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HUMAN GENETICS

Role of Neurotransmitter System Genes in Chronic Obstructive Pulmonary Disease

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Abstract—Chronic obstructive pulmonary disease (COPD) is a complex chronic inflammatory disease of the respiratory system. Smoking is a major risk factor of COPD. Neurotransmitter system genes were tested for association with COPD using DNA samples from COPD patients ($N = 601$) and healthy subjects ($N = 617$). SNPs of *GRIK3* (rs534131), *GRIN2B* (rs7301328 and rs1805476), *GRIA1* (rs2195450), *GRIN1* (rs6293), *GABBR2* (rs3750344), *BDNF* (rs6265 and rs11030107), and *ANKK1* (rs1800497) were genotyped by real-time polymerase chain reaction (PCR). Significant associations were detected for *GRIK3* (rs534131) ($P = 0.009$, OR = 1.42 in a dominant model), *GRIA1* (rs2195450) ($P = 0.015$, OR = 1.35 in a dominant model), *GRIN1* (rs6293) ($P = 0.036$, OR = 0.79 in a log-additive model), and *GRIN2B* (rs7301328) ($P = 0.0009$, OR = 0.54 in a recessive model). *GRIK3* (rs534131) ($P = 0.0001$, OR = 1.68 in a dominant model) and *GRIN2B* (rs7301328) ($P = 0.001$, OR = 0.52 in a recessive model) were significantly associated with COPD only in smokers. Associations of *GRIN1* (rs6293) ($P = 0.0001$, OR = 0.36 in a dominant model), *GRIA1* (rs2195450) ($P = 0.0018$, OR = 2.40 in a log-additive model), and *BDNF* (rs11030107) ($P = 0.005$, OR = 2.86 for TT) with COPD were detected in nonsmokers. Lower smoking index were observed in carriers of the *GRIK3* (rs534131) genotype GG and *GRIA1* (rs2195450) genotype GG ($P = 0.028$ and 0.0001, respectively). The level of nicotine dependence was significantly higher in carriers of the rare allele A of *GRIK3* (rs534131A>G) and the genotype GC of *GRIN2B* (rs7301328C>G) ($P = 0.011$ and 0.023, respectively). Informative genotype and allele combinations significantly associated with COPD were identified using the APSampler algorithm and included *GRIN2B* rs2268132**T*, *GRIN2B* rs7301328**G*, *GRIN2B* rs1805476**C*, *ANKK1* rs1800497**G*, *GABBR2* rs3750344**A*, *CHRNA5* rs16969968**T*, *CHRNA3* rs1051730**A*, *HTR2A* rs6313**CC*, and *GRIA1* rs2195450**G*.

Keywords: chronic obstructive pulmonary disease, glutamate receptors, nicotine dependence, smoking, gene–environmental interactions

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a multifactorial heterogeneous chronic inflammatory disease of the respiratory system; it involves predominantly distal airway regions and lung parenchyma and is the fourth leading cause of death in the world, causing more than 3 million deaths annually [1]. Smoking is a main risk factor of many disorders, including COPD [2]. Tobacco smoking is associated with the development of pathological processes in the lungs, systemic inflammatory reactions, oxidative stress, dysfunction of the vascular endothelium, and elevated activity of procoagulant factors [1]. Tobacco dependence is an important problem of public health; nicotine is a major component of tobacco; and nicotine dependence develops in the majority of regular smokers [2]. There is convincing evidence that genetic factors contribute to nicotine dependence [3]. Genome-wide association studies have identified several loci that are associated with nicotine dependence and quantitative parameters of smoking behavior. The loci include the *CHRNA3/CHRNA5/CHRNB4* gene cluster for nicotinic cholinergic receptors in chromosome 15q25, LOC100188947 in chromosome 10q25, *EGLN2* in chromosome 9q13, and *BDNF* in chromosome 11p13 [4]. The hereditary basis of COPD has been addressed in many studies, and certain genes have been associated with both COPD and nicotine dependence [5]. The genes are involved in neurotransmission, and some of them have showed significant associations with smoking, implicating the neurotransmitter genes in smoking and nicotine dependence [6]. We previously showed that polymorphic variants of the nicotinic cholinergic receptor *CHRNA5*

(rs16969968) and *CHRNA3* (rs1051730), glutamate receptor *GRIK5* (rs8099939) and *GRIN2B* (rs2268132), and serotonin receptor *HTR2A* (rs6313) genes are associated with COPD in smokers of the Tatar population [7, 8]. In this work, the set of candidate genes was expanded to include the genes for ionotropic receptor kainate type subunit 3 (*GRIK3*), ionotropic AMPA type receptor 1 (*GRIA1*), glutamate receptor NMDA type subunit 1 (*GRIN1*), γ-aminobutyric acid type B receptor subunit 2 (*GABBR2*), the brain-derived neurotrophic factor (*BDNF*), and a protein kinase (*ANKK1*). The objectives were to test SNPs of *GRIK3*, *GRIN2B*, *GRIA1*, *GRIN1*, *GABBR2, BDNF*, and *ANKK1* for association with COPD; to estimate their contributions to the variance in nicotine dependence parameters and smoking index; and to evaluate the gene by environmental interaction in COPD development.

