ISSN 2304-3415, Russian Open Medical Journal
2022. Volume 11. Issue 3 (September). Article CID e0305
DOI: 10.15275/rusomj.2022.0305

Original article

Association of inflammation gene polymorphism with increased risk of metabolic syndrome in tatar ethnic group

Olga V. Kochetova [1], Diana S. Avzaletdinova [2], Gulnaz F. Korytina [1,2]

[1] Ufa Federal Research Center of the Russian Academy of Sciences, Ufa, Russia
[2] Bashkir State Medical University, Ufa, Russia

Received 18 September 2021, Revised 15 March 2022, Accepted 17 May 2022

Abstract: *Background and objective* — Chronic low-grade inflammation plays an important role in pathophysiology of metabolic syndrome (MetS). The aim of our study was to determine the associations of polymorphic variants of inflammation genes with MetS and serum levels of high-sensitivity C-reactive protein (hsCRP) and tumor necrosis factor-α (TNF-α) in Tatar patients (Bashkortostan).
Methods — In our case-control cross-sectional study, 271 MetS patients and 327 healthy Tatars were genotyped for the SNPs in *CRP*, *TNFA*, *LTA*, *TNFRSF1B* genes.
Results — *TNFRSF1B* (rs1061624) was associated with the MetS [odds ratio (OR)=0.49, p^{ADJ}=0.0034] and TNF-α level (p=0.033). *TNFA* (rs1800629) was associated with TNF-α (p=0.015), albuminuria (p=0.013). *CRP* (rs2794521) was associated with fasting (p=0.0096) and postprandial (p=0.01) insulin, HOMA-IR (homeostasis model assessment of insulin resistance, p=0.0019), hsCRP (p=0.036), waist-hip ratio (WHR, p=0.007), body mass index (BMI, p=0.039).
The participants having the C-C haplotype of *CRP* rs2794521-rs1130864 were more common among MetS patients (OR=1.99, p=0.032). T-T haplotype in *CRP* was associated with hsCRP (p=0.0043), low-density lipoprotein cholesterol (p=0.025), HOMA-IR (p=0.00029), glycated hemoglobin (p=0.006), postprandial (p=0.0006) and fasting insulin (p=0.00031), WHR (p=0.00012), BMI (p=0.00024).
Conclusions — The data confirms that the variants of inflammation genes *CRP*, *TNFA*, *TNFRSF1B* are associated with levels of TNF-α, hsCRP. Novel association of *TNFRSF1B* (rs1061624) with MetS had been identified.

Keywords: Tatar population; metabolic syndrome; genetic polymorphisms; C-reactive protein; tumor necrosis factor.

Cite as Kochetova OV, Avzaletdinova DS, Korytina GF. Association of inflammation gene polymorphism with increased risk of metabolic syndrome in tatar ethnic group. *Russian Open Medical Journal* 2022; 11: e0305.

Correspondence to Diana S. Avzaletdinova. Phone: +79173417006. E-mail: hyppocrat@mail.ru.

Introduction

Metabolic syndrome (MetS) is a group of cardiometabolic risk factors that are linked to central fat accumulation and an increased risk of cardiovascular disease and type 2 diabetes (T2D) [1]. The 'deadly quartet' consisting of obesity, glucose intolerance, hypertriglyceridemia, and hypertension that is not fully understood. The pathophysiology of MetS is not agreed upon, but it seems uncontroversial that the syndrome results from the complex interactions between genetic and environmental factors. Low-grade inflammation and oxidative stress are the major risk factors for MetS. Tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), nitric oxide synthase, and C-reactive protein (CRP) are inflammation molecules produced by adipose tissue that play a key role in the pathogenesis of insulin resistance and are involved in a pathological process of the MetS [2-6].

Genetic polymorphisms of immune response genes were found to be associated with the MetS, obesity, and obesity complications. Previous results confirmed that CRP was a marker of systemic inflammation, cardiovascular disease, and T2D [7]. It is known that CRP expression is regulated by TNF-α. Its level correlates with body mass index (BMI), serum levels of triglycerides, total cholesterol, and fasting glycaemia. CRP contributes to the transcription of numerous inflammation genes.

However, some studies yielded controversial results of the analysis of the *CRP* gene polymorphic variants [8]. The rs2794521 variant of the *CRP* gene is located in the promoter. This variant is associated with increased transcriptional gene activity [9]. Some authors reported that carriers of allele A of -717A>G (rs2794521) variant had a nearly seven-fold increased risk of cardiovascular diseases compared with G-allele carriers. Other studies have described that G-allele carriers had a lower of risk myocardial infarction and ischemic stroke and the decreased high sensitivity CRP (hsCRP) levels during acute myocardial infarction [10].

TNF-α and lymphotoxin-α (LTA) genes are members of the tumor necrosis factor (TNF) superfamily. Both cytokines belong to the cluster located on 6p21.3 a chromosome. TNF-α is a pro-inflammatory cytokine that is primarily produced by monocytes, macrophages, and adipocytes. It is known that overexpression of TNF-α plays a major role in the pathophysiology of insulin resistance. The increased level of TNF-α is related to insulin resistance and leads to the development of T2D [11].

Table 1. Clinical Characteristics of the Studied Cohorts

Characteristics	*Control Group Members, n=327*	*MetS patients, n=271*	*p*
Sex, women/men (*n*)	245/82	195/76	0.41[a]
Age, years	55.8±7.2	57.9±6.9	0.15[b]
Height, cm	167.5±10.5	168.9±12.9	0.08[b]
Weight, kg	70±7.5	89.2±5.5	0.0001[b]
BMI, kg/m^2	24.1±1.4	31.1±2.5	0.00001[b]
Waist circumference, cm	81.5±5.8	106.5±9.5	0.0001[b]
Hip circumference, cm	96.6±8.1	109.6±8.1	0.0001[b]
WHR	0.85±0.80	1.37±1.35	0.0001[b]
Fasting blood glucose, mmol/L	4.45±0.87	5.37±1.28	0.0001[b]
Glucose at 2 h (OGTT), mmol/L	-	6.45±2.20	-
Fasting insulin, mU/L	13.35±5.34	10.86±7.21	0.0001[b]
Insulin at 2 h (OGTT), mU/L	-	15.23±10.95	-
hsCRP, mg/L	1.70±1.01	3.40±1.82	0.0001[b]
HbA_{1c}, % (mmol/moL)	4.8±0.6 (29.0±3.6)	5.3±1.0 (34.0±12.7)	0.0001[b]
HOMA-IR	1.66±1.01	2.71±2.41	0.0001[b]
Cholesterol, mmol/L	5.02±0.64	5.89±0.72	0.0001[b]
TG, mmol/L	1.40±0.70	1.71±0.51	0.003[b]
HDL, mmol/L	1.09±0.37	1.01±0.12	0.3205[b]
LDL, mmol/L	2.9±1.01	3.17±0.19	0.04[b]
TNF-α, pg/mL	9.03±0.50	18.41±0.89	0.0001[b]
Hypertension, %	13.5%	100%	0.00001[a]
Duration of hypertension, years	-	6.98±3.49	-
Systolic BP, mm Hg	115.5±10.1	152.63±12.06	0.0001[b]
Diastolic BP, mm Hg	80±6.4	88.30±5.59	0.0001[b]
Pre-diagnosed T2D (yes/no)	0/327	80/191	-
T2D duration, years	NA	5.43±4.10	-

M (±SEM), the mean and the standard error of the mean; BMI, body mass index; WHR, waist-hip ratio; OGTT, oral glucose tolerance test; hsCRP, high-sensitivity CRP; HbA1c, glycated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance; TG, triglycerides; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; BP, blood pressure. P^a – Pearson's chi-squared test; P^b – statistical significance of the Mann-Whitney U test.

