Kh.A. Kurtanov, L.A. Sydykova, N.I. Pavlova, N.P. Filippova,
G.A. Apsolikhova, N.A. Solovyova, V.V. Dodokhov,
M.A. Varlamova, A.T. D'jakonova, L.M. Neustroeva, N.V. Borisova

RESEARCH OF ADIPONUTRIN GENE *(PNPLA3)* IN INDIGENOUS PEOPLE OF THE REPUBLIC OF SAKHA (YAKUTIA) WITH TYPE 2 DIABETES

ABSTRACT

Polymorphism of the *I148M G*ene of the *PNPLA3 G*ene was studied in patients with type 2 diabetes of the Yakut nationality. The predominance of the *GG G*enotype (58.5%) with a *G*-allele frequency of 74.1% was revealed. The normally functionin*G* protein of the *PNPLA3 G*ene re*G*ulates the activity of tri*G*lyceride hydrolase and lysophosphatidic acid acyltransferase. It is likely that the hi*G*h frequency of the mutant allele *G* in the Yakuts with type 2 diabetes may be one of the causes of the lipid metabolism mechanism disorder in the liver.

Keywords: diabetes mellitus type 2, insulin resistance, adiponutrin *G*ene, polymorphism, *PNPLA3*, rs738409, *I148M*.

INTRODUCTION

The most significant indicator of a person's adaptation to living conditions is the duration of his life. The same indicator is one of the generally accepted for assessing the quality of life in general. On the inhabitants of the North, the 20th century had a strong influence in the form of a drastic change in the social and environmental conditions of residence, the image and diet of traditional food. Undoubtedly, these changes have led to changes in the quality of health of its indigenous inhabitants. In particular, among the causes in the medical and social disadaptation of man, a large proportion is occupied by so-called diseases with complex inheritance (caused by a combination of genes and environmental factors).

One of the most socially significant pathologies in the Republic of Sakha (Yakutia) is type 2 diabetes mellitus (DM 2). According to Rosstat, the total number of patients with diabetes in the republic is 21,677 people, of which 20,508 with type 2 diabetes, 1099 with type 1 diabetes, and 70 with diabetes of other types [7].

Epidemiological data indicate the frequent combination of type 2 diabetes mellitus and non-alcoholic fatty liver disease (NAFLD) [1]. Patients with CD2 are insulin resistant, often obese, have dyslipidemia and increased activity of liver enzymes, they tend to accumulate fat in the liver regardless of BMI (body mass index), thus they have a higher risk of developing severe liver disease compared to patients without diabetes [15].

Non-alcoholic fatty liver disease refers to the most common chronic liver disease. The incidence of this disease is 20-30 % in the general population and 67-75 % in the population of obese people [2]. The prevalence of non-alcoholic steatosis of the liver among residents of the economically developed countries of the world is on average 20-35 %, non-alcoholic steatohepatitis – 3 %. In the United States, 34 % of the adult population has liver disease, 29 % in Japan. In Russia, according to the screening program for detecting the prevalence of NAFLD and its clinical forms, conducted in 2007 and covering 30,754 people, NAFLD was detected in 27 % of the examined, 80.3 of whom had steatosis, 16.8 - steatohepatitis and 2.9 % - cirrhosis of the liver [3].

The prevalence of NAFLD among patients with type 2 diabetes is 60-80 %, and the incidence of NASH is 12-40 % [8].

Genetic, as well as environmental factors play an important role in the development of NAFLD [19]. One of the candidate genes involved in the pathogenesis of NAFLD is the PNPLA3 gene, which codes for the synthesis of the adiponucleus protein. In this case, this genetic feature is most common in Latinos and rarely in representatives of the Negroid race [5]. The association of the PNPLA3 gene with the development of NAFLD revealed a number of studies [14].

The full-genomic search for associations (GWAS) has shown that SNP in the PNPLA3 gene affects the levels of liver enzymes in the plasma. The G allele of polymorphism rs738409 of the PNPLA3 gene is strongly associated with NAFLD, as well as with increasing AST and ALT, ferritin level and fibrosis stage in patients with NAFLD [11].

