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Analysis of the Association of 5-Hydroxytryptamine Receptor 2A Gene Variants in Nicotine Addiction in the Yakut Population

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Abstract

The aim of this study was to investigate the relationship between the *HTR2A* 1438A/G (rs6311) SNP and the risk of nicotine addiction in Yakuts.

Methods and Results: A total of 292 people of Yakut nationality were tested (77 women and 215 men). Two groups of examined persons were formed: smokers (n=141) and non-smokers (NS, n=151). To determine the association of the studied SNP with the degree of smoking, the smoker group was divided into two subgroups: Heavy smokers (HS) and Light smokers (LS). Subjects who were smokers were classified based on their consumption of cigarettes per day (cpd), as follows: LS (n=10), those who consumed between 1 and 10 cpd, and HS (n=131), those who consumed ≥20 cpd. The study of the *HTR2A* 1438A/G (rs6311) SNP was performed by PCR and RFLP analysis. The A allele of the *HTR2A* 1438A/G (rs6311) SNP was associated with more of a risk for the HS group than the LS. Using the A allele as a risk factor, RR analysis showed a significant association with a risk factor when comparing HS with LS (RR=1.086; 95% CI=1.032-1.142; *P*=0.049). The analysis of the OR and RR between the HS and LS showed that the AA and AG genotypes were associated with an increased risk for heavy smoking (OR=9.714; 95% CI=1.196-78.874; RR =1.126; 95% CI=1.027-1.235; *P*=0.026). We cannot dismiss the possibility that our results may reflect the small sample size. Despite this potential limitation, the significant associations should not be disregarded and merit further investigation for clarification of these results.(**International Journal of Biomedicine. 2022;12(1):151-154.**)

Key Words: HTR2A • rs6311 • SNP • addictive behavior

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Introduction

Smoking is one of the causes leading to the development of serious diseases that significantly reduce life expectancy. Worldwide, more people die from smoking than from alcohol, cocaine, and heroin, as well as from AIDS, violent death, car and plane crashes combined. The state of dependence on nicotine, alcohol, and drugs is included in one category of ICD-10: "mental and behavioral disorders due to the use of psychoactive substances." According to the World Health Organization, in 2018, Russia ranked 34th in the world in number of tobacco users, which is 28.3% of Russians (34.2 million inhabitants) over 15 years old.(1) As for the regions of Russia, according to Rosstat for 2019, Chukotka has the largest percentage of smokers (39%) of the adult population. In second place is the Jewish Autonomous Region - 37.3%. In the third place - the Trans-Baikal Territory (36.3%). In the Republic of Sakha (Yakutia), the share of the smoking population was 34.4%, that is, in fifth place among the smoking population in Russia. Nicotine addiction is a chronic disease, similar to other types of substance dependence, requiring repeated interventions to achieve and maintain sustainable smoking cessation. The nicotine contained

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in tobacco causes the development of a strong commitment to smoking. Research suggests that nicotine is more addictive than cocaine, ethanol, heroin, caffeine, and marijuana, both in terms of physical and psychological dependence. Nicotine acts as a full agonist at nicotinic acetylcholine receptors (n-AChR) in the central nervous system, activates dopaminergic pathways in the mesolimbic system of the brain, and thus promotes craving and addiction.[2,3] Therefore, genes encoding receptors involved in dopaminergic and serotonergic pathways are potential candidates in the mechanisms of nicotine dependence.

