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HUMAN GENETICS

An Analysis of the Associations of Polymorphic Variants of the *LEPR* (rs1137100), *LRP5* (rs3736228), and *LPL* (rs320) Genes with the Risk of Developing Type 2 Diabetes Mellitus

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Abstract—Diabetes mellitus is a hereditary predisposed multifactorial disease. However, the genetic mechanisms of its development have not been fully revealed yet. We conducted a search for associations of polymorphic variants of the *LEPR* (rs1137100), *LRP5* (rs3736228), and *LPL* (rs320) genes involved in the development of obesity with the development of type 2 diabetes mellitus. The association with development of the disease was established for the *T* allele of the *LRP5* locus (rs3736228) ($p = 0.029$, OR = 1.46). The rs1137100 locus ($p = 0.032$) of the *LEPR* gene was shown to be associated with the body mass index (BMI), but it was not connected with the presence of type 2 diabetes mellitus. Risk markers of development of type 2 diabetes included the *T* allele of the rs3736228 locus of the *LRP5* gene (OR = 1.74, $p = 0.012$) and the *G* allele of the rs320 locus of the *LPL* gene (OR = 1.39, $p = 0.027$). Statistically significant association was only found in the group of nonobese patients. A decrease in the level of low-density lipoprotein was observed in individuals with the *TT* genotype of the *LPL* locus (rs320) ($p = 0.04$). Individuals with the *GT* and *GG* genotypes of this locus had a lower cholesterol level ($p = 0.027$). A decrease in the level of BMI ($p = 0.012$) and a decrease in the concentration of triglycerides in the blood ($p = 0.00000004$) were detected in carriers of the *CC* genotype of the *LRP5* rs3736228 locus.

Keywords: diabetes mellitus 2, lipoprotein lipase, leptin receptor, Wnt signaling gene, polymorphic marker
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INTRODUCTION

In recent years, the incidence of type 2 diabetes mellitus (T2DM) has increased in almost all countries of the world. According to experts of the World Health Organization, there are 180 million patients with type 2 diabetes in the world now, and it is predicted that by 2025 their number will reach 330 million. The growth of this pathology is also noted in Russia. The prevalence, early disability of patients, and high mortality from complications determine the relevance of research [1]. It is believed that obesity leads to the formation of T2DM. Recently, the molecular genetic mechanisms of the etiopathogenesis of T2DM have been the subject of large-scale research worldwide [2].

The gene candidate analyses conducted revealed several genes associated with T2DM and quantitative parameters that determine type 2 diabetes. One of these genes is the *LEPR* gene, which encodes leptin receptors. Leptin is a hormone produced by adipocytes and several other tissues, such as the mucous membrane of the stomach. It acts as a signaling molecule that is involved in the regulation of body weight. Reaching the brain, leptin affects the hypothalamic receptors, leading to a decrease in appetite, stimulating energy intake and loss of body weight [3]. Leptin performs its function by binding to leptin receptors belonging to the family of cytokine receptors [4]. These receptors are located mainly in the hypothalamus. They can also be found in the tissues and cells that regulate glucose homeostasis, for example, in the beta cells of the pancreas. In this case, leptin receptors affect the inhibition of insulin secretion mediated by leptin. Obviously, the *LEPR* gene is a candidate gene for the estimation of the risk of T2DM development [5].

The *LRP5* gene encodes a protein related to low density lipoprotein (LDL) receptors, as well as being a receptor for the Wnt signaling pathway. LRP5 is responsible for the growth and development of cells and is considered a key regulator of tissue development and homeostasis. In addition, it is known that the Wnt signaling pathway plays a key role in the regulation of

Abbreviations used: T2DM—type 2 diabetes mellitus, BMI—body mass index.

Table 1. Characteristics of the samples examined

Parameter	Control group	T2DM patients
N	*N* = 444	*N* = 486
Men, % (*N*)	40.5 (180)	32.6 (158)
Women, % (*N*)	59.5 (262)	67.4 (328)
Age, years	56.3	60.9
Weight, kg	78.9	80.4
Height, cm	173.2	161.6
BMI, kg/m^2	25.4	30.9
Systolic pressure, mmHg	120.4	142.6
Diastolic pressure, mmHg	80.2 ± 7.8	105.1 ± 11.6
Blood glucose on an empty stomach, mmol/L	4.9 ± 0.8	7.1 ± 1.9
Cholesterol, mmol/L	5.01 ± 9.6	5.44 ± 1.13
Triglycerides, mmol/L	1.4 ± 0.5	1.7 ± 1.3
High density lipoproteins (HDL), mmol/L	2.9 ± 1.01	3.1 ± 1.4
Low density lipoproteins (LDL), mmol/L	1.08 ± 0.3	1.2 ± 0.5
HbA1c, %	4.8 ± 0.6	6.7 ± 1.3
C-peptide, ng/mL	2.26 ± 0.9	2.3 ± 1.2

