ORIGINAL RESEARCHES

A.T. Diakonova, N.I. Pavlova, N.A. Solovyeva,
M.A. Varlamova, T.N. Alexandrova, Kh.A. Kurtanov

MOLECULAR-GENETIC ANALYSIS OF THE CONNECTION OF THE SLC6A3 GENE WITH NICOTINE ADDICTION IN YAKUTIA

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ABSTRACT

The purpose of this work is to study the relationship of the gene *SLC6A3* rs27072 with nicotine addiction in the smoking population living in Yakutia. In total, we examined 100 people (men and women aged 20 to 70 years). The status of smoking was revealed at the questionnaire. Smoking cigarettes, like many other addictives, has a genetic component. The *SLC6A3* gene is an important candidate for predisposition to nicotine addiction. Analysis of the polymorphism association of *SLC6A3* rs27072 with nicotine addiction testified the absence of statistically significant differences between carriers of different genotypes, not only in the study group as a whole, but also separately in men and women. Probably, this is due to the small sampling and difficulties in determining the status of smoking using only questionnaires.

Keywords: smoking, nicotine addiction, *SLC6A3* gene, Yakutia, polymorphism.

Introduction. Cigarette smoking remains widespread, a risk factor for more than two dozen diseases and is the biggest cause of death all over the world. Smoking damages virtually all systems of the human body and is a habit that can be got rid of. Smoking of tobacco causes psychological and physiological addiction and, in addition, is closely related to social and cultural factors. Despite widely recognized risks, about one third of the world's adult population continues to smoke tobacco [1].

According to the World Health Organization (WHO), smoking causes nearly 6 million deaths each year, of which more than five million occur among smokers. The Fig. 1 shows the prevalence of smoking in the world. Smoking prevalence is prevalent in Asian countries [2].

Also among non-smoking people, more than 600,000 are exposed to second-hand tobacco smoke. WHO estimates that tobacco contains more than 7,000 chemical compounds, 60 of which are known or suspected carcinogens, i.e. cause changes in the cells of the body leading to the development of cancer, and 250 have a proven cytotoxic effect. Eleven substances contained in tobacco smoke (2-naphthylamine, 4-aminobiphenyl, benzene, vinyl chloride, ethylene oxide, arsenic, beryllium, nickel compounds, chromium, cadmium and polonium-210), the International Agency for Research on Cancer refers to the first group of carcinogens ie with a proven carcinogenic effect) [2].

It is assumed that there is a central pathophysiological mechanism for maintaining addiction on psychoactive substances, under genetic control. This mechanism is independent of the specific type of psychoactive substance and causes neurochemical changes in the future patient even before the meeting with psychoactive substances, and this includes nicotine addiction. Which determines the biological basis of the predisposition proper. However, the conclusions between previous studies are difficult to explain because smoking cigarettes is a very complex process, influenced by various factors such as age, sex, environment [1].

The dopamine transporter gene (*SLC6A3*) localized in the short arm of chromosome 5 (5p15.3) participates in the control of dopaminergic transmission. The rs27072 polymorphism of the *SLC6A3* gene is associated with more severe symptoms with an alcohol withdrawal syndrome, such as convulsions. Many authors report that the gene of the dopamine transporter (*SLC6A3*) is associated with the syndrome of hyperactivity and attention deficit disorder (ADHD). A link was also found between the transporter gene of dopamine and the age of onset with regard to the use of tobacco and alcohol. Studies conducted predominantly in the European population have revealed that the allele of this polymorphism increases the risk of smoking, while studies in the Japanese population have shown a link between this genotype and smoking. Previously, the influence of polymorphisms on ethnicity was also considered. In association studies between the variant *ANKK1 / DRD2* and *SLC6A3* alleles and smoking, it was suggested that the presence of the *ANKK1 / DRD2* Taq I allele along with the A *SLC6A3* allele increases the craving for cigarettes, causing addiction. In addition, several studies have suggested that, compared to non-carriers, carriers of the A *SLC6A3* allele have a lower risk with early onset of smoking [3, 4, 6].

