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Gene-gene and gene-environment interactions of the inflammatory gene variants in the development of chronic obstructive pulmonary disease

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Abstract

Chronic obstructive pulmonary disease (COPD) is a complex chronic inflammatory disease that is characterized by partly reversible airflow limitation, chronic inflammation, fibrosis of small airways, and destruction of lung parenchyma. We aimed to assess the association of the inflammatory gene loci singly and in combinations with COPD in smokers and non-smokers in ethnic Tatar from Russia to evaluate the gene-gene and gene-environment interactions in COPD development. Eleven loci of inflammatory genes, including *IL19*, *IL20*, *IL24*, *PPBP*, *IL4*, *IL4RA,* C5, *FAS, FASLG*, and *TGFb1*, were genotyped in 484 smoking COPD patients, 517 healthy smokers, 117 non-smoking COPD patients, and 100 healthy non-smokers. Significant associations with COPD in smokers were identified for *IL19* (rs2243193), *IL4* (rs2243250), *IL4* (rs2070874), and *PPBP* (rs352010). In non-smokers, associations were established for *IL24* (rs291107), *IL4* (rs2070874), and *PPBP* (rs352010). Associations of inflammatory genes loci *IL19* (rs2243193), *IL4* (rs2070874), *TGFb1* (rs1800469), *PPBP* (rs352010), and *FASLG* (rs763110) and smoking index were determined. Associations of *FAS* (rs1800682), *FASLG (*rs763110), *IL4 (*rs2243250), *IL4RA* (rs1805010), and *PPBP* (rs352010) loci with pulmonary function variables were observed. The results of gene-gene interactions analysis showed distinctive patterns of association of inflammatory gene loci with COPD in groups stratified by smoking status. The combination of A allele of *IL19* (rs2243193), C allele of *IL4* (rs2243250), and T allele of *PPBP* (rs352010) was the main component of the majority of protective gene-gene combination associated with COPD in smokers. The highest risk of COPD was conferred by TT genotype of *PPBP* (rs352010) in combination with A allele of *FAS* (rs1800682). While in non-smokers, the most commonly featured was *IL24* (rs291107) C allele in protective patterns and *IL24* (rs291107) T allele in predisposing combinations. The highest risk of COPD in non-smokers was detected in a gene-gene combination consisting of A allele of *IL12RB2* (rs3762317) together with G allele of *IL12A* (rs2243115), C allele of *IL4* (rs2070874), and A allele of *IL4RA* (rs1805010).

Keywords: Chronic obstructive pulmonary disease; Cytokine; IL19; IL24; PPBP

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1. Introduction

Chronic obstructive pulmonary disease (COPD) is a complex lung disease characterized by progressive airflow limitation and abnormal inflammatory response of the lungs to the inhaled noxious particles[1,2]. COPD is one of the most common chronic respiratory diseases with high morbidity and mortality rates[3]. Tobacco smoking, environmental pollution, and genetic predisposition are the principal risk factors of COPD with genetic contributions from multiple genes[4,5]. COPD pathogenesis was linked to oxidative stress and systemic inflammation[6]. To date, comprehensive studies of COPD genetic background are being conducted[7]. There is evidence that the genes of cytokines and inflammatory mediators play an important roles in the development of COPD[8-10].

Cytokines are secreted proteins with key functions that regulate immune responses[11,12]. IL19, IL20, and IL24 are proinflammatory cytokines of the IL20 subfamily members within the IL10 cytokine family, which can bind the IL20 receptor complex IL-20R1/IL-20R2[13-15]. IL4 is cytokine produced by activated T cells and binds to the IL4 receptor. As an important cytokine for tissue repair, IL4 also promotes allergic airway inflammation, as well as mediates and regulates a variety of human host responses[16].

Chemokines play a key role to the formation of immune responses[17]. Pro-platelet basic protein (PPBP) is a platelet-derived growth factor or platelet-derived chemokine ligand 7 chemokine (CXCL7), which is a member of the CXC-chemokine family[18]. This growth factor is a potent chemoattractant and activator of neutrophils[19]. CXCL chemokines are produced by inflammatory cells and endothelial cells, and CXCL7 displays chemotactic activity on neutrophils. The receptors CXCR1 and CXCR2 for CXCL7 are expressed on neutrophils, endothelial cells, and macrophages[20].

The FASL/FAS system is considered a major pathway for apoptosis in cells and tissues. FAS plays a central role in the physiological regulation of programmed cell death[21], as well as in pulmonary inflammation, injury, and fibrosis[22]. The transforming growth factor beta (TGF-β) superfamily consists of secreted growth factors involved in the regulation of different cellular processes[23]. TGF-β1 plays a key role in tissue injury and airway remodeling in smokers[24]. C5 (complement C5) is a component of the complement system, which is a part of the innate immune system[25].

Previously, we identified the association of chemokines genes with COPD risk in the Tatar population from Russia[26]. In this study, we aimed to assess the association of single nucleotide polymorphisms (SNPs) of *IL19*, *IL20*, *IL24*, *PPBP*, *IL4*, *IL4RA*, C5, *FAS, FASLG*, and *TGFb1* genes singly and in combinations with COPD in smokers and non-smokers to evaluate the gene-gene and gene-environment interactions in COPD development.

2. Materials and methods

2.1. Study population

All participants of this study provided written informed consent and the study was approved by the Local Research Ethics Committee (Ufa, Protocol No 17, December 07, 2010). We included unrelated subjects of self-reported ethnic Tatar population; all details have been previously described[26,27]. The participants were recruited through the pulmonary departments of Ufa City Hospitals No. 21 (Ufa, Russia) during the 2010 – 2020 period. The smoker group includes 484 COPD patients and 517 healthy individuals, while the non-smoker group includes 117 COPD patients and 100 healthy individuals. Details of inclusion and exclusion criteria for case and control groups have been previously described[24,26]. Briefly, to determine pulmonary function, measures of forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) were derived in accordance with American Thoracic Society/European Respiratory Society criteria[28]. COPD was diagnosed according to the Tenth Revision of the International Classification of Diseases (ICD 10) (http://www.who.int/classifications/icd/en/) and in accordance with the recommendations of the Global Initiative on COPD (GOLD; http www.goldcopd.org)[26,27]. Baseline demographics and clinical characteristics of studied groups are presented in Table 1.