MATERIALS AND METHODS

The study was designed as a candidate gene case–control study. DNA samples were collected from unrelated subjects who were Tatars in ethnicity and resided in the Republic of Bashkortostan. The COPD group included 601 patients (522 (86.85%) males and 79 (13.15%) females) with a mean age of 63.38 ± 11.81 years. There were 484 (80.53%) smokers and former smokers and 117 (19.47%) nonsmokers in the COPD group. The smoking index was 44.58 ± 25.92 pack/years in the smokers and former smokers. The control group included 617 subjects (548 (88.88%) males and 69 (11.12%) females) with a mean age of 58.44 ± 14.79. There were 517 (83.79%) smokers and former smokers and 100 (16.21%) nonsmokers in the group; the smoking index was 38.54 ± 23.12 pack/years in the smokers. Inclusion and exclusion criteria for the COPD and control groups and a method to estimate the nicotine dependence level by the Fagerström Test for Nicotine Dependence (FTND) were as described previously [8]. The study was approved by the Ethics Committee at the Institute of Biochemistry and Genetics.

Genotyping

DNA was isolated from peripheral blood leukocytes by phenol–chloroform extraction. The SNPs selected for analysis were functionally significant or earlier associated with psychoactive substance dependence and mental disorders. The rare allele frequencies in Caucasian populations were obtained from the National Center for Biotechnology Information database [9]. The set included SNPs of the following genes: *GRIK3* (rs534131, g.20608A>G), *GRIN2B* (rs7301328, c.366C>G, p.Pro122=), *GRIN2B* (rs1805476, c.*1354C>A), *GRIA1* (rs2195450, c.–750G>A), *GRIN1* (rs6293, c.789A>G, p.Pro263=), *GABBR2* (rs3750344, c.360A>G, p.Ala120=), *BDNF* (rs6265, c.283G>A, p.Val66Met), *BDNF* (rs11030107, c.–21-14703T>C), and *ANKK1* (rs1800497, c.2137G>A, p.Glu713Lys). The functional significance of the SNPs was verified using RegulomeDB version 1.1 (https://regulomedb.org), SNPinfo Web Server (https://snpinfo.niehs.nih.gov), and HaploReg v3 [10]. SNP genotyping was performed by real-time polymerase chain reaction (PCR) using commercial kits for fluorescence detection (DNK-Sintez, Russia; https://www.oligos.ru) and a BioRad CFX96™ instrument (Bio-Rad Laboratories, United States). The methods of analysis were described in detail previously [7].

Statistical Analyses

Statistical analyses of the results were performed using the software packages Statistica v. 6.0 (StatSoft, United States) and PLINK v. 1.07 [11]. The methods were described in detail previously [7, 8]. Linear regression was used to estimate the contribution of the allelic variants of a candidate gene to the variance in quantitative traits (smoking index and FTND score). Association analyses of allele or genotype combinations with COPD were carried out using the program APSampler 3.6.1 (http://sourceforge.net/projects/apsampler/). The main algorithm was described in [12]. The Benjiamini–Hochberg correction for multiple testing was performed using special software (http://www.sdmproject.com/utilinies/?show=FDR) to decrease the false discovery rate (FDR) and to obtain $P_{\text{FDR-cor}}$.

RESULTS

Prior to testing the SNPs of the candidate genes for association with COPD, genotype frequency distributions were tested for correspondence to the Hardy–Weinberg equilibrium. The following results were obtained for the control group: *GRIK3* (rs534131) $P_{\text{H–W}} = 0.082$, *GRIN2B* (rs7301328) $P_{\text{H–W}} = 0.66$, *GRIN2B* (rs1805476) $P_{\text{H–W}} = 0.11$, *GRIA1* (rs2195450) $P_{\text{H–W}} = 0.15$, *GRIN1* (rs6293) $P_{\text{H–W}} = 0.14$, *GABBR2* (rs3750344) $P_{\text{H–W}} = 0.08$, *BDNF* (rs6265) $P_{\text{H–W}} = 0.67$, *BDNF* (rs11030107) $P_{\text{H–W}} = 0.16$, and *ANKK1* (rs1800497) $P_{\text{H–W}} = 0.88$. Table 1 summarizes the data on the allele and genotype frequency distributions of the loci under study, the significance of between-group differences, and the odds ratios (ORs) calculated for the rare alleles of all loci. Statistically significant results of a regression analysis are shown in Table 2. The frequency of the rare allele A of *GRIK3* (rs534131) was significantly increased in the COPD group ($P = 0.017$, OR = 1.21, 95%CI 1.04–1.57). An association of *GRIK3* (rs534131) with COPD was established in the dominant ($P = 0.009$, $P_{\text{cor-FDR}} = 0.044$, OR = 1.42, 95%CI 1.10–1.83) and log-additive ($P = 0.018$, $P_{\text{cor-FDR}} = 0.044$, OR = 1.25, 95%CI 1.07–1.51) models. The fre-

