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Research paper

Associations of the NRF2/KEAP1 pathway and antioxidant defense gene polymorphisms with chronic obstructive pulmonary disease

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ABSTRACT

Background and objective: Chronic obstructive pulmonary disease (COPD) is a complex chronic inflammatory disease of the respiratory system affecting primarily distal respiratory pathways and lung parenchyma. This work was designed as a case-control study aimed at investigating the association of the NRF2/KEAP1 signaling system, and antioxidant defense gene polymorphisms with COPD in population from Russia.

Methods: Ten SNPs: *NFE2L2* (rs35652124), *KEAP1* (rs1048290), *MPO* (rs2333227), *PRNP* (rs1799990), *PTGR1* (rs2273788), *HSPA1A* (rs1008438), *TXNRD2* (rs1139793), *GSR* (rs1002149), *SIRT2* (rs10410544), and *PTGS1* (rs1330344) were genotyped by the real-time polymerase chain reaction (TaqMan assays) in a case-control study (425 COPD patients and 457 controls, from the same region of Russia, representatives of Tatar population). Logistic regression was used to detect the association of SNPs in different models. Linear regression analyses were performed to estimate the relationship between SNPs and lung function parameters and smoking pack-years.

The results: In our population, a significant associations of *KEAP1* (rs1048290) (P = 0.0015, OR = 0.72 in additive model), *HSPA1A* (rs1008438) (P = 0.006, OR = 2.26 in recessive model), *GSR* (rs1002149) (P = 0.037, OR = 1.31 in additive model) with COPD were revealed. *NFE2L2* (rs35652124), *PRNP* (rs1799990), and *HSPA1A* (rs1008438) were significantly associated with COPD only in smokers. In nonsmokers, significant association was established for *GSR* (rs1002149). *KEAP1* (rs1048290) was associated with COPD in both groups.

The relationship between *KEAP1* (rs1048290), *NFE2L2* (rs35652124), and *HSPA1A* (rs1008438) and smoking pack-years was found (P = 0.005, P = 0.0028, P = 0.015). A significant genotype-dependent variation of forced vital capacity and forced expiratory volume in 1 s was observed for *SIRT2* (rs10410544) (P = 0.04), *NFE2L2* (rs35652124) (P = 0.028), and *PRNP* (rs1799990) (P = 0.044).

1. Introduction

Chronic obstructive pulmonary disease (COPD) is one of the most widespread diseases worldwide; COPD is one of the most important causes of death in most countries (Rabe et al., 2007). According to the current definition, COPD is a multifactorial heterogeneous chronic inflammatory disease of the respiratory system predominantly affecting the lower respiratory pathways and the lung parenchyma (Rabe et al., 2007; Vestbo et al., 2013). Tobacco smoking is the principal risk factor of COPD; however, only 10–20% of smokers develop signs of

Abbreviations: COPD, chronic obstructive pulmonary disease; ICD 10, the International Classification of Diseases tenth revision; GOLD, The Global Initiative for Chronic Obstructive Lung Disease (Global Strategy for the Diagnosis, Management and Prevention of Chronic Obstructive Pulmonary Disease; BMI, body mass index; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; Post, post-bronchodilator; PY, pack-years, smoking index; OR, odds ratio; 95% CI, 95% confidence interval; FDR, false discovery rate; MAF, minor allele frequencies; HWE, Hardy-Weinberg-Equilibrium

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respiratory obstruction. Presumably, exposure to tobacco smoke induces pathological processes in the lungs, leading to systemic inflammatory response and endothelial dysfunction (Rabe et al., 2007; Vestbo et al., 2013; Barnes, 2017).

In the recent years, genetic mechanisms that underlie the development of COPD have become a subject of extensive research. Genome-wide association studies (GWAS) have identified several chromosome loci associated with COPD: 15q25.1 (*CHRNA3*, *CHRNA5*, *IREB2*, *PSMA4*, *HYKK*), 4q31.21 (*HHIP*), 4q22.1 (*FAM13A*, *GPRIN3*), and 19q13 (*EGLN2*, *RAB4B*) (Boueiz et al., 2017; Sakornsakolpat et al., 2018).

Increased oxidative stress is a key mechanism in the pathogenesis of COPD (Kirkham and Barnes, 2013; Barnes, 2017). The levels of oxidative stress biomarkers are increased in the exhaled breath condensates, in the blood serum, and in the systemic circulation of COPD patients; this is caused not only by cigarette smoke and other irritants inhaled into the respiratory tract, but also by activation of inflammatory response cells (Domej et al., 2014; Choudhury and MacNee, 2017). Oxidative stress-driven pulmonary inflammation and the spread of pulmonary inflammation to the systemic circulation play a central role in the pathophysiology of COPD (Rahman and Adcock, 2006; Barnes et al., 2015; Choudhury and MacNee, 2017).

One of the most important redox-sensitive system of the cell is the NRF2/KEAP1 signaling pathway, which activates gene transcription due to interaction of the NRF2 transcription factor (also known as NFE2L2, nuclear factor (erythroid-derived 2)-like 2) with the *cis*-regulatory antioxidant-responsive elements (ARE) in the target gene promoters. The AREs are represented by 5′-A/GTGAC/TnnnGCA/G-3′ sequence motifs (Hasselbalch et al., 2014). NRF2 is a redox-sensitive transcription factor of the Cap'n'Collar (CNC) subfamily of basic leucine zipper proteins (Hasselbalch et al., 2014). It is encoded by the *NFE2L2* gene located on chromosome 2q31.2 (Cho, 2013); *NFE2L2* is extensively expressed in the lung tissue, a site of continual detoxification activity (Zhao et al., 2017). NRF2 (NFE2L2) is a short-lived protein whose stability is regulated by KEAP1 (Kelch-like ECH associating protein 1) - dependent ubiquitination and 26S proteasome degradation. KEAP1 acts as an adapter protein to mediate the interaction between NRF2 and cullin-3-based ubiquitin–ligase complex E3 (Cul3-E3 ligase) (Canning et al., 2015). Normal ubiquitination also requires RING-box protein 1 (Rbx1), which, together with Cul3, forms the catalytic component of the enzyme complex and interacts with ubiquitin ligase E2 to transfer ubiquitin onto NRF2 (Hasselbalch et al., 2014; Canning et al., 2015; Zenkov et al., 2013). In the absence of stimulation, NRF2 mainly remains in the cytoplasm as a complex with the regulatory KEAP1 subunit. As a result of oxidative stress, KEAP1–NRF2 complexes disintegrate and NRF2 translocates into the nucleus, where it interacts with ARE sequences in the target gene promoters and induces the expression of antioxidant defense genes (Hasselbalch et al., 2014; Canning et al., 2015; Zenkov et al., 2013). Recent research has shown that NRF2, in addition to antioxidant defense, responds to proinflammatory stimuli and protects the cell from inflammation-induced damage (Zenkov et al., 2013).

