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Polymorphic Variants of Glutamate Receptor (*GRIK5*, *GRIN2B*) and Serotonin Receptor (*HTR2A*) Genes Are Associated with Chronic Obstructive Pulmonary Disease

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Abstract—Chronic obstructive pulmonary disease (COPD) is a complex chronic inflammatory disease of the respiratory system that affects primarily distal respiratory pathways and lung parenchyma. Smoking tobacco is a major risk factor for COPD. The relationship of *HTR4* (rs3995090), *HTR2A* (rs6313), *GRIK5* (rs8099939), *GRIN2B* (rs2268132), and *CHRNB4* (rs1948) gene polymorphisms and COPD, as well as the contribution of these polymorphisms to the variations in quantitative characteristics that describe respiratory function, smoking behavior, and nicotine dependence was assessed in an ethnically homogeneous Tatar population. The polymorphisms of *HTR2A* (rs6313) ($P = 0.026$, $OR = 1.42$ for the CC genotype) and *GRIN2B* (rs2268132) ($P = 0.0001$, $OR = 2.39$ for the TT genotype) were significantly associated with increased risk of COPD. The AA genotype of *GRIK5* (rs8099939) had a protective effect ($P = 0.02$, $OR = 0.61$). Importantly, the *HTR2A* (rs6313), *GRIN2B* (rs2268132), and *GRIK5* (rs8099939) polymorphisms were only associated with COPD in smokers. Smoking index (pack-years) was significantly higher in carriers of the *GRIK5* genotype AC (rs8099939) ($P = 0.0027$). The TT genotype of *GRIN2B* (rs2268132) was associated with COPD in subjects with high nicotine dependence according to the Fagerström test ($P = 0.002$, $OR = 2.98$). The TT genotype of *HTR2A* (rs6313) was associated with a reduced risk of the disease in the group with moderate nicotine dependence ($P = 0.02$, $OR = 0.22$). The CC genotype of *HTR2A* (rs6313) and the TT genotype of *GRIN2B* (rs2268132) were associated with higher levels of nicotine dependence according to the Fagerström test ($P = 0.0011$ and $P = 0.037$). Our results may provide insight into potential molecular mechanisms that involve the glutamate (*GRIK5*, *GRIN2B*) and serotonin (*HTR2A*) receptor genes in the pathogenesis of COPD.

Keywords: chronic obstructive pulmonary disease, association, smoking status, nicotine dependency, cholinergic nicotinic receptors, serotonin receptors, glutamate receptors

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory disease of the respiratory system that affects primarily distal respiratory pathways and lung parenchyma [1]. COPD is one of the most common diseases and, if treated inefficaciously, can considerably reduce the patients' quality of life and may even lead to a lethal outcome [1]. This disease is currently the fourth most common cause of mortality and is primarily due to widespread smoking habits [1, 2]. Similar to dependences on many other psychoactive substances, the complex mechanism of tobacco (nicotine) dependence involves several principal neuromediator systems (adrenergic, cholinergic, and serotonergic), hormone systems (androgenic, estrogenic, glucocorticoid, and gestagenic), immune factors (tobacco antibodies), and hypoxia-related factors (carboxymyoglobin, aberrations of blood microcirculation) [3, 4]. It has been convincingly demonstrated that genetic factors contribute to the development of nicotine dependence and patterns of smoking behavior [5].

Genome-wide association studies [6] and subsequent meta-analysis [7] led to the identification of several loci associated with nicotine dependence and quantitative characteristics of smoking behavior, among them genes of cholinergic nicotinic receptors on chromosome 15q25, several loci on chromosome 10q25, and *EGLN2* on chromosome 9q13 [7]. Genes that encode glutamate receptors (*GRIK5*, *GRIN2B*, *GRIK2*, *GRM3*), gamma-aminobutyric acid receptor

Abbreviations: COPD, chronic obstructive pulmonary disease; VC, vital capacity; FVC, forced vital capacity; FEV1, forced expiration volume in 1 second; FTND, Fagerström test for nicotine dependence.

B (*GABBR2*), neurexins (*NRXN3*, *NRXN1*), and serotonin receptor (*HTR2A*) also contribute to smoking behavior [8].

Genome-wide association studies of COPD have identified several disease-associated loci located in the chromosomal regions 15q25.1 (*CHRNA3*, *CHRNA5*, *CHRNB4*, *IREB2*), 4q31.21 (*HHIP*), 4q22.1 (*FAM13A*), and 5q31-q33 (*HTR4*). Single-nucleotide polymorphisms of these genes were associated with COPD risk, respiratory function parameters, and with smoking dependence in Caucasian and Mongoloid populations [9–12].

Genes of the *CHRNA3*/*CHRNA5*/*CHRNB4* cluster encode cholinergic nicotinic receptors; they are expressed in the central nervous system and the bronchial epithelium and play a central role in the development of nicotine dependence [5]. Polymorphisms of these genes are associated with smoking intensity and with diseases for which smoking is a major risk factor [4, 13, 14].