quency of the rare allele A of *GRIA1* (rs2195450) in the COPD patients was significantly higher than in the controls (22.13% vs. 18.15%, $P = 0.016$, OR = 1.28, 95%CI 1.05–1.56). *GRIA1* (rs2195450) was associated with COPD in the dominant ($P = 0.015$, $P_{cor\text{-}FDR} = 0.044$, OR = 1.35, 95%CI 1.07–1.71) and log-additive ($P = 0.02$, $P_{cor\text{-}FDR} = 0.044$, OR = 1.26, 95%CI 1.01–1.57) models. The allele frequency distribution of *GRIN1* (rs6293) was found to significantly differ between the two groups ($P = 0.04$, OR = 0.81, 95%CI 0.66–0.98). *GRIN1* (rs6293) was associated with COPD in the dominant ($P = 0.047$, $P_{cor\text{-}FDR} = 0.0705$, OR = 0.78, 95%CI 0.62–0.98) and log-additive ($P = 0.036$, $P_{cor\text{-}FDR} = 0.061$, OR = 0.79, 95%CI 0.63–0.99) models by a regression analysis, but the differences did not reach statistical significance after the FDR correction. The frequency of the rare allele C of *GRIN2B* (rs7301328) in the COPD patients was significantly lower than in the controls (36.36% vs. 41.17%, $P = 0.021$, OR = 0.81, 95%CI 0.69–0.96). *GRIN2B* (rs7301328) was associated with COPD in the recessive ($P = 0.0009$, $P_{cor\text{-}FDR} = 0.0108$, OR = 0.54, 95%CI 0.37–0.78) and log-additive ($P = 0.022$, $P_{cor\text{-}FDR} = 0.044$, OR = 0.81, 95%CI 0.68–0.98) models; homozygosity for the rare allele C (genotype CC) of *GRIN2B* (rs7301328) was identified as a marker of COPD resistance. Comparisons of the allele and genotype frequency distributions between the COPD and control groups did not detect significant differences in the case of the following SNPs: *GRIN2B* (rs1805476), *GABBR2* (rs3750344), *BDNF* (rs6265), *BDNF* (rs11030107), and *ANKK1* (rs1800497) (Table 1).

Analyses of Associations of the Candidate Genes with COPD in the Groups Differentiated by Smoking Status

The association with COPD was confirmed in the smoker subgroup for *GRIK3* (rs534131) and *GRIN2B* (rs7301328) (Table 3). As in the total sample, *GRIK3* (rs534131) showed a significant association with COPD in the dominant ($P = 0.0001$, $P_{cor\text{-}FDR} = 0.00065$, OR = 1.68, 95%CI 1.27–2.24) and log-additive ($P = 0.003$, $P_{cor\text{-}FDR} = 0.0078$, OR = 1.31, 95%CI 1.10–1.56) models. The association of *GRIN2B* (rs7301328) with COPD in the smoker subgroup was demonstrated in the recessive ($P = 0.001$, $P_{cor\text{-}FDR} = 0.004$, OR = 0.52, 95%CI 0.36–0.77) and log-additive ($P = 0.021$, $P_{cor\text{-}FDR} = 0.027$, OR = 0.80, 95%CI 0.67–0.96) models. In the nonsmokers, a regression analysis associated *GRIN1* (rs6293) with COPD in the dominant ($P = 0.0001$, $P_{cor\text{-}FDR} = 0.00065$, OR = 0.36, 95%CI 0.20–0.63) and log-additive ($P = 0.02$, $P_{cor\text{-}FDR} = 0.027$, OR = 0.51, 95%CI 0.31–0.84) models; COPD risk was associated with the genotype AA of *GRIN1* (rs6293) (OR = 2.8, 95%CI 1.57–4.97). *GRIA1* (rs2195450) was significantly associated with COPD in the nonsmokers in the dominant ($P = 0.0082$, $P_{cor\text{-}FDR} = 0.015$, OR = 2.57, 95%CI 1.27–5.19), recessive ($P = 0.0096$, $P_{cor\text{-}FDR} = 0.015$, OR = 6.40, 95%CI 1.31–31.36), and log-additive ($P = 0.0018$, $P_{cor\text{-}FDR} = 0.005$, OR = 2.40, 95%CI 1.35–4.25) models. A significant association with COPD was observed only in the nonsmoker subgroup in the case of the genotype TT of *BDNF* (rs11030107) ($P = 0.005$ $P_{cor\text{-}FDR} = 0.01$, OR = 2.86, 95%CI 1.40–5.81).

