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Association of Genes Involved in Nicotine and Tobacco Smoke Toxicant Metabolism (*CHRNA3/5*, *CYP2A6*, and *NQO1*) and DNA Repair (*XRCC1*, *XRCC3*, *XPC*, and *XPA*) with Chronic Obstructive Pulmonary Disease

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Abstract—Polymorphisms of the *CHRNA5/A3*, *CYP2A6*, *NQO1*, *XPC*, *XRCC1*, *CRCC3*, *XPD*, and *XPA* genes were tested for association with the development and progression of chronic obstructive pulmonary disease (COPD) in ethnic Tatars. *CHRNA5* (rs16969968) ($P = 0.0001$, OR = 2.24) and *CHRNA3* (rs1051730) ($P = 0.0001$, OR = 2.72) polymorphisms were associated with COPD development in the recessive model. The nondeletion variant of *CYP2A6* (del) was associated with COPD risk ($P = 0.00001$, OR = 2.77). *NQO1* (rs113341), *XRCC1* (rs25487), *XRCC3* (rs86539), *XPC* (rs2228001), and *XPA* (rs1800975) were associated with COPD in the additive model ($P = 0.000001$, OR = 2.67; $P = 0.00001$, OR = 0.51; $P = 0.0003$, OR = 1.76; $P = 0.0004$, OR = 0.54; and $P = 0.007$, OR = 0.74, respectively). A gene-by-environment interaction was observed for *XPA* (rs1800975) and the smoking status ($P_{interact} = 0.002$), and the rs16969968 and rs1051730 polymorphisms of the *CHRNA3/5* gene cluster showed an association with COPD only in smokers. Carriers of the *CYP2A6* deletion (*CYP2A6*4*) had a lower smoking index ($P = 0.0019$). *XRCC3* (rs861539) genotype TT was characterized by lower indices of pulmonary function, including vital capacity (VC) ($P = 0.0487$), forced vital capacity (FVC) ($P = 0.0032$), and forced expiratory volume in 1 second (FEV1) ($P = 0.02$). FVC was found to depend on the genotype at *XPA* (rs1800975) ($P = 0.0028$).

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a multifactorial chronic inflammatory disease of the respiratory system that affects predominantly distal airways and lung parenchyma and leads to emphysema. COPD manifests itself as partly reversible bronchial obstruction with the development and progression of respiratory insufficiency [1]. COPD is one of the major causes of deaths and the fourth leading cause of morbidity in both developed and developing countries [1]. It is thought conventionally that smoking often leads to COPD, which is diagnosed in 20–30% of smokers [1, 2]. The genetic mechanisms of COPD development have recently come to be the subject of large-scale studies worldwide [2–4].

Genome-wide association studies (GWASs) have identified several loci associated with chronic lung obstruction in Caucasian and Mongoloid populations [3, 4]. The *CHRNA3/CHRNA5/CHRNB4* gene cluster codes for cholinergic nicotine receptors, is expressed in the central nervous systems and bronchial epithelium, and plays a major role in nicotine dependence [5, 6]. *CHRNA3/5* and *CHRNB4* polymorphisms have been associated with smoking intensity and disorders where smoking is a major risk factor [5–10].

In 2011, a new COPD-associated marker has been revealed in chromosome 19q13 in addition to known markers as a result of whole-genome studies. Region 19q13 harbors *CYP2A6* [4], whose protein product is involved in metabolizing many drugs, coumarin, tobacco smoke procarcinogens, and nicotine, which is a main substance that induces and maintains tobacco dependence [11–13].

Tobacco smoke-induced oxidative stress plays an important role in COPD by damaging lung tissue. NAD(P)H–quinone oxidoreductase 1 (NQO1) cata-

Abbreviations: GWAS, genome-wide association study; MAF, minor allele frequency; GOLD, Global Initiative for Chronic Obstructive Lung Disease; NQO1, NAD(P)H–quinone oxidoreductase 1; COPD, chronic obstructive pulmonary disease; VC, vital capacity; FVC, forced vital capacity; ROS, reactive oxygen species; FEV1, forced expiratory volume in 1 second.

lyzes two-electron reduction of quinone compounds and prevents the generation of semiquinone free radicals and reactive oxygen species (ROS), thus protecting the cell from oxidative stress [14]. On the other hand, NQO1 activates certain carcinogens, such as nitrosamines and heterocyclic amines, which are present in tobacco smoke [14, 15].

Inflammatory cells release large amounts of ROS, which can damage proteins, lipids, and nucleic acids [6]. DNA damage has been identified as a cause of pulmonary emphysema [17]. DNA repair genes, which determine the individual toxicogenetic sensitivity to environmental factors, have several hundreds of polymorphic variants [18, 19], but their role in nontumor disorders of the respiratory system, including COPD, is still poorly understood. There is evidence that DNA repair is distorted in COPD [20, 21]. The distortion may be related, to a considerable extent, to an oxidant–antioxidant imbalance, permanent oxidative stress, and cyclic activation and proliferation of lymphocytes [22]. It cannot also be excluded that a lower efficiency of double-strand DNA repair is a cause [17]. *XRCC1*, *OGG1*, *XRCC3*, and *XRCC4* polymorphisms are associated with the extent of DNA damage and COPD [17, 22–24].