Genome-wide association studies, meta-analysis studies, and several replicated works have conclusively demonstrated that *HTR4* polymorphisms are associated with COPD in smokers [12], as well as with respiratory function parameters [9, 10]. Subsequent research elucidated the possible role of HTR4 serotonin receptor in the pathogenesis of COPD and its association with the characteristics of lung function [15].

The goal of the present study was to investigate the relationship between the polymorphisms of genes that encode serotonin receptors (*HTR4*, rs3995090; *HTR2A*, rs6313), glutamate receptors (*GRIK5*, rs8099939; *GRIN2B*, rs2268132), and the cholinergic nicotinic receptor (*CHRNB4*, rs1948) and the risk of COPD, as well as to determine whether these polymorphisms contribute to the variations in traits that describe disease progression, nicotine dependence, and the smoking index in a Tatar population.

EXPERIMENTAL

Subjects. The study was performed in a population sample of unrelated individuals of Tatar ethnicity, residents of Bashkortostan. It included 425 COPD patients, among them 369 men (86.8%) and 56 women (13.2%), aged 63.4 ± 11.8 years. The diagnosis of COPD was established according to IDC-10 and taking into account the guidelines proposed by the Working Group for the Global Strategy for the Diagnosis, Management, and Prevention of COPD (2011 Update) [1, 16]. The patients did not have a history of exposure to occupational hazards. Subjects with a history of allergy, bronchial asthma, malignant tumors, or specific pulmonary infections (tuberculosis) were not included in the study. The group included 331 smokers and ex-smokers (77.9%) and 94 nonsmokers (22.1%). The smoking index was calculated in pack-year (PY) units using the conventional formula [1]. The smoking index in smokers and ex-smokers was 44.58 ± 25.92 PY. In all patients, the respiratory function was assessed by spirometry to determine vital capacity (VC), forced vital capacity (FVC), forced expiration volume in 1 s (FEV1), and the FEV1/VC ratio. In the group of COPD patients, these parameters (in percents relative to the normal level) were as follows: FEV1 = 38.85 ± 16.62, FVC = 46.06 ± 16.32, VC = 49.02 ± 15.54, and FEV1/FVC = 54.21 ± 11.40.

Control group consisted of 457 healthy individuals with no history of respiratory system pathology and without professional exposure to hazardous chemical compounds. The group included 406 men (88.84%) and 51 women (11.16%) aged 58.44 ± 14.79 years; participants with smoking status included 322 smokers and ex-smokers (70.46%), along with 135 nonsmokers (29.54%); the smoking index in smokers was 37.71 ± 14.12 PY. In the control group, the respiratory function parameters (percent of the normal level) were as follows: FEV1 = 102.7 ± 52.1, FVC = 107.1 ± 32.05, VC = 105.3 ± 42.87, FEV1/FVC = 87.94 ± 10.69.

Nicotine dependence was assessed using the Fagerström test for nicotine dependence (FTND), which includes six questions that pertain to different aspects of a smoker's behavior. The overall score is graded from 0 to 10. A score of 0–3 corresponds to a low level of nicotine dependence, 4–5 reflects moderate dependence, and 6–10 means a high level of dependence [17]. In the total population sample studied, 306 subjects (34.69%) had a low-level dependence, 177 (20.07%) had moderate dependence, and 399 subjects (45.24%) had a high level of nicotine dependence. The study was approved by the Ethical Committee of the Institute of Biochemistry and Genetics. All subjects gave their voluntary informed consent to the use of their biological material in the proposed research.

Genotyping. DNA was isolated from peripheral blood leukocytes using phenol–chloroform extraction. Polymorphisms of *HTR4* (rs3995090, c.1077-327T>G), *HTR2A* (rs6313, c.102C>T), *GRIK5* (rs8099939, c.1871+4345A>C), *GRIN2B* (rs2268132, c.411+30893G>T), and *CHRNB4* (rs1948, c.594T>C) were genotyped by real-time PCR using commercial FLASH/RTAS kits (TestGene, Russia) and a BioRad CFX96™ detection system (Bio-Rad Laboratories, United States). End-point fluorescence measurement and genotype discrimination were performed using CFX Manager software according to the BioRad CFX96 protocol.