The most significant polymorphism in the PNPLA3 gene is I148M. It consists in replacing the nucleotide cytosine by guanine, leading to a change in the amino acid isoleucine to methionine at position 148. This replacement leads to a violation of the mechanism of lipid metabolism in the liver. Polymorphism I148M is associated with susceptibility to NAFLD and affects the histological pattern and development of fibrosis in children and adolescents with obesity [17].

According to the National Center for Biotechnological Information (NCBI), the frequency of the allele G of the polymorph I148M of the PNPLA3 gene (rs738409) varies from 19.6 % (African population AFD_AFR_PANEL ss24098326) to 43.2 % (Asian population HapMap-JPT ss76896972) (Fig. 1).

The polymorphism association rs738409 I148M with type 2 diabetes mellitus and NAFLD was confirmed in several ethnic and geographical groups, but to date no frequency assessment has been performed in the populations of Yakutia. The Yakuts are one of the many peoples of the Far East and Siberia. The Yakut population is very interesting in a genetic sense. It is remarkable that this population is formed by an admixture of two or more ancestral populations, so it gives a unique opportunity to study the interaction between gene polymorphisms, ethnic genetic background and ecological contributions to the disease. The aim of the study was to investigate the relationship between the variants of the PNPLA3 gene (rs738409 C> G) and type 2 diabetes mellitus in patients of the Yakut nationality.

Materials and methods of research. The study was conducted in the laboratory of hereditary pathology of the department of molecular genetics of the YSC of the ILC. Informed consent to genetic research was obtained from each patient. For the study, DNA samples from the YMC KMB biomaterial collection were used. The sample includes 106 pa-

tients of the Yakut nationality of the endocrinology department of the Republic of Belarus No. 2-Center for Emergency Medical Care diagnosed with diabetes 2 (79 women and 27 men) aged 31 to 82 years. All patients underwent polymorphism rs738409 of the PNPLA3 gene. Samples of genomic DNA were isolated from the whole blood of patients by a standard phenol-chloroform method. The single nucleotide polymorphism (SNP) of rs738409 I148M was determined by a PCR-RFLP method.

Amplification of the region of the PNPLA3 gene containing the polymorphic variant was carried out by standard primer pairs (forward primer: 5’-CCGGCCTGAAGTCCGAGTTT-3 ‘and reverse primer: 5’-GCGACACCAAAGCCCTGCGG-3’) (Biotech-Industry Ltd., Moscow). The composition of the reaction mixture for PCR (total volume of the reaction mixture is 25 μl): 13 μl ddH2O, 2.5 μl 10x PCR buffer, 2.5 μl 25 mM MgCl2, 2.5 μl 2.5 mM dNTP Mix, 1.5 μl 10 pkmol / μl) of each oligonucleotide primer, 0.3 units. (1.5 units) of the «hotstart» Taq polymerase and 3 μl of DNA. PCR was carried out in a thermal cycler MJ Mini Gradient Thermal Cycler («BioRad»).

Temperature conditions PCR: 95° C - 5 min, then 37 cycles at 94° C - 30 s, 66 ° C - 30 s, and 72 ° C - 40 s and the final elongation at 72° C - 5 min. The PCR products were then cut with a BstF5 I restriction enzyme (SibEnzyme LLC, Novosibirsk) overnight at 65 ° C. The cut PCR products were subjected to horizontal electrophoresis in 1.5 % agarose gels stained with ethidium bromide in 1 x TBE buffer at 120 V for 1 h and visualized using a gel documenting system (Vilber Lourmat, France).

The detection of RFLP products was carried out by horizontal electrophoresis in a plate of 4 % agarose gel stained with ethidium bromide, using a standard tris-acetate buffer at 120 V for 1 h. Visualized in UV-rays using a gel documenting system (Fig. 2).

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

Statistical analysis of the results of medical genetic research was carried out with the help of programs: «Office Microsoft Excel 2010», «Statistica 8.0». The frequency rs738409 was determined by direct counting. The results are considered significant when the value of «p» is less than 0.05 (p <0.05).