2.2. Gene and SNPs selection and genotyping

Eleven SNPs of inflammatory genes (Table 2), including *FASLG* (rs763110), *IL19* (rs2243193), *IL20* (rs2981573), *IL24* (rs291107), *PPBP* (rs352010), *IL4* (rs2243250), *IL4* (rs2070874), C5 (rs17611), *FAS* (rs1800682), *IL4RA* (rs1805010), and *TGFb1* (rs1800469), were selected for the study based on the following criteria[26,27]: (i) Associations with complex human diseases; (ii) functional effect of SNP on gene expression or relation to non-synonymous substitutions; and (iii) minor allele frequency (MAF) of ≥5% in the European populations (http://www.internationalgenome.org/). The SNPs regulatory potential and effect on the gene expression were assessed using HaploReg (v 4.1; http://archive.broadinstitute.org/mammals/haploreg/haploreg.php)[29], SNPinfo Web Server (https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.html), RegulomeDB Version 1.1 (https://regulomedb.org), PolyPhen-2 prediction of functional effects of human nsSNPs (http://genetics.bwh.harvard.edu/pph2/), and GTExportal (http://www.gtexportal.org/). According to

Table 1. Baseline demographics and clinical characteristics of studied group

Parameters	Smokers		Non-smokers	
	Patients *n*=484	Healthy individuals *n*=517	Patients *n*=117	Healthy individuals *n*=100
Gender, *n* (%)				
Male	465 (96.18)	444 (85.85)	56 (47.86)	76 (76.00)
Female	19 (3.82)	73 (14.15)	61 (52.14)	24 (24.00)
Age, years (mean±SD)	62.89±11.36	57.66±10.92	66.03±11.93	58.92±13.19
BMI (kg/m^2) (mean±SD)	25.87±5.50	26.62±3.55	25.77±4.05	27.01±3.23
Smoking index in pack/years (mean±SD)	44.57±25.82	38.74±23.17	NA	NA
Lung function				
Post-FEV1% (mean±SD)	38.70±17.29	136.59±38.33	46.34±15.94	141.21±39.75
Post-FEV1/FVC ratio (mean±SD)	61.15±18.92	89.12±10.07	74.76±23.69	86.23±9.95
FVC % (mean±SD)	53.61±21.49	128.26±3 0.87	49.46±18.58	132.91±28.44
GOLD status (stage)				
2, *n* (%)	112 (23.12)	NA	47 (40.17)	NA
3, *n* (%)	144 (29.70)	NA	31 (26.49)	NA
4, *n* (%)	228 (47.16)	NA	39 (33.34)	NA
MMRC, *n* (%)				
0	29 (6.00)	NA	20 (17.10)	NA
1	55 (11.33)	NA	20 (17.10)	NA
2	152 (31.34)	NA	19 (16.23)	NA
3	184 (38.00)	NA	58 (49.57)	NA
4	64 (13.33)	NA	0 (0)	NA
CAT (mean±SD)	23.02±8.64	NA	21.33±10.65	NA

BMI, body mass index; CAT, COPD assessment test; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; GOLD, global initiative for chronic obstructive lung disease; Post, post-bronchodilator; MMRC, modified medical research council dyspnea scale; NA, not available.

functional analysis, *TGFb1* (rs1800469) has regulatory score 1a and affects gene expression; rs1800469 is located at 2 kb upstream region in the binding sites for transcriptional factor NR2F1. *IL19* (rs2243193) has regulatory score 2b, showing the high probability of this SNP as a regulatory player for this gene; rs2243193 is located in 3' UTR, and it leads to a change in the microRNA-binding sites (hsa-miR-1259, hsa-miR-135b, hsa-miR-27a, hsa-miR-27b, hsa-miR-450b-5p, and hsa-miR-641). *IL20* (rs2981573) is located at the region of promoter histone marks (NHEK). *IL24* (rs291107) is located at the region hypersensitive to DNase I in five different tissues. *IL4* (rs2070874) has regulatory score 2b and affects gene expression; rs2070874 is located in 5' UTR, and it changes the binding sites for transcription factors BARX1, BSX, DLX1, and HOXA3. *IL4* (rs2243250) is located at 2 kb upstream region and alters the binding sites of transcriptional factors. *FASLG* (rs763110) has regulatory score 2a; rs763110 alters expression of *FASLG* as it is located at the transcription factor binding site (CEBPZ, CEBPD, CEBPE, CEBPG, CEBPB, and CEBPA). Furthermore, according to GTEx (https://www.gtexportal.org), rs763110 is associated with changes in gene expression in lung tissue. *FAS* (rs1800682) is located in the promoter region and alters the transcriptional factor binding sites (STAT, ZBRK1, PAX3, and SP-1). *PPBP* (rs352010) is located at 2 kb upstream region and alters transcriptional factor binding sites (ARNT and HIF). C5 (rs17611) and *IL4RA* (rs1805010) are missense variants. *IL4RA* (rs1805010) is located in the region of promoter and enhancer histone marks (HSMM) and is hypersensitive to DNase I in five different tissues. Thus, all selected loci of inflammatory genes have significant regulatory potential.

DNA was extracted from whole blood by the phenol-chloroform protocol as described earlier[26,27]. Genotyping was performed using TaqMan SNP Genotyping Assays (https://www.thermofisher.com). Details of genotyping procedure and quality control have been previously described[26,27].

2.3. Statistical analysis

Statistical analysis was performed using the software Graph Pad Prism software version 8.4.3, PLINK[30], and Haploview 4.2. Sample size and statistical power for each SNP were

Table 2. Baseline characteristics of studied inflammatory genes loci

No.	Gene	Chromosome Position	Consequence	HGVS Names	RefSNP	Minor allele	Minor allele frequency in COPD	Minor allele frequency in control	*P*-value for Hardy-Weinberg equilibrium in control
1	*FASLG*	1q24.3 172658358	2 kb upstream variant	c.-844C > T	rs763110	T	0.3469	0.3549	0.24
2	*IL19*	1q32.1 206842880	3' UTR variant	c.*258A > G	rs2243193	A	0.3644	0.4546	0.084
3	*IL20*	1q32.1 206867232	Intron variant	c. 379-152A > G	rs2981573	G	0.3053	0.3282	0.7
4	*IL24*	1q32.1 206901826	Intron variant	c. 108-172T > C	rs291107	C	0.4443	0.4765	0.31
5	*PPBP*	4q13.3 73989514	2 kb upstream variant	c.-1411T > C	rs352010	T	0.2163	0.2237	0.19
6	*IL4*	5q31.1 132673462	2kb upstream variant	c.-589C > T	rs2243250	T	0.2745	0.2310	0.54
7	*IL4*	5q31.1 132674018	5' UTR variant	c.-33C > T	rs2070874	T	0.1597	0.2277	0.81
8	*C5*	9q33.2 121006922	Missense variant	c. 2422G > A, p.Val802Ile	rs17611	A	0.4376	0.4344	0.23
9	*FAS*	10q23.31 88990206	Intron variant	c.-671A > G	rs1800682	G	0.4268	0.4489	0.43
10	*IL4RA*	16p12.1 27344882	Missense variant	c. 223A > G, p.Ile75Val	rs1805010	G	0.368	0.4408	0.15
11	*TGFb1*	19q13.2 41354391	2 kb upstream variant	c.-1347T > C	rs1800469	T	0.3178	0.3558	0.46

HGVS names for rs2243193 is c.*258A > G. Source: https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?do_not_redirect&rs = rs2243193.

estimated using Quanto 1.2.4[31]. Details of statistical analysis procedure have been previously described[26,27].

