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HUMAN GENETICS

Association of *CRP*, *CD14*, Pro-Inflammatory Cytokines and Their Receptors (*TNFA*, *LTA*, *TNFRSF1A*, *TNFRSF1B*, *IL1B*, and *IL6*) Genes with Chronic Obstructive Pulmonary Disease Development

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Abstract—The aim of the present study was to investigate the association of COPD and frequent exacerbator COPD phenotype with *CRP*, *CD14*, and pro-inflammatory cytokines and their receptors (*TNFA*, *LTA*, *TNFRSF1A*, *TNFRSF1B*, *IL1B*, and *IL6*) genes. It was found that COPD was associated with allele *A* of the *TNFA* gene (rs1800629G>A) ($P = 0.002$, OR = 1.45); the association was established in the log-additive model ($P = 0.0022$, $P_{cor\text{-}FDR} = 0.01705$, OR = 1.47); this association was confirmed in the frequent exacerbator COPD phenotype group ($P = 0.001$, $P_{cor\text{-}FDR} = 0.007$, OR = 1.59). Allele *G* of the *LTA* gene (rs909253A>G) ($P = 0.002$, OR = 1.33) was also shown to be a marker for COPD risk; the association was established in the log-additive model ($P = 0.0021$, $P_{cor\text{-}FDR} = 0.01705$, OR = 1.31) and it was confirmed in patients with rare exacerbations ($P = 0.003$, $P_{cor\text{-}FDR} = 0.0084$, OR = 1.39). The genotype *GG* of the *TNFRSF1B* gene (rs1061622T>G) was a marker of resistance to the development of the frequent exacerbator COPD phenotype ($P = 0.003$, $P_{cor\text{-}FDR} = 0.0084$, OR = 0.46). The genotype *CC* of the *CD14* gene (rs2569190T>C) was associated with higher forced expiratory volume in 1 s ($P = 0.006$); subjects with genotype *AA* of the *TNFRSF1B* gene (rs1061624A >G) and genotype *GG* of the *LTA* gene (rs909253A>G) exhibited lower forced expiratory volume in 1 s ($P = 0.04$ and $P = 0.01$, respectively). Genotype *AA* of the *TNFRSF1A* gene (rs767455A>G) was associated with higher smoking pack-years ($P = 0.0036$).

Keywords: chronic obstructive pulmonary disease (COPD), tumor necrosis factor, pro-inflammatory cytokines, C-reactive protein, CD14

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a multifactorial chronic inflammatory disease of the respiratory system. COPD is characterized by the development of systemic effects leading to severe complications, further worsening the course of the disease [1]. One of the reasons for difficulties in identifying COPD markers is phenotypic heterogeneity. A certain proportion of patients with COPD are more prone to the development of frequent exacerbations of the disease, which cause a sharp progression of airway obstruction and an unfavorable outcome of the disease [2]. Owing to a change in the strategy for the diagnosis and prevention of COPD in 2017, researchers are currently focusing on identifying biomarkers of various phenotypes of the disease and effectively identifying patients with an increased risk of exacerbations—COPD with frequent exacerbations (frequent exacerbator COPD phenotype) [1–3]. It is well known that COPD is based on a long-lasting inflammatory process that affects all structures of lung tissue [1]. The role of inflammation in the pathogenesis of COPD has been widely studied, but only recently have researchers paid attention to the systemic nature of COPD and the possible relationship between chronic systemic inflammation and the development of exacerbations in COPD [2, 3]. Studies on the association of genetic markers with the development of a phenotype with frequent exacerbations are not enough, but it has been clinically shown that this COPD phenotype is a homogeneous stable group, which indicates a certain genetic predisposition [4].

CD14 protein is a co-receptor in the *CD14/TLR4/MD2* cell receptor complex that recognizes bacterial lipopolysaccharide (LPS) and it is expressed by monocytes, macrophages, and neutrophils [5]. The *CD14* gene is located on chromosome 5q31.1; polymorphism rs2569190 (c.–260T>C) in the

promoter region of the gene reduces the affinity of the promoter for the transcription factor Sp1 [6]. The formation of the receptor complex is necessary for the activation of monocytes and neutrophils, which leads to the migration of leukocytes to the focus of inflammation and the activation of monocytes and granulocytes, which begin the production of the key pro-inflammatory cytokines IL1, TNFA, IL6, and IL8 [5]. The main role of pro-inflammatory cytokines is to trigger an inflammatory reaction, enhance expression of adhesion molecules on endothelial cells and leukocytes, activate oxygen metabolism of cells, and stimulate the production of other cytokines. The release of excessively secreted pro-inflammatory cytokines into the circulation contributes to the manifestation of systemic effects of inflammation and the development of frequent exacerbations [5]. TNFA and LTA are secreted by many types of cells, mainly macrophages, dendritic cells, T lymphocytes, monocytes, and B cells. The *TNFA* and *LTA* genes are located on chromosome 6p21.3 [7]. Several functional SNPs of the *TNFA* gene have been described, some of which are associated with increased gene expression [8]. TNFA and LTA bind to the same receptors on cell surfaces (TNFR1 and TNFR2) [5]. The *TNFRSF1A* gene is localized on chromosome 12p13.31 and encodes type 1 receptor for TNFA, member 1A (TNFR1) of the TNFA receptor superfamily; the *TNFRSF1B* gene is localized on chromosome 1p36.22 and encodes receptor 1B (TNFR2) of the TNFA receptor superfamily [7]. The interaction of the *TNFA*, *LTA*, *TNFRSF1A*, and *TNFRSF1B* genes triggers the production of other cytokines and the activation of NF-kB [5, 9]. IL1, a cytokine with a wide range of biological and physiological effects, is a trigger interleukin for a cascade of pro-inflammatory cytokines [5]. The biological effect of IL1 is associated with the activation of nuclear transcription factors NF-kB and AP-1, which, in turn, stimulate the synthesis of a number of molecules involved in the regulation of the inflammatory response [10]. The *IL1B* gene is mapped in the 2q14 region. Individual variation in *IL1B* gene expression depends on functional polymorphic loci [11]. Interleukin 6 (IL6) is one of the key participants in the cytokine network [5]. IL6 is secreted mainly by monocytes, alveolar macrophages, airway epithelial cells, lymphocytes, and endotheliocytes [5]. The *IL6* gene is located on chromosome 7p21 [7]; the polymorphic locus (c.–237C>G, rs1800795) of the *IL6* gene determines a different constitutive and inducible level of gene expression; in carriers of the allele *C*, gene expression is inhibited compared to carriers of the allele *G* [12]. The induction of the cascade of pro-inflammatory cytokines leads to an increase in the level of C-reactive protein (CRP), which is a sensitive marker of acute and systemic inflammation [5, 13]. CRP expression is mainly induced by IL6 in hepatocytes. The *CRP* gene is located on chromosome 1q23.2 [7]. The polymorphic locus rs2794521 is associated with increased expression and production of CRP; another polymorphism (rs1205), located in the 3'UTR region of the gene, is associated with a change in CRP level [7, 14].

