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A STUDY OF THE POLYMORPHISM RS9939609 OF THE *FTO* GENE AND RS738409 OF THE *PNPLA3* GENE AS RISK FACTORS FOR THE DEVELOPMENT OF NAFLD IN THE YAKUT POPULATION OF TYPE 2 DIABETES MELLITUS

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ABSTRACT

In order to study the frequency distribution of the polymorphism alleles rs9939609 of the *FTO* gene and polymorphism rs738409 of the *PNPLA3* gene among the Yakuts, 132 DNA samples of patients with type 2 diabetes and 70 DNA samples of healthy volunteers were tested.

The study of the frequency distribution of the polymorphism alleles rs9939609 of the *FTO* gene and polymorphism rs738409 of the *PNPLA3* gene in both groups showed no significant differences. Analysis of the frequency distribution of alleles and genotypes of the polymorphic variant of the *FTO* gene (rs9939609) in the group of patients with type 2 diabetes and healthy revealed a predominance of the T allele and a homozygous genotype of *TT*, except for a group of practically healthy men, which despite the prevalence of the *T* allele was characterized by the highest level of the heterozygous genotype *AT*. When analyzing the frequency distribution of the alleles and genotypes of the polymorphic version of the *PNPLA3* gene (rs738409), the allele *G* and the homozygous genotype *GG* prevailed in both groups. In the men and both groups studied, the *G* allele significantly prevailed over the *C* allele ($p < 0.05$).

Keywords: *FTO* gene, adiponadine gene, type 2 diabetes mellitus, NAFLD, overweight, Yakuts.

Introduction

Currently, obesity is an actual problem, which is associated with its progressive spread and the severity of complications, which often cause the death of patients at a young age. To date, according to the World Health Organization in the world - 39% of adults are overweight, and 13% are obese. In Russia, among the able-

bodied population, 25% are overweight and 30% obese [4].

Increased consumption of food, with the advantage of fats and carbohydrates, leads to the accumulation of excess weight and a violation of the natural physiological transformation of energy. Metabolic disorders in obese people with overeating lead to a relative insufficiency of insulin. As a result, type 2 diabetes is formed, which further exacerbates obesity. Genetic predisposition is one of the important factors in the pathogenesis of obesity [2]. It is estimated that 40-70% of the dispersion of the body mass index (BMI) can be attributed to direct or indirect genetic factors [8].

The full-genomic search for associations (GWAS) showed that the single nucleotide polymorphism (SNP) rs9939609, located in the first intron of the FTO gene (Fat Mass and Obesity Associated Gene), associated with a set of fat mass was significantly associated with obesity in various ethnic populations in both children and in adults [11]. The FTO gene encodes one of the lipolysis regulators, is involved in the control of adipocyte differentiation, energy homeostasis, leptin-independent appetite control. According to the results of previous studies, allele A of the FTO gene is associated with reduced lipolysis, a violation of appetite control, a lack of satiety after adequate food intake. The phenotypic manifestation of the FTO gene allele A is overweight, obesity due to overeating, which in turn is one of the common risk factors for the development of non-alcoholic fatty liver disease (NAFLD). Epidemiological data indicate a frequent combination of type 2 diabetes and NAZHBP characterized by the accumulation of lipids both in the hepatocytes themselves and in the intercellular space [1]. Patients with type 2 diabetes are characterized by insulin resistance, often obese, have dyslipidemia and increased activity of hepatic enzymes, they tend to accumulate fat in the liver regardless of BMI values, which causes a higher risk of developing severe liver pathology compared to patients not suffering from CD [7]. NAFLD is considered as an important medical and social problem because it includes a range of clinical and morphological concepts: liver steatosis, non-alcoholic steatohepatitis (NASH), which leads to the development of fibrosis and cirrhosis of the liver.