The results of population studies in different countries are presented in Table 1. In the world, the frequency of allele A

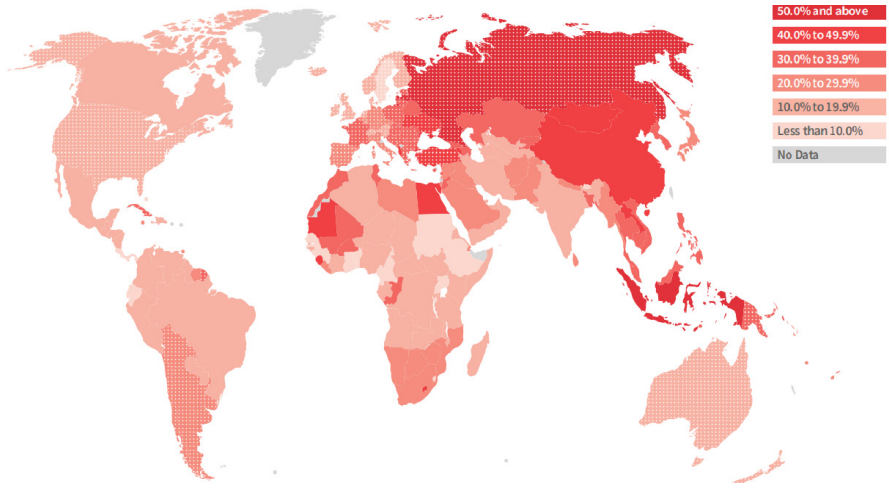

Fig.1. Prevalence of tobacco smoking in the world: light to dark shows the increase in the percentage of smoking population by country (WHO data on trends in tobacco prevalence, 2015)

was 21%. Studies were conducted in the Chinese population of Han (South China) in which the percentage of allele A was 31%. Also in the Chinese population of Dai (China) was A - 31%, which is the highest rate in the world. In the population of African descent (South-West USA), the lowest A is 10%. In the Yoruba population (Nigeria), the percentage of allele A was 13%, which is also one of the least.

The authors who studied the *SLC6A3* gene relationship with the nicotinic addiction insisted that it had an effect on smoking cessation [4, 8].

The **purpose** of this work was to study the relationship of the gene *SLC6A3* rs27072 with nicotine addiction in the smoking population living in Yakutia.

Materials and methods of the research. The experimental part of the genotyping of polymorphism rs27072 of the *SLC6A3* 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 were used for the study using the UMU "Genome of Yakutia" (registration #USU_507512). The study involved residents of the Republic of Sakha (Yakutia). The study was conducted with the written consent of the participants. A total of 97 DNA samples, 45 males and 52 females, were examined.

Genomic DNA was extracted from the peripheral blood of each participant using the Excell Biotech DNA Excellence Kit (Russia) in accordance with the manufacturer's instructions. The DNA concentration in each sample was determined on an Implen Nano Photometer (Germany) spectrophotometer for measurement in microvolumes. Single nucleotide polymorphisms (SNP) were determined by polymerase chain reaction (PCR-RFLP). Amplification of the region of a gene containing a polymorphic variant was carried out by standard pairs of primers produced by Biotech-Industriya LLC, Moscow. Reaction mixture primer direct and reverse 1 µl; Dream Taq PCR master mix -12.5 µL; 9.5 µl of desionized water and 1 µl of DNA. The total volume of the reaction mixture for amplification was 25 µl. The restriction mixture was 20 µl, 7 µl of amplification, 10.9 µl of desionized water, 2 µl of restriction buffer, and 0.1 µl of restriction endonuclease MspI.

The temperature-time mode for conducting PCR is optimized to amplify this nucleotide sequence and is presented in Table 2.

The detection of PCR products was carried out by horizontal electrophoresis in a 2% agarose gel plate with the addition of ethidium bromide-a specific intercalating fluorescent DNA (RNA) dye-using a standard tris-acetate buffer at a field strength of ~ 20 V / cm for 30 minutes.