The aim of this study was to identify the association of polymorphic variants of the C-reactive protein (*CRP*), *CD14* receptor, and pro-inflammatory cytokines and their receptors (*TNFA*, *LTA*, *TNFRSF1A*, *TNFRSF1B*, *IL1B*, and *IL6*) genes with chronic obstructive pulmonary disease and the development of various phenotypes of the disease.

MATERIALS AND METHODS

Study design is a candidate study on a case–control basis. We used DNA samples from unrelated Tatars by ethnicity living in the Republic of Bashkortostan. The group of patients included 601 individuals (522 men (86.85%) and 79 women (13.15%)); the average age was 63.38 ± 11.81 years. A diagnosis of COPD was made by pulmonologists at the Department of Pulmonology of the City Clinical Hospital No. 21 of Ufa (Republic of Bashkortostan) according to the International Classification of Diseases of the tenth revision and taking into account the recommendations of the working group on the Global Strategy for the Diagnosis, Treatment, and Prevention of Chronic Obstructive Pulmonary Disease (2017 revision) [1, 15]. The examined patients were not previously exposed to a complex of harmful production factors. Candidates with symptoms of allergic diseases, asthma, oncological diseases, and specific infectious diseases of the respiratory system (tuberculosis) were excluded. Among patients with COPD there were 484 smokers and former smokers (80.53%) and 117 nonsmokers (19.47%). Calculation of the smoking index in conventional units (number of packs per year, pack-years, PY) was carried out according to the generally accepted formula [1]. The smoking index in smokers and former smokers was 44.58 ± 25.92 pack-years. The function of external respiration was examined in all patients by spirometry; vital capacity (VC), forced vital capacity (FVC), forced expiratory volume in the first second (FEV1), and the ratio of this volume to lung vital capacity (FEV1/VC) were evaluated as well. In the group of patients, the indices (in % of the norm) were FEV1 = 41.68 ± 19.32, FVC = 44.22 ± 17.88, VC = 49.02 ± 15.54, and FEV1/FVC = 58.66 ± 13.66. In order to identify genetic markers associated with COPD phenotypes, the control group and patients differentiated according to the modern classification (GOLD, 2017) were compared [1]. Two phenotypes were identified: group 1—COPD with frequent exacerbations (frequent exacerbator) (N = 293), one or more exacerbations, which led to hospitalization during a year; group 2—COPD with rare exacerbations (N = 308), no more than one exacerbation, which did not lead to hospitalization.

The control group ($N = 617$) included essentially healthy individuals without pathology of the respiratory system and without chronic diseases (including cardiovascular, metabolic diseases, allergic, and oncological) in their history, without professional contact with harmful chemicals, selected by age (58.44 ± 14.79), gender (548 men, 88.88%, and 69 women, 11.12%), and smoking status (smokers and former smokers—517 (83.79%) and nonsmokers—100 (16.21%)); the smoking index for smokers was 38.54 ± 23.12 pack-years. In the control group the indices of the function of external respiration (in % of the norm) were FEV1 = 102.7 ± 52.1, FVC = 107.1 ± 32.05, VC = 105.3 ± 42.87, and FEV1/FVC = 87.94 ± 10.69.

The study was approved by the Ethics Committee of the Institute of Biochemistry and Genetics, Ufa Federal Research Centre, Russian Academy of Sciences. From all participants of the study, informed voluntary consent to the use of biological material was received.

Genotyping. DNA was extracted from peripheral blood leukocytes using the phenol-chloroform method. Polymorphic variants of the *TNFA* (c.–488G>A, rs1800629), *LTA* (c.–9–198A>G, rs909253), *TNFRSF1A* (c.36A >G, p.Pro12 = rs767455), *TNFRSF1B* (c.*188A>G, rs1061624), *TNFRSF1B* (c.587T>G, p.Met196Arg, rs1061622), *IL1B* (c.–598C>T, rs16944), *IL6* (c.–237C>G, rs1800795), *CRP* (c.*1082C>T, rs1205), *CRP* (c.–821G>A, rs2794521), and *CD14* (c.–260T>C, rs2569190) genes were analyzed by real-time polymerase chain reaction (PCR) with commercial fluorescence detection kits (FLASH/RTAS) (http://test-gen.ru, LLC Test-Gen, Russia) using a BioRadCFX-96™ system (Bio-Rad Laboratories, Inc., United States) [7]. Allelic discrimination analysis was carried out in plates for 96 samples. Each cell contained a DNA sample and a PCR mixture with a total volume of 10 μL. Amplification curves and the results of each allelic discrimination were analyzed using the graphical software CFX96 Touch™ Real-Time PCR Detection System. To control the quality of the reaction (PCR) in real time, at each genotyping of the plate for 96 samples, positive controls provided by the kit supplier and negative controls without including a DNA matrix were used, and also 5% of samples were selectively duplicated in each experiment.