The objective of this work was to test the polymorphic variants of genes coding for cholinergic nicotine receptors (*CHRNA5* and *CHRNA3*), DNA repair enzymes (*XRCC1, XRCC3*, *XPC, XPD*, and *XPA*), and enzymes involved in metabolizing nicotine and tobacco smoke toxicants (*CYP2A6* and *NQO1*) with COPD in ethnic Tatars.

EXPERIMENTAL

DNA samples were obtained from unrelated ethnic Tatars ($N = 882$) from the Republic of Bashkortostan (Russia). COPD was diagnosed according to the International Classification of Diseases, 10th revision, and recommendations of the Global Initiative for Chronic Obstructive Lung Disease (GOLD) of 2011 [1, 25]. The patients denied any previous exposure to occupational hazards. Candidates with signs of allergic disorders, bronchial asthma, oncology, or specific infections of respiratory organs (tuberculosis) were excluded from the analysis.

A test sample comprised 425 patients, including 369 males (86.82%) and 56 females (13.18%) with a mean age of 63.38 ± 11.81 years. Of all the patients, 331 were current or former smokers (77.88%) and 94 were nonsmokers (22.12%). The smoking index was conventionally expressed in pack years (PY) and calculated as $PY = (N \times n)/20$, where N is the number of cigarettes smoked per one day, n is the smoking history (years), and 20 is the number of cigarettes per pack [1]. The smoking indices were 43.52 ± 28.42 in the current and former smokers taken together and 48.15 ± 27.41 in the current smokers alone.

The patients were tested for pulmonary function by spirometry with measuring the vital capacity (VC), forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1), and FEV1/VC ratio. The patients had FEV1 = 38.85 ± 16.62%, FVC = 46.06 ± 16.32%, VC = 49.02 ± 15.54%, and FEV1/FVC = 54.21 ± 11.40% relative to the corresponding normal values. A control group ($N = 457$) included healthy individuals without a history of respiratory pathology or occupational exposure to chemical hazards and matched the patient sample in age (58.44 ± 14.79 years), gender (406 males, 88.84% and 51 females, 11.16%), smoking status (322 current and former smokers, 70.46% and 135 nonsmokers, 29.54%), and smoking index (PY = 18.64 ± 13.27 in the current and former smokers taken together and PY = 37.71 ± 14.12 in the current smokers alone). The pulmonary function parameters in the control group were FEV1 = 130 ± 52.1, FVC = 128.1 ± 32.05, VC = 131 ± 42.87, and FEV1/FVC = 87.94 ± 10.69.

The study was approved by the Ethics Committee at the Institute of Biochemistry and Genetics. All of the subjects voluntarily gave their informed consent to their biological material being used for the study.

Genotyping. DNA was isolated from peripheral blood leukocytes by phenol–chloroform extraction. The following polymorphisms were examined: *CHRNA5* (rs16969968), *CHRNA3* (rs1051730 and rs6495309), *CYP2A6* (whole deletion, *CYP2A6*4*), *NQO1* (rs1131341), *XPC* (rs2228001 and poly(AT)), *XRCC1* (rs25487 and rs25489), *XRCC3* (rs861539), *XPD* (rs13181), and *XPA* (rs1800975). The rs16969968, rs1051730, rs6495309, and rs1131341 were examined by the real-time polymerase chain reaction (PCR), with the use of commercial kits and fluorescence detection (FLASH/RTAS, http://testgen.ru, Test-Gen, Russia) with a BioRad CFX96 instrument (BioRad Laboratories, United States). End-point fluorescence and genotype discrimination were determined according to the BioRad CFX96 protocol, using CFX Manager software. The *CYP2A6* (del, *CYP2A6*4*), *XPC* (rs2228001 and poly(AT)), *XRCC1* (rs25487 and rs25489), *XRCC3* (rs861539), *XPD* (rs13181), and *XPA* (rs1800975) polymorphisms were examined by PCR with subsequent digestion of the product with Bst2UI, PvuII, MspI, RsaI, NcoI, PstI, or MspI (SibEnzyme, Russia; Thermo Scientific, Germany) as recommended by the manufacturer. PCR was run in standard conditions, using a T100 thermal cycler (BioRad Laboratories, United States) and *Thermus aquaticus* DNA polymerase (Thermo Scientific, Germany). Primer sequences and allele identification followed published protocols [11, 26, 27]. The amplification and restriction products were examined via vertical electrophoresis in 6–8% polyacrylamide gel. Gels were stained with 0.1 μg/mL ethidium bromide for 15 min and photographed in transmitted UV light. The product lengths were estimated against a marker 100-bp ladder (SibEnzyme, Russia).

Statistical analysis. Minor allele frequencies (MAFs) were calculated, genotype frequencies were tested for correspondence to the Hardy–Weinberg equilibrium (χ^2), an association was checked via the basal allele test, the odds ratio (OR) was computed for each of the loci under study, and differences in allele and genotype frequencies were tested for statistical significance (χ^2 test for sample homogeneity and P-value for the test) with the use of the program PLINK v. 1.07 [28]. Logistic regression was used to test the polymorphisms for association in various models (additive, dominant, and recessive) with due account of quantitative and binary characters (gender, age, smoking status, and smoking index), which were included as independent variables in the regression equation. In the additive model, the effect of a genetic marker on the disease risk depends on the minor allele dosage, which is 0 in the case of homozygotes for the major allele, 1 in the case of heterozygotes, and 2 in the case of homozygotes for the minor allele, and each minor allele copy changes (increases or decreases) the disease risk by OR. In the dominant model, one copy of the minor allele is enough for the risk to change, and the risk is associated with both homozygosity for the minor allele and heterozygosity. When C is the minor allele and T is the major allele, the dominant model is a comparison of (CC + CT) versus TT. The recessive model tests homozygosity for the minor allele for association with a disease (i.e., two copies of the minor allele are necessary to affect the risk, whereas one copy is insufficient) and consists in a comparison of CC versus (CT + TT). The exponent value of a particular regression coefficient (β) was interpreted as OR in the logistic model, and a 95% confidence interval was calculated. The hypothesis that the resulting model is significant with all variables taken into account was checked using the likelihood ratio test and its significance P_{adj}. The Akaike information criterion (AIC) was used to select the best model. Models with the lowest AIC values were selected among statistically significant models ($P_{adj} < 0.05$) for each locus.