β-cell function of the pancreas [6]. The *LRP5* gene is given considerable attention in the study of osteoporosis. However, a few researchers have shown the role of this gene in the development of predisposition to T2DM. It has been established that the Wnt/LRP5 signaling pathway is a link between adipogenesis and osteogenesis [7]. Studies conducted on model animals have shown that LRP5 affects cholesterol and glucose metabolism. It is shown that polymorphic variants of this gene are associated with the risk of obesity and dyslipidemia [7]. Obesity, in turn, is associated with the development of insulin resistance and is involved in the T2DM pathogenesis. Studies on this gene in T2DM are scarce.

LPL cleaves the triglycerides of the largest and lipid-rich plasma lipoproteins (chylomicrons) and very low density lipoproteins into free fatty acids and monoacylglycerol [8]. LPL deficiency leads to the development of atherosclerosis, obesity, dyslipidemia, insulin resistance, and T2DM. In T2DM, LPL activity is usually insufficient and contributes to an increase in serum triglyceride levels. The various polymorphic variants found in the *LPL* gene reduce or increase the activity of the enzyme, thereby changing the level of cholesterol and lipoproteins. The polymorphic rs320 locus of the *LPL* gene is known to be associated with triglyceride and high-density lipoprotein levels and total plasma cholesterol concentration [9]. The interrelation of polymorphic variants of the *LPL* gene with insulin resistance has been shown [10].

Thus, according to the analysis of published data, a possible association of polymorphic variants of the *LEPR* (rs1137100), *LRP5* (rs3736228), and *LPL* (rs320) genes with the risk of obesity and quantitative parameters characterizing T2DM was established [7, 10]. However, the results obtained by researchers in different populations are quite contradictory. This may be due to differences in the distribution patterns of the genotypes and alleles in populations of the world. Our study included subjects of Tatar ethnicity living in the Republic of Bashkortostan. Earlier studies on the gene pool of the population of Tatars showed that they have haplogroups characteristic of the peoples of Western Eurasia in 70% of cases. The proportion of the eastern component accounts for about 30–20% of haplogroups [11].

The aim of this study was to identify the association of polymorphic variants of the leptin receptor gene *LEPR* (rs1137100), the *LRP5* gene (rs3736228), and the lipoprotein lipase *LPL* (rs320) gene with the development of type 2 diabetes in the population of Tatars.

MATERIALS AND METHODS

We studied the DNA samples of 930 unrelated individuals, Tatars by ethnicity, living in the territory of the Republic of Bashkortostan. Among these, 486 patients had type 2 diabetes and 444 patients had no clinical or laboratory signs of diabetes. The samples are described in Table 1.

Genotyping. DNA was isolated from peripheral blood leukocytes using phenol-chloroform purification. Polymorphic loci of the *LEPR* (rs1137100), *LRP5* (rs3736228), and *LPL* (rs320) genes were studied using PCR with subsequent cleavage of the product with the appropriate restrictases *Hae*III, *Dra*III, and *Hin*dIII. The conditions of PCR and the sequences of primers are presented in Table 2.

Table 2. Polymorphic markers included in the study, their localization, nucleotide sequences of primers, restriction nuclease and alleles

Gene	Polymorphism, localization	Primers, restrictase	Allele, fragments sizes, bp
LEPR	c.326A>G rs1137100 Lys109Arg exon 2	F: 5'-TTTCCACTGTTGCTTTCGGA-3' R: 5'-AAACTAAAGAATTTACTGTTGAAACAAATGGC-3' *Hae*III	*A*—100, *G*—70, 30
LRP5	c.2246C>T rs3736228 p.Ala749Val exon 18	F: 5'-GACTGTCAGGACCGCTCACACG-3' R: 5'-AAGGTTTTCAGAGCCCCTAC-3' *Dra*III	*C*—143, *T*—119
LPL	g.27496T>G intron 8	F: 5'-AGATGCTACCTGGATAATCAAAG-3' R: 5'-AATTTGTCAATCCTAACTTAGAG-3' *Hin*dIII	*T*—139, 90, *G*—229

The results of amplification and restriction were analyzed using vertical electrophoresis in a 6–8% polyacrylamide gel. The gel was stained with ethidium bromide solution (0.1 μg/mL) for 15 min and photographed in transmitted ultraviolet light. A 100 bp molecular weight marker was used to determine the product size (SibEnzyme, Russia).