Homozygotes GG at *GRIA1* (rs2195450) had a lower smoking index (29.54 pack/years, $P = 0.028$) (Table 4). Heterozygotes and homozygotes carrying the rare allele A of *GRIK3* (rs534131) had a significantly higher smoking index (32.88 pack/years, $P = 0.0001$). The FTND score, which characterizes the level of nicotine dependence, was also significantly higher in carriers of the rare allele A of *GRIK3* (rs534131) (FTND = 5.51, $P = 0.011$) and the heterozygous genotype GC of *GRIN2B* (rs7301328) (FTND = 5.44, $P = 0.023$).

Associations of Allele and Genotype Combinations with COPD

The APSampler (Allelic Pattern Sampler) program was used to find the informative predictors of COPD development. In addition to the nine SNPs examined in this work, eight other SNPs, which were studied previously, were included in the analysis. These were *CHRNA5* (rs16969968), *CHRNA3* (rs1051730, rs6495309), *CHRNB4* (rs1948), *HTR4* (rs3995090), *HTR2A* (rs6313), *GRIK5* (rs8099939), and *GRIN2B* (rs2268132) [7, 8]. Genetic patterns significantly associated with COPD were identified. Table 5 summarizes the most significant combinations with $P_{FDR} < 0.05$ and OR > 2.5 in the case of risk-associated combinations or OR < 0.33 in the case of protective combinations. The majority of the most significant combinations associated with COPD included the genotype *GRIN2B* rs2268132*TT or allele *GRIN2B* rs2268132*T, *GRIN2B* rs7301328*G, and *GRIN2B* rs1805476*C. The allele *GRIN2B* rs2268132*G was a mandatory element of the combinations that exerted a protective effect. The alleles *ANKK1* rs1800497*G and *GABBR2* rs3750344*A were observed in several combinations associated with COPD risk. The risk-associated combinations included *CHRNA5* rs16969968*T, *CHRNA3* rs1051730*A, and *CHRNB4* rs1948*C along with *HTR2A* rs6313*CC and *HTR4* rs3995090*A, which were associated with COPD in our sample [7, 8]. The allele *GRIK3* rs534131*G was found only in combinations associated with a lower risk of COPD. The analysis of the allele and genotype combinations of the SNPs under study showed an association for *ANKK1* (rs1800497) and *GABBR2* (rs3750344), which showed their effect only in combinations with the glutamate or nicotinic cholinergic receptor genes.

Table 1. Genotype and allele frequency distributions of the neurotransmitter gene SNPs in the COPD and control groups

Gene, SNP	Rare allele	Genotypes, alleles	COPD *n* (%) (*N* = 601)	Control *n* (%) (*N* = 617)	*P*	OR (95%CI)
GRIK3 rs534131 A>G	A	GG/GA/AA	141/341/119 (23.46/56.74/19.80)	187/326/104 (30.31/52.84/16.86)	**0.022**	–
		G/A	623/579 (51.83/48.17)	700/534 (56.73/43.27)	**0.017**	1.21 (1.04–1.57)
GRIA1 rs2195450 G>A	A	GG/GA/AA	367/202/32 (61.06/33.61/5.32)	419/172/26 (67.91/27.88/4.21)	**0.044**	–
		G/A	936/266 (77.87/22.13)	1010/224 (81.85/18.15)	**0.016**	1.28 (1.05–1.56)
GRIN1 rs6293 A>G	G	AA/AG/GG	386/198/17 (64.23/32.95/2.83)	361/231/25 (58.51/37.44/4.05)	0.096	–
		A/G	970/232 (80.70/19.30)	953/281 (77.23/22.77)	**0.04**	0.81 (0.66–0.98)
GRIN2B rs7301328 C>G	C	GG/GC/CC	225/315/61 (37.44/52.41/10.15)	217/292/108 (35.17/47.33/17.50)	**0.001**	–
		G/C	765/437 (63.64/36.36)	726/508 (58.83/41.17)	**0.021**	0.81 (0.69–0.96)
GRIN2B rs1805476 C>A	C	AA/AC/CC	163/265/173 (27.12/44.09/28.79)	170/286/161 (27.55/46.35/26.09)	0.55	–
		A/C	591/611 (49.17/50.83)	626/608 (50.73/49.27)	0.465	1.06 (0.91–1.24)
GABBR2 rs3750344 A>G	G	AA/AG/GG	403/170/28 (67.05/28.29/4.66)	399/183/35 (64.67/29.66/5.67)	0.587	–
		A/G	976/226 (81.20/18.80)	981/253 (79.50/20.50)	0.315	0.89 (0.74–1.09)
BDNF rs6265 G>A	A	GG/GA/AA	446/141/14 (74.21/23.46/2.33)	457/147/13 (74.07/23.82/2.11)	0.958	–
		G/A	1033/169 (85.94/14.06)	1061/ 173 (85.98/14.02)	0.975	1.00 (0.79–1.26)
BDNF rs11030107 T>C	C	TT/TC/CC	461/136/4 (76.71/22.63/0.67)	475/138/4 (76.99/22.37/0.65)	0.993	–
		T/C	1058/144 (88.02/11.98)	1088/146 (88.17/11.83)	0.960	1.01 (0.79–1.29)
ANKK1 rs1800497 G>A	A	GG/GA/AA	336/237/28 (55.91/39.43/4.66)	363/220/34 (58.83/35.66/5.51)	0.359	–
		G/A	909/293 (75.62/24.38)	946/288 (76.66/23.34)	0.580	1.05 (0.83–1.27)