Bonferroni correction for multiple comparisons was used to minimize type 1 error; i.e., P was multiplied by the total polymorphic loci ($n = 10$) involved in the association analysis to obtain P_{cor}. A regression analysis was carried out to assess the interaction of a polymorphic locus and smoking (the smoking status and smoking index), using the software packages PLINK v. 1.07 and SNPStats [28, 29]. The contributions the genotypes of the loci under study made to the total variation of quantitative traits characterizing the disease severity (VC, FVC, and FEV1 as parameters of external respiration) and smoking (the smoking index in pack years) were estimated by Kruskal–Wallis (in the case of three groups) or Mann–Whitney (in the case of two groups) test, using the program Statistica v. 6.0 (StatSoft, United States) [30].

RESULTS

At the first step, genotype frequencies of the polymorphisms under study were tested for correspondence to the Hardy–Weinberg proportion and MAFs estimated for the pooled patient sample, control sample, and particular groups taken separately. The results obtained for the control group were as follows: *CHRNA5* (rs16969968) ($P = 0.44$, MAF = 0.268), *CHRNA3* (rs1051730) ($P = 0.43$, MAF = 0.234), *CHRNA3* (rs6495309) ($P = 0.20$, MAF = 0.208), *NQO1* (rs1131341) ($P = 1.00$, MAF = 0.689), *XPC* (rs2228001) ($P = 0.41$, MAF = 0.337), *XPC* (poly(AT)) ($P = 0.0001$, MAF = 0.268), *XRCC1* (rs25487) ($P = 0.038$, MAF = 0.514), *XRCC1* (rs25489) ($P = 0.14$, MAF = 0.079), *XRCC3* (rs861539) ($P = 0.24$, MAF = 0.291), *XPD* (rs13181) ($P = 0.018$, MAF = 0.325), and *XPA* (rs1800975) ($P = 1.0$, MAF = 0.510). The *XPC* (poly(AT)) and *XPD* (rs13181) polymorphisms showed a deviation from Hardy–Weinberg equilibrium and were excluded from further association analysis.

Association of the Polymorphisms with COPD

The allele and genotype frequency distributions of the loci under study, the significance of between-group differences in genotype and allele frequency distributions, and the OR values calculated for the minor allele of each locus are summarized in Tables 1 and 2. Significant differences between the two groups were observed for the following polymorphisms: *CHRNA5* (rs16969968), *CHRNA3* (rs1051730), *CYP2A6* (del), *NQO1* (rs1131341), *XRCC1* (rs25487), *XRCC3* (rs861539), *XPC* (rs2228001), and *XPA* (rs1800975).

Table 3 summarizes the significant results of testing the loci for association with the disease (with regression coefficient β, the exponent of which was interpreted as OR, calculated for the logistic model along with its 95% confidence interval) and the results of testing the significance level in various models with the gender, age, smoking status, and smoking index considered.

In the case of *CHRNA5* (rs16969968), the frequency of minor allele A was higher in the patients ($P = 0.0001$, OR = 1.46). The locus showed a significant association with the disease in the recessive ($P = 0.0001$, $P_{cor} = 0.001$, OR = 2.24) and additive ($P = 0.0001$, $P_{cor} = 0.001$, OR = 1.65) models. Homozygosity for the minor *CHRNA5* (rs16969968) allele was identified as a risk factor, occurring at a frequency of 14.69% in the patients versus 7.11% in the controls.

In the case of *CHRNA3* (rs1051730), minor allele T was associated with the disease ($P = 0.0001$, OR = 1.52). *CHRNA3* (rs1051730) genotype TT was identified as a risk marker; its frequency was 10.19% in the patients versus 4.0% in the controls ($P = 0.0001$, $P_{cor} = 0.001$, OR = 2.72). The association of *CHRNA3* (rs1051730) with the disease was more informative in the additive model ($P = 0.0001$, $P_{cor} = 0.001$, OR = 1.63), suggest-

Table 1. Genotype and allele frequency distributions of *CHRNA5*, *CHRNA3*, *CYP2A6*, and *NQO1* in COPD patients and controls