After PCR amplification was subjected to restriction with the use of endonuclease Msp I (OOO SibEnzim, Novosibirsk) for 3 hours at 37 ° C. The detection of RFLP products was carried out by horizontal electrophoresis in a plate of 4% agarose gel with the addition of ethidium bromide using standard tris-acetate buffer at a field strength of ~ 20 V / cm for 45 minutes (Figure 2)

Interpretation of genotyping results was performed on the basis of different band patterns: GG genotype 137, 80 bp, AG genotype 217, 137 and 80 bp, AA genotype 217 bp.

Statistical analysis of the results of the research was carried out using the program: "Office Microsoft Excel 2010". The correlation of the genotype distributions with the expected values at the Hardy-Weinberg equilibrium and the comparison of the frequencies of the allelic variants / genotypes was carried out using the Pearson method for the 2x2 conjugacy tables, OR, 95% confidence interval (95% CI) . Differences were considered reliable at P <0.05.

Results and discussion. As a result of genotyping polymorphism rs27072 of the *SLC6A3* gene, it was established that the incidence rate of the GG genotype among all the examined individuals is 69.1%. The allele frequency G was 80.9% (Table 3).

In our sample, smoking frequency of allele A was 17, 34%, in the non-smoking sample- 20, 83%. Also, with the gender division in the groups of smokers (women) and non-smokers (men), the frequency of allele A was 8.7%, and in the groups not smoking (women) and smoking (men) - 22.4%. The results of the association evaluation of rs27072 polymorphism of the *SLC6A3* gene showed that the incidence of the A allele in the sample of smokers and non-smokers is not significantly different. However, in the sample of smokers, the number of carriers of the homozygous AA genotype exceeded the number with such a genotype in the non-

Table 1

Frequency distribution of alley polymorphism rs27072 gene *SLC6A3*

Allele	Population				
	All	Southern Han Chinese, China	Chinese Dai, China	African in Southwest, US	Yorubain Ibadan,
Allele A (%)	Nigeria	31	31	10	13
Allele G (%)	79	69	69	90	87

Table 2

PCR conditions

Gene	Amplificate	PCR conditions
SLC6A3	217 п.н.	1. 95°C – 5 min 2. (94°C – 30 sec; 62°C – 30 sec; 72°C – 1 min)-35 cycles 3. 72°C – 7 min

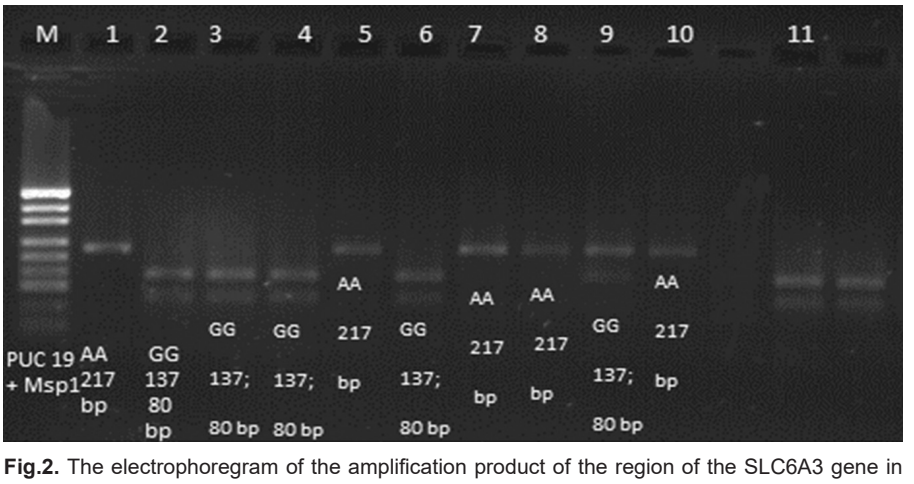

Fig.2. The electrophoregram of the amplification product of the region of the SLC6A3 gene in a 4% agarose gel: tracks number 1, 5, 7, 8, 10, - genotype AA; № 2, 3,4, 6 and 9 - genotype of GG; M - marker PUC19 / + Msp I, Bp - base pairs.

smoking sample by a factor of two. Allele in smokers A allele reaches 17.3%, which is almost the same as the percentage of allele A in the sample of non-smokers. The analysis of statistical data between samples of men and women showed similar results in the two groups. This is most likely due to the fact that at a small sample it is necessary carefully refine the unreliability of the detection of smoking in the survey, adding aspects such as length, the number of cigarettes smoked.

