 0.05$  was only 6-24% for rs846910: G>A (dominant model), and 7-33% for rs45487298: insA variant (recessive model). To detect effect sizes of OR = 1.2-2.0, sample sizes of nearly 200-3200 and 150-2000 subjects in each group would be required, for rs846910: G>A and rs45487298: insA polymorphism, respectively.

Clinical and metabolic characteristics of patients with MetS according to the genotypes of rs846910: G>A and rs45487298: insA polymorphisms are shown in Table 3. No significant associations between disease-associated traits and rs45487298: insA were found. Regarding rs846910: G>A variant, heterozygous patients (G/A) had significantly lower systolic ( $P = 0.017$ ) and diastolic blood pressure ( $P = 0.015$ ), lower HOMA-IR index ( $P = 0.011$ ) and

**TABLE 1.** Clinical and biochemical characteristics of MetS patients and controls.

	<b>MetS patients (N = 43)</b>	<b>Controls (N = 43)</b>	<b>P</b>
Age (years)	49 (40-56)	45 (41-51)	0.206
Males/Females	19/24	10/33	<b>0.040</b>
BMI (kg/m <sup>2</sup> )	33.0 (29.2-35.5)	24.7 (22.2-27.4)	<b>&lt;0.001</b>
Waist circumference (cm)	110 (96-120)	83 (78-90)	<b>&lt;0.001</b>
Systolic BP (mm Hg)	143 (130-158)	120 (110-125)	<b>&lt;0.001</b>
Diastolic BP (mm Hg)	90 (80-100)	78 (70-80)	<b>&lt;0.001</b>
Fasting insulin (mU/L)	10.6 (8.0-13.9)	7.3 (6.4-10.1)	<b>0.010</b>
Fasting glucose (mmol/L)	8.4 (5.5-11.7)	5.0 (4.7-5.2)	<b>&lt;0.001</b>
HOMA-IR	4.1 (2.7-6.1)	1.6 (1.4-2.3)	<b>&lt;0.001</b>
HbA1c (mmol/mol)	43 (37-55)	38 (29-42)	<b>&lt;0.001</b>
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)	<b>&lt;0.001</b>
Triglycerides (mmol/L)	2.26 (1.77-3.27)	1.17 (0.76-1.45)	<b>&lt;0.001</b>
hsCRP (mg/L)	5.0 (3.0-6.0)	1.3 (0.8-4.0)	<b>&lt;0.001</b>

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. All differences were tested using Mann-Whitney test.

**TABLE 2.** Genotype and variant allele frequencies for rs846910: G>A and rs45487298: insA polymorphisms.

Polymorphism	Genotype	MetS patients		Controls		P <sup>1</sup>
		N (%)	Mutated allele frequency	N (%)	Mutated allele frequency	
rs846910: G>A	G/G	35 (83.3%)		34 (79.1%)		0.615
	G/A	7 (16.7%)	0.08	9 (20.9%)	0.10	
	A/A	0 (0%)		0 (0%)		
	Total	42		43		
	p <sup>2</sup>	0.841		0.745		
rs45487298: insA	N/N3	26 (63.4%)		25 (58.1%)		0.930
	N/insA	14 (34.1%)	0.20	16 (37.2%)	0.23	
	insA/insA	1 (2.4%)		2 (4.7%)		
	Total	41		43		
	p <sup>2</sup>	0.856		0.962		

<sup>1</sup>significance of  $\chi^2$  / Fisher's exact test for comparison of genotype frequencies between MetS patients and controls. <sup>2</sup>P-value for Hardy-Weinberg equilibrium. <sup>3</sup>N – wild-type allele of rs45487298: insA.