N is the number of individuals included in the analysis; *P* is the significance of between-group differences in allele and genotype frequencies (χ^2 test for sample homogeneity); OR is the odds ratio for the rare allele (basic allele test).

DISCUSSION

Our study associated the polymorphic variants of the glutamate receptor genes *GRIK3*, *GRIN2B*, *GRIN1*, and *GRIA1* with COPD with the smoking index and the level of nicotine dependence in the total sample and groups differentiated by smoking status. Risk of COPD was associated with the rare allele A of *GRIK3* (rs534131) in our sample. The association was confirmed only in the smoker group. Moreover, the smoking index and the level of nicotine dependence were significantly increased in carriers of the rare

Table 2. Statistically significant results of the analysis of the association of candidate gene SNPs with COPD (log-regression analysis)

Gene, SNP	Rare allele	Model	COPD *n* (%) (*N* = 601)	Control *n* (%) (*N* = 617)	OR_{adj} (95%CI)	P_{adj}	$P_{cor\text{-}FDR}$
GRIK3 rs534131	*A*	*GG* *GA* + *AA* Dominant	141 (23.46) 460 (76.54)	187 (30.31) 430 (69.69)	1.00 1.42 (1.10–1.83)	0.009	0.044
		GG + *GA* *AA* Recessive	482 (80.20) 119 (19.80)	513 (83.16) 104 (16.86)	1.00 1.22 (0.88–1.70)	0.21	0.264
		Log-additive	–	–	1.25 (1.07–1.51)	0.018	0.044
GRIA1 rs2195450	*A*	*GG* *GA* + *AA* Dominant	367 (61.06) 234 (38.94)	419 (67.91) 198 (32.09)	1.00 1.35 (1.07–1.71)	0.015	0.044
		GG + *GA* *AA* Recessive	569 (94.68) 32 (5.32)	591 (95.79) 26 (4.21)	1.00 1.27 (0.75–2.17)	0.438	0.445
		Log-additive	–	–	1.26 (1.01–1.57)	0.02	0.044
GRIN1 rs6293	*G*	*AA* *AG* + *GG* Dominant	386 (64.23) 215 (35.77)	361 (58.51) 256 (41.49)	1.00 0.78 (0.62–0.98)	0.047	0.0705
		AA + *AG* *GG* Recessive	584 (97.17) 17 (2.83)	592 (95.95) 25 (4.05)	1.00 0.65 (0.32–1.30)	0.22	0.264
		Log-additive	–	–	0.79 (0.63–0.99)	0.036	0.061
GRIN2B rs7301328	*C*	*GG* *GC* + *CC* Dominant	225(37.44) 376 (62.56)	217 (35.17) 400 (64.83)	1.00 0.91 (0.70–1.17)	0.445	0.445
		GG + *GC* *CC* Recessive	540 (89.85) 61(10.15)	509 (82.50) 108(17.50)	1.00 0.54 (0.37–0.78)	0.0009	0.0108
		Log-additive	–	–	0.81 (0.68–0.98)	0.022	0.044

P_{adj} is the significance of the log-regression model by the likelihood ratio test with allowance for the age, body mass index, and gender; OR_{adj} is the odds ratio with allowance for all factors; 95%CI is the 95% confidence interval of OR; $P_{cor\text{-}FDR}$ is the significance after the FDR correction; in the log-additive model per rare allele dosage, the rare allele dosage increases in the following order: homozygote for the common allele (0)–heterozygote (1)–homozygote for the rare allele (2).

allele A. The alternative allele *GRIK3* rs534131*G was a component of the combinations that were associated with a lower risk of COPD, along with the genes for nicotinic cholinergic (*CHRNA5* and *CHRNA3*) and serotonin (*HTR2A*) receptor genes. *GRIK3* codes for glutamate ionotropic receptor kainate type subunit 3 [9]. In accordance with HaploReg v. 4.1, the polymorphic locus *GRIK3* (rs534131) is in a DNA region that binds with regulatory proteins. Polymorphic variants of the gene are associated with schizophrenia, alcohol dependence, severe depressive disorders, and suicidal behavior [13]. The contribution to COPD has not been studied for *GRIK3* polymorphic variants. The genotype CC of *GRIN2B* (rs7301328) was identified as a marker of COPD resistance. The *GRIN2B* gene codes for glutamate ionotropic receptor *N*-methyl D-aspartate type subunit 2B. The significance of the association was confirmed in the smoker group, where carriers of the genotype CC had lower levels of nicotine dependence. As for the COPD-associated combinations of polymorphic variants of the candidate genes under study, the allele *GRIN2B* rs7301328*G was observed in the four most significant combinations and the most informative combinations included *GRIN2B* (rs2268132), which was examined earlier [8], and *ANKK1* (rs1800497) and *GABBR2* (rs3750344).

