Вавиловский журнал генетики и селекции. 2023;27(5):512-521
DOI 10.18699/VJGB-23-62

Original Russian text https://vavilovj-icg.ru/

Role of PI3K/AKT/mTOR signaling pathway and sirtuin genes in chronic obstructive pulmonary disease development

G.F. Korytina[1, 2] ✉, L.Z. Akhmadishina[1], V.A. Markelov[1, 2], Y.G. Aznabaeva[2], O.V. Kochetova[1], T.R. Nasibullin[1], A.P. Larkina[1], N.N. Khusnutdinova[1], N.Sh. Zagidullin[2], T.V. Victorova[2]

[1] Institute of Biochemistry and Genetics – Subdivision of the Ufa Federal Research Centre of the Russian Academy of Sciences, Ufa, Russia
[2] Bashkir State Medical University, Ufa, Russia
✉ guly_kory@mail.ru

Abstract. Chronic obstructive pulmonary disease (COPD) is a multifactorial disease of the respiratory system which develops as a result of a complex interaction of genetic and environmental factors closely related to lifestyle. We aimed to assess the combined effect of the PI3K/AKT/mTOR signaling pathway (*PIK3R1*, *AKT1*, *MTOR*, *PTEN*) and sirtuin (*SIRT1*, *SIRT3*, *SIRT6*) genes to COPD risk. SNPs of *SIRT1* (rs3758391, rs3818292), *SIRT3* (rs3782116, rs536715), *SIRT6* (rs107251), *AKT1* (rs2494732), *PIK3R1* (rs10515070, rs831125, rs3730089), *MTOR* (rs2295080, rs2536), *PTEN* (rs701848, rs2735343) genes were genotyped by real-time polymerase chain reaction (PCR) among 1245 case and control samples. 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. Significant associations with COPD were identified for *SIRT1* (rs3818292) ($P = 0.001$, OR = 1.51 for AG), *SIRT3* (rs3782116) ($P = 0.0055$, OR = 0.69) and *SIRT3* (rs536715) ($P = 0.00001$, OR = 0.50) under the dominant model, *SIRT6* (rs107251) ($P = 0.00001$, OR = 0.55 for CT), *PIK3R1*: (rs10515070 ($P = 0.0023$, OR = 1.47 for AT), rs831125 ($P = 0.00001$, OR = 2.28 for AG), rs3730089 ($P = 0.0007$, OR = 1.73 for GG)), *PTEN*: (rs701848 ($P = 0.0015$, OR = 1.35 under the log-additive model), and rs2735343 ($P = 0.0001$, OR = 1.64 for GC)). A significant genotype-dependent variation of lung function parameters was observed for *SIRT1* (rs3818292), *SIRT3* (rs3782116), *PIK3R1* (rs3730089), and *MTOR* (rs2536). Gene-gene combinations that remained significantly associated with COPD were obtained; the highest risk of COPD was conferred by a combination of G allele of the *PIK3R1* (rs831125) gene and GG of *SIRT3* (rs536715) (OR = 3.45). The obtained results of polygenic analysis indicate the interaction of genes encoding sirtuins *SIRT3*, *SIRT2*, *SIRT6* and *PI3KR1*, *PTEN*, *MTOR* and confirm the functional relationship between sirtuins and the PI3K/AKT/mTOR signaling pathway.
Key words: chronic obstructive pulmonary disease; PI3K/AKT/mTOR signaling pathway; sirtuins; cellular senescence; oxidative stress.