As a transcription factor, NRF2 regulates the expression of numerous antioxidant defense genes that can be considered as candidate genes of COPD (Zhao et al., 2017). In the present study, we investigated a set of NRF2-regulated genes. Glutathione-disulfide reductase (GSR) is the enzyme that reduces the disulfide bond of oxidized glutathione (GSSG) to produce the sulfhydryl form (GSH), an important intracellular antioxidant; it is encoded by the *GSR* gene located on chromosome 8p21.1 (Han et al., 2017; Zhu et al., 2018). Sirtuin 2 (SIRT2) belongs to the family of evolutionary conserved NAD-dependent proteins with deacetylase or ADP-ribosyltransferase activity; the corresponding gene *SIRT2* is located on chromosome 19q13 (Taka et al., 2017). SIRT2 participates in different intracellular processes associated with DNA repair, cell cycle and metabolism, apoptosis, and ageing (Gomes et al., 2015; Wei et al., 2014); it also contributes to inflammatory signaling mediated by NF-kB, which regulates the expression of a number of inflammatory response genes (Hall et al., 2013; Taka et al., 2017). *MPO* gene is located on chromosome 17q23–q24; the *MPO* promoter region contains a functional polymorphism c.–643G > A, (rs2333227) that affects gene expression (Wang et al., 2017). *PRNP* encodes the PRNP prion protein, the main role of which is to protect the tissue from oxidative stress (Doeppner et al., 2015); the *PRNP* promoter was shown to contain an Nrf2-binding site (Cichon and Brown, 2014). Prostaglandin-endoperoxide synthase 1 (PTGS1) is encoded by the *PTGS1* gene located on chromosome 9q32–q33.3; the rs1330344 (c.–1676C > T) polymorphism in the 5′-untranslated region of *PTGS1* affects the level of the gene expression (Cao et al., 2014). *PTGR1* located on chromosome 9q31.3 encodes prostaglandin reductase 1; expression of *PTGR1* is regulated by NRF2 (Sánchez-Rodríguez et al., 2017). Heat shock 70 kDa protein 1A (HSPA1A) is a serum and intracellular heat shock protein; it is involved in the mechanisms of cell adaptation and protection from a wide range of stress factors, including oxidative stress (Dulin et al., 2012). Hacker et al. found that HSPA1A levels were increased in the serum of COPD patients and acted as a marker of immune activation and cell damage (Hacker et al., 2009). Dong et al. showed that elevated HSPA1A expression was strongly associated with COPD severity and smoking status, and can be considered as a biomarker of inflammation and disease progression (Dong et al., 2013). The *TXNRD2* gene located on chromosome 22q11.21, encodes thioredoxin reductase 2, a mitochondrial protein that maintains the reduced state of thioredoxin, contributing importantly to the regulation of the redox balance in the cell (Cunniff et al., 2014).

Since NRF2 is a key regulatory element of the antioxidant defense system, polymorphisms of *NFE2L2*, *KEAP1*, and NRF2- target genes may contribute significantly to the development COPD (Figarska et al., 2014). We selected genes involved in the downregulation of NRF2 (*KEAP1)*, genes known to be regulated by NRF2 (*MPO*, *PRNP*, *PTGR1*, *HSPA1A*, *TXNRD2*, *GSR*, *SIRT2*, and *PTGS1*), we also genotyped functional SNP in *NFE2L2* gene. The frequency distribution of the *NFE2L2*, *KEAP1*, *MPO*, *PRNP*, *PTGR1*, *HSPA1A*, *TXNRD2*, *GSR*, *SIRT2*, and *PTGS1* polymorphisms and their association with COPD hadn't been investigated yet in populations of Russia. Association studies of polymorphic markers in such candidate genes affecting development and progression of cancers, type 2 diabetes mellitus, cardiovascular diseases, and ageing were published (Figarska et al., 2014; Wei et al., 2014; Wang et al., 2017; Cao et al., 2014; Dulin et al., 2012; He et al., 2009; Edvardsen et al., 2013; Kariž et al., 2015; Kopp et al., 2017; Hartikainen et al., 2015; Smid et al., 2013; Soerensen et al., 2012; Liu et al., 2018). To avoid possible problems arising from population stratification, in our study, we analyzed the association of SNP markers with COPD in ethnically homogenous group - ethnic Tatars, historically dispersed over the territory of the Volga-Ural region of Russia. According to previous population genetic studies of our colleagues, the Tatar population of the Volga-Ural region of Russia has mostly West Eurasian genetic component (cluster) in their structure (Yunusbayev et al., 2015).

The aim of this study was to investigate the association of *NFE2L2* (rs35652124), *KEAP1* (rs1048290), *MPO* (rs2333227), *PRNP (*rs1799990), *PTGR1* (rs2273788), *HSPA1A* (rs1008438), *TXNRD2* (rs1139793), *GSR* (rs1002149), *SIRT2* (rs10410544), and *PTGS1* (rs1330344) polymorphisms with COPD in a Tatar population from Russia.

2. Materials and methods

Prior to implementation, present study was approved by the Local Ethical 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). Written informed consent was obtained from all individuals. All DNA samples used in the

study were anonymous.

2.1. Patients and controls

This work was designed as a case - control study. The total number of 882 DNA samples of unrelated individuals from the same region of Russia Federation, representatives of Tatar population, historically dispersed over the territory of the Volga-Ural region of Russia, have been analyzed in this study. Ethnic origin (up to the third generation) of all the participants was derived by direct interviews with examined persons.

The COPD (N = 425) for our candidate gene approach study were accurately selected and collected from 2010 to 2015 years in the pulmonary departments of Ufa City Hospitals №21 (Ufa, Russia). The COPD patients were recruited randomly according to the International Classification of Diseases tenth revision (ICD 10) (http://www.who.int/classifications/icd/en/) and following the recommendations of the Global Initiative for Chronic Obstructive Lung Disease (Global Strategy for the Diagnosis, Management and Prevention of Chronic Obstructive Pulmonary Disease, 2011–2015) (http://goldcopd.org). For all patients with COPD the diagnosis was detected by the hospital specialists on the basis of the medical histories and the results of general, clinical, and special tests (chest X-ray, spirometry measures, and fibrobronchoscopy), physical examination, and laboratory approaches. Patients were excluded from the study if they had diagnosis of asthma and lung cancer. Subjects performed standardized pre-bronchodilator and post-bronchodilator spirometry in accordance with American Thoracic Society/European Respiratory Society (Miller et al., 2005). The spirometry was done in the in pulmonary departments of Ufa City Hospitals №21 (Ufa, Russia) by the hospital specialists. All COPD patients had post-bronchodilator FEV1/FVC values of < 70%.

The control group is comprised of 457 unrelated age-, sex- and ethnicity matched to the cases healthy residents of Ufa (Russia) with no history of chronic diseases such as respiratory system pathology and allergic diseases in the anamnesis. All the control subjects were collected among those individuals who attended Ufa City Hospitals 21 (Russia) for regular medical examination. All individuals from control group were unrelated to patients and independent of one another. Control subjects demonstrated normal lung function (FEV1/FVC > 70%, FEV1 > 80%). Summary is given in Table 1.

2.2. Genotyping

Genomic DNA was isolated from peripheral blood leukocytes using the standard phenol–chloroform extraction procedure (Mathew, 1985). We worked with so called candidate genes approach, meaning that we have chosen for consideration only polymorphisms in genes with known functions and previously shown association with other complex diseases. Minor allele frequency (MAF) of ≥5% in the Caucasian population, parameters set by the SNP database of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/projects/SNP/), were also reviewed. For the current study, ten most widely studied SNPs on *NFE2L2* (ID:4780) (rs35652124), *KEAP1* (ID: 9817) (rs1048290), *MPO* (ID: 4353) (rs2333227), *PRNP* (ID:5621) (rs1799990), *PTGR1* (ID: 22949) (rs2273788), *HSPA1A* (ID: 3303) (rs1008438), *TXNRD2* (ID: 10587) (rs1139793), *GSR* (ID: 2936) (rs1002149), *SIRT2* (ID: 22933) (rs10410544), *PTGS1* (ID: 5742) (rs1330344) were examined by the real-time polymerase chain reaction (PCR), with the use of TaqMan SNP discrimination assays (Applied Biosystems, Foster City, CA). Accumulation of specific PCR-product by hybridization and cleavage of double-labelled fluorogenic probe during amplification was detected with a BioRad CFX96 instrument (Bio-Rad Laboratories Inc., USA). End-point fluorescence and genotype discrimination were determined according to the BioRad CFX96 protocol, using CFX Manager software. For quality control, 5% dummy duplicates, blank and positive controls were also taken up along with the samples in each experiment. The genotyping was blind to case or control status of the samples. Quality control of genotyping data was assessed by subject and by marker. A priori of the association analysis we run strict quality control on our data to exclude genotyping errors, SNPs and individuals with law call rates and other important quality characteristics. Subject data were excluded after examining missingness, reproducibility, and inbreeding. All subjects with a genotype call rate of < 95% were removed. Subsequently SNPs were filtered according to their proportion of missing, minor allele frequency (MAF) or deviation from Hardy-Weinberg-Equilibrium (HWE).