**TABLE 3.** Comparison of clinical and biochemical characteristics between different genotypes of rs846910: G>A and rs45487298: insA polymorphisms in the MetS patients group.

	rs846910: G>A		P	rs45487298: insA		P
	G/G (N = 35)	G/A (N = 7)		N/N (N = 26)	N/InsA+ InsA/insA (N = 15)	
Age (years)	52 (40-56)	45 (36-47)	0.154	51 (40-54)	49 (40-56)	0.925
BMI (kg/m <sup>2</sup> )	33.0 (29.4-36.1)	30.0 (26.5-34.3)	0.331	33.0 (27.3-36.5)	30.8 (29.8-35.5)	0.989
Waist circumference (cm)	110 (102-121)	96 (88-121)	0.265	110 (96-121)	110 (96-116)	0.790
Systolic BP (mm Hg)	145 (140-160)	130 (120-140)	<b>0.017</b>	140 (140-160)	140 (130-150)	0.403
Diastolic BP (mm Hg)	90 (80-100)	80 (70-90)	<b>0.015</b>	90 (80-105)	90 (80-95)	0.344
Fasting insulin (mU/L)	10.9 (8.1-14.1)	9.6 (8.0-10.6)	0.272	10.2 (7.6-14.1)	10.9 (8.1-13.9)	0.713
Fasting glucose (mmol/L)	8.6 (5.9-12.0)	5.6 (4.6-9.4)	0.063	8.5 (5.5-12.2)	6.8 (5.0-9.9)	0.171
HOMA-IR	4.6 (3.3-7.7)	2.5 (2.1-3.7)	<b>0.011</b>	4.0 (2.7-7.8)	4.1 (3.1-4.8)	0.563
HbA1c (mmol/mol)	43 (37-56)	41 (37-48)	0.483	42 (37-55)	43 (22-53)	0.649
Total cholesterol (mmol/L)	5.7 (5.1-6.2)	6.0 (4.8-7.5)	0.398	5.6 (5.1-6.2)	5.7 (5.0-6.4)	0.872
LDL-cholesterol (mmol/L)	3.10 (2.59-3.87)	4.35 (2.98-5.42)	<b>0.049</b>	3.21 (2.64-4.11)	3.11 (2.77-3.69)	0.863
HDL-cholesterol (mmol/L)	1.04 (0.88-1.39)	1.11 (0.88-1.23)	0.985	1.00 (0.86-1.28)	1.19 (0.96-1.66)	0.150
Triglycerides (mmol/L)	2.36 (2.00-3.85)	1.72 (1.69-2.96)	0.113	2.38 (1.96-3.42)	1.88 (1.70-2.41)	0.187
hsCRP (mg/L)	4.5 (3.0-6.0)	5.3 (1.0-7.0)	0.894	4.0 (3.0-5.7)	6.0 (2.3-8.5)	0.312

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. All differences were tested using Mann-Whitney test.

higher LDL-cholesterol levels ( $P = 0.049$ ), compared to the wild-type homozygotes.

Comparisons of clinical and metabolic parameters between genotypes in the control group are shown in Table 4. The carriers of rs45487298: insA polymorphism had significantly lower fasting plasma insulin ( $P = 0.041$ ), lower HOMA-IR index ( $P = 0.041$ ), and lower diastolic blood pressure ( $P = 0.048$ ), compared to the wild-type homozygotes (Table 4). Significant differences between rs846910: G>A genotypes in control subjects were not detected (Table 4).

In addition, the haplotype analysis was also performed. The rs846910: G>A and rs45487298: insA polymorphisms were in moderate linkage disequilibrium ( $D' = 0.418$ ). Reconstructed haplotype and diplotype frequencies are shown in Table 5. There was no significant difference in distribution of haplotype frequencies between MetS patients and controls ( $P = 0.90$ ). As AN and AInsA haplotypes were rare, only the associations of GN and GInsA

haplotypes with clinical and biochemical markers were tested. By comparing the effects of GN/GInsA and GInsA/GInsA diplotypes together, to complete wild-type diplotype, we examined the potential effect of the InsA polymorphism solely (without rs846910: G>A). In MetS patients group, significant associations were not found (data not shown). In the control group, analysis confirmed the above mentioned results: carriers of GN/GInsA and GInsA/GInsA diplotypes, as compared to GN/GN individuals, had lower fasting insulin level (data presented as median (1<sup>st</sup>-3<sup>rd</sup> quartile): 8.1 (7.0-13.9) mU/L vs. 6.2 (4.7-7.1) mU/L;  $P = 0.011$ ) and lower HOMA-IR index (1.8 (1.4-2.8) vs. 1.3 (0.9-1.6);  $P = 0.016$ ).