The G-308A (rs1800629) variant has been largely studied, but results have been inconsistent. The variant has been correlated with increased transcriptional gene activity. The A allele carriers had higher levels TNF-α compared with the carriers of G allele [12]. The other authors demonstrated that A allele of the TNF-α gene increases the binding of a transcription factor to the promoter region [13]. It was shown that G-308A variant directly correlated with obesity, hypertension and increased insulin level. Pyrzak B. et al. (2006) described a rs1800629 variant association with MetS [14]. However, some authors did not demonstrate an association between the rs1800629 variant and MetS [15, 16].

LTA is a pro-inflammatory cytokine. It plays a key role in the immunologic response. The genes coding LTA and the TNF-α are located within the cluster in the major histocompatibility complex (MHC) region on chromosome 6p21.3. It was reported that rs909253 variant of LTA gene was located in the 1 intron and may have been associated with the increased transcriptional activity of the gene. Genetic variants of the LTA and the TNF-α genes have been investigated for associations with obesity, metabolic traits, T2D, but the results were inconsistent.

One of the most important candidates of the activation of TNF-TNFR axis is tumor necrosis factor receptor superfamily member 1B (*TNFRSF1B* or TNFR2). TNFR2 is involved in metabolic and inflammatory pathways, and in lipid and glucose metabolism. Increased TNFR2 levels associated with obesity, insulin resistance and cardiovascular diseases [13].

Though *CRP*, *TNFA*, *LTA* and *TNFRSF1B* genes are strong biological candidates for studying the pathogenesis of MetS, their association with metabolic disorders and overlapping phenotypes (T2D) has been contradictory. In this study, we examined the association of SNPs *TNFRSF1B* rs1061624, *TNFA* rs1800629, *LTA* rs909253 and *CRP* rs2794521, rs1130864 with MetS. Also, we investigated their association with serum levels of hsCRP and TNF-α in MetS patients in ethnic homogeneous group of Tatars. Previous genetic studies reported that Tatar populations were related to the Caucasian population [17].

Material and Methods

Study design

This is a cross-sectional case-control study.

Participants

The study group consisted of 271 Tatar patients with MetS (affected group) and 327 healthy individuals without clinical symptoms of diabetes and obesity (unaffected group). Both patients and controls were ethnic Tatars, living in the Republic of Bashkortostan (Russia).

The inclusion criteria for the affected group were the following: aged 40 years or older; the MetS diagnosis established according to the Consensus definition [18]; living in the Republic of Bashkortostan since their birth; Tatars by ethnicity; all studied DNA of unrelated individuals; written informed consent provided.

The inclusion criteria for the unaffected group were as follows: aged 40 years or older; absence of any clinical or laboratory symptoms of metabolic disorders; absence of the family history of diabetes; living in the Republic of Bashkortostan since their birth; Tatars by ethnicity; all studied DNA of unrelated individuals; written informed consent provided. MetS patients and control group members were matched according to their age and sex. Clinical characteristics are described in *Table* 1.

Table 2. Genotypes and Alleles Frequency Distribution by CRP, TNFA, LTA and TNFRSF1B Gene Loci in MetS Patients and Control Group Members

Gene/SNP Chromosome location	*Genotypes Alleles*	*MetS (N=271) N/%*	*Controls (N=271) N/%*	p^a	p^b	*OR (95% CI)*
CRP	TT	168/62.0	168/62.0			1.00
rs2794521	CT	98/36.1	94/34.7	0.54	0.73	1.04 (0.73-1.48)
	CC	5/1.9	9/3.3			0.55 (0.18-1.68)
1:159715306	T	434/80.1	430/79.3	0.82	-	1.04 (0.78-1.40)
c.-821G>A	C	108/19.9	112/20.7			
CRP	CC	140/51.7	128/47.2			1.00
rs1130864	CT	108/39.9	115/42.4	0.58	0.30	0.86 (0.60-1.20)
	TT	23/10.0	28/10.3			0.77 (0.42-1.42)
1:159713301	C	388/71.6	371/68.5	0.29	-	1.16 (0.89-1.50)
c.*22+202C>T	T	154/28.4	171/31.6			
TNFA	GG	207/76.4	210/77.5			1.00
rs1800629	AG	60/22.1	58/21.4	0.91	0.72	1.08 (0.71-1.64)
	AA	4/1.5	3/1.1			1.36 (0.30-6.17)
6:31575254	G	474/87.5	478/88.2	0.78	-	0.91 (0.65-1.34)
c.-488G>A	A	68/12.5	64/11.8			
LTA	AA	173/63.8	151/55.7			1.00
rs909253	AG	84/31.0	110/40.6	0.055	0.17	0.66 (0.46-0.95)
	GG	14/5.2	10/3.7			1.21 (0.52-3.81)
6:31572536	A	430/79.3	412/76.0	0.21	-	1.20 (0.91-1.61)
g.5438A>T	G	112/20.7	130/24.0			
TNFRSF1B	GG	87/32.1	75/27.7			1.00
rs1061624	AG	156/57.6	143/52.8	0.012	0.018	0.94 (0.64-1.38)
	AA	28/10.3	53/19.5			0.46 (0.27-0.81)
1:12207208	G	330/60.9	293/54.1	0.03	-	1.31 (1.03-1.67)
c.*188A>G	A	212/39.1	249/45.9			

p^a – chi-squared test for genotype frequency difference between the MetS and control group, p^b – Cochran–Armitage trend test, OR with 95 % CI for a minor allele in basic allele test.

The exclusion criteria for both the affected and the unaffected groups were the following: concomitant treatment with non-steroidal anti-inflammatory drugs and systemically administered glucocorticoids; chronic inflammatory diseases such as rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, psoriatic arthritis or inflammatory bowel disease.

The patients with MetS had been enrolled between 2012 and 2017 in the Clinic of Bashkir State Medical University (Ufa, Russia). The control group members were selected between 2012 and 2017 in the Republic Center of Blood Transfusion (Ufa, Russia). The experimental work was performed in the Department of Genomics of IBG UFRC RAS. Blood samples (4 ml) were collected both in affected and unaffected groups.