Statistical analysis of the data was performed using Statistica v. 6.0 (StatSoft Inc., United States) [19] and PLINK v. 1.07 [18]. Allele and genotype frequencies were calculated, and their agreement with the Hardy–Weinberg equilibrium was verified using the χ^2 test and the corresponding P_{HW}; the significance of differences between groups in the allele and genotype frequency distributions was assessed using the χ^2 test for sample homogeneity and the corresponding *P*

value; pairwise comparison of allele and genotype frequencies in the groups of patients and controls was performed using Fisher's two-sided test. Association of polymorphisms with COPD was analyzed using logistic regression; the exponential of the regression coefficient (beta) was interpreted as the odds ratio (*OR*), and the 95% confidence interval was calculated. To minimize a type-1 error, the false discovery rate (FDR) was calculated using the Benjamini–Hochberg procedure with an online service (http://www.sdm-project.com/utilities/?show=FDR) to obtain the corrected $P_{cor\text{-}FDR}$ value. The contribution of genotypes by the candidate loci to the variations in quantitative traits, such as respiratory function characteristics that describe the severity of COPD (VC, FVC, FEV1), smoking index, and Fagerström test scores, was evaluated using the Kruskal–Wallis test (for three groups) or the Mann–Whitney test (for two groups).

RESULTS

Before analyzing the associations of candidate gene polymorphisms with COPD, we verified whether the corresponding genotype frequency distributions agreed with the Hardy–Weinberg equilibrium. In the control group, the following results were obtained: $P_{HW} = 0.051$ for *HTR4* (rs3995090), $P_{HW} = 0.68$ for *HTR2A* (rs6313), $P_{HW} = 0.098$ for *GRIK5* (rs8099939), $P_{HW} = 0.36$ for *GRIN2B* (rs2268132), and $P_{HW} = 0.66$ for *CHRNB4* (rs1948). The *HTR4* locus rs3995090 with a genotype distribution deviating from the Hardy–Weinberg equilibrium was excluded from the further analysis of associations.

Association of Gene Polymorphisms with COPD

Table 1 shows the data on the association of COPD with polymorphisms of four candidate genes: *HTR2A* (rs6313), *GRIK5* (rs8099939), *GRIN2B* (rs2268132), and *CHRNB4* (rs1948).

The C allele of the *HTR2A* polymorphism rs6313 (c.102C>T) was associated with an increased risk of COPD ($P = 0.01$, $P_{cor\text{-}FDR} = 0.03$; $OR = 1.29$, 95%CI 1.07–1.55); its frequency in the patients' group was 59.06% vs. 52.84% in the control group. The group of patients included 34.82% of CC homozygotes, as opposed to 27.35% in the control group ($P = 0.026$; $P_{cor\text{-}FDR} = 0.039$; $OR = 1.42$; 95%CI 1.07–1.89). In contrast, carriers of the T allele had a lower risk of COPD ($P = 0.01$, $P_{cor\text{-}FDR} = 0.03$; $OR = 0.77$, 95%CI 0.64–0.93).

The frequency of the T allele of the *GRIN2B* polymorphism rs2268132 (c.411+30893G>T) was higher in COPD patients than in healthy subjects (38.47% vs 27.02%; $P = 0.0001$, $P_{cor\text{-}FDR} = 0.00045$; $OR = 1.69$, 95%CI 1.39–2.06). The genotype frequency distributions differed significantly between the groups of patients and controls ($\chi^2 = 24.707$, $P = 0.00001$). In COPD patients, the frequency of the TT genotype by *GRIN2B* (rs2268132) was 18.12% compared to 8.32% in the control group ($P = 0.0001$, $P_{cor\text{-}FDR} = 0.00045$; $OR = 2.39$, 95%CI 1.52–3.76). Carriers of the GG genotype by *GRIN2B* (rs2268132) had a decreased risk of COPD ($P = 0.0005$, $P_{cor\text{-}FDR} = 0.0015$; $OR = 0.59$, 95%CI 0.45–0.77).

The genotype frequency distributions of the *GRIK5* polymorphism rs8099939 (c.1871+4345A>C) were significantly different in the groups of COPD patients and healthy controls ($\chi^2 = 6.425$; $P = 0.040$). The frequency of the *GRIK5* rs8099939 allele C was higher in COPD patients than in control subjects (64.47% vs 59.63%; $P = 0.041$; $P_{cor\text{-}FDR} = 0.052$; $OR = 1.22$; 95%CI 1.01–1.49). The AA genotype by *GRIK5* (rs8099939) was a marker of resistance to COPD ($P = 0.02$, $P_{cor\text{-}FDR} = 0.036$; $OR = 0.61$, 95%CI 0.41–0.93).

The allele and genotype frequency distributions for the *CHRNB4* polymorphism rs1948 (c.594T>C) did not exhibit significant differences between the groups of COPD patients and healthy subjects ($\chi^2 = 0.061$; $P = 0.971$) (Table 1).

Effect of Interactions between Environmental and Genetic Factors on the Risk of COPD

To analyze the interactions between genetic and environmental factors, we compared the *OR* values for the candidate genes in groups differentiated by exposure to tobacco smoke (smoking status). Table 2 shows the data on the associations of COPD with candidate gene polymorphisms *HTR2A* (rs6313), *GRIK5* (rs8099939), *GRIN2B* (rs2268132), and *CHRNB4* (rs1948) in groups differentiated by smoking status.