Statistical processing of results. Statistical data processing was performed using the application software packages of Statistica v. 6.0 (StatSoft Inc., United States) and PLINK v. 1.07 [12]. The association between the polymorphic variants of the studied genes and obesity was assessed using the Pearson χ^2 criterion. The groups of patients with type 2 diabetes and the individuals in the control group were compared in pairs. The frequencies of alleles and genotypes and the correspondence of the distribution of genotype frequencies to Hardy–Weinberg equilibrium ($\chi^2$and p) were calculated. Logistic regression was used to identify the association of polymorphic variants of the studied genes with the development of T2DM. The exponent of a separate regression coefficient (beta) was interpreted as an odds ratio (OR) with a 95% confidence interval calculated.

The contribution of allelic variants of the studied candidate genes to the variability of quantitative clinical and biochemical parameters (glucose, lipids, etc.) was determined using the Kruskal–Wallis test (in the case of three groups) or Mann–Whitney test (in the case of two groups); the calculations were performed in Statistica v. 6.0 (StatSoft Inc., United States) [13].

RESULTS

At the first stage of work, we checked the correspondence of the distribution of frequencies of genotypes of polymorphic loci to the Hardy–Weinberg equilibrium; we also evaluated the minor allele frequency (MAF) in both patients and control samples. The following results were obtained upon study of the control group: *LEPR* (rs1137100) (p = 0.82, MAF = 0.306), *LRP5* (rs3736228) (p = 0.34, MAF = 0.081), *LPL* (rs320) (p = 0.89, MAF = 0.226). The resulting frequency distribution corresponds to the minor allele frequency in the HapMap-CEU population (MAF = 0.338, MAF = 0.138, MAF = 0.270, respectively).

Table 3 presents data on the distribution of frequencies of genotypes and alleles of the studied loci, the significance of differences between groups of patients with type 2 diabetes, and also the odds ratios calculated for the minor allele of each locus. Statistically significant differences between the studied groups were detected for the polymorphic rs3736228 locus of the *LRP5* gene among patients with type 2 diabetes (p = 0.029).

Table 4 shows statistically significant results of the analysis of the association with the T2DM development (with the determination of the regression coefficient, beta, the exponent of which was interpreted as OR for the logistic model with a 95% confidence interval (95% CI)) and the results of the analysis of the significance level taking into account age and body mass index (BMI) in various models.

An association of the minor *T* allele of the *LRP5* locus (rs3736228) with the development of T2DM (p = 0.029, OR = 1.46) was discovered. In this case, the additive model is more informative (p = 0.02, OR = 1.43). It shows that each dose of the minor *T* allele increases the risk of disease.

At the trend level, an association of the *LPL* locus (rs320) was revealed owing to the increase in the frequency of the minor *G* allele in the group of patients to 26.65% compared to 22.64% in the control group (p = 0.051, OR = 1.24). The additive model (p = 0.046, OR = 1.24) indicating that each dose of the minor *G* allele increases the risk of disease is more informative.

Table 3. Distribution of frequencies of polymorphic variants of genes *LRP5*, *LEPR*, and *LPL* in the group of patients with T2DM and the control group

Gene, polymorphism	Genotypes, alleles	Chromosome	T2DM ($N = 486$) %/N	Control ($N = 444$) %/N	p (χ^2 for genotypes)	p (χ^2 for alleles)
LRP5, rs3736228 c.2246C>T p.Ala749Val	*CC*	11:68433827	78.40/381	84.01/373	0.06	0.029
	CT		20.78/101	15.7/770		
	TT		0.82/4	0.23/1		
	C		88.79/863	91.89/816		
	T		11.21/109	8.11/72		
LEPR, rs1137100 c.326A>G p.Lys109Arg	*AA*	1:65570758	44.44/216	47.75/212	0.13	0.88
	AG		48.97/238	43.24/192		
	GG		6.58/32	9.01/40		
	G		68.93/670	69.37/616		
	A		31.07/302	30.63/272		
LPL, rs320 g.27496T>G	*TT*	8:19961566	54.32/264	59.68/265	0.12	0.051
	GT		38.07/185	35.36/157		
	GG		7.61/37	4.95/22		
	T		73.35/713	77.36/687		
	G		26.65/259	22.64/201		

Table 4. The results of the analysis of the association of polymorphic loci of the *LRP5*, *LEPR*, and *LPL* genes in the group of patients with T2DM and the control group