The *HTR2A* gene is located on chromosome 13 and includes three exons and two introns with a length of more than 63 kb, and it contains more than 200 single nucleotide polymorphisms (SNPs). This gene has two known functional SNPs, T102C (rs6313) and A1438G (rs6311), which have been associated with smoking addiction. Some studies have found that SNPs rs6311 (1438A/G) in the promoter region and rs6313 (102T/C) in exon 1 may be associated with a higher risk of cigarette smoking among Caucasians. [4-6]

We have studied for the first time the association of the *SLC6A3* rs27072 SNP with nicotine addiction in the smoking population and the *ABCB1* C3435T SNP in Yakuts associated with addictive behavior. An analysis of the association of the *SLC6A3* rs27072 with nicotine addiction indicated 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.[7] It has been shown that nicotine alters the expression of ABCB1 and that 4-methylnitrosamino-1-3-pyridyl-1-butanone, which is an ingredient in cigarette smoke, induces the *ABCB1* gene by expressing mRNA.[8,9] Therefore, it can be hypothesized that *ABCB1* genetic polymorphisms may interact with mechanisms associated with substance addiction, including nicotine addiction.[10]

The aim of this study was to investigate the relationship between the HTR2A 1438A/G (rs6311) SNP and the risk of nicotine addiction in Yakuts.

Materials and Methods

The study was carried out in the Department of Molecular Genetics at the Yakut Scientific Center for Complex Medical Problems (YSC CMP). A total of 292 people of Yakut nationality were tested (77 women and 215 men). Two groups of examined persons were formed: smokers (n=141) and non-smokers (NS, n=151). To determine the association of the studied SNP with the degree of smoking, the smoker group was divided into two subgroups: Heavy smokers (HS) and Light smokers (LS). Subjects who were smokers were classified based on their consumption of cigarettes per day (cpd), as follows: LS (n=10), those who consumed between 1 and 10 cpd, and HS (n=131), those who consumed ≥20 cpd.

The study was approved by the Ethics Committee of the Yakut Science Center of Complex Medical Problems (YSC CMP). Written informed consent was obtained from each research participant.

Genomic DNA samples were isolated from the peripheral blood leukocytes using a commercial kit for DNA isolation Excell biotech (Yakutsk, Russia). The study of the *HTR2A* 1438A/G (rs6311) SNP was performed by PCR and RFLP analysis. Primer sequences, conditions for amplification, restriction enzymes, and the lengths of the restoration fragments are presented in Table 1.

Table 1.

The primers and restriction enzymes used for detection of the HTR2A 1438A/G (rs6311) SNP using PCR-RFLP methods

Primers	Annealing temperature (˚C)	Restriction enzyme	PCR-RFLP results (bp)
5'- AAC CAA CTT ATT TCC TAC CAC -3'	57 °C	MspI	Allele A: 468 Allele G: 244 and 224
5'-AAG CTG CAA GGT AGC AAC AGC -3'			

bp - base pair

PCR products were detected 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 voltage of ~ 20V/cm for 30 minutes. After PCR, the amplificate was subjected to restriction using MspI endonuclease (OOO SibEnzim, Novosibirsk) for 3 hours at 37°C. RFLP products were detected by horizontal electrophoresis in 4% agarose gel stained with ethidium bromide using a standard Tris-acetate buffer at 120V for 1 hour.

Fragments containing the uncut A allele had a 468-bp band; fragments containing the G allele had two bands of 244 and 224 bp.

Statistical analysis was performed using Microsoft Excel 2010. The comparison of the frequencies of allelic variants/genotypes was performed using the chi-square test with Yates correction. The odds ratio (OR), relative risk (RR) and the corresponding 95% CI were calculated to estimate the strength of the association. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

An analysis of the frequency distribution of alleles and genotypes of the *HTR2A* 1438A/G (rs6311) SNP showed that in the studied sample of Yakuts (men and women), the G allele was predominant (75.2%) and the frequency of occurrence of the GG genotype was 53.4%. The frequency of the AA genotype in women was three times higher than in men: 6.5% and 1.9%, respectively (P=0.046) (Table 2).

The frequency distribution of alleles and genotypes of the *HTR2A* 1438A/G (rs6311) SNP did not differ significantly between smokers and non-smokers (Table 3). The A allele frequency for non-smokers was 22.6%-23.7%, while in the smoking group it was 23.7%-31.5%. The highest frequency of the AA genotype was found in female smokers (8.7%), while in male smokers it was the lowest (1.1%). The frequency of the heterozygous AG genotype in all subgroups of smokers was higher than in non-smokers.

