ASSOCIATION OF FTO GENE VARIANT (RS8050136) WITH TYPE 2 DIABETES AND MARKERS OF OBESITY, GLYCAEMIC CONTROL AND INFLAMMATION

POVEZANOST VARIJANTE FTO GENA (RS8050136) SA TIPOM 2 DJIABETESA I MARKERIMA GOJAZNOSTI, GLIKEMIJSKE KONTROLE I INFLAMACIJE

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Summary

Background: FTO, a gene recently discovered in genomewide associated studies for type 2 diabetes mellitus (T2D), play an important role in the management of energy homeostasis, nucleic acid demethylation and regulation of body fat mass by lipolysis. The aim of this study was to analyze the association of FTO rs8050136 A>C genetic variant with clinical and biochemical parameters of T2D in the population of West Balkan region (Bosnians and Herzegovinians and Kosovars).

Methods: The study included 638 patients with T2D and prediabetes and 360 healthy controls, aged from 40 to 65 years. Patients were recruited at the Clinical Centre University of Sarajevo, University Hospital of Clinical Centre in Banja Luka, General Hospital in Tesanj and Health Centre in Prizren. Genotyping of analyzed FTO polymorphism rs8050136 A>C was performed by qPCR allelic discrimination.

List of abbreviations: T2D, type 2 diabetes; FTO, alpha-ketoglutarate dependent dioxygenase; 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.
Results: Genotype frequencies of the analyzed polymorphism were comparable between patients with T2D, prediabetic patients, and healthy population. Logistic regression analyses didn’t show significant association of FTO rs8050136 A allele with increased risk of T2D. However, risk A allele was significantly associated with higher levels of HbA1c, insulin, HOMA-IR index, diastolic blood pressure, and inflammatory markers (fibrinogen and leukocytes) as well as showed tendency of association with increased values of obesity markers (BMI, waist and hip circumference).

Conclusions: Results of our study showed a significant association of FTO genetic variant rs8050136 A>C with the major markers of insulin resistance, obesity and inflammation, opening new avenues for solving many unclear questions in the pathogenesis of T2D.

Keywords: FTO gene, Type 2 diabetes, obesity, inflammation, gene variant

Introduction

Type 2 diabetes (T2D) is a chronic complex disease characterized by hyperglycemia which occurs as a result of reduced insulin secretion, inadequate response of pancreatic cells on progressive development of insulin resistance in peripheral tissues or impaired glucose regulation in the liver (1). Global prevalence of T2D is increasing with average value of 8.7%. Bosnia and Herzegovina belongs to a group of countries with the highest prevalence in Europe of 12.0%. As far as Kosovo is concerned, there are no prevalence data yet available (2). It is well known that the risk of developing T2D is associated with obesity which is now a major global problem, considering that approximately 1.1 billion people worldwide are overweight, while 312 million are obese (3, 4). GWA (genome-wide associated) studies identified around 12 candidate genes responsible for an increase in the risk of developing T2D (10–15). Results of several meta-analyses showed a significant association of rs8050136 gene variant with increased T2D and obesity risk (3, 8, 16–18). Studies performed up to now have shown that FTO gene variant rs8050136 A>C is significantly associated with major markers of obesity (BMI, waist and hip circumference) (13, 19–20), markers of glucose homeostasis (glucose, HbA1c, insulin) and insulin resistance (HOMA-IR) in different population studies (21–23). However, studies carried out on the Russian, Mexican Mestizos, Lebanese and Omani population have not confirmed the association of this polymorphism with T2D (24–28). Also, results of large DiaGene study did not confirmed impact of rs8050136 A>C with increased risk of T2D in Netherland population (29). This is the first study that investigated the impact of FTO candidate gene polymorphism rs8050136 A>C on T2D and its related traits in populations of West Balkan region (Bosnians and Herzegovinians and Kosovars).

Materials and Methods

Subjects

The study included 998 participants: 638 patients with T2D and prediabetes, and 360 healthy controls of both sexes, aged from 40 up to 65 years. T2D and prediabetes were diagnosed by endocrinologists and diabetologists according to definitions of International Diabetes Federation (IDF) (30). Diabetic patients and healthy subjects were recruited at the
Clinic for Endocrinology and Diabetes, University Clinical Centre of Sarajevo, Department of Endocrinology and Internal Medicine, University Hospital of Clinical Centre in Banja Luka, Department of Internal Medicine, General Hospital in Tešanj, and Health Centre in Prizren. Patients treated with insulin and patients with acute and chronic gastrointestinal diseases, chronic kidney disease, endocrine disorders, acute infection and/or inflammation and hormonal therapy were excluded. All patients included in the study were taking heterogeneous therapy (antihypertensive therapy, glucose-lowering drugs, and lipid-lowering drugs). Individuals in control group were not taking any medication during the course of the study.