Table 1
Characteristics of groups.

	COPD (N = 425)	Controls (N = 457)	P-value
Male (%)	369 (86.82)	406 (88.84)	0.51[a]
Female (%)	56 (13.18)	51 (11.16)	
Age (± SD)	63.38 ± 11.81	58.44 ± 14.79	0.09[b]
BMI (± SD)	25.81 ± 5.92	27.06 ± 3.84	0.06[b]
Smoking index, pack-years for smokers (± SD)	44.58 ± 25.92	38.54 ± 23.12	0.06[b]
Smoking status:			0.318[a]
Current and former smokers (%)	331 (77.88)	322 (70.46)	
Non-smokers (%)	94 (22.12)	135 (29.54)	
Post-FEV1% (± SD)	41.68 ± 19.32	102.7 ± 52.1	0.0001[b]
Post-FEV1/FVC ratio (± SD)	58.66 ± 13.66	87.94 ± 10.69	
FVC % (± SD)	44.22 ± 17.88	107.1 ± 32.05	
GOLD status:			
GOLD 2 (%)	149 (29.16)	–	–
GOLD 3 (%)	139 (27.20)		
GOLD 4 (%)	223 (43.64)		

Abbreviations: Values are means ± SD for continuous data, BMI, body mass index; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; Post, post-bronchodilator; GOLD, global initiative for chronic obstructive lung disease, Smoking index, pack-years PY = (number of cigarettes per day X number of years smoked)/20.

[a] Pearson's X^2-test.

[b] Mann-Whitney *U* test.

2.3. Statistical analysis

The sample size was calculated by Quanto software (http://biostats.usc.edu/software). We examined ten candidate genes and used the most significant reported SNPs with a high minor allele frequency for each gene. On the basis of our calculations using the Power and Sample Size software program, our sample (N = 882) was considered adequate to study the selected SNPs. The sample size (N = 425 for case group and N = 457 for control group) was sufficient to detect the association of examined SNPs and COPD with > 80% power (Power: 95.53%, Disease prevalence, 7%, error: 5%). For the quantitative traits, the mean values and standard deviations (M ± SD) were calculated; the group comparison was performed with a nonparametric Mann–Whitney *U* test. The frequencies of qualitative traits were compared using the Pearson's X^2. Statistical analysis was carried out with the Statistica v. 6.0 program (StatSoft Inc., Tulsa, OK, USA). A minor allele frequencies (MAF) and the agreement of the genotype distribution to the Hardy–Weinberg equilibrium (X^2), the association analysis using the basic allele test and the calculation of the odds ratio (OR) for the rare allele of each locus and the significance of intergroup differences in allele and genotype frequencies (X^2 test for sample heterogeneity and the P-value), and Cochran-Armitage trend test were performed with PLINK v. 1.07 (Purcell et al., 2007). Differences were considered significant if their corresponding *P*-values were < 0.05. To control Type I error rate false discovery rate (FDR) (Benjamini Hochberg) was calculated using the online software program http://www.sdmproject.com/utilities/?show=FDR. Logistic regression was used to detect the association of SNPs loci in different models, accounting for quantitative and binary

Table 2
Location, allele frequency, and quality control information for *KEAP1*, *NFE2L2* and antioxidant gene polymorphisms.

Gene	SNP	Alleles		MAF (COPD)	MAF (control)	HWE (control) P-value
		Minor	Major			
NFE2L2	rs35652124	G	A	0.4235	0.4661	0.22
KEAP1	rs1048290	C	G	0.3988	0.4773	0.68
MPO	rs2333227	A	G	0.2835	0.2823	0.073
PRNP	rs1799990	G	A	0.318	0.279	0.52
PTGR1	rs2273788	A	G	0.2518	0.2319	0.47
HSPA1A	rs1008438	C	A	0.2247	0.1783	0.21
TXNRD2	rs1139793	T	C	0.2424	0.2341	0.66
GSR	rs1002149	A	C	0.1965	0.1575	0.17
SIRT2	rs10410544	A	G	0.3706	0.3753	0.15
PTGS1	rs1330344	C	T	0.3624	0.3162	0.55

Note: MAF, minor allele frequency, HWE, Hardy-Weinberg equilibrium P-value.

traits (gender, age, pack-years, smoking status, body mass index). The significance of the obtained model accounting for all variables was verified by the significance of the likelihood ratio test (P_{adj}). The best model was chosen using the Akaike's information criterion (AIC). For each significant locus ($P < 0.05$), the model with lowest AIC was chosen. We used a logistic regression approach implemented in the software package PLINK v. 1.07 to assess the effects of genotype × smoking interactions. Linear regression analyses were performed to estimate the relationship between SNPs and quantitative phenotypes, such as lung function parameters and pack-years. The regression analysis was performed with PLINK v. 1.07 (Purcell et al., 2007).

3. Results

Before candidate gene polymorphisms were analyzed for associations with COPD, we checked whether their genotype frequency distributions agreed with the Hardy-Weinberg equilibrium (HWE) and evaluated minor allele frequencies (MAF) both in the combined group of patients and healthy subjects and in either group individually (see Table 2).

3.1. Association of candidate polymorphic loci with COPD

Data on the allele and genotype frequency distributions for the loci in question, the significance of their differences between the groups, and odd ratio values calculated for the minor allele, and Cochran-Armitage trend test of each locus are shown in Tables 2 and 3. Significant differences between the groups studied were identified for the following polymorphic loci: *KEAP1* (c.1413C > G, rs1048290), *PRNP* (c.385A > G, rs1799990), *HSPA1A* (c.-326A > C, rs1008438), *GSR* (c.-386C > A, rs1002149), *PTGS1* (c.-1676C > T, rs1330344) (see Tables 3 and 4). At the next stage, the association was analyzed using various models.

Table 5 presents the characteristics of the detected significant associations with COPD: the regression coefficient (beta), its exponent interpreted as odds ratio (OR) in the logistic model, the corresponding 95% confidence intervals, and the level of significance, calculated while taking into account the patients' sex, age, smoking status, BMI, and smoking index in different models.

The frequency of the minor C allele of *KEAP1* (c.1413C > G, rs1048290) was significantly lower in COPD patients than in controls ($P = 0.001$, OR = 0.72). The more frequent G allele was the marker associated with the disease ($P = 0.001$, OR = 1.37) (Table 3). The portion of CC homozygotes in the group of COPD patients was lower in contrast to healthy subjects ($P_{adj} = 0.0047$, $P_{cor\text{-}FDR} = 0.028$, OR = 0.60, in the recessive model). Significant association with COPD was established in the additive ($P_{adj} = 0.0015$, $P_{cor\text{-}FDR} = 0.018$, OR = 0.72) and in the dominant model ($P_{adj} = 0.016$, $P_{cor\text{-}FDR} = 0.048$,

Table 3
Allele and genotype frequencies of the *NFE2L2*, *KEAP1*, *MPO*, *PRNP*, and *PTGR1* polymorphisms in COPD patients and control subjects.