Briefly, correspondence of the studied SNPs to the Hardy-Weinberg equilibrium (HWE) was checked by the Chi-square test. The SNPs were analyzed for associations with COPD using logistic regression in additive, dominant, and recessive models with adjustment for covariates. The linkage disequilibrium structure and haplotype frequencies were calculated with Haploview 4.2. Multilocus analysis and gene-gene interaction were performed using Allelic Pattern Sampler (APSampler) version 6.0 software (http://apsampler.sourceforge.net/)[32]. False discovery rate (FDR)[33] was applied to adjust the results for multiple comparisons using the online software program (http://www.sdmproject.com/utilities/?show=FDR).

3. Results

3.1. Single-locus association analysis of COPD risk in smokers and non-smokers

Table 2 presents data on MAF and results of test to deviation from HWE within the control group (Table 2). All studied SNPs were in HWE. Tables 3 and 4 summarize the data on the allele and genotype frequency distributions of the studied loci of inflammatory genes in smoker and non-smoker groups. Results of association analysis of inflammatory gene loci and COPD in groups stratified by smoking status are shown in Table 5.

Significant association of *IL19* (rs2243193) with COPD in smokers was established ($P = 0.00001$, OR =0.67 for basic allele test), in the log-additive (P_{adj} =0.0003, $P_{cor\text{-}FDR}$ =0.00112, OR =0.72), and dominant (P_{adj} =0.00001, $P_{cor\text{-}FDR}$ =7.5 × 10^{-5}, OR =0.48) models (Tables 3 and 5). We analyzed the haplotype frequencies of *IL19* (rs2243193), *IL20* (rs2981573), and *IL24* (rs291107) loci since they are located in the same linkage block on chromosome 1q32.1. The pair-wise linkage disequilibrium values for rs2243193, rs2981573, and rs291107 loci were calculated (Figure 1). The strong level of linkage disequilibrium was observed only between *IL19* (rs2243193) and *IL20* (rs2981573) loci (D' =0.828, r^2 = 0.458) (Figure 1). Smoking patients differ significantly from smoking healthy individuals in their haplotype frequency distribution by *IL19* (rs2243193) and *IL20* (rs2981573) loci ($P = 0.00001$; Table 6). The frequency of the G-A haplotype by *IL19* (rs2243193) and *IL20* (rs2981573) loci was higher among patients (61.13% in COPD vs. 50.81% in control, P_{adj} =2.12 × 10^{-6}, OR =2.42), and A-A haplotype was lower in COPD group (P_{adj} =1.07 × 10^{-7}, OR =0.42).

Table 3. Distributions of genotypes and alleles of studied inflammatory gene loci in smokers

Gene, SNP	Minor allele	Genotypes and alleles	COPD patients, *n* (%) (N=484)	Healthy individuals, *n* (%) (N=517)	*P*-value[a]	*P*-value[b]	OR (95% CI)
FASLG, rs763110 C>T	T	CC/CT/TT	225/183/76 (46.49/37.81/15.70)	221/229/67 (42.75/44.29/12.96)	0.11	0.88	0.99 (0.82 – 1.18)
		C/T	633/335 (65.39/34.61)	671/363 (64.89/35.11)	0.852	-	0.97 (0.81 – 1.17)
IL19, rs2243193 A>G	A	GG/GA/AA	237/149/98 (48.97/30.79/20.25)	163/239/115 (31.53/46.23/22.24)	0.00001	0.0003	0.72 (0.60 – 0.86)
		G/A	623/345 (64.36/35.64)	565/469 (54.64/45.36)	0.00001	-	0.67 (0.56 – 0.80)
IL20, rs2981573 A>G	G	AA/AG/GG	243/190/51 (50.21/39.26/10.54)	241/216/60 (46.62/41.78/11.61)	0.57	0.31	0.90 (0.74 – 1.10)
		A/G	676/292 (69.83/30.17)	698/336 (67.50/32.50)	0.283	-	0.88 (0.74 – 1.08)
IL24, rs291107 T>C	C	TT/TC/CC	155/214/115 (32.02/44.21/23.76)	156/237/124 (30.17/45.84/23.98)	0.82	0.66	0.96 (0.81 – 1.15)
		T/C	524/444 (54.13/45.87)	549/485 (53.09/46.91)	0.674	-	0.96 (0.80 – 1.14)
PPBP, rs352010 T>C	T	CC/CT/TT	353/76/55 (72.93/15.70/11.36)	319/170/28 (61.70/32.88/5.42)	0.00001	0.21	0.88 (0.72 – 1.07)
		C/T	782/186 (80.79/19.21)	808/226 (78.14/21.86)	0.160	-	0.85 (0.68 – 1.06)
IL4, rs2243250 C>T	T	CC/CT/TT	253/192/39 (52.27/39.67/8.06)	306/188/23 (59.19/36.36/4.45)	0.038	0.014	1.33 (1.06 – 1.66)
		C/T	698/270 (72.11/27.89)	800/234 (77.37/22.63)	0.003	-	1.32 (1.08 – 1.62)
IL4, rs2070874 C>T	T	CC/CT/TT	324/158/2 (66.94/32.64/0.41)	308/183/26 (59.57/35.40/5.03)	0.00001	0.0006	0.66 (0.52 – 0.84)
		C/T	806/162 (83.26/16.74)	799/235 (77.27/22.73)	0.00001	-	0.68 (0.55 – 0.85)
C5, rs17611 G>A	A	GG/GA/AA	152/231/101 (31.40/47.73/20.87)	171/238/108 (33.08/46.03/20.89)	0.86	0.74	1.03 (0.86 – 1.24)
		G/A	535/433 (55.27/44.73)	580/454 (56.09/43.91)	0.744	-	1.03 (0.87 – 1.23)
FAS, rs1800682 A>G	G	AA/AG/GG	169/214/101 (34.92/44.21/20.87)	156/250/111 (30.17/48.36/21.47)	0.32	0.29	0.91 (0.75 – 1.09)
		A/G	552/416 (57.02/42.98)	562/472 (54.35/45.65)	0.247	-	0.90 (0.75 – 1.07)
IL4RA, rs1805010 A>G	G	AA/AG/GG	166/206/112 (34.30/42.56/23.14)	163/247/107 (31.53/47.78/20.70)	0.32	0.89	0.99 (0.83 – 1.18)
		A/G	538/430 (55.58/44.42)	573/461 (55.42/44.58)	0.798	-	0.99 (0.83 – 1.19)
TGFb1, rs1800469 T>C	T	CC/CT/TT	229/201/54 (47.31/41.53/11.16)	222/230/65 (42.94/44.49/12.57)	0.43	0.21	0.88 (0.72 – 1.07)
		C/T	659/309 (68.08/31.92)	674/360 (65.18/34.82)	0.185	-	0.88 (0.73 – 1.06)

[a]*P*-value for Chi-square test; [b]*P*-value for Cochran-Armitage trend test. CI, confidence interval; OR, odds ratio.