For continuous traits, the study had only 25% and 34% power to detect moderate effect sizes of 0.5 at the  $\alpha$  level 0.05, for rs846910: G>A and rs45487298: insA variants, respectively. Sample sizes of 200 and 140 subjects would be needed to detect effect sizes of 0.5 with 80% power, for rs846910: G>A and rs45487298: insA polymorphism, respectively.

**TABLE 4.** Comparison of clinical and biochemical characteristics between different genotypes of rs846910: G>A and rs45487298: insA polymorphisms in the control group.

	rs846910: G>A		P	rs45487298: insA		P
	G/G (N = 34)	G/A (N = 9)		N/N (N = 25)	N/InsA+ InsA/InsA (N = 18)	
Age (years)	45 (41-52)	46 (45-49)	0.756	46 (41-52)	45 (42-50)	0.705
BMI (kg/m <sup>2</sup> )	24.8 (21.4-28.5)	23.3 (22.4-26.3)	0.958	24.5 (22.3-28.5)	24.8 (21.5-26.9)	0.707
Waist circumference (cm)	83 (78-90)	83 (82-91)	0.565	82 (78-90)	85 (78-91)	0.802
Systolic BP (mm Hg)	120 (110-130)	120 (120-125)	0.780	120 (120-130)	120 (110-125)	0.137
Diastolic BP (mm Hg)	79 (70-80)	75 (70-85)	0.914	80 (75-84)	75 (70-80)	<b>0.048</b>
Fasting insulin (mU/L)	7.3 (6.3-10.5)	6.9 (5.7-8.8)	0.535	7.4 (6.9-11.6)	6.6 (5.5-7.1)	<b>0.041</b>
Fasting glucose (mmol/L)	4.9 (4.5-5.2)	5.0 (4.8-5.3)	0.483	5.0 (4.7-5.2)	4.9 (4.5-5.4)	0.685
HOMA-IR	1.6 (1.3-2.5)	1.5 (1.3-2.0)	0.564	1.7 (1.4-2.7)	1.4 (1.2-1.6)	<b>0.041</b>
HbA1c (mmol/mol)	38 (27-42)	39 (32-41)	0.899	38 (27-42)	38 (30-41)	0.978
Total cholesterol (mmol/L)	5.6 (5.1-6.5)	6.3 (5.8-7.0)	0.086	5.7 (5.0-6.3)	6.1 (5.3-6.8)	0.191
LDL-cholesterol (mmol/L)	3.20 (2.84-4.09)	4.19 (3.26-5.15)	0.055	3.37 (2.87-4.09)	3.51 (2.93-4.57)	0.808
HDL-cholesterol (mmol/L)	1.70 (1.41-1.87)	1.48 (1.29-1.90)	0.337	1.62 (1.39-1.81)	1.74 (1.37-1.98)	0.588
Triglycerides (mmol/L)	1.08 (0.78-1.39)	1.27 (0.67-1.56)	0.588	1.08 (0.85-1.32)	1.32 (0.67-1.94)	0.416
hsCRP (mg/L)	1.3 (0.8-4.3)	1.2 (0.9-1.9)	0.731	1.6 (0.8-4.0)	1.2 (0.8-2.1)	0.454

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. All differences were tested using Mann-Whitney test.

**TABLE 5.** Haplotype and diplotype frequencies of rs846910: G>A and rs45487298: insA variants in MetS patients and controls.

Haplotype <sup>1</sup>	Frequency		Diplotype	Frequency	
	MetS patients	Controls		MetS patients	Controls
GN <sup>2</sup>	0.758	0.717	GN/GN	0.571	0.535
GlnsA	0.159	0.178	GN/GlnsA	0.262	0.209
AN	0.047	0.062	GN/AN	0.071	0.070
AlnsA	0.036	0.043	GN/AlnsA	0.071	0.140
			GlnsA/GlnsA	0	0.047
			GlnsA/AlnsA	0.024	0

<sup>1</sup>corresponding to the rs846910: G>A and rs45487298: insA polymorphism, respectively. <sup>2</sup>N – wild-type allele of rs45487298: insA.