The C allele of the *HTR2A* polymorphism rs6313 was a marker associated with COPD risk in smokers ($P = 0.003$, $P_{cor\text{-}FDR} = 0.012$; $OR = 1.31$, 95%CI 1.01 = 1.64). The frequency of the CC genotype by *HTR2A* (rs6313) was 36.25% in smokers with COPD and 28.88% in smokers of the control group ($P = 0.054$). The most significant associations were observed for the polymorphism of the glutamate receptor gene *GRIN2B* (rs2268132). The TT genotype by this polymorphism was associated with COPD risk in smokers ($P = 0.0001$, $P_{cor\text{-}FDR} = 0.0006$; $OR = 3.06$, 95%CI 1.71–5.48). The GG genotype was a marker of resistance to COPD in smokers ($P = 0.0005$, $P_{cor\text{-}FDR} = 0.002$; $OR = 0.55$, 95%CI 0.40–0.75).

The frequency of the C allele of the *GRIK5* polymorphism rs8099939 in the subgroup of smokers with COPD was 63.9% in contrast to 58.4% in the control group ($P = 0.047$, $P_{cor\text{-}FDR} = 0.065$; $OR = 1.26$, 95%CI 1.01–1.57). The AA genotype of *GRIK5* rs8099939 was associated with resistance to COPD in smokers ($P = 0.005$, $P_{cor\text{-}FDR} = 0.015$; $OR = 0.54$, 95%CI 0.34–0.87).

In the group of nonsmokers, the studied polymorphisms of *HTR2A* (rs6313), *GRIK5* (rs8099939), *GRIN2B* (rs2268132), and *CHRNB4* (rs1948) did not

Table 1. Allele and genotype frequency distribution of *HTR4*, *HTR2A*, *GRIN2B*, *GRIK5*, and *CHRNB4* polymorphisms in groups of COPD patients and healthy subjects; analysis of association with the disease

Gene, polymorphism	Genotypes, alleles	COPD abs. (%) ($n = 425$)	Control abs. (%) ($n = 457$)	[3]P	$P_{cor\text{-}FDR}$	OR (95% CI)
[1]*HTR4* rs3995090 c.1077-327T>G	TT GT GG	144 (33.88) 180 (42.35) 101 (23.76)	164 (35.89) 200 (43.76) 93 (20.35)	–	–	–
		$\chi^2 = 1.522$, [2]$P = 0.467$				
	T G	468 (55.06) 382 (44.94)	528 (57.77) 386 (42.23)	0.279	–	–
HTR2A rs6313 c.102C>T	CC TC TT	148 (34.82) 206 (48.47) 71 (16.71)	125 (27.35) 233 (50.98) 99 (21.66)	0.026 0.074 0.084	0.039 – –	1.42 (1.10–1.89) 0.90 (0.69–1.17) 0.72 (0.52–1.01)
		$\chi^2 = 7.058$, $P = 0.029$				
	C T	502 (59.06) 348 (40.94)	483 (52.84) 431 (47.16)	0.01	0.03	1.29 (1.07–1.55) 0.77 (0.64–0.93)
GRIN2B rs2268132 c.411+30893G>T	GG TG TT	175 (41.18) 173 (40.71) 77 (18.12)	248 (54.27) 171 (37.42) 38 (8.32)	0.0005 0.325 0.0001	0.0015 – 0.00045	0.59 (0.45–0.77) 1.15 (0.87–1.51) 2.39 (1.52–3.76)
		$\chi^2 = 24.707$, $P = 0.00001$				
	G T	523 (61.53) 327 (38.47)	667 (72.98) 247 (27.02)	0.0001	0.00045	0.59 (0.48–0.72) 1.69 (1.39–2.06)
GRIK5 rs8099939 c.1871+4345A>C	CC AC AA	175 (41.18) 198 (46.59) 52 (12.24)	172 (37.64) 201 (43.98) 84 (18.38)	0.34 0.503 0.02	– 0.036	1.15 (0.88–1.52) 1.11 (0.85–1.44) 0.61 (0.41–0.93)
		$\chi^2 = 6.425$, $P = 0.040$				
	C A	548 (64.47) 302 (35.53)	545 (59.63) 369 (40.37)	0.041	0.052	1.22 (1.01–1.49) 0.81 (0.67–0.98)
CHRNB4 rs1948 c.594T>C	CC TC TT	173 (40.71) 191 (44.94) 61 (14.35)	188 (41.14) 206 (45.08) 63 (13.79)	0.971	–	–
		$\chi^2 = 0.061$, $P = 0.971$				
	C T	537 (63.18) 313 (36.82)	582 (63.68) 332 (36.32)	0.866	–	–

[1] *HTR4* polymorphism rs3995090 was excluded from the association study, because the genotype distribution in the control group deviated from the Hardy–Weinberg equilibrium: $P_{HW} = 0.051$. [2] P value for the χ^2 test for sample homogeneity. [3] P value for the two-sided Fisher's test for pairwise comparison of allele and genotype frequencies between the groups of patients and controls; *OR*, odds ratio; CI95%, 95% confidence interval for *OR*; $P_{cor\text{-}FDR}$, test significance after the false discovery rate (FDR) correction.

exhibit significant association with COPD (Table 2). In an analysis of the contribution of candidate gene polymorphisms to variations in the smoking index (PY), a quantitative trait that describes the intensity and history of smoking, significant differences were found between smoking individuals with different genotypes by the *GRIK5* polymorphism rs80999390 (Table 3). In the total group of smokers, heterozygosity by this polymorphism was associated with a higher smoking index ($P = 0.0027$).

