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POLYMORPHISM RS738409 OF THE ADIPONUTRIN GENE (*PNPLA3*) AMONG THE INDIGENOUS RESIDENTS OF THE NORTH

ABSTRACT
In this paper, we analyzed the polymorphism rs738409 of the adiponuclein gene *(PNPLA3)* among the indigenous inhabitants of the North of the Republic of Sakha (Yakutia). Under the constant influence of low temperatures, the human body needs a high level of energy metabolism, which in turn is accompanied by a significant consumption of lipids. Epidemiological data indicate the frequent combination of type 2 diabetes and non-alcoholic fatty liver disease (NAFLD) characterized by accumulation of lipids both in the hepatocytes themselves and in the intercellular space. Recently, great importance is attached to the genetic conditionality of NAFLD.
Keywords: diabetes mellitus type 2, insulin resistance, adiponutrin gene, polymorphism.

Introduction

The problem of adaptation to the conditions of the North is being actively studied in the world. The achievements of Russian science are related to the identification of physiological, mental, biochemical features of the organism, the fundamental differences in the state of the organism of the northerners and inhabitants of the middle latitudes. By the present time it is an established fact that when a person adapts to the extreme natural conditions of the North, all kinds of metabolism of proteins, fats, carbohydrates, vitamins, macro- and microelements are restructured. Under the constant influence of low temperatures, the human body needs a high level of energy metabolism, which in turn is accompanied by a significant consumption of lipids.

Metabolism of the organism passes to a qualitatively new level of homeostasis, characterized by greater use of fats and proteins for energy needs and less use of carbohydrates.

High-calorie nutrition, excessive intake of (saturated) fats correlate with increased body weight and obesity, and recently their relationship with NAFLD has been revealed. Non-alcoholic fatty liver disease (NAFLD) is usually associated with obesity, metabolic syndrome and type 2 diabetes mellitus (DM 2), is one of the most common chronic liver diseases [1]. Epidemiological data indicate a fre-

quent combination of type 2 diabetes and NAFLD characterized by accumulation of lipids both in the hepatocytes themselves and in the intercellular space [1].

Patients with type 2 diabetes mellitus are insulin resistant, often obese, have dyslipidemia and increased activity of liver enzymes, they tend to accumulate fat in the liver regardless of BMI, thereby they have a higher risk of developing severe liver disease compared to patients without diabetes [3]. Recently, great importance is attached to the genetic conditionality of NAFLD. The value of the PNPLA3 gene, which codes for the synthesis of the protein-enzyme adiponase, is isolated.

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 allele G of the polymorphism rs738409 of the PNPLA3 gene is strongly associated with NAFLD, as well as with the increase in ACT and ALT, ferritin level, and fibrosis stage in patients with NAFLD [4]. With the objectiveto clarify the genetic background of NAFLD in the Yakut population of patients with type 2 diabetes, the present study analyzes the polymorphism of rs738409 of the PNPLA3 gene.

PNPLA3 is mainly expressed in the liver and has lipase activity of triacylglycerol. The mutation I148M is associated with a decreased activity of triglyceride lipase, as shown in studies with the recombinant PNPLA3 protein. This leads to the accumulation of triglycerides in the liver cells, but reduces the release of very low density lipoproteins (VLDL) into the circulation. Reduced lipid concentrations in the blood can reduce the deposition of lipids in the wall of blood vessels. There is a natural reaction of the body to the cold, the so-called «peripheral vasoconstriction», which is to maintain the internal temperature of the body by narrowing the blood vessels.

Materials and methods of research

The experimental part of the genotyping of polymorphism rs 738409 of the PNPLA3 gene was performed in the laboratory of hereditary pathology of the department of molecular genetics of the Yakut Scientific Center of Complex Medical Problems (YSC CMP). DNA samples from the collection of the YMC biomaterial (UNU «Genome of Yakutia», reg. No.USU_507512) were used for the study. The indigenous residents of North, living in the territory of the RS (Ya) participated in the study. The study was conducted with the written consent of the participants. 153 DNA samples of patients with a diagnosis of type 2 diabetes were tested, 105 of which belonged to women, 48 to men. The comparison group was a sample of 84 healthy volunteers, men (n = 26) and women (n = 58).

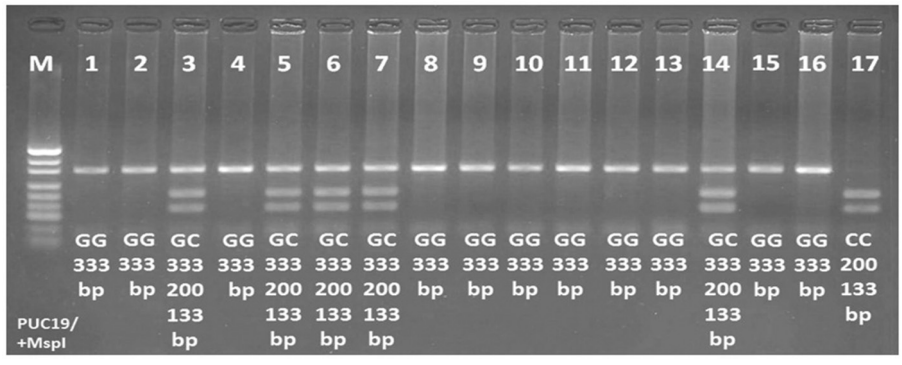

Fig. 1. Electrophoregram of the amplification product of the *PNPLA3* gene site in a 4% agarose gel. 17 - genotype CC, 3, 5, 6, 7, 14 - genotype GC, 1, 2, 4, 8, 9, 10, 11, 12, 13, 15, 16 - genotype of GG. M - marker PUC19 / + Msp I. bp - base pairs.