The research was carried out in accordance with ethics principles outlined in the Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects (initiated June 1964, last amended October 2000). The study was approved by Ethics Committee of the International University of Sarajevo and each patient has given a written informed consent.

Anthropometrical and biochemical measurements

Waist circumference, height, weight, systolic and diastolic blood pressure were measured in all participants. BMI was calculated as weight (kg)/(height (m))². For analysis of all biochemical parameters, IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) standard protocols were used. Serum levels of fasting glucose, triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol, HbA1c, fibrinogen and C reactive protein (CRP) were determined by using 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) × fasting glucose (mmol/L)/22.5 (31).

Genetic testing

Blood samples were collected from all participants under fasting conditions from antecubital vein into siliconized tubes with EDTA (BD Vacutainer Systems, Plymouth, UK) and stored at −20 °C. For isolation of genomic DNA, Miller’s protocol and QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany) were used (32). Purity and concentration of isolated DNA was determined by UV/VIS spectrophotometer NanoDrop ND-1000. After extraction, DNA samples were stored at −20 °C. Genotyping of FTO gene polymorphism (rs8050136 A>C) was performed by hydrolyzing probes and real-time PCR using TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA), ID C_2031259_10, in cooperation with the Department of Clinical Chemistry, Faculty of Pharmacy, University of Ljubljana (Ljubljana, Slovenia) on LightCycler® 480 Real-Time PCR System (Roche Diagnostics, Switzerland) and Charles University in Prague/University Hospital Hradec Kralove, Czech Republic) on Rotor gene Q machine (Qiagen, Netherlands).

Statistical analysis

Statistical analysis was done using SPSS Statistics v.19.0. Normality evaluation of data distribution was done using Kolmogorov-Smirnov test and Shapiro-Wilk test, respectively (for prediabetes group of patients due its smaller size). Variables that were not normally distributed have been log-transformed. Chi-square (χ²) 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 T2D or prediabetes, respectively. Logistic regression analysis was used to calculate the odds ratio (OR) with confidence intervals (95% CI) with adjustments to age and gender. Significance of differences of biochemical and anthropometrical measurements according to genotypes of analyzed polymorphisms, sex and age was estimated using ANCOVA test (analysis of covariance) adjusted for sex, age and BMI. A p-value ≤ 0.05 was considered statistically significant.

Results

Clinical and biochemical characteristics of patients with T2D, patients with T2D without treatment (have not use oral hypoglycemics), prediabetic patients and healthy controls are presented in Table I. The most of the measured anthropometric and metabolic parameters were significantly different between the treated T2D patients, T2D patients without treatment, prediabetic patients and healthy controls (Table I).

Allele and genotype frequencies for FTO gene polymorphism rs8050136 for patients with T2D, prediabetic patients and healthy controls are presented in Table II. The allele frequencies for FTO polymorphism – rs8050136 A>C were in Hardy-Weinberg equilibrium in all groups of subjects studied (p>0.05). However, no significant differences in analyzed genotype frequencies were found between T2D, pre-diabetic patients, and healthy controls (Table II). Odds ratios that were adjusted for age and gender did not confirm expected association of FTO rs8050136 with diabetes risk (OR=1.084, 95% CI 0.758–1.551, p=0.659). In the healthy subjects (Table III), FTO gene polymorphism rs8050136 showed a significant association with most important markers of glucose
**Table I** Metabolic and anthropometric characteristics of patients with T2D, patients with T2D without treatment (have not use oral hypoglycemics), prediabetic patients and healthy controls.

