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HUMAN GENETICS

# The Role of Serum Amyloid A1, Adhesion Molecules, Chemokines, and Chemokine Receptors Genes in Chronic Obstructive Pulmonary Disease

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**Abstract**—Chronic obstructive pulmonary disease (COPD) is a multifactorial chronic inflammatory disease of the respiratory system. A key phenomenon of COPD pathogenesis is inflammation. The goal of the present study was to investigate the association of COPD with alleles and genotypes of the genes that encode chemokines and chemokine receptors (*CCL11*, *CX3CR1*, *CCR5, CCL5*, *CXCL12*, *CCL2*, and *CCL17*), adhesion molecules (*PECAM1* and *ICAM1*), and serum amyloid A1 (*SAA1*). It was found that COPD was associated with the C allele and the TC genotype of *SAA1* (rs1136743C>T) ($P = 0.0001$, $OR = 1.58$ and $P = 0.00001$, $OR = 2.15$, respectively); this association was confirmed in the subgroups differentiated by smoking status. Markers of COPD risk were also the CG genotype of *PECAM1* (rs281865545G>C) ($P = 0.028$, $OR = 1.36$) and the GG genotype of *ICAM1* (rs5498A>G), which were significantly associated with the disease in smokers ($P = 0.005$, $OR = 1.66$). The AA genotype of *CCL2* (rs1024611A>G) was associated with the disease in nonsmokers ($P = 0.037$, $OR = 1.82$). The GG genotype of *PECAM1* (rs281865545G>C) and the AA genotype of *CX3CR1* (rs3732378A>G) were associated with higher vital capacity ($P = 0.014$ and $P = 0.04$, respectively). Subjects with the GG genotype of *ICAM1* (rs5498A>G) exhibited lower forced expiration volume in 1 s and lower forced vital capacity ($P = 0.025$ and $P = 0.029$, respectively).

*Keywords:* chronic obstructive pulmonary disease (COPD), acute-phase protein, adhesion molecules, chemokines, systemic inflammation markers

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## INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is one of the most widespread diseases worldwide; because of delayed diagnosis and low treatment efficacy, it represents a major public health issue [1]. According to the currently accepted definition, COPD is a multifactorial chronic heterogeneous inflammatory disease of the respiratory system primarily affecting the lower respiratory pathways and the lung parenchyma [1]. Smoking is the principal risk factor of COPD; however, only 10–20% of habitual smokers develop signs of respiratory obstruction. It is assumed that exposure to tobacco smoke provokes pathological processes in the lungs, systemic inflammation, and endothelial dysfunction [1, 2].

The genetic mechanisms of COPD are currently a subject of extensive research worldwide. Genome-wide association studies (GWAS) have identified a number of loci associated with COPD: *CHRNA3*, *CHRNA5*, *IREB2*, and *PSMA4* on chromosome 15 (15q25.1), *HHIP* and *FAM13A* on chromosome 4 (4q31.21 and 4q22.1, respectively), and rs7937 on 19q13 [3]. At the same time, the mechanisms that underlie COPD progression and the development of advanced inflammation in the lungs remain poorly understood.

Inflammation is the key phenomenon of COPD pathogenesis. Patients with COPD, especially those with severe or acute disease, exhibit increased levels of systemic inflammation markers, circulating cytokines and chemokines, acute-phase proteins, and leukocyte counts [4]. We supposed that allelic variants of the genes that encode factors involved in inflammatory response may contribute to the development of COPD and disease progression. This work was focused on polymorphisms of the genes of acute-phase proteins, adhesion molecules, chemokines, and chemokine receptors.

Serum amyloid A1 (SAA1), a key factor of acute inflammation, promotes chemotaxis, migration, and adhesion of proinflammatory cells, especially monocytes and macrophages [5]. Some studies suggest that

SAA is involved in smoking-induced lung tissue damage [6].

ICAM1 (intercellular adhesion molecule 1), a single-chain membrane-bound glycoprotein, is expressed on different types of epithelial cells, fibroblasts, and tissue macrophages [7]. Numerous studies have demonstrated the involvement of ICAM1 in the pathogenesis of inflammatory and autoimmune diseases [7–9]. PECAM1 (platelet and endothelial cell adhesion molecule 1) is a cell adhesion molecule of the immunoglobulin family; it is expressed mainly by endothelial cells, but is also detected on platelets, monocytes, and neutrophils. It was shown that PECAM1 participates in inflammatory reactions and in the interaction of leukocytes with endothelial cells [10].