Selection of SNPs genotyped in this study

We selected a subset of SNPs based on the functional SNP selection approach: a) the functional significance of studied SNPs; b) disease-associated SNPs known in the literature (association with T2D, diabetes complications, obesity, cardiovascular diseases), c) minor allele frequency (MAF) of more than 5% in the Caucasian population (NCBI).

Regulatory scores of SNPs were assessed according to the RegulomeDB (http://regulomedb.org/) database. The polymorphisms of *TNFRSF1B* rs1061624, *TNFA* rs1800629, and *LTA* rs909253 genes had regulatory scores 5, 1d and 4, respectively. Only SNP rs1800629 of *TNFA* gene deleteriously affected gene expression.

The functional significance of silent SNPs was analyzed using HaploReg (v4.1) program. It was revealed that *CRP* rs2794521 is located in regulatory DNA motifs region which are binding sites of five transcriptional factors, including Ras-responsive element-binding protein 1 which promotes brown adipocyte differentiation (https://www.genecards.org). *CRP* rs1130864 is located in the region of promoter and enhancer histone marks.

In *TNFRSF1B,* we selected rs1061624 SNP, which resides in the 3'-untranslated region (UTR) and it has MAF of 0.468 according to the 1000 genomes project. Previous studies demonstrated that this SNP was involved in inflammatory bowel disease, tuberculosis, sporadic breast cancer, colorectal cancer, Crohn's disease [19-23].

In *TNFA,* we studied rs1800629 G-308A (located in the regulatory region). It had MAF of 0.164 in the CEU population (1000Genomes). The associations between *TNF-308* haplotypes and the susceptibility to gestational diabetes mellitus, coronary artery disease, rheumatoid arthritis have been described [24-29]. The association of the rs1800629 variant with affected gene expression was initially discovered in previous studies [30].

The rs909253 polymorphism is an A to C substitution in the intron 1 of *LTA* gene, MAF of 0.306 in the CEU population (1000Genomes) [28]. The rs909253 variant was associated with myocardial infarction [28-31]. A recent functional study showed that variants in *TNFA* and LTA genes could affect many inflammatory diseases [32].

The rs2794521 SNP is located in the upstream region of the *CRP* gene (MAF of 0.285 in the CEU population according to 1000 Genomes). *CRP* rs2794521 has been associated with hemorrhagic stroke in men, ischemic stroke, preeclampsia [29-36].

We investigated that SNP rs1130864 had C to T substitution located in the 3'UTR region, MAF was 0.327 (1000 Genomes). The SNP is associated with serum CRP concentrations as suggested by previous studies [35]. The variant of *CRP* is associated with increased serum CRP levels while others are associated with decreased ones [37-39].

The novel aspects of this study are: 1) the analysis of associations of those inflammation gene polymorphisms which

previously associated with separate compounds of the MetS with the MetS risk; 2) the analysis of associations of the inflammation gene polymorphisms with hsCRP and TNF-α serum levels in Tatar ethnic group, Russia, previously associated with the serum levels of hsCRP and TNF-α in other populations.

Genotyping SNPs

Total genomic DNA was isolated from 6 mL of venous blood using standard phenol–chloroform extraction. Genotyping of five SNPs in *CRP* rs2794521, *CRP* rs1130864, *TNFA* rs1800629, *LTA* rs909253, *TNFRSF1B* rs1061624 genes were performed by real-time polymerase chain reaction (PCR), using TaqMan SNP assays (Applied Biosystems, Foster City, CA). Specific PCR-product accumulation by hybridization and cleavage of double-labeled fluorogenic probe during amplification was detected using BioRad CFX96 instrument (Bio-Rad Laboratories Inc., USA). End-point fluorescence and genotype discriminations were determined according to the BioRad CFX96 protocol, using CFX Manager software. For quality control, 5 per cent of dummy samples and blank control samples were also taken in each experiment. The genotyping was blind to case or control status of the samples. Quality control of genotyping data was assessed by the subject and by marker. SNPs were subsequently analyzed according to their proportion of missing, MAF (2% threshold) or deviation from Hardy–Weinberg equilibrium (HWE) ($p \geq 0.05$).

Anthropometric measurements

Body weight and height were measured in light indoor clothing barefoot. Waist and hip circumferences and blood pressure were measured.

Clinical measures

Blood samples were collected after a 12 h overnight fast and 2 h after oral intake of 75 grams of glucose (OGTT was performed).

HbA_1c level was measured by high-performance liquid chromatography. Plasma glucose was measured by glucose oxidase method.

The level of total cholesterol, TG, HDL and LDL was evaluated by the spot metering method with an Olympus biochemical analyzer (Abbott, Germany) using the Beckman Coulter kits.

The insulin resistance index HOMA-IR was calculated using the HOMA2 Calculator v2.2.3 software (http://dtu.ox.ac.homa).

Fasting and postprandial insulin content in the blood serum was evaluated by radio-immunoassay method (RIA) (RIA Diagnostic Corporation, Los Angeles, CA) with a sensitivity of 0.5 mUI/L (reference range is 0.5-30 mUI/L).

The CRP level was measured by immunoturbidimetry (Roche Diagnostics GmbH, Mannheim, Germany), with a normal range of 0-7 mg/L and analytical sensitivity of 0.5 mg/L.

The TNF-α blood level was evaluated by multiplex analysis method (flow cytometry) with a two-array laser automatic analyzer Bio-Plex Protein Assay System using “Bio-Rad” kits (USA).

Statistical analysis

The sample size was calculated by Quanto software (http://biostats.usc.edu/software). The sample size (n=271 for the affected group and n=327 for the unaffected group) was determined to be sufficient to detect the association between the five studied polymorphisms and MetS with more than 80% power (power: 95.53%, disease prevalence, 7%, error: 5%, OR, 2.0, and significance level <0.05 for each model).

Power calculation for the study was performed basing on the MAF of five candidate SNPs (*CRP* rs2794521, *CRP* rs1130864, *TNFA* rs1800629, *LTA* rs909253, *TNFRSF1B* rs1061624*)* in European (http://www.ncbi.nlm.nih.gov/projects/SNP/).

Statistical analysis was performed using Statistica v. 6.0 software (StatSoft Inc., Tulsa, OK, USA). The clinical and biochemical features of study people were presented as mean ± SD. Comparison between two groups was performed by Mann -Whitney U test for the data without normal distribution. Categorical variables were compared using Pearson’s chi-squared test. All SNPs were tested for departure from Hardy–Weinberg equilibrium in the control group HWE ($\chi 2$). Associations of the studied SNP and MetS were analyzed the basic allele test, the calculation of the odds ratio (OR) for the minor allele and the Cochran–Armitage trend test were performed using PLINK v. 1.07. Logistic regression analysis was carried out to detect associations under dominant and additive models. The significance of the obtained model accounting for all variables was verified by the significance of the likelihood ratio test (p^{ADJ}). The best model was chosen using the Akaike’s information criterion (AIC). A model with the lowest AIC was chosen for each significantly associated locus ($p<0.05$). Linear regression analysis was performed to detect associations between the studied SNPs and quantitative phenotypes, such as body weight. The regression analysis was performed using PLINK software v. 1.07. Correction for multiple testing was conducted using the false discovery rate (FDR) method.