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

The isolation of DNA from peripheral blood lymphocytes was carried out by a standard phenol-chloroform extraction method. The single nucleotide polymorphism (SNP) of I148M (rs738409) was determined by a PCR-RFLP method.

Amplification of the region of the gene containing the polymorph variant was carried out by standard primer pairs (forward primer: 5'-TGGGCCTGAAGTCCGAGGGT-3 'and reverse primer: 5'-CCGACACCAGTGCCCTGCAG-3') (Biotech Industry Ltd., Moscow) for polymorphism rs738409. The composition of the reaction mixture for PCR (total volume of the reaction mixture was 25 μl): 13 μl of ddH2O, 2.5 μl of 10x PCR buffer, 2.5 μl of 25 mMMgCl2, 2.5 μl of 2.5 mMdNTPMix, 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 performed by the MJMiniGradientThermalCycler (BioRad).

The temperature conditions of the PCR were as follows: 95 ° C for 5 minutes, then 37 cycles at 94 ° C for 30 seconds, 66 ° C for 30 seconds, and 72 ° for 40 seconds, and the final elongation at 72 ° C for 5 minutes. The PCR products were then cut with BstF5 I restriction enzyme (SibEnzyme LLC, Novosibirsk) for 16 hours at 65 ° C. PCR-cut 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 hour and visualized using a gel-documenting system(VilberLourmat, 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 volts for 1 hour. Visualization of restriction products was carried out in UV-rays using a gel-documenting system (VilberLourmat, France) (Figure 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, GG genotype -333 bp.

Statistical analysis of the results of the study was carried out using the program: OfficeMicrosoftExcel 2010, Statistica 8.0. The distribution of genotypes by the investigated polymorphisms was checked for compliance with the Hardy-Weinberg equilibrium using the exact Fisher test. To compare the frequencies of the alleles between different groups, we used the χ2 criterion with the Yates correction for continuity. The expected heterozygosity was calculated by Nei. The results were considered significant, with a value of p <0.05 (p <0.05).

Results and discussion

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

The revealed high frequency of the allele G polymorphism rs738409 of the PNPLA3 gene associated with fat accumulation in the liver in the samples studied is probably related to the adaptive qualities of the organism to the extreme natural conditions of the North. Since it is known that fatty acids entering the bloodstream

Table 1

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

	n		Genotype, %			Allele		χ^2	p
			CC	CG	GG	C	G		
	Patientswithdiabetes 2								
Women	105	H	8,57	30,48	60,95	0,238	0,762	2,688	0,101
		O	5,67	36,28	58,05				
Men	48	H	16,67	25,00	58,33	0,292	0,708	7,488	0,006
		O	8,51	41,32	50,17				
	Healthy								
Women	58	H	6,90	36,21	56,90	0,250	0,750	0,069	0,793
		O	6,25	37,50	56,25				
Men	26	H	23,08	15,38	61,54	0,308	0,692	10,613	0,001
		O	9,47	42,60	47,93				

enter the liver and muscles, where glycogen is the main source of energy. By acting on the process of the decomposition of glycogen, they act as a dissociation factor for oxidation and phosphorylation, causing a smaller terminal yield of ATP and a larger final heat yield. According to the «1000 genomes» project in Asia, the high frequency of G allele is found in Japanese (42.3%). In their studies of the Japanese population of patients with type 2 diabetes, M. Ueyama, N. Nishida (2015) and KanH. with co-authors [5], note the high frequency of the G allele (48-48.8%).The low frequency of G allele is noted in the African American population (19%),in patients with type 2 diabetes - 13.7% [7].

At comparing the mean values of biochemical blood indices in carriers of different genotypes of the PNPLA3 gene (rs738409) in the group of patients with type 2 diabetes, an increased level of triglycerides, fasting glucose and glycated (glycosylated) hemoglobin was observed, the remaining parameters were within the norm (Table 2).

The highest content of triglycerides in the bloodof the investigated sample of diabetes patients was revealed in the GG genotype carriers in comparison with the carriers of the CC and CG genotypes, that is consistent with the findings of research Jean-MichelPetit et al. [7], who found the association of the polymorphism PNPLA3 rs738409 with the fat content in the liver independent of general and visceral obesity and insulin resistance. They believe that adiponutrincan be an important key 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 [7].