Statistical analysis. Statistical data processing was carried out using Statistica v. 6.0 software (StatSoft Inc., United States) and PLINK v. 1.07 [16, 17]. We calculated the frequencies of alleles and genotypes and the correspondence of the frequency distribution of genotypes to the Hardy–Weinberg equilibrium (χ^2 and $P_{H\text{-}W}$-value for the test); the statistical significance of differences between groups in the distribution of allele and genotype frequencies was evaluated (χ^2 test for sample homogeneity and P-value for the test). Logistic regression was used to identify the association of polymorphic variants of the studied genes with the development of COPD; the exponent of an individual regression coefficient (beta) was interpreted as the odds ratio (OR) with the calculation of the 95% confidence interval, taking into account gender, age, body mass index, status, and smoking index. Multiple testing was performed using the FDR method (False Discovery Rate) (Benjiamini Hochberg), using the online program (http//www.sdmproject.com/utilinies/?show=FDR), and a new $P_{FDR\text{-}cor}$ value was obtained. The contribution of the allelic variants of the candidate genes studied to the variability of the quantitative traits characterizing the indices of the function of external respiration (VC, FVC, FEV1) and the smoking index were determined using the Kruskal–Wallis (in the case of three groups) or Mann–Whitney test (in the case of two groups); calculations were performed using Statistica v. 6.0 software (StatSoft Inc., United States) [16].

RESULTS

Before proceeding to analysis of the association of allelic variants of candidate genes with the development of COPD, the correspondence of the frequency distribution of genotypes to the Hardy–Weinberg equilibrium was checked. The following results were obtained for the control group: *TNFA* (rs1800629G>A) ($P_{H\text{-}W} = 0.67$), *LTA* (rs909253A>G) ($P_{H\text{-}W} = 0.77$), *TNFRSF1A* (rs767455A>G) ($P_{H\text{-}W} = 0.22$), *TNFRSF1B* (rs1061624A>G) ($P_{H\text{-}W} = 0.44$), *TNFRSF1B* (rs1061622T>G) ($P_{H\text{-}W} = 0.27$), *IL1B* (rs16944C>T) ($P_{H\text{-}W} = 0.08$), *IL6* (rs1800795C>G) ($P_{H\text{-}W} = 0.46$), *CRP* (rs1205G>A) ($P_{H\text{-}W} = 0.45$), *CRP* (rs2794521G>A) ($P_{H\text{-}W} = 0.99$), and *CD14* (rs2569190T>C) ($P_{H\text{-}W} = 0.67$).

Association Analysis of the Allelic Variants of Candidate Genes with the Development of COPD

Data on the distribution of genotypes and alleles frequencies of the studied loci, the significance of differences between groups in the frequencies of genotypes and alleles, the odds ratio calculated for the rare allele of each locus, and the results of analysis of the association with the development of COPD, with the calculation of the regression coefficient (beta), the exponent of which was interpreted as the odds ratio (OR) for the logistic model, with the calculation of the 95% confidence interval, are shown in Tables 1 and 2.

Statistically significant differences were found between the COPD and the control groups according to the frequency distribution of the genotypes of the polymorphic locus of the *TNFA* gene (rs1800629G>A) ($P = 0.004$, $P_{cor\text{-}FDR} = 0.0206$). The frequency of the rare allele *A* of the *TNFA* gene (rs1800629G>A) was significantly higher in the group of patients with COPD ($P = 0.002$, $P_{cor\text{-}FDR} = 0.01705$;

Table 1. The association analysis of the tumor necrosis factor family and their receptors polymorphic genes (*TNFA*, *LTA, TNFRSF1A*, *TNFRSF1B)* with the development of COPD

Gene, polymorphic locus	Rare allele	Genotypes, alleles, model	COPD, abs. (%) ($N = 601$)	Control, abs. (%) ($N = 617$)	P (P_{adj})	$P_{cor\text{-}FDR}$	OR (OR_{adj}) (95%CI)
TNFA c.–488G>A rs1800629	*A*	*GG/GA/AA*	423/166/12 (70.38/27.62/2.00)	485/123/9 (78.61/19.94/1.46)	0.004	0.0206	–
		G/A	1 012/190 (84.19/15.81)	1 093/141 88.57/11.43	0.002	0.01705	1.45 (1.15–1.83)
		Log-additive	–	–	0.0022	0.01705	1.47 (1.15–1.88)
LTA c.–9–198A>G rs909253	*G*	*AA/AG/GG*	282/269/50 (46.92/44.76/8.32)	339/248/30 (54.94/40.19/4.86)	0.004	0.0206	–
		A/G	833/369 (69.30/30.70)	926/308 (75.04/24.96)	0.002	0.01705	1.33 (1.11–1.59)
		Log-additive	–	–	0.0021	0.01705	1.31 (1.09–1.56)
TNFRSF1A c.–542A>G rs767455	*G*	*AA/AG/GG*	235/252/114 (39.10/41.93/18.97)	222/283/112 (35.98/45.87/18.15)	0.373	0.6938	–
		A/G	722/480 (60.07/39.93)	727/507 (58.91/41.09)	0.591	0.7268	0.95 (0.81–1.12)
		Log-additive	–	–	0.6	0.7268	0.95 (0.79–1.14)
TNFRSF1B c.*188A>G rs1061624	*G*	*AA/AG/GG*	147/295/159 (24.46/49.08/26.46)	153/320/144 (24.80/51.86/23.34)	0.434	0.6938	–
		A/G	589/613 (49.00/51.00)	626/608 (50.73/49.27)	0.417	0.6938	1.07 (0.91–1.25)
		Log-additive	–	–	0.47	0.6938	1.07 (0.89–1.30)
TNFRSF1B c.587T>G, p.Met196Arg rs1061622	*G*	*TT/TG/GG*	248/286/67 (41.26/47.59/11.15)	257/270/90 (41.65/43.76/14.59)	0.151	0.4255	–
		T/G	782/420 (65.06/34.94)	784/450 (63.53/36.47)	0.457	0.6938	0.93 (0.79–1.10)
		Log-additive	–	–	0.51	0.7186	0.93 (0.76–1.14)