For citation: Korytina G.F., Akhmadishina L.Z., Markelov V.A., Aznabaeva Y.G., Kochetova O.V., Nasibullin T.R., Larkina A.P., Khusnutdinova N.N., Zagidullin N.Sh., Victorova T.V. Role of PI3K/AKT/mTOR signaling pathway and sirtuin genes in chronic obstructive pulmonary disease development. *Vavilovskii Zhurnal Genetiki i Selektsii = Vavilov Journal of Genetics and Breeding*. 2023;27(5):512-521. DOI 10.18699/VJGB-23-62

Роль генов PI3K/AKT/mTOR-сигнального каскада и сиртуинов в развитии хронической обструктивной болезни легких

Г.Ф. Корытина[1, 2] ✉, Л.З. Ахмадишина[1], В.А. Маркелов[1, 2], Ю.Г. Азнабаева[2], О.В. Кочетова[1], Т.Р. Насибуллин[1], А.П. Ларкина[1], Н.Н. Хуснутдинова[1], Н.Ш. Загидуллин[2], Т.В. Викторова[2]

[1] Институт биохимии и генетики – обособленное структурное подразделение Уфимского федерального исследовательского центра Российской академии наук, Уфа, Россия
[2] Башкирский государственный медицинский университет, Уфа, Россия
✉ guly_kory@mail.ru

Аннотация. Хроническая обструктивная болезнь легких (ХОБЛ) – многофакторное заболевание дыхательной системы, развивающееся в результате комплексного взаимодействия молекулярно-генетических и средовых факторов, тесно связанных с образом жизни. Цель исследования – анализ комбинированного вклада генов PI3K/AKT/mTOR-сигнального каскада (*PIK3R1*, *AKT1*, *MTOR*, *PTEN*) и сиртуинов (*SIRT1*, *SIRT3*, *SIRT6*) в риск развития хронической обструктивной болезни легких. В работе использованы образцы ДНК 1245 индивидов. Полиморфные варианты генов *SIRT1* (rs3758391, rs3818292), *SIRT3* (rs3782116, rs536715), *SIRT6* (rs107251), *AKT1* (rs2494732), *PIK3R1* (rs10515070, rs831125, rs3730089), *MTOR* (rs2295080, rs2536), *PTEN* (rs701848, rs2735343) анализировали методом полимеразной цепной реакции в реальном времени. Логистическую ре-

грессию использовали для выявления ассоциации полиморфных локусов в различных моделях. Проводили линейный регрессионный анализ для оценки вклада генотипов изучаемых локусов в вариабельность показателей функции внешнего дыхания и индекса курения. Установлена ассоциация генов: *SIRT1* (rs3818292) (*P* = 0.001, OR = 1.51 для генотипа AG), *SIRT3* (rs3782116) (*P* = 0.0055, OR = 0.69) и *SIRT3* (rs536715) (*P* = 0.00001, OR = 0.50) в доминантной модели; *SIRT6* (rs107251) (*P* = 0.00001, OR = 0.55 для генотипа CT), *PIK3R1*: (rs10515070 (*P* = 0.0023, OR = 1.47 для генотипа AT), rs831125 (*P* = 0.00001, OR = 2.28 для генотипа AG), rs3730089 (*P* = 0.0007, OR = 1.73 для генотипа GG)) и *PTEN*: (rs701848 (*P* = 0.0015, OR = 1.35 в лог-аддитивной модели) и rs2735343 (*P* = 0.0001, OR = 1.64 для генотипа GC)). Обнаружена вариабельность показателей функции легочного дыхания в зависимости от полиморфных вариантов генов *SIRT1* (rs3818292), *SIRT3* (rs3782116), *PIK3R1* (rs3730089) и *MTOR* (rs2536). Идентифицированы ген-генные сочетания, ассоциированные с ХОБЛ; наибольший риск развития ХОБЛ определялся сочетанием аллеля G гена *PIK3R1* (rs831125) с генотипом GG гена *SIRT3* (rs536715) (OR = 3.45). Полученные результаты полигенного анализа указывают на взаимодействие генов, кодирующих сиртуины *SIRT3*, *SIRT2*, *SIRT6* и *PI3KR1*, *PTEN*, *MTOR*, и находят подтверждение в функциональной взаимосвязи сиртуинов и PI3K/AKT/mTOR-сигнального каскада.
Ключевые слова: хроническая обструктивная болезнь легких; PI3K/AKT/mTOR-сигнальный каскад; сиртуины; клеточное старение; окислительный стресс.