Table 2.

The frequency of occurrence of genotypes and alleles of the HTR2A 1438A/G (rs6311) SNP in the Yakut population

Group	Genotype frequency			Allele frequency	
	AA	AG	GG	A	G
Men and women (n=292)	3.1	43.5	53.4	24.8	75.2
Men (n=215)	1.9	43.7	54.4	23.7	76.3
P-value	0.046	>0.05	>0.05	>0.05	>0.05
Women (n=77)	6.5	42.9	50.6	27.9	72.1

The A allele of the *HTR2A* 1438A/G (rs6311) SNP was associated with more of a risk for the HS group than the LS. Using the A allele as a risk factor, RR analysis showed a significant association with a risk factor when comparing HS with LS (RR=1.086; 95% CI=1.032-1.142; *P*=0.049). The analysis of the OR and RR between the HS and LS showed that the AA and AG genotypes were associated with an increased risk for heavy smoking (OR=9.714; 95% CI=1.196-78.874; RR =1.126; 95% CI=1.027-1.235; *P*=0.026). This is consistent with the data of Pérez-Rubio et al.,[4] who found in the Mexican mestizo population that the A allele of the *HTR2A* 1438A/G (rs6311) SNP was associated with cigarette smoking; in addition, the heterozygous GA genotype and the homozygous AA genotype were associated with the risk in the comparison between HS and NS. According to Z. Verde,[11] the allele A of the *HTR2A* 1438A/G (rs6311) SNP, among all smokers, was associated with the number of packs smoked per year (*P*=0.02).

A potential limitation of this study is that smokers self-reported smoking and cigarette consumption levels. Ideally, the amount of nicotine consumed should be measured by serum cotinine levels. This measure is relevant because cigarette consumption affects the level of nicotine in the body. According to power retrospective analysis, the *HTR2A* 1438A/G (rs6311) SNP has low statistical power in our study, probably because the A allele frequency has a relatively rare occurrence that needs to be taken into account and possibly requires later population validation.

We cannot dismiss the possibility that our results may reflect the small sample size. Despite this potential limitation, the significant associations should not be disregarded and merit further investigation for clarification of these results.

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Competing Interests

The authors declare that they have no competing interests.

Table 3.

OR and 95% CI for HTR2 rs6311 (dominant effect of the A allele, AA+AG vs GG) in relation to smokers

Groups and subgroups	Genotype frequency (%)			Allele frequency (%)		OR	RR	*P*	OR (AA+AG)	RR (AA+AG)	*P*
	AA	AG	GG	A	G						
All smokers Men and women	3.5	45.4	51.1	26.2	73.8	1.578 (0.753-3.308)	1.242 (0.947-1.631)	0.303	1.184 (0.746-1.878)	1.099 (0.867-1.393)	0.435
NS Men and women	2.6	41.7	55.6	23.5	76.5						
Women smokers	8.7	45.7	45.7	31.5	68.5	1.578 (0.753-3.308)	1.242 (0.947-1.631)	0.303	1.648 (0.657-4.134)	1.222 (0.844-1.769)	0.286
NS Women	3.2	38.7	58.1	22.6	77.4						
Men smokers	1.1	45.3	53.7	23.7	76.3	0.996 (0.637-1.559)	1.040 (0.804-1.345)	0.922	1.054 (0.614-1.810)	1.030 (0.762-1.392)	0.848
NS Men	2.5	42.5	55	23.7	76.3						
HS Men and women	3.8	48.1	48.1	27.9	72.1	1.257 (0.860-1.836)	1.127 (0.929-1.366)	0.278	1.353 (0.846-2.164)	1.175 (0.915-1.510)	0.207
NS Men and women	2.6	41.7	55.6	23.5	76.5						
HS Men and women	3.8	48.1	48.1	27.9	72.1	7.339 (0.965-55.820)	1.086 (1.032-1.142)	0.049	9.714 (1.196-78.874)	1.126 (1.027-1.235)	0.026
LS Men and women	0	10	90	5	95						