This association was further confirmed in the subgroup of smokers with COPD; subjects heterozygous by rs8099939 had a mean smoking index of 47.7 PY, while in CC and AA homozygotes, it was 39.54 PY ($P = 0.014$). We also analyzed the association of COPD with candidate gene polymorphisms in groups with

Table 2. Association of candidate gene polymorphisms with COPD in groups differentiated by smoking status

Gene, polymorphism	Genotypes, alleles	Smokers		*P*	$P_{cor\text{-}FDR}$	*OR* (95% CI)[1]	Nonsmokers		*P*
		COPD abs. (%) ($n = 331$)	Control abs. (%) ($n = 322$)				COPD abs. (%) ($n = 94$)	Control abs. (%) ($n = 135$)	
HTR2A rs6313 c.102C>T	CC	120 (36.25)	93 (28.88)	0.054	–	1.40 (0.99–1.97)	25 (26.60)	34 (25.19)	0.77
	TC	156 (47.13)	156 (48.45)	0.796	–	–	53 (56.30)	74 (54.81)	0.92
	TT	55 (16.62)	73 (22.67)	0.06	–	0.67 (0.44–1.03)	16 (17.10)	27 (20.00)	0.69
	C	396 (59.82)	342 (53.11)	0.003	0.012	1.31 (1.10–1.64)	103 (54.79)	142 (52.59)	0.71
	T	266 (40.18)	302 (46.89)			0.76 (0.61–0.94)	85 (45.21)	128 (47.41)	
GRIN2B rs2268132 c.411+30893G>T	GG	138 (41.69)	182 (56.52)	0.0005	0.002	0.55 (0.40–0.75)	38 (40.4)	67 (49.63)	0.24
	TG	132 (39.88)	118 (36.65)	0.308	–	1.15 (0.83–1.57)	46 (49.0)	47 (34.81)	0.054
	TT	61 (18.43)	22 (6.83)	0.0001	0.0006	3.06 (1.71–5.48)	10 (10.6)	21 (15.56)	0.38
	G	408 (61.63)	482 (74.84)	0.00001	0.00012	0.53 (0.43–0.68)	122 (64.89)	181 (67.04)	0.71
	T	254 (38.37)	162 (25.16)			1.85 (1.46–2.31)	66 (35.11)	89 (32.96)	
GRIK5 rs8099939 c.1871+4345A>C	CC	132 (39.88)	119 (36.96)	0.49	–	–	45 (47.87)	52 (38.52)	0.24
	AC	159 (48.04)	138 (42.85)	0.211	–	–	31 (32.98)	62 (45.93)	0.062
	AA	40 (12.08)	65 (20.19)	0.005	0.015	0.54 (0.34–0.87)	18 (19.15)	21 (15.56)	0.63
	C	423 (63.90)	376 (58.39)	0.047	0.065	1.26 (1.01–1.57)	121 (64.36)	166 (61.48)	0.597
	A	239 (36.10)	268 (41.61)			0.79 (0.63–0.99)	67 (35.64)	104 (38.52)	
CHRNB4 rs1948 c.594T>C	CC	132 (39.88)	131 (40.68)	0.81	–	–	43 (45.74)	57 (42.2)	0.63
	TC	151 (45.62)	148 (45.96)	0.96	–	–	38 (40.43)	58 (43.0)	0.81
	TT	48 (14.50)	43 (13.35)	0.63	–	–	13 (13.83)	20 (14.8)	0.86
	C	415 (62.69)	410 (63.66)	0.75	–	–	124 (65.96)	172 (63.70)	0.691
	T	247 (37.31)	234 (36.34)			–	64 (34.04)	98 (36.30)	

[1] Only the data with significant differences between groups are shown.