Introduction

Chronic obstructive pulmonary disease (COPD) is a multifactorial respiratory system disease that affects the distal parts of the respiratory tract (bronchi, bronchioles) and lung parenchyma with lung emphysema development (Chuchalin et al., 2022). Chronic obstructive pulmonary disease develops as a result of complex interaction between molecular genetic and environmental factors, closely related to lifestyle, and smoking is considered the main cause of COPD (Ragland et al., 2019). Published data suggest that the COPD pathogenesis may involve dysregulation of stress responses that inhibit cellular senescence, which includes a wide range of signaling cascades and their regulators (Ryter et al., 2018).

The PI3K/AKT/mTOR intracellular signaling pathway is a universal pathway controlling cell growth, metabolism, and proliferation (Ersahin et al., 2015). The key components of this signaling pathway are phosphatidylinositol-3 kinase (PI3K), serine/threonine protein kinase (AKT), and serine/threonine kinase (mammalian target of rapamycin, mTOR) (Ersahin et al., 2015). Signal transduction through the PI3K/AKT/mTOR signaling cascade is essential for cellular senescence. This signaling pathway is inhibited by the tyrosine phosphatases PTEN (phosphatase and tensin homolog) and SHIP-1 (inositol polyphosphate-5-phosphatase D). Both enzymes have oxidation-sensitive cysteine residue in the active region (Worby, Dixon, 2014).

Oxidative stress is the main mechanism that causes accelerated senescence through its damaging effects on DNA and the PI3K/AKT/mTOR signaling pathway activation (Wang et al., 2013). In COPD and other age-associated diseases, the expression of genes encoding endogenous antioxidant molecules is reduced, which leads to an increased level of oxidative stress and cellular senescence activation (Kirkham, Barnes, 2013). NAD-dependent protein deacetylases from the sirtuins family are considered as potential factors that decrease senescence (Ito, Barnes, 2009).

We aimed to assess the combined effect of the PI3K/AKT/mTOR signaling pathway (*PIK3R1*, *AKT1*, *MTOR*, *PTEN*) and sirtuins (*SIRT1*, *SIRT3*, *SIRT6*) genes on COPD risk.

Materials and methods

DNA samples were collected from unrelated subjects who were Tatars in ethnicity and resided in the Republic of Bashkortostan. The study was approved by the Ethics Committee at the Institute of Biochemistry and Genetics (Protocol No 17, December 7, 2010). All participants of this study provided written informed consent. The COPD group included 621 patients (539 (86.79 %) males and 82 (13.21 %) females) with a mean age of 64.42 ± 10.71 years. There were 510 (82.13 %) smokers and former smokers and 111 (17.87 %) nonsmokers in the COPD group. The smoking index was 45.34 ± 23.84 pack years in the smokers and former smokers. The control group included 624 subjects (555 (88.94 %) males and 69 (11.06 %) females) with a mean age of 59.67 ± 12.31. There were 526 (84.29 %) smokers and former smokers and 98 (15.71 %) nonsmokers in the group; the smoking index was 38.75 ± 24.87 pack years in the smokers.

In all patients, spirometry was performed to assess lung function, including vital capacity (VC), forced vital capacity (FVC), forced expiration volume in the first second (FEV1), and the FEV1/FVC ratio. The group of patients had the following parameters (% of normal levels): FEV1 = 40.75 ± 18.33; FVC = 45.01 ± 18.22; VC = 49.32 ± 14.34; FEV1/FVC = 59.5 ± 12.34. Inclusion and exclusion criteria for the COPD and control have been previously described (Korytina et al., 2019).

Genotyping. DNA was isolated from peripheral blood leukocytes by phenol-chloroform extraction. The set included SNPs of the following genes: *SIRT1* (rs3758391, rs3818292), *SIRT3* (rs3782116, rs536715), *SIRT6* (rs107251), *AKT1* (rs2494732), *PIK3R1* (rs10515070, rs831125, rs3730089), *MTOR* (rs2295080, rs2536), *PTEN* (rs701848, rs2735343) (Supplementary Material 1)[1].