Gene	Polymorphism	Minor allele	Genotype/model	T2DM $N = 486$ N (%)	Control $N = 444$ N (%)	OR (CI 95%), p
LRP5	rs3736228	*T*	*CC* *TC-TT* Dominant	381 (78.3) 105 (21.6)	373 (84) 71 (16)	1.45 (1.04–2.03) 0.027
			CC-TC *TT* Recessive	482 (99.2) 4 (0.8)	443 (99.8) 1 (0.2)	3.68 (0.41–32.98) 0.2
			Log-additive	–	–	1.46 (1.06–2.01) 0.02
LEPR	rs1137100	*A*	*AA* *GA-GG* Dominant	216 (44.4) 270 (55.6)	212 (47.9) 231 (52.1)	1.15 (0.89–1.49) 0.3
			AA-GA *GG* Recessive	454 (93.4) 32 (6.6)	403 (91) 40 (9)	0.71 (0.44–1.15) 0.16
			Log-additive	–	–	1.02 (0.83–1.26) 0.81
LPL	rs320	*G*	*TT* *GT-GG* Dominant	264 (54.3) 222 (45.7)	265 (59.7) 179 (40.3)	1.24 (0.96–1.62) 0.099
			TT-GT *GG* Recessive	449 (92.4) 37 (7.6)	422 (95) 22 (5)	1.58 (0.92–2.72) 0.094
			Log-additive	–	–	1.24 (1.00–1.53) 0.046

Table 5. Analysis of the association of polymorphic loci of the *LRP5*, *LEPR*, and *LPL* genes with the development of T2DM in groups differentiated by the level of BMI

Group	Gene, polymorphic locus	Minor allele	Genotype or model	*p*	OR (CI 95%)
Obesity T2DM	*LRP5* rs3736228	*T*	Log-additive *CC* (0) *TC* (1) *TT* (2)	0.19	1.31 (0.87–1.97)
	LEPR rs1137100	*A*	Log-additive *AA* (0) *GA* (1) *GG* (2)	0.84	0.97 (0.76–1.25)
	LPL rs320	*G*	Log-additive *TT* (0) *GT* (1) *GG* (2)	0.12	1.23 (0.94–1.60)
Norm T2DM	*LRP5* rs3736228	*T*	Log-additive *CC* (0) *TC* (1) *TT* (2)	0.012	1.74 (1.12–2.68)
	LEPR rs1137100	*A*	Log-additive *AA* (0) *GA* (1) *GG* (2)	0.55	0.92 (0.70–1.21)
	LPL rs320	*G*	Log-additive *TT* (0) *GT* (1) *GG* (2)	0.027	1.39 (1.04–1.85)

Analysis of the Impact of BMI and Gender Differences on T2DM Development

When analyzing the selected genes, the relationship between the BMI and T2DM was shown only for the *LEPR* locus (rs1137100) ($p = 0.032$). This locus had a statistically significant association only with the BMI level and was not associated with the development of T2DM. Analysis of the quantitative indicator of BMI depending on the allelic variants of this locus showed a significant association with the level of BMI in the group of obese patients and patients with a normal BMI level. The association in the recessive model ($p = 0.004$) indicating the protective effect of the *GG* variant in the formation of obesity is more informative. No significant interactions were shown for other loci.

An analysis of the interactions of genotypes with external factors (BMI level and gender) was also carried out by comparing the odds ratio (OR) values calculated for individual genotypes in groups differentiated by the level of BMI and gender. The association for the rs3736228 locus of the *LRP5* gene is shown in a BMI-differentiated analysis. The association with the development of T2DM was detected only in the group of patients with T2DM with normal body weight (OR = 1.74, $p = 0.012$). No association was found in the group of patients with obesity (OR = 1.31, $p = 0.19$). In the group of patients with a normal BMI level, an association was identified for the *LPL* locus (rs320) (OR = 1.39, $p = 0.027$) (Table 5).

An assessment of gender differences revealed no statistically significant variations.

Analysis of Association of Polymorphic Loci of the Studied Genes with the Quantitative Signs of Metabolic Disorders

No associations of polymorphic loci *LEPR* (rs1137100), *LRP5* (rs3736228), and *LPL* (rs320) with biochemical and anthropometric parameters were detected in the control group. Further analysis was carried out in the group of patients. No associations of the polymorphic rs1137100 locus of the *LEPR* gene with biochemical parameters in patients with type 2 diabetes were detected either. An analysis of the association of quantitative parameters with the polymorphic variants of the genes studied is presented in Table 6.

The association with the BMI level was detected for the rs1137100 locus of the *LEPR* gene ($p = 0.0067$). It was found that carriers of the *GG* genotype had a lowered BMI level, reaching 26.9 kg/m^2, unlike carriers of the *AA* and *AG* genotypes, having a BMI of 28.9 kg/m^2.

An analysis of quantitative anthropometric indicators revealed an association of the *LRP5* gene rs3736228 locus with the risk of developing obesity. In carriers of the *CC* genotype, the BMI level was 28.53 kg/m^2, and in subjects with the *TC* and *TT* genotypes, it was 29.9 kg/m^2 ($p = 0.012$). An association of the *LRP5* gene rs3736228 polymorphic locus with the level of triglycerides (TG) was detected. It was shown that the carriers of the *CC* genotype had a TG level of 1.52 mmol/L, while the carriers of the *CT* and *TT* genotypes had their TG level at 2.32 mmol/L ($p = 0.00000004$).