Table 3. Statistically significant results of the analysis of the association of candidate gene SNPs with COPD in the groups differentiated by smoking status

Gene, SNP	Rare allele	Model	COPD absolute (%)	Control absolute (%)	OR (95%CI)	P	$P_{cor\text{-}FDR}$
Smokers			($N = 484$)	($N = 517$)			
GRIK3 rs534131	A	GG GA + AA Dominant	106 (21.90) 378 (78.10)	166 (32.11) 351 (67.89)	1.00 1.68 (1.27–2.24)	0.0001	0.00065
		GG + GA AA Recessive	388 (80.17) 96 (19.83)	430 (83.17) 87(16.83)	1.00 1.21 (0.84–1.72)	0.25	0.27
		Log-additive	–	–	1.31 (1.10–1.56)	0.003	0.0078
GRIN2B rs7301328	C	GG GC + CC Dominant	180 (37.19) 304 (62.81)	176 (34.04) 341 (65.96)	1.00 0.87 (0.66–1.15)	0.34	0.34
		GG + GC CC Recessive	438 (90.50) 46 (9.50)	431 (83.37) 86 (16.63)	1.00 0.52 (0.36–0.77)	0.001	0.004
		Log-additive	–	–	0.80 (0.67–0.96)	0.021	0.027
Nonsmokers			($N = 117$)	($N = 100$)			
GRIN1 rs6293	G	AA AG + GG Dominant	88 (75.21) 29 (24.79)	52 (52.00) 48 (48.00)	1.00 0.36 (0.20–0.63)	0.0001	0.00065
		AA + AG GG Recessive	113 (96.58) 4(3.42)	100 (100.00) 0	1.00 NA (0.00–NA)	0.037	0.043
		Log-additive	–	–	0.51 (0.31–0.84)	0.02	0.027
GRIA1 rs2195450	A	GG GA + AA Dominant	59 (50.42) 58 (49.58)	72 (72.00) 28 (28.00)	1.00 2.57 (1.27–5.19)	0.0082	0.015
		GG + GA AA Recessive	101 (86.32) 116 (13.68)	98 (98.00) 2 (2.00)	1.00 6.40 (1.31–31.36)	0.0096	0.015
		Log-additive	–	–	2.40 (1.35–4.25)	0.0018	0.005
BDNF rs11030107	C	TT TC	103 (88.03) 14 (11.97)	72 (72.00) 28 (28.00)	2.86 (1.40–5.81) 0.34 (0.17–0.71)	0.005	0.010

Grucza et al. [14] (2010) associated *GRIN2B* SNPs with the development of nicotine dependence and the age at onset of smoking. Vink et al. [15] (2009) associated several genes involved in glutamate signaling with the age at onset of smoking. Associations with COPD have been established for polymorphic variants of *GRIA1* (rs2195450), which codes for ionotropic AMPA type receptor 1 and is in chromosome 5q33.2 [9]. GRIA1 is expressed mostly in the forebrain and hippocampus, in the areas involved in the formation of memory [9]. The rare allele A of *GRIA1* (rs2195450) is associated with a higher risk of COPD. The association with COPD was confirmed in the nonsmoker group. On the other hand, a higher smoking index was observed in carriers of the rare allele A. In accordance with RegulomeDB version 1.1 and SNPinfo Web Server (https://snpinfo.niehs.nih.gov), the *GRIA1* (rs2195450) locus is in a 2-kb region and harbors binding sites for several transcription factors. *GRIA1* was not tested earlier for association with COPD or nicotine dependence. An association with COPD in nonsmokers was observed for *GRIN1* (rs6293A>G). *GRIN1* codes for glutamate receptor NMDA type subunit 1 and is in chromosome 9q34.3 [9]. The *GRIN1* (rs6293) genotype AA, which is homozygous for the rare allele A, is a marker of COPD risk. A functional

Table 4. Contributions of the genotypes at the candidate gene SNPs to the variance in smoking index and the level of nicotine dependence