The SNPs were selected for the study based on the following criteria: functional effect of SNP on gene expression or relation to non-synonymous substitutions, and/or associations with complex human diseases; minor allele frequency

[1] Supplementary Materials 1 and 2 are available in the online version of the paper: http://vavilov.elpub.ru/jour/manager/files/Suppl_Korytina_Engl_27_5.pdf

(MAF) of ≥ 5 % in the European populations according to the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/projects/SNP/). The functional significance of the SNPs was verified using RegulomeDB Version 1.1 (https://regulomedb.org), SNPinfo Web Server (https://snpinfo.niehs.nih.gov), and HaploReg v3 (Ward, Kellis, 2016). Data were presented in Supplementary Material 2. SNP genotyping was performed by real-time polymerase chain reaction (PCR) using commercial kits for fluorescence detection (https://www.oligos.ru, DNK-Sintez, Russia) and a BioRad CFX96™ instrument (Bio-Rad Laboratories, United States). The methods of analysis were described in detail previously (Korytina et al., 2019).

Statistical analyses. Statistical analyses of the results were performed using the software packages IBM SPSS Statistics 22.0 and PLINK v. 1.07 (Purcell et al., 2007). The methods were described in detail previously (Korytina et al., 2019). Association analyses of allele or genotype combinations with COPD were carried out using the APSampler 3.6.1 program (http://sourceforge.net/projects/apsampler/). The Benjiamini–Hochberg correction for multiple testing was performed using special software (http://www.sdmproject.com/utilinies/?show=FDR) to decrease the false discovery rate (FDR) and to obtain $P_{cor\text{-}FDR}$. The linkage disequilibrium structure LD (D′) and haplotype frequencies were calculated with Haploview 4.2.

Results

Before analyzing the association of candidate gene alleles with COPD, we verified whether the genotype frequency distributions corresponded to the Hardy–Weinberg equilibrium and evaluated minor allele frequencies (MAF) both in the combined group of patients and healthy subjects and in either group individually (see Supplementary Material 1). All studied SNPs were in Hardy–Weinberg equilibrium in the control group: *SIRT1* (rs3758391) ($P_{X\text{-}B}$ = 0.24), *SIRT1* (rs3818292) ($P_{X\text{-}B}$ = 0.47), *SIRT3* (rs3782116) ($P_{X\text{-}B}$ = 0.5), *SIRT3* (rs536715) ($P_{X\text{-}B}$ = 0.75), *SIRT6* (rs107251) ($P_{X\text{-}B}$ = 0.67), *AKT1* (rs2494732) ($P_{X\text{-}B}$ = 0.2), *PIK3R1* (rs10515070) ($P_{X\text{-}B}$ = 0.65), *PIK3R1* (rs831125) ($P_{X\text{-}B}$ = 0.25), *PIK3R1* (rs3730089) ($P_{X\text{-}B}$ = 0.22), *MTOR* (rs2295080) ($P_{X\text{-}B}$ = 0.15), *MTOR* (rs2536) ($P_{X\text{-}B}$ = 0.24), *PTEN* (rs701848) ($P_{X\text{-}B}$ = 0.85), *PTEN* (rs2735343) ($P_{X\text{-}B}$ = 0.06).

The groups of COPD patients and healthy controls differed significantly in the genotypes and/or alleles frequency distributions of *SIRT1* (rs3818292), *SIRT3* (rs3782116, rs536715), *SIRT6* (rs107251), *AKT1* (rs2494732), *PIK3R1* (rs10515070, rs831125, rs3730089), and *PTEN* (rs701848, rs2735343) (Table 1). Statistically significant results of association analysis of the studied gene loci and COPD are shown in Table 2.