Gene polymorphism location refSNP	Chr. location	Minor allele	N	Genotypes, alleles	COPD n (%)	Control n (%)	P[a]	P[b]	OR (95% CI)
NFE2L2 c.-1946A > G rs35652124	2q31	G	882	AA/AG/GG	146/198/81 (34.35/46.59/19.06)	138/212/107 (30.20/46.39/23.41)	0.208	0.11	0.86 (0.71–1.04)
				A/G	490/360 (57.65/42.35)	488/426 (53.39/46.61)	0.08	–	0.84 (0.69–1.01)
KEAP1 c.1413C > G rs1048290	19p13.2	C	882	GG/GC/CC	152/207/66 (35.76/48.71/15.53)	127/223/107 (27.79/48.80/23.41)	0.003	0.0015	0.71 (0.58–0.86)
				G/C	511/339 (60.12/39.88)	461/421 (52.27/47.73)	0.001	–	0.72 (0.60–0.87)
MPO c.-643G > A rs2333227	17q23.1	A	882	GG/GA/AA	232/145/48 (54.59/34.12/11.29)	244/168/45 (53.39/36.76/9.85)	0.628	0.99	1.00 (0.81–1.23)
				G/A	609/241 (71.65/28.35)	656/258 (71.77/28.23)	0.996	–	1.01 (0.82–1.24)
PRNP c.385A > G rs1799990	20p13	G	882	AA/AG/GG	210/165/50 (49.41/38.82/11.76)	234/191/32 (51.20/41.79/7.00)	0.05	0.16	1.17 (0.94–1.45)
				A/G	585/265 (68.82/31.18)	659/255 (72.10/27.90)	0.145	–	1.17 (0.95–1.44)
PTGR1 c.210-172G > A rs2273788	9q31.3	A	882	GG/GA/AA	243/150/32 (57.18/35.29/7.53)	266/170/21 (58.21/37.20/4.60)	0.181	0.37	1.11 (0.88–1.40)
				G/A	636/214 (74.82/25.18)	702/212 (76.81/23.19)	0.36	–	1.11 (0.89–1.38)

[a] X^2 test for allele or genotypes frequency difference between COPD and control.
[b] Cochran-Armitage trend test, OR with 95% CI for minor allele in basic allele test or Cochran-Armitage trend test, Chr. – Chromosome location.

Table 4
Allele and genotype frequencies of the *HSPA1A*, *TXNRD2*, *GSR*, *SIRT2*, and *PTGS1* polymorphisms in COPD patients and control subjects.

Gene polymorphism location refSNP	Chr. location	Minor allele	N	Genotypes, alleles	COPD n (%)	Control n (%)	P[a]	P[b]	OR (95% CI)
HSPA1A c.-326A > C rs1008438	6p21.3	C	882	AA/AC/CC	272/115/38 (64.00/27.06/8.94)	313/125/19 (68.49/27.35/4.16)	0.014	0.035	1.23 (1.05–1.62)
				A/C	659/191 (77.53/22.47)	751/163 (82.17/17.83)	0.018	–	1.33 (1.06–1.69)
TXNRD2 c.1106 T > C p.Ile370Thr rs1139793	22q11.21	T	882	CC/CT/TT	245/154/26 (57.65/36.24/6.12)	270/160/27 (59.08/35.01/5.91)	0.911	0.69	1.05 (0.23–1.35)
				C/T	644/206 (75.76/24.24)	700/214 (76.59/23.41)	0.727	–	1.04 (0.84–1.30)
GSR c.-386C > A rs1002149	8p21.1	A	882	CC/CA/AA	281/121/23 (66.12/28.47/5.41)	329/112/16 (71.99/24.51/3.50)	0.121	0.042	1.31 (1.07–1.65)
				C/A	683/167 (80.35/19.65)	770/144 (84.25/15.75)	0.037	–	1.31 (1.02–1.67)
SIRT2 c.-48-1025A > G rs10410544	19q13	A	882	GG/GA/AA	174/187/64 (40.94/44.00/15.06)	187/197/73 (40.92/43.11/15.97)	0.924	0.95	0.99 (0.81–1.21)
				G/A	535/315 (62.94/37.06)	571/343 (62.47/37.53)	0.878	–	0.98 (0.81–1.19)
PTGS1 c.-1676C > T rs1330344	9q32-q33.3	C	882	TT/TC/CC	180/182/63 (42.35/42.82/14.82)	217/191/49 (47.48/41.79/10.72)	0.119	0.067	1.21 (0.99–1.49)
				T/C	542/308 (63.76/36.24)	625/289 (68.38/31.62)	0.046	–	1.22 (1.01–1.49)

[a] X^2 test for allele or genotypes frequency difference between COPD and control.
[b] Cochran-Armitage trend test, OR with 95% CI for minor allele in basic allele test or Cochran-Armitage trend test, Chr. – Chromosome location.

OR = 0.69) (Table 5).

The frequency of the GG genotype of *PRNP* (c.385A > G, rs1799990) polymorphism was higher in COPD patients than in controls (11.76% vs 7.00%; P_{adj} = 0.023, OR = 1.77); however, the difference became insignificant after the FDR-correction ($P_{cor\text{-}FDR}$ = 0.055).

The minor allele C of *HSPA1A* (c.-326A > C, rs1008438) was also shown to be associated with COPD (P = 0.018, OR = 1.33) (Tables 4 and 5). A regression analysis established rs1008438 association with COPD in the recessive model (P_{adj} = 0.006, $P_{cor\text{-}FDR}$ = 0.024, OR = 2.26) (Table 5).

The frequency of the A allele of *GSR* (c.-386C > A, rs1002149) was significantly higher in COPD patients than in controls (P = 0.037, OR = 1.31) (Table 4). Association with COPD was established in the additive model (P_{adj} = 0.04, OR = 1.29) however, the difference became insignificant after the FDR-correction ($P_{cor\text{-}FDR}$ = 0.075) (Table 5).

The minor C allele of *PTGS1* (c.-1676C > T, rs1330344) was the marker associated with the disease (P = 0.046, OR = 1.22) (Table 4). However, regression analysis failed to detect a significant association between rs1330344 and COPD.

3.2. Analysis of gene-environment interactions in COPD

We investigated the relationship between the candidate gene polymorphisms and smoking index (in pack-years) in smoking subjects (Table 6). The smoking index was affected by the genotypes of *KEAP1* (c.1413C > G, rs1048290), *NFE2L2* (c.-1946A > G, rs35652124), and *HSPA1A* (c.-326A > C, rs1008438) (Table 6). In particular, the smoking index was significantly higher in carriers GG genotype of *KEAP1* (c.1413C > G, rs1048290) (P = 0.005). The AA genotype by *NFE2L2* (c.-1946A > G, rs35652124) was associated with higher smoking index values (P = 0.0028). The AA genotype by *HSPA1A* (c.-326A > C, rs1008438) was associated with lower smoking index values (P = 0.031), whereas the homozygous CC of *HSPA1A* (c.-326A > C, rs1008438) was associated with an elevated smoking index (P = 0.015) (Table 6).

