27. Burton GJ. [et al.] Pre-eclampsia: pathophysiology and clinical implications. *BMJ.* 2019; 366: l2381

28. Nakimuli A. [et al.] Pregnancy, parturition and preeclampsia in women of African ancestry. *Am J Obstet Gynecol*. 2014; 210(6): 510-520. doi: 10.1016/j.ajog.2013.10.879.

29. Tanaka M. [et al.] Racial disparity in hypertensive disorders of pregnancy in New York State: a 10-year longitudinal population-based study. *Am J Public Health.* 2007; 97(1): 163-70. doi: 10.2105/AJPH.2005.068577.

30. Gerhardt A. [et al.] The G20210A prothrombin-gene mutation and the plasminogen activator inhibitor (PAI-1) 5G/5G genotype are associated with early onset of severe preeclampsia. *J Thromb Haemost.* 2005; 3(4): 686-691.

31. Seremak-Mrozikiewicz A. [et al.] The significance of genetic polymorphisms of factor V Leiden and prothrombin in the preeclamptic Polish women. *J Thromb Thrombolysis.* 2010; 30(1): 97-104. doi: 10.1007/s11239-009-0432-1.

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POLYMORPHISM OF THE *NOS3* GENE IN THE YAKUT POPULATION

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Several studies confirm the role of nitric oxide in the development of inflammatory and fibrous changes in liver parenchyma. However, the role of nitric oxide synthase – *NOS3* gene in LF formation remains unclear. The **aim** of our research was to study the polymorphism of rs1799983 of the NOS3 gene in healthy individuals of the Yakut population. Materials and methods of research: The experimental part of the work was carried out in the Laboratory of Hereditary pathology of the Department of Molecular Genetics of the Yakut Science Center of Complex Medical Problems (YSC CMP). A total of 146 DNA samples of healthy volunteers of Yakut ethnicity from the YSC CMP biomaterial collection were examined. Single nucleotide polymorphism (SNP) was determined by polymerase chain reaction (amplification of specific gene sites) followed by RFLP analysis. As a result of genotyping of the *NOS3* gene polymorphism rs1799983 among the Yakut population, the prevalence of the *G allele* (91.44%) was determined. An analysis of the frequency of occurrence of genotypes of the polymorphic variant rs1799983 of the *NOS3* gene revealed that carriers of the *GG* homozygous genotype prevailed among all examined individuals (83.56%), the heterozygous *GT* genotype was 15.75%, while the homozygous genotype of the *T* allele was found only in 0.69 % of cases. Thus, the study of the *NOS3* gene polymorphism rs1799983 in various ethnic groups may have a perspective in the development of personalized medicine, for the prediction of pathological conditions associated with endothelial dysfunction: liver fibrosis, cardiovascular diseases, obstetric and gynecological pathology, dysfunction of various organs and systems.

Keywords: nitric oxide synthase 3 gene, nitric oxide, polymorphism, *NOS3*, *G894T*, endothelial dysfunction, liver fibrosis, Yakut population.

Introduction. Liver fibrosis (LF) is a key element in the pathogenesis of liver diseases, specifically the degree of LF is clearly associated with the progression of clinical manifestations of socially significant diseases such as chronic viral hepatitis (CVH) [12]. Despite the whole range of preventive and therapy measures being taken to combat viral hepatitis, the number of people with CVH is steadily growing. Thus, from 2013 to 2016 in the Republic of Sakha (Yakutia), the hospitalization rate of people with CVH increased by 130%, with liver cirrhosis - 141%. There is a predisposition of indigenous people to the progressive course of HCV with frequent formation of cirrhosis and liver cancer, mainly in the outcome of HDV infection-52.2 % [2].

Therefore, the urgent issue is the search for new non-invasive methods for diagnosing and predicting fibrotic changes in liver tissue. It is known that the pathogenesis of LF is closely associated with oxidative stress, due to which there is an increase in the production of free radicals, one of which is nitrogen oxide (NO). NO is an active free radical that acts as a key mediator of vasodilation and contributes to the inflammation in liver tissue affected by hepatitis virus [8, 12].