It was shown that carriers of the *TT* genotype of the rs320 locus of the *LPL* gene had a reduced level of low density lipoprotein (2.84 mmol/L), while in individuals with the genotypes *GT* and *GG* the LDL level reached 3.24 mmol/L ($p = 0.04$). A statistically significant variability of total cholesterol was found depend-

Table 6. The association of polymorphic variants of the *LRP5*, *LEPR*, and *LPL* genes with clinical and biochemical parameters of T2DM

Parameters	*LRP5* rs3736228 *M* (SE)			*p*	*LEPR* rs1137100 *M* (SE)			*p*	*LPL* rs320 *M* (SE)			*p*
	CC	*TC*	*TT*		*AA*	*GA*	*GG*		*TT*	*GT*	*GG*	
T2DM debut age, years	54.94 (0.77)	57 (1.66)	46 (6)	0.21	54.42 (0.99)	55.7 (1.05)	57.5 (2.41)	0.45	59.76 (2.25)		54.81 (0.72)	0.04
Weight, kg	80.04 (1.14)	85.38 (3.28)	84 (12)	0.17	82.04 (1.6)	80.08 (1.66)	81.71 (4.35)	0.69	82.08 (1.54)	80.9 (1.83)	75.47 (3)	0.27
BMI, kg/m^2	28.53 (0.23)	29.9 (0.48)		**0.012**	28.94 (0.21)		26.95 (0.74)	**0.0067**	28.53 (0.26)	29.09 (0.33)		0.18
WS, cm	98.64 (0.96)	101.38 (2.42)	99 (10)	0.5	99.22 (1.16)	99.36 (1.62)	98 (2.94)	0.92	99.22 (1.22)	98.8 (1.41)	101.21 (3.37)	0.8
Glucose on an empty stomach, mmol/L	7.21 (0.16)	7.42 (0.36)	7.35 (1.05)	0.85	7.17 (0.18)	7.43 (0.24)	6.54 (0.3)	0.27	7.4 (0.2)	7.15 (0.25)	6.65 (0.23)	0.32
HbA1c, %	7.51 (0.09)	7.38 (0.08)	7.25 (0.35)	0.73	7.39 (0.08)	7.63 (0.13)	7.16 (0.1)	0.12	7.49 (0.1)	7.54 (0.13)	7.25 (0.11)	0.58
C-peptide, ng/mL	2.31 (0.09)	2.17 (0.24)	1.11 (0.35)	0.34	2.18 (0.1)	2.36 (0.14)	2.26 (0.51)	0.62	2.22 (0.11)	2.28 (0.13)	2.53 (0.46)	0.63
Cholesterol, mmol/L	5.44 (0.09)	5.43 (0.21)	5 (1.1)	0.87	5.46 (0.11)	5.4 (0.13)	5.44 (0.18)	0.94	5.19 (0.1)	5.57 (0.11)		0.027
TG, mmol/L	1.52 (0.07)	2.32 (0.39)		**0.00000004**	1.72 (0.15)	1.63 (0.14)	1.64 (0.19)	0.89	1.65 (0.1)	1.72 (0.17)	1.65 (0.22)	0.94
LDL, mmol/L	3.04 (0.11)	3.18 (0.24)	2.49 (0.71)	0.73	3.13 (0.14)	3.07 (0.15)	2.5 (0.3)	0.29	2.84 (0.14)	3.24 (0.14)		**0.047**
HDL, mmol/L	1.18 (0.04)	1.31 (0.08)	1.23 (0.6)	0.42	1.19 (0.05)	1.22 (0.05)	1.2 (0.16)	0.91	1.26 (0.06)	1.13 (0.05)	1.2 (0.07)	0.29

M (SE)—Mean values and standard error of the mean, *p*—significance level for the Mann–Whitney or Kruskal–Wallis tests, WS—waist size, HbA1c—glycated hemoglobin, TG—triglycerides, HDL—high density lipoproteins, LDL—low density lipoproteins.

ing on the genotype of the rs320 locus of the *LPL* gene. A decrease in cholesterol level to 5.19 mmol/L was observed in individuals with the *GT* and *GG* genotypes compared to the *TT* genotype. The carriers of this genotype had cholesterol values of 5.57 mmol/L ($p = 0.047$). Statistically significant differences in the age of onset of the disease depending on the genotypes of this locus were shown. In carriers of the *TT* and *GT* genotypes, diabetes was manifested earlier (on average at the age of 54.8 years) as compared with patients with the *GG* genotype (59.76 years, $p = 0.04$).