Analysis of the association of *SLC6A3* rs27072 polymorphism with nicotine addiction indicated that there was no statistically significant difference between carriers of different genotypes, not only in the study group as a whole, but also separately in men and women (Table 3).

The conclusion. The results of a study of polymorphism in the smoking population among residents of the Republic of Sakha (Yakutia) found that among the surveyed persons prevalence was 81% among smokers and in non-smoking 76% of the G. allele, which is not associated with nicotine addiction. In the sample of smokers, the number of carriers of the homozygous AA genotype exceeded the number with such a genotype in the non-smoking sample by a factor of two. But on the whole, according to the statistical data, it turned out that the reliability of the samples was not found.

Thus, as a result of this study, we found that polymorphism rs27072 of the *SLC6A3* gene did not reveal a connection with nicotinic addiction in the studied sample. This probably depends on the small sample size and the difficulty in determining smoking using questionnaires. Since we were based only on the honesty of our respondents, in the future, when composing samples, it is necessary to group them according to the age and age of the beginning of smoking.

The research was carried out within the framework of the R & D study of the genetic structure and cargo of the hereditary pathology of populations of the Republic of Sakha (Yakutia).

Table 3

Frequency distribution of alleles and genotypes of rs27072 polymorphism of the *SLC6A3* gene

Comparable groups	Frequency of occurrence of genotypes, abs. (%)			Frequency of occurrence of alleles,%		OR (95% CI), P	*p*
	AA	AG	GG	A	G	Allele of risk A	
Smoking	5 (10.2)	7 (14.28)	37 (75.51)	17 (17.34)	81 (82.65)	1.247 (0.389-1.636)	0.537
Do not smoke	2 (4.16)	16 (33.33)	30 (62.5)	20 (20.83)	76 (79.16)		
Smoking Women	1 (4.4)	2 (8.7)	20 (86.9)	4 (8.7)	42 (91.3)	0.991 (0.100-1.091)	0.061
Do not smoke Women	1 (3.5)	11 (37.9)	17 (58.6)	13 (22.4)	45 (77.6)		
Smoking Men	4 (13.8)	5 (17.3)	20 (68.9)	13 (22.42)	45 (77.58)	9.125 (0.916-10.041	0.061
Do not smoke Men	1 (4.34)	2 (8.69)	20 (86.95)	4 (8.7)	42 (91.3)		

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The authors

YSC CMP, Yakutsk, Russia:

1. D'yakonova Aleksandra Timofeevna – junior researcher of the laboratory of heritable pathology Tel.: 8 (914) 238 68 93. E-mail: dyakonovaa@bk.ru;

2. Pavlova Nadezhda Ivanovna – PhD, temporary acting chief scientific officer - head of the laboratory of heritable pathology. Tel.: +7 (914) 289 39 36. E-mail: solnishko_84@inbox.ru;

3. Solov'eva Natal'ya Alekseevna – PhD, temporary acting chief scientific officer - head of the Laboratory of Population Genetics. Tel.: 8 (924) 171 34 89. E-mail: sonata608@yandex.ru;

4. Varlamova Marina Alekseevna –researcher of the laboratory of heritable pathology Tel.: 8 (914) 298 87 60. E-mail: varlamova.m@yandex.ru.

5. Aleksandrova Tujara Nikonovna - Junior researcher of the laboratory of heritable pathology E-mail: alexandrova_tyara@mail.ru

6. Kurtanov Khariton Alekseevich – PhD, Chief Scientific Officer - Head of the Department of Molecular Genetics. Tel.: +7 (914) 106 00 30. E-mail: hariton_kurtanov@mail.ru.