Chronic systemic inflammation in COPD involves constant production of proinflammatory factors, such as cytokines, fibrinogen, C-reactive proteins, and chemokines, by alveolar macrophages, neutrophils, and other activated cells [11]. Chemokines are polypeptides with a molecular weight of 5–20 kDa, which act as chemoattractant cytokines via the corresponding receptors [12, 13]. Chemokines are classified into four groups differing in target range. The best-studied group are CC chemokines, which attract monocytes and lymphocytes [12, 13].

The goal of the present study was to investigate associations between allele variants of genes that encode chemokines and chemokine receptors (*CCL11*, *CX3CR1*, *CCR5*, *CCL5*, *CXCL12*, *CCL2*, and *CCL17*), adhesion molecules (*PECAM1* and *ICAM1*), and serum amyloid protein A1 (*SAA1*) and COPD.

## MATERIALS AND METHODS

*Subjects.* The candidate gene study was performed using the case–control design. DNA specimens were collected from unrelated individuals of Tatar ethnic origin, residents of Bashkortostan. The group of patients (case) ($N = 425$) included 369 men (86.82%) and 56 women (13.18%) at the age of 63.4 ± 11.8 years. The diagnosis of COPD was established according to ICD10 and the guidelines proposed by the Global Strategy for the Diagnosis, Management, and Prevention of COPD (2017 Update) [1, 14]. The patients did not have a history of exposure to occupational hazards. Subjects with allergic diseases, bronchial asthma, malignant tumors, and specific pulmonary infections (tuberculosis) were excluded from the analysis. Among COPD patients, there were 331 smokers and ex-smokers (77.88%) and 94 nonsmokers (22.12%). The smoking index was calculated using the conventional pack-year (PY) units as recommended in [1]. The smoking index in smokers and ex-smokers was 44.58 ± 25.92 PY. In all patients, spirometry was performed to assess lung function, including vital capacity (VC), forced vital capacity (FVC), forced expiration volume in the first second (FEV1), and the FEV1/FVC ratio. The group of patients had the following parameters (% of normal levels): FEV1 = 38.85 ± 16.62, FVC = 46.06 ± 16.32, VC = 49.02 ± 15.54, FEV1/FVC = 54.21 ± 11.40.

The control group ($N = 457$) included healthy individuals with no history of respiratory pathology or professional contact with hazardous chemical compounds. The group was matched by age (58.44 ± 14.79 years), sex (406 men (88.84%) and 51 women (11.16%)), and smoking status (322 smokers and ex-smokers (70.46%) and 135 nonsmokers (29.54%)); the smoking index in smokers was 37.71 ± 14.12 PY. In the control group, lung function parameters were as follows (% of normal): FEV1 = 102.7 ± 52.05, FVC = 107.1 ± 32.1, VC = 105.3 ± 42.87, FEV1/FVC = 87.94 ± 10.69.

*Genotyping.* DNA was isolated from peripheral blood leukocytes using the phenol–chloroform extraction procedure. Polymorphisms of *CCL11* (c.-426C>T, rs16969415), *SAA1 (*c.209C>T, rs1136743), *CX3CR1* (c.839G>A, rs3732378), *PECAM1* (c.373G>C, rs281865545), *ICAM1* (c.1405A>G, rs5498), *CCR5* (*del32*), *CCL5* (c.-471G>A, rs2107538), *CXCL12* (c.*519G>A, rs1801157), *CCL2* (g.2493A>G, rs1024611), and *CCL17* (c.-59-372T>C, rs223828) [15] were analyzed by real-time PCR using commercial FLASH/RTAS kits with fluorescence-based detection (Test-Gen, Russia) and a BioRad CFX96™ system (Bio-Rad Laboratories, United States). Measurements of end-point fluorescence and genotype discrimination were performed according to the BioRad CFX96™ protocol using the CFX Manager™ software program.

*Statistical analysis* was performed using the PLINK v. 1.07 [16] and Statistica v. 6.0 (StatSoft, United States) [17] software packages. For all polymorphic loci concerned, the allele and genotype frequencies were calculated and their agreement with the Hardy–Weinberg equilibrium was verified using the $\chi^2$ test and the corresponding $P_{HW}$ value; the significance of differences between the groups in the distribution of genotype and allele frequencies was assessed by the $\chi^2$ test for sample homogeneity and the corresponding $P$ value. Pairwise comparison of the allele and genotype frequencies between the groups of patients and controls was performed using the two-sided Fisher's test. Association of the candidate gene polymorphisms with COPD was assessed using logistic regression; the exponent of an individual regression coefficient (beta) was interpreted as the odds ratio (*OR*) and the 95% confidence interval was determined. To minimize the risk of type I error, the Benjamini–Hochberg correction for false discovery rate (FDR) was calculated to obtain the $P_{FDR\text{-}cor}$ value using the online service at http://www.sdmproject.com/utilities/?show=FDR. The contribution of the candidate gene alleles to the variation of quantitative parameters characterizing the course of the disease (lung function parameters VC, FVC, and FEV1) was evaluated using the Kruskal–