A number of studies confirm the role of NO in the development of inflammatory and fibrous changes in liver parenchyma [10-11]. However, the role of nitric oxide synthase – *NOS3* gene (*eNOS*, *nitric oxide synthase 3*) in LF formation remains unclear. Nitric oxide synthase induces the conversion of L-arginine to endogenous nitrogen monoxide. At this point, polymorphisms at 11 loci have been studied, 8 *NOS3* polymorphisms associated with cardiovascular diseases have been described, one of which is the single nucleotide polymorphism rs1799983 *(Glu-298Asp, E298D, G894T),* the action of the risk allele *T* can be associated with endothelial dysfunction [3, 13].

The rs1799983 polymorphism of the *NOS3* gene has been studied in many human populations, but this polymorphism has not been sufficiently studied in the Yakut population.

The aim of our research was to study the polymorphism of rs1799983 of the *NOS3* gene in healthy individuals of the Yakut population.

Materials and methods of research: The experimental part of the work was carried out in the Laboratory of Hereditary pathology of the Department of Molecular Genetics of the Yakut Science Center of Complex Medical Problems (YSC CMP). DNA samples from the YSC CMP biomaterial collection were used for the research. A total of 146 DNA samples of healthy volunteers of Yakut ethnicity were examined (including Yakuts in the third generation). Of these, 31 DNA samples belonged to male individuals and 115 samples to female individuals. The average age of the study subjects was 30.51 ± 10.92. Criteria for exclusion from the study were: age under 18, chronic cardiovascular disease, oncological diseases, viral hepatitis, alcohol abuse. All the studied individuals filled out a questionnaire with informed consent to genetic research, approved by the Local Committee on biomedical ethics at the YSC CMP.

DNA was extracted by a standard method from frozen whole blood. Single nucleotide polymorphism (SNP) was determined by polymerase chain reaction (amplification of specific gene sites) followed by RFLP analysis.

Table 1 presents the amplification protocol, the sequences of oligonucleotide primers used, the PCR temperature and amplification length.

The restriction conditions, the used restriction endonuclease and the length of the restriction fragments are presented in table 2. For restriction, 5 µl of amplification, 1 µl of restriction buffer, 0.2 µl of restriction endonuclease *MboI* (New England Biolabs Inc., USA) and 8.8 µl of deionized water were used.

The resulting genotypes were determined by analysis of the lengths of restriction fragments (restricts) by gel

Table 1

PCR primers and conditions

SNP	Primer Sequence	Temperature	Amplicon size
rs1799983	F: TCACGGAGACCCAGCCAATGAG R: TCCATCCCACCCAGTCAATCCC	1. 95°C – 5 min 2. (95°C – 35 s; 64°C – 35 s; 72°C – 35 s) * 35 cycles 3. 72°C – 5 min	292 bp

Table 2

Restriction conditions

SNP	Endonuclease	Conditions	Restriction fragments size
rs1799983	*MboI*	37°C - 15 min	292 bp - *GG* 197 and 95 bp - *TT* 292. 197. and 95 bp - *GT*

electrophoresis on a 2% agarose gel with ethidium bromide (3,8-Diamino-5-ethyl-6-phenylphenanthridium bromide, Ethidium bromide) at 120 V in standard tris- acetate buffer for 1 hour. The results were visualized using a gel-documenting system in UV rays (Vilber Lourmat, France) (Fig. 1).

The research data were processed by statistical programs "Office Microsoft Excel 2010" and "IBM SPSS Statistics 23". Frequency of alleles and genotypes of rs1799983 polymorphism of the *NOS3* gene was checked for compliance with Hardy - Weinberg equilibrium. Frequency of alleles and genotypes between groups of men and women was compared using the criterion χ2 with Yates correction for continuity. The results were considered significant, with a value of *p* less than 0.05 ($p<0.05$).