Recently, there have been studies that prove hereditary mechanisms for the development of NAFLD. Genetic risk factors for the development and progression of NAFLD have been found. The involvement of the *PNPLA3* gene in the formation of cirrhosis and primary liver cancer has been proven. Polymorphism of this gene is a predictor of the progressing course of NAFLD and the main risk factor for the transformation of NAFLD into cirrhosis. Molecular genetic studies have shown that the *PNPLA3* gene located on the long arm of chromosome 22q13.31 is expressed in the membranes of hepatocytes and is responsible for intrahepatic lipid metabolism by coding the synthesis of adiponuclein, a protein regulating the activity of triacylglycerol lipase in adipocytes [10]. The most significant polymorphism in the *PNPLA3* gene is I148M (rs738409). Polymorphism I148M is the replacement of cytosine by guanine, which leads to a change in the amino acid isoleucine to methionine at position 148. This replacement causes a violation of the mechanism of lipid metabolism in the liver.

The purpose of our study was to study the frequency distribution of polymorphism alleles rs9939609 of the *FTO* gene and the polymorphism rs738409 of the *PNPLA3* gene among people suffering from type 2 diabetes of the Yakut population.

Materials and methods of research

The experimental part of the genotyping of polymorphism rs9939609 of the FTO gene and the polymorphism rs738409 of the PNPLA3 gene was carried out in the laboratory of hereditary pathology of the department of molecular genetics of the Yakutsk Scientific Center of Complex Medical Problems. DNA samples from the YMC KMB biomaterial collection using the UMU "Genome of Yakutia" (registration No. USU_507512) were used for the study. Tested 132 DNA samples of patients diagnosed with type 2 diabetes, 91 of which belonged to women, 41 to men. The average age of participants in the study was 58.8 ± 0.43 years. As a comparison group, a sample was collected with normal BMI and no diabetes mellitus (n = 70) from 22 men and 48 women, whose mean age was 27.2 ± 0.47 years. All participants in the study on ethnicity were Yakuts and lived on the territory of the RS (Y). The study was conducted with the written consent of the participants.

The criteria for inclusion in the study were: the absence of liver damage by chronic viral hepatitis; all subjects were excluded: autoimmune hepatitis, primary biliary cholangitis, primary sclerosing cholangitis, hereditary hemochromatosis, Wilson-Konovalov's disease, and lack of alcohol abuse (> 30 g / l).

For PCR and RFLP analysis, genomic DNA samples were isolated from the whole blood of patients by a standard phenol-chloroform method. Single nucleotide polymorphisms (SNP) were determined by polymerase chain reaction (PCR).

To carry out PCR, specific primers were used by Biotech-Industry Ltd., Moscow:

rs9939609 F: 5'-TGGGCCTGAAGTCCGAGGGT-3'

rs9939609 R: 5'-CCGACACCAGTGCCCTGCAG-3';

rs738409 F: 5'-AACTGGCTCTTGAATGAAATAGGATTCAGA-3'

rs738409 R: 5'-AGAGTAACAGAGACTATCCAAGTGCAGTAC-3'

After PCR, the amplification of rs9939609 of the FTO gene was subjected to restriction with the use of ZrmI endonuclease (SibEnzyme LLC, Novosibirsk) for 3 hours at 37 ° C. The detection of RFLP products was carried out by horizontal electrophoresis in a 4% agarose gel plate with the addition of ethidium bromide-specific intercalating fluorescent DNA (RNA) dye-using a standard tris-acetate buffer at a field strength of ~ 20 V / cm for 30 minutes (figure - 1).

The amplification of rs738409 of the PNPLA3 gene was subjected to restriction with the use of endonuclease BstF5 I (SibEnzyme LLC, Novosibirsk) for 3 hours at 65 ° C. The detection of RFLP products was carried out by horizontal electrophoresis in a 4% agarose gel plate with the addition of ethidium bromide-specific intercalating fluorescent DNA (RNA) dye-using a standard tris-acetate buffer at a field strength of ~ 20 V / cm for 30 minutes (figure - 2).

Interpretation of the results of

Table 1

PCR conditions

Gene	Amplificate	Length of restriction fragments	PCR conditions
FTO	182 b.p.	AA – 154, 28 AT – 154, 28, 182 TT – 182	1. 95 °C – 4 min 2. (94 °C – 30 sec; 58 °C – 30 sec; 72 °C – 1 min)*35 3. 72°C – 10 min
PNPLA3	333 b.p.	CC: 200 и 133 b.p. GC: 333, 200 и 133 b.p. GG: 333 b.p	1. 95 °C – 5 min 2. (94 °C – 30 sec; 66 °C – 30 sec; 72 °C – 40 sec)*37 3. 72 °C – 5 min

genotyping was performed on the basis of different patterns of bands: CC genotype 200 and 133 bp, CG genotype - 333, 200 and 133 bp, GG genotype -333 bp.