The linkage disequilibrium (LD) between *TNFA*, *LTA* and *CRP* loci analyses were performed using HaploView 4.2 software [40].

Results

Before proceeding to the analysis of the association of genes controlling immune response with MetS, the frequency distribution of the genotypes of polymorphic gene variants was tested for the Hardy–Weinberg equilibrium, and evaluated MAF in the total study group and in the affected and unaffected groups individually. For the control group, the following results were obtained: *CRP* rs2794521 (p=0.46, MAF=20.66), *CRP rs*1130864 (p=0.89, MAF=31.55), *TNFA* rs1800629 (p=1.00, MAF=11.81), *LTA* rs909253 (p=0.54, MAF=23.99), *TNFRSF1B* rs1061624 (p=0.27, MAF=45.74). The frequency distribution of the rare allele does not differ from the European population. None of the study participants were excluded.

We genotyped five SNPs in the *CRP* (rs2794521, rs1130864), *TNFA* (rs1800629), *LTA* (rs909253), *TNFRSF1B* (rs1061624) genes in MetS patients and in the control group. *Table* 2 shows the distribution of the genotypes and alleles of the studied polymorphic markers.

Statistically significant differences were found in the polymorphic locus *TNFSF1B rs1061624* c.*188A>G. The frequency of the minor A allele of *TNFSF1B* (c.*188A>G, *rs1061624*) was significantly lower in MetS patients than in controls (p=0.03). A more frequent G allele was associated with the disease (p=0.03, OR=1.31).

The frequency of AA homozygotes in the group of MetS patients was lower than in the healthy subjects (p_{adj}=0.0034, OR^{ADJ}=0.49, in the recessive model). Association with the MetS was found under the additive model (p_{adj}=0.017, OR^{ADJ}=0.73) (*Table* 3).

No association was revealed between *CRP* rs2794521 and rs1130864, *TNFA* rs1800629, *LTA* rs909253 loci and MetS.

We examined quantitative clinical-demographic characteristics of the MetS and candidate genes in MetS patients (*Table* 4). *CRP* rs2794521 and *LTA* rs909253 polymorphisms were not significantly associated with quantitative characteristics of MetS. People with *AA* genotype variant *TNFA* rs1800629 had higher TNF-a level (38.27 pg/mL) compared with patients with GG (18.51 pg/mL) and AG (16.9 pg/mL) genotypes (p=0.015). Patients with AA genotype had higher level of albumin in urine – 36.5 mg/g vs. 22.58 mg/g and 21.86 mg/g in patients with GG and AG genotypes (p=0.013).

We established that TT genotype *CRP* rs2794521 was associated with increased levels of hsCRP (p=0.036), HOMA-IR (p=0.0019), OGTT (p=0.01), fasting insulin (p=0.0096), WHR (p=0.007), BMI (p=0.039), height (p=0.041).

Patients with TT genotype had higher WHR (0.96) than the patients with other genotypes (0.93, p=0.007), their BMI (32.56 kg/m^2) was also more than in CC (31.0 kg/m^2) and CT (31.83 kg/m^2) carriers (p=0.039), and they had greater height (p=0.041).

The variant rs1061624 of the *TNFRSF1B* gene was associated with a decreased TNF-a level in the blood. The patients homozygous for the *A* allele of *TNFRSF1B* had a low level of TNF-a (13.58 pg/mL) when compared with GG (20 pg/mL) and AG (18.13 pg/mL) genotype carriers (p=0.033).

Haplotype analysis

The genes *TNFA* and *LTA* are located within the MHC class I region of chromosome 6 p21.3.

Using Haploview 4.1 program, we performed the linkage disequilibrium test between *TNFA* rs1800629, *LTA* rs909253 and *CRP* rs2794521, rs1130864 loci. We detected linkage between *TNFA* rs1800629 and *LTA* rs909253 (D'=0.59, r^2=0.41). We did not find association between haplotypes of tow SNPs (*TNFA* rs1800629, *LTA* rs909253) with MetS.

Table 3. Analysis of the *TNFRSF1B* (rs1061624) Genes Polymorphism Association with the MetS

Gene SNP	Test/Model	MetS N (%)	Controls N (%)	OR^{ADJ} (95% CI)	p_{adj}	p_{FDR}
	Dominant					
	GG	87 (32.1)	75 (27.7)	0.81 (0.56-1.17)	0.26	0.26
	AG-AA	184 (67.9)	196 (72.3)			
TNFRSF1B	Recessive					
rs1061624	GG-AG	243 (89.7)	219 (80.8)	0.49 (0.30-0.80)	0.0034	0.01
	AA	28 (10.3)	52 (19.2)			
	Log-additive	---	---	0.73 (0.56-0.95)	0.017	0.02

OR^{ADJ}, odds ratio adjusted for sex and age; p_{adj} – significance in the likelihood ratio test for the regression model adjusted for sex and age; p_{FDR} – significance after the FDR correction.

Table 4. Analysis of the *CRP, TNFA, LTA* and *TNFRSF1B* Genes Polymorphisms Association with Clinical Characteristics in MetS Patients

Gene SNP	*Characteristics*	*Genotype model*	*Mean (SD)*	*p^b*	*Beta (CI 95%)*
TNFA rs1800629	TNF-a, pg/mL	GG	18.51 (1.07)	0.015	19.76 (5.80-33.72)
		AG	16.9 (1.42)		
		AA	38.27 (10.32)		
	Microalbumin test, mg/g	GG	22.58 (1)	0.013	13.92 (0.78-27.07)
		AG	21.86 (1.52)		
		AA	36.5 (6.65)		
CRP rs1130864	hsCRP, mg/L	CC	3.32 (0.22)	0.036	1.12 (0.23-2.01)
		CT	3.37 (0.21)		
		TT	4.45 (0.45)		
	HOMA-IR	CC	2.46 (0.23)	0.0019	2.07 (0.91-3.22)
		CT	2.67 (0.2)		
		TT	4.53 (0.98)		
	Insulin at 2 h (OGTT), mU/L	CC	13.78 (0.95)	0.01	8.28 (2.97-13.60)
		CT	16.23 (1.34)		
		TT	22.06 (3.49)		
	Fasting insulin, mU/L	CC	10.52 (0.86)	0.0096	5.49 (1.89-9.09)
		CT	10.99 (0.71) 1		
		TT	6.01 (2.54)		
	WHR	CC	0.93 (0)	0.007	0.03 (0.01-0.05)
		CT	0.93 (0)		
		TT	0.96 (0.01)		
	BMI, kg/m^2	CC	31.00 (0.3)	0.039	1.37 (0.25-2.00)
		CT	31.83 (0.27)		
		TT	32.56 (0.59)		
	Height, cm	CC	167.78 (0.81)	0.041	-3.96 (-7.01--0.90)
		CT	166.47 (0.7)		
		TT	164 (1.54)		
TNFRSF1B rs1061624	TNF-a, pg/mL	GG	20.00 (1.8)	0.033	-2.80 (-5.37--0.24)
		AG	18.13(0.99)		
		AA	13.58 (1.33)		

Beta – coefficient of regression; p^b – statistical significance of regression coefficient.