The association of *IL4* (rs2243250) and COPD in smokers was detected (P = 0.003, OR =1.32 for basic allele test), in the log-additive (P_{adj} =0.014, $P_{cor\text{-}FDR}$ =0.019, OR =1.33), and recessive (P_{adj} =0.036, $P_{cor\text{-}FDR}$ =0.041, OR =1.85) models (Tables 3 and 5). The frequency of minor T allele of *IL4* (rs2070874) in patients group was decreased (16.74% in COPD vs. 22.73% in control P = 0.00001, OR =0.68 for basic allele test). *IL4* (rs2070874) was associated with COPD in smokers in the log-additive (P_{adj} =0.0006, $P_{cor\text{-}FDR}$ =0.0018, OR =0.66) and recessive (P_{adj} =0.00001, $P_{cor\text{-}FDR}$ =7.5 × 10^{-5}, OR =0.08) models (Table 5).

Moderate level of linkage disequilibrium between the rs2243250 and rs2070874 loci of *IL4* gene was observed (D' =0.46, r^2 = 0.15) (Figure 1). We have established significant differences in the haplotype frequencies distribution of *IL4* gene loci between patients and healthy individuals groups (P = 0.00001) (Table 6). Significant association of T-C haplotype by rs2243250 and rs2070874 loci of *IL4* gene with COPD risk in smokers was observed (P_{adj} =0.024, OR =1.44). The C-T haplotype by rs2243250 and rs2070874 of *IL4* gene was identified as protective (P_{adj} =0.001, OR =0.52; Table 6).

Association of *PPBP* (rs352010) with the development of COPD in smokers was detected in the recessive (P_{adj} =0.0008, $P_{cor\text{-}FDR}$ =0.002, OR =2.26), and dominant (P_{adj} =0.0002, $P_{cor\text{-}FDR}$ =0.001, OR =0.60) models (Table 5). A significant interaction of the *PPBP* (rs352010) and smoking status was detected in the regression analysis ($P_{interact}$ =0.017, OR =0.60, 95% CI =0.46 – 0.79 in the dominant model). *PPBP* (rs352010) was predominantly associated with COPD in smokers.

Table 4. Distributions of genotypes and alleles of studied inflammatory genes loci in non-smokers

Gene, SNP	Minor allele	Genotypes, alleles	COPD patients, *n* (%) (N=117)	Healthy individuals, *n* (%) (N=100)	*P*-value[a]	*P*-value[b]	OR (95% CI)
FASLG, rs763110 C>T	T	CC/CT/TT	54/44/19 (46.15/37.61/16.24)	43/39/18 (43.00/39.00/18.00)	0.88	0.61	0.90 (0.60–1.35)
		C/T	152/82 (64.96/35.04)	125/75 (62.50/37.50)	0.667	-	0.89 (0.61–1.33)
IL19, rs2243193 A>G	A	GG/GA/AA	51/40/26 (43.59/34.19/22.22)	32/44/24 (32.00/44.00/24.00)	0.28	0.22	0.78 (0.51–1.17)
		G/A	142/92 (60.68/39.32)	108/92 (54.00/46.00)	0.191	-	0.76 (0.52–1.11)
IL20, rs2981573 A>G	G	AA/AG/GG	50/58/9 (42.74/49.57/7.69)	39/52/9 (39.00/52.00/9.00)	0.85	0.57	0.87 (0.53–1.42)
		A/G	158/76 (67.52/32.48)	130/70 (65.00/35.00)	0.651	-	0.89 (0.60–1.33)
IL24, rs291107 T>C	C	TT/TC/CC	44/60/13 (37.61/51.28/11.11)	20/58/22 (20.00/58.00/22.00)	0.011	0.0028	0.49 (0.30–0.79)
		T/C	148/86 (63.25/36.75)	98/102 (49.00/51.00)	0.004	-	0.60 (0.41–0.89)
PPBP, rs352010 T>C	T	CC/CT/TT	60/33/24 (51.28/28.21/20.51)	59/32/9 (59.00/32.00/9.00)	0.086	0.075	1.45 (0.96–2.19)
		C/T	153/81 (65.38/34.62)	150/50 (75.00/25.00)	0.038	-	1.59 (1.05–2.41)
IL4, rs2243250 C>T	T	CC/CT/TT	67/41/9 (57.26/35.04/7.69)	56/38/6 (56.00/38.00/6.00)	0.89	0.99	1.00 (0.59–1.71)
		C/T	175/59 (74.79/25.21)	150/50 (75.00/25.00)	0.952	-	1.01 (0.65–1.56)
IL4, rs2070874 C>T	T	CC/CT/TT	90/27/0 (76.92/23.08/0)	60/37/3 (60.00/37.00/3.00)	0.011	0.0058	0.43 (0.23–0.80)
		C/T	207/27 (88.46/11.54)	157/43 (78.50/21.50)	0.007	-	0.47 (0.28–0.80)
C5, rs17611 G>A	A	GG/GA/AA	49/43/25 (41.88/36.75/21.37)	34/49/17 (34.00/49.00/17.00)	0.26	0.77	0.94 (0.62–1.42)
		G/A	141/93 (60.26/39.74)	117/83 (58.50/41.50)	0.785	-	0.92 (0.63–1.36)
FAS, rs1800682 A>G	G	AA/AG/GG	42/57/18 (35.90/48.72/15.38)	38/44/18 (38.00/44.00/18.00)	0.81	0.91	0.98 (0.62 – 1.53)
		A/G	141/93 (60.26/39.74)	120/80 (60.00/40.00)	0.965	-	0.98 (0.67 – 1.45)
IL4RA, rs1805010 A>G	G	AA/AG/GG	47/43/27 (40.17/36.75/23.08)	40/39/21 (40.00/39.00/21.00)	0.97	0.98	1.01 (0.68 – 1.48)
		A/G	137/97 (58.55/41.45)	119/81 (59.50/40.50)	0.918	-	1.04 (0.7 – 1.52)
TGFb1, rs1800469 T>C	T	CC/CT/TT	62/37/18 (52.99/31.62/15.38)	39/44/17 (39.00/44.00/17.00)	0.17	0.14	0.72 (0.47 – 1.12)
		C/T	161/73 (68.80/31.20)	122/78 (61.00/39.00)	0.11	-	0.71 (0.48 – 1.05)

CI, confidence interval; OR, odds ratio.