Here and in Tables 2 and 3: P—value for the χ^2 test on the homogeneity of the samples; OR—indicator of the odds ratio for the basic allele test (for the rare allele of each locus); 95%CI—95% confidence interval for OR. The number of individuals included in the regression analysis is 218–1218; P_{adj}—significance for the likelihood ratio test taking into account age, gender, status, and smoking index; and OR_{adj}—odds ratio taking into account all factors are shown for the log-regression model; $P_{cor\text{-}FDR}$—test significance after FDR correction.

Table 2. The association analysis of the *IL1B*, *IL6*, *CRP*, and *CD14* polymorphic genes with the development of COPD

Gene, polymorphic locus	Rare allele	Genotypes, alleles, model	COPD, abs. (%) ($N = 601$)	Control, abs. (%) ($N = 617$)	P (P_{adj})	$P_{cor\text{-}FDR}$	OR (OR_{adj}) (95%CI)
IL1B c.–598C>T rs16944	*T*	*CC/CT/TT*	204/302/95 (33.94/50.25/15.81)	209/278/130 (33.87/45.06/21.07)	0.043	0.1666	–
		C/T	710/492 (59.07/40.93)	696/538 (56.40/43.60)	0.197	0.43621	0.89 (0.76–1.05)
		CC+CT *TT* Recessive	506 (84.19) 95 (15.81)	487 (78.93) 130 (21.07)	0.022	0.0974	1.00 0.70 (0.52–0.94)
		Log-additive	–	–	0.19	0.43621	0.89 (0.75–1.06)
IL6 c.–237C>G rs1800795	*C*	*GG/GC/CC*	208/294/99 (34.61/48.92/16.47)	174/339/104 (28.20/54.94/16.86)	0.06	0.2066	–
		G/C	710/492 (59.07/40.93)	687/547 (55.67/44.33)	0.099	0.3069	0.87 (0.74–1.03)
		Log-additive	–	–	0.173	0.4362	0.91 (0.83–1.15)
CRP c.*1082C>T rs1205	*T*	*CC/CT/TT*	241/280/80 (40.10/46.59/13.31)	249/296/72 (40.36/47.97/11.67)	0.675	0.7268	–
		C/T	762/440 (63.39/36.61)	794/440 (64.34/35.66)	0.656	0.7268	1.04 (0.88–1.22)
		Log-additive	–	–	0.68	0.7268	1.04 (0.85–1.27)
CRP c.–821G>A rs2794521	*G*	*AA/AG/GG*	349/228/24 (58.07/37.94/3.99)	375/213/29 (60.78/34.52/4.70)	0.426	0.6938	–
		A/G	926/276 (77.04/22.96)	963/271 (78.04/21.96)	0.587	0.7268	1.05 (0.87–1.28)
		Log-additive	–	–	0.62	0.7268	1.05 (0.86–1.30)
CD14 c.–260T>C rs2569190	*T*	*CC/CT/TT*	167/284/150 (27.79/47.25/24.96)	170/302/145 (27.55/48.95/23.50)	0.454	0.6938	–
		C/T	618/584 (51.41/48.59)	642/592 (52.03/47.97)	0.794	0.794	1.02 (0.87–1.20)
		Log-additive	–	–	0.76	0.7853	1.03 (0.86–1.23)

OR = 1.45, 95%CI 1.15–1.83). Association with the development of COPD and the *TNFA* gene (rs1800629G>A) was established in the log-additive model ($P = 0.0022$, $P_{cor\text{-}FDR} = 0.01705$; OR = 1.47, 95%CI 1.15–1.88).

The group of patients with COPD statistically significantly differed in the frequency distribution of the genotypes of the polymorphic locus of the *LTA* gene (rs909253A>G) from the control group ($P = 0.004$, $P_{cor\text{-}FDR} = 0.0206$). The frequency of the rare allele *G* of the *LTA* gene (rs909253A>G) was significantly higher in the group of patients with COPD (30.70% versus 24.96% in the control, $P = 0.002$, $P_{cor\text{-}FDR} = 0.01705$; OR = 1.33, 95%CI 1.11–1.59). The *LTA* gene (rs909253A>G) was associated with the development of COPD in the log-additive model ($P = 0.0021$, $P_{cor\text{-}FDR} = 0.01705$; OR = 1.31, 95%CI 1.09–1.56).

Significant differences were found in the frequency distribution of the genotypes of the *IL1B* polymorphic locus (rs16944C>T) between the studied groups ($P = 0.043$, $P_{cor\text{-}FDR} = 0.1666$), which were due with a decrease in the frequency of the genotype *TT* of the *IL1B* gene (rs16944C>T) in the COPD group (15.81% against 21.07% in the control, $P = 0.022$, $P_{cor\text{-}FDR} = 0.0974$; OR = 0.70, 95%CI 0.52–0.94).