Table 3. Contribution of the *GRIK5* polymorphism rs8099939 to variations in smoking index in smokers

Gene, polymorphism	Genotype	*n*	$M \pm SEM$	P^a
Smoking index (PY) in the whole group of smokers ($n = 653$)				
GRIK5 rs8099939 c.1871+4345A>C	CC	251	31.23 (1.45)	0.01
	AC	297	37.79 (2)	
	AA	105	29.93 (2.56)	
	CC	251	31.23 (1.45)	0.052
	(AC + AA)	402	35.82 (1.64)	
	(CC + AA)	356	30.86 (1.27)	0.0027
	AC	297	37.79 (2)	
Smoking index (PY) in smoking COPD patients ($n = 331$)				
GRIK5 rs8099939 c.1871+4345A>C	CC	132	38.66 (1.96)	0.039
	AC	159	47.7 (2.82)	
	AA	40	42.49 (4.43)	
	CC	132	38.66 (1.96)	0.018
	(AC + AA)	199	46.65 (2.42)	
	(CC + AA)	172	39.54 (1.82)	0.014
	AC	159	47.7 (2.82)	

$M \pm SEM$, mean and standard error of the mean. [a] *P* is significance of differences by the Mann–Whitney or Kruskal–Wallis test.

Table 4. Association of candidate gene polymorphisms with COPD in groups differentiated by nicotine dependence level

Gene, polymorphism	Genotype	Level of nicotine dependence as determined by the Fagerström test					
		low (0–3 points) Smoking index, 1.74 ± 3.35 PY (n = 306)		moderate (4–5 points) Smoking index, 16.7 ± 2.6 PY (n = 177)		high (6–10 points) Smoking index (46.6 ± 23.6) (n = 399)	
		OR (CI95%)	*P*	*OR* (CI95%)	*P*	*OR* (CI95%)	*P*
HTR2A rs6313 c.102C>T	CC	1.15 (0.62–2.12)	0.76	1.10 (0.48–2.51)	0.82	1.36 (0.76–2.40)	0.29
	TC	1.01 (0.59–1.74)	1.01	1.78 (0.80–3.91)	0.15	0.95 (0.56–1.61)	0.84
	TT	0.82 (0.41–1.66)	0.58	0.22 (0.05–0.79)	0.02	0.71 (0.37–1.36)	0.31
	C	1.13 (0.76–1.64)	0.62	1.46 (0.83–2.57)	0.23	1.28 (0.88–1.87)	0.225
	T	0.89 (0.61–1.30)		0.68 (0.38–1.20)		0.77 (0.53–1.13)	
GRIN2B rs2268132 c.411+30893G>T	GG	0.62 (0.36–1.07)	0.07	0.85 (0.39–1.80)	0.67	0.44 (0.25–0.77)	0.0039
	GT	1.22 (0.71–2.09)	0.47	1.01 (0.46–2.20)	0.99	1.41 (0.79–2.50)	0.24
	TT	1.89 (0.85–4.22)	0.12	1.58 (0.45–5.47)	0.48	2.98 (1.34–7.79)	0.002
	G	0.75 (0.50–1.11)	0.19	0.82 (0.46–1.47)	0.62	0.47 (0.30–0.73)	0.00001
	T	1.33 (0.89–1.98)		1.29 (0.72–2.34)		2.12 (1.36–3.28)	
GRIK5 rs8099939 c.1871+4345A>C	CC	1.36 (0.79–2.36)	0.32	1.13 (0.53–2.41)	0.75	1.43 (0.79–2.57)	0.22
	CA	0.90 (0.52–1.56)	0.72	1.22 (0.57–2–62)	0.61	0.89 (0.51–1.54)	0.67
	AA	0.66 (0.30–1.47)	0.29	0.58 (0.21–1.67)	0.30	0.65 (0.31–1.39)	0.28
	C	1.31(0.88–1.96)	0.21	1.12 (0.65–1.92)	0.77	1.32 (0.89–1.96)	0.194
	A	0.75 (0.51–1.13)		0.89 (0.52–1.57)		0.75 (0.51–1.11)	
CHRNB4 rs1948 c.594T>C	CC	1.14 (0.68–1.94)	0.61	1.41 (0.66–3.01)	0.37	0.90 (0.52–1.54)	0.71
	TC	0.86 (0.51–1.47)	0.59	0.79 (0.37–1.67)	0.53	1.02 (0.60–1.73)	0.95
	TT	1.01 (0.46–2.24)	0.98	0.82 (0.29–2.37)	0.71	1.19 (0.54–2.58)	0.66
	C	1.08 (0.70–1.59)	0.78	1.26 (0.72–2.18)	0.48	0.90 (0.61–1.33)	0.698
	T	0.92 (0.62–1.38)		0.79 (0.45–1.37)		1.10 (0.74–1.620)	

different levels of nicotine dependence determined using FTND. Subjects were divided into three groups characterized with low (FTND score 0–3), moderate (FTND score 4–5), and high (FTND score 6–10) levels of nicotine dependence (Table 4).

In the subgroup with low nicotine dependence (FTND score 0–3, smoking index 1.74 ± 3.35 PY), the allele and genotype frequency distributions of the polymorphic markers *HTR2A* (rs6313), *GRIK5* (rs8099939), *GRIN2B* (rs2268132), and *CHRNB4* (rs1948) did not differ significantly between COPD patients and control subjects.

