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Analysis of Polymorphisms of Genes Associated with Immune Response and Tissue Remodeling in Occupational Chronic Bronchitis

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Abstract—The involvement of polymorphisms of genes encoding immune response-associated molecules (*LTA, TNFA, IL1B, ILRN, IL8, IL10, VDBP*), matrix metalloproteinases (*MMP1, MMP2, MMP3*, *MMP9*, *MMP12, ADAM33*), and tissue and serum inhibitors of metalloproteinases (*TIMP2, TIMP3, SERPINA1*, *SERPINA3*) in the predisposition to occupational chronic bronchitis was assessed by PCR–RFLP analysis in groups of patients ($n = 122$) and healthy workers ($n = 166$). It was found that occupational chronic bronchitis was associated with polymorphisms of *VDBP* ($P_{adj} = 0.00005$, $OR_{adj} = 2.06$), *MMP1* ($P_{adj} = 0.00002$, $OR_{adj} = 2.57$), *ADAM33* ($P_{adj} = 0.0004$, $OR_{adj} = 2.52$), and *IL8* ($P_{adj} = 0.0058$, $OR_{adj} = 2.87$). The most significant association was observed for the *VDBP* polymorphism *1296T>G*. The *VDBP* haplotype GC*1S by the loci *1296T>G* and *1307C>A* was an informative susceptibility marker ($P_{adj} = 0.0001$, $OR_{adj} = 2.60$, 95%CI (1.62–4.19)). There was also a significant interaction between the *VDBP* polymorphism *1307C>A* and the duration of occupational exposure to hazardous factors ($P_{interaction} = 0.02$). Apparently, the investigated polymorphisms of *VDBP, MMP1, ADAM33*, and *IL8* contribute to the genetic susceptibility to chronic bronchitis induced by dust and toxic agents.

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INTRODUCTION

Occupational chronic bronchitis occupational chronic bronchitis is progressive bronchial inflammation induced by industrial dust and aerosols of different chemical composition that develops as a bilateral diffuse dystrophic sclerosing process accompanied by airflow restriction, bronchospastic or dyskinetic impairment of bronchial motility, and progressive respiratory dysfunction [1].

Inflammation is considered central to the pathogenesis of chronic respiratory diseases. It can be induced by tobacco smoke and solid particles of polluted air, as well as by bacteria and viruses [2]. Cytokines are a group of polypeptide mediators involved in generating and regulating an organism's defense reactions. This group includes interferons, colony-stimulating factors, interleukins, chemokines, transforming growth factors, tumor necrosis factors, etc. [3]. Along with adhesion molecules, arachidonates, acute-phase proteins, and antibacterial peptides, cytokines are important signaling agents moderating the initiation, progression, and efficiency of the immune response in the lungs [4]. It was shown that polymorphisms of cytokine genes are associated with autoimmune, allergic, and infectious diseases, as well as with their clinical presentation and severity [5, 6].

Vitamin D-binding protein (VDBP) can bind not only vitamin D but also with extracellular actin and plays an important role in inflammatory reactions. VDBP enhances neutrophil chemotaxis to complement factors C5 and C5 des-Arg; it can also stimulate C5-induced chemotaxis of monocytes and fibroblasts. The VDBP-encoding gene comprises 13 exons and is mapped to chromosome 4 region q12. Three major VDBP isoforms identified previously by isoelectric focusing, GC*1F, GC*1S, and GC*, result from two single-nucleotide substitutions in *VDBP* exon 11 [7]. Under consideration of VDBP function as an inflammation mediator, these polymorphisms can be expected to have an important effect on the level of damage to lung parenchyma.

In the late 20th century, the view implicating a misbalance in the proteolysis–antiporteolysis system in the pathogenesis of lung emphysema and chronic bronchitis became widely acknowledged [2]. A lack of equilibrium between proteolytic enzymes and their inhibitors can under certain conditions result in excessive activity of proteolytic enzymes, causing the destruction of thin interalveolar walls and a confluence of alveoli into larger emphysematous cavities with a gradual reduction of the total respiratory area of the lungs [8]. Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes; in humans, it includes 26 zinc-containing endopeptidases [9]. MMPs regu-

late cell migration in the course of tissue regeneration, wound healing, and inflammation serving for leukocyte migration across blood vessel walls, and tissue remodeling processes. In the lungs, MMPs destroy extracellular matrix molecules, such as growth factors, chemokines, proteases, and cell adhesion molecules [10]. MMPs are secreted by several types of cells, among them alveolar macrophages and epithelial cells. MMP-encoding genes have been mapped to chromosomes 1, 8, 11, 14, 16, 20, and 22; most of them constitute a cluster on the long arm of chromosome 11: *MMP1, MMP3, MMP7, MMP8, MMP10, MMP12, MMP13,* and *MMP20* [11]. Disintegrin metalloprotease 33 (ADAM33) is a member of the ADAM (a disintegrin and metalloprotease), or adamolysin, family of membrane-bound proteolytic enzymes of the metzincin (Zn-dependent metalloproteinase) subclass. ADAMs participate in cell interactions and proteolysis [12]. *ADAM33* polymorphisms have been associated with accelerated lung function decline, as well as with chronic obstructive lung disease (COPD), respiratory hyperreactivity, and with aggravated inflammatory response in COPD. MMP and ADAM activity depends on the enzymes' interactions with tissue inhibitors of matrix metalloproteinases (TIMPs) and with serum inhibitors of proteolytic enzymes. The largest and the most widespread superfamily of serum protease inhibitors are serine protease inhibitors, or serpins. Serpins are involved in the regulation of coagulation and inflammation by inhibiting chemotrypsin-like serine proteases, such as thrombin, trypsin, and neutrophil elastase, as well as MMPs. Another group of inhibitors includes locally produced tissue inhibitors. The TIMP family includes the products of four genes: *TIMP1, TIMP2, TIMP3* and *TIMP4* [13]. They play a key role in maintaining the extracellular matrix homeostasis by regulating MMP activity. Polymorphisms of MMP-encoding genes and *ADAM33* were found to contribute to predisposition to bronchial asthma and COPD [14, 15].