References

1. World Health Organization. 01.17.2022. Age-standardized estimates of current tobacco use, tobacco smoking and cigarette smoking. Data by country. Available from: https://apps.who.int/gho/data/node.main.TOBAGESTDCURR?lang=en
2. Federal State Statistics Service. Selective observation of behavioral factors affecting the health status of the population 2018. Available from: https://burstat.gks.ru/pfczn. [In Russian].
3. Ramos Neto ES, Mágulas JO, Sousa JJ, Moura AC, Pinto GR, Yoshioka FK, Canalle R, Motta FJ. Study of polymorphic variants of the serotonin 2A receptor gene (5-HT2A) and its possible effects on smoking habits of a population from northeastern Brazil. Genet Mol Res. 2014 Oct 20;13(4):8268-77. doi: 10.4238/2014.October.20.3.
4. Pérez-Rubio G, Ramírez-Venegas A, Noé Díaz V, García Gómez L, Elvira Fabián K, García Carmona S, et al. Polymorphisms in HTR2A and DRD4 Predispose to Smoking and Smoking Quantity. PLoS One. 2017 Jan 19;12(1):e0170019. doi: 10.1371/journal.pone.0170019.
5. Ramos Neto ES, Mágulas JO, Sousa JJ, Moura AC, Pinto GR, Yoshioka FK, Canalle R, Motta FJ. Study of polymorphic variants of the serotonin 2A receptor gene (5-HT2A) and its possible effects on smoking habits of a population from northeastern Brazil. Genet Mol Res. 2014 Oct 20;13(4):8268-77. doi: 10.4238/2014.October.20.3.
6. Polina ER, Contini V, Hutz MH, Bau CH. The serotonin 2A receptor gene in alcohol dependence and tobacco smoking. Drug Alcohol Depend. 2009 Apr 1;101(1-2):128-31. doi: 10.1016/j.drugalcdep.2008.11.001.
7. Diakonova AT, Pavlova NI, Solovyeva NA, Varlamova MA, Alexandrova TN, Kurtanov KhA. Molecular-genetic analysis of the connection of the *SLC6A3* gene with nicotine addiction in Yakutia. Yakut Medical Journal. 2018;4(64):6-8. doi: 10.25789/YMJ.2018.64.01. [Article in Russian].
8. Levran O, O'Hara K, Peles E, Li D, Barral S, Ray B, Borg L, Ott J, Adelson M, Kreek MJ. ABCB1 (MDR1) genetic variants are associated with methadone doses required for effective treatment of heroin dependence. Hum Mol Genet. 2008 Jul 15;17(14):2219-27. doi: 10.1093/hmg/ddn122.
9. Hsin CH, Stoffel MS, Gazzaz M, Schaeffeler E, Schwab M, Fuhr U, Taubert M. Combinations of common SNPs of the transporter gene ABCB1 influence apparent bioavailability, but not renal elimination of oral digoxin. Sci Rep. 2020 Jul 27;10(1):12457. doi: 10.1038/s41598-020-69326-y.
10. Pavlova NI, Diakonova AT, Alekseev VA, Borisova YP, Dodokhov VV, Dmitrieva TI, Kurtanov KhA. C3435T Polymorphism of the *ABCB1* Gene in the Yakut Population. International Journal of Biomedicine 11(3) (2021) 367-371. doi: 10.21103/Article11(3) OA15.
11. Verde Z, Santiago C, Chicharro LM, Bandrés F, Gómez-Gallego F, Rodríguez González-Moro JM, de Lucas P. Association of HTR2A-1438G/A Genetic Polymorphism With Smoking and Chronic Obstructive Pulmonary Disease. Arch Bronconeumol (Engl Ed). 2019 Mar;55(3):128-133. doi: 10.1016/j.arbres.2018.07.021.