**Table 1.** The frequency distributions of alleles and genotypes by candidate gene polymorphisms in the groups of COPD patients and controls and their association with COPD

| Gene, polymorphism | Genotype, allele | COPD abs (%) ($N = 425$) | Control abs (%) ($N = 457$) | $P$ | $P_{cor\text{-}FDR}$ | $OR$ (95% CI) |
|---|---|---|---|---|---|---|
| *CCL11* c.-426C>T rs16969415 | CC | 379 (89.18) | 406 (88.84) | 0.959 | – | 1.03 (0.67–1.58) |
| | CT | 43 (10.12) | 49 (10.72) | 0.855 | – | 0.93 (0.61–1.44) |
| | TT | 3 (0.71) | 2 (0.44) | 0.935 | – | 1.61 (0.27–1.71) |
| | C | 801 (94.24) | 861 (94.20) | 0.945 | – | 1.0 (0.67–1.50) |
| | T | 49 (5.76) | 53 (5.80) | | | 0.99 (0.66–1.48) |
| *SAA1* c.209C>T p.Ala70Val rs1136743 | TT | 57 (13.41) | 159 (34.79) | 0.00001 | 0.00005 | 0.31 (0.22–0.43) |
| | TC | 287 (67.53) | 225 (49.23) | 0.00001 | 0.00005 | 2.15 (1.54–2.84) |
| | CC | 81 (19.06) | 73 (15.97) | 0.45 | – | 1.16 (0.81–1.65) |
| | T | 401 (47.18) | 543 (59.41) | 0.0001 | 0.0003 | 0.63 (0.52–0.76) |
| | C | 449 (52.82) | 371 (40.59) | | | 1.58 (1.31–1.91) |
| *CX3CR1* c.839G>A p.Thr280Met rs3732378 | GG | 266 (62.59) | 269 (58.86) | 0.288 | – | 1.16 (0.89–1.53) |
| | GA | 141 (33.18) | 172 (37.64) | 0.189 | – | 0.82 (0.62–1.08) |
| | AA | 18 (4.24) | 16 (3.50) | 0.696 | – | 1.21 (0.61–2.42) |
| | G | 673 (79.18) | 710 (77.68) | 0.481 | – | 1.09 (0.87–1.37) |
| | A | 177 (20.82) | 204 (22.32) | | | 0.91 (0.72–1.14) |
| *PECAM1* c.373G>C p.Val125Leu rs281865545 | CC | 145 (34.12) | 161 (35.23) | 0.783 | – | 0.95 (0.72–1.26) |
| | CG | 223 (52.47) | 205 (44.86) | 0.028 | 0.043 | 1.36 (1.04–1.77) |
| | GG | 57 (13.41) | 91 (19.91) | 0.013 | 0.022 | 0.62 (0.43–0.89) |
| | C | 513 (60.35) | 527 (57.66) | 0.271 | – | 1.12 (0.92–1.35) |
| | G | 337 (39.65) | 387 (42.34) | | | 0.89 (0.73–1.08) |
| *ICAM1* c.1405A>G p.Lys469Glu rs5498 | AA | 117 (27.53) | 201 (43.98) | 0.00001 | 0.00005 | 0.48 (0.36–0.64) |
| | AG | 216 (50.82) | 191 (41.79) | 0.009 | 0.021 | 1.44 (1.10–1.88) |
| | GG | 92 (21.65) | 65 (14.22) | 0.005 | 0.014 | 1.66 (1.17–2.36) |
| | A | 450 (52.94) | 593 (64.88) | 0.0001 | 0.0003 | 0.60 (0.50–0.73) |
| | G | 400 (47.06) | 321 (35.12) | | | 1.64 (1.35–1.98) |
| *CCR5 del32* | NN | 332 (78.12) | 380 (83.15) | 0.071 | – | 0.72 (0.51–1.01) |
| | ND | 89 (20.94) | 73 (15.97) | 0.069 | – | 1.39 (0.98–1.96) |
| | DD | 4 (0.94) | 4 (0.88) | 0.801 | – | 1.07 (0.26–4.32) |
| | N | 753 (88.59) | 833 (91.14) | 0.09 | – | 0.75 (0.55–1.03) |
| | D | 97 (11.41) | 81 (8.86) | | | 1.32 (0.97–1.8) |
| *CCL5* c.-471G>A rs2107538G>A | GG | 259 (60.94) | 290 (63.46) | 0.483 | – | 0.89 (0.68–1.17) |
| | GA | 147 (34.59) | 153 (33.48) | 0.782 | – | 1.05 (0.79–1.38) |
| | AA | 19 (4.47) | 14 (3.06) | 0.084 | – | 1.96 (0.97–3.97) |
| | G | 665 (78.24) | 733 (80.20) | 0.339 | – | 0.88 (0.71–1.11) |
| | A | 185 (21.76) | 181 (19.80) | | | 1.12 (0.89–1.41) |
| *CXCL12* c.*519G>A rs1801157A>G | GG | 220 (51.76) | 243 (53.17) | 0.726 | – | 3.23 (2.54–4.11) |
| | GA | 170 (40.00) | 177 (38.73) | 0.752 | – | 1.05 (0.80–1.38) |
| | AA | 35 (8.24) | 37 (8.10) | 0.962 | – | 1.01 (0.62–1.65) |
| | G | 610 (71.76) | 663 (72.54) | 0.757 | – | 0.96 (0.78–1.18) |
| | A | 240 (28.24) | 251 (27.46) | | | 1.03 (0.84–1.27) |