A significant difference between the COPD and control groups was identified for *IL24* (rs291107) (P = 0.004, OR =0.60 for basic allele test), only in non-smoking group (Table 4). The frequency of major T allele was significantly higher in patient group (63.25% vs. 49.00% in control, P = 0.004, OR =1.79, 95% CI =1.22 – 2.63). We have identified the association of *IL24* (rs291107) with COPD in non-smokers in the log-additive model (P_{adj} =0.0028, $P_{cor\text{-}FDR}$ =0.006, OR =0.49), and dominant models (P_{adj} =0.0082, $P_{cor\text{-}FDR}$ =0.013, OR =0.40). A significant interaction of the *IL24* (rs291107) and smoking status was detected in the regression analysis in the log-additive model ($P_{interact}$ =0.0068, OR =0.41 95% CI =0.21 – 0.80) and in the dominant model ($P_{interact}$ =0.028, OR =0.43, 95% CI =0.19 – 0.96).

Association of *IL4* (rs2070874) and COPD in non-smokers was established in basic allele test (P = 0.007, OR =0.47) and in the log-additive model (P_{adj} =0.0058, $P_{cor\text{-}FDR}$ =0.0108, OR =0.43).

The minor T allele and TT genotype of *PPBP* (rs352010) were also associated with COPD in non-smokers (P = 0.038, OR =1.59 for basic allele test) and (P_{adj} =0.027, $P_{cor\text{-}FDR}$ =0.033, OR =2.65 for recessive model) (Tables 4 and 5).

3.2. Association of inflammatory genes loci and quantitative phenotypes

We investigated the effect of the inflammatory genes polymorphisms and smoking index in smoking subjects (Table 7). The carriers of the GG genotype of *IL19* (rs2243193) (P = 0.04), CC genotype of *TGFb1* (rs1800469) (P = 0.042), and CC genotype of *FASLG* (rs763110) (P = 0.049) had a significantly higher smoking index. In the carriers of the TT genotype of *IL4* (rs2070874) (P = 0.0075), and TT genotype of *PPBP* (rs352010) (P = 0.0021), the smoking index was significantly lower.

We have studied the contribution of studied SNPs to the respiratory variables (FVC, FEV1, and FEV1/FVC ratio) (Table 7). Individuals that presented the AA genotype of *FAS* (rs1800682), TT genotype of *IL4* (rs2243250), AA genotype of *IL4RA* (rs1805010), and TT genotype of *FASLG* (rs763110) showed significant decrease in their FVC (Table 7). Meanwhile, carriers of the *PPBP* (rs352010)

Table 5. Significant association of inflammatory genes loci with COPD in smokers and non-smokers

Gene, SNP	Minor allele	*n*	Model	OR_{adj} (95% CI)	*P*-value	$P_{cor\text{-}FDR}$
Smokers						
IL19, rs2243193 A>G	A	1001	GG GA + AA Dominant	1.00 0.48 (0.37 – 0.64)	0.00001	7.5×10-5
			Log-additive	0.72 (0.62 – 0.86)	0.0003	0.00112
IL4, rs2243250 C>T	T	1001	CC + CT TT Recessive	1.00 1.85 (1.03 – 3.32)	0.036	0.041
			Log-additive	1.33 (1.06 – 1.66)	0.014	0.019
IL4, rs2070874 C>T	T	1001	CC + CT TT Recessive	1.00 0.08 (0.02 – 0.35)	0.00001	7.5×10-5
			Log-additive	0.66 (0.52 – 0.84)	0.0006	0.0018
PPBP, rs352010 T>C	T	1001	CC CT + TT Dominant	1.00 0.60 (0.45 – 0.79)	0.0002	0.001
			CC + CT TT Recessive	1.00 2.26 (1.38 – 3.69)	0.0008	0.002
			Log-additive	0.88 (0.72 – 1.07)	0.21	0.21
Non-smokers						
IL4, rs2070874 C>T	T	217	CC CT + TT Dominant	1.00 0.44 (0.23 – 0.85)	0.012	0.018
			Log-additive	0.43 (0.23 – 0.80)	0.0058	0.0108
IL24, rs291107 T>C	C	217	TT TC + CC Dominant	1.00 0.40 (0.20 – 0.80)	0.0082	0.013
			Log-additive	0.49 (0.30 – 0.79)	0.0028	0.006
PPBP, rs352010 T>C	T	217	CC + CT TT Recessive	1.00 2.65 (1.08 – 6.47)	0.027	0.033
			Log-additive	1.45 (0.96 – 2.19)	0.075	0.0803

P, *P* value for the likelihood ratio test for the regression model adjusted for covariates (age, gender, pack-year [in smokers], BMI); $P_{cor\text{-}FDR}$, *P* value after the FDR test. CI, confidence interval; OR, odds ratio.

CC genotype exhibited higher FVC value. Heterozygous genotypes of the *IL4RA* (rs1805010) and *FASLG* (rs763110) were associated with higher FEV1 value.

3.3. Analysis of gene-gene interaction of inflammatory gene loci and COPD risk in groups stratified by smoking status

We have identified gene-gene combinations significantly associated with the development of COPD in smokers and non-smokers. To identify significant interactions of functionally related inflammatory genes, five other cytokine gene loci: *IL12RB2* (rs3762317), *IL12B* (rs3212227), *IL12A* (rs568408), *IL12A* (rs2243115), and *IL13* (rs20541)[34] were included in the analysis in addition to the eleven gene loci studied in this work. SNPs of *IL12A* (rs568408, rs2243115), *IL12B* (rs3212227), *IL13* (rs20541), and *IL12RB2* (rs3762317) genes were analyzed for association with COPD in the same cohort of COPD patients and controls in our previous work[34]. As a result, statistically significant associations with COPD and *IL12A* (rs568408), *IL12A* (rs2243115), and *IL13* (rs20541) in the study group were identified[34].