We did not observe significant gene-environment interactions in the logistic regression analysis of *KEAP1* (rs1048290), *MPO* (rs2333227), *PRNP* (rs1799990), *PTGR1* (rs2273788), *HSPA1A* (rs1008438), *TXNRD2* (rs1139793), *GSR* (rs1002149), *SIRT2* (rs10410544), *PTGS1* (rs1330344) polymorphisms with smoking status. A significant gene-environment interaction of *NFE2L2 (*rs35652124) polymorphism and smoking status was detected in the logistic regression analysis ($P_{interact}$ = 0.0034, OR = 0.58 CI95% 0.38–0.89 under the assumption of dominant model).

Gene-environment interactions were also analyzed by comparing odds ratio values calculated for the candidate genes in subgroups formed according to the presence or absence of the environmental factor. The significant associations of the candidate polymorphisms with COPD observed in the subgroups stratified by smoking status are shown in Tables 7 and 8. *NFE2L2* (c.-1946A > G, rs35652124), *PRNP* (c.385A > G, rs1799990), and *HSPA1A* (c.-326A > C, rs1008438) polymorphisms were significantly associated with COPD only in the smokers. *NFE2L2* (c.-1946A > G, rs35652124) was associated with COPD in smokers in the additive (P_{adj} = 0.035, $P_{cor\text{-}FDR}$ = 0.042, OR = 0.79) and in the dominant model (P_{adj} = 0.019, $P_{cor\text{-}FDR}$ = 0.037, OR = 0.65); the frequency of the AA genotype of *NFE2L2* (c.-1946A > G, rs35652124) was significantly higher in COPD smokers than in controls (36.56% vs 27.64%; P = 0.019) (Table 7). *HSPA1A* (c.-326A > C, rs1008438) was associated with COPD in smokers in the additive model (P_{adj} = 0.016, $P_{cor\text{-}FDR}$ = 0.036, OR = 1.41). Significant associations were established for *PRNP* (c.385A > G, rs1799990) in the recessive model (P_{adj} = 0.017, $P_{cor\text{-}FDR}$ = 0.036, OR = 2.67) (Table 7). The risk of COPD in smokers was associated with *KEAP1* (c.1413C > G, rs1048290) in the additive (P_{adj} = 0.005, $P_{cor\text{-}}$

Table 5
Association between *KEAP1*, *PRNP*, *GSR*, and *HSPA1A* polymorphisms and COPD.

Gene, SNP	Minor allele	N	Genotype/model	COPD абс. (%)	Control абс. (%)	OR_{adj} (CI95%)	P_{adj}[a]	$P_{cor\text{-}FDR}$
KEAP1 c.1413C > G rs1048290	C	882	GG GC + CC Dominant	152 (35.76)	127 (27.79)	1.00	0.016	0.048
				273 (64.24)	330 (72.21)	0.69 (0.51–0.93)		
			GG + GC	359 (84.47)	350 (76.59)	1.00	0.0047	0.028
			CC	66 (15.53)	107 (23.41)	0.60 (0.42–0.86)		
			Recessive					
			Log-additive	–	–	0.72 (0.59–0.88)	0.0015	0.018
PRNP c.385A > G rs1799990	G	882	AA	210 (49.41)	234 (51.20)	1.00	0.61	0.61
			AG + GG	215 (50.59)	223 (48.8)	1.08 (0.81–1.42)		
			Dominant					
			AA + AG	375 (88.24)	425 (93.00)	1.00	0.023	0.055
			GG	50 (11.76)	32 (7.00)	1.77 (1.07–2.93)		
			Recessive					
			Log-additive	–	–	1.17 (0.94–1.45)	0.16	0.192
HSPA1A c.-326A > C rs1008438	C	882	AA	272 (64.00)	313 (68.49)	1.00	0.2	0.21
			AC + CC	153 (36.00)	144 (31.51)	1.22 (0.90–1.66)		
			Dominant					
			AA + AC	397 (91.06)	438 (95.84)	1.00	0.006	0.024
			CC	38 (8.94)	19 (4.16)	2.26 (1.21–4.21)		
			Recessive					
			Log-additive	–	–	1.28 (1.01–1.63)	0.039	0.075
GSR c.-386C > A rs1002149	A	882	CC	281 (66.12)	329 (71.99)	1.00	0.071	0.10
			CA + AA	144 (33.88)	128 (28.01)	1.32 (0.98–1.79)		
			Dominant					
			CC + CA	402 (94.79)	441 (96.5)	1.00	0.15	0.19
			AA	23 (5.41)	16 (3.50)	1.66 (0.83–3.33)		
			Recessive					
			Log-additive	–	–	1.29 (1.00–1.66)	0.04	0.075

[a] P_{adj}, significance in the likelihood ratio test for the regression model adjusted for age, sex, BMI, smoking status and pack-years; OR_{adj}, adjusted odds ratio and CI, 95% confidence interval; $P_{cor\text{-}FDR}$, significance after the FDR correction.

$_{FDR}$ = 0.031, OR = 0.72), and recessive model (P_{adj} = 0.023, $P_{cor\text{-}FDR}$ = 0.036, OR = 0.62); in COPD patients, the frequency of homozygous carriers of the major G allele of *KEAP1* (c.1413C > G, rs1048290) was higher (35.95% vs. 27.02% in control, P_{adj} = 0.022, $P_{cor\text{-}FDR}$ = 0.037) (Table 7).

In nonsmokers, significant associations were established for *KEAP1* (c.1413C > G, rs1048290) in the recessive model (P_{adj} = 0.015, $P_{cor\text{-}FDR}$ = 0.036, OR = 0.34), and *GSR* (c.-386C > A, rs1002149) in the additive model (P_{adj} = 0.027, $P_{cor\text{-}FDR}$ = 0.039, OR = 1.76) (Table 8).

There was no significant association of *MPO* (rs2333227), *PTGR1* (rs2273788), *TXNRD2* (rs1139793), *SIRT2* (rs10410544), *PTGS1* (rs1330344) gene polymorphisms with COPD in the subgroups stratified by smoking status.

3.3. Association between lung function parameters and candidate gene polymorphisms

We investigated the relationship between the NRF2/KEAP1 signaling system and antioxidant defense gene polymorphisms and lung function values in COPD patients (Table 9). *KEAP1* (rs1048290), *MPO*

Table 6
The relationship between *KEAP1*, *NFE2L2* and antioxidant gene polymorphisms and smoking index (pack-years).

Gene, SNP	Model/genotypes	M ± S.E	P[a]	Beta (CI 95%)
NFE2L2	AA	41.11 (2.16)	0.0028	0.00
rs35652124	(AG + GG)	34.21 (1.25)		−6.90 (−11.49 - -2.31)
PRNP	AA	34.97 (1.46)	0.23	0.00
rs1799990	(AG + GG)	37.6 (1.67)		2.64 (−1.71–6.98)
SIRT2	(GG + GA)	36.46 (1.24)	0.97	0.00
rs10410544	AA	36.59 (2.51)		0.13 (−5.77–6.02)
GSR	CC	36.43 (1.37)	0.87	0.00
rs1002149	(CA + AA)	36.03 (1.87)		−0.40 (−5.06–4.26)
KEAP1 rs1048290	GG	40.72 (2.13)	0.005	0.00
	(GC + CC)	34.25 (1.26)		−6.47 (−11.05 - -1.89)
PTGS1	TT	36.3 (1.72)	0.98	0.00
rs1330344	(TC + CC)	36.35 (1.44)		0.05 (−4.31–4.41)
TXNRD2 rs1139793	(CC + CT)	36.65 (1.18)	0.72	0.00
	TT	38.25 (3.92)		1.60 (−7.06–10.26)
HSPA1A	AA	34.81 (1.33)	0.031	0.00
rs1008438	(AC + CC)	39.94 (2.14)		5.14 (0.39–9.88)
	(AA + AC)	35.7 (1.16)	0.015	0.00
	CC	46 (4.81)		10.30 (1.95–18.65)
PTGR1	GG	34.89 (1.41)	0.095	0.00
rs2273788	(GA + AA)	38.68 (1.82)		3.79 (−0.65–8.23)
MPO	GG	36.74 (1.62)	0.63	0.00
rs2333227	(GA + AA)	35.68 (1.5)		−1.07 (−5.35–3.83)

Notes: Data presented are beta, mean and standard error with two-sided *P* values.
[a] Linear regression analysis adjusting for age, gender, BMI.