**Table 1.** (Contd.)

| Gene, polymorphism | Genotype, allele | COPD abs (%) ($N = 425$) | Control abs (%) ($N = 457$) | $P$ | $P_{cor\text{-}FDR}$ | $OR$ (95% CI) |
|---|---|---|---|---|---|---|
| *CCL2* g.2493A>G rs1024611A>G | AA | 204 (48.00) | 193 (42.23) | 0.313 | – | 0.86 (0.67–1.12) |
| | AG | 178 (41.88) | 191 (41.79) | 0.967 | – | 1.00 (0.77–1.31) |
| | GG | 43 (10.12) | 73 (15.97) | 0.013 | 0.022 | 0.52 (0.39–0.88) |
| | A | 586 (68.94) | 577 (63.13) | 0.012 | 0.022 | 1.30 (1.06–1.58) |
| | G | 264 (31.06) | 337 (36.87) | | | 0.77 (0.63–0.94) |
| *CCL17* c.-59-372T>C rs223828T>C | CC | 320 (75.29) | 339 (74.18) | 0.762 | – | 1.06 (0.78–1.43) |
| | CT | 94 (22.12) | 111 (24.29) | 0.495 | – | 0.88 (0.64–1.21) |
| | TT | 11 (2.59) | 7 (1.53) | 0.384 | – | 1.71 (0.65–4.44) |

*P*, significance of the two-sided Fisher's test for pairwise comparison of allele and genotype frequencies between the groups of patients and controls; *OR*, odds ratio; 95% CI, 95% confidence interval for *OR*; $P_{cor\text{-}FDR}$, significance after correction for false discovery rate (FDR).

Wallis test (for three groups) or the Mann–Whitney test (for two groups); the calculations were performed with Statistica v. 6.0 [17].

## RESULTS

Before analyzing the association of candidate gene alleles with COPD, we verified whether the genotype frequency distributions corresponded to the Hardy–Weinberg equilibrium. In the control group, the obtained *P* values were as follows: *CCL11* (rs16969415C>T), $P_{HW} = 0.99$; *SAA1* (rs1136743C>T), $P_{HW} = 0.52$; *CX3CR1* (rs3732378A>G), $P_{HW} = 0.11$; *PECAM1* (rs281865545G>C), $P_{HW} = 0.13$; *ICAM1* (rs5498A>G), $P_{HW} = 0.11$; *CCR5* (*del32*), $P_{HW} = 0.99$; *CCL5* (rs2107538G>A), $P_{HW} = 0.33$; *CXCL12* (rs1801157A>G), $P_{HW} = 0.61$; *CCL2* (rs1024611A>G), $P_{HW} = 0.074$; *CCL17* (rs223828T>C), $P_{HW} = 0.22$.

### *Association of Candidate Gene Alleles with COPD*

A COPD association analysis was performed for the alleles and genotypes of the following candidate gene polymorphisms: *CCL11* (rs16969415C>T), *SAA1* (rs1136743C>T), *CX3CR1* (rs3732378A>G), *PECAM1* (rs281865545G>C), *ICAM1* (rs5498A>G), *CCR5* (*del32*), *CCL5* (rs2107538G>A), *CXCL12* (rs1801157A>G), *CCL2* (rs1024611A>G), and *CCL17* (rs223828T>C) (Table 1).