The purpose of the present study was to analyze associations of occupational chronic bronchitis with polymorphisms of genes encoding immune response-related molecules (*LTA, TNFA, IL1B, ILRN, IL8, IL10, VDBP*), metalloproteinases (*MMP1, MMP2, MMP3, MMP9, MMP12, ADAM33*), as well as tissue and serum protease inhibitors (*TIMP2, TIMP3, SERPINA1, SERPINA3*).

MATERIALS AND METHODS

Subjects. The study was performed with DNA specimens obtained from unrelated individuals who were residents of Bashkortostan. The group of occupational chronic bronchitis patients included 122 individuals: 87 men (71.31%) and 35 women (28.69%) aged 55.69 ± 9.46 years (mean ± SD); the duration of exposure to occupational hazards was 21.70 ± 8.27 years. The occupational chronic bronchitis group included 37 smokers and ex-smokers (30.33%) and 85 nonsmokers (69.67%); the smoking index in smokers was 19.97 ± 12.32. The patients' ethnicity was distributed as follows: 45 Russians (36.89%) and 77 Tatars (63.11%). For all patients, the history included the data on the nature of the etiologic agent involved and their occupational activity, as well as the clinical features of the disease. Among the 122 occupational chronic bronchitis patients included in the study, 85 patients (69.67%) had dust bronchitis and 37 (30.33%) had toxic dust bronchitis. The patients' diagnosis was established in the Research Institute for Labor Medicine and Human Ecology (Ufa). The control group included 166 healthy workers with similarly long work experience in the same industries of Bashkortostan, among them 158 men (95.18%) and 8 women (4.82%) aged 46.01 ± 6.99 years; the duration of exposure to occupational hazards was 16.57 ± 6.77 years. There were 104 smokers and ex-smokers (62.65%) and 62 nonsmokers (37.35%); the smoking index in smokers was 18.62 ± 12.55. The control group included 84 Russians (50.60%) and 82 Tatars (49.40%).

The study design was approved by the Ethics Committee of the Institute of Biochemistry and Genetics. All subjects gave their informed consent for the use of their biological material in the study.

Genotyping. DNA was isolated from peripheral blood leukocytes using phenol–chloroform extraction. The study concerned functional polymorphisms of genes for which the products might be involved in the pathogenesis of respiratory diseases. Genes and loci were selected with consideration of their allele frequencies, the functional significance of polymorphisms and their effects on the gene expression levels, and protein activity and abundance. The association with occupational chronic bronchitis was analyzed for the following polymorphic loci: *MMP1* (*–1607G>GG*, rs1799750 and *–519A>G*, rs494379), *MMP2* (*–735C>T*, rs2285053), *MMP3* (*–11715A>6A*, rs35068180), *MMP9* (*–1562C>T*, rs3918242 and *2660A>G*, rs17576), *MMP12* (*–82A>G*, rs2276109), *ADAM33* (*12418A>G*, rs2280091 and *13491C>G*, rs2787094), *TIMP2* (*–418G>C*, rs8179090), *TIMP3* (*–1296T>C*, rs9619311), *SERPINA1* (*1237G>A*, rs2073333, *2313A>T*, rs17580 and *4628A>G*, rs28929474), *SERPINA3* (*25G>A,* rs4934), *VDBP* (*1296T>G*, rs7041 and *1307C>A*, rs4588), *LTA* (*252A>G*, rs909253), *TNFA* (*–308G>A*, rs1800629), *IL1B* (*3539C>T*, rs1143634 and *–511C>T*, rs16944), *ILRN* (*VNTR* intron 2, rs71941886), *IL8* (*–251T>A*, rs4073), *IL10* (*–627C>A*, rs1800872). Polymorphisms were genotyped by PCR and subsequent cleavage with restriction endonucleases *Mro*XI, *Kpn*I, *Hin*fI, *Tth*111I, *Sph*I, *Sma*I, *Pvu*II, *Nco*I, *Eco*88I, *Alu*BI, *Taq*I, *Hin*fI, *Hae*III, *Sty*I, *Bsp*19I, *Ama*87I, *Taq*I and *Rsa*I (SibEnzyme, Russia; Thermo Scientific, Germany) according to the manufacturers' instructions. PCR was performed according to the standard protocol using *Taq* DNA polymerase (Thermo Scientific, Germany) in a T100^{TM} thermal cycler (Bio-Rad Laboratories, Inc., United States). Primer sequences and

allele identification protocols were as described previously in [15–17]. The results of PCR–RFLP were evaluated by PAGE in 6–8% gels; gels were stained with 0.1 μg/mL ethidium bromide for 15 min and photographed using a UV transilluminator. Alleles were identified using a 100 bp ladder molecular weight marker (SibEnzyme).