Table 5. Association between CRP Haplotypes and Clinical-Demographic Characteristics of MetS Patients

Characteristics	*TC (63.2%)*	*TT (17.3%)*	*CC (11.9%)*	*CT (7.6%)*
hsCRP (mg/L)	Reference	0.59 (0.19-0.99)	-0.17 (-0.72-0.38)	-0.36 (-1.07-0.35)
p	---	0.0043	0.54	0.32
LDL (mmol/L)	Reference	-0.05 (-0.09--0.01)	-0.1 (-0.17--0.02)	0.02 -0.04-0.08)
p	---	0.025	0.012	0.51
HDL (mmol/L)	Reference	0 (-0.02-0.03)	0.05 (0.01-0.1)	-0.04 (-0.08-0)
p	---	0.86	0.03	0.044
HOMA-IR	Reference	1.06 (0.49-1.62)	0.03 (-0.94-0.99)	-0.33 (-1.09-0.42)
p	---	0.00029	0.39	0.96
HbA1c (%)	Reference	0.32 (0.09-0.55)	0.22 (-0.2-0.63)	-0.1 (-0.42-0.23)
p	---	0.006	0.3	0.56
Insulin at 2 h (OGTT), mU/L	Reference	4.45 (1.94-6.97)	-1.23 (-5.88-3.43)	-0.84 (-4.25-2.56)
p	---	0.0006	0.61	0.63
Fasting insulin (mU/L)	Reference	2.99 (1.38-4.6)	-0.29 (-3.08-2.5)	-1.08 (-3.26-1.1)
p	---	0.00031	0.84	0.33
Fasting glucose, mmol/L	Reference	0.34 (0.03-0.64)	0.28 (-0.27-0.82)	-0.2 (-0.61-0.2)
p	---	0.03	0.32	0.32
WHR	Reference	-0.03 (-0.04--0.01)	-0.06 (-0.08--0.04)	0 (-0.02-0.02)
p	---	0.00012	0.0001	0.86
Waist circumference (cm)	Reference	-0.86 (-2.99-1.28)	-4.81 (-8.48--1.14)	1.93 (-1.02-4.89)
p	---	0.43	0.011	0.2
BMI, kg/m^2	Reference	1.03 (0.49-1.58)	-0.36 (-1.33-0.61)	0.32 (-0.42-1.06)
p	---	0.00024	0.47	0.4

The data present the values of β (estimated coefficient of regression model), (CI 95%).

Also, linkage disequilibrium was detected between SNP of the *CRP* gene (rs2794521 and rs1130864) (D'=0.60, r^2=0.45). High frequency of the *C-C* haplotype was found in MetS patients (7.68%, OR=1.99, CI 95% (1.06-3.71), *p*=0.032) compared to the controls (3.81%).

We evaluated the linear regression of two haplotypes in the *CRP* gene, and in the *TNFA* and *LTA* genes, according to clinical and demographic characteristics of MetS patients. The data are presented in *Table* 5.

CRP rs2794521 T-rs1130864 T haplotype was associated with increased a hsCRP serum level (p=0.0043), HOMA-IR (p=0.00029), HbA1c (p=0.006), postprandial insulin (p=0.0006), fasting insulin (p=0.03) and BMI (p=0.00024). T-T haplotype was associated with decreased levels of LDL (p=0.025) and WHR (waist-hip ratio) (p=0.00012).

CRP rs2794521 C-rs1130864 C haplotype was associated with higher HDL levels (p=0.03) and lower LDL levels (p=0.012), WHR (p=0.0001), and this haplotype was associated with low waist circumference (p=0.011) and BMI (p=0.012).

Discussion

We identified the association between *TNFSF1B* (c.*188A>G, rs1061624) polymorphism and MetS, and we found the association with decreased levels of TNF-a in Tatar population.

TNF-α is a pro-inflammatory cytokine, TNF-α binds to two transmembrane receptors, TNFRSF1A/TNFR1 (p55/60) and TNFRSF1B/TNFR2 (p75/80) [41]. The genes coding TNF-α and TNFRSF1B are very polymorphic. The SNP rs1061624 is the located in 3'UTR region. This DNA site may contribute to differences in expression levels of the gene through miRNA binding [42].

The data about the association of the *TNFRSF1B* genes with MetS is absent. We found study reporting the association of *TNFRSF1B* with Crone disease, tuberculosis, arterial hypertension [43, 44]. The association studies of *TNFRSF1B* were controversial. For example, AA genotype was associated with the risk of tuberculosis in Asian populations (Xu F. et al., 2014). This finding proposed that the *TNFRSF1B* rs1061624 *AA* genotype has a protective effect against MetS in Tatar population. Recent studies shown association with inflammatory bowel diseases, tuberculosis, Crohn's disease in non-Asian populations [19, 45]. The rs1061624 polymorphism in the *TNFRSF1B* gene would break the miRNA-mRNA binding sites for miR-516a-3p, miR-720 and miR-328 and can disturb the expression of the gene [46].

A polymorphism in TNF rs1800629 was associated with high level of TNF-α in blood and albumin in urine, induced adipocytes apoptosis and stimulates insulin resistance [46, 4].

Our results showed no significant differences in the distribution of genotypes in *TNFA* rs1800629 among MetS patients and healthy controls. However, they demonstrated association with high TNF-α in serum and albumin in urine. Sookoian S.C. and co-authors (2005) suggested that the genetic risk for high fasting insulin level, arterial hypertension, obesity, high HOMA-IR increased for patients with genotype AG and AA [47]. In accordance with our results, Ribeiro C.M. et al. (2019) showed significantly higher TNF-α serum levels in rheumatoid arthritis patients with AA and GA genotype [48].

Several meta-analyses of the rs1800629 (G-308A) SNP did not detect a significant association with T2D and with MetS [49, 50].

The number of studies exhibited that TNF-α involved in the pathogenesis of obesity and obesity-related traits [51]. Yaribeygi H. et al. (2019) suggested that the glucose directly increased levels of pro-inflammatory cytokines IL-6, monocyte chemotactic protein 1, and TNF-α [52]. The association of rs1800629 SNP with high levels of the cytokine could be used in clinical practice for appropriate TNF modulating therapy in MetS patients.