DISCUSSION

As a result of the study, the association of polymorphic loci of the *LRP5* (rs3736228) and *LPL* (rs320) genes with the development of T2DM and quantitative parameters characterizing T2DM in the population of Tatars was confirmed. These data are consistent with data from other authors for various populations of the world, for example, in the Chinese [14] and Spaniards [15]. The most significant association was obtained by analyzing the level of triglycerides. High levels of triglycerides can cause T2DM and diseases of the heart and blood vessels. A number of authors showed the relationship of polymorphic variants of the *LRP5* gene with plasma cholesterol levels in model animals [16]. The involvement of the Wnt/LRP5 signaling pathway in adipogenesis, insulin secretion, and glucose tolerance was also shown. Turning off the *LRP5* gene in model mice fed a high fat diet led to an increase in plasma cholesterol levels. When keeping mice with *LRP5* deficiency on a normal diet, a marked impairment of glucose tolerance was observed, with a marked decrease in intracellular ATP and Ca^{2+} in response to glucose administration and deterioration of insulin-dependent secretion. It was shown that LRP5-deficient pancreatic islets lacked Wnt stimulated insulin secretion. These data suggest that Wnt-LRP5 signaling promotes glucose-induced insulin secretion in β-islands [16]. The Wnt/LRP5 signaling pathway is involved in the metabolism of fatty acids, as was shown in model animals. Magoori et al. showed that LRP5 activity modulates plasma triglyceride levels [17]. The rs3736228T polymorphic variant of the *LPR5* gene causes the loss of the functions of this protein, which in turn leads to a change in lipid metabolism [18]. In this regard, polymorphic variants of the *LRP5* gene are considered as potential risk factors in the development of metabolic disorders [16, 17].

Then we showed the association of the rs320 polymorphic locus of the *LPL* gene with levels of blood plasma lipids. Since this polymorphism occurs in intron, the association is connected not with this polymorphic variant, but with the polymorphic locus of exon 9 (Ser447Ter) that is fully linked with it [19]. It is believed that the rs320 locus of the *LPL* gene leads to a decrease in enzyme activity. This polymorphic marker caused a discussion in contemporary scientific literature. A number of studies showed an association with the risk of developing metabolic disorders with the minor *G* allele [20, 21], whereas, according to other authors, the risk allele was the *T* allele [22]. According to Munshi et al., no association with the rs320 locus of the *LPL* gene was shown in the Indian population [23]. Tao He et al. have suggested that the *G* allele exerts a protective role in the populations of Asia, but not Europe, since Europeans have a reverse relationship with the *G* allele [22, 24].

We have shown that the variant of the *G* allele is a risk factor for the development of T2DM in individuals with normal body weight. The relationship of this variant with elevated levels of cholesterol and LDL has also been shown. These data are consistent with the results obtained by others such as Bushueva et al. [24] in the Russian population, Javorský et al. [20] in the Slovak population, and Vardarl et al. [21] in the Turkish population. They showed that the *GG* genotype of the rs320 locus of the *LPL* gene is a marker for the development of T2DM and dyslipidemia [20–24]. No possible relationship with gender was identified.

The leptin receptor (LEPR) is known to play a key role in controlling body weight, energy metabolism, and insulin sensitivity. We analyzed the associations of *LEPR* genetic variants with the development of T2DM. Our study established the relationship of polymorphic variants of the *LEPR* rs1137100 locus with the body mass index. It was shown that the *GG* genotype is a protective marker of obesity but is not related to the development of T2DM. A large meta-analysis by Yang and Niu on the K109R locus (rs1137100) also showed the absence of a statistically significant relationship between the polymorphic variants of this marker and the risk of developing T2DM in different populations around the world [25]. According to GWAS, the *G* allele causes a decrease in the level of the soluble form of the leptin receptor (sOb-R) [26]. According to various studies, there is an inverse relationship between the receptor level in serum and body weight, as well as the development of obesity. On the other hand, Dolgushena et al. showed that elevated leptin levels were detected in Russian carriers of the *G* allele of the K109R locus [27]. The association of the *LEPR* rs1137100 locus for allele *A* with obesity was shown by Rosmond et al. for Swedes and by Okada et al. for Mexicans [28, 29]. Other authors found no statistically significant differences concerning this locus in obesity [30]. The obtained contradictory results indicate the presence of environmental factors (for example, gender) and interpopulation differences in the frequency distribution of the polymorphic locus of the *LEPR* gene.

We identified an association of polymorphic variants of the Wnt signaling gene (*LRP5*) and the lipoprotein lipase gene (*LPL*) with the development of T2DM in the population of Tatars. The presented association analysis confirms the role of these genes in

the development of type 2 diabetes. It has been shown that polymorphic variants of the *LEPR* gene (rs1137100) are associated only with obesity but are not markers for T2DM. Pathogenetically significant interactions of the studied markers with lipid metabolism in patients were determined. The results obtained are essential for understanding the molecular mechanisms of the development of T2DM.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement of compliance with standards of research involving humans as subjects. The study was approved by the Ethics Committee of the Institute of Biochemistry and Genetics. All participants in the study gave their informed voluntary consent to the use of the biological material.