Statistical analysis. For quantitative parameters, the mean values, standard deviations, and standard errors were calculated; the groups were compared using the nonparametric Mann–Whitney U-test. For categorical traits, the frequencies were compared using the Pearson's χ^2 test. Statistical analysis was performed using the BIOSTAT software (Primer of Biostatistics, version 4.03) [18]. The frequencies of rare alleles, the correspondence of genotype distributions to the Hardy–Weinberg equilibrium (χ^2), the significance of intergroup differences in allele and genotype frequencies for the χ^2 test of sample homogeneity, and the corresponding P-value were determined using the PLINK v. 1.07 program [19]; differences were considered significant at $P < 0.05$. The association of polymorphisms and haplotypes by linked loci was studied by log regression in different models (additive, dominant, and recessive) with an account of quantitative and binary traits introduced into the regression formula as independent variables (sex, age, ethnicity, smoking status, smoking index, and duration of employment). The significance of individual factors was tested using the *t*-statistics coefficient ($t = \beta/SE$, where β is a standardized equivalent of the regression coefficient for an independent variable and SE is the standard error) and the corresponding significance value P. The exponent of an individual regression coefficient (β) was interpreted as the odds ratio (OR) in the logistic model, and a 95% confidence interval (95%CI) was calculated. The significance of the resulting model, which accounted for all variables, was evaluated using the likelihood ratio test and its significance, P_{adj}. The best models were chosen using the Akaike's information criterion (AIC): for each significant locus ($P_{adj} < 0.05$), the model with the lowest AIC value was selected. To minimize type I error, Bonferroni correction for multiple comparisons was introduced by multiplying the *P* value by the number of polymorphic loci analyzed ($n = 20$) to obtain $P_{cor\text{-}Bf}$; alternatively, the Benjamini–Hochberg false discovery rate (FDR) was computed using the online calculator at http://www.sdmproject.com/utilities/?show=FDR to obtain the $P_{cor\text{-}FDR}$ value. Since both groups of subjects were ethnically heterogeneous, the Cochran–Mantel–Haenszel test and the Breslow–Day test, as well as the test for OR homogeneity (PLINK v. 1.07), were applied to evaluate occupational chronic bronchitis association with polymorphic alleles in stratified samples. Interactions between candidate gene polymorphisms and environmental factors (work duration, smoking status, and smoking index) were studied by means of regression analysis performed using the PLINK v. 1.07 and SNPStats program packages [19, 20]. Haplotype frequencies and the standardized linkage disequilibrium coefficient D', as well as intergroup differences in haplotype frequencies, were calculated with Haploview 4.2 [21].

RESULTS

At the first stage, we analyzed occupational chronic bronchitis associations with candidate genes using 22 polymorphic loci. The locus with a rare allele frequency of less than 0.01 (*SERPINA1*, rs28929474) and the one for which the allele distribution in the control group deviated significantly ($P < 0.001$) from the Hardy–Weinberg equilibrium (*MMP1*, rs1799750) were excluded from the further analysis. The allele and genotype frequency distributions in the occupational chronic bronchitis group and in the control group of healthy workers are shown in Tables 1 and 2. Significant differences between the groups were observed for three loci: *VDBP* (*1296T>G*), *MMP1* (*–519A>G*), and *ADAM33* (*13491C>G*). Further analysis including the calculation of OR and P values for occupational chronic bronchitis risk was performed for these individual loci (Table 3).

Association of Candidate Gene Polymorphisms with Occupational Chronic Bronchitis

Among occupational chronic bronchitis patients, there was a higher portion of heterozygotes and GG homozygotes by the rare allele of the *VDBP* polymorphism *1296T>G* ($P_{adj} = 0.00005$, $OR_{adj} = 2.06$ in the additive model; Table 3). This association remained significant after correction for multiple comparisons ($P_{cor\text{-}Bf} = 0.008$, $P_{cor\text{-}FDR} = 0.0022$).

The *VDBP* loci *1307C>A* and *1296T>G* were significantly linked, with the standardized linkage disequilibrium coefficient $D' = 0.75$. occupational chronic bronchitis was associated with the haplotypes C-G (GC*1S) and A-T (GC*2) by *1307C>A* and *1296T>G* ($P_{adj} = 0.0001$, $OR_{adj} = 2.60$, 95%CI 1.62–4.19 and $P_{adj} = 0.04$, $OR_{adj} = 1.76$, 95%CI 1.04–2.98). At the same time, the frequency of the TT genotype by *VDBP* (*1296T>G*) was significantly higher in healthy workers (63.86% vs. 40.16% in the occupational chronic bronchitis group; $P_{adj} = 0.000053$, OR = 0.37, 95%CI 0.23–0.61), while the C-T haplotype (GC*1F) by *VDBP* was associated with resistance to hazardous factors ($P_{adj} = 0.00001$, $OR_{adj} = 0.43$, 95%CI 0.35–0.67).