A significant association was observed with hsCRP, HOMA-IR, Insulin at 2 h, WHR, BMI, height and 3'UTR region of *CRP* gene. Probably the association is due to the fact that the rs1130864 polymorphism was in linkage disequilibrium to −286T>A; rs3091244 polymorphism of *CRP* gene. Besides, the 3'UTR region is

associated with increased stability of mRNA in carriers of T-allele of SNP rs1130864 and therefore increased *CRP* expression. The same result was reported by Martínez-Calleja A. et al (2012), these authors showed that TT genotype was associated with increased hsCRP level and higher BMI level [53]. We confirmed the data for *CRP* rs1130864 association with hsCRP level obtained in different populations [8, 54-56]. Also, we detected the expected associations between *CRP* haplotypes and hsCRP serum levels and MetS, HOMA-IR, HbA_{1c}, fasting glucose, LDL, BMI. Our results demonstrate a possible relationship between *CRP* haplotypes and MetS. The results were confirmed published data.

Conclusion

The results of our study suggested that *CRP, TNFA* and *TNFRSF1B* genes are involved in the pathogenesis of MetS. Our results confirmed that low-grade inflammation is a common in patients with MetS.

Limitations

The limitation of the study data on the therapy are not available and were not analyzed.

Ethical approval

The study was approved by the Ethics Committee at the Institute of Biochemistry and Genetics, Ufa Federal Research Center of Russian Academy of Sciences (IBG UFRC RAS) (Protocol No 8, March 14, 2012). Written informed consent was obtained from each participant in accordance with Helsinki declaration outlining the principles for medical research involving human subjects.

Funding

The study was supported by Russian Foundation for Basic Research (Grant No. 20-013-00261), the Ministry of Science and Higher Education of Russian Federation (№ AAAA-A16-116020350031-4), the mega-grant from the Government of Russian Federation No. 075-15-2021-595.

Conflict of interests

The authors declare no conflict of interest.

References

1. Ford ES. The metabolic syndrome and mortality from cardiovascular disease and all-causes: findings from the National Health and Nutrition Examination Survey II Mortality Study. *Atherosclerosis* 2004; 173(2): 307-314. https://doi.org/10.1016/j.atherosclerosis.2003.12.022.
2. Dandona P, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. Trends Immunol 2004; 25(1): 4-7. https://doi.org/10.1016/j.it.2003.10.013.
3. Hussain M, Rafique MA, Iqbal J, Akhtar L. Effect of sitagliptin and glimepiride on C-reactive protein (CRP) in overweight Type-2 diabetic patients. *Pak J Med Sci* 2019; 35(2): 383-387. https://doi.org/10.12669/pjms.35.2.645.
4. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. *J Clin Invest* 2006; 116(7): 1793-1801. https://doi.org/10.1172/jci29069.
5. Zeyda M, Farmer D, Todoric J, Aszmann O, Speiser M, Gyori G, et al. An adipose tissue macrophages are of an anti-inflammatory phenotype but capable of excessive pro-inflammatory mediator production. *Int J Obes (Lond)* 2007; 31(9): 1420-1428. https://doi.org/10.1038/sj.ijo.0803632.
6. Lumeng CN, DelProposto JB, Westcott DJ, Saltiel AR. Phenotypic switching of adipose tissue macrophages with obesity is generated by spatiotemporal differences in macrophage subtypes. *Diabetes* 2008; 57(12): 3239-3246. https://doi.org/10.2337/db08-0872.
7. Kahn SE, Zinman B, Haffner SM, Haffner SM, O'Neill C, Kravitz BG. Obesity is a major determinant of the association of C-reactive protein levels and the metabolic syndrome in type 2 diabetes. *Diabetes* 2006; 55(8): 2357-2364. https://doi.org/10.2337/db06-0116.
8. Miller DT, Zee RY, Suk Danik J, Kozlowski P, Chasman DI, Lazarus R, et al. Association of common CRP gene variants with CRP levels and cardiovascular events. *Ann Hum Genet* 2005; 69(Pt 6): 623-638. https://doi.org/10.1111/j.1529-8817.2005.00210.x.
9. Wang L, Lu X, Li Y, Li H, Chen S, Gu D. Functional analysis of the C-reactive protein (CRP) gene -717A>G polymorphism associated with coronary heart disease. *BMC Med Genet* 2009; 10: 73. https://doi.org/10.1186/1471-2350-10-73.
10. Wang Q, Ding H, Tang JR, Zhang L, Xu YJ, Yan JT, et al. C-reactive protein polymorphisms and genetic susceptibility to ischemic stroke and hemorrhagic stroke in the Chinese Han population. *Acta Pharmacol Sin* 2009; 30(3): 291-298. http://doi.org/10.1038/aps.2009.14.
11. Swaroop JJ, Rajarajeswari D, Naidu JN. Association of TNF-α with insulin resistance in type 2 diabetes mellitus. *Indian J Med Res* 2012; 135(1): 127-130. https://doi.org/10.4103/0971-5916.93435.
12. Hameed I, Masoodi SR, Malik PA, Mir SA, Ghazanfar K, Ganai BA. Genetic variations in key inflammatory cytokines exacerbates the risk of diabetic nephropathy by influencing the gene expression. *Gene* 2018; 661: 51-59. http://doi.org/10.1016/j.gene.2018.03.095.
13. You T, Nicklas BJ, Ding J, Penninx BW, Goodpaster BH, Bauer DC, et al. The metabolic syndrome is associated with circulating adipokines in older adults across a wide range of adiposity. *J Gerontol Ser A Biol Sci Med Sci* 2008; 63(4): 414-419. https://doi.org/10.1093/gerona/63.4.414.
14. Pyrzak B, Wiśniewska A, Rymkiewicz-Kluczyńska B. Tumor necrosis factor alpha (TNF-alpha) gene G-308A polymorphism relationship to insulin resistance and lipid abnormalities in children with obesity. *Endokrynol Diabetol Chor Przemiany Materii Wieku Rozw* 2006; 12(3): 171-174. https://pubmed.ncbi.nlm.nih.gov/17020650.
15. Ranjith N, Pegoraro RJ, Naidoo DP, Shanmugam R, Rom L. Genetic variants associated with insulin resistance and metabolic syndrome in young Asian Indians with myocardial infarction. *Metab Syndr Relat Disord* 2008; 6(3): 209-214. https://doi.org/10.1089/met.2008.0023.
16. Martínez-García MÁ, Moncayo S, Insenser M, Montes-Nieto R, Fernández-Durán E, Álvarez-Blasco F, et al. Postprandial inflammatory responses after oral glucose, lipid and protein challenges: Influence of obesity, sex and polycystic ovary syndrome. *Clin Nutr* 2020; 39(3): 876-885. http://doi.org/10.1016/j.clnu.2019.03.027.
17. Yunusbayev B, Metspalu M, Metspalu E, Valeev A, Litvinov S, Valiev R, et al. The genetic legacy of the expansion of Turkic-speaking nomads across Eurasia. *PLoS Genet* 2015; 11(4): e1005068. https://doi.org/10.1371/journal.pgen.1005068.
18. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. International Diabetes Federation Task Force on Epidemiology and Prevention, National Heart, Lung, and Blood Institute, American Heart Association, World Heart Federation, International Atherosclerosis Society, International Association for the Study of Obesity: 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(16): 1640-1645. https://doi.org/10.1161/circulationaha.109.192644.
19. Ferguson LR, Han DY, Huebner C, Petermann I, Barclay ML, Gearry RB, et al. Tumor necrosis factor receptor superfamily, member 1B haplotypes increase or decrease the risk of inflammatory bowel diseases in a New Zealand caucasian population. *Gastroenterol Res Pract* 2009; 2009: 591704. https://doi.org/10.1155/2009/591704.