Table 8 summarizes the gene-gene combinations significantly associated with COPD in groups stratified by smoking status with P_{FDR} <0.05 and OR >1.5 in the case of risk combinations, or OR <0.4 in the case of protective combinations. We have identified various patterns of gene-gene interactions associated with the development of

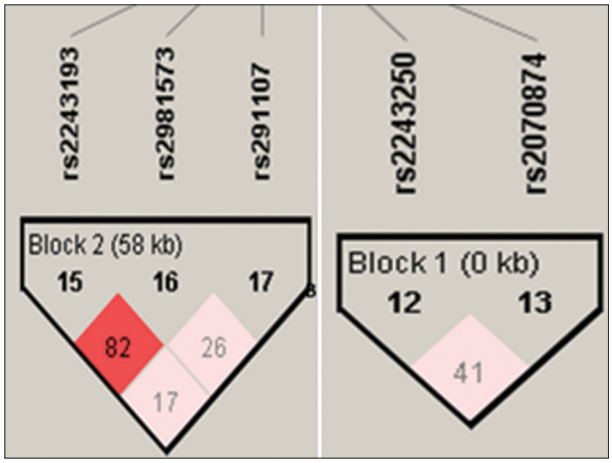

Figure 1. Visualization of linkage disequilibrium between the *IL19* (rs2243193), *IL20* (rs2981573), and *IL24* (rs291107) loci (A), and rs2243250 and rs2070874 of *IL4* gene (B). Linkage disequilibrium values are presented as D' value of normalized linkage disequilibrium coefficient (Lewontin's coefficient) and linkage disequilibrium block. Linkage disequilibrium between the *IL19* (rs2243193) and *IL20* (rs2981573) was D' =0.828, r^2 = 0.458; linkage disequilibrium between the rs2243250 and rs2070874 of *IL4* gene was D' =0.46, r^2 = 0.15.

Table 6. Association of *IL19*, *IL20*, and *IL4* gene loci haplotypes with COPD in smokers

Haplotype	Haplotype association with COPD in smokers (*n*=1001)		
	Frequency in patients/controls	OR (95% CI)	*P*-value
IL19 (rs2243193 A>G) - *IL20* (rs2981573 A>G)			
G-A	0.6113/0.5081	2.42 (1.84 – 3.18)	2.12×10-6
A-G	0.2839/0.2857	0.91 (0.74 – 1.12)	0.36
A-A	0.0787/0.1662	0.42 (0.30 – 0.58)	1.07×10-7
G-G	0.0261/0.04	0.59 (0.34 – 1.03)	0.063
P-value			0.00001
IL4 (rs2243250C>T) - (rs2070874C>T)			
C-C	0.6693/0.659	0.896 (0.74 – 1.14)	0.3711
T-C	0.1631/0.1135	1.44 (1.05 – 1.97)	0.024
T-T	0.1076/0.11	0.91 (0.65 – 1.27)	0.6002
C-T	0.0559/0.1175	0.52 (0.36 – 0.77)	0.001
P-value			0.00001

P, *P* value for the likelihood ratio test adjusted for covariates (gender, age, pack-years, BMI).

COPD in smokers and non-smokers. The number of gene-gene combinations significantly associated with COPD in smokers was significantly higher than in non-smokers, which is partly due to the predominance of smokers among the study groups.

The combination of A allele of *IL19* (rs2243193), C allele of *IL4* (rs2243250), and T allele of *PPBP* (rs352010) was the main component of the majority of protective gene-gene combinations associated with COPD in smokers. The highest risk of COPD was conferred by TT genotype of *PPBP* (rs352010) in combination with A allele of *FAS* (rs1800682) (OR =3.49).

While in non-smokers, the most commonly featured was C allele of *IL24* (rs291107) in protective patterns and T allele of *IL24* (rs291107) in risk combinations. The highest risk of COPD in non-smokers was conferred by A allele of *IL12RB2* (rs3762317) together with G allele of *IL12A* (rs2243115), C allele of *IL4* (rs2070874), and A allele of *IL4RA* (rs1805010) (OR =18.5).

The analysis on the gene-gene interaction of inflammatory gene loci established an association of *IL20* (rs2981573), *C5* (rs17611), *FASLG* (rs763110), *TGFb1* (rs1800469), and *FAS* (rs1800682) only in combinations with the *PPBP* (rs352010) and *IL19* (rs2243193) genes.

4. Discussion

To identify significant gene-gene and gene-environment interactions of functionally related inflammatory genes associated with the development of COPD, we analyzed the association of 11 SNPs of *IL19*, *IL20*, *IL24*, *PPBP*, *IL4*, *IL4RA*, C5, *FAS, FASLG*, and *TGFb1* genes in combination with previously studied SNPs of cytokines genes (*IL12A* [rs568408, rs2243115], *IL12B* [rs3212227], *IL13* [rs20541), *and IL12RB2* [rs3762317])[34] with COPD in groups stratified by smoking status in ethnic Tatar from Russia.

We established association of the *IL19* (rs2243193) with COPD in smokers. Moreover, *IL19* (rs2243193) was associated with smoking index. The strong level of LD between *IL19* (rs2243193) and *IL20* (rs2981573) was observed in our study, and haplotype G-A by *IL19* (rs2243193) and *IL20* (rs2981573) was a risk marker of COPD in smokers. The A allele of *IL19* (rs2243193) was observed in the majority of gene-gene interaction patterns associated with low COPD risk in smokers together with C allele of *IL4* (rs2243250) and T allele of *PPBP* (rs352010). IL19 is released by macrophages and monocytes after their activation by extracellular pathogens[35]. *IL19*, *IL20*, and *IL24* genes are located at chromosome 1q32.1, and are *IL10* family genes[14,15,35]. Upregulation of the IL19 results in the reduction of the inflammatory response by suppressing the expression of TNFA, IL6, and IL12[14,15,35]. Rong *et al.* showed that increased serum levels of IL19 were correlated with COPD progression[36]. The role of IL20 cytokine subfamily in COPD development is currently unclear.