|                           | T2D patients n=476 | T2D patients without treatment n=109 | Pre-diabetic patients n=53 | Healthy controls n=360 | p T2D+/T2D- | p T2D+/preD | p T2D+/ctrl | p T2D-/preD | p T2D-/ctrl | p preD/ctrl |
|---------------------------|---------------------|--------------------------------------|----------------------------|------------------------|-------------|------------|-------------|-------------|-------------|------------|
| Male/Female ratio         | 169 / 277           | 46 / 63                              | 26 / 27                    | 128 / 209              |             |            |             |             |             |            |
| Age (years)               | 55.30 ± 0.478       | 56.89 ± 1.015                        | 52.00 ± 1.448              | 48.95 ± 0.477          | 0.362       | 0.851      | <0.001      | 0.299       | <0.001      | 0.004      |
| Fasting glucose (mmol/L)  | 9.30 ± 0.190        | 8.76 ± 0.275                         | 8.05 ± 1.319               | 5.12 ± 0.503           | 0.686       | <0.001     | 0.003       | <0.001      | <0.001      | <0.001     |
| HbA1c (%)                 | 7.61 ± 0.082        | 7.78 ± 0.140                         | 6.04 ± 0.138               | 5.53 ± 0.055           | 0.510       | <0.001     | <0.001      | <0.001      | <0.001      | <0.001     |
| Fasting insulin (mU/L)    | 15.47 ± 0.761       | 17.54 ± 1.871                        | 13.92 ± 1.289              | 10.21 ± 0.589          | 0.728       | 1.000      | <0.001      | 0.931       | <0.001      | 0.085      |
| BMI (kg/m²)               | 31.02 ± 0.343       | 30.32 ± 0.565                        | 29.14 ± 0.687              | 26.75 ± 0.389          | 0.830       | 0.701      | <0.001      | 0.974       | <0.001      | 0.070      |
| Waist circumference (cm)  | 102.28 ± 0.779      | 103.08 ± 1.127                       | 101.90 ± 1.184             | 94.35 ± 0.807          | 1.000       | 0.988      | <0.001      | 0.985       | <0.001      | 0.001      |
| Hip circumference (cm)    | 109.41 ± 0.988      | 108.62 ± 0.975                       | 109.02 ± 1.086             | 106.67 ± 0.808         | 0.308       | 0.788      | 0.003       | 0.990       | 0.796       | 0.738      |
| Systolic BP (mm Hg)       | 137.25 ± 1.378      | 134.43 ± 1.682                       | 132.94 ± 2.352             | 122.33 ± 1.093         | 0.077       | 0.108      | <0.001      | 0.971       | <0.001      | 0.004      |
| Diastolic BP (mm Hg)      | 88.14 ± 0.521       | 83.75 ± 0.873                        | 84.50 ± 1.346              | 79.85 ± 0.498          | 0.001       | 0.088      | <0.001      | 0.982       | 0.005       | 0.014      |
| Triglycerides (mmol/L)    | 2.43 ± 0.129        | 2.35 ± 0.159                         | 1.95 ± 0.138               | 1.66 ± 0.565           | 0.984       | 0.229      | <0.001      | 0.484       | <0.001      | 0.258      |
| Total cholesterol (mmol/L)| 5.22 ± 0.076        | 5.46 ± 0.126                         | 5.11 ± 0.124               | 5.47 ± 0.077           | 0.868       | 0.811      | 0.010       | 0.582       | 0.564       | 0.075      |
| HDL cholesterol (mmol/L)  | 1.05 ± 0.016        | 1.16 ± 0.059                         | 1.06 ± 0.042               | 1.30 ± 0.027           | 0.324       | 0.961      | <0.001      | 0.428       | <0.001      | <0.001     |
| LDL-cholesterol (mmol/L)  | 3.34 ± 0.064        | 3.36 ± 0.120                         | 3.22 ± 0.110               | 3.54 ± 0.059           | 0.998       | 0.100      | 0.050       | 0.999       | 0.441       | 0.572      |
| VLDL-cholesterol (mmol/L) | 1.400 ± 0.159       | 0.987 ± 0.063                        | 0.755 ± 0.065              | 0.746 ± 0.049          | 0.182       | 0.004      | <0.001      | 0.230       | 0.003       | 0.972      |
| hsCRP (mg/L)              | 5.40 ± 0.507        | 5.04 ± 1.110                         | 3.60 ± 0.833               | 3.51 ± 0.189           | 0.767       | 0.014      | 0.002       | 0.192       | 0.478       | 0.640      |
| Fibrinogen (g/L)          | 3.74 ± 0.092        | 3.92 ± 0.101                         | 3.52 ± 0.164               | 3.87 ± 0.407           | 0.791       | 0.743      | 0.258       | 0.361       | 0.031       | 0.999      |
| Leucocytes (10^9/L)       | 7.43 ± 0.125        | 7.92 ± 0.682                         | 6.62 ± 0.275               | 6.42 ± 0.109           | 0.999       | 0.230      | <0.001      | 0.277       | <0.001      | 0.648      |
| Sedimentation (mm/h)      | 16.62 ± 2.083       | 18.16 ± 2.145                        | 8.56 ± 1.621               | 11.61 ± 1.039          | 0.959       | 0.018      | 0.284       | 0.004       | 0.079       | 0.295      |