20. Mokrousov I, Wu XR, Vyazovaya A, Feng WX, Sun L, Xiao J, et al. Polymorphism of 3'UTR region of TNFR2 coding gene and its role in clinical tuberculosis in Han Chinese pediatric population. *Infect Genet Evol* 2011; 11(6): 1312-1318. https://doi.org/10.1016/j.meegid.2011.04.025.
21. Xu F, Zhou G, Han S, Yuan W, Chen S, Fu Z, et al. Association of TNF-α, TNFRSF1A and TNFRSF1B gene polymorphisms with the risk of sporadic breast cancer in northeast Chinese Han women. PLoS One 2014; 9(7): e101138. https://doi.org/10.1371/journal.pone.0101138.
22. Yu Y, Zheng S, Zhang S, Jin W, Liu H, Jin M, et al. Polymorphisms of inflammation-related genes and colorectal cancer risk: a population-based case-control study in China. *Int J Immunogenet* 2014; 41(4): 289-97. http://doi.org/10.1111/iji.12119.
23. Qasem A, Ramesh S, Naser SA. Genetic polymorphisms in tumour necrosis factor receptors (TNFRSF1A/1B) illustrate differential treatment response to TNFα inhibitors in patients with Crohn's disease. *BMJ Open Gastroenterol* 2019; 6(1): e000246. https://doi.org/10.1136/bmjgast-2018-000246.
24. Feng Y, Jiang CD, Chang AM, Shi Y, Gao J, Zhu L, et al. Interactions among insulin resistance, inflammation factors, obesity-related gene polymorphisms, environmental risk factors, and diet in the development of gestational diabetes mellitus. *J Matern Fetal Neonatal Med* 2019; 32(2): 339-347. https://doi.org/10.1080/14767058.2018.1446207.
25. Kumari R, Kumar S, Ahmad MK, Singh R, Kant Kumar S, Pradhan A, et al. Promoter variants of TNF-α rs1800629 and IL-10 rs1800871 are independently associated with the susceptibility of coronary artery disease in north Indian. *Cytokine* 2018; 110: 131-136. https://doi.org/10.1016/j.cyto.2018.04.035.
26. Cadena-Sandoval D, Alemán-Ávila I, Barbosa-Cobos RE, Becerril-Mendoza LT, Fragoso JM, Ramírez-Bello J. Tumor necrosis factor (TNF) and TNFR1 polymorphisms are not risk factors for rheumatoid arthritis in a Mexican population. *Mol Biol Rep* 2018; 45(3): 227-232. https://doi.org/10.1007/s11033-018-4155-2.
27. Couceiro J, Matos I, Mendes JJ, Baptista PV, Fernandes AR, Quintas A. Inflammatory factors, genetic variants, and predisposition for preterm birth. *Clin Genet* 2021; 100(4): 357-367. https://doi.org/10.1111/cge.14001.
28. Yamada Y, Ichihara S, Nishida T. Molecular genetics of myocardial infarction. Genomic medicine 2008; 2(1-2): 7-22. https://doi.org/10.1007/s11568-008-9025-x.
29. Boraska V, Zeggini E, Groves CJ, Rayner NW, Skrabić V, Diakite M, et al. Family-based analysis of tumor necrosis factor and lymphotoxin-alpha tag polymorphisms with type 1 diabetes in the population of South Croatia. *Hum Immunol* 2009; 70(3): 195-199. http://doi.org/10.1016/j.humimm.2008.12.010.
30. Boraska V, Rayner NW, Groves CJ, Frayling TM, Diakite M, Rockett KA, et al. Large-scale association analysis of TNF/LTA gene region polymorphisms in type 2 diabetes. *BMC Med Genet* 2010; 11: 69. https://doi.org/10.1186/1471-2350-11-69.
31. Pooja S, Francis A, Bid HK, Kumar S, Rajender S, Ramalingam K, et al. Role of ethnic variations in TNF-α and TNF-β polymorphisms and risk of breast cancer in India. *Breast Cancer Res Treat* 2011; 126(3): 739-747. http://doi.org/10.1007/s10549-010-1175-6.
32. Castro-Giner F, Kogevinas M, Mächler M, de Cid R, Van Steen K, Imboden M, et al. TNFA -308G>A in two international population-based cohorts and risk of asthma. *Eur Respir J* 2008; 32(2): 350-561. https://doi.org/10.1183/09031936.00155607.
33. Xue Y, Zhang L, Fan Y, Li Q, Jiang Y, Shen C. C-reactive protein gene contributes to the genetic susceptibility of hemorrhagic stroke in men: A case-control study in Chinese Han population. *J Mol Neurosci* 2017; 62(3-4): 395-401. https://doi.org/10.1007/s12031-017-0945-6.
34. Wu Z, Huang Y, Huang J, Fan L. Impact of CRP gene and additional gene-smoking interaction on ischemic stroke in a Chinese Han population. *Neurol Res* 2017; 39(5): 442-447. http://doi.org/10.1080/01616412.2017.1297905.
35. Wang X, Fan Y, Wang L, Chen B, Lu Y, Luo D. The association between the C-reactive protein gene +1444C/T polymorphism and Parkinson's disease susceptibility in a Chinese population. *Gene* 2020; 753:144808. https://doi.org/10.1016/j.gene.2020.144808.
36. Wang Y, Wang Q, Guo C, Wang S, Wang X, An L, et al. Association between CRP gene polymorphisms and the risk of preeclampsia in Han Chinese women. *Genet Test Mol Biomarkers* 2014; 18(11): 775-780. https://doi.org/10.1089/gtmb.2014.0142.
37. Cipriani V, Hogg RE, Sofat R, Moore AT, Webster AR, Yates JR, et al. Association of C-reactive protein genetic polymorphisms with late age-related macular degeneration. *JAMA Ophthalmol* 2017; 135(9): 909-916. https://doi.org/10.1001/jamaophthalmol.2017.2191.
38. Navarro P, de Dios O, Gavela-Pérez T, Soriano-Guillen L, Garcés C. Relationship between polymorphisms in the CRP, LEP and LEPR genes and high sensitivity C-reactive protein levels in Spanish children. *Clin Chem Lab Med* 2017; 55(11): 1690-1695. https://doi.org/10.1515/cclm-2017-0134.
39. Atisha-Fregoso Y, Lima G, Carrillo-Maravilla E, Posadas-Sánchez R, Pérez-Hernández N, Baños-Peláez M, et al. C-reactive protein (CRP) polymorphisms and haplotypes are associated with SLE susceptibility and activity but not with serum CRP levels in Mexican population. *Clin Rheumatol* 2018; 37(7): 1817-1824. https://doi.org/10.1007/s10067-018-4059-5.
40. Morita A, Nakayama T, Doba N, Hinohara S, Soma M. Polymorphism of the C-reactive protein (CRP) gene is related to serum CRP Level and arterial pulse wave velocity in healthy elderly Japanese. *Hypertens Res* 2006; 29(5): 323-331. https://doi.org/10.1291/hypres.29.323.
41. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005; 21(2): 263-265. https://doi.org/10.1093/bioinformatics/bth457.
42. Karakas Celik S, Cakmak Genc G, Dursun A. A bioinformatic approach to investigating cytokine genes and their receptor variants in relation to COVID-19 progression. *Int J Immunogenet* 2021; 48(2): 211-218. https://doi.org/10.1111/iji.12522.
43. Mokrousov I, Wu XR, Vyazovaya A, Feng WX, Sun L, Xiao J, et al. Polymorphism of 3'UTR region of TNFR2 coding gene and its role in clinical tuberculosis in Han Chinese pediatric population. *Infect Genet Evol* 2011; 116: 1312-1318. https://doi.org/10.1016/j.meegid.2011.04.025.
44. Moskalenko MI, Ponomarenko IV, Milanova SN, Verzilina IN, Efremova OA, Polonikov AV. Polymorphic locus rs1061624 of the TNFR2 gene is associated with the development of arterial hypertension in males. *Kardiologiia* 2020; 60(8): 78-83. Russian. https://doi.org/10.18087/cardio.2020.8.n996.
45. Qasem A, Ramesh S, Naser SA. Genetic polymorphisms in tumour necrosis factor receptors (TNFRSF1A/1B) illustrate differential treatment response to TNFα inhibitors in patients with Crohn's disease. *BMJ Open Gastroenterol* 2019; 6(1): e000246. https://doi.org/10.1136/bmjgast-2018-000246.
46. Stacey D, Redlich R, Büschel A, Opel N, Grotegerd D, Zaremba D, et al. TNF receptors 1 and 2 exert distinct region-specific effects on striatal and hippocampal grey matter volumes (VBM) in healthy adults. *Genes Brain Behav* 2017; 16(3): 352-360. https://doi.org/10.1111/gbb.12318.
47. Sookoian SC, González C, Pirola CJ. Meta-analysis on the G-308A tumor necrosis factor alpha gene variant and phenotypes associated with the metabolic syndrome. *Obes Res* 2005; 13(12): 2122-2131. https://doi.org/10.1038/oby.2005.263.
48. Ribeiro CM, Oliveira SR, Alfieri DF, Flauzino T, Kaimen-Maciel DR, Maes M, et al. Tumor necrosis factor alpha (TNF-α) and its soluble receptors are associated with disability, disability progression and clinical forms of multiple sclerosis. *Inflamm Res* 2019; 68(12): 1049-1059. https://doi.org/10.1007/s00011-019-01286-0.
49. Boraska V, Rayner NW, Groves CJ, Frayling TM, Diakite M, Rockett KA, et al. Large-scale association analysis of TNF/LTA gene region polymorphisms in type 2 diabetes. *BMC Med Genet* 2010; 11: 69. https://doi.org/10.1186/1471-2350-11-69.