Results and discussion:

Analysis of the frequency distribution of alleles and genotypes of the polymorphic version of the FTO gene (rs9939609) in the group of patients with type 2 diabetes and healthy did not reveal significant differences, in both groups allele T (p = 0.252) and homozygous genotype TT (p = 0.820) prevailed, practically healthy men, which despite the predominance of the T allele (p = 0.08) was characterized by the highest level of heterozygous AT genotype (Ho = 0.546).

The results of the analysis of the frequency distribution of alleles and genotypes of polymorphism rs9939609 of the FTO gene are presented in Table 2.

The analysis of BMI indices, characterizing the correspondence of the body weight of a person and its growth, showed that among patients with type 2 diabetes (n = 122), overweight (n = 33; 27%) and obesity prevailed (n = 81, 66.4 %). Compliance with normal BMI was noted in only 8 patients (6.6%).

The results of analysis of the frequency distribution of alleles and genotypes of polymorphism of the FTO gene (rs9939609) depending on the BMI parameters are presented in Table 3.

Table 3 shows that, regardless of gender, among patients with type 2 diabetes having an overweight and obesity, the allele T (p = 0.199) prevailed. Analysis of the frequency distribution of genotypes showed the following, in the overweight group both in women and men, the heterozygous genotype of AT prevailed (p = 0.754), whereas in obese patients the homozygous genotype of TT (p = 0.844) was more prevalent. In studies Shilina NM. (2017), in contrast, notes in women 27 years and older the connection with obesity in carriers of genotypes of AT + AA in comparison with carriers of the genotype of TT [5].

Analysis of the allele and genotype distribution of the polymorphic variant of the PNPLA3 gene (rs738409) in the group of patients with type 2 diabetes and healthy did not reveal significant differences, in both groups allele G (p <0.001) and homozygous genotype GG prevailed (Table 4). In the men and both groups studied, the G allele significantly prevailed over the C allele (p <0.05).

According to the data of the “1000 genomes” project, the frequency of distribution of the allele G of the PNPLA3 gene (rs738409) is characterized by heterogeneity. When comparing

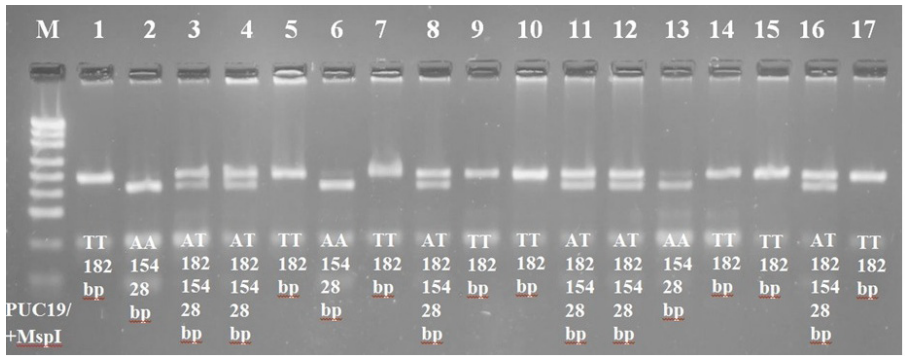

Fig. 1 Electrophoregram of the amplification product of the *FTO* gene site in a 4% agarose gel.
Note. Tracks No. 1, 5, 7, 9, 10, 14, 15 and 17 are the genotype of TT; № 2, 6 and 13 - genotype AA; № 3, 4, 8, 11, 12 and 16 - the genotype of AT; M - marker PUC19 / + Msp I. Bp - base pairs.