Results and discussion. As a result of genotyping of the *NOS3* gene polymorphism rs1799983 among the Yakut population, the prevalence of the *G allele* (91.44%) was determined, which corresponds to the previously published results of a study conducted by Mestnikova E.N. et al. (2018) [1], according to which the Yakut population was also characterized by the predominance of the G *allele.* An analysis of the frequency of occurrence of genotypes of the polymorphic variant rs1799983 of the *NOS3* gene revealed that carriers of the *GG* homozygous genotype prevailed among all examined individuals (83.56%), the heterozygous *GT* genotype was 15.75%, while the homozygous genotype of the *T* allele was found only in 0.69 % of cases (Table 3).

Analysis of genotype distribution depending on gender revealed no significant differences (χ2=1.131, *p*=0.288). It was found that among women, as well as men, carriers of the homozygous genotype *GG* prevailed (83.87%, 83.48%, respectively). The GT genotype was found in 16.13% of men and 15.65% of women. The incidence of the T allele was 8.07% in the male group and 8.7% in the female group, without statistically significant differences (table 4).

According to open sources of the project "1000 genomes" [15], a high incidence of *allele* T was observed in the European population (indigenous people of Northern Europe - 36%, Finns - 23%, British - 34%, Spaniards - 38%, Tuscans - 40%), as well as in Colombians – 27%, Mexicans – 20%, Puerto Ricans – 28%. Most rarely the *T* allele is found in the African population (Kenyans - 4%, residents of Sierra Leone - 5%, Yoruba, Nigeria - 6%). Among Asian populations, the *T* allele is more common among Vietnamese (16%), Chinese (15%), more rarely - among Japanese (8%) (figure 2).

When compared with world populations according to the "1000 genomes", the *T* allele among Yakuts is less common than among the population of North America, South America, Europe, India, Oceania and China. The prevalence of this allele is comparable to Japan, where the *T* allele is found in 8% of the population [15].

When comparing the results of this study, allele *T* polymorphism of rs1799983 gene *NOS3* in the Yakut population was less common than in previous population studies. Thus, in the research by *Bae* et al (2010), the incidence of the *T* allele in the indigenous people of Korea was 18.6% [7]. In the Japanese population, the *T* allele was found in 15.6%, according to the research by Tamemoto et al (2008) [14]. In 2015 Huo et al. examined 420 young healthy Chinese and found the *T* allele in 28.09% of those examined [5]. Similar results were obtained by Indonesian geneticists Thaha et al. The *T* allele was found in 28.84% [6], while in the Asian population of India - 32.89% [4] (table 5).

Thus, the study of the *NOS3* gene polymorphism rs1799983 in various ethnic groups may have a perspective in the development of personalized medicine, for the prediction of pathological conditions associated with endothelial dysfunction: liver fibrosis, cardiovascular diseases, obstetric and gynecological pathology, dysfunction of various organs and systems.

Conclusion. The results of this research showed that the healthy population of the Yakuts living in the territory of the Sakha Republic (Yakutia), is characterized by a significant predominance