Gene, SNP	Genotype	*n*	$M \pm$ S.E.	P^a	β (95%CI)
Smoking index (pack/years) in the pooled smoker sample ($N = 1001$)					
GRIA1 rs2195450	*GG*	653	29.54 (1.04)	0.028	0.00
	GA + AA	348	33.56 (1.58)		4.02 (0.44–7.61)
GRIK3 rs534131	*GG*	277	25.12 (1.38)	0.0001	0.00
	GA + AA	724	32.88 (1.07)		7.76 (3.97–11.55)
	GG + AA	458	27.69 (1.12)	0.0013	0.00
	GA	543	33.31 (1.29)		5.62 (2.21–9.04)
Level of nicotine dependence by FTND score ($N = 1218$)					
GRIK3 rs534131	*GG*	329	4.99 (0.16)	0.011	0.00
	GA + AA	889	5.51 (0.11)		0.51 (0.12–0.91)
GRIN2B rs7301328	*GG + GC*	1045	5.42 (0.09)	0.042	0.00
	CC	173	5.00 (0.24)		–0.16 (–0.31 to –0.01)
	GG + CC	613	5.28 (0.12)	0.023	0.00
	GC	605	5.44 (0.12)		0.12 (0.02–0.23)

$M \pm$ S.E. is the mean $\pm$ standard error of the mean; P^a is the significance level for the regression equation; β (95%CI) is the regression coefficient (95% confidence interval of the coefficient).

analysis showed that *GRIN1* (rs6293) has regulatory rank 1f and coefficient 0.55436 according to RegulomeDB version 1.1, indicating that the SNP affects gene expression. According to HaploReg v. 3 and SNPinfo Web Server (https://snpinfo/niehs.nih.gov), SNP rs6293 is in a DNA region that has histone marks of enhancer and promoters (GM12878 and K562) and a DNA region that binds the ZNF263 regulatory protein and is hypersensitive to DNase I in 30 different tissues. Orihara et al. [16] (2018) showed that functional NMDA receptors activate CD4+ T cells, thus affecting cytokine production and cell proliferation and viability. The effect of glutamate on immunocompetent cells may play an important role in the pathogenesis of various disorders associated with systemic inflammation, including COPD.

We showed that the genotype TT of *BDNF* (rs11030107) is significantly associated with COPD in nonsmokers. The allele *BDNF* rs11030107*T is involved in an informative COPD risk combination together with alleles of *GRIN2B* (rs2268132 and rs7301328) and *GABBR2* (rs3750344). *BDNF* codes for the brain-derived neurotrophic factor and is in chromosome 11p13 [9]. BDNF belongs to the family of neurotrophins, which play a key role in regulating neurogenesis and general neuroplasticity [9]. In a functional analysis, rs11030107 has regulatory rank 2b, indicating that the SNP affects gene expression. According to GTEx (https://www.gtexportal.org), rs11030107 is associated with changes in gene expression in various tissues, including lung tissue, and gene expression is significantly higher in TT homozygotes. *BDNF* (rs6265) was earlier associated with nicotine dependence and higher serum BDNF levels [17]. Ohmoto et al. [18] (2019) associated *BDNF* (rs6265) with nicotine dependence and age at onset of smoking in the Japanese population.

The *GABBR2* gene is in chromosome 9q22.1-q22.3 and codes for a receptor of γ-aminobutyric acid, which acts as a main inhibitory neurotransmitter and is involved in regulating many physiological and psychological processes in the brain [9]. Beuten et al. [19] (2005) observed that *GABAB2* polymorphisms, including rs3750344, are associated with nicotine dependence in Afro-American and Caucasian populations. There is convincing evidence that genes of the GABAergic signaling pathway are involved in nicotine and alcohol dependences [20]. We did not associate *GABBR2* (rs3750344) with COPD, but the polymorphic variant *GABBR2* rs3750344*A was a component of three significant combinations associated with a higher risk of COPD, along with the alleles *GRIN2B* rs2268132*T, *GRIN2B* rs7301328*G, *ANKK1* rs1800497*G, *BDNF* rs11030107*T, *CHRNA5* rs16969968*T, and *CHRNA3* rs1051730*A. The *GABBR2* gene was not tested for association with COPD earlier.

The dopaminergic system plays a crucial role in various dependences, including nicotine dependence. *DRD2* is in chromosome 11q23.2 and has been studied most comprehensively [21]. The so-called Taq1A polymorphism of *DRD2* (rs1800497) was studied earlier; more recent studies showed that the polymorphism occurs in exon 8 of the adjacent *ANKK1* protein kinase gene and causes the p.Glu713Lys amino acid substitution [22]. According to HaploReg v. 3, *ANKK1*

Table 5. Significant COPD-associated combinations of alleles and genotypes at SNPs of the neurotransmitter system genes