Gene, polymorphic locus	Minor allele	*N*	Genotypes, alleles	COPD, subjects (%)	Control, subjects (%)	*P*	OR (95% CI)
CHRNA5 (*c.1192G>A*) rs16969968	A	872	AA/AG/GG	62/171/189 (14.69/ 40.52/44.79)	32/178/240 (7.11/39.56/53.33)	0.0001	–
			A /G	295/549 (34.95/65.05)	242/658 (26.89/73.11)	0.0001	1.46 (1.19–1.79)
CHRNA3 (*c.645C>T*) rs1051730	T	872	TT/TC/CC	43/182/197 (10.19/43.13/46.68)	18/175/257 (4.00/38.89/57.11)	0.0001	–
			T/C	268/576 (31.75/68.25)	211/689 (23.44/76.56)	0.0001	1.52 (1.23–1.88)
CHRNA3 (*c.–2109G>A*) r6495309	A	872	AA/AG/GG	19/123/280 (4.50/29.15/66.35)	14/159/277 (3.11/35.33/61.56)	0.107	–
			A/G	161/683 (19.08/80.92)	187/713 (20.78/79.22)	0.407	0.89 (0.71–1.13)
CYP2A6 (whole deletion, *CYP2A6*4*)	del	875	del/n	29/396 (6.82/83.11)	76/374 (16.89/83.11)	0.00001	0.36 (0.23–0.56)
NQO1 (*c.415C>T*) rs1131341	T	875	TT/TC/CC	6/104/315 (1.41/24.47/74.12)	2/58/390 (0.44/12.89/86.67)	0.000001	–
			T/C	116/734 (13.65/86.35)	62/838 (6.89/93.11)	0.0000012	2.14 (1.54–2.95)

Here and in Table 2, *N* is the sample size, *P* shows the significance of between-group differences in allele and genotype frequencies (χ^2 test for sample homogeneity), and OR is the odds ratio for the minor allele (basic allele test).

ing a higher risk of the disease for both heterozygotes and homozygotes for the minor allele.

In the case of *CYP2A6* (del), the proportion of subjects carrying the deletion in the patients was lower than in the controls (6.82% vs. 16.89%; $P = 0.00001$, $P_{cor} = 0.0001$, OR = 0.36 for the minor variant, which contained the deletion). The major genotype without the deletion was identified as a risk marker (OR = 2.77, 95% CI 1.76–4.35).

In the case of *NQO1* (rs1131341), the minor allele was associated with the disease ($P = 0.0000012$, OR = 2.14). Its frequency reached 13.65% in the patients, while not exceeding 7% in the controls. A regression analysis associated *NQO1* (rs1131341) with the disease in both dominant ($P = 0.000001$, $P_{cor} = 0.00001$, OR = 2.34) and additive ($P = 0.000001$, $P_{cor} = 0.00001$, OR = 2.67) models. The association was due to a higher proportion of heterozygotes and homozygotes for minor allele T in the patients.

In the case of *XRCC1* (rs25487), the frequency of minor allele G in the patients was lower than in the controls (41.65% vs. 51.44%; $P = 0.00001$, OR = 0.67). A subsequent regression analysis associated *XRCC1* (rs25487) with the disease in the recessive model ($P = 0.00001$, $P_{cor} = 0.0001$, OR = 0.52). The most informative association was observed for *XRCC1* (rs25487) in the additive model ($P = 0.00001$, $P_{cor} = 0.0001$, OR = 0.51). Homozygosity for major allele A was identified as a risk marker, occurring at a frequency of 30.35% in the patients versus 20.22% in the controls ($P = 0.00002$, $P_{cor} = 0.0002$, OR = 1.72, 95% CI 1.26–2.34).

In the case of *XRCC3* (rs861539), minor allele T was associated with the disease ($P = 0.0001$, OR = 1.48). The disease risk is higher in homozygotes for minor allele T ($P = 0.001$, $P_{cor} = 0.01$, OR = 1.94). The additive model was more informative ($P = 0.0003$, $P_{cor} = 0.003$, OR = 1.76), indicating that each minor allele T copy increases the disease risk.

The *XPC* (rs2228001) locus also showed a significant association with the disease ($P = 0.0001$, OR = 0.64 for minor allele C). A regression analysis showed the most informative model for the association of *XPC* (rs2228001) with the disease ($P = 0.0001$, $P_{cor} = 0.001$, OR = 0.60 in the dominant model). The proportion of homozygotes for *XPC* (rs2228001) major allele A in the patients was significantly higher than in the controls (54.9% vs. 43.6%; OR = 1.65, 95% CI 1.27–2.16).

In the case of *XPA* (rs1800975), the frequency of minor allele G in the patients was lower than in the controls (42.82% vs. 51.00%; $P = 0.0001$, OR = 0.71). The association with *XPA* (rs1800975) was the most significant in the recessive model ($P = 0.003$, $P_{cor} = 0.03$, OR = 0.59). Homozygosity for major allele A in

Table 2. Genotype and allele frequency distributions of the polymorphisms of DNA repair genes in COPD patients and controls

Gene, polymorphic locus	Minor allele	*N*	Genotypes, alleles	COPD, subjects (%)	Control, subjects (%)	*P*	OR (95% CI)
XRCC1 (*c.1196A>G*) rs25487	G	875	GG/AG/AA	58/238/129 (13.65/56.00/30.35)	104/255/91 (23.11/56.67/20.22)	0.00001	–
			G/A	354/496 (41.65/58.35)	463/437 (51.44/48.56)	0.00001	0.67 (0.56–0.81)
XRCC1 (*c.839G>A*) r25489	A	875	AA/AG/GG	1/62/362 (0.24/14.59/85.18)	5/61/384 (1.11/13.56/ 85.33)	0.271	–
			A/G	64/786 (7.53/92.47)	71/829 (7.89/92.11)	0.848	0.95 (0.67–1.35)
XRCC3 (*c.722C>T*) rs861539	T	875	TT/TC/CC	77/168/180 (18.12/39.53/42.35)	46/170/234 (10.22/ 37.78/52.00)	0.0001	–
			T/C	322/528 (37.88/62.12)	262/638 (29.11/70.89)	0.0001	1.48 (1.22–1.81)
XPC (*c.2815C>A*) rs2228001	C	882	CC/CA/AA	17/175/233 (4.00/41.18/54.82)	44/220/193 (9.63/48.14/42.23)	0.0001	–
			C/A	209/641 (24.59/75.41)	308/606 (33.70/66.30)	0.0001	0.64 (0.52–0.79
XPA (*c.–4A>G*) rs1800975	G	877	GG/GA/AA	71/222/132 (16.71/52.24/31.06)	114/233/105 (25.22/51.55/23.23)	0.002	–
			G/A	364/486 (42.82/57.18)	461/443 (51.00/49.00)	0.0001	0.71 (0.59–0.86)