An association of *SIRT1* (rs3818292) with COPD was established in the dominant model (P_{adj} = 0.0066, OR = 1.40). The risk of COPD was increased in heterozygous individuals (P_{adj} = 0.001, OR = 1.51). An association of COPD with the heterozygous genotype (P_{adj} = 0.0052, OR = 0.69) and in the dominant model of *SIRT3* (rs3782116) and in the dominant (P_{adj} = 0.00001, OR = 0.50), log-additive (P_{adj} = 0.00001, OR = 0.66) models and with the heterozygous genotype (P_{adj} = 0.00001, OR = 0.48) of *SIRT3* (rs536715). It should be noted that in both cases the COPD risk was associated with the frequent G allele (rs3782116 – OR = 1.21 95 % CI 1.03–1.43 и rs536715 – OR = 1.58 95 % CI 1.32–1.91) and the GG genotype (rs3782116 – OR = 1.44 95 % CI 1.16–1.81 и rs536715 – OR = 1.99 95 % CI 1.58–2.51).

We carried out a linkage disequilibrium analysis of the rs3758391 and rs3818292 loci of the *SIRT1*, rs3782116 and rs536715 of the *SIRT3*, which showed the absence of linkage disequilibrium between the loci of the *SIRT1* gene (D′ = 0.168, r^2 = 0.097) and the *SIRT3* gene (D′ = 0.28, r^2 = 0.011). Based on the obtained data, haplotypes association analysis was not performed. Association of *SIRT6* (rs107251) with the development of COPD was detected in the dominant model (P_{adj} = 0.0005, OR = 0.65), but the association with the heterozygous CT genotype was more significant (P_{adj} = 0.00001, OR = 0.55). The risk of COPD was associated with the CC genotype of *SIRT6* (rs107251) (OR = 1.54 95 % CI 1.23–1.93).

We have identified the association SNPs of the *PIK3R1* gene (rs10515070, rs831125, rs3730089) with COPD. The risk of COPD was associated with heterozygous genotypes of the studied SNPs of the *PIK3R1* gene: rs10515070 (P_{adj} = 0.0023, OR = 1.47), rs831125 (P_{adj} = 0.00001, OR = 2.28) and with the GG genotype of rs3730089 (P_{adj} = 0.0007, OR = 1.73). We showed the absence of linkage disequilibrium between rs10515070, rs831125, rs3730089 of the *PIK3R1* gene: for rs10515070 and rs831125 (D′ = 0.02, r^2 = 0.00), for rs10515070 and rs3730089 (D′ = 0.127, r^2 = 0.008), for rs831125 and rs3730089 (D′ = 0.155, r^2 = 0.005).

Based on the obtained data, haplotypes association analysis was not performed. Association of *PTEN* (rs701848) and COPD was established in the dominant (P_{adj} = 0.0035, OR = 1.52), recessive (P_{adj} = 0.028, OR = 1.44) and log-additive models P_{adj} = 0.0015, OR = 1.35). We have identified the association of *PTEN* (rs2735343) with COPD in the log-dominant model (P_{adj} = 0.01, OR = 1.42) and for the heterozygous genotype (P_{adj} = 0.0001, OR = 1.64). Linkage disequilibrium between the rs701848 and rs2735343 was not detected (D′ = 0.234, r^2 = 0.035), thus, haplotype association analysis was not performed.

Association of the studied genes loci and quantitative phenotypes with lung function parameters and smoking index

Lung function decline is a key clinical feature of airway obstruction in COPD that indicates progression of the disease. We investigated the relationship between the studied genes loci and lung function parameters: Forced Vital Capacity (FVC), Forced Expiration Volume in 1 s (FEV1), and FEV1/FVC ratio in COPD patients (Table 3). The heterozygous genotype of *PIK3R1* (rs3730089) (P = 0.013) and the TT genotype of *MTOR* (rs2536) (P = 0.013) were associated with a decrease in the FVC value. Carriers of the *SIRT3* (rs3782116) AA genotype exhibited a higher FVC value (P = 0.0015).