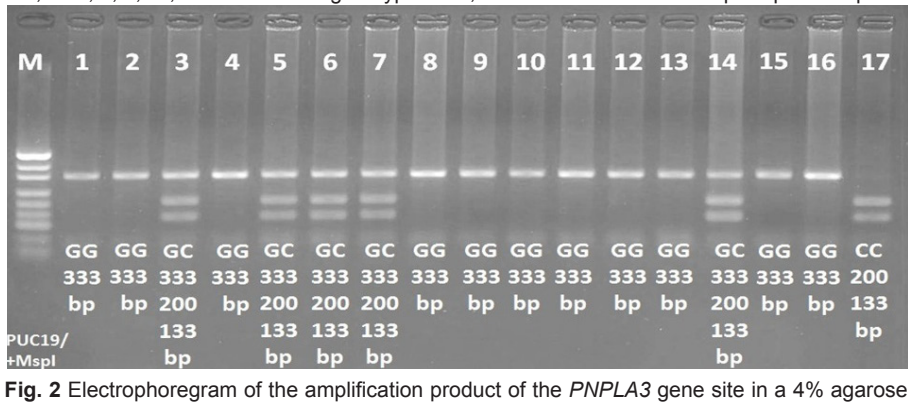

Fig. 2 Electrophoregram of the amplification product of the *PNPLA3* gene site in a 4% agarose gel.
Note. Tracks № 17 - genotype CC, № 3, 5, 6, 7, 14 - genotype GC, № 1, 2, 4, 8, 9, 10, 11, 12, 13, 15, 16 - genotype of GG. M - marker PUC19 / + Msp I. bp - base pairs.

Table 2

Frequency distribution of alleles and genotypes of polymorphism rs9939609 of the *FTO* gene in the group of patients with type 2 diabetes and healthy individuals

	n		Genotype, %			Allele		H_o	H_e	X^2	p
			TT	AT	AA	T	A				
Patients with diabetes 2											
Women	91	H	52,8	42,9	4,4	0,742	0,258	0,429	0,383	1,282	0,258
		O	55,1	38,3	6,7						
Men's	41	H	48,8	39,0	12,2	0,683	0,317	0,390	0,433	0,401	0,527
			46,6	43,3	10,1						
Healthy											
Women	48	H	58,3	37,5	4,2	0,771	0,229	0,375	0,353	0,180	0,67
		O	59,4	35,3	5,3						
Men's	22	H	45,5	54,6	0,0	0,727	0,273	0,546	0,397	3,09	0,08
			52,9	39,7	7,4						

Note: n-quantity; O - observable; E is the expected; X2 is the chi-square; Ho -observed heterozygosity; He -expected heterozygosity.

Table 3

Frequency distribution of alleles and genotypes of the *FTO* gene (rs9939609) depending on BMI and sex in the group of patients with type 2 diabetes

ИМТ		Genotype, %			Allele		H_o	H_e	X^2	p
		TT	AT	AA	T	A				
Women (n=79)										
Overweight (BMI ≥ 25 кг/м2)	O	36,8	52,6	10,5	0,632	0,368	0,526	0,465	0,326	0,568
	E	39,9	46,5	13,6						
Obesity (BMI≥ 30 кг/м2.)	O	51,7	45,0	3,3	0,742	0,258	0,450	0,383	1,824	0,177
	E	55,0	38,3	6,7						
Men’s (n=35)										
Overweight (BMI ≥ 25 кг/м2)	O	28,6	64,3	7,1	0,607	0,393	0,643	0,477	1,691	0,193
	E	36,9	47,7	15,4						
Obesity (BMI≥ 30 кг/м2.)	O	52,4	33,3	14,3	0,690	0,310	0,333	0,427	1,018	0,313
	E	47,7	42,7	9,6						

Table 4

Distribution of frequencies of alleles and genotypes of polymorphism rs738409 of *PNPLA3* gene in the group of patients with type 2 diabetes and healthy individuals

	n		Genotype, %			Allele		H_o	H_e	X^2	p
			CC	CG	GG	C	G				
Patients with diabetes 2											
Women	91	O	8,8	33	58,2	0,253	0,747	0,33	0,378	1,473	0,225
		E	6,4	37,8	55,8						
Men's	41	O	19,5	22	58,5	0,305	0,695	0,22	0,424	9,529	0,002
		E	9,3	42,4	48,3						
Healthy											
Women	49	O	4,1	38,8	57,1	0,235	0,765	0,388	0,359	0,309	0,578
		E	5,5	35,9	58,6						
Men's	24	O	25,0	16,7	58,3	0,333	0,667	0,167	0,444	9,375	0,002
		E	11,1	44,4	44,4						