Table 7
Association analysis of the *KEAP1*, *NFE2L2* and antioxidant genes polymorphisms with COPD in smokers (N = 653).

Gene, SNP	Test/model	COPD (N = 331) n (%)	Control (N = 322) n (%)	P_{adj} [a]	$P_{cor\text{-}FDR}$ [c]	OR_{adj} (CI95%)
NFE2L2 rs35652124	AA	121 (36.56)	89 (27.64)	0.019	0.037	1.00
	AG + GG	210 (63.44)	233 (72.36)			0.65 (0.39–0.87)
	Dominant					
	Log-additive	–	–	0.035	0.042	0.79 (0.63–0.99)
KEAP1 rs1048290	GG + GC	273 (82.48)	244 (75.78)	0.023	0.037	1.00
	CC	58 (17.52)	78 (24.22)			0.62 (0.41–0.94)
	Recessive					
	Log-additive	–	–	0.005	0.031	0.72 (0.57–0.91)
HSPA1A rs1008438	AA	211 (63.75)	232 (72.05)	0.029	0.039	1.00
	AC + CC	120 (36.25)	90 (27.95)			1.47 (1.02–2.13)
	Dominant					
	AA + AC	299 (90.33)	306 (95.03)	0.03	0.039	1.00
	CC	32 (9.67)	16 (4.97)			2.04 (1.10–3.81)
	Recessive					
	Log-additive	–	–	0.016	0.036	1.41 (1.06–1.87)
PRNP rs1799990	AA + AG	293 (88.52)	303 (94.10)	0.017	0.036	2.67 (1.17–3.67)
	GG	38 (11.48)	19 (5.90)			
	Recessive					
GSR rs1002149	CC	223 (67.37)	229 (71.12)	0.35	–	1.00
	CA + AA	108 (32.63)	93 (28.88)			1.19 (0.83–1.70)
	Dominant					
	Log-additive	–	–	0.26	–	1.19 (0.88–1.60)
SIRT2 rs10410544	GG	132 (39.88)	131(40.68)	0.86	–	1.00
	GA + AA	199 (60.12)	191 (59.32)			1.03 (0.73–1.45)
	Dominant					
	Log-additive	–	–	0.98	–	1.00 (0.79–1.26)
PTGS1 rs1330344	TT	142 (42.9)	150 (46.58)	0.42	–	1.00
	TC + CC	189 (57.1)	172 (53.42)			1.15 (0.82–1.60)
	Dominant					
	Log-additive	–	–	0.21	–	1.17 (0.92–1.49)
TXNRD2 rs1139793	CC	185 (55.89)	189 (58.7)	0.58	–	1.00
	CT + TT	146 (44.11)	133 (41.3)			1.10 (0.78–1.54)
	Dominant					
	Log-additive	–	–	0.72	–	1.05(0.80–1.37)
PTGR1 rs2273788	GG	187 (56.5)	184 (57.14)	0.96	–	1.00
	GA + AA	144 (43.5)	138 (42.86)			0.99 (0.71–1.39)
	Dominant					
	Log-additive	–	–	0.50	–	1.10 (0.84–1.44)
MPO rs2333227	GG	177 (53.47)	177 (54.97)	0.68	–	1.00
	GA + AA Dominant	154 (46.53)	145 (45.03)			1.07 (0.77–1.50)
	Log-additive	–	–	0.74	–	1.04 (0.82–1.33)

[a] P_{adj}, significance in the likelihood ratio test for the regression model adjusted for age, sex, and smoking index in pack-years, BMI; OR_{adj}, adjusted odds ratio and CI, 95% confidence interval; $P_{cor\text{-}FDR}$, significance after the FDR correction.

(rs2333227), *PTGR1* (rs2273788), *HSPA1A* (rs1008438), *TXNRD2* (rs1139793), *GSR* (rs1002149), *PTGS1* (rs1330344) gene polymorphisms were not significantly associated with lung function values. Carriers of the *SIRT2* (rs10410544) AA genotype exhibited higher FVC (P = 0.022) and FEV1 (P = 0.04) values. The presence of AA genotype for *NFE2L2* (rs35652124) was associated with a decrease in FEV1% predicted (P = 0.028) (Table 9). Patients homozygous for the A allele of *PRNP* (rs1799990) had higher FEV1 than those with GG and AG genotypes (P = 0.044) (Table 9).

4. Discussion

The mechanisms of COPD pathogenesis are still being extensively studied worldwide, but there can be no doubt concerning the key role of oxidative stress (Domej et al., 2014). Numerous studies on genetic association and comparative gene expression analysis have revealed the relation between the genes that encode antioxidant enzymes and COPD (Bentley et al., 2008; Blake et al., 2010; Pierrou et al., 2007; Taylor et al., 2008). In the present study, we investigated the association of the NRF2/KEAP1 signaling system and antioxidant defense gene polymorphisms with COPD in a Tatar population from Russia.

NFE2L2, or NRF2, is a crucial factor of lung tissue protection against the damage (Taylor et al., 2008). Several studies demonstrated the involvement of *NFE2L2* and *KEAP1* polymorphisms, which encode NRF2 transcription factor and its repressor, respectively, in atherosclerosis, bronchial asthma, COPD, and ageing (Zhao et al., 2017; Figarska et al., 2014; Sandford et al., 2012; Marzec et al., 2007; Siedlinski et al., 2009). *NFE2L2* polymorphisms may modulate the expression of the transcription factor or affect its ability to translocate into the nucleus and bind to the ARE site in target gene promoters (Marzec et al., 2007). Marzec et al. (2007) showed that two functional *NFE2L2* SNPs (rs6721961 and rs6706649) suppressed the promoter activity by > 50%; the rs35652124 variant inhibited *NFE2L2* expression by 50%. Decreased *NFE2L2* expression was associated with a risk of acute lung injury accompanied with lung edema and inflammation (Marzec et al., 2007). Taking into account the low minor allele frequencies of *NFE2L2* SNPs rs6721961 and rs6706649, we chose to perform our study using the functional *NFE2L2* polymorphism rs35652124 (c.–1946A > G). Our study showed that this SNP was associated with COPD in smokers in the additive model and the frequency of the AA genotype of *NFE2L2* (rs35652124) was significantly higher in COPD smokers than in controls (Table 7). This polymorphism was also characterized with significant interaction with the smoking index, which was significantly higher in subjects with the AA genotype (Table 6). The same AA genotype of *NFE2L2* (rs35652124) was associated with a decrease in FEV1 value, a crucial characteristic of COPD progression (Table 9).

The first study of *NFE2L2* polymorphisms in COPD was performed in a Japanese population in 2004, but it did not reveal any significant

Table 8
Association analysis of the *KEAP1*, *NFE2L2* and antioxidant genes polymorphisms with COPD in non-smokers (N = 229).