Heterozygotes and the rare allele *G* homozygotes by the *MMP1* polymorphism *–519A>G* were more common among occupational chronic bronchitis patients ($P_{adj} = 0.00002$, $OR_{adj} = 2.57$ in the dominant model, and $P_{adj} = 0.0001$, $OR_{adj} = 2.04$ in the additive model). These associations remained significant after correction for multiple comparisons (Table 3).

The group of occupational chronic bronchitis patients had a higher portion of heterozygotes by *ADAM33* (*13491C>G*) (50.0% vs. 35.54% in the con-

Table 1. Frequency distribution of polymorphic variants of immune response-associated genes in groups of OBC patients and healthy workers

Gene, polymorphic locus	Rare allele	Genotypes, alleles	Occupational chronic bronchitis patients, abs. (%)	Healthy workers, abs. (%)	χ^2	*P*
IL10 −627C>A rs1800872	*A*	*AA/AC/CC*	9/58/55 (7.38/47.54/45.08)	12/67/87 (7.23/40.36/52.41)	1.60	0.45
		A/C	76/168 (31.15/68.85)	91/241 (27.41/72.59)	0.95	0.33
IL1B 3539C>T rs1143634	*T*	*TT/TC/CC*	7/41/74 (5.74/33.61/60.66)	10/53/103 (6.02/31.93/62.05)	0.09	0.95
		T/C	55/189 (22.54/77.46)	73/259 (21.99/78.01)	0.025	0.87
ILRN VNTR intron 2 rs71941886	*2*	*22/24/44*	5/44/73 (4.10/36.07/59.84)	11/58/97 (6.63/34.94/58.43)	0.86	0.65
		2/4	54/190 (22.13/77.87)	80/252 (24.10/75.90)	0.30	0.58
VDBP 1307C>A rs4588	*A*	*AA/AC/CC*	10/45/67 (8.20/36.89/54.92)	9/60/97 (5.42/36.14/58.43)	0.98	0.61
		A/C	65/179 (26.64/73.36)	78/254 (23.49/76.51)	0.75	0.39
***VDBP 1296T>G* rs7041**	***G***	***GG/GT/TT***	**20/53/49 (16.39/43.44/40.16)**	**12/48/106 (7.23/28.92/63.86)**	**16.88**	**0.00022**
		G/T	**93/151 (38.11/61.89)**	**72/260 (21.69/78.31)**	**18.57**	**0.000016**
IL8 −251T>A rs4073	*A*	*AA/AT/TT*	29/62/31 (23.77/50.82/25.41)	28/82/56 (16.87/49.40/33.73)	3.34	0.19
		A/T	120/124 (49.18/50.82)	138/194 (41.57/58.43)	3.30	0.07
LTA 252A>G rs909253	*G*	*GG/GA/AA*	8/55/59 (6.56/45.08/48.36)	8/66/92 (4.82/39.76/55.42)	1.53	0.47
		G/A	71/173 (29.10/70.90)	82/250 (24.70/75.30)	1.40	0.24
TNFA −308G>A rs1800629	*A*	*AA/AG/GG*	1/30/91 (0.82/24.59/74.59)	3/29/134 (1.81/17.47/80.72)	2.57	0.28
		A/G	32/212 (13.11/86.89)	35/297 (10.54/89.46)	0.91	0.34

Here and in Table 2, loci for which significant differences were detected are shown in bold. *P* is the significance level.

trol group, $P_{adj} = 0.0004$, $OR_{adj} = 2.52$). The association of this locus with occupational chronic bronchitis remained significant after correction for multiple comparisons ($P_{cor\text{-}Bf} = 0.008$, $P_{cor\text{-}FDR} = 0.0022$).

Occupational chronic bronchitis was also associated with the *IL8* locus *−251T>A* ($P_{adj} = 0.0058$, $OR_{adj} = 2.87$; $P_{cor\text{-}Bf} = 0.116$, $P_{cor\text{-}FDR} = 0.028$).

Association of Candidate Genes with Occupational Chronic Bronchitis with Consideration of Patient Ethnicity

The Cochran–Mantel–Haenszel test and the Breslow–Day test for OR homogeneity in stratified samples showed that OR values for *MMP1* (*−519A>G*) did not differ between Russians and Tatars. This locus was significantly associated with occupational chronic bronchitis in Tatars ($P_{adj} = 0.0003$, $P_{cor\text{-}Bf} = 0.006$, $OR_{adj} = 3.07$ in the additive model; Table 4).