the patients was more frequent than in the controls (31.06% vs. 23.33%; $P = 0.011$, $P_{cor} = 0.11$, OR = 1.48, 95% CI 1.10–2.00).

Gene-by-Environment Interactions in COPD

A statistically significant interaction with the smoking status was observed only for *XPA* (rs1800975) ($P_{interact} = 0.002$). We did not observe any significant interaction of the loci under study with the smoking index, which characterizes the intensity of exposure to the environmental factor. To further study the gene-by-environment interactions, OR values were compared for groups formed by the presence or absence of exposure to a particular factor. The two loci of the genes for cholinergic nicotine receptors 3 and 5, *CHRNA5* (rs16969968) and *CHRNA3* (rs1051730), showed a significant association with the disease only in smokers ($P = 0.001$, $P_{cor} = 0.01$, OR = 1.54 and $P = 0.00012$, $P_{cor} = 0.0012$, OR = 1.78, respectively) (Table 4).

In the case of *CYP2A6* (del), *NQO1* (rs1131341), *XRCC1* (rs25487), *XRCC3* (rs861539), *XPC* (rs2228001), and *XPA* (rs1800975), the association was significant regardless of the smoking status. *CYP2A6* (del) was associated with the disease in both nonsmokers ($P = 0.00001$, $P_{cor} = 0.0001$, OR = 0.25 for the deletion variant) and smokers ($P = 0.0001$, $P_{cor} = 0.001$, OR = 0.47). *NQO1* (rs1131341) was associated with the disease in the nonsmokers ($P = 0.012$, $P_{cor} = 0.12$, OR = 2.11), but its association in the smokers was more significant ($P = 0.00001$, $P_{cor} = 0.0001$, OR = 2.94). In the case of *XRCC1* (rs25487), comparable significance levels of the association were obtained for the smokers ($P = 0.002$, $P_{cor} = 0.002$, OR = 0.53) and nonsmokers ($P = 0.0003$, $P_{cor} = 0.003$, OR = 0.48). The association of *XRCC3* (rs861539) and *XPC* (rs2228001) with the disease remained significant after a correction for multiple comparisons only in the smokers ($P = 0.0001$, $P_{cor} = 0.001$, OR = 1.44 and $P = 0.0003$, $P_{cor} = 0.003$, OR = 0.59, respectively) (Table 4). *XPA* (rs1800975) was associated with the disease in the dominant model in the smokers ($P = 0.0005$, $P_{cor} = 0.001$, OR = 0.48), while the recessive model was more informative in the nonsmokers ($P = 0.017$, $P_{cor} = 0.1$, OR = 0.41).

We examined the contributions the loci make to the variation of the smoking index as a quantitative trait characterizing the smoking intensity and found that the index depends on the *CYP2A6* genotype (Table 5). Carriers of the *CYP2A6* deletion (*CYP2A6*4*) had a significantly lower smoking index as compared with carriers of the genotypes without the deletion in both

Table 3. Association analysis of the polymorphic loci of *CHRNA5*, *CHRNA3*, *NQO1*, and DNA repair genes with COPD (log-regression analysis)