*Values represent mean ± standard error of mean (SEM), p T2D+/T2D- significance between T2D patients and T2D without treatment, p T2D+/preD- significance between T2D patients (treated) and prediabetic patients, p T2D+/ctrl- significance between T2D patients (treated) and healthy controls, p T2D-/preD- significance between T2D without treatment and prediabetic patients, p T2D-/ctrl- significance between T2D without treatment and healthy controls, p preD/ctrl- significance between prediabetic patients and healthy controls. All differences were tested using ANOVA 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. HOMA – IR (homeostasis model assessment insulin resistance index) was calculated using the formula: fasting insulin (mU/L) x fasting glucose (mmol/L)/22.5
Table II  Genotype and variant allele frequencies for FTO gene polymorphism rs8050136: A>C.

| Polymorphism | Genotype | T2D patients | Mutated allele frequency | Prediabetic patients | Mutated allele frequency | Healthy controls | Mutated allele frequency | p  |
|--------------|----------|--------------|--------------------------|---------------------|--------------------------|------------------|--------------------------|-----|
| rs8050136    | AA       | 130 (24.7%)  | 0.50                     | 17 (41.3%)          | 0.46                     | 84 (26.0%)       | 0.51                     | p preD/ctrl = 0.100 |
|              | AC       | 264 (50.1%)  |                           | 16 (30.4%)          | 150 (46.4%)              |                 |                           | p T2D/preD = 0.576 |
|              | CC       | 133 (25.2%)  |                           | 13 (28.3%)          |                         | 89 (27.6%)       |                           | P preD/ctrl = 0.224 |
|              | Total    | 527          | p1=0.999                 | 46                  | p2=0.127                 | 323             | p3=0.443                 | p=0.258 |

p – significance of the 2 test for comparison of genotype frequencies between T2D, prediabetic patients and healthy controls
ppreD/ctrl – significance of the 2 test for comparison of genotype frequencies between T2D and prediabetic patients
p T2D/preD – significance of the 2 test for comparison of genotype frequencies between T2D patients and healthy controls
P1 – value for Hardy-Weinberg equilibrium for T2D patients group
P2 – value for Hardy-Weinberg equilibrium for prediabetic patients group
P3 – value for Hardy-Weinberg equilibrium for healthy controls group

Table III  Comparison of clinical and biochemical characteristics between different genotypes of FTO gene polymorphism rs8050136: A>C in healthy subjects.

| FTO rs8050136 A>C | AA (n=79) | AC (n=140) | CC (n=87) | p-value AA/AC | p-value AA/CC | p-value AC/CC |
|-------------------|-----------|------------|-----------|---------------|---------------|---------------|
| Fasting glucose (mmol/L) | 5.02 ± 0.138 | 5.18 ± 0.055 | 5.15 ± 0.117 | 0.996 | 0.561 | 0.502 |
| HbA1c (%)         | 9.37 ± 3.811 | 5.47 ± 0.048 | 5.39 ± 0.062 | 0.119 | 0.016 | 0.246 |
| Fasting insulin (mU/L) | 11.27 ± 2.227 | 11.17 ± 1.105 | 10.443 ± 0.801 | 0.674 | 0.486 | 0.751 |
| +HOMA-IR          | 2.212 ± 0.206 | 2.629 ± 0.282 | 2.49 ± 0.217 | 0.744 | 0.618 | 0.842 |
| BMI (kg/m²)       | 29.41 ± 2.661 | 26.91 ± 0.589 | 27.02 ± 0.805 | 0.133 | 0.221 | 0.880 |
| Waist circumference (cm) | 98.55 ± 1.638 | 94.07 ± 1.012 | 92.78 ± 2.008 | 0.163 | 0.076 | 0.630 |
| Hip circumference (cm) | 109.25 ± 1.498 | 106.27 ± 0.884 | 106.55 ± 2.574 | 0.414 | 0.635 | 0.194 |
| Systolic BP (mm Hg) | 123.05 ± 2.181 | 120.43 ± 1.803 | 126.75 ± 2.107 | 0.375 | 0.175 | 0.108 |
| Diastolic BP (mm Hg) | 82.65 ± 1.128 | 79.13 ± 0.665 | 80.43 ± 1.112 | 0.020 | 0.362 | 0.179 |
| Triglycerides (mmol/L) | 1.75 ± 0.137 | 1.66 ± 0.082 | 1.64 ± 0.112 | 0.781 | 0.835 | 0.600 |
| Total cholesterol (mmol/L) | 5.37 ± 0.167 | 5.44 ± 0.106 | 5.36 ± 0.157 | 0.521 | 0.679 | 0.850 |
| HDL cholesterol (mmol/L) | 1.21 ± 0.054 | 1.34 ± 0.040 | 1.27 ± 0.051 | 0.040 | 0.169 | 0.585 |
| LDL-cholesterol (mmol/L) | 3.51 ± 0.119 | 3.45 ± 0.088 | 3.48 ± 0.115 | 0.426 | 0.414 | 0.914 |
| VLDL-cholesterol (mmol/L) | 0.735 ± 0.118 | 0.822 ± 0.089 | 0.687 ± 0.066 | 0.152 | 0.576 | 0.365 |
| hsCRP (mg/L)       | 3.72 ± 0.433 | 3.39 ± 0.228 | 3.66 ± 0.428 | 0.896 | 0.964 | 0.933 |
| Fibrinogen (g/L)   | 5.30 ± 1.584 | 3.81 ± 0.601 | 3.11 ± 0.083 | 0.035 | 0.013 | 0.439 |
| Leukocytes (10³/L) | 6.77 ± 0.192 | 6.19 ± 0.170 | 6.44 ± 0.235 | 0.006 | 0.133 | 0.330 |
| Sedimentation (mm/h) | 12.65 ± 2.893 | 11.13 ± 1.320 | 10.06 ± 2.518 | 0.820 | 0.801 | 0.937 |