REFERENCES

1. Dedov, I.I., Shestakova, M.V., and Suntsov, Yu.I., *Sakharnyi diabet v Rossii: problemy i resheniya* (Diabetes in Russia: Problems and Solutions), Moscow, 2008.
2. Belousova, O.N., Sirotina, S.S., Yakunchenko, T.I., and Zhernakova, N.I., Molecular and genetic mechanisms of the pathogenesis of type 2 diabetes, *Nauch. Vedomosti Belgorod. Gos. Univ., Ser. Med. Farm.*, 2015, vol. 31, no. 16, p. 213.
3. Oswal, A. and Yeo, G., Leptin and the control of body weight: a review of its diverse central targets, signaling mechanisms, and role in the pathogenesis of obesity, *Obesity*, 2010, vol. 18, no. 2, pp. 221—229. doi 10.1038/oby.2009.228
4. Winick, J.D., Stoffel, M., and Friedman, J.M., Identification of microsatellite markers linked to the human leptin receptor gene on chromosome 1, *Genomics*, 1996, vol. 36, no. 1, pp. 221—222. doi 10.1006/geno.1996.0455
5. Emilsson, V., Liu, Y.L., and Cawthorne, M.A., Expression of the functional leptin receptor mRNA in pancreatic islets and direct inhibitory action of leptin on insulin secretion, *Diabetes*, 1997, vol. 46, no. 2, pp. 313—316. https://doi.org/.10.2337/diab.46.2.313
6. Foer, D., Zhu, M., Cardone, R.L., et al., Impact of gain-of-function mutations in the low-density lipoprotein receptor-related protein 5 (LRP5) on glucose and lipid homeostasis, *Osteoporosis Int.*, 2017, vol. 28, no. 6, pp. 2011—2017. doi 10.1007/s00198-017-3977-4
7. Guo, Y.F., Xiong, D.H., Shen, H., Zhao, L.J., et al., Polymorphisms of the low-density lipoprotein receptor-related protein 5 (LRP5) gene are associated with obesity phenotypes in a large family-based association study, *J. Med. Genet.*, 2006, vol. 43, no. 10, pp. 798—803. doi 10.1590/S0100-879X2000001100006
8. Cho, Y.S., Go, M.J., Han, H.R., et al., Association of lipoprotein lipase (LPL) single nucleotide polymorphisms with type 2 diabetes mellitus, *Exp. Mol. Med.*, 2008, vol. 40, no. 5, p. 523. doi 10.3858/emm.2008.40.5.523
9. Ahn, Y.I., Kamboh, M.I., Hamman, R.F., et al., Two DNA polymorphisms in the lipoprotein lipase gene and their associations with factors related to cardiovascular disease, *J. Lipid Res.*, 1993, vol. 34, no. 3, pp. 421—428.
10. Goodarzi, M.O., Guo, X., Taylor, K.D., et al., Lipoprotein lipase is a gene for insulin resistance in Mexican Americans, *Diabetes*, 2004, vol. 53, no. 1, pp. 214—220. https://doi.org/.10.2337/diabetes.53.1.214
11. Trofimova, N.V., Litvinov, S.S., Khusainova, R.I., et al., Genetic characteristic of the Volga Tatars based on uniparental markers, *Izv. Samar. Nauchn. Tsentra Ross. Akad. Nauk*, 2013, no. 15, pp. 3—5.
12. Purcell, S., Neale, B., Todd-Brown, K., et al., PLINK: a toolset for whole genome association and population based linkage analysis, *Am. J. Hum. Genet.*, 2007, vol. 81, no. 3, pp. 559—575. doi 10.1086/519795
13. Statistica v. 6.0 Program, StatSoft. http:// www.statistica.com.
14. Xuan, M., Wang, Y., Wang, W., et al., Association of LRP5 gene polymorphism with type 2 diabetes mellitus and osteoporosis in postmenopausal women, *Int. J. Clin. Exp. Med.*, 2014, vol. 7, no. 1, p. 247.
15. Ariza, M.J. Sánchez-Chaparro, M.Á., Barón, F.J., et al., Additive effects of LPL, APOA5 and APOE variant combinations on triglyceride levels and hypertriglyceridemia: results of the ICARIA genetic sub-study, *BMC Med. Genet.*, 2010, vol. 11, no. 1, p. 66.
16. Fujino, T., Asaba, H., Kang, M.J., et al., Low-density lipoprotein receptor-related protein 5 (LRP5) is essential for normal cholesterol metabolism and glucose-induced insulin secretion, *Proc. Natl. Acad. Sci. U.S.A.*, 2003, vol. 100, no. 1, pp. 229—234. doi 10.1073/pnas.0133792100
17. Magoori, K., Kang, M.J., Ito, M.R., et al., Severe hypercholesterolemia, impaired fat tolerance, and advanced atherosclerosis in mice lacking both low density lipoprotein receptor-related protein 5 and apolipoprotein E, *J. Biol. Chem.*, 2003, vol. 278, no. 13, pp. 11331—11336. doi 10.1074/jbc.M211987200
18. Borrell-Pagès, M., Romero, J.C., Badimon, L., LRP5 deficiency down-regulates Wnt signalling and promotes aortic lipid infiltration in hypercholesterolaemic mice, *J. Cell. Mol. Med.*, 2015, vol. 19, no. 4, pp. 770—777. doi 10.1111/jcmm.12396
19. Rip, J., Nierman, M.C., Ross, C.J., et al., Lipoprotein lipase S447X: a naturally occurring gain-of-function mutation, *Arterioscler., Thromb., Vasc. Biol.*, 2006, vol. 26, no. 6, pp. 1236—1245. doi 10.1161/01.ATV.0000219283.10832.43