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 the alleles and genotypes of the PNPLA3 gene (rs738409) is in accordance with the Hardy-Weinberg law. In patients with type 2 diabetes, a high incidence of allele G (69.5-74.7%) was found with a predominance of the GG genotype (55.8-58.2%).

Thus, it has been established that the frequency of the mutant allele of functional polymorphism of the gene rs738409 PNPLA3 is higher than in other known world populations. The normally functioning protein of the PNPLA3 gene regulates the activity of triglyceride hydrolase and acetyltransferaselisophosphatidic acid. Therefore, it can be assumed that the high frequency of the mutant allele G of the I148M polymorphism of the PNPLA3 gene in Yakuts with type 2 diabetes may be adaptation of the organism to low temperatures. The study of the adiponutrin gene can be an important key to understanding the mechanisms of adaptation to low temperatures and metabolic processes in the indigenous population of the North.

Table 2

The average clinical indices of patients with type 2 diabetes, depending on the genotype

Indicators	CC (n=16)	CG (n=40)	GG (n=78)	Norm
Cholesterol, mmol / l	5,38±0,37	4,96±0,73	5,04±0,52	within3,2-5,6
Triglycerides, mmol / l	2,04±0,31	1,71±0,59	2,32±0,11	within 0,41-1,8
HDL, mmol / l	1,17±0,08	1,32±0,45	1,33±0,40	0,78-1,81
LDL, mmol / L	2,89±0,32	3,02±0,87	2,86±0,59	1,71-3,5
AST, U / L	18,82±1,83	21,53±0,48	19,70±0,39	Women -31 Men – 37
ALT, U / l	20,38±3,42	22,69±0,57	22,86±0,32	Women – 34 Men- 45
AST / ALT	1,11±0,15	1,04±0,39	0,97±0,53	0,91-1,75
Hb. A 1c, %	8,48±0,56	8,34±0,59	8,71±0,55	4-6,2 %
Totalbilirubin, μmol / l	9,47±1,09	9,41±0,62	10,15±0,26	3,4-17,1
Glucose on an empty stomach, mmol / l	8,51±0,96	8,84±0,76	9,19±0,53	3,89 - 5,83

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II. ISSUES OF THE MANIFESTATIONS AND TREATMENT OF COLD TRAUMA

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ACTION OF COLD ON THE ORGANISM. CRYOPROTECTORS AND MEANS OF ANTI-ISCHEMIC TISSUE PROTECTION

ABSTRACT

Temperature is the most important environmental factor affecting humans and animals. The effect of low temperatures on biological objects depends on the degree of phylogenetic maturity of the organism and is realized through various mechanisms including in vitro and in vivo conditions. The report discusses the mechanisms of cold and ischemic damage to biological objects, examines the mechanisms of damage to tissues and organs after heating, and cryoprotection products of biological objects. Particular attention is paid to preparations of pharmacological protection of tissues with antioxidant properties from cold ischemia.

Keywords: mechanisms of cold action on the body, preparations preventing cryo-damage of biological objects.

1.1 Mechanisms of cryo-damage of biological objects

During action of a low temperature on biological objects, including the organism as a whole, there are two main mechanisms of the damaging effect of cold [1, 22, 25, 28, 28, 29, 30].The first, most obvious mechanism, is a direct cryo-damage. Cold impact is crucial when a low temperature leads to frostbite or is used to conserve cells, cell suspensions and tissues.

Damage to biological objects develops both during cooling and during heating. Even before the transformation of water into a solid phase, the rate of metabolic processes slows down, and the cells undergo deep changes in the activity of enzymes. In the development of cryo-damages, the formation of ice is of great importance, which occurs in different ways depending on the freezing rate [12]. In the case of slow cooling, crystallization of water first occurs in extracellular fluids, since they have a higher freezing point than protoplasm and the nucleus. This process leads to an increase in the concentration of salts and other substances in the extracellular space and the disturbance of osmotic equilibrium. The release of water from the cells begins, the mass of ice outside the cells gradually grows, and cells lose water and undergo osmotic compression. This process up to a certain limit contributes to the preservation of cells' life, as the loss of water, in turn, increases the concentration of salts and colloids in the protoplasm, preventing its freezing.However, the continuation of the process leads to an «osmotic shock», disrupting the permeability of the membranes. After reaching the temperature of tissues -21.2 ° C, salts begin to crystallize, membranes break and the death of cells occurs.

In conditions of rapid cooling, the dehydration processes do not have time to develop, small crystals are formed both inside and outside the cells. Microscopic examination reveals minimal changes, however, the protoplasm of cellular elements is already in a state of severe disorganization, leading to cell death, mainly due to damage to membrane structures.

Thus, generalizing the factors that cause cell damage in the direct effect of cold (freezing), we can distinguish the following:

1) cell compression by extracellular ice;

2) increase in the concentration of extra- and intracellular electrolytes;

3) disturbanceof membrane permeability as a result of changes in cell lipoproteins (phase transition and separation of lipids and proteins in the plane of the membrane);

4) rupture of membranes due to rapid loss of water;