In the subgroup with moderate nicotine dependence (FTND score 4–5, smoking index 16.66 ± 2.64 PY), the TT genotype of the *HTR2A* polymorphism rs6313 was a marker of resistance to COPD (P = 0.02, $P_{\text{cor-FDR}}$ = 0.036; *OR* = 0.22, 95% CI 0.05–0.79).

The *GRIN2B* polymorphism rs2268132 was significantly associated with COPD in the subgroup with high nicotine dependence (FTND score was 6–10; smoking index was 46.59 ± 23.60 PY). An increased risk of COPD in this subgroup was associated with the T allele (P = 0.00001; $P_{\text{cor-FDR}}$ = 0.00012; *OR* = 2.12, 95% CI 1.36–3.28) and the TT genotype (P = 0.002, $P_{\text{cor-FDR}}$ = 0.01; *OR* = 2.98, 95% CI 1.34–7.79).

We also analyzed the relationship between FTND scores and the genotypes of candidate gene polymorphisms *HTR2A* (rs6313), *GRIK5* (rs8099939), *GRIN2B* (rs2268132), and *CHRNB4* (rs1948) in the total population sample studied. Polymorphisms of *HTR2A* (rs6313) and *GRIN2B* (rs2268132) exhibited significant association with FTND scores (Table 5). Higher FTND scores were observed in CC homozygotes by the *HTR2A* polymorphism rs6313 (P = 0.0011) and in TT homozygotes by the *GRIN2B* polymorphism rs2268132 (P = 0.037).

Table 5. Contribution of *HTR2A* and *GRIN2B* polymorphisms to the total FTND variations in the whole population sample studied

Gene, polymorphism	Genotype	*n*	*M* ± *SEM*	*P*
FTND (*n* = 882)				
HTR2A rs6313 c.102C>T	CC	273	5.5 (0.24)	0.0035
	TC	441	4.48 (0.19)	
	TT	168	4.75 (0.3)	
	CC	273	5.5 (0.24)	0.0011
	(TC + TT)	609	4.56 (0.16)	
	(CC + TT)	441	5.22 (0.19)	0.0059
	TC	441	4.48 (0.19)	
GRIN2B rs2268132 c.411+30893G>T	GG + GT	767	4.73 (0.14)	0.037
	TT	115	5.5 (0.37)	

M ± *SEM* is mean and standard error of the mean; *P* is significance in Mann–Whitney or Kruskal–Wallis test.

Contribution of Genotypes by Candidate Gene Polymorphisms to Variations in Quantitative Traits of Respiratory Function

The study analyzed the relationship between quantitative parameters of respiratory function, i.e., VC, FVC, and FEV1, and genotypes of candidate gene polymorphisms. It was found that the polymorphisms of *HTR4* (rs3995090), *HTR2A* (rs6313), *GRIK5* (rs8099939), *GRIN2B* (rs2268132), and *CHRNB4* (rs1948) did not exhibit significant associations with the quantitative characteristics of the respiratory function either in the total population sample studied (patients and controls) or within the group of COPD patients.

DISCUSSION

In this work, we investigated the association of COPD with the serotonin receptors *HTR4* (rs3995090) and *HTR2A* (rs6313), the glutamate receptors *GRIK5* (rs8099939) and *GRIN2B* (rs2268132), and the cholinergic nicotinic receptor *CHRNB4* (rs1948) genes polymorphisms. We also evaluated the contribution of the corresponding genotypes to the variations in quantitative traits that characterize disease progression, nicotine dependence, and smoking behavior. For the first time, it has been shown that polymorphisms of *HTR2A* (rs6313, c.102C>T), *GRIN2B* (rs2268132, c.411+30893G>T), and *GRIK5* (rs8099939, c.1871+4345A>C) are associated with COPD, the smoking index, and the level of nicotine dependence.

The most significant associations with COPD risk were observed for the rs2268132 (c.411+30893G>T) polymorphism of *GRIN2B* both in the general group and in the subgroups differentiated by smoking status and nicotine dependence level. This gene is located on chromosome 12p12 and encodes subunit 2B of glutamate ionotropic receptor of the N-methyl D-aspartate (NDMA) type [20]. The T allele and the TT genotype of this polymorphism were markers of an increased risk of COPD; this association remained significant after correction for multiple comparisons, but was observed in smokers and in subjects with high FTND scores of 6–10. In addition, the *GRIN2B* polymorphism rs2268132 (c.411+30893G>T) was significantly associated with FTND score characterizing the level of nicotine dependence: individuals with the TT genotype had significantly higher FTND scores.

The rs8099939 (c.1871+4345A>C) polymorphism of *GRIK5*, which is located on chromosome 19q13.2 and encodes glutamate ionotropic receptor of the kainate type (GluK5, or GRIK5) [20], also exhibited association with COPD. The risk of COPD was associated with the C allele of *GRIK5* (rs809993), while the AA genotype was a marker of resistance to COPD, both in the general sample and the group of smokers. In addition, the smoking index, i.e., the parameter that describes the history and intensity of smoking, was also associated with this locus; in both the group of all smokers and the subgroup of smoking COPD patients, subjects heterozygous by *GRIK5* (rs8099939) tended to have a higher smoking index.