## Discussion

A number of studies have investigated an association of 11 $\beta$ -HSD1 with obesity and insulin resistance in humans. Most of them demonstrated a correlation of adipose *HSD11B1* expression with obesity (7-16). Lindsay *et al.* (14) reported a positive correlation of 11 $\beta$ -HSD1 activity with measures of adiposity, fasting plasma glucose, insulin, and insulin resistance by the homeostasis model. Another study did not find relationship between 11 $\beta$ -HSD1 and insulin sensitivity (33). The role of 11 $\beta$ -HSD1 in the pathogenesis of visceral obesity and insulin resistance has been corroborated by experimental studies with enzyme inhibitors. However, several studies which explored the relationship between *HSD11B1* polymorphisms, obesity and type 2 diabetes, gave conflicting results.

In this study, we did not find the association of two investigated *HSD11B1* gene polymorphisms, rs846910: G>A in 5' upstream region and rs45487298: insA in intron 3, with MetS in a sample of Bosnian population. However, our study was preliminary and of limited statistical power due to the insufficient sample size. Our negative findings possibly reflect statistical type 2 errors and the role of investigated genetic variants in MetS risk cannot be ruled out. Analysis of anthropometric and biochemical markers in regards to the genotypes of rs846910: G>A showed significant associations of the mutated A allele with lower systolic (P = 0.017) and diastolic blood pressure (P = 0.015), lower HOMA-IR index (P = 0.011) and higher LDL-cholesterol levels (P = 0.049) in patients with MetS. These results might reflect the influence of

rs846910: G>A variant on metabolic control in MetS patients. However, majority of patients in our cohort received therapy and therefore, these associations have to be taken with particular caution. In the control group, rs846910: G>A did not show any significant associations. Since this allele was rare, we could not confirm these results by haplotype analysis. Only a few studies have examined the effect of rs846910: G>A on the metabolic phenotypes. In a study of Pima Indians, mutated A allele was associated with type 2 diabetes and higher fasting insulin concentrations in healthy subjects, independently of obesity (15). In another study, also in Pima Indians, rs846910: G>A was associated with hypertension, although these effects were modified by birth era of subjects (22). However, in a recent study, no effect of rs846910: G>A variation on *HSD11B1* promoter activity *in vitro* was found (34), suggesting that this polymorphism may not have direct functional consequences on the 11 $\beta$ -HSD1 activity *in vivo*.

Analysis of rs45487298: insA polymorphism, showed a significant association between the variant allele and lower fasting plasma insulin levels (P = 0.041), lower HOMA-IR index (P = 0.041), and lower diastolic blood pressure (P = 0.048), in the control group. In MetS patients group, no significant associations were observed. Haplotype analysis confirmed beneficial effect of rs45487298: insA on markers of insulin resistance in controls with even higher significance (P = 0.011 for fasting plasma insulin and P = 0.016 for HOMA-IR). This polymorphism, as well as another SNP in intron 3, located

40 bp downstream, rs12086634: T>G, reduce HSD11B1 transcriptional activity 2.5 x in vitro (35). These two mutations are in 100% linkage disequilibrium. Interestingly, in the above mentioned study of Pima Indians, the wild-type allele of rs12086634: T>G was associated with type 2 diabetes (15). It was also associated with higher insulin-mediated glucose disposal rate (15). The authors suggest that the mechanism of association with markers of insulin resistance, independently of obesity, could be modulation of 11 $\beta$ -HSD1 expression in pancreatic beta cells, that is, a tissue-specific regulation of HSD11B1 (15). On the contrary, in a study sample of healthy overweight and normal weight children from different racial backgrounds, mutated homozygotes for rs45487298: insA had greater BMI, waist circumference and insulin resistance indices than heterozygote or wild-type children (23). Although no significant differences were found between the heterozygote and wild-type children in this study, heterozygotes had actually the lowest BMI, waist circumference, insulin concentration, and HOMA-IR index (23). This is partially in accordance with our results. Since there were only 2 homozygous mutant subjects in our study, we were not able to differentiate between the effects of mutated hetero- and homozygous state on phenotype.