Comparative analysis of the genotypes and alleles frequency distribution between groups of patients with COPD and control by polymorphic variants of the *TNFRSF1A* (rs767455A>G), *TNFRSF1B* (rs1061624A>G), *TNFRSF1B* (rs1061622T>G), *IL6* (rs1800795C>G), *CRP* (rs1205G>A), *CRP* (rs2794521G>A), and *CD14* (rs2569190T>C) genes did not give statistically significant differences (Tables 1, 2).

Association Analysis of the Polymorphic Variants of Candidate Genes with COPD Phenotypes

The results of the association analysis of polymorphic variants of candidate genes with COPD phenotypes are shown in Table 3.

Statistically significant differences were found in the distribution of the genotypes and alleles frequencies of the *TNFA* polymorphic locus (rs1800629G>A) between the group of patients with COPD with frequent exacerbations and control ($P = 0.004$, $P_{cor\text{-}FDR} = 0.009$). An association with the development of this COPD phenotype was obtained in the allelic test; the frequency of the rare allele *A* reached 17.06% in the group of patients versus 11.43% in the control ($P = 0.001$, $P_{cor\text{-}FDR} = 0.007$; OR = 1.60, 95%CI 1.21–2.11). The significance of the association is confirmed in the log-additive regression model ($P = 0.001$, $P_{cor\text{-}FDR} = 0.007$; OR = 1.59, 95%CI 1.21–2.11). In the group of COPD patients with frequent exacerbations, a decrease in the frequency of the genotype *TT* of the *IL1B* gene (rs16944C>T) was revealed ($P = 0.03$, $P_{cor\text{-}FDR} = 0.046$; OR = 0.65, 95%CI 0.44–0.97 for the recessive model). Statistically significant differences were found between the group of patients with chronic obstructive pulmonary disease with frequent exacerbations and control in the frequency distribution of the *TNFRSF1B* locus genotypes (rs1061622T>G) ($P = 0.005$, $P_{cor\text{-}FDR} = 0.01$). An association with the development of this phenotype of the disease was detected with the *TNFRSF1B* locus (rs1061622T>G) in the recessive regression model ($P = 0.003$, $P_{cor\text{-}FDR} = 0.0084$; OR = 0.46, 95%CI 0.29–0.77), which is associated with a decrease in the proportion of homozygotes for the rare allele *G* of the gene in the patient group (7.51% versus 14.59% in the control).

A comparative analysis of the group of patients with COPD with rare exacerbations and the control group revealed statistically significant differences in the frequency distribution of the genotypes of the polymorphic locus of the *LTA* gene (rs909253A>G) ($P = 0.006$, $P_{cor\text{-}FDR} = 0.0105$). The association with the development of this COPD phenotype was established in the basic allelic test ($P = 0.003$, $P_{cor\text{-}FDR} = 0.0084$; OR = 1.39, 95%CI 1.13–1.72) and the log-additive regression model ($P = 0.003$, $P_{cor\text{-}FDR} = 0.0084$; OR = 1.39, 95%CI 1.12–1.72).

Analysis of the Contribution of Allelic Variants of Candidate Genes to the Variability of the Lung Function Values and the Smoking Index

Analysis of quantitative parameters of lung function values reflecting the progression of airway obstruction in patients with COPD (VC, FVC, and FEV1) and the smoking index characterizing the intensity and length of smoking depending on the polymorphic variants of the studied candidate genes was performed (Table 4). Genotype *CC* of the *CD14* gene (rs2569190T>C) is associated with higher FEV1 ($P = 0.006$), while individuals with genotype *AA* of the *TNFRSF1B* gene (rs1061624A>G) and genotype *GG* of the *LTA* gene (rs909253A>G) showed a significant decrease in FEV1 ($P = 0.04$ and $P = 0.01$). For individuals with the genotype *GG* of the *TNFRSF1A* gene (rs767455A>G), lower FVC values were observed ($P = 0.0019$). A relationship between the smoking index and the polymorphic variants of the *TNFRSF1A* locus (rs767455A>G) was revealed. Thus, a statistically significant increase in the smoking index ($P = 0.0036$) was observed in carriers of the genotype *AA* (Table 4).

DISCUSSION

Analysis of the association of polymorphic variants of the genes of the C-reactive protein (*CRP*), receptor *CD14*, and pro-inflammatory cytokines and their receptors (*TNFA, LTA, TNFRSF1A, TNFRSF1B, IL1B,* and *IL6*) with the development of COPD and various disease phenotypes differentiated on the basis of the frequency of exacerbations was performed. The

Table 3. Statistically significant results of the association analysis of polymorphic loci of candidate genes with the development of COPD in groups differentiated by the phenotype of the disease on the basis of the number of exacerbations per year (according to GOLD 2017)