The groups of COPD patients and healthy controls differed significantly in the genotype frequency distributions of *SAA1* (rs1136743C>T) ($\chi^2 = 50.89$; $P = 0.00001$), *PECAM1* (rs281865545G>C) ($\chi^2 = 8.254$; $P = 0.016$), *ICAM1* (rs5498A>G) ($\chi^2 = 27.242$; $P = 0.00001$), and *CCL2* (rs1024611A>G) ($\chi^2 = 7.37$; $P = 0.025$).

For the *SAA1* (rs1136743C>T) locus, significant associations with COPD were established for the C allele ($P = 0.0001$, $P_{cor\text{-}FDR} = 0.0003$; $OR = 1.58$, 95% CI 1.31–1.91) and the TC genotype ($P = 0.00001$, $P_{cor\text{-}FDR} = 0.00005$; $OR = 2.15$, 95% CI 1.54–2.84).

The frequency of the CG genotype of *PECAM1* (rs281865545G>C) was significantly higher in COPD patients (52.47% vs. 44.86% in the control group; $P = 0.028$, $P_{cor\text{-}FDR} = 0.043$; $OR = 1.36$, 95% CI 1.04–1.77).

The G allele and the GG genotype of *ICAM1* (rs5498A>G) were associated with COPD: $P = 0.0001$, $P_{cor\text{-}FDR} = 0.0003$, $OR = 1.64$, 95% CI 1.35–1.98 and $P = 0.005$, $P_{cor\text{-}FDR} = 0.014$, $OR = 1.66$, 95% CI 1.17–2.36, respectively.

The risk of COPD was also associated with the A allele of *CCL2* (rs1024611A>G): $P = 0.012$, $P_{cor\text{-}FDR} = 0.022$, $OR = 1.30$, 95% CI 1.06–1.58.

There were no significant differences between the groups of COPD patients and healthy controls in the allele and genotype frequency distributions by the polymorphisms *CCL11* (rs16969415C>T), *CX3CR1* (rs3732378A>G), *CCR5* (*del32*), *CCL5* (rs2107538G>A), *CXCL12* (rs1801157A>G), and *CCL17* (rs223828T>C) (Table 1).

### *Interaction of Genetic and Environmental Risk Factors for COPD*

Interaction between environmental and genetic factors was analyzed by comparing the odds ratio values for candidate gene alleles between subgroups based on the presence or absence of an environmental risk factor (smoking status). The significant associations of the candidate polymorphisms with COPD observed in the subgroups differentiated by smoking status are shown in Table 2.

**Table 2.** Association of candidate gene alleles with COPD in subgroups differentiated by smoking status

| Gene, polymorphism | Genotype, allele | COPD abs (%) | Control abs (%) | *P* | $P_{cor\text{-}FDR}$ | OR (95% CI) |
|---|---|---|---|---|---|---|
| Smokers | | | | | | |
| | | ($N = 331$) | ($N = 322$) | | | |
| *PECAM1* c.373G>C p.Val125Leu rs281865545 | CC | 112 (33.84) | 117 (36.33) | 0.557 | – | 0.89 (0.64–1.24) |
| | CG | 172 (51.96) | 139 (43.16) | 0.03 | 0.037 | 1.42 (1.01–2.00) |
| | GG | 47 (14.20) | 66 (20.51) | 0.043 | 0.046 | 0.64 (0.42–0.60) |
| | C | 396 (59.82) | 373 (57.92) | 0.411 | – | 1.08 (0.86–1.34) |
| | G | 266 (40.18) | 271 (42.08) | | | 0.92 (0.74–1.15) |
| *ICAM1* c.1405A>G rs5498 | AA | 100 (30.21) | 143 (44.41) | 0.0007 | 0.0015 | 0.54 (0.39–0.74) |
| | AG | 164 (49.55) | 138 (42.86) | 0.102 | – | 1.31 (0.96–1.78) |
| | GG | 67 (20.24) | 41 (12.73) | 0.013 | 0.022 | 1.75 (1.09–2.81) |
| | A | 364 (54.98) | 424 (65.84) | 0.0001 | 0.0003 | 0.63 (0.51–0.79) |
| | G | 298 (45.02) | 220 (34.16) | | | 1.58 (1.26–1.97) |
| *SAA1* c.209C>T p.Ala70Val rs1136743 | TT | 47 (14.20) | 117 (36.34) | 0.0001 | 0.0003 | 0.28 (0.19–0.42) |
| | TC | 229 (69.18) | 151 (46.89) | 0.0001 | 0.0003 | 2.55 (1.80–3.60) |
| | CC | 55 (16.62) | 54 (16.77) | 0.625 | – | 0.88 (0.58–1.33) |
| | T | 323 (48.79) | 385 (59.78) | 0.00001 | 0.00007 | 0.64 (0.51–0.79) |
| | C | 339 (51.21) | 259 (40.22) | | | 1.56 (1.25–1.94) |
| Non-smokers | | | | | | |
| | | ($N = 94$) | ($N = 135$) | | | |
| *SAA1* c.209C>T p.Ala70Val rs1136743 | TT | 10 (10.64) | 42 (31.11) | 0.0001 | 0.0003 | 0.26 (0.12–0.55) |
| | TC | 58 (61.70) | 74 (54.81) | 0.367 | – | 1.32 (0.77–2.27) |
| | CC | 26 (27.66) | 19 (14.07) | 0.017 | 0.026 | 2.33 (1.20–4.53) |
| | T | 78 (41.49) | 158 (58.52) | 0.00001 | 0.00007 | 0.50 (0.34–0.73) |
| | C | 110 (58.51) | 112 (41.48) | | | 1.99 (1.36–2.90) |
| *CCL2* g.2493A>G rs1024611 | AA | 53 (56.38) | 56 (41.48) | 0.037 | 0.042 | 1.82 (1.07–3.10) |
| | AG | 36 (38.30) | 57 (42.22) | 0.647 | – | 0.84 (0.49–1.45) |
| | GG | 5 (5.32) | 22 (16.30) | 0.02 | 0.027 | 0.28 (0.10–0.70) |
| | A | 142 (75.53) | 169 (62.59) | 0.005 | 0.009 | 1.84 (1.22–2.79) |
| | G | 46 (24.47) | 101 (37.41) | | | 0.54 (0.36–0.82) |