Polymorphic locus	Minor allele	*N*	Genotypes, model	COPD, subjects (%)	Control, subjects (%)	OR_{adj} (CI95%)	P_{adj}
CHRNA5 (*c.1192G>A*) rs16969968	A	872	GG	189 (44.79)	240 (53.33)	1.00	
			GA+AA Dominant	233 (55.21)	210 (46.67)	1.41 (1.08–1.83)	0.013
			GG+GA	360 (85.31)	418 (92.89)	1.00	
			AA Recessiv	62 (14.69)	32 (7.11)	2.24 (1.44–3.52)	0.0001
			Additive	–	–	1.65 (1.21–1.72)	0.0001
CHRNA3 (*c.645C>T*) rs1051730	T	872	CC	197 (46.68)	257 (57.11)	1.00	
			CT+TT Dominant	225 (53.32)	193 (42.89)	1.52 (1.16–1.98)	0.0027
			CC+CT	379 (89.81)	432 (96.00)	1.00	
			TT Recessive	182 (10.19)	18 (4.00)	2.72 (1.54–4.72)	0.0001
			Additive	–	–	1.63 (1.32–2.21)	0.0001
NQO1 (*c.415C>T*) rs1131341	T	875	CC	315 (74.12)	390 (86.67)	1.00	
			CT+TT Dominant	110 (25.88)	60 (13.33)	2.34 (1.68–3.23)	0.000001
			CC+CT	419 (98.59)	448 (99.56)	1.00	
			TT Decessiv	6 (1.41)	2 (0.44)	3.21 (0.64–15.9)	0.251
			Additive	–	–	2.67 (1.81–4.02)	0.000001
XRCC1 (*c.1196A>G*) rs25487	G	875	AA	129 (30.35)	91 (20.22)	1.00	
			AG+GG Dominant	296 (69.65)	359 (79.78)	0.58 (0.43–0.79)	0.00002
			AA+AG	367 (86.35)	346 (76.89)	1.00	
			GG Recessiv	58 (13.65)	104 (23.11)	0.52 (0.36–0.74)	0.00001
			Additive	–	–	0.51 (0.38–0.66)	0.00001
XRCC3 (*c.722C>T*) rs861539	T	875	CC	180 (42.35)	234 (52.00)	1.00	
			CT+TT Dominant	245 (57.65)	216 (48.00)	1.47 (1.13–1.92)	0.005
			CC+CT	348 (81.88)	404 (89.78)	1.00	
			TT Recessiv	77 (18.12)	46 (10.22)	1.94 (1.31–2.87)	0.001
			Additive	–	–	1.76 (1.37–2.03)	0.0003
XPC (*c.2815C>A*) rs2228001	C	882	AA	233 (54.82)	193 (42.23)	1.00	
			AC+CC Dominant	192 (45.18)	264 (57.77)	0.60 (0.46–0.78)	0.0001
			AA+AC	408 (96.00)	413 (90.37)	1.00	
			CC Recessive	17 (4.00)	44(9.63)	0.39 (0.22–0.69)	0.002
			Additive	–	–	0.54 (0.32–0.74)	0.0004
XPA (*c.–4A>G*) rs1800975	G	877	AA	132 (31.06)	105 (23.23)	1.00	
			AG+GG Dominant	293 (68.94)	347 (76.77)	0.67 (0.49–0.91)	0.011
			AA+AG	354 (83.29)	338 (74.78)	1.00	
			GG Recessive	71 (16.71)	114 (25.22)	0.59 (0.42–0.82)	0.003
			Additive	–	–	0.74 (0.59–0.92)	0.007

N is the sample size in the regression analysis; P_{adj} shows the significance for the likelihood ratio test of the log-regression model accounting for the age, gender, smoking status, and smoking index; OR_{adj} is the odds ratio obtained with all of the factors taken into account; 95% CI is the 95% confidence interval of OR; the additive model for minor allele dosage suggests that the dosage increases from major allele homozygote (0) through heterozygote (1) to minor allele homozygote (2).

Table 4. Statistically significant results obtained in testing the polymorphisms of the candidate genes for association with COPD in groups differentiated by smoking status (log-regression analysis)

Group	Gene, polymorphic locus	Minor allele	*N*	Genotype or model	P_{adj}	OR_{adj} (CI95%)
Smokers	*CHRNA5* (c.1192G>A) rs16969968	A	643	GG(0) GA(1) AA(2) (additive)	0.001	1.54 (1.18–2.01)
	CHRNA3 (c.645C>T) rs1051730	T	643	CC(0) CT(1) TT(2) (additive)	0.00012	1.78 (1.25–2.15)
	NQO1 (c.415C>T) rs1131341	T	646	CC(0) TC(1) TT(2) (additive)	0.00001	2.94 (2.24–5.07)
	CYP2A6 (*CYP*2A6*4)[a]	del	646	Del vs. n	0.0001	0.47 (0.34–0.78)
	XRCC1 (c.1196A>G) rs25487	G	646	AA(0) AG(1) GG(2) (additive)	0.0002	0.53 (0.37–0.78)
	XRCC3 (c.722C>T) rs861539	T	646	CC(0) TC(1) TT(2) (additive)	0.0001	1.44 (1.24–1.83)
	XPC (c.2815C>A) rs2228001	C	653	AA(0) AC(1) CC(2) (additive)	0.0003	0.59 (0.48–0.74)
	XPA (*c*.–4A>G) rs1800975	G	648	(GG + AG) vs. AA (dominant)	0.0005	0.48 (0.31–0.76)
Nonsmokers	*NQO1* (c.415C>T) rs1131341	T	229	CC(0) TC(1) TT(2) (additive)	0.012	2.11 (1.15–4.90)
	CYP2A6 (*CYP*2A6*4)[a]	D	229	Del vs. n	0.00001	0.25 (0.17–0.56)
	XPC (c.2815C>A) rs2228001	C	229	AA(0) AC(1) CC(2) (additive)	0.019	0.58 (0.35–0.96)
	XRCC1 (c.1196A>G) rs25487	G	229	AA(0) AG(1) GG(2) (additive)	0.0003	0.48 (0.34–0.80)
	XRCC3 (c.722C>T) rs861539	T	229	TT vs. (CT + CC) (recessive)	0.012	2.66 (1.24–5.73)
	XPA (c.–4A>G) rs1800975	G	229	GG vs. (AA + AG) (recessive)	0.017	0.41 (0.17–0.99)

The most informative models identified in the log-regression analysis are shown. *N* is the sample size in the regression analysis; P_{adj} shows the significance for the likelihood ratio test of the log-regression model accounting for the age, gender, and smoking index in smokers and the age and gender in nonsmokers; OR_{adj} is the odds ratio obtained with the corresponding factors taken into account; 95% CI is the 95% confidence interval of OR; the additive model for minor allele dosage suggests that the dosage increases from major allele homozygote (0) through heterozygote (1) to minor allele homozygote (2).