Gene, SNP	Rare allele	Genotypes, alleles, model	COPD, abs. (%)	Control, abs. (%)	OR (OR_{adj}) (95%CI)	P (P_{adj})	$P_{cor\text{-}FDR}$
COPD phenotype with frequent exacerbations			($N = 293$)	($N = 617$)			
TNFA rs1800629	*A*	*GG/GA/AA*	201/84/8 (68.60/28.67/2.73)	485/123/9 (78.61/19.94/1.46)	–	0.004	0.009
		G/A	486/100 (82.94/17.06)	1 093/141 (88.57/11.43)	1.60 (1.21–2.11)	0.001	0.007
		Log-additive	–	–	1.59 (1.21–2.11)	0.001	0.007
IL1B rs16944	*T*	*CC/CT/TT*	105/144/44 (35.84/49.14/15.02)	209/278/130 (33.87/45.06/21.07)	–	0.093	0.1302
		C/T	354/232 (60.41/39.59)	696/358 (56.40/43.60)	0.85 (0.69–1.04)	0.117	0.14
		CC + CT *TT* Recessive	249 (84.98) 44 (15.02)	487 (78.93) 130 (21.07)	1.00 0.65 (0.44–0.97)	0.03	0.046
		Log-additive	–	–	0.85 (0.69–1.04)	0.12	0.14
TNFRSF1B rs1061622	*G*	*TT/TG/GG*	121/150/22 (41.30/51.19/7.51)	257/270/90 (41.65/43.76/14.59)	–	0.005	0.01
		T/G	392/194 (66.89/33.11)	784/450 (64.53/36.47)	0.86 (0.71–1.06)	0.177	0.177
		TT + TG *GG* Recessive	271 (92.49) 22 (7.51)	527 (85.65) 90 (14.59)	1.00 0.46 (0.29–0.77)	0.003	0.0084
		Log-additive	–	–	0.85 (0.66–1.10)	0.17	0.177
COPD phenotype with rare exacerbations			($N = 308$)	($N = 617$)			
LTA rs909253	*G*	*AA/AG/GG*	140/141/27 (45.45/45.78/8.77)	339/248/30 (54.94/40.19/4.86)	–	0.006	0.0105
		A/G	421/195 (68.34/31.66)	926/308 (75.04/24.96)	1.39 (1.13–1.72)	0.003	0.0084
		Log-additive	–	–	1.39 (1.12–1.72)	0.003	0.0084

Table 4. Contribution of candidate genes genotypes to the variability of quantitative parameters characterizing the lung function and smoking index

Gene, polymorphic locus	Genotype	*N*	Me (25%; 75%)*	*P*
FEV1 (forced expiratory volume in the first second) ($N = 601$)				
LTA rs909253	*AA* + *AG*	551	38 (26.0; 56.0)	0.01
	GG	50	32 (22.0; 50.25)	
CD14 rs2569190	*CC*	167	42 (31.0; 57.0)	0.006
	CT + *TT*	434	35 (26.0; 48.0)	
TNFRSF1B rs1061624	*GG* + *GA*	454	37.5 (28.0; 51.75)	0.04
	AA	147	34 (25.0; 45.0)	
FVC (forced vital capacity) ($N = 601$)				
TNFRSF1A rs767455	*AA* + *AG*	487	55 (43.0; 69.0)	0.0019
	GG	114	44 (30.0; 59.0)	
Smoking index (pack-years) in the common group of smokers ($N = 1001$)				
TNFRSF1A rs767455	*AA*	390	25 (13.0; 45.25)	0.0036
	AG + *GG*	611	22.5 (12.0; 40.0)	
	AA + *AG*	830	24 (12.0; 44.25)	0.005
	GG	171	23 (12.0; 34.0)	

P—significance level for the Mann–Whitney test. * Median and interquartile range.

contribution of allelic variants of the studied loci of the candidate genes to the variability of parameters characterizing the progression of airway obstruction and the intensity and length of smoking is analyzed.

As a result of our study, we have found an association of polymorphic variants of the tumor necrosis factor family genes *TNFA* and *LTA* and their receptors *TNFRSF1A* and *TNFRSF1B* with the development of COPD, various COPD phenotypes, lung function, and smoking index. The risk of the COPD development in our study was associated with the allele *A* of the *TNFA* gene (c.–488G>A, rs1800629); association with COPD is established in the log-additive model. The presence of the allele *A* at position c.–488 of the *TNFA* gene promoter is associated with a doubly increased cytokine production compared to the allele *G* [8]. Further analysis showed that this association was significant only in the COPD group with frequent exacerbations of the disease. Recently, several meta-analyses have been performed that confirm the association of the allele *A* in various populations [18]. Considering the data obtained data, one can assume that genetically determined increased *TNFA* gene expression is a marker of adverse COPD development. TNFA induces the expression and secretion of matrix metalloproteinases by interacting with proteinase-activating receptors (PAR) [5]. Its excessive secretion activates macrophages and neutrophils; this is followed by the synthesis of a cascade of interleukins and the development of a systemic inflammatory response [5].

The risk of COPD development was associated with the allele *G* of the *LTA* gene (rs909253A>G); an association with the development of the disease was established by the log-additive model. The significance of the association was confirmed in the COPD group with rare exacerbations. In addition, we showed the dependence of the indices of the external respiration function on the genotypes of the *LTA* gene (rs909253A>G); thus, in homozygotes for the rare allele *G* of the *LTA* gene (rs909253A>G), lower FEV1 values were established, which is consistent with the results of the analysis of the association with the development of the disease. Allele *G* at position rs909253 of the *LTA* gene leads to an increased level of *LTA* expression in peripheral blood monocytes [19]. The allele *G* of the *LTA* gene was described in [20] as a marker of the protracted course of sarcoidosis. Associations of the polymorphic locus *252G>A* and other polymorphic loci of the *LTA* gene with the development of COPD and bronchiectasis were not detected in populations of Italy, Spain, and Mexico [21–23].

We found a significant decrease in the proportion of homozygotes for the rare allele *G* of the *TNFRSF1B* gene (rs1061622T>G) in the COPD group with frequent exacerbations; this genotype is a marker of resistance to the development of the COPD phenotype with frequent exacerbations. Polymorphism rs1061622 (c.587T>G) is localized in exon 6 of the *TNFRSF1B* gene; T to G substitution leads to a functional substitution in the amino acid sequence of p.Met196Arg; this variant is responsible for the transition of TNFRSF1B to a soluble form, which as a result worsens NF-kB signaling and affects TNFA-induced apoptosis [9]. Carriers of the genotype *GG* of the *TNFRSF1A* polymorphic locus (rs767455A>G) showed lower rates of forced vital capacity (FVC) and decreased smoking index. The rs767455 polymor-

phism (c.36A>G) is localized in exon 1 near the *TNFRSF1A* gene promoter, leading to a synonymous p.Pro12 substitution, which can generate ectopic mRNA splicing, alter the mRNA structure, and affect the folding of the protein molecule [9]. The contribution of allelic variants of the *TNFRSF1A* and *TNFRSF1B* genes to the development of COPD was not studied previously, but there are published data on the role of TNFR2 as an early and sensitive marker of systemic inflammation and the development of COPD in smokers and former smokers [24].