Robitaille *et al.* showed no association of rs45487298: insA and rs12086634: T>G polymorphisms with MetS in French-Canadian men (24). Variants were not associated with components of the MetS, except for higher plasma apolipoprotein B levels (24). Several other studies found also no association of rs45487298: insA or rs12086634: T>G with metabolic characteristics (36-39).

As mentioned above, cohort of several hundreds of subjects, depending on variant allele frequency, would be required to detect moderate effect sizes of two analyzed HSD11B1 polymorphisms, and thousands of individuals would be needed to detect potentially small contributions of these variants to the complex phenotype of MetS. In general, many genetic association studies fail to replicate initial positive findings (40). Quality of these investigations depends on methodological issues related to phenotyping and genotyping, and issues related to the study design (40). The key de-

terminant of the quality and the most critical issue is the sample size (40,41). Beside this, the proper definition of phenotype/disease is essential for the quality of a genetic association study (40). This is especially important for studies of MetS, since criteria for diagnosis were changing throughout the last decade and population of MetS patients represent very heterogeneous group.

The results of our preliminary study indicated a positive effect of rs45487298: insA variation on blood pressure and markers of insulin sensitivity in the control group. However, these positive findings have to be replicated in a larger cohort of subjects. Several genetic association studies performed so far, yielded inconsistent results. The discrepancy might be also attributed to the ethnic and environmental differences between studied cohorts. Furthermore, other, yet unidentified genetic variants in HSD11B1 gene or mutations in H6PD, the gene encoding the hexose-6-phosphate dehydrogenase (enzyme needed for 11 $\beta$ -HSD1 reductase activity), can also influence expression or activity of 11 $\beta$ -HSD1. Nonetheless, the role of 11 $\beta$ -HSD1 in insulin sensitivity and obesity seems to be complex and may be explained by its tissue-specific expression patterns.

In conclusion, this is the first study performed in population from Bosnia and Herzegovina, in which a significance of genetic variations of HSD11B1 gene was investigated in the pathogenesis of metabolic syndrome. We found a significant association between rs45487298: insA, HSD11B1 gene polymorphism, and lower fasting plasma insulin and HOMA-IR index, as measures of insulin resistance in the control subjects. However, associations of this SNP with BMI and waist circumference, as markers of obesity, were not found. Future investigations including larger cohorts of subjects, as well as studies in other populations, are needed to substantiate these findings.

### Acknowledgements

We greatly appreciate the technical assistance of Mr. Nermin Kotoric, Mr. Hajrudin Mujic and Ms. Elma Topic.

### Potential conflict of interest

None declared.