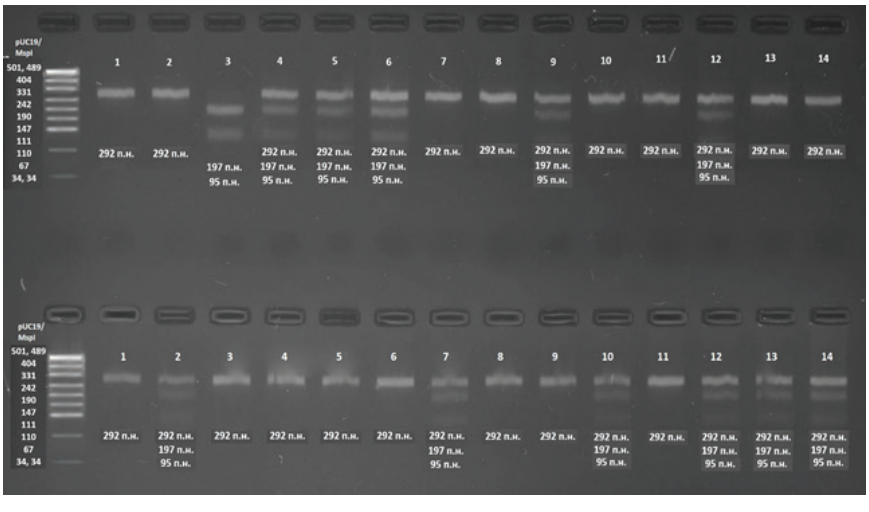

Fig. 1 Electrophoregram of PCR- RFLP. M - marker PUC19/+Msp I. Series 1. 1, 2, 7, 8, 10, 11, 13, 14 – GG genotype, 4, 5, 6, 9, 12 – GT genotype, 3 – TT genotype. Series 2. 1, 3, 4, 5, 6, 8, 9, 11 – GG genotype, 2, 7, 10, 12, 13, 14 – GT genotype. П.н. – bp.

Table 3

The frequency of occurrence of genotypes and alleles of polymorphism rs1799983 of the NOS3 gene among the Yakut population

Group	Распр	Genotype			Allele		χ2	p
		GG	GT	TT	G	T		
n=146	H	122 (83.56)	23 (15.75)	1 (0.69)	267 (91.44)	25 (8.56)	0.06	0.94
	O	122.07 (83.61)	22.86 (15.66)	1.07 (0.73)				

Note: O is the observed distribution, E is the expected distribution.

Table 4

The prevalence of genotypes and alleles in groups of men and women

Groups	Genotype			Allele		χ2	p
	GG	GT	TT	G	T		
Men (n=31)	26 (83.87)	5 (16.13)	0	57 (91.93)	5 (8.07)	1.131	0.288
Women (n=115)	96 (83.48)	18 (15.65)	1 (0.87)	210 (91.3)	20 (8.7)		

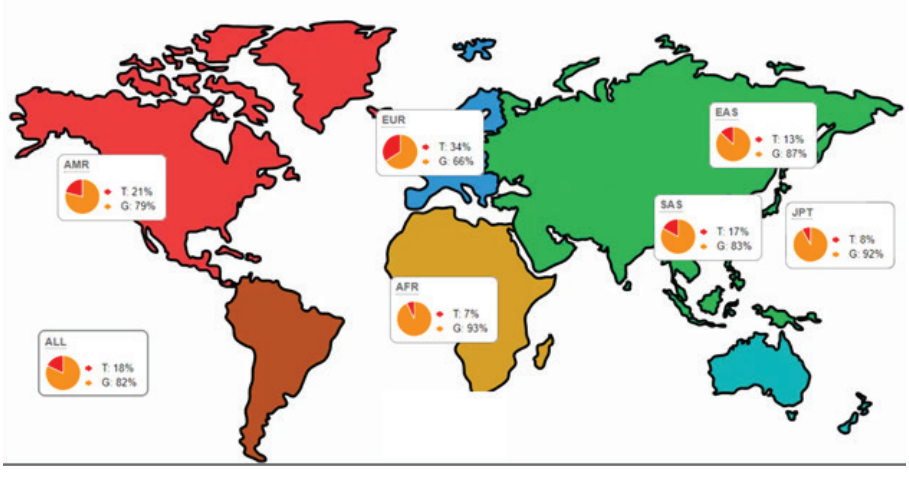

Рис. 2. Частота встречаемости аллелей, по данным «1000 геномов»

of carriers of allele *G* (91,44%) and *GG* genotype (83,56%) polymorphic variant rs1799983 *NOS3* gene.