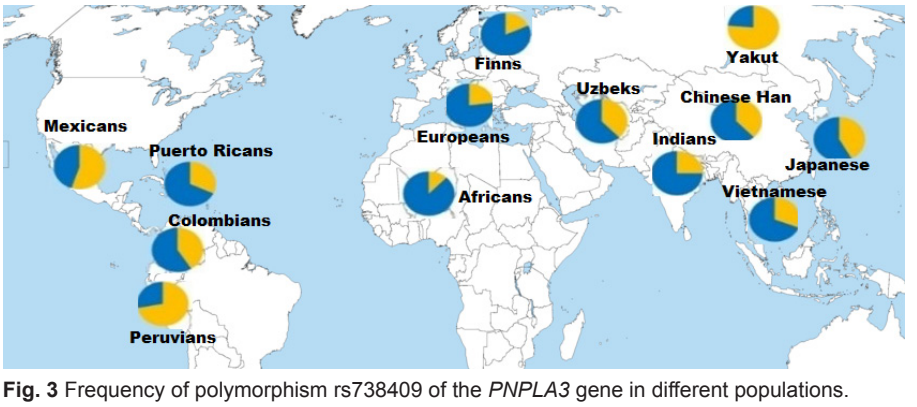

Fig. 3 Frequency of polymorphism rs738409 of the *PNPLA3* gene in different populations.

frequencies among healthy and patients with type 2 diabetes in different populations, the prevalence of G allele frequency in the Yakut population was established (Fig. 3).

In their studies of the Japanese population of patients with type 2 diabetes, M. Ueyama, N. Nishida (2015) and Kan H. et al. (2016), noted the high frequency of the G allele (48-48.8%) [12 , 13]. In studies among the European population of patients with type 2 diabetes, Jean-Michel Petit et al. (2010), the allele frequency G was - 29.6% [11]. According to Cox A.J. (2011), the lowest frequency of occurrence of the G allele (13.7%) at the GG genotype frequency was 1.5%, had a population of African American patients with type 2 diabetes [6].

According to the researchers, the pathogenesis of NAFLD is played by the theory of two-stage lesion. At the first stage, against the background of visceral obesity and insulin resistance (IR) lipolysis increases, the concentration of free fatty acids (FFA) in the blood serum increases due to an increase in synthesis and inhibition of their oxidation in mitochondria with accumulation of triglycerides and a decrease in the excretion of fats by liver cells. So, there are conditions for the formation of fatty liver dystrophy - steatosis. At the same time, fatty hepatosis, regardless of the cause, can contribute to high insulin levels due to reduced insulin clearance [3]. Despite the active study of the problem of NAFLD, absolute algorithms for the treatment of this disease have not been developed.

Conclusion

Today, the problem of NAFLD is very relevant and increasingly attracts the attention of researchers and practitioners.

The detection of the polymorphism markers of the PNPLA3 (rs738409) and FTO (rs9939609) genes associated with the development of NAFLD will allow the formation of risk groups with the goal of carrying out preventive and therapeutic activities.

Analysis of the frequency distribution of alleles and genotypes of the polymorphic version of the FTO gene (rs9939609) in the group of patients with type 2 diabetes and healthy did not reveal significant differences, in both groups allele T (p = 0.252) and homozygous genotype TT (p = 0.820) prevailed, practically healthy men, which despite the predominance of the T allele (p = 0.08) was characterized by the highest level of heterozygous AT genotype (Ho = 0.546).

When analyzing the frequency distribution of alleles and genotypes of the polymorphic version of the PNPLA3 gene (rs738409), there were no significant differences in the group of patients with type 2 diabetes and healthy, in both groups allele G (p <0.001) and homozygous genotype GG prevailed. In the men and both groups studied, the G allele significantly prevailed over the C allele (p <0.05).