Results and discussion. A comparative analysis of the frequency distribution of alleles and genotypes of the polymorphic gene PNPLA3 (rs738409) in the sample of Yakuts suffering from type 2 diabetes revealed a prevalence of the GG genotype (58.49 %), significantly less often the CC genotype (10.38 %). The heterozygous genotype GC was observed in 31.13% of patients. Analysis of the allele distribution of the polymorphic locus PNPLA3 (rs738409) showed a higher allele frequency G of 74.1 %. Allele C is found in Yakuts with a frequency of 25.9 %. Population-genetic analysis of the distribution of polymorphisms of the adiponuclear gene PNPLA3 (rs738409) in the Yakuts showed that the level of observed heterozygosity was Ho = 0.311 level of the expected heterozygosity He = 0.387. The distribution of the rs738409 polymorphism genotypes in the sample (p> 0.05) was in the Hardy-Weinberg equilibrium (Table 1).

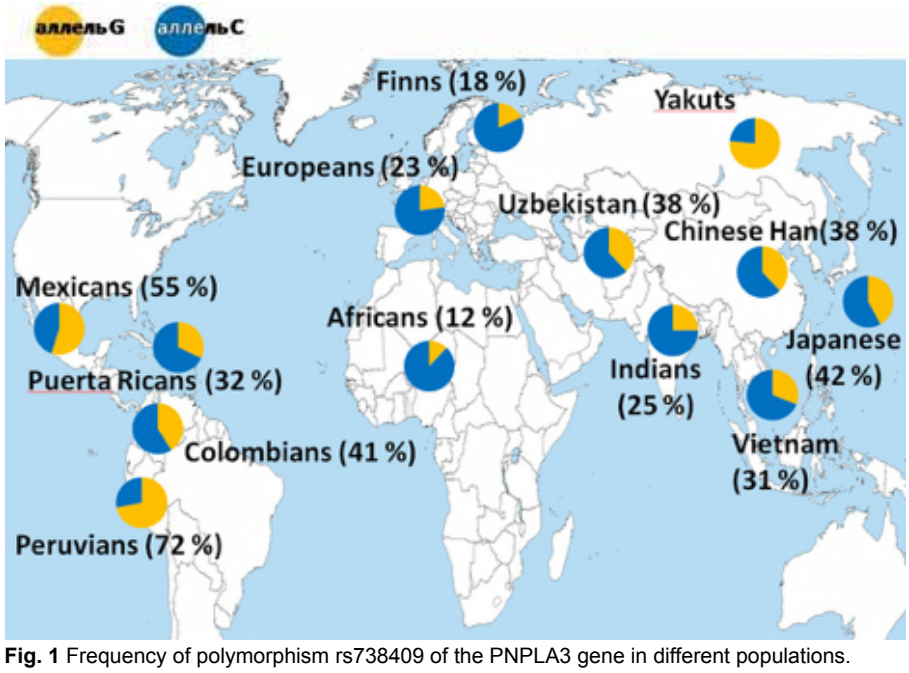

Fig. 1 Frequency of polymorphism rs738409 of the PNPLA3 gene in different populations. Note: yellow color - allele G, blue color - allele C. The data are obtained from the database of the project «1000 genomes» and from literary sources.