Credits. *The study was carried out within the framework of the research project Study of the genetic structure and burden of congenital defects of the populations of the Sakha Republic (Yakutia) using the UNU "Genome of Yakutia" (reg. No. USU_507512).*

Table 5

The prevalence of the G allele of the polymorphic variant rs1799983 of the *NOS3* gene in Asian populations of the world

Populations	Genotypes			p	Authors
	GG	GT	TT		
Yakutia (n=146)	122	23	1	-	-
South Korea (n=161)	131	30	0	0,192	[7]
Japan (n=211)	181	27	3	0,101	[14]
China (n=420)	315	92	13	0,047	[5]
Indonesia (n=104)	76	26	2	0,845	[6]
India (n=152)	114	46	2	0,263	[4]

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References

1. Местникова Е.Н. [и др.]. Взаимосвязь полиморфизма генов ACE и NOS3 с уровнем артериального давления у спортсменов Республики Саха (Якутия). *Педагогико-психологические и медико-биологические проблемы физической культуры и спорта*. 2018; 18(2): 244-251. [Mestnikova EN [et al.]. The relationship of ACE and NOS3 gene polymorphism with the level of blood pressure in athletes of the Republic of Sakha (Yakutia). *Pedagogiko-psihologicheskie i mediko-biologicheskie problemy fizicheskoj kul'tury i sporta*. 2018; 18(2): 244-251 (In Russ.)].

2. Софронова А.Р. [и др.]. Причины прогрессирования хронических вирусных гепатитов в Якутии. *Вестник Северо-Восточного федерального университета им. М.К. Аммосова. Серия: Медицинские науки*. 2016; 3 (4): 92-95. [Sofronova A.R. [et al.]. Causes of the progression of chronic viral hepatitis in Yakutia. *Vestnik Severo-Vostochnogo federal'nogo universiteta im. M.K. Ammosova. Seriya: Medicinskie nauki*. 2016; 3 (4): 92-95 (In Russ.).]

3. Acute-on-chronic liver disease enhances phenylephrine-induced endothelial nitric oxide release in rat mesenteric resistance arteries through enhanced PKA, PI3K/AKT and cGMP signalling pathways. Caracuel L [et al.]. *Scientific Reports*. 2019; 9(1): 69-93. DOI: 10.1038/s41598-019-43513-y

4. Narne P [et al.]. Association Analysis of Polymorphisms in Genes Related to Oxidative Stress in South Indian Type 2 Diabetic Patients with Retinopathy. *Ophthalmic Genetics*. 2016; 37(1): 1–8. DOI: 10.3109/13816810.2014.895015

5. Huo P [et al.]. Association between genetic polymorphisms of ACE & eNOS and diabetic nephropathy. *Molecular Biology Reports*. 2015; 42(1): 27–33. DOI: 10.1007/s11033-014-3736-y

6. Thaha M [et al.]. Association of endothelial nitric oxide synthase Glu298Asp polymorphism with end-stage renal disease. *Clin Nephrol*. 2008; 70: 144–154. DOI: 10.5414/CNP70144

7. Bae J [et al.]. Association of endothelial nitric oxide synthase polymorphisms with coronary artery disease in Korean individuals with or without diabetes mellitus. *Exp Ther Med*. 2010; 1(4): 719–724. DOI: 10.3892/etm_00000111

8. Dong J, Ping Y, Wang Y, Zhang Y. The roles of endothelial nitric oxide synthase gene polymorphisms in diabetes mellitus and its associated vascular complications: a systematic review and meta-analysis. *Endocrine*. 2018; 62(2): 412–422. DOI: 10.1007/s12020-018-1683-4

9. Heidari MM, Khatami M, Tahamtan Y. Molecular Analysis of rs2070744 and rs1799983 Polymorphisms of NOS3 Gene in Iranian Patients With Multiple Sclerosis. *Basic Clin Neurosci*. 2017; 8(4): 279–284. DOI: 10.18869/nirp.bcn.8.4.279