Timely detection of risk factors for NAFLD, maintenance of appropriate preventive work, adequate pharmacotherapy will reduce insulin resistance, obesity, including abdominal, incidence of diabetes, arterial hypertension and atherosclerosis leading to coronary heart disease, and reduce mortality from cirrhosis and increase life expectancy of the population.

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ANTI-INFLAMMATORY ACTIVITY OF THE COMPLEX HERBAL REMEDY

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ABSTRACT

During experiments on white rats of the Wistar line, anti-inflammatory activity of the extract of dry complex herbal remedy (*Juglans regia L.* Rich ex Kimth., *Corylus avellana L., Agrimonia eupatória L., Bidens tripartita L., Xanthium strumarium L., Urtica dioica L. Lemna minor L., Cichorium intybus L., Onopordum acanthium L.*) was examined on models of acute exudative (carrageenan and formalin), chronic alterative and proliferative inflammation. It is found that phytoextract in doses of 100-300 mg/kg has antiexudative activity, reducing the exudation caused by phlogogenic agents. The tested extract has an anti-alterative effect, limiting the alteration of tissues with acetic acid and enhancing regenerative processes.

Keywords: complex remedy (*Juglans regia* L. Rich ex Kimth., *Corylus avellana* L., *Agrimónia eupatória* L., *Bidens tripartita* L., *Xanthium strumarium* L., *Urtica dioica* L. *Lemna minor* L., *Cichorium intybus* L., *Onopordum acanthium* L.), anti-inflammatory activity.

Introduction

Currently, hypothyroidism is one of the most common diseases of the endocrine system. According to epidemiological studies, the incidence of this disease in the population is about 2%; while in the group of women over 74 years old it reaches 21% [3]. Patients with hypothyroidism, against the decrease in the level of basal metabolism and anabolic processes in general, as well as the activation of free radical oxidation and the weakening of the body's antioxidant defense, show a slowdown in the regeneration of damaged tissues and recovery processes. Posttraumatic intoxication in turn aggravates disorders of thyroid regulation of intracellular metabolism [11].

To treat hypothyroidism, thyroid hormone preparations, drugs containing iodine, and preparations that affect the immune system (immunosuppressors and immunomodulators), as well as efferent therapy are used. Drug treatment methods for hypothyroidism, which are part of the scope of evidence-based medicine, allow for achieving clinical results, while not always achieving the proper life quality for a particular patient, require compulsory medical supervision and often have side effects [5].

Of particular interest in the treatment of hypothyroidism are herbal remedies that, due to the synergism of biologically active substances, have systemic exposure on the body: normalize the level of hormones, manifest antioxidant, anti-inflammatory, psychotropic, cardioprotective and other actions, and thus contribute to delaying the administration of hormone replacement therapy or reducing the dose of hormones during its administration [4]. In the light of the development of personalized medicine, a complex herbal remedy consisting of: *Juglans regia L. Rich ex Kimth., Corylus avellana L., Agrimonia eupatória Ldb, Bidens tripartita L., Xanthium strumarium L., Urtica dioica L. Lemna , L., Onopordum aconthium* L is of interest. Earlier in animal experiments, it was found that this complex remedy exerts a pronounced pharmacotherapeutic efficacy in experimental hypothyroidism, increasing the synthesis of thyroid hormones, peripheral conversion of fT4 to fT3, normalizing the cardiovascular parameters, increasing animal resistance to hypoxia [14].

The **study objective** was to evaluate the anti-inflammatory activity of the dry complex herbal remedy extract with thyroid-stimulating activity.

Study materials and methods

the experiments were performed on Wistar rats of both sexes with an initial mass of 180-190 g. The animals were kept in standard vivarium conditions with the same care and nutrition, light and temperature conditions in accordance with Order No. 708H of the Ministry of Health of the Russian Federation of August 23, 2010 "On Approval of Laboratory Practice Regulations". Experimental studies were carried out in accordance with the "Work code for using experimental animals" (Appendix to the Order of the Ministry of Health of the USSR No. 755 of 12.08.77) and "Rules adopted in the European Convention for the Protection