We have showed that the genotype *TT* of the *IL1B* gene (rs16944C>T) is a marker of resistance to the development of COPD; the obtained association is confirmed only in the COPD group with frequent exacerbations of the disease. The rs16944 polymorphism (c.–598C>T) is localized in the promoter region of the *IL1B* gene and leads to the loss of the transcription factor AP2 binding site and an almost 3-fold increase in LPS-induced secretion of IL1B [11]. The results of the analysis of the association of the polymorphic variants of the *IL1B* gene (rs16944C>T) with COPD are contradictory; therefore, several meta-analyses were performed that showed that the allele *T* of the *IL1B* gene (rs16944C>T) in the populations of East Asia is a risk marker for COPD [25]; in [26], associations of polymorphic variants of the *IL1B* gene with COPD were not detected. Studies in groups with the COPD phenotype with frequent exacerbations were not conducted previously; it is possible that the obtained conflicting data are associated with the heterogeneity of the studied groups with COPD. Isolation of a homogeneous group with frequent exacerbations (frequent exacerbator) reveals genetic markers associated with the development of this COPD phenotype.

In this work, we established the dependence of the volume of forced expiration in the first second (FEV1) on polymorphic variants of the *CD14* gene (c.–260T>C, rs2569190); individuals with the genotype *CC* had significantly higher FEV1; on the other hand, in carriers of the rare allele *T* FEV1, reflecting the progression of airway obstruction, was significantly lower. CD14 is a marker of activation of monocytes/macrophages and bronchial epithelial cells, which initiate the production of proinflammatory cytokines IL1B, TNFA, IL6, and IL8 involved in the pathogenesis of COPD [5]. Polymorphism c.–260T>C in the promoter region of the *CD14* gene leads to a change in gene expression; in homozygotes *TT*, the expression of *CD14* is significantly higher than in heterozygotes and homozygotes for the allele *C*. An association of the allele *C* of the *CD14* gene (rs2569190) with the development of COPD in a sample from India has been established [27]. In [28], it was shown that the genotype *TT* of the *CD14* gene (rs2569190) was associated with a decrease in lung function parameters (FEV1) in patients with occupational bronchitis. It can be suggested that CD14 may play a role in the progression of airway obstruction in patients with COPD.

Our results are of interest for understanding the molecular mechanisms of the development of COPD and phenotypic heterogeneity of the disease. It was found that polymorphic variants of the *TNFA*, *LTA*, and *IL1B* genes are risk markers for the development of COPD in the population of Tatars. The development of the phenotype of COPD with frequent exacerbations is associated with polymorphic variants of the *TNFA*, *IL1B*, and *TNFRSF1B* genes. Polymorphic variants of the *LTA*, *CD14*, *TNFRSF1A*, and *TNFRSF1B* genes are associated with lung function parameters, reflecting the progression of airway obstruction in COPD.

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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 carried out in a study with the participation of people comply with the ethical standards of the institutional and/or national research ethics committee and the 1964 Helsinki Declaration and its subsequent changes or comparable standards of ethics. Informed voluntary consent was obtained from each of the participants in the study.

REFERENCES

1. From the Global Strategy for the Diagnosis, Management and Prevention of COPD, Global Initiative for Chronic Obstructive Lung Disease (GOLD), 2017. http://goldcopd.org.
2. Shaw, J.G., Vaughan, A., Dent, A.G., et al., Biomarkers of progression of chronic obstructive pulmonary disease (COPD), *J. Thorac Dis.*, 2014, vol. 6, no. 11, pp. 1532—1547. https://doi.org/10.3978/j.issn.2072-1439.2014.11.33
3. Geerdink, J.X., Simons, S.O., Pike, R., et al., Differences in systemic adaptive immunity contribute to the 'frequent exacerbator' COPD phenotype, *Respir Res.*, 2016, vol. 17, no. 1, p. 140. https://doi.org/10.1186/s12931-016-0456-y