Gene, SNP	Test/model	COPD (N = 94) n (%)	Control (N = 135) n (%)	P_{adj} [a]	$P_{cor\text{-}FDR}$ [c]	OR_{adj} (CI95%)
NFE2L2 rs35652124	AA	25 (26.6)	49 (36.3)	0.15	–	1.00
	AG + GG	69 (73.4)	86 (63.7)			1.57 (0.85–2.93)
	Dominant					
	Log-additive	–	–	0.44	–	1.15 (0.81–1.62)
KEAP1 rs1048290	GG + GC	86 (91.49)	106 (78.52)	0.015	0.036	1.00
	CC	8 (8.51)	29 (21.48)			0.34 (0.14–0.78)
	Recessive					
	Log-additive	–	–	0.075	–	0.67 (0.44–1.05)
HSPA1A rs1008438	AA	61 (64.89)	81 (60.00)	0.69	–	1.00
	AC + CC	33 (35.11)	54 (40.00)			0.88 (0.47–1.65)
	Dominant					
	Log-additive	–	–	0.97	–	1.04 (0.60–1.71)
PRNP rs1799990	AA + AG	82 (87.23)	122 (90.37)	0.46	–	1.00
	GG	10 (12.77)	13 (9.63)			1.44 (0.56–3.70)
	Recessive					
	Log-additive	–	–	0.98	–	1.00 (0.64–1.56)
GSR rs1002149	CC	58 (61.7)	100 (74.07)	0.064	–	1.00
	CA + AA	36 (38.3)	35 (25.93)			1.80 (0.97–3.36)
	Dominant					
	Log-additive	–	–	0.027	0.039	1.76 (1.10–2.82)
SIRT2 rs10410544	GG	42 (44.68)	56 (41.48)	0.66	–	1.00
	GA + AA	52 (55.32)	79 (58.52)			0.88 (0.48–1.59)
	Dominant					
	Log-additive	–	–	0.56	–	0.88 (0.56–1.36)
PTGS1 rs1330344	TT	38 (40.42)	67 (49.63)	0.21	–	1.00
	TC + CC	56 (59.58)	68 (50.37)			1.46 (0.81–2.64)
	Dominant					
	Log-additive	–	–	0.14	–	1.36 (0.90–2.06)
TXNRD2 rs1139793	CC	60 (63.83)	81 (60.0)	0.59	–	1.00
	CT+ TT	34 (36.17)	54 (40.0)			0.85 (0.46–1.56)
	Dominant					
	Log-additive	–	–	0.58	–	0.86 (0.50–1.47)
PTGR1 rs2273788	GG	56 (59.57)	82 (60.74)	0.87	–	1.00
	GA + AA	38 (40.43)	53 (39.29)			1.05 (0.57–1.94)
	Dominant					
	Log-additive	–	–	0.99	–	1.00 (0.59–1.69)
MPO rs2333227	GG	55 (58.51)	67 (49.63)	0.22	–	1.00
	GA + AA Dominant	39 (41.49)	68 (50.37)			0.70 (0.39–1.25)
	Log-additive	–	–	0.62	–	0.90 (0.58–1.39)

[a] P_{adj}, significance in the likelihood ratio test for the regression model adjusted for age, sex, BMI; OR_{adj}, adjusted odds ratio and CI, 95% confidence interval; $P_{cor\text{-}FDR}$, significance after the FDR correction.

associations (Yamamoto et al., 2004). Later, Siedlinski et al. (2009) detected an association between *NFE2L2* polymorphism rs2364723 and the level of lung function in a Caucasian population. They found that the effect of rs2364723 and *NFE2L2* haplotypes was significant only in smokers (Siedlinski et al., 2009). In another study, *NFE2L2* polymorphism rs6726395 was associated with decreased lung function and exhibited a significant gene-by-environment interaction with smoking status in a Japanese population (Masuko et al., 2011). At the same time, associations of *NFE2L2* polymorphisms with COPD or pulmonary function decline were not confirmed by Sandford et al. (2012). Since NRF2 transcription factor plays a key role in preventing damage induced by oxidative stress, it appears promising to develop pharmacological means of stimulating NRF2 activity, which could provide a new approach to the treatment of a whole range of diseases associated with oxidative stress, including COPD (Schumacher et al., 2016; Copple et al., 2017).

Data obtained in our study confirmed the association of *KEAP1* polymorphism rs1048290 (c.1413C > G) with COPD both in smokers and in non-smoking (Tables 7, 8). Moreover, *KEAP1* (rs1048290) was associated with smoking index, which was significantly higher in GG homozygotes (Table 5). *KEAP1* polymorphism, rs1048290, was found to be associated with drug resistant epilepsy (Liu et al., 2015). A further study showed that *KEAP1* polymorphisms, including rs1048290, were associated with the efficacy of radio- and chemotherapy, as well as with overall survival rates in patients with breast cancer (Hartikainen et al., 2015). Previously, Siedlinski et al. studied several *KEAP1* polymorphisms in a general Caucasian population of the Vlagtwedde-Vlaardingen cohort and did not reveal any significant associations of the rs1048290 with COPD (Siedlinski et al., 2009).

According to our data, *HSPA1A* polymorphism rs1008438 (c.-326A > C) was associated with COPD in the general population sample and in the subgroup of smokers (Tables 5, 7). It was also found that the smoking index was significantly higher in subjects homozygous by the rare C allele of rs1008438 (Table 6). Previous studies showed that *HSPA1A* promoter activity was higher for the A allele of c.-326A > C (rs1008438) than for the C allele (Qi et al., 2009). It was also shown that the AA genotype of rs1008438 was associated with higher intracellular HSPA1A levels and thus acted as protective against atherosclerosis (Dulin et al., 2012). Our data on the association of *HSPA1A* (rs1008438) with COPD agree with the previously published results concerning other diseases related to oxidative stress, such as atherosclerosis and coronary heart disease (Dulin et al., 2012; He et al., 2009), and confirm that HSPA1A is involved in the pathogenesis of COPD.

In our study *PRNP* (rs1799990) polymorphism was associated with COPD development in smokers, and lung function decline that reflects disease progression (Tables 7, 9). The contribution of *PRNP* variants to COPD has not been studied previously. At the same time, effects of *PRNP* rs1799990 (c.385A > G) have been fairly extensively investigated in neurodegenerative diseases: Creutzfeldt–Jakob disease, Alzheimer's disease, and Parkinson's disease (Smid et al., 2013; He et al., 2013).

Table 9
The relationship between *KEAP1*, *NFE2L2* and antioxidant gene polymorphisms and lung function values.