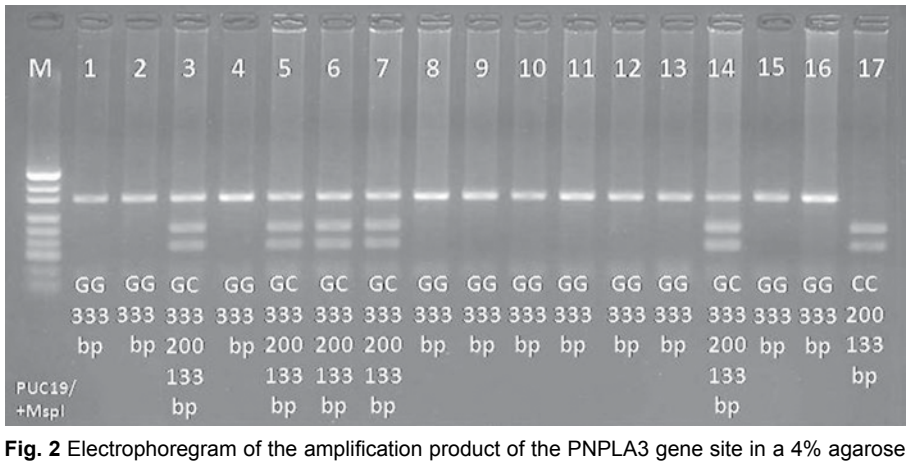

Fig. 2 Electrophoregram of the amplification product of the PNPLA3 gene site in a 4% agarose gel. 17 - genotype CC, 3, 5, 6, 7, 14 - genotype GC, 1, 2, 4, 8, 9, 10, 11, 12, 13, 15, 16 - genotype of GG. M - marker PUC19 / + Msp I. bp - base pairs.

High frequency of allele G (74.1 %) in patients with type 2 diabetes is associated with a high frequency of its occurrence among a healthy population of Yakuts (76.8 %) [4]. According to the “1000 genomes” project, in Asia the high frequency of G allele is found in Japanese (43.2 %). In studies of the Japanese population of patients with type 2 diabetes, M. Ueyama, N. Nishida (2015) and Kan H. et al. (2016), note the high incidence of allele G (48-48.8 %) [13, 16]. In the African American population, the frequency of the G allele is low (19 %) [18], and it is also low (13.7 %) at a genotype GG frequency of 1.5 % in patients with type 2 diabetes [10]. In the European population, the G allele frequency is on average 22.6 % [20], in patients with type 2 diabetes - 29.6 % [12].

As many domestic and foreign re-

searchers note, patients with type 2 diabetes mellitus carry PNPLA3 gene allele (rs738409) as a whole are more susceptible to liver diseases (NAFLD, NASH) with a high risk of developing cirrhosis and hepatocellular carcinoma [6].

The distribution of the frequency of alleles and genotypes of polymorphism rs738409 of the PNPLA3 gene in patients, depending on age, revealed a high frequency (83 %) in the groups of patients up to 35 and up to 65 years (Table 2). The high prevalence rate of the homozygous genotype GG was observed in the group of patients from 55 to 65 years old and was 70 %.

Patients with type 2 diabetes and NAFLD have a higher risk of cardiovascular disease, as well as mortality, due to the depletion of hepatic glycogen stores and a decrease in reserve regulation of glucose homeostasis, and the acceleration of the development of vascular complications. According to the pathogenesis of NAFLD, in the issue of pathogenesis, researchers adhere to 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.

In his studies, Jean-Michel Petit et al. [12], found the relationship of polymorphism PNPLA3 rs738409 with the fat content in the liver independent of general and visceral obesity and insulin resistance. They believe that adiponadine can be an important clue to understanding the mechanisms associated with the difference between fatty liver and fatty liver without metabolic effects, so the accumulation of fat in the liver can be metabolically benign.

The conclusion

As a result of the investigation of the PNPLA3 gene in the Yakuts with type 2 diabetes, it was established that the frequency distribution of alleles and genotypes of the PNPLA3 gene (rs738409) is in accordance with the Hardy-Weinberg law. In patients with type 2 diabetes, a high frequency of G allele (74.1 %) was found with a predominance of the GG genotype (58.49 %).

Thus, it has been established that the frequency of the mutant allele of functional polymorphism rs738409 of the PNPLA3 gene is higher than in other populations of the world. The normally functioning protein of the PNPLA3 gene regulates the activity of triglyceride hydrolase and acyltransferase of lysophosphatidic acid. Therefore, it can be assumed that the high frequency of the mutant allele G of the polymorphism I148M of the PNPLA3 gene in the Yakuts with type 2 diabetes may be one of the causes of the disturbance of the lipid metabolism mechanism in the liver, which requires careful research on larger samples of the populations of Yakutia, and further investigation of the gene is necessary PNPLA3 in Yakuts with type 2 diabetes mellitus.