Association of *IL24* (rs291107) with COPD in non-smokers has been observed. *IL24* codes for one of the members of the IL10 family cytokines[14,15,35]. The major T allele of *IL24* (rs291107) was a key component of the two

Table 7. Association of inflammatory gene loci with lung function parameters and smoking index

Gene, SNP	Model	Mean±S.E.	*P*-value	b (95% CI)
FVC				
FAS, rs1800682 A>G	AA AG + GG Dominant	50.97 (1.59) 55.59 (1.44)	0.041	0.00 4.62 (0.20 – 9.03)
IL4, rs2243250 C>T	CC + CT TT Recessive	56.34 (1.27) 46.33 (3.04)	0.017	0.00-10.01 (−18.18 – −1.89)
IL4RA, rs1805010 A>G	AA AG + GG Dominant	51.29 (1.78) 56.15 (1.35)	0.031	0.00 4.87 (0.45 – 9.28)
	AA + GG AG	52.00 (1.34) 57.66 (1.73)	0.009	0.00 5.66 (1.43 – 9.90)
PPBP, rs352010 T>C	CC CT + TT Dominant	55.15 (1.3) 49.36 (1.72)	0.011	0.00–5.79 (−10.23 – −1.35)
FASLG, rs763110 C>T	CC + CT TT Recessive	54.56 (1.19) 48.79 (2.35)	0.049	0.00–5.77 (−11.49 – −0.05)
	CC + TT CT	51.73 (1.29) 56.65 (1.85)	0.025	0.00 4.92 (0.63 – 9.20)
FEV1				
IL4RA, rs1805010 A>G	AA + GG AG	38.67 (1.07) 42.37 (1.25)	0.024	0.00 3.70 (0.49 – 6.92)
FASLG, rs763110 C>T	CC + TT CT	38.46 (0.99) 41.89 (1.36)	0.038	0.00 3.43 (0.19 – 6.67)
Smoking index in pack-years				
IL19, rs2243193 A>G	GG GA + AA Dominant	33.52 (1.27) 29.98 (1.12)	0.04	0.00–3.54 (−6.91 – −0.17)
IL4, rs2070874 C>T	CC + CT TT Recessive	31.72 (0.84) 18.7 (2.39)	0.0075	0.00–13.01 (−22.53 – −3.49)
TGFb1, rs1800469 T>C	CC CT + TT Dominant	32.89 (1.31) 29.43 (1.1)	0.042	0.00–3.46 (−9.67 – −0.51)
PPBP, rs352010 T>C	CC CT + TT Dominant	32.49 (1) 28.72 (1.36)	0.029	0.00–3.77 (−7.15 – −0.40)
	CC + CT TT Recessive	32.67 (0.95) 26.91 (1.52)	0.0021	0.00–5.76 (−9.43 – −2.09)
FASLG, rs763110 C>T	CC CT + TT Dominant	32.97 (1.31) 29.72 (1.04)	0.049	0.00–3.25 (−6.48 – −0.02)

b, beta coefficient; mean±S.E., mean and standard error; *P*, two-sided *P* values for linear regression analysis adjusting for covariates (age, gender, BMI, and smoking status for lung function parameters). FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity.

patterns associated with increased risk of COPD in non-smokers. While the C allele of *IL24* (rs291107) was observed in protective patterns. The study by Vargas-Alarcón *et al.* revealed the association of *IL24* functional SNPs with cardiovascular disease in the Mexican population[37].

We established the association of *PPBP* (rs352010) with COPD both in smokers and non-smokers. At the same time, the significance of the association in smokers was higher. TT genotype of *PPBP* (rs352010) was associated with increased risk of COPD and

Table 8. Gene-gene combinations of inflammatory gene loci associated with COPD in patients stratified by smoking status

Pattern	COPD patients	Healthy individuals	*P*-value	P_{FDR}	OR	95% CI
Smokers						
IL19 rs2243193*A + *IL4* rs2243250*C + *PPBP* rs352010*T	0.081	0.255	9.11e-11	2.77e-07	0.25	0.16 – 0.40
IL20 rs2981573*AA + *IL19* rs2243193*A	0.046	0.233	2.36e-10	2.39e-07	0.21	0.12 – 0.36
IL12A rs568408*G + *IL4* rs2243250*C + *PPBP* rs352010*TC	0.109	0.423	3.13e-10	1.90e-07	0.29	0.19 – 0.44
IL19 rs2243193*A + *IL4* rs2243250*C + *PPBP* rs352010*TC	0.071	0.288	4.86e-10	2.11e-07	0.25	0.15 – 0.40
IL12B rs3212227*A + *IL12A* rs2243115*T + *IL4* rs2243250*C + *PPBP* rs352010*T	0.121	0.328	1.36e-09	2.43e-07	0.28	0.18 – 0.43
IL19 rs2243193*A + *PPBP* rs352010*T + *FASLG* rs763110*C	0.086	0.238	1.38e-09	2.33e-07	0.30	0.19 – 0.45
IL19 rs2243193*A + *IL12A* rs2243115*T + *C5* rs17611*G + *PPBP* rs352010*T	0.075	0.228	2.03e-09	2.8e-07	0.27	0.17 – 0.43
IL12A rs2243115*T + *IL4* rs2243250*C + *TGFb1* rs1800469*C + *PPBP* rs352010*T	0.133	0.314	5.39e-09	3.81e-07	0.33	0.22 – 0.49
IL12A rs2243115*G + *FAS* rs1800682*AA	0.12	0.039	1.35e-05	6.46e-05	3.33	1.87 – 5.92
IL4 rs2070874*CC + *PPBP* rs352010*TT	0.10	0.034	4.57e-05	0.00017	3.14	1.74 – 5.65
FAS rs1800682*A + *PPBP* rs352010*TT	0.081	0.025	0.000134	0.0004	3.49	1.74 – 6.98
IL4 rs2243250*T + *IL24* rs291107*T	0.395	0.31	0.00615	0.011	1.56	1.19 – 1.97
Non-smokers						
IL12RB2 rs3762317*A + *IL12A* rs2243115*G + *IL4* rs2070874*C + *IL4*RA rs1805010*A	0.333	0.026	1.3e-06	0.022	18.5	4.09 – 83.63
IL12A rs2243115*G + *IL4*RA rs1805010*A + *IL24* rs291107*T	0.35	0.062	1.37e-05	0.022	8.04	2.83 – 22.84
IL12A rs2243115*T + *IL24* rs291107*C + *PPBP* rs352010*C	0.415	0.763	1.97e-05	0.017	0.22	0.10 – 0.45
IL19 rs2243193*A + *IL12A* rs2243115*T + *IL24* rs291107*C + *FASLG* rs763110*C	0.175	0.519	3.45e-05	0.017	0.19	0.08 – 0.44
IL12A rs2243115*T + *IL24* rs291107*C	0.492	0.802	7.74e-05	0.016	0.23	0.11 – 0.49
IL19 rs2243193*G + *IL12A* rs568408*A + *IL24* rs291107*T + *PPBP* rs352010*C	0.52	0.202	0.0002	0.023	4.26	1.92 – 9.41

P, *P* value for Fisher's test, $P_{cor\text{-}FDR}$, *P* value after the FDR test.

decreased smoking index in smokers. The data obtained indicate a possible interaction of *PPBP* (rs352010) with smoking. Moreover, CT and TT genotypes of *PPBP* (rs352010) were associated with low levels of FVC, which characterize the degree of airway obstruction in COPD and disease progression. Analysis of gene-gene interaction of inflammatory genes loci with COPD risk in groups stratified by smoking status determined that the T allele or CT genotype of *PPBP* (rs352010) gene was the key components of several combinations associated with low risk of COPD in smokers, while the TT genotype of *PPBP* (rs352010) along with the CC genotype of *IL4* (rs2070874) or with A allele of *FAS* (rs1800682) genes was associated with increased risk of COPD in smokers. *PPBP* codes for PPBP and is located at chromosome 4q13.3[18]. PPBP (CXCL7) has been identified as a biomarker in lung cancer[20]. Bdeir *et al.* demonstrated the role of CXCL7 to the pathogenesis of acute lung injury[38]. Information about the relationship between COPD and PPBP (CXCL7) is limited.