## References

1. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* 2009;120:1640-5.
2. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2005;112:2735-52.
3. Grundy SM. Metabolic syndrome pandemic. *Arterioscler Thromb Vasc Biol* 2008;28:629-36.
4. Tomlinson JW, Walker EA, Bujalska IJ, Draper N, Lavery GG, Cooper MS, et al. 11beta-hydroxysteroid dehydrogenase type 1: a tissue-specific regulator of glucocorticoid response. *Endocr Rev* 2004;25:831-66.
5. Seckl JR, Morton NM, Chapman KE, Walker BR. Glucocorticoids and 11beta-hydroxysteroid dehydrogenase in adipose tissue. *Recent Prog Horm Res* 2004;59:359-93.
6. Walker BR. Cortisol--cause and cure for metabolic syndrome? *Diabet Med* 2006;23:1281-8.
7. Paulsen SK, Pedersen SB, Fisker S, Richelsen B. 11Beta-HSD type 1 expression in human adipose tissue: impact of gender, obesity, and fat localization. *Obesity (Silver Spring)* 2007;15:1954-60.
8. Desbriere R, Vuaroqueaux V, Achard V, Boullu-Ciocca S, Labuhn M, Dutour A, Grino M. 11beta-hydroxysteroid dehydrogenase type 1 mRNA is increased in both visceral and subcutaneous adipose tissue of obese patients. *Obesity (Silver Spring)* 2006;14:794-8.
9. Li X, Lindquist S, Chen R, Myrnas T, Angsten G, Olsson T, Hernell O. Depot-specific messenger RNA expression of 11 beta-hydroxysteroid dehydrogenase type 1 and leptin in adipose tissue of children and adults. *Int J Obes (Lond)* 2007;31:820-8.
10. Engeli S, Bohnke J, Feldpausch M, Gorzelniak K, Heintze U, Janke J, et al. Regulation of 11beta-HSD genes in human adipose tissue: influence of central obesity and weight loss. *Obes Res* 2004;12:9-17.
11. Michailidou Z, Jensen MD, Dumesic DA, Chapman KE, Seckl JR, Walker BR, Morton NM. Omental 11beta-hydroxysteroid dehydrogenase 1 correlates with fat cell size independently of obesity. *Obesity (Silver Spring)* 2007;15:1155-63.
12. Kannisto K, Pietilainen KH, Ehrenborg E, Rissanen A, Kaprio J, Hamsten A, Yki-Jarvinen H. Overexpression of 11beta-hydroxysteroid dehydrogenase-1 in adipose tissue is associated with acquired obesity and features of insulin resistance: studies in young adult monozygotic twins. *J Clin Endocrinol Metab* 2004;89:4414-21.
13. Wake DJ, Rask E, Livingstone DE, Soderberg S, Olsson T, Walker BR. Local and systemic impact of transcriptional up-regulation of 11beta-hydroxysteroid dehydrogenase type 1 in adipose tissue in human obesity. *J Clin Endocrinol Metab* 2003;88:3983-8.
14. Lindsay RS, Wake DJ, Nair S, Bunt J, Livingstone DE, Permana PA, et al. Subcutaneous adipose 11 beta-hydroxysteroid dehydrogenase type 1 activity and messenger ribonucleic acid levels are associated with adiposity and insulinemia in Pima Indians and Caucasians. *J Clin Endocrinol Metab* 2003;88:2738-44.
15. Nair S, Lee YH, Lindsay RS, Walker BR, Tataranni PA, Bogardus C, et al. 11beta-Hydroxysteroid dehydrogenase Type 1: genetic polymorphisms are associated with Type 2 diabetes in Pima Indians independently of obesity and expression in adipocyte and muscle. *Diabetologia* 2004;47:1088-95.
16. Mlinar B, Marc J, Jensterle M, Bokal EV, Jerin A, Pfeifer M. Expression of 11beta-hydroxysteroid dehydrogenase type 1 in visceral and subcutaneous adipose tissues of patients with polycystic ovary syndrome is associated with adiposity. *J Steroid Biochem Mol Biol* 2011;123:127-32.
17. Tomlinson JW, Sinha B, Bujalska I, Hewison M, Stewart PM. Expression of 11beta-hydroxysteroid dehydrogenase type 1 in adipose tissue is not increased in human obesity. *J Clin Endocrinol Metab* 2002;87:5630-5.
18. Goedecke JH, Wake DJ, Levitt NS, Lambert EV, Collins MR, Morton NM, et al. Glucocorticoid metabolism within superficial subcutaneous rather than visceral adipose tissue is associated with features of the metabolic syndrome in South African women. *Clin Endocrinol (Oxf)* 2006;65:81-7.
19. Alberti L, Girola A, Gilardini L, Conti A, Cattaldo S, Michelletto G, Invitti C. Type 2 diabetes and metabolic syndrome are associated with increased expression of 11beta-hydroxysteroid dehydrogenase 1 in obese subjects. *Int J Obes (Lond)* 2007;31:1826-31.
20. Gathercole LL, Stewart PM. Targeting the pre-receptor metabolism of cortisol as a novel therapy in obesity and diabetes. *J Steroid Biochem Mol Biol* 2010;122:21-7.
21. Anagnostis P, Athyros VG, Tziomalos K, Karagiannis A, Mikhailidis DP. Clinical review: The pathogenetic role of cortisol in the metabolic syndrome: a hypothesis. *J Clin Endocrinol Metab* 2009;94:2692-701.
22. Franks PW, Knowler WC, Nair S, Koska J, Lee YH, Lindsay RS, et al. Interaction between an 11betaHSD1 gene variant and birth era modifies the risk of hypertension in Pima Indians. *Hypertension* 2004;44:681-8.
23. Gelernter-Yaniv L, Feng N, Sebring NG, Hochberg Z, Yanovski JA. Associations between a polymorphism in the 11 beta hydroxysteroid dehydrogenase type I gene and body composition. *Int J Obes Relat Metab Disord* 2003;27:983-6.
24. Robitaille J, Brouillette C, Houde A, Despres JP, Tchernof A, Vohl MC. Molecular screening of the 11beta-HSD1 gene in men characterized by the metabolic syndrome. *Obes Res* 2004;12:1570-5.
25. Ku YH, Koo BK, Kwak SH, Cho YM, Shin HD, Lee HK, et al. Regulatory effect of common promoter polymorphisms on the expression of the 11beta-hydroxysteroid dehydrogenase type 1 gene. *Horm Res* 2009;72:25-32.
26. Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. *World Health Organ Tech Rep Ser* 1995;854:1-452.
27. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412-9.

28. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;16:1215.
29. Dujic T, Ostaneck B, Marc J, Causevic A, Mlinar B. Optimization of high-resolution melting analysis for simultaneous genotyping of two 11beta-hydroxysteroid dehydrogenase type 1 gene polymorphisms. *Genet Test Mol Biomarkers* 2011;15:43-9.
30. Zhao JH. 2LD, GENECOUNTING and HAP: Computer programs for linkage disequilibrium analysis. *Bioinformatics* 2004;20:1325-6.
31. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* 2001;68:978-89.
32. Gauderman W, Morrison J. QUANTO 1.1: A computer program for power and sample size calculations for genetic epidemiology studies, <http://hydra.usc.edu/gxe>. 2006.
33. Kerstens MN, Riemens SC, Sluiter WJ, Pratt JJ, Wolthers BG, Dullaart RP. Lack of relationship between 11beta-hydroxysteroid dehydrogenase setpoint and insulin sensitivity in the basal state and after 24h of insulin infusion in healthy subjects and type 2 diabetic patients. *Clin Endocrinol (Oxf)* 2000;52:403-11.
34. Malavasi EL, Kelly V, Nath N, Gambineri A, Dakin RS, Paggotto U, et al. Functional effects of polymorphisms in the human gene encoding 11 beta-hydroxysteroid dehydrogenase type 1 (11 beta-HSD1): a sequence variant at the translation start of 11 beta-HSD1 alters enzyme levels. *Endocrinology* 2010;151:195-202.
35. Draper N, Walker EA, Bujalska IJ, Tomlinson JW, Chalder SM, Arlt W, et al. Mutations in the genes encoding 11beta-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase interact to cause cortisone reductase deficiency. *Nat Genet* 2003;34:434-9.
36. Caramelli E, Strippoli P, Di Giacomi T, Tietz C, Carinci P, Pasquali R. Lack of mutations of type 1 11beta-hydroxysteroid dehydrogenase gene in patients with abdominal obesity. *Endocr Res* 2001;27:47-61.
37. White PC. Genotypes at 11beta-hydroxysteroid dehydrogenase type 11B1 and hexose-6-phosphate dehydrogenase loci are not risk factors for apparent cortisone reductase deficiency in a large population-based sample. *J Clin Endocrinol Metab* 2005;90:5880-3.
38. San Millan JL, Botella-Carretero JJ, Alvarez-Blasco F, Luque-Ramirez M, Sancho J, Moghetti P, Escobar-Morreale HF. A study of the hexose-6-phosphate dehydrogenase gene R453Q and 11beta-hydroxysteroid dehydrogenase type 1 gene 83557insA polymorphisms in the polycystic ovary syndrome. *J Clin Endocrinol Metab* 2005;90:4157-62.
39. Smit P, Dekker MJ, de Jong FJ, van den Beld AW, Koper JW, Pols HA, et al. Lack of Association of the 11beta-hydroxysteroid dehydrogenase type 1 gene 83,557insA and hexose-6-phosphate dehydrogenase gene R453Q polymorphisms with body composition, adrenal androgen production, blood pressure, glucose metabolism, and dementia. *J Clin Endocrinol Metab* 2007;92:359-62.
40. Simundic AM. Methodological issues of genetic association studies. *Clin Chem Lab Med* 2010;48 Suppl 1:S115-8.
41. Simundic AM, Nikolac N, Topic E. Methodological issues in genetic association studies of inherited thrombophilia: original report of recent practice. *Clin Appl Thromb Hemost* 2009;15:327-33.