Table 1

Distribution of frequencies of alleles and genotypes of polymorphism rs738409 of *PNPLA3* gene

Genotypes	Observed	Expected	Alleles		Ho	He	X^2	p
GG	58,49	54,91	*G*	0,741	0,311	0,387	4,123	0,05
GC	31,13	38,38	*C*	0,259				
CC	10,38	6,71						

Note: p> 0.05; X2 is the chi-square; Ho - observed heterozygosity; He is the expected heterozygosity.

Table 2

Frequency distribution of alleles and genotypes of polymorphism rs738409 of the *PNPLA3* gene in patients, depending on age

Age	n		Genotypes, %			Alleles		X^2	Ho	He	p
			CC	*GC*	*GG*	*C*	*G*				
under 35 years old	3	H	0,00	33,33	66,67	0,167	0,833	0,000	0,333	0,278	0,729
		O	2,79	27,82	69,39						
under 55 years old	24	H	16,66	41,67	41,67	0,375	0,625	0,535	0,417	0,469	0,586
		O	14,06	46,88	39,06						
up to 65 years	50	H	4,00	26,00	70,00	0,170	0,830	1,100	0,260	0,282	0,578
		O	2,89	28,22	68,89						
after 65 years	29	H	17,24	31,03	51,73	0,328	0,672	2,872	0,310	0,441	0,112
			10,76	44,08	45,16						

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

The study was supported by Federal Agency for Scientific Organizations program for support the bioresource collections (0556-2017-0003).

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The authors:

Yakutsk, Republic Sakha (Yakutia), Russia:

Kurtanov Khariton Alekseevich - Candidate of Medical Science, Chief Researcher, Head of the Department of Molecular Genetics;

Sydykova Lyubovy Akhmedovna - Candidate of Medical Science, Head of Department of propaedeutic and faculty therapy with endocrinology and physical therapy, North-Eastern Federal University, Medical Institute;

Pavlova Nadejda Ivanovna – Candidate of Biological Sciences, Leading Researcher, Head of the Laboratory of Hereditary Pathology;

Filippova Natalya Pavlovna – Candidate of Biological Sciences, Senior Researcher of the Laboratory of Hereditary Pathology;

Dodokhov Vladimir Vladimirovich – Candidate of Biological Sciences, Senior Researcher of the Laboratory of Hereditary Pathology;

Apsolikhova Galina Aleksandrovna – junior researcher of the laboratory of hereditary pathology;

Solovieva Natalya Alekseevna – Candidate of Biological Sciences, Leading Researcher, Head of the Laboratory of Population Genetics;

Dyakonova Aleksandra Timofeyevna – researcher of the laboratory of hereditary pathology; Neustroyeva Lena Mikhaylovna – researcher of the Laboratory of Population Genetics;

Varlamova Marina Alekseyevna – researcher of the Laboratory of hereditary pathology;

Natalya Vladimirovna Borisova - doctor of Medical Sciences, Professor of the Department «Normal and Pathological Physiology», North-Eastern Federal University, Medical Institute.

Sofronova S.I.

ARTERIAL HYPERTENSION AND METABOLIC SYNDROME IN SMALL INDIGENOUS PEOPLE OF THE NORTH OF YAKUTIA

ABSTRACT

The research was conducted in the north of Yakutia in places of compact residence of indigenous people of the North. High prevalence of hypertension in the adult population was revealed, its highest rate was observed in Anabarsky district. We studied the frequency of metabolic syndrome (MS) in the indigenous people of Yakutia. The highest frequency of MS was identified in the Evenks and the lowest among the Chukchi. In women MS was observed significantly more often than in men.

Keywords: small indigenous people of the North, arterial hypertension, metabolic syndrome.

Cardiovascular diseases are the first leading cause (45.4%) of mortality of the population in Yakutia, as in Russia in total. According to the Federal State Statistics Service from 2013 to 2015 the circulatory diseases morbidity rate of the population remains on the same level, and the mortality decreases slightly by 0.9% that makes 45.4% [2]. Despite the fact there is