For the *VDBP* polymorphism *1296T>G*, the association with occupational chronic bronchitis differed significantly between the groups of Russians and Tatars ($P_{CMH} = 0.00000887$, $OR_{CMH} = 2.335$, 95%CI 1.59–3.417 in the Cochran–Mantel–Haenszel test; $P_{BD} = 0.04602$ in the Breslow–Day test, and $P = 0.052$ in the test for OR homogeneity in stratified samples). In Tatars, the most significant association with occupational chronic bronchitis was observed for the *1296T>G* locus of *VDBP* ($P_{adj} = 0.0001$, $P_{cor\text{-}Bf} = 0.002$, $OR_{adj} = 3.93$ in the additive model) and the C-G (GC*1S) haplotype by *1307C>A* and *1296T>G* poly-

Table 2. Frequency distribution of polymorphic variants of metalloproteinase and protease inhibitor genes in groups of occupational chronic bronchitis patients and healthy workers

Gene, polymorphic locus	Rare allele	Genotypes, alleles	Occupational chronic bronchitis patients, abs. (%)	Healthy workers, abs. (%)	χ^2	*P*
MMP1 *−519A>G* rs494379	*G*	*GG/GA/AA*	12/45/65 (9.84/36.89/53.28)	10/33/123 (6.02/19.88/74.10)	13.51	0.001
		G/A	69/175 (28.28/71.72)	53/279 (15.96/84.04)	12.78	0.0004
MMP3 *−11715A>6A* rs35068180	*5A*	*5A5A/5A6A/6AA*	0/5/117 (0/4.10/95.90)	0/9/157 (0/5.42/94.58)	0.06	0.81
		5A/6A	5/239 (2.05/97.95)	9/323 (2.71/97.29)	0.26	0.61
MMP12 *−82A>G* rs2276109	*G*	*GG/AG/AA*	0/29/93 (0/23.77/76.23)	0/43/123 (0/25.90/74.10)	0.08	0.78
		G/A	29/215 (11.89/88.11)	43/289 (12.95/87.05)	0.15	0.70
SERPINA1 *1237G>A* rs2073333	*A*	*AA/AG/GG*	0/19/103 (0/15.57/84.43)	0/19/147 (0/11.45/88.55)	0.72	0.40
		A/G	19/225 (7.79/92.21)	19/313 (5.72/94.28)	0.97	0.32
SERPINA1 *2313A>T* rs17580	*T*	*TT/TA/AA*	0/2/120 (0/1.64/98.36)	0/5/161 (0/3.01/96.99)	0.13	0.72
		T/A	2/242 (0.82/99.18)	5/327 (1.51/98.49)	0.55	0.46
SERPINA3 *25G>A* rs4934	*A*	*AA/AG/GG*	22/58/42 (18.03/47.54/34.43)	43/78/45 (25.90/46.99/27.11)	3.18	0.20
		A/G	102/142 (41.80/58.20)	164/168 (49.40/50.60)	3.26	0.07
MMP2 *−735C>T* rs2285053	*T*	*TT/TC/CC*	0/27/95 (0/22.13/77.87)	0/49/117 (0/29.52/70.48)	1.61	0.20
		T/C	27/217 (11.07/88.93)	49/283 (14.76/85.24)	1.68	0.20
TIMP2 *−418G>C* rs8179090	*C*	*CC/CG/GG*	0/4/118 (0/3.28/96.72)	0/8/158 (0/4.82/95.18)	0.12	0.73
		C/G	4/240 (1.64/98.36)	8/324 (2.41/97.59)	0.41	0.52
ADAM33 ***13491C>G*** **rs2787094**	***C***	***CC/CG/GG***	**27/61/34 (22.13/50.00/27.87)**	**50/59/57 (30.12/35.54/34.34)**	**6.14**	**0.05**
		C/G	115/129 (47.13/52.87)	159/173 (47.89/52.11)	0.03	0.86
MMP9 *−1562C>T* rs3918242	*T*	*TT/TC/CC*	2/26/94 (1.64/21.31/77.05)	3/33/130 (1.81/19.88/78.31)	0.10	0.95
		T/C	30/214 (12.30/87.70)	39/293 (11.75/88.25)	0.04	0.84
MMP9 *2660A>G* rs17576	*G*	*GG/GA/AA*	4/39/79 (3.28/31.97/64.75)	10/39/117 (6.02/23.49/70.48)	3.29	0.19
		G/A	47/197 (19.26/80.74)	59/273 (17.77/82.23)	0.21	0.65
TIMP3 *−1296T>C* rs9619311	*C*	*CC/CT/TT*	33/36/53 (27.05/29.51/43.44)	31/57/78 (18.67/34.34/46.99)	2.92	0.23
		C/T	102/142 (41.80/58.20)	119/213 (35.84/64.16)	2.11	0.15

Table 3. Association of occupational chronic bronchitis with candidate gene polymorphisms

Gene, polymorphic locus SNP	Rare allele	Genotypes (model)	P	OR (CI 95%)	P_{adj}	OR_{adj} (CI 95%)	$P_{cor\text{-}Bf}$	$P_{cor\text{-}FDR}$
VDBP 1296T>G rs7041	*G*	*GT* vs. *GG, TT*	0.0005	2.99 (1.85–4.82)	0.0004	2.76 (1.55–3.82)	0.008	0.0022
		GG, GT vs. *TT* (dominant)	0.000067	2.63 (1.62–4.25)	0.000053	2.67 (1.50–4.05)	0.0011	0.00068
		TT (0) *GT* (1) *GG*(2) (additive)	0.000055	2.05 (1.43–2.93)	0.00005	2.06 (1.36–3.13)	0.001	0.00068
MMP1 −519A>G rs494379	*G*	*GA* vs. *GG, AA*	0.003	2.35 (1.38–4.00)	0.0001	2.52 (1.36–4.69)	0.002	0.00078
		GG, GA vs. *AA* (dominant)	0.0002	2.50 (1.52–4.12)	0.00002	2.57 (1.47–4.49)	0.0004	0.00068
		AA (0) *GA* (1) *GG*(2) (additive)	0.001	1.87 (1.28–2.73)	0.0001	2.04 (1.48–3.81)	0.002	0.00078
ADAM33 13491C>G rs2787094	*C*	*CG* vs. *CC, GG*	0.014	1.81 (1.13–2.92)	0.0004	2.52 (1.40–4.52)	0.008	0.0022
IL8 −251T>A rs4073	*A*	*AA, AT* vs. *TT* (dominant)	0.014	2.07 (1.14–3.76)	0.0058	2.87 (1.32–6.22)	0.116	0.028