Gene, SNP	Model/ genotypes	M ± S.E	P[a]	Beta (CI 95%)
Forced expiratory volume in 1 s (FEV1, %)				
NFE2L2	AA	37.61 (1.59)	0.028	0.00
rs35652124	(AG + GG)	42.17 (1.27)		4.56 (0.52–8.59)
PRNP	AA	42.74 (1.51)	0.044	0.00
rs1799990	(AG + GG)	38.66 (1.34)		−4.08 (−8.04 to −0.12)
SIRT2	(GG + GA)	39.68 (1.09)	0.04	0.00
rs10410544	AA	45.46 (2.65)		5.78 (0.29–11.27)
GSR	CC	40.49 (1.28)	0.92	0.00
rs1002149	(CA + AA)	40.28 (1.58)		−0.21 (−4.27–3.86)
KEAP1 rs1048290	GG	38.29 (1.63)	0.11	0.00
	(GC + CC)	41.63 (1.26)		3.34 (−0.75–7.43)
PTGS1	TT	38.98 (1.59)	0.19	0.00
rs1330344	(TC + CC)	41.64 (1.29)		2.66 (−1.32–6.65)
TXNRD2 rs1139793	(CC + CT)	41.16 (1.05)	0.26	0.00
	TT	36.74 (3.96)		−4.42 (−12.04–3.20)
HSPA1A	AA	40.41 (1.31)	0.77	0.00
rs1008438	(AC + CC)	41.03 (1.67)		0.62 (−3.53–4.78)
PTGR1	GG	41.09 (1.41)	0.55	0.00
rs2273788	(GA + AA)	39.89 (1.5)		−1.26 (−5.35–2.83)
MPO	GG	39.76 (1.3)	0.42	0.00
rs2333227	(GA + AA)	41.39 (1.58)		1.63 (−2.34–5.6)
Forced vital capacity (FVC, %)				
NFE2L2	AA	49.52 (1.74)	0.31	0.00
rs35652124	(AG + GG)	51.96 (1.63)		2.44 (−2.23–7.12)
PRNP	AA	51.44 (1.81)	0.65	0.00
rs1799990	(AG + GG)	50.35 (1.61)		−1.09 (−5.83–3.65)
SIRT2	(GG + GA)	49.62 (1.31)	0.022	0.00
rs10410544	AA	57.1 (2.98)		7.47 (1.13–13.82)
GSR	CC	51.04 (1.56)	0.81	0.00
rs1002149	(CA + AA)	50.43 (1.76)		−0.60 (−5.44–4.23)
KEAP1 rs1048290	GG	49.66 (1.88)	0.5	0.00
	(GC + CC)	51.34 (1.53)		1.68 (−3.19–6.54)
PTGS1	TT	48.33 (1.82)	0.061	0.00
rs1330344	(TC + CC)	52.84 (1.56)		4.51 (−0.19–9.20)
TXNRD2 rs1139793	(CC + CT)	49.56 (1.65)	0.2	0.00
	TT	52.67 (1.76)		3.11 (−1.64–7.86)
HSPA1A	AA	50.53 (1.31)	0.69	0.00
rs1008438	(AC + CC)	54.96 (3.77)		1.00 (−3.95–5.96)
PTGR1	GG	51.64 (1.64)	0.65	0.00
rs2273788	(GA + AA)	50.54 (1.86)		−1.11 (−5.95–3.73)
MPO	GG	50.97 (1.6)	0.94	0.00
rs2333227	(GA + AA)	51.16 (1.81)		0.19 (−4.6–4.98)

Notes: Data presented are beta, mean and standard error with two-sided P values.

[a] Linear regression analysis adjusting for age, gender, BMI, and smoking status.

Our study revealed an association of *GSR* polymorphism (c.–386C > A, rs1002149) and COPD in the general population sample (Table 4); this association was also confirmed in the subgroup of non-smokers (Table 8). The rs1002149 polymorphism is located in the *GSR* promoter region and may affect the level of the gene expression (Soerensen et al., 2012). Glutathione-disulfide reductase (GSR), the enzyme encoded by *GSR*, reduces oxidized glutathione (Han et al., 2017). Most biological functions of glutathione rely on the transformation of its reduced form (GSH) into the oxidized form (GSSG) by glutathione peroxidase and its subsequent reversal to GSH mediated by NADPH-dependent glutathione reductase. The GSH/GSSG ratio is determined by the activity of these two enzymes and specifies the oxidative status of the cell; thus, GSR is a key enzyme of antioxidant defense (Soerensen et al., 2012; Han et al., 2017). It was found that GSR may play a key role in the regulation of drug resistance in tumor cells (Zhu et al., 2018). Previously, Pierrou et al. (2007) reported elevated GSR expression in the bronchial epithelium of COPD patients (Pierrou et al., 2007). Kopp et al. (2017) revealed an association between the *GSR* (rs1002149) and the level of the enzyme activity in erythrocytes, which was higher in carriers of the rare c.-386 A variant (Kopp et al., 2017). It was also shown that rare alleles of several genes, including *GSR* (rs1002149), were risk factors for certain age-associated diseases (Soerensen et al., 2012).

We also analyzed the intronic *SIRT2* polymorphism (c.–48–1025A > G, rs10410544), which, presumably, lies close to a functional gene variant (Gomes et al., 2015; Wei et al., 2014). It was found that homozygous carriers of the more frequent A allele of rs10410544 had significantly higher levels of FVC and FEV1 than heterozygotes and minor allele homozygotes (Table 9). The role of *SIRT2* in the development of COPD has not been studied previously. Sirtuins (SIRT1 and SIRT2) are involved in the development of age-associated diseases, such as type 2 diabetes mellitus, cardiovascular, COPD and neurodegenerative diseases (Liu et al., 2018; Hall et al., 2013; Porcelli et al., 2013; Taka et al., 2017; Xia et al., 2014; Y. Yang et al., 2017; W. Yang et al., 2017; Crocco et al., 2016). In particular, the rs10410544 polymorphism of *SIRT2* was found to be associated with Alzheimer's disease in Caucasian populations and in a Han Chinese population (Porcelli et al., 2013; Xia et al., 2014). The studies by W. Yang et al. (2017) and by Liu et al. (2018) revealed associations of the *SIRT2* functional promoter polymorphisms with acute myocardial infarction and type 2 diabetes mellitus (W. Yang et al., 2017; Liu et al., 2018). Crocco et al. (2016) reported data on the association of *SIRT2* polymorphisms with human longevity (Crocco et al., 2016). The results of our study, in agreement with the previously published data, indicate that certain *SIRT2* variants may constitute an important risk factor for diseases associated with chronic systemic inflammation and oxidative stress, such as COPD.

Our study also had some potential limitations. Present study was only restricted to a population of Tatars from Russia (the Volga-Ural region of Russia). Further large sample size studies with more diverse ethnic populations are required to replicate our results. Second, although our study suggested that *KEAP1* (rs1048290), *PRNP* (rs1799990), *HSPA1A* (rs1008438), *GSR* (rs1002149), *NFE2L2* (rs35652124), and *SIRT2* (rs10410544) were associated with the risk of COPD, more biological background data and functional studies are needed to explain the results.

In summary, we analyzed the associations of the NRF2/KEAP1 signaling pathway and antioxidant defense gene polymorphisms with COPD in population from Russia. For the first time, the data obtained indicate the contribution of *SIRT2*, *GSR*, *PRNP*, *HSPA1A*, and *KEAP1* polymorphisms to this disease. The association of *NFE2L2* polymorphisms with COPD was confirmed in a Tatar population from Russia. Further research aimed at elucidating the mechanisms of COPD pathogenesis should improve our understanding of hereditary predisposition to this disease and lead to identification of novel therapeutic targets.

CRediT author statement/authors' contributions

All authors critically commented and approved the manuscript.

Declaration of interest

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

CRediT authorship contribution statement

Gulnaz F. Korytina: Conceptualization, Funding acquisition, Writing - original draft, Writing - review & editing, Methodology, Project administration. **Leysan Z. Akhmadishina:** Methodology, Investigation, Validation. **Yulia G. Aznabaeva:** Methodology, Investigation, Resources. **Olga V. Kochetova:** Methodology, Investigation, Validation. **Naufal Sh. Zagidullin:** Methodology, Formal analysis, Visualization. **Julia G. Kzhyshkowska:** Writing - original draft, Writing - review & editing. **Shamil Z. Zagidullin:** Conceptualization, Supervision, Writing - review & editing. **Tatyana V. Viktorova:** Conceptualization, Supervision, Writing - review & editing.

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