*P*, significance of the two-sided Fisher's test for pairwise comparison of allele and genotype frequencies between the groups of patients and controls; *OR*, odds ratio; 95% CI, 95% confidence interval for *OR*; $P_{cor\text{-}FDR}$, significance after correction for false discovery rate (FDR).

The *PECAM1* genotype CG (rs281865545G>C) was a marker of COPD risk in smokers ($P = 0.03$, $P_{cor\text{-}FDR} = 0.037$; $OR = 1.42$, 95% CI 1.01–2.00). The frequency of the G allele of *ICAM1* (rs5498A>G) was as high as 45.02% in smoking COPD patients, in contrast to 34.16% in healthy smokers ($P = 0.0001$, $P_{cor\text{-}FDR} = 0.0002$; $OR = 1.58$, 95% CI 1.26–1.97). The risk of COPD in smokers was associated with the GG genotype of *ICAM1* (rs5498A>G) ($P = 0.013$, $P_{cor\text{-}FDR} = 0.022$; $OR = 1.75$, 95% CI 1.09–2.81). The frequency of the C allele of *SAA1* (rs1136743C>T) in smokers with COPD was 51.21% vs. 40.22% in smokers of the control group ($P = 0.00001$, $P_{cor\text{-}FDR} = 0.00007$; $OR = 1.56$, 95% CI 1.25–1.94). The risk of COPD in smokers was associated with the TC genotype ($P = 0.0001$, $P_{cor\text{-}FDR} = 0.0003$; $OR = 2.55$, 95% CI 1.80–3.60).

In the group of nonsmokers, the risk of COPD was associated with the CC genotype of *SAA1* (rs1136743C>T): $P = 0.017$, $P_{cor\text{-}FDR} = 0.026$; $OR = 2.33$, 95% CI 1.20–4.53. The A allele and the AA genotype of *CCL2* (rs1024611A>G) were significantly associated with COPD only in nonsmokers: $P = 0.005$, $P_{cor\text{-}FDR} = 0.009$; $OR = 1.84$, 95% CI 1.22–2.79 and $P = 0.037$, $P_{cor\text{-}FDR} = 0.042$; $OR = 1.82$, 95% CI 1.07–3.10, respectively.

### *Contribution of Candidate Gene Alleles to Variation of Lung Function Parameters*

It was analyzed how the candidate gene alleles affect variation in quantitative lung function parameters that characterize disease progression: VC, FVC,