50. Szkup M, Chełmecka E, Lubkowska A, Owczarek AJ, Grochans E. The influence of the TNFα rs1800629 polymorphism on some inflammatory biomarkers in 45-60-year-old women with metabolic syndrome. *Aging (Albany NY)* 2018; 10(10): 2935-2943. https://doi.org/10.18632/aging.101600.
51. de Luis DA, Aller R, Izaola O, González Sagrado M, Conde R, Romero E. Influence of G308A polymorphism of tumor necrosis factor alpha gene on insulin resistance in obese patients after weight loss. *Med Clin (Barc)* 2007; 129(11): 401-404. https://doi.org/10.1157/13110463.
52. Yaribeygi H, Atkin SL, Pirro M, Sahebkar A. A review of the anti-inflammatory properties of antidiabetic agents providing protective effects against vascular complications in diabetes. *J Cell Physiol* 2019; 234(6): 8286-8294. https://doi.org/10.1002/jcp.27699.
53. Martínez-Calleja A, Quiróz-Vargas I, Parra-Rojas I, Muñoz-Valle JF. Haplotypes in the CRP Gene Associated with Increased BMI and Levels of CRP in Subjects with Type 2 Diabetes or Obesity from Southwestern Mexico. *Exp Diabetes Res* 2012; 2012: 982683. https://doi.org/10.1155/2012/982683.
54. Kathiresan S, Larson MG, Vasan RS, Guo CY, Gona P, Keaney Jr, et al. Contribution of clinical correlates and 13 C-reactive protein gene polymorphisms to interindividual variability in serum C-reactive protein level. *Circulation* 2006; 113(11): 1415-1423. https://doi.org/10.1161/circulationaha.105.591271.
55. Sheu WH, Wang WC, Wu KD, He CT, Hwu CM, Quertermous T, et al. CRP-level-associated polymorphism rs1205 within the CRP gene is associated with 2-hour glucose level: The SAPPHIRe study. *Sci Rep* 2017; 7(1): 7987. https://doi.org/10.1038/s41598-017-08696-2.
56. Eiriksdottir G, Smith AV, Aspelund T, Hafsteinsdottir SH, Olafsdottir E, Launer LJ, et al. The interaction of adiposity with the CRP gene affects CRP levels: age, gene/environment susceptibilty-Reykjavik study. *Int J Obes (Lond)* 2009; 33(2): 267-272. https://doi.org/10.1038/ijo.2008.274.

Authors:
Olga V. Kochetova – PhD, Associate Professor, Institute of Biochemistry and Genetics, Ufa Federal Research Center of the Russian Academy of Sciences, Ufa, Russia. http://orcid.org/0000-0003-2071-0969.
Diana S. Avzaletdinova – PhD, Associate Professor, Bashkir State Medical University, Ufa, Russia. http://orcid.org/0000-0002-1590-6433.
Gulnaz F. Korytina – PhD, Professor, Institute of Biochemistry and Genetics, Ufa Federal Research Center of the Russian Academy of Sciences; Bashkir State Medical University, Ufa, Russia. http://orcid.org/0000-0002-1695-5173.