Values represent mean ± standard error mean (SEM), p-values show the significance of the differences of clinical and biochemical characteristics between the stated genotypes of FTO gene polymorphism rs8050136: A>C. All differences were tested using ANCOVA test (adjusted for age, sex and BMI).
homeostasis, dyslipidemia, inflammation and obesity. Carriers of the risk AA genotype of rs8050136 had higher levels of HbA1c (p=0.016) and fibrinogen (p=0.013) when compared to the carriers of CC genotype. In addition, carriers of AA genotype had significantly higher levels of diastolic BP (p=0.020) and leucocytes (p=0.006), and lower HDL cholesterol levels than carriers of AC genotype (p=0.040).

Importantly, tendency of association of AA genotype with higher waist circumference (p=0.076) was demonstrated when compared to the CC genotype.

In T2D patients group (Table IV), carriers of AA genotype of rs8050136 had lower VLDL cholesterol levels compared to carriers of AC and CC genotypes (retrospectively p=0.011; p=0.021, Figure IA).

Table IV Comparison of clinical and biochemical characteristics between different genotypes of FTO gene polymorphism rs8050136: A>C in T2D patients treated with hypoglycemics.

| FTO rs8050136 A>C | AA (n=104) | AC (n=203) | CC (n=107) | p-value AA/AC | p-value AA/CC | p-value AC/CC |
|-------------------|-----------|------------|------------|--------------|--------------|--------------|
| Fasting glucose (mmol/L) | 8.86 ± 0.361 | 9.50 ± 0.280 | 9.16 ± 0.392 | 0.124 | 0.830 | 0.185 |
| HbA1c (%) | 7.57 ± 0.172 | 7.74 ± 0.124 | 7.53 ± 0.154 | 0.639 | 0.794 | 0.435 |
| Fasting insulin (mU/L) | 16.95 ± 2.283 | 15.29 ± 0.990 | 15.65 ± 1.449 | 0.856 | 0.480 | 0.291 |
| +HOMA-IR | 6.96 ± 1.238 | 6.30 ± 0.434 | 6.65 ± 0.716 | 0.532 | 0.603 | 0.186 |
| BMI (kg/m²) | 30.76 ± 0.727 | 30.98 ± 0.411 | 30.98 ± 0.805 | 0.377 | 0.980 | 0.381 |
| Waist circumference (cm) | 101.09 ± 2.021 | 103.32 ± 0.859 | 101.77 ± 1.814 | 0.346 | 0.268 | 0.731 |
| Hip circumference (cm) | 107.83 ± 2.821 | 110.92 ± 0.945 | 107.23 ± 2.060 | 0.139 | 0.067 | 0.503 |
| Systolic BP (mm Hg) | 134.48 ± 2.878 | 138.50 ± 2.275 | 136.87 ± 1.973 | 0.154 | 0.654 | 0.049 |
| Diastolic BP (mm Hg) | 88.37 ± 1.088 | 88.09 ± 0.763 | 87.16 ± 1.054 | 0.516 | 0.322 | 0.621 |
| Triglycerides (mmol/L) | 2.38 ± 0.259 | 2.31 ± 0.100 | 2.78 ± 0.428 | 0.390 | 0.209 | 0.557 |
| Total cholesterol (mmol/L) | 5.21 ± 0.130 | 5.17 ± 0.118 | 5.29 ± 0.167 | 0.484 | 0.616 | 0.899 |
| HDL-cholesterol (mmol/L) | 1.11 ± 0.036 | 1.03 ± 0.022 | 1.03 ± 0.031 | 0.295 | 0.083 | 0.341 |
| LDL-cholesterol (mmol/L) | 3.29 ± 0.118 | 3.33 ± 0.089 | 3.36 ± 0.155 | 0.841 | 0.740 | 0.855 |
| VLDL-cholesterol (mmol/L) | 0.80 ± 0.064 | 1.51 ± 0.211 | 1.71 ± 0.438 | 0.011 | 0.021 | 0.988 |
| hsCRP (mg/L) | 6.31 ± 1.292 | 4.69 ± 0.557 | 5.64 ± 1.091 | 0.182 | 0.172 | 0.821 |
| Fibrinogen (g/L) | 3.60 ± 0.149 | 3.60 ± 0.114 | 3.76 ± 0.137 | 0.761 | 0.666 | 0.393 |
| Leukocytes (10⁹/L) | 7.12 ± 0.225 | 7.57 ± 0.169 | 7.50 ± 0.311 | 0.132 | 0.407 | 0.609 |
| Sedimentation (mm/h) | 13.38 ± 2.941 | 18.58 ± 4.092 | 16.19 ± 2.940 | 0.543 | 0.178 | 0.356 |