**Table 3.** Association of quantitative lung function characteristics with candidate gene alleles

| Gene, polymorphism | Genotype | *N* | *M* ± *S.E.* | *P* |
|---|---|---|---|---|
| VC (vital capacity) (*N* = 425) | | | | |
| *PECAM1* c.373G>C p.Val125Leu rs281865545 | CC | 136 | 51.79 (2.09) | 0.041 |
| | CG | 238 | 50.36 (1.41) | |
| | GG | 51 | 59.17 (3.01) | |
| | (CC+CG) | 374 | 50.88 (1.17) | 0.014 |
| | GG | 51 | 59.17 (3.01) | |
| *CX3CR1* c.839G>A p.Thr280Met rs3732378 | (GG+GA) | 412 | 51.74 (1.03) | 0.04 |
| | AA | 13 | 62.69 (5.13) | |
| FEV1 (forced expiration volume in 1 s) (*N* = 425) | | | | |
| *ICAM1* c.1405A>G rs5498 | AA | 102 | 37.5 (2.06) | 0.005 |
| | AG | 221 | 43.47 (1.5) | |
| | GG | 102 | 36.1 (1.91) | |
| | (AA+AG) | 23 | 41.55 (1.23) | 0.025 |
| | GG | 102 | 36.1 (1.91) | |
| | (AA+GG) | 204 | 36.8 (1.4) | 0.0013 |
| | AG | 221 | 43.47 (1.5) | |
| FVC (forced vital capacity) (*N* = 425) | | | | |
| *ICAM1* c.1405A>G rs5498 | AA | 110 | 47.25 (2.53) | 0.0045 |
| | AG | 221 | 54.66 (1.77) | |
| | GG | 94 | 45.42 (2.45) | |
| | (AA+AG) | 331 | 52.18 (1.47) | 0.029 |
| | GG | 94 | 45.42 (2.45) | |
| | (AA+GG) | 204 | 46.42 (1.77) | 0.0012 |
| | AG | 221 | 54.66 (1.77) | |

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

and FEV1. The GG genotype of *PECAM1* (rs281865545G>C) and the AA genotype of *CX3CR1* (rs3732378A>G) were associated with higher VC values ($P = 0.014$ and $P = 0.04$) (Table 3). Individuals with the *ICAM1* (rs5498A>G) genotype GG exhibited decreased FEV1 and FVC ($P = 0.025$ and $P = 0.029$), while AG heterozygotes had higher FEV1 and FVC values ($P = 0.0013$ and $P = 0.0012$).

## DISCUSSION

In this work, the association of COPD with polymorphic variants of the genes that encode chemokines and chemokine receptors, *CCL11* (rs16969415C>T), *CX3CR1* (rs3732378A>G), *CCR5* (*del32*), *CCL5* (rs2107538G>A), *CXCL12* (rs1801157A>G), *CCL2* (rs1024611A>G), and *CCL17* (rs223828 T>C); adhesion molecules, *PECAM1* (rs281865545G>C) and *ICAM1* (rs5498A>G); and serum amyloid A1, *SAA1* (rs1136743C>T), was investigated in a Tatar population.

The most significant association with the disease, both in the general sample and in the subgroups stratified by smoking status, was observed for the alleles of *SAA1* (rs1136743), which encodes serum amyloid A1 [5]. The human genome contains four *SAA* genes located on chromosome 11p15.1: *SAA1*, *SAA2*, *SAA3*, and *SAA4*. Among them, *SAA1* and *SAA2* encode serum amyloid A, an acute-phase protein; *SAA4* encodes a SAA-related protein that is expressed constitutively and is not affected by proinflammatory stimuli; and *SSA3* is a pseudogene. *SAA1* comprises

four exons, and its coding sequence corresponds to exons 2, 3, and a part of exon 4. The rs1136743 (209C>T) polymorphism lies in *SAA1* exon 3 and results in a valine for alanine substitution in the protein sequence (p.Ala70Val) [5, 15]. We found that the C allele and the TC genotype of the *SAA1* polymorphism rs1136743C>T were markers of COPD risk; this association remained significant after correction for multiple comparisons and was confirmed in the subgroups differentiated by smoking status. The associations of *SAA1* polymorphisms with COPD were not studied previously, but it was observed that the production of C-reactive protein and SAA was elevated in the lung parenchyma and bronchi of COPD patients [18]. It was also shown that SAA is extensively produced by endothelial cells and macrophages of normal lung tissue [19]. It was demonstrated that the vascular system of the lungs is the principal site of SAA and C-reactive protein production and the source of systemic inflammation in COPD [18].