[a] In the case of *CYP2A6* (whole deletion, *CYP2A6*4*), modeling was not performed, and the results obtained in the basic test for the minor allele are shown.

Table 5. Smoking index as dependent on the *CYP2A6* genotype

Gene, polymorphic locus	Genotype	*N*	Smoking index, pack years ($M \pm$ S.E.)	*P*
Total sample ($N = 875$)				
CYP2A6 (whole deletion, *CYP2A6*4*)	n	770	17.23 (0.78)	0.0019
	del	105	10.57 (1.33)	
Smokers ($N = 646$)				
CYP2A6 (whole deletion, *CYP2A6*4*)	n	575	24.59 (0.93)	0.0171
	del	71	17.92 (1.69)	

$M \pm$ S.E. is the mean with the standard error of the mean; *P* is the significance level in the Mann–Whitney test.

Table 6. Parameters of pulmonary function as dependent on the genotypes at the *XRCC3* and *XPA* polymorphisms in COPD patients

Gene, polymorphic locus	Genotype	*N*	$M \pm$ S.E.	*P*
VC (vital capacity)				
XRCC3 (*c.722C>T*) rs861539	CC	182	50.84 (1.68)	0.0421
	CT	171	53.76 (1.57)	
	TT	72	47.72 (0.26)	
	CC + CT	353	52.27 (1.16)	0.0487
	TT	72	47.72 (0.26)	
FVC (forced vital capacity)				
XRCC3 (*c.722C>T*) rs861539	CC	170	50.99 (1.88)	0.0107
	CT	183	52.73 (1.88)	
	TT	72	41.67 (2.7)	
	CC + CT	353	51.89 (1.33)	0.0032
	TT	72	41.67 (2.7)	
XPA (*c.−4A>G*) rs1800975	AA	136	53.44 (2.25)	0.008
	AG	204	45.46 (1.52)	
	GG	85	51.06 (2.66)	
	GG + AA	221	52.54(1.72)	0.0028
	AG	204	45.46 (1.74)	
FEV_1 (forced expiratory volume in 1 second)				
XRCC3 (*c.722C>T*) rs861539	CC+CT	353	41.29 (1.11)	0.02
	TT	72	35.12 (2.45)	

$M \pm$ S.E. is the mean with the standard error of the mean; *P* is the significance level in the Mann–Whitney or Kruskall–Wallis test.

the total sample ($P = 0.0019$) and in the smokers ($P = 0.0171$).

Contributions of the Genotypes at the Polymorphisms of the Candidate Genes to the Variation of the Quantitative Parameters of External Respiration

We analyzed the three external respiration parameters—VC, FVC, and FEV1—as dependent on the genotypes at the loci under study. All of the parameters characterize the disease severity according to the GOLD criteria of 2011 [1]. An association with the variation of the external respiration parameters was not observed for *CHRNA5* (rs16969968), *CHRNA3* (rs1051730 and rs6495309), *CYP2A6* (whole deletion, *CYP2A6*4*), *NQO1* (rs1131341), *XPC* (rs2228001 and poly(AT)), *XRCC1* (rs25487 and rs25489), and *XPD* (rs13181).

Genotype TT of *XRCC3* (rs861539) was associated with lower values of VC ($P = 0.0487$), FVC ($P = 0.0032$), and FEV1 ($P = 0.02$) (Table 6), supporting

the association of the locus with COPD. A significant genotype-dependent variation of FVC was observed for *XPA* (rs1800975). Carriers of genotype AG had a lower FVC as compared with carriers of genotypes AA and GG ($P = 0.0028$).

DISCUSSION

Our study confirmed the association of *CHRNA5* rs16969968 and *CHRNA3* rs1051730 with COPD in the Tatar population. This finding agrees with the data obtained for Caucasian populations [3, 4, 6]. A meta-analysis has identified allele T of *CHRNA3* rs1051730 as a risk factor not only for COPD, but also for emphysematous destruction of lung parenchyma [6]. Cholinergic nicotine receptors are expressed in key regions of the brain and lungs and play an important role in the smoking control [5, 6]. Every copy of the higher-risk rs1051730 allele T is associated with an increase in smoking intensity [6]. We did not reveal any significant gene-by-environment interactions when analyzing the smoking status and smoking index with the *CHRNA3/5* loci. However, *CHRNA5* rs16969968 and *CHRNA3* rs1051730 were associated with COPD only in smokers.

The major genotype (normal variant) of *CYP2A6* was identified as a COPD risk marker in the Tatar population. The association of *CYP2A6* (whole deletion, *CYP2A6*4*) was significant in the groups differentiated by smoking status. Carriers of the *CYP2A6* deletion (*CYP2A6*4*) had a lower smoking index, which characterizes the smoking intensity. The finding testifies again that the deletion variant of *CYP2A6* exerts a protective effect in COPD. *CYP2A6* polymorphism is an important factor that affects the individual characteristics of coumarin and nicotine metabolism, smoking intensity, and addiction to smoking [11, 12]. In addition, the CYP2A6 protein is involved in activating nitrosamines and tobacco smoke procarcinogens [11]. The gene is expressed mostly in the liver and, to a lower extent, bronchial epithelial cells [11, 31]. The *CYP2A6* expression level in smokers is significantly lower than in nonsmokers [32]. Smoking has been assumed to inhibit *CYP2A6* expression in bronchial epithelial cells [31]. The extended deletion (allele *CYP2A6*4*) blocks *CYP2A6* expression and leads to loss of *CYP2A6* activity [11]. Lung cancer has been associated with polymorphic variants of *CYP2A6* [13]. A relationship has been observed between smoking intensity and *CYP2A6* polymorphism [32]. Our findings fully agree with earlier studies, confirming the protective role of the *CYP2A6* deletion in the disorders where smoking plays a leading pathogenetic role [13, 32, 33].