Values represent mean ± standard error mean (SEM), p-values show the significance of the differences of clinical and biochemical characteristics between the stated genotypes of FTO gene polymorphism rs8050136: A>C. All differences were tested using ANCOVA test (adjusted for age, sex and BMI).
Carriers of AC genotype had significantly higher systolic BP compared to CC genotype carriers (p=0.049, Figure IB).

In T2D patients group without treatment (i.e. without hypoglycemic therapy), FTO rs8050136 was significantly associated with the markers of glucose homeostasis, insulin resistance, and obesity (Table V). Here, carriers of AA and AC genotypes of rs8050136: A>C had significantly higher insulin levels than carriers of CC genotype (retrospectively p=0.016, p=0.044, Figure IC). Carriers of the risk A allele had significantly higher HOMA-IR levels as compared to the carriers of C allele (p=0.018, Figure ID). It is important to mention the tendency of association of AC genotype with higher levels of BMI (p=0.063) as compared to the CC genotype.

Table V Comparison of clinical and biochemical characteristics between different genotypes of FTO gene polymorphism rs8050136 in T2D patients without hypoglycemic therapy.

|                       | FTO rs8050136 A>C |                  |                  |                  |                  |                  |
|-----------------------|-------------------|------------------|------------------|------------------|------------------|------------------|
|                       | AA (n=25)         | AC (n=55)        | CC (n=23)        | p-value AA/AC    | p-value AA/CC    | p-value AC/CC    |
| Fasting glucose (mmol/L) | 9.36 ± 0.658      | 8.27 ± 0.242     | 9.67 ± 0.919     | 0.182            | 0.848            | 0.138            |
| HbA1c (%)              | 7.89 ± 0.270      | 7.75 ± 0.177     | 7.95 ± 0.386     | 0.882            | 0.862            | 0.728            |
| Fasting insulin (mU/L) | 26.53 ± 8.243     | 15.95 ± 1.607    | 14.15 ± 2.442    | 0.044            | 0.016            | 0.343            |
| +HOMA-IR              | 14.04 ± 7.317     | 5.84 ± 0.604     | 4.76 ± 0.887     | 0.062            | 0.018            | 0.264            |
| BMI (kg/m²)           | 30.24 ± 1.297     | 30.64 ± 0.551    | 28.93 ± 0.405    | 0.868            | 0.141            | 0.063            |
| Waist circumference (cm) | 101.52 ± 2.957    | 103.70 ± 1.447   | 105.20 ± 2.211   | 0.311            | 0.217            | 0.649            |
| Hip circumference (cm) | 108.44 ± 2.403    | 109.15 ± 1.365   | 109.46 ± 1.637   | 0.825            | 0.708            | 0.825            |
| Systolic BP (mm Hg)   | 139.12 ± 4.085    | 134.81 ± 2.361   | 130.65 ± 3.069   | 0.218            | 0.106            | 0.490            |
| Diastolic BP (mm Hg)  | 83.32 ± 1.583     | 83.65 ± 1.356    | 83.91 ± 1.692    | 0.897            | 0.863            | 0.745            |
| Triglycerides (mmol/L)| 2.24 ± 0.294      | 2.08 ± 0.140     | 3.00 ± 0.447     | 0.986            | 0.155            | 0.091            |
| Total cholesterol (mmol/L) | 5.13 ± 0.268    | 5.44 ± 0.161     | 5.84 ± 0.277     | 0.284            | 0.091            | 0.340            |
| HDL-cholesterol (mmol/L) | 1.10 ± 0.067     | 1.16 ± 0.036     | 1.23 ± 0.146     | 0.452            | 0.302            | 0.628            |
| LDL-cholesterol (mmol/L) | 3.04 ± 0.252     | 3.37 ± 0.147     | 3.64 ± 0.266     | 0.218            | 0.103            | 0.456            |
| VLDL-cholesterol (mmol/L) | 0.88 ± 0.115     | 0.89 ± 0.052     | 1.28 ± 0.225     | 0.544            | 0.072            | 0.114            |
| hsCRP (mg/L)          | 8.67 ± 4.271     | 4.03 ± 0.515     | 3.73 ± 0.450     | 0.559            | 0.940            | 0.659            |
| Fibrinogen (g/L)      | 3.86 ± 0.270     | 4.00 ± 0.127     | 3.73 ± 0.216     | 0.558            | 0.756            | 0.329            |
| Leukocytes (10⁹/L)    | 9.87 ± 2.930     | 7.33 ± 0.272     | 7.38 ± 0.307     | 0.527            | 0.696            | 0.883            |
| Sedimentation (mm/h)  | 21.27 ± 6.159    | 18.43 ± 3.147    | 14.33 ± 2.404    | 0.504            | 0.523            | 0.893            |