Combination	COPD, %	Control, %	P_{value}	P_{FDR}	OR	95%CI
Risk						
GRIN2B rs2268132*TT + *GRIN2B* rs7301328*G + *ANKK1* rs1800497*G	14.84	1.32	8.09e-11	1.35e-08	13.02	4.62–36.66
GRIN2B rs2268132*TT + *GRIN2B* rs7301328*G	14.24	1.58	3.04e-10	3.47e-08	10.46	4.10–26.67
CHRNA5 rs16969968*T + *HTR2A* rs6313*CC	15.84	2.89	3.78e-09	1.82e-07	6.74	3.16–4.40
GRIN2B rs7301328*G + *HTR2A* rs6313*CC	28.36	10.87	6.57e-09	2.95e-07	3.24	2.135–4.92
GRIN2B rs2268132*T + *GRIN2B* rs7301328*G + *ANKK1* rs1800497*G + *GABBR2* rs3750344*A	46.58	26.07	9.68e-08	2.79e-06	2.57	1.75–3.47
GRIN2B rs2268132*T + *GRIN2B* rs7301328*G + *GABBR2* rs3750344*A + *BDNF* rs11030107*T	48.97	29.49	9.36e-07	1.78e-05	2.59	1.63–3.22
GRIN2B rs2268132*T + *GRIN2B* rs1805476*C + *GABBR2* rs3750344*A + *CHRNA5* rs16969968*T + *CHRNA3* rs1051730*A	21.51	7.31	1.12e-05	0.00013	3.47	1.90–6.33
GRIN2B rs2268132*T + *ANKK1* rs1800497*G + *CHRNA5* rs16969968*T + *GRIK5* rs8099939*C	19.62	8.22	2.71e-05	0.0002	2.96	1.72–5.11
GRIN2B rs2268132*T + *GRIN2B* rs1805476*C + *CHRNA5* rs16969968*T + *GRIA1* rs2195450*G	24.15	9.80	3.3e-05	0.0003	2.92	1.70–5.02
GRIN2B rs1805476*C + *CHRNA3* rs1051730*A + *HTR4* rs3995090*A + *GRIA1* rs2195450*G	19.44	6.63	3.54e-05	0.0003	3.39	1.81–6.34
GRIN2B rs2268132*T + *CHRNA5* rs16969968*T + *CHRNB4* rs1948*C + *GRIK5* rs8099939*C + *GRIA1* rs2195450*G	18.24	6.45	5.69e-05	0.0004	3.23	1.74–6.01
Protective						
GRIN2B rs2268132*G + *CHRNA5* rs16969968*C + *HTR2A* s6313*T	48.20	78.73	$6.56e^{-15}$	1.64e-11	0.251	0.17–0.36
CHRNA5 rs16969968*C + *CHRNA3* rs1051730*G + *HTR2A* rs6313*T + *GRIK3* rs534131*G	43.29	75.21	3.31e-13	4.16e-10	0.252	0.17–0.37
GRIN2B rs2268132*G + *CHRNA3* rs1051730*G + *HTR2A* rs6313*T	47.53	75.24	4.57e-13	3.83e-10	0.298	0.21–0.41
CHRNA5 rs16969968*C + *HTR2A* rs6313*T + *GRIK3* rs534131*G	45.79	75.90	1.2e-12	3.01e-09	0.268	0.18–0.39

P_{value} is the significance level by Fisher's test; P_{FDR} is the significance after the FDR correction; OR is the odds ratio; 95%CI is the 95% confidence interval of OR.

(rs1800497) is in a DNase I-hypersensitive DNA region in six different tissues. Apart from a tight linkage of *ANKK1* rs1800497 with *DRD2*, a functional association with the dopaminergic system was observed for ANKK1 [23]. Liu et al. [24] (2020) associated the polymorphic variants of the *ANKK1/DRD2* gene cluster with nicotine dependence in Chinese males. We did not associate rs1800497 of *ANKK1* with COPD. Significant associations with COPD were observed only for the polymorphic variant *ANKK1* rs1800497*G as part of informative combinations with the polymorphic variants *GRIN2B* rs2268132*TT and *GRIN2B* rs7301328*G, *GABBR2* rs3750344*A, *CHRNA5* rs16969968*T, and *GRIK5* rs8099939*C.

To conclude, our findings support the assumption that neurotransmitter system genes play a substantial role in predisposition to COPD, which depends on the smoking status as a main factor. The most significant associations with COPD were detected for the glutamate receptor genes *GRIK3*, *GRIN2B*, *GRIA1*, and *GRIN1*. In the case of *BDNF*, *GABBR2*, and *ANKK1*, associations with COPD were observed only in informative combinations with polymorphic variants of glutamate receptor (*GRIN2B* and *GRIK5*) and nicotinic cholinergic receptor (*CHRNA5* and *CHRNA3*) genes, suggesting their potential synergistic effect. Pathogenetically important interactions of the *GRIA1*, *GRIK3*, and *GRIN2B* polymorphic variants were observed with the level of nicotine dependence and the smoking index. Our results provide for a better understanding of the molecular mechanisms of smoking-associated disorders.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interest.

Statement of compliance with standards of research involving humans as subjects. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants involved in the study.

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