We associated *NQO1* (rs1131341) with COPD. The heterozygous genotype at *NQO1* (rs1131341) was identified as a risk marker in smokers. *NQO1* expression is induced by oxidative stress because *NQO1* harbors an antioxidant response element (ARE) in its promoter and its transcription is regulated by the Nrf2 (nuclear erythroid 2 p45-related factor 2) transcription factor, which is involved in regulating expression of many antioxidant genes [14]. A protective role NQO1 plays in pulmonary emphysema has been demonstrated in mice. Treatment of NQO1-deficient mice with the antioxidant *N*-acetylcysteine reduces emphysematous alterations [15]. Expression of the Nrf2 target genes is substantially lower in lung tissue of human patients, and changes in Nrf2 gene expression distinctly correlate with damage to the lungs and the disease severity [14]. *NQO1* polymorphic variants have been associated with oxidative stress intensity and respiratory diseases in many studies [34, 35].

There is convincing evidence that oxidative damage to DNA is involved in the pathogenesis of atherosclerosis, neurodegenerative disorders, senescence, and carcinogenesis [18, 36]. Considering that oxidative stress plays a key role in COPD and causes damage to all cell structures and especially DNA and that DNA repair is less efficient in patients [37], we studied the seven polymorphisms of the genes involved in nucleotide excision repair (*XPC* (rs2228001 and poly(AT) in intron 9), *XPD* (rs13181), and *XPA* (rs1800975)), base excision repair (*XRCC1* (rs25487 and rs25489)), and double-strand break repair (*XRCC3* (rs861539)). *XPC* (rs2228001), *XPA* (rs1800975), *XRCC1* (rs25487), and *XRCC3* (rs861539) showed a significant association with COPD in the Tatar population.

The most significant association was observed for *XRCC1* (rs25487). The association remained significant in the groups differentiated by the smoking status. Our findings agree with the published data demonstrating the contribution of the *XRCC1* (rs25487) locus, the extent of DNA damage [23], and an association of the locus with the disease [24]. The protein product of *XRCC1* protects the cell from the aggressive effect of DNA base-modifying agents [38]. Loss of its function increases the cell sensitivity to ionizing radiation, oxidative stress, and alkylating agents [38, 39]. The *XRCC1* (rs25487 and rs25489) polymorphic variants code for a conformationally changed protein, which has lower affinity for a multicomponent protein complex involved in repair [36].

XRCC3 codes for an enzyme involved in double-strand break repair and, in particular, nonhomologous end joining. We associated *XRCC3* (rs861539) with COPD, especially in smokers. The *XRCC3* allelic variants are responsible for the variation of the pulmonary function parameters that characterize the disease severity. VC, FVC, and FEV1 were lower in patients homozygous for minor allele T of *XRCC3* (rs861539), pointing to a substantial contribution of the gene to COPD. *XRCC3* have already been associated with the extent of DNA damage and COPD [23]. The majority of relevant studies have focused on the role *XRCC3* (rs861539) plays in lung cancer [40].

The *XPC* (rs2228001) locus, which codes for a nucleotide excision repair enzyme, was associated

with COPD, and the association was confirmed in the analysis of the sample stratified by smoking status. Among all *XPC* polymorphisms, rs2228001 of exon 15 and poly(AT) in intron 9 have been studied most comprehensively. A meta-analysis has been performed on the basis of many association studies of the *XPC* polymorphisms with lung cancer [18, 41].

The *XPA* (rs1800975) polymorphism is due to the substitution c.–4A>G in the 5' gene region and changes transcription of the gene [42]. We found that *XPA* (rs1800975) is associated with COPD and contributes to the variation of the pulmonary function parameters, FVC being lower in patients heterozygous at *XPA* (rs1800975).

A significant gene-by-environment interaction was observed between *XPA* (rs1800975) and the smoking status. Other studies have confirmed that the locus contributes to lung cancer and other oncology diseases [43, 44].

Several works have shown that oxidative stress-induced damage to nucleic acids in alveolar fibroblasts is involved in the pathogenesis of lung emphysema [16]. Our findings that the DNA repair genes are associated with COPD and interact with smoking as an environmental factor confirm the hypothesis that DNA damage in airway epithelial cells plays an important role in the pathogenesis of COPD. DNA damage leads to activation of local immunity factors, which recognize the endogenous intracellular molecules resulting from damage to cell structures, and the factors trigger the inflammation cascade in lung and airway tissues [37].

To summarize, we tested the genes for the α-type cholinergic nicotine receptors, enzymes metabolizing nicotine and tobacco smoke toxicants, and enzymes involved in DNA excision repair for association with COPD in the Tatar population of Russia, and our analysis confirmed that the *CHRNA3/5*, *CYP2A6*, *XRCC1*, and *XRCC3* polymorphisms play a role in disease development. We were the first to associate the *NQO1*, *XPC*, and *XPA* polymorphisms with COPD. Pathogenetically important interactions of genetic loci with smoking were identified for COPD.

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