Thus, this work has established associations between polymorphisms of the glutamate receptor genes *GRIN2B* and *GRIK5* and COPD risk in smokers, the level of nicotine dependence, and the smoking index. These data suggest that glutamate receptors and neurotransmission-related factors play an important role in the development of multifactorial diseases associated with smoking. Glutamate is the most common excitatory neurotransmitter in the central nervous system of mammals; it is responsible for signal transmission via glutamatergic synapses in the brain [21]. The pleiotropic effects of glutamate, as well as those of other neurotransmitters, are mediated by dif-

ferent types of receptors [22–24]. The contribution of polymorphisms of glutamate receptor genes to the pathogenesis of COPD was not studied previously. On the other hand, the available data suggest that *GRIK5* and *GRIN2B* polymorphisms are associated with bipolar disorder, mental illness and nervous system diseases, and mental retardation [25–29]. It was previously shown that *GRIN2B* (rs2268132) variants are somehow implicated in smoking status and quantitative characteristics of smoking behavior [6]. There are also data that suggest that *GRIN2B* is involved in the development of nicotine dependence and interacts with the age of onset of smoking [7]. Our results, together with these data, confirm that polymorphisms of glutamate receptor genes *GRIN2B* and *GRIK5* may be important risk factors for smoking-associated diseases, such as COPD.

Our study is the first to analyze the polymorphisms of *HTR4* (rs3995090, c.1077-327T>G) and *HTR2A* (rs6313, c.102C>T), which encode two key membrane receptors of 5-hydroxytryptamine (serotonin). Serotonin acts as a neuromediator and a hormone and interacts with a wide range of pharmaceutical agents and psychoactive compounds, including nicotine [30]. From the chemical point of view, serotonin is a biogenic amine; it is one of the principal transmitters in the central nervous system and also acts as an important endocrine regulator, playing a key role in the functioning of the cardiovascular and the respiratory system [30]. The activation of serotonin receptors triggers intracellular cascades that affect the functioning of other mediator systems (glutamatergic, dopaminegic, and GABA-ergic) [31].

It was found that the C allele and the CC genotype of the *HTR2A* polymorphism rs6313 (c.102C>T) were significantly associated with COPD. This association was also confirmed in the group of smokers. In addition, carriers of the CC genotype of *HTR2A* rs6313 (c.102C>T) had higher FTND scores, which reflect the level of nicotine dependence. *HTR2A* is located on chromosome 13q14-q21; the gene comprises three exons and two introns [20]. The two best-studied functional variants of this gene are rs6313 (c.102C>T) in exon 1 and rs6311 (c.–998G>A) in the promoter region [32]. The G allele of rs6311 is associated with decreased promoter activity [32], while the C allele of rs6313 is associated with decreased protein synthesis [33, 34]. The rs6313 and rs6311 polymorphisms of *HTR2A* were previously shown to be associated with nicotine dependence and smoking behavior [33–35]. Functional studies showed that the activation of serotonin receptors regulates the release of interleukin-6 and chemokine CXCL8 [36]. It was also found that serotonin stimulates different signaling pathways and regulates cytokine release in epithelial cells of the respiratory pathways; thus, it is involved in the pathogenesis of bronchial asthma [36]. Our data invite the hypothesis that, in addition to their involvement in the initial stages of nicotine dependence, serotonin receptors in COPD patients trigger a cascade of inflammatory reactions induced by tobacco smoke, which requires further detailed investigation.

We also investigated the relationship between the polymorphism rs1948 (c.594T>C) of the cholinergic receptor gene *CHRNB4* and the risk of COPD in the population of Tatars, but did not detect any significant association of rs1948 variants with COPD or any quantitative parameters that describe nicotine dependence, smoking behavior, or disease progression. The *CHRNA3/CHRNA5/CHRNB4* cluster of genes that encode cholinergic nicotinic receptors is expressed in the central nervous system and the bronchial epithelium; these genes play a central role in the development of nicotine dependence and smoking-associated diseases [12, 14]. Our previous work confirmed the association of the polymorphisms *CHRNA5* (rs16969968) and *CHRNA3* (rs1051730) with COPD in the Tatar population of Russia [37].

The obtained results contribute to our understanding of the molecular mechanisms of COPD pathogenesis. We found that glutamate receptors (*GRIK5*, *GRIN2B*) and a serotonin receptor (*HTR2A*) genes polymorphisms were risk factors for COPD in smokers. The *GRIK5* polymorphism was associated with the smoking index, while *GRIN2B* and *HTR2A* variants were associated with FTND scores. Apparently, glutamate and serotonin receptors represent important elements in the development of nicotine dependence and smoking habits, while exposure to tobacco smoke acts as environmental trigger of COPD risk.

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