P, significance in the likelihood ratio test for the log-regression model; P_{adj}, significance in the test accounting for the effects of age, sex, ethnicity, smoking status and index, and work duration; OR, odds ratio; OR_{adj}, odds ratio after accounting for all factors; CI 95%, 95% confidence interval for OR; $P_{cor\text{-}Bf}$, significance after Bonferroni correction; $P_{cor\text{-}FDR}$, significance after FDR correction; the additive model for the rare allele dose implies an increase in the rare allele dose in the following sequence: frequent allele homozygote (0) — heterozygote (1) — rare allele homozygote (2).

morphisms of *VDBP* (P_{adj} = 0.00001, OR_{adj} = 4.86, 95%CI 2.46– 9.62).

In the group of Tatars, occupational chronic bronchitis was found to be associated with the *IL8* polymorphism *−251T>A* (P_{adj} = 0.02, $P_{cor\text{-}Bf}$ = 0.4, OR_{adj} = 2.70 in the recessive model).

For the *TIMP3* polymorphism *−1296T>C*, the significance values in the Breslow–Day test and the OR homogeneity test were P_{BD} = 0.053, P_{hom} = 0.053, respectively; in ethnically differentiated groups, the rare *C* allele of *TIMP3* (*−1296T>C*) was associated with occupational chronic bronchitis risk in Russians (P_{adj} = 0.006, OR_{adj} = 2.16). However, after correction for multiple comparisons was introduced, the association was no longer significant ($P_{cor\text{-}Bf}$ = 0.12).

The association between occupational chronic bronchitis and *ADAM33* (*13491C>G*) was confirmed only in Russians (P_{adj} = 0.0033, $P_{cor\text{-}Bf}$ = 0.066, OR_{adj} = 3.30 for the heterozygous genotype). The portion of homozygotes by the rare *C* allele was significantly higher in the healthy control group (P_{adj} = 0.007, OR_{adj} = 0.26).

Interactions of Environmental and Genetic Factors in Occupational Chronic Bronchitis Pathogenesis

The duration of employment in hazardous industries is an important environmental factor contributing to the risk of professional diseases, as it indicates the extent of subject's exposure to certain risk factors; in particular, it plays a central role in occupational chronic bronchitis pathogenesis. The polymorphisms of *IL1RN* (*VNTR*) and *VDBP* (*1307C>A*) showed a significant interaction with work duration ($P_{interaction}$ = 0.03 and 0.02, respectively). At the same time, none of the loci studied showed a significant interaction with smoking status or smoking index.

Genotype-by-environment interactions were also analyzed by comparing the OR values obtained for different candidate genes in groups of subjects differing by smoking status. The significant results of association analysis in groups with different smoking status are shown in Table 5. In particular, the association of *VDBP* (*1296T>G*) with occupational chronic bronchitis in the additive model was more significant in nonsmokers (P_{adj} = 0.0008, $P_{cor\text{-}Bf}$ = 0.016, OR_{adj} = 2.46). In both groups, the C-G (*1S) haplotype by *VDBP* loci *1307C>A* and *1296T>G* was a marker of an increased occupational chronic bronchitis risk, whereas the C-T (*1F) haplotype was an occupational chronic bronchitis resistance marker (Table 5). Both in smokers and nonsmokers, the portion of homozygous and heterozygous carriers of the rare *G* allele of *MMP1* (*−519A>G*) was significantly higher among occupational chronic bronchitis patients (P_{adj} = 0.02, $P_{cor\text{-}Bf}$ = 0.4, OR_{adj} = 2.57 and P_{adj} = 0.006, $P_{cor\text{-}Bf}$ = 0.12, OR_{adj} = 2.66, respectively). The heterozygous genotype by the *ADAM33* polymorphism *13491C>G* was a marker of occupational chronic bronchitis risk in smokers (P_{adj} = 0.02, $P_{cor\text{-}Bf}$ = 0.4, OR = 2.80). We also found that the *MMP9* polymorphism *2660A>G* was associated with occupational chronic bronchitis in nonsmoking workers (P_{adj} = 0.003, $P_{cor\text{-}Bf}$ = 0.06, OR_{adj} = 4.14).