20. Javorský, M., Gašperíková, D., Ukropec, J., et al., Lipoprotein lipase HindIII polymorphism influences HDL-cholesterol levels in statin-treated patients with coronary artery disease, *Wiener Klin. Wochenschrift*, 2007, vol. 119, nos. 15—16, pp. 476—482. doi 10.1007/s00508-007-0824-1

21. Vardarl, A.T., Harman, E., Çetintaş, V.B., et al., Polymorphisms of lipid metabolism enzyme-coding genes in patients with diabetic dyslipidemia, *Anatolian J. Cardiol.*, 2017, vol. 17, no. 4, p. 313. doi 10.14744/AnatolJCardiol.2.7142

22. He, T., Wang, J., Deng, W.S., and Sun, P., Association between lipoprotein lipase polymorphism and the risk of stroke: a meta-analysis, *J. Stroke Cerebrovasc. Dis.*, 2017, vol. 26, no. 11, pp. 2570—2578. doi 10.1016/j.jstrokecerebrovasdis.2017.06.003

23. Munshi, A., Babu, M.S., Kaul, S., et al., Association of LPL gene variant and LDL, HDL, VLDL cholesterol and triglyceride levels with ischemic stroke and its subtypes, *J. Neurol. Sci.*, 2012, vol. 31, no. 1, pp. 51—54. doi 10.1016/j.jns.2012.04.006

24. Bushueva, O.Yu., Stetskaya, T.A., Korogodina, T.V., et al., Study of the interrelationship between the HindIII polymorphisms of the *LPL* gene and the Taq1b *CETP* gene with the risk of atherothrombotic stroke in Central Russia, *Ther. Arkh.*, 2015, vol. 87, no. 8, pp. 86—91. doi 10.17116/terarkh201587886-91

25. Yang, Y. and Niu, T., A meta-analysis of associations of LEPR Q223R and K109R polymorphisms with type 2 diabetes risk, *PLoS One*, 2018, vol. 13, no. 1. e0189366. doi 10.1371/journal.pone.0189366

26. Sun, Q., Cornelis, M.C., Kraft, P., et al., Genome-wide association study identifies polymorphisms in LEPR as determinants of plasma soluble leptin receptor levels, *Hum. Mol. Genet.*, 2010, vol. 19, no. 9, pp. 1846—1855. doi 10.1093/hmg/ddq056

27. Dolgushina, N.V., Desyatkova, N.V., and Donnikov, A.E., et al., The role of adipokines and adipokin regulator genes in the effectiveness of assisted reproductive technology programs in overweight patients, *Akush. Ginekol.*, 2017, vol. 2, pp. 71—78. https://dx.doi.org/.10.18565/aig.2017.2.71-8

28. Rosmond, R., Chagnon, Y.C., Holm, G., et al., Hypertension in obesity and the leptin receptor gene locus, *J. Clin. Endocrinol. Metab.*, 2000, vol. 85, no. 9, pp. 3126—3131. doi 10.1210/jcem.85.9.6781

29. Okada, T., Ohzeki, T., Nakagawa, Y., et al., Impact of leptin and leptin-receptor gene polymorphisms on serum lipids in Japanese obese children, *Acta Pædiatr.*, 2010, vol. 99, no. 8, pp. 1213—1217. doi 10.1111/j.1651-2227.2010.01778.x

30. Hollensted, M., Ahluwalia, T.S., Have, C.T., et al., Common variants in LEPR, IL6, AMD1, and NAMPT do not associate with risk of juvenile and childhood obesity in Danes: a case–control study// *BMC Med. Genet.*, 2015, vol. 16, no. 1, p. 105. doi 10.1186/s12881-015-0253-3

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