Values represent mean ± standard error mean (SEM). p-values show the significance of the differences of clinical and biochemical characteristics between the stated genotypes of FTO gene polymorphism rs8050136: A>C. All differences were tested using ANCOVA test (adjusted for age, sex and BMI).
In the group of patients with prediabetes results of our study did not show any association of rs8050136: A>C gene variant FTO with analyzed biochemical and anthropometric parameters (data not shown).

**Discussion**

Obesity is one of today’s biggest global problems (4). Overweight is very important in pathophysiology of T2D and one of major risk factors for developing this complex disease (33). The increasing global prevalence of T2D is tied to rising rates of obesity – which is in part a consequence of social trends toward higher energy intake and reduced energy expenditure (34). However, the mechanisms that underlie individual differences in the predisposition to obesity remain obscure (1). This is a reason why it is important to find out association of different genetic variants of candidate genes of obesity and to find a link with their role in pathophysiology of T2D. For genetic epidemiology of complex diseases, replication studies at various ethnic groups are essential to support the genotype – phenotype linkage to correctly suggest subjects for further (e.g. mechanism uncovering) studies.

Recently, FTO was indicated by GWA study as the candidate gene for development of T2D (35). In this study, association of FTO gene polymorphism (rs8050136) with the traits of T2D was studied for the first time in the West Balkan region population (Bosnians and Herzegovinians and Kosovars), showing associations of genotypes of rs8050136 polymorphism with certain clinical and biochemical parameters of T2D, especially with glucose, insulin and HOMA IR levels, as well as BMI, waist and hips circumference, as indicators of visceral obesity.

Our results demonstrated that no significant differences in analyzed genotype frequencies were found between patients with T2D, prediabetes, and...
healthy controls. Results of logistic regression analysis did not confirm a significant association of FTO genetic variation (rs8050136: A>C) with diabetes risk, probably due to the low number of subjects in our cohort (OR=1.084, 95% CI 0.758–1.551, p=0.659). Several studies conducted on different populations have confirmed the impact of this genetic variant on the increased risk of developing T2D (10–14), although there are those made on Russian, Mexican Mestizo, Netherlands, Lebanese and Omani population, which do not confirm this association (24–28).

Results of our study did not also confirm the previously published significant association of FTO polymorphism rs8050136 A>C with most important markers of obesity. However, risk A allele showed a tendency of association with higher values of BMI, waist circumference and hip circumference in different analyzed groups of our study. Numerous studies have shown association of risk A allele of rs8050136 A>C genetic variant with higher values of obesity biomarkers. In a large study in Indian population, significant association rs8050136 A>C of with higher values of BMI, waist circumference, as well as the relationship with waist-hip ratio has been demonstrated (13). Similar results of association of this genetic variant with BMI were demonstrated in Han Chinese adolescent study (19). Xiao et al. (18) found a significant association of risk A allele of rs805016 gene variant with higher levels of BMI in Uygur population from northwest China. Large meta-analysis which analyzed over 34 000 participants aged from 18 to 100 years, came to finding of greater influence of rs8050136 A>C on elevated BMI in younger patients than in older (20).