Table 4. Association of occupational chronic bronchitis with candidate gene polymorphisms in different ethnic groups

Ethnic group	Gene, polymorphic locus	Rare allele	Genotypes, alleles, models	Occupational chronic bronchitis patients, abs. (%)	Control group, abs. (%)	P_{adj}	OR_{adj} (CI 95%)
Russians	*TIMP3 −1296T>C* rs9619311	*C*	*CC/CT/TT*	16/16/13 (35.56/35.56/28.89)	15/28/41 (17.86/33.33/48.81)	0.04	–
			C/T	48/42 (53.33/46.67)	58/110 (34.52/65.48)	0.006	2.16 (1.24–3.78)
			CC vs. *CT, TT*	–	–	0.03	2.54 (1.11–5.80)
			TT (0) *CT*(1) *CC* (2)	–	–	0.01	1.83 (1.15–2.93)
	ADAM33 13491C>G rs2787094	*C*	*CC/CG/GG*	5/26/14 (11.11/57.78/31.11)	25/31/28 (29.76/36.90/33.33)	0.02	–
			C/G	36/54 (40.00/60.00)	81/87 (48.21/51.79)	0.26	–
			CG vs. *CC, GG*	–	–	0.0033	3.30 (1.45–7.51)
			CC vs. *CG, GG*	–	–	0.007	0.26 (0.09–0.77)
Tatars	*VDBP 1296T>G* rs7041	*G*	*GG/GT/TT*	13/33/31 (16.88/42.86/40.26)	2/21/59 (2.44/25.61/71.95)	0.0001	
			G/T	59/95 (38.31/61.69)	25/139 (15.24/84.76)	0.0005	3.45 (1.95–6.11)
			TT (0) *GT* (1) *GG*(2)	–	–	0.0001	3.93 (1.83–8.46)
	MMP1 −519A>G rs494379	*G*	*GG/GA/AA*	9/27/41 (11.69/35.06/53.25)	5/14/63 (6.10/17.07/76.83)	0.008	
			G/A	45/109 (29.22/70.78)	24/140 (14.63/85.37)	0.006	2.40 (1.33–4.35)
			AA (0) *GA* (1) *GG*(2)	–	–	0.0003	3.07 (1.23–5.47)
	IL8 −251T>A rs4073	*A*	*AA/ AT/TT*	21/37/19 (27.27/48.05/24.68)	31/81/47 (19.50/50.94/29.56)	0.04	–
			A/T	79/75 (51.30/48.70)	143/175 (44.97/55.03)	0.23	–
			AA vs. *AT, TT*	–	–	0.02	2.70 (1.18–6.19)

Table 5. Association of occupational chronic bronchitis with candidate gene polymorphisms in groups differentiated by smoking status

Locus	Model, genotypes, haplotypes	Association with occupational chronic bronchitis in smokers ($N = 141$)		Association with occupational chronic bronchitis in nonsmokers ($N = 147$)	
		P_{adj}	OR_{adj} (CI 95%)	P_{adj}	OR_{adj} (CI 95%)
VDBP (1296T>G)	*TT* (0) *TG* (1) *GG* (2) (additive)	0.1	–	0.0008	2.46 (1.41–4.30)
VDBP (1296T>G)	*GG, TG* vs. *TT*	0.06	–	0.0005	3.55 (1.69–7.43)
VDBP (1307C>A and *1296T>G)*	C-T (*1F)	0.002	0.41 (0.24–0.72)	0.0008	0.44 (0.27–0.71)
VDBP (1307C>A and *1296T>G)*	C-G (*1S)	0.006	2.44 (1.30–4.57)	0.0009	2.84 (1.55–5.20)
VDBP (1307C>A and *1296T>G)*	A-T (*2)	0.04	2.09 (1.06–4.15)	0.14	1.63 (0.86–3.07)
MMP1 (–519A>G)	*AG*	0.05	2.40 (1.00–5.74)	0.02	2.67 (1.15–6.19)
MMP1 (–519A>G)	*GG, AG* vs. *AA* (dominant)	0.04	2.43 (1.05–5.60)	0.006	2.85 (1.32–6.15)
ADAM33 (13491C>G)	*CG*	0.02	2.80 (1.19–6.57)	0.09	–
MMP9 (2660A>G)	*AG*	–	–	0.003	4.14 (1.53–11.18)

P_{adj}, significance in the likelihood ratio test for a log regression model accounting for age, sex, ethnicity, and work experience; OR_{adj}, odds ratio after accounting for all factors; *N*, the number of individuals for which the association was studied.

DISCUSSION

We analyzed the association of occupational chronic bronchitis with polymorphisms of genes encoding immune response-associated molecules (*LTA, TNFA, IL1B, ILRN, IL8, IL10, VDBP*), metalloproteinases (*MMP1, MMP2, MMP3, MMP9, MMP12, ADAM33*), and tissue and serum metalloproteinase inhibitors (*TIMP2, TIMP3, SERPINA1, SERPINA3*). We also studied the interaction of these candidate gene polymorphisms with smoking and the duration of work in hazardous environments.

The most significant association with occupational chronic bronchitis was observed for the *VDBP* polymorphism *1296T>G*. This association was further confirmed in the subgroups of Tatars and nonsmokers. The C-G (GC*1S) haplotype by *VDBP* loci *1307C>A* and *1296T>G* was an informative marker of occupational chronic bronchitis risk. In addition, *1307C>A* showed a significant interaction with the duration of exposure to occupational hazards. Our data suggest that *VDBP* polymorphisms constitute an important component of genetic susceptibility to respiratory diseases induced by the toxic agents and dust present in industrial environments.