10. Iwakiri Y. Nitric oxide in liver fibrosis: The role of inducible nitric oxide synthase. *Clinical and Molecular Hepatology*. 2015; 21(4): 319–325 DOI: 10.3350/cmh.2015.21.4.319

11. Zhang X [et al.]. Nitric oxide inhibits autophagy and promotes apoptosis in hepatocellular carcinoma. *Cancer Science*. 2019; 110(3): 1054–1063. DOI: 10.1111/cas.13945

12. Li H [et al.]. Regulation of endothelial-type NO synthase expression in pathophysiology and in response to drugs. *Nitric Oxide*. 2002; (7): 149–164

13. Jin L [et al.]. Role and regulation of autophagy and apoptosis by nitric oxide in hepatic stellate cells during acute liver failure. *Liver International: Official Journal of the International Association for the Study of the Liver*. 2017; 37(11): 1651–1659. DOI: 10.1111/liv.13476

14. Tamemoto H, Ishikawa S-E, Kawakami M. Association of the Glu298Asp polymorphism of the eNOS Gene with ischemic heart disease in Japanese diabetic subjects. *Diabetes Research and Clinical Practice*. 2008; 80(2): 275–279. DOI: 10.1016/j.diabres.2007.12.019

15. Сайт «1000 геномов» [Электронный ресурс]: http://www.internationalgenome.org/

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ETHNIC DIFFERENCES IN DISTRIBUTIONS OF ALLELIC AND GENOTYPIC FREQUENCIES OF *NAT2* POLYMORPHIC VARIANTS IN PATIENTS WITH PULMONARY TUBERCULOSIS

Ethnic differences in allele frequencies and genotype distribution for polymorphic variants of *NAT2* gene (NAT2*5, NAT2*6, NAT2*7) were studied among ethnic Yakuts and Russians with newly diagnosed pulmonary tuberculosis.

This is the first study to establish allele and genotype frequencies for polymorphic variants of *NAT2* gene (NAT2*5, *6, *7) among ethnic Yakuts and Russians, who permanently reside in the Sakha Republic (Yakutia). Prevalence of NAT2*5, *6, *7 polymorphisms among Yakuts and Russians was determined, and ethnic differences were shown in allele frequencies and genotype distribution of NAT2 polymorphic variants: rs1801280 (341T>C), rs1799930 (590G>A), and rs1799931 (857G>A).

Keywords: *NAT2* gene polymorphism, Yakuts, Russians, tuberculosis, adverse drug effects

Introduction. Treatment success indicators are remaining at low levels in Russia. Based on official statistical data, in 2016, effective chemotherapy outcomes were registered in 74.3% out of all new pulmonary TB cases treated with regimens I–III (irrespective of microscopy results at case notification), or in 64.3% of cases with positive sputum microscope results [4]. Clinically, low treatment effectiveness in new TB cases resulted from nonadherence to chemotherapy duration, treatment interruptions, temporary cancellation of drugs due to adverse drug effects (ADE) (up to 61% cases [3] treated for drug-sensitive TB). Development of ADE is associated with variations in drug kinetics, and can be stemming from both transient (inhibition of biotransformation enzymes, as a result of drug interactions, eating habits) and constant causes (sex, concomitant diseases, genetic mutations in genes encoding enzymes involved in drug metabolism) [15, 24].

Genetic polymorphisms have been established and thoroughly researched for N-acetyltransferase (NAT) 2. NAT2 is an enzyme participating in biotransformation phase II, and is responsible for acetylation of more than 70% of xenobiotics, including therapeutic agents [21]. Single-nucleotide substitutions in the structural region of NAT2 gene determine genetic variations in enzyme activity [1,12], and have been linked to decreased or increased rates of xenobiotic metabolism [2]. NAT2 polymorphisms considered the most clinically important in terms of effective and safe TB chemotherapy are: *4, *5, *6, *7, *12, *13, *14. Patients with slow acetylation alleles (NAT2*5, *6, *7, *14) have been shown to be at risk for