Interestingly, our results showed that the risk A allele of rs8050136 A>C polymorphism was significantly associated with decreased HDL cholesterol levels in control subjects. Results of several studies, including large meta-analysis, confirmed the association of rs8050136 A>C with the increased risk of developing metabolic syndrome, which is characterized by higher levels of glucose, waist circumference, triglycerides and total cholesterol, and lower levels of HDL cholesterol (11, 17, 36, 37). The associations of rs8050136 A>C with the markers observed in our study can be explained by the significant effects of metabolic syndrome on the metabolic and clinical parameters including higher values of anthropometric parameters (BMI, waist and hip circumference) and lower HDL cholesterol levels. It is very important to emphasize that most patients within T2D group had developed metabolic syndrome. The results of our study showed a significant association with higher diastolic pressure, which is also one of diagnostic parameters and one of major risk factors of the metabolic syndrome. In T2D patients group, risk A allele showed association with lower values of systolic pressure, which again can be explained by the fact that the most of patients in the study used antihypertensive drugs. Therefore, results obtained for this group of patients need to be interpreted with a special caution.

Interestingly, our results showed a significant association of A allele of rs8050136 A>C with higher HbA1c levels in healthy control group as well as with elevated insulin levels and HOMA IR index in T2D patients without treatment. The presence of the risk A allele appears to lead to the increased levels of HbA1c, insulin, and HOMA IR, pointing out the pronounced insulin resistance in newly diagnosed patients with T2D. Namely, increased insulin levels in the initial stage of developing T2D represent a defence mechanism of the organism against the elevated glucose and Hba1c levels.

Our findings are consistent with the results of studies which have analyzed association of FTO gene polymorphism, rs8050136 A>C with insulin sensitivity. Result of a study analysing influence of rs8050136 A>C with insulin sensitivity in women with advanced ovarian polycystic syndrome (PCOS), showed a significant association with insulin sensitivity, in women without PCOS, indicated a direct effect of rs8050136 A>C on insulin sensitivity, not associated with PCOS (21). A large study of Wang and colleagues confirmed the influence of rs8050136 A>C on the parameters of obesity and glucose homeostasis in various populations in the USA (22). Another study also showed the significant association of the risk A allele with the elevated levels of glucose, C-peptide, and BMI (23). Thus, in our study we confirmed results of the above-mentioned studies of significant association of rs8050136 A>C with important markers of glycaemic control and insulin sensitivity, such as elevated HbA1c, insulin, and HOMA IR levels.

The results of our study also showed significant effects of rs8050136 A>C polymorphism on the increased levels of inflammatory markers (fibrinogen and number of leukocytes). To our knowledge, this is one of the first studies analyzing association of rs8050136 A>C polymorphism in T2D with the inflammatory markers. A recent large meta-analysis examining the impact of candidate genes of metabolic syndrome on the inflammatory processes demonstrated an important association of FTO intron gene variant with the CRP levels (38).

A major limitation of our study is related to the relatively small number of our population cohort, particularly in regards to the patients with prediabetes and patients with newly diagnosed T2D who were not taking any medications. Nevertheless, these categories of patients are very important in terms of analysis of the associations of genetic variants with biomarkers related to the pathophysiology of T2D. Importantly, our results showed a significant association of FTO genetic variant rs8050136 A>C with the
major markers of insulin resistance, obesity, T2D, and inflammation, opening new avenues for solving many unclear questions in the pathogenesis of this complex disease. These findings may lead to the new possibilities for prevention, diagnosis, and personalized medical treatment of T2D. Especially, seeing this genetic association through the perspective of obesity-related T2D pathophysiology suggests that obesity prevention and increase in physical activity in the genetically risky subgroups may be a valuable contribution to the T2D prevention.

Acknowledgements. The authors thank all subjects who participated in the study, as well as medical doctors and paramedical staff from the Health Centre of Sarajevo Canton, Clinic for Endocrinology and Diabetes, University Clinical Centre of Sarajevo, University Hospital of Clinical Centre in Banja Luka, General Hospital in Té{anj, and Health Centre in Prizren who assisted in the study.

The work was supported by the research grant for EU-FP7 project preparation by the Council of Ministers of Bosnia and Herzegovina (BH)/Ministry of Civil Affairs BH, awarded to S.S. and by the grant MH CZ – DRO (UHHK, 00179906).

Conflict of interest statement

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

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