The results obtained in our work indicate that allelic variants of the adhesion molecule genes *ICAM1* and *PECAM1* are associated with COPD, as well as with characteristics of lung function and disease progression. The risk of COPD was associated with the G allele and the GG genotype of *ICAM1* (rs5498A>G). Moreover, it was shown that lung function parameters depended on the genotype of this gene. In particular, GG homozygotes by *ICAM1* (rs5498A>G) exhibited lower FEV1 and FVC levels, which is consistent with the general association between this genotype and the disease. *ICAM1* is located on chromosome 19p13.2 [15]; it encodes a membrane-bound protein containing five Ig-like extracellular domains. ICAM1 expression is induced by proinflammatory mediators, such as IL-1, TNFα, and IFNγ [9]. The rs5498A>G polymorphism in exon 6 results in the amino acid substitution p.Lys469Glu [7]. COPD was also found to be associated with the rs281865545 (c.373G>C) polymorphism of *PECAM1*. The heterozygous genotype was associated with the disease both in the general sample and in smokers. At the same time, GG homozygotes exhibited higher VC values and decreased risk of COPD. *PECAM1* is located on chromosome 17q23.3 and encodes the cell adhesion molecule PECAM1 [15]. PECAM1 mediates intercellular leukocyte migration through vascular endothelium [10]. The rs281865545 (c.373G>C) substitution in *PECAM1* exon 3 results in a leucine for valine substitution in codon 125 (p.Val125Leu), which affects PECAM1 functioning [15]. The contribution of adhesion molecule gene polymorphisms to COPD has not been investigated previously. The transformation of local lung tissue inflammation into chronic systemic inflammation in COPD may be related to a higher permeability of blood vessels in the patient's lungs, resulting in a release of proinflammatory factors into the bloodstream [2, 11]. Along with the previously published data, our results suggest that allele variants of the adhesion molecule genes *ICAM1* and *PECAM1* may represent an important risk factor for the development and progression of diseases involving chronic systemic inflammation, such as COPD.

Owing to their ability to produce proinflammatory cytokines and chemokines, macrophages act as a major inflammation-promoting factor in COPD [11–13]. Monocytes recruited to the lungs by CCL2 secreted by macrophages also subsequently differentiate to macrophages. Among proinflammatory CC chemokines, CCL2 and CCL5 are critically involved in the initiation and development of inflammatory processes primarily due to monocyte activity [13]. We found that the A allele of the *CCL2* polymorphism (rs1024611A>G) was significantly associated with COPD. A more detailed analysis showed that the AA genotype was associated with COPD only in nonsmokers. *CCL2* is located on chromosome 17q11.2-q12 [15]. CCL2, also known as monocyte chemoattractant protein 1 (MCP-1), is produced by activated monocytes, macrophages, and dendritic cells. CCL2 plays a central role in the recruitment of macrophages, basophils, and T cells to the inflammation focus and participates in the activation of matrix metalloproteases, key factors of lung tissue remodeling in COPD [13, 20]. The polymorphism rs1024611A>G (–2518A>G) lies in the regulatory region of *CCL2* [20]. It was shown that the G allele is associated with increased CCL2 levels in the serum and plasma [20]. An association of the GG genotype of *CCL2* (rs1024611A>G) with COPD was previously reported in a Chinese population [21]. On the other hand, studies [22] and [23] performed in Taiwan and France, respectively, did not detect any association between *CCL2* (rs1024611A>G) alleles and COPD.

It was found that allelic variants of *CX3CR1* (rs3732378G>A) affected quantitative variation in lung function (specifically, in VC). Subjects with the AA genotype had significantly higher VC values. CX3CR1 is a chemokine receptor expressed on monocytes, activated T cells, natural killers, and dendritic cells [13]. The protein encoded by *CX3CR1* (3p22.2) is a specific receptor for CX3CL1 chemokine (fractalkine), which acts as a chemoattractant promoting migration, adhesion, and proliferation of activated inflammatory cells [15]. The rs3732378G>A transition in the *CX3CR1* sequence results in the amino acid substitution p.Thr280Met [15]. In a previous study, it was shown that the A allele, which corresponds to methionine in the amino acid sequence of the receptor molecule, is associated with decreased cell adhesion levels and leukocyte chemotaxis [24]. Monocytes and macrophages carrying the CX3CR1 receptor readily migrate through the vascular endothelium in the lungs and accumulate in the blood vessel walls and the parenchyma [13, 24]. Infiltration by the immune cells induces proinflammatory mediator release and lung tissue remodeling, stimulating proliferation of inflammatory cells [2].

The results obtained in this study contribute to the understanding of the molecular mechanisms of COPD pathogenesis. Polymorphisms of *SAA1* (serum amyloid A1), *PECAM1* and *ICAM1* adhesion molecule genes, and *CCL2* were shown to be markers of COPD risk in the Tatar population. It was found that the genotypes of *PECAM1*, *ICAM1*, and *CX3CR1* were associated with lung function parameters that reflect COPD progression.

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## COMPLIANCE WITH ETHICAL STANDARDS

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

*Statement of compliance with standards of research involving humans as subjects*. The study was approved by the Ethics Committee of the Institute of Biochemistry and Genetics. All participants gave their informed consent to the use of their biological materials in the proposed experiments.

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