Significant associations of *IL4* (rs2243250, rs2070874) loci and COPD were observed. *IL4* (rs2243250) was associated with COPD only in smokers, and minor T allele and TT genotype were identified as COPD risk markers. Haplotype analysis demonstrated that frequency of T-C haplotype by the rs2243250 and rs2070874 of *IL4* gene was higher among smoking patients with COPD. Moreover, *IL4* (rs2243250) TT genotype was associated with decreased FVC value. Gene-gene interaction analysis showed that the C allele of *IL4* (rs2243250) was observed in several patterns associated with low risk of COPD in smokers and in one combination in non-smokers. *IL4* (rs2070874) was associated with COPD both in smokers and non-smokers; minor T allele and TT genotype were identified as protective markers. TT genotype of rs2070874 was associated with decreased smoking index. The CC genotype and C allele of *IL4* (rs2070874) were detected as component of gene-gene combination associated with COPD risk in smokers and non-smokers. The *IL4* gene is located at chromosome 5q31.1 together with *IL3*, *IL5*,

IL13, and *CSF2* genes[16]. Numerous reports associated *IL4* gene polymorphisms with allergic rhinitis[39], bronchial asthma[40], and COPD[41,42]. IL4 is produced by activated immune cells and binds to the IL4 receptor[16]. The human *IL4R* (*IL4RA*) is located at chromosome 16p12.1 and encodes the alpha chain of the IL4 receptor[43]. Analysis of gene-gene interaction of inflammatory genes loci to COPD risk showed that A allele of *IL4RA* (rs1805010) was a part of two informative combinations associated with COPD risk in non-smokers. These combinations included functionally related cytokine genes *IL12RB2* (rs3762317), *IL12A* (rs2243115), *IL4* (rs2070874), and *IL24* (rs291107). We detected significant association of *IL4RA* (rs1805010) to the respiratory variables in COPD patients; the FVC level was significantly lower in homozygous carriers of the more frequent A allele of the *IL4RA* (rs1805010) locus and heterozygous carriers had higher FEV1 level.

We also analyzed the *FASLG* (rs763110) and *FAS* (rs1800682) polymorphisms. Significantly low level of pulmonary function was detected in carriers of AA genotype of *FAS* (rs1800682) (for FVC) and TT genotype of *FASLG* (rs763110) (for FVC and FEV1). Moreover, the smoking index was significantly increased in carriers of CC genotype of *FASLG* (rs763110). *FAS* (rs1800682) A allele and AA genotype were associated with COPD risk in smokers only in combination with *IL12A* (rs2243115) and *PPBP* (rs352010) genes. Allele C of *FASLG* (rs763110) was a part of two informative combinations associated with low COPD risk in smokers and non-smokers. FAS is a member of the tumor necrosis factor receptor superfamily and plays a central role in regulation of programmed cell death[21]. The *FAS* gene is located at chromosome 10q23.31 and widely expressed in in most tissues, including lung[44]. FASLG is a member of the tumor necrosis factor superfamily[21]. The *FASLG* gene is located at chromosome 1q24.3 and expressed in activated immune cells[44]. Several functional SNPs of *FAS* and *FASLG* genes were associated with susceptibility to non-small cell lung cancer[45].

The *TGFb1* gene is located at chromosome 19q13.2 and encodes a secreted ligand of the TGF-β superfamily of proteins[23]. *TGFb1* is highly expressed in patients with COPD, asthma, and lung fibrosis[46]. Published meta-analyses failed to support the association of *TGFb1* gene loci with COPD, and demonstrated a race-specific and stage-dependent association between *TGFb1* loci and COPD[47,48]. We did not associate *TGFb1* (rs1800469) with COPD in smokers and non-smokers. At the same time, the C allele of *TGFb1* (rs1800469) was a part of significant protective gene-gene combination associated with a low risk of COPD in smokers. Moreover, *TGFb1* (rs1800469) was associated with smoking index.

We also analyzed the C5 (rs17611) polymorphism which are missense variant according to functional analysis in SNPinfo Web Server (https://snpinfo/niehs.nih.gov). *C5* gene is located at chromosome 9q33.2. Significant association with COPD was observed only for G allele of *C5* (rs17611) as part of informative protective gene-gene combinations in smokers together with A allele of *IL19* (rs2243193), T allele of *IL12A* (rs2243115), and T allele of *PPBC* (rs352010). C5 plays an important role in the inflammatory response, host homeostasis, and host defense[25]. Effects of C5 gene polymorphisms, including rs17611, have been extensively investigated in cardiovascular disease[49], artery atherosclerosis[50], bacterial meningitis[51], and rheumatoid arthritis[52].

There are several limitations in this study. The sample size of this study is limited and restricted to Tatar population from Russia. We will further expand the sample size to verify the results of this study. The strength of this study is the careful selection of the gene panel based on hypothesized biological pathways.

5. Conclusion

The single locus and multilocus analysis showed distinctive patterns of association of inflammatory genes loci with COPD in groups stratified by smoking status. To the best of our knowledge, this is the first study portraying the contribution of *IL19* (rs2243193), *IL24* (rs291107), and *PPBP* (rs352010) polymorphisms to COPD. Our study provides new insights into the interactions between the inflammatory genes and the environmental factors of COPD in the Tatar population from Russia.

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Conflict of interest

None of the authors have conflicts of interest to report with regard to this manuscript.

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Ethics approval and consent to participate

The study was approved by the Ethics Committee of Institute of Biochemistry and Genetics of Ufa Scientific Center of Russian Academy of Sciences (IBG USC RAS), Ufa, Russia (Ufa, Protocol No 17, December 7, 2010).

All procedures carried out in the study involving the participation of people are in compliance 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.

Consent for publication

Written informed consent for the publication of any associated data was obtained from each of the participants in the study.

Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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