## Povezanost između polimorfizama gena 11β-hidroksisteroidne dehidrogenaze tipa 1 i metaboličkog sindroma u Bosanskoj populaciji

### Sažetak

**Uvod:** Enzim 11β-hidroksisteroidne dehidrogenaza tipa 1 (engl. *11β-hydroxysteroid dehydrogenase type 1*, 11β-HSD1) katalizira pretvorbu hormonalno neaktivnog kortizona u aktivan kortizol te na taj način omogućuje aktivaciju receptora glukokortikoda u ciljnim tkivima. Povišena ekspresija 11β-HSD1 u adipoznom tkivu povezuje se s pretilošću i inzulinskom rezistencijom. U ovom istraživanju ispitali smo povezanost dvaju polimorfizama gena 11β-HSD1 (HSD11B1) s metaboličkim sindromom (MetS) i njegovim karakteristikama u Bosanskoj populaciji.

**Materijali i metode:** U istraživanje je bilo uključeno 86 ispitanika: 43 bolesnika s dijagnozom metaboličkog sindroma i 43 zdrava ispitanika. Načinjena je genotipizacija analizom krivulje taljenja DNK visoke rezolucije za dva polimorfizma gena *HSD11B1*: rs846910:G>A i rs45487298:insA. Ispitana je i raspodjela genotipova te utjecaj genotipova na kliničke i biokemijske parametre.

**Rezultati:** Nije nađena statistički značajna razlika u frekvencijama mutiranih alela za dva polimorfizma gena *HSD11B1* između skupina bolesnika s MetS i kontrolnih ispitanika. Kod bolesnika s MetS nisu primijećene statistički značajne veze između značajki povezanih s bolešću i rs45487298: insA. Što se tiče polimorfizma rs846910: G>A, heterozigotni su bolesnici (G/A) imali statistički značajno niži sistolički (P = 0,017) i dijastolički (P = 0,015) krvni tlak, niži HOMA-IR indeks (P = 0,011) i višu koncentraciju LDL-kolesterola (P = 0,049) u usporedbi s divljim tipom. U skupini zdravih ispitanika polimorfizam rs45487298: insA bio je povezan s nižim koncentracijama inzulina natašte (P = 0,041), nižim homeostatskim modelom procjene inzulinske rezistencije - HOMA-IR indeksom (P = 0,041) i nižim dijastoličkim tlakom (P = 0,048). Značajne razlike između rs846910: G>A genotipova kod kontrola nisu nađene. Analiza halotipova potvrdila je povezanost rs45487298: insA s biljezima inzulinske rezistencije kod kontrolnih ispitanika.

**Zaključak:** Naši rezultati pokazuju da čest polimorfizam rs45487298: insA gena *HSD11B1* može imati zaštitni učinak protiv inzulinske rezistencije.

**Ključne riječi:** metabolički sindrom; inzulinska rezistencija; pretilost; 11-beta-hidroksisteroid dehidrogenaza tipa 1; genetički polimorfizam; analiza krivulje taljenja DNK visoke rezolucije