VDBP acts as an immunomodulating agent, affecting macrophage activation and neutrophil chemotaxis [22], and considerably affects the level of inflammatory damage to lung parenchyma. Analysis of the VDBP role in COPD performed in several European and Asian populations found that the GC*1F variant was associated with COPD in the Japanese and the Chinese, which is probably related to its high frequency in Mongoloid populations [7, 23–25]. At the same time, the frequency distribution of *VDBP* alleles and genotypes in a Caucasian population of Canada suggested that *VDBP* variants did not affect the risk of COPD in this population [7]. The GC*1F variant was associated with chronic bronchial hypersecretion in patients from Iceland [24], while *VDBP* polymorphisms in Belgian COPD patients were associated with disease severity [26]. *VDBP* variants were found to correlate with different COPD phenotypes [27]. In our previous study, polymorphic *VDBP* loci were shown to be associated with COPD in Tatars [16].

Our data indicate an association between occupational chronic bronchitis and the *MMP1* locus – *519A>G*, which was, however, confirmed only in Tatars; this SNP was also associated with occupational chronic bronchitis in nonsmoking workers.

MMP1 is the enzyme that specifically hydrolyzes interstitial collagens type I, II,and III, the major fibrillar components of the extracellular matrix in the lungs. Under normal conditions, MMP1 is produced in very small amounts, but certain cytokines and growth factors can upregulate its expression significantly; in particular, this occurs in different pathologies [28]. It is known that functional polymorphisms of the *MMP1* promoter considerably affect the collagenase 1 gene transcription level. The *MMP1* variant *–755G>T* generates a potential binding site for the AP-1 transcription factor; the *MMP1* (*–519A>G*) site analyzed in our work is located in close vicinity to *MMP1* (*–755G>T*) and lies within the AP-1 binding area [28, 29]. According to Imai et al., significant amounts of MMP1 were expressed and secreted in lung tissues of patients with emphysema but not of healthy subjects [30]. Fomina and Kuz'mina reported that the *MMP1* polymorphism *–1607G>GG* was associated with professional respiratory diseases [31].

Our results indicate a significant association between the *13491C>G* polymorphism of *ADAM33* and the risk of occupational chronic bronchitis. An analysis performed in groups stratified by ethnicity and by smoking status showed that *13491C>G* was associated with occupational chronic bronchitis in the subgroup of Russians and in smokers only. *ADAM33* is located on

chromosome 20p13; it comprises 22 exons and is expressed primarily in lung fibroblasts in bronchial muscle cells [12]. In addition to augmenting inflammatory response and proteolysis in the lungs, *ADAM33* is also involved in angiogenesis [32]. Several studies have previously reported that *ADAM33* polymorphisms were associated with COPD and bronchial asthma [12, 32–34]; our own data confirmed such an association in COPD patients from Bashkortostan [15]. Our results suggest that *ADAM33* is an important factor involved in the pathogenesis of respiratory diseases induced by smoking or industrial dust and aerosols.

For *IL8* (*–251T>A*), an association with occupational chronic bronchitis was observed in the ethnic group of Tatars. IL8 is a proinflammatory cytokine acting as a selective neutrophil chemoattractor [8, 35]. It is secreted by macrophages, neutrophils, and epithelial cells of respiratory pathways. *IL8* is located to the chromosome 4 region 4q12-13; it is known to contain several SNPs, including the *–251A>T* polymorphism in the promoter region [36]. A British study showed that the rare *A* allele was associated with increased IL8 production, which was a risk factor for bronchiolitis in children with viral infections [36, 37]. IL8 levels were significantly increased in the sputum of COPD patients [35]. In addition, increased IL8 concentrations in bronchoalveolar lavage correlated with decreased FEV1 values [8]. The *IL8* (*–251T>A*) variant was also associated with an increased risk of idiopathic lung fibrosis [38]. A study from China showed that *IL8* variants can contribute significantly to COPD risk in residents of regions with high air pollution levels [39].

An analysis of *TIMP3* (*–1296T>C*) allele and genotype distributions in subgroups stratified by ethnicity showed that the frequency of the *C* allele was increased in Russian occupational chronic bronchitis patients. Among other members of the TIMP family, TIMP3 inhibits a particularly wide range of proteolytic enzymes [13]. TIMP3 also regulates the TGFβ1 and TNFα release in response to tissue damage or in the course inflammation [40]. *TIMP3* is located on chromosome 22 at site 22q12.1-q13.2; the (*–1296T>C*) polymorphisms in the gene promoter is thought to affect the level of *TIMP3* transcription [13]. It was reported that TIMP3 contributes to the resolution of acute inflammatory damage to lung tissue [41]; the data from our previous studies suggested that *TIMP3* locus was associated with COPD and with lingering childhood pneumonia [15, 42].

Thus, the risk of occupational chronic bronchitis in workers depends not only on the composition of industrial aerosols and the duration of exposure to occupational hazards but also on individual susceptibility: the disease primarily affects individuals with a certain genetic constitution and depends on the interaction between genetic and environmental factors. Our study revealed associations between the risk of occupational chronic bronchitis and polymorphisms of *VDBP, MMP1, ADAM33*, and *IL8*, as well as pathogenetically significant interactions between genetic loci and the duration of occupational exposure to hazardous factors.

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