4. Santibáñez, M., Garrastazu, R., Ruiz-Nuñez, M., et al., Predictors of hospitalized exacerbations and mortality in chronic obstructive pulmonary disease, *PLoS One*, 2016, vol. 11, no. 6. e0158727. https://doi.org/10.1371/journal.pone.0158727.eCollection 2016
5. Yarilin, A.A., *Immunologiya: uchebnik* (Immunology: Tutorial), Moscow: GEOTAR-Media, 2010.
6. Hubacek, J.A., Rothe, G., Pit'ha, J., et al., C(-260)>T polymorphism in the promoter of the CD14 monocyte receptor gene as a risk factor for myocardial infarction, *Circulation*, 1999, vol. 99, no. 25, pp. 3218—3220.
7. Open database of single nucleotide polymorphisms (SNPs) and multiple small-scale variations that include insertions/deletions, microsatellites, and non-polymorphic variants, Bethesda (MD): The National Center for Biotechnology Information Advances Science and Health by Providing Access to Biomedical and Genomic Information (U.S.). http://www.ncbi.nlm.nih.gov/projects/SNP/.
8. Kroeger, K.M., Carville, K.S., and Abraham, L.J., The −308 tumor necrosis factor-alpha promoter polymorphism effects transcription, *Mol. Immunol.*, 1997, vol. 3, pp. 391—399.
9. Xu, F., Zhou, G., Han, S., et al., Association of TNF-α, TNFRSF1A and TNFRSF1B gene polymorphisms with the risk of sporadic breast cancer in northeast Chinese Han women, *PLoS One*, 2014, vol. 9, no. 7. e101138. https://doi.org/10.1371/journal.pone.0101138.eCollection 2014
10. Dinarello, C.A., Immunological and inflammatory functions of the interleukin-1 family, *Ann. Rev. Immunol.*, 2009, vol. 27, pp. 519—550. https://doi.org/10.1146/annurev.immunol.021908.132612
11. Hall, S.K., Perregaux, D.G., Gabel, C.A., et al., Correlation of polymorphic variation in the promoter region of the interleukin-1 beta gene with secretion of interleukin-1 beta protein, *Arthritis Rheum.*, 2004, vol. 50, pp. 1976—1983. https://doi.org/10.1002/art.20310
12. Fishman, D., Faulds, G., Jeffery, R., et al., The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis, *Clin. Invest.*, 1998, vol. 102, pp. 1369—1376. https://doi.org/10.1172/JCI2629
13. Danesh, J., Wheeler, J.G., Hirschfield, G.M., et al., C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease, *N. Engl. J. Med.*, 2004, vol. 350, pp. 1387—1397. https://doi.org/10.1056/NEJMoa032804
14. Miller, D.T., Zee, R.Y., Suk Danik, J., et al., Association of common CRP gene variants with CRP levels and cardiovascular events, *Ann. Hum. Genet.*, 2005, vol. 69, no. 6, pp. 623—638. https://doi.org/10.1111/j.1529-8817.2005.00210.x
15. International Statistical Classification of Diseases and Related Health Problems, Tenth Revision (ICD-10). http://www.who.int/classifications/icd/en/.
16. STATISTICA v. 6.0, 1996. http://www.statistica.com.
17. Purcell, S., Neale, B., Todd-Brown, K., et al., PLINK: a toolset for whole-genome association and population-based linkage analysis, *Am. J. Hum. Genet.*, 2007, vol. 81, pp. 559—575. https://doi.org/10.1086/519795
18. Salimi Asl, M., Ahmadi, A., Salimian, J., et al., TNF-α -308 G/A variant and susceptibility to chronic obstructive pulmonary disease: a systematic review and meta-analysis, *Cytokine*, 2019, vol. 123, article 154763. https://doi.org/10.1016/j.cyto.2019.154763
19. Messer, G., Spengler, U., and Jung, M.C., Polymorphic structure of the tumor necrosis factor (TNF) locus: an NcoI polymorphism in the first intron of the human TNF-beta gene correlates with a variant amino acid in position 26 and a reduced level of TNF-beta production, *J. Exp. Med.*, 1991, vol. 173, no. 1, pp. 209—219. https://doi.org/10.1084/jem.173.1.209
20. Yamaguchi, E., Itoh, A., Hizawa, N., et al., The gene polymorphism of tumor necrosis factor-beta, but not that of tumor necrosis factor-alpha, is associated with the prognosis of sarcoidosis, *Chest*, 2001, vol. 119, no. 3, pp. 753—761. https://doi.org/10.1378/chest.119.3.753
21. Reséndiz-Hernández, J.M., Ambrocio-Ortiz, E., Pérez-Rubio, G., et al., *TNF* promoter polymorphisms are associated with genetic susceptibility in COPD secondary to tobacco smoking and biomass burning, *Int. J. Chronic Obstruct. Pulm. Dis.*, 2018, vol. 13, pp. 627—637. https://doi.org/10.2147/COPD.S147688.eCollection 2018
22. Patuzzo, C., Gile, L.S., Zorzetto, M., et al., Tumor necrosis factor gene complex in COPD and disseminated bronchiectasis, *Chest*, 2000, vol. 117, no. 5, pp. 1353—1358. https://doi.org/10.1378/chest.117.5.1353
23. Córdoba-Lanús, E., Baz-Dávila, R., de-Torres, J.P., et al., TNFA-863 polymorphism is associated with a reduced risk of chronic obstructive pulmonary disease: a replication study, *BMC Med. Genet.*, 2011, vol. 12, p. 132. https://doi.org/10.1186/1471-2350-12-132
24. Caram, L.M.O., Ferrari, R., Nogueira, D.L., et al., Tumor necrosis factor receptor 2 as a possible marker of COPD in smokers and ex-smokers, *Int. J. Chronic Obstruct. Pulm. Dis.*, 2017, vol. 12, pp. 2015—2021. https://doi.org/10.2147/COPD.S138558.eCollection2017
25. Xie, Z.K., Huang, Q.P., Huang, J., and Xie, Z.F., Association between the IL1B, IL1RN polymorphisms and COPD risk: a meta-analysis, *Sci. Rep.*, 2014, vol. 4, p. 6202. https://doi.org/10.1038/srep06202
26. Smolonska, J., Wijmenga, C., Postma, D.S., and Boezen, H.M., Meta-analyses on suspected chronic obstructive pulmonary disease genes: a summary of 20 years' research, *Am. J. Respir. Crit. Care Med.*, 2009, vol. 180, no. 7, pp. 618—631. https://doi.org/10.1164/rccm.200905-0722OC
27. Bose, P., Bathri, R., De, S., and Maudar, K.K., CD14 C-159T polymorphism and its association with chronic lung diseases: a pilot study on isocyanate exposed population of Central India, *Indian J. Hum. Genet.*, 2013, vol. 19, no. 2, pp. 88—95. https://doi.org/10.4103/0971-6866.116124
28. LeVan, T.D., Von Essen, S., Romberger, D.J., et al., Polymorphisms in the CD14 gene associated with pulmonary function in farmers, *Am. J. Respir. Crit. Care Med.*, 2005, vol. 171, pp. 773—779. https://doi.org/10.1164/rccm.200404-530OC

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