Individuals that presented the AA genotype of *SIRT1* (rs3818292) (P = 0.017), the GG genotype of *PIK3R1* (rs3730089) (P = 0.025), and the AA genotype of *SIRT3* (rs3782116) (P = 0.0028) showed a significant increase in their FVC. Meanwhile, carriers of the heterozygous genotypes of *SIRT1* (rs3818292) (P = 0.04), *MTOR* (rs2295080) (P = 0.025), and *SIRT3* (rs3782116) (P = 0.016) exhibited a lower FVC

Table 1. Genotypes and alleles distribution of the studied PI3K/AKT/mTOR signaling pathway and sirtuins genes loci in COPD and control

Gene, SNP	Rare allele	Genotypes and alleles	COPD, *n* (%) (*N* = 621)	Control, *n* (%) (*N* = 624)	*P*
SIRT1 rs3758391 T>C	C	TT/TC/CC	184/265/172 (29.63/42.67/27.70)	168/294/162 (26.92/47.12/25.96)	0.283
		T/C	633/609 (50.97/49.03)	630/618 (50.48/49.52)	0.84
SIRT1 rs3818292 A>G	G	AA/AG/GG	322/273/26 (51.85/43.96/4.19)	375/213/36 (60.10/34.13/5.77)	0.001
		A/G	917/325 (73.83/26.17)	963/285 (77.16/22.84)	0.059
SIRT3 rs3782116 G>A	A	GG/GA/AA	294/230/97 (47.34/37.04/15.62)	239/287/98 (38.30/45.99/15.71)	0.003
		G/A	818/424 (65.86/34.14)	765/483 (61.30/38.70)	0.02
SIRT3 rs536715 G>A	A	GG/GA/AA	424/148/49 (68.28/23.83/7.89)	324/249/51 (51.92/39.90/8.17)	0.00001
		G/A	996/246 (80.19/19.81)	897/351 (71.88/28.13)	0.00001
SIRT6 rs107251 C>T	T	CC/CT/TT	396/160/65 (63.77/25.76/10.47)	333/243/48 (53.37/38.94/7.69)	0.0001
		C/T	952/290 (76.65/23.35)	909/339 (72.84/27.16)	0.032
AKT1 rs2494732 T>C	C	TT/CT/CC	193/313/115 (31.08/50.40/18.52)	159/329/136 (25.48/52.72/21.79)	0.062
		T/C	699/543 (56.28/43.72)	647/601 (51.84/48.16)	0.029
PIK3R1 rs10515070 T>A	A	TT/TA/AA	175/351/95 (28.18/56.52/15.30)	228/293/103 (36.54/46.96/16.51)	0.001
		T/A	701/541 (56.44/43.56)	749/499 (60.02/39.98)	0.077
PIK3R1 rs831125 A>G	G	AA/AG/GG	299/274/48 (48.15/44.12/7.73)	441/161/22 (70.67/25.80/3.53)	0.00001
		A/G	872/370 (70.21/29.79)	1 043/205 (83.57/16.43)	0.00001
PIK3R1 rs3730089 G>A	G	AA/AG/GG	230/257/134 (37.04/41.38/21.58)	270/269/85 (43.27/43.11/13.62)	0.0001
		A/G	717/525 (57.73/42.27)	809/439 (64.82/35.18)	0.0001
MTOR rs2295080 T>G	G	TT/TG/GG	220/304/97 (35.43/48.95/15.62)	218/318/88 (34.94/50.96/14.10)	0.686
		T/G	744/498 (59.90/40.10)	754/494 (60.42/39.58)	0.825
MTOR rs2536 T>C	C	TT/TC/CC	580/41/0 (93.40/6.60/0)	567/55/2 (90.87/8.81/0.32)	0.124
		T/C	1 201/41 (96.70/3.30)	1 189/59 (95.27/4.73)	0.087
PTEN rs701848 T>C	C	TT/CT/CC	156/324/141 (25.12/52.17/22.71)	212/306/106 (33.97/49.04/16.99)	0.0001
		T/C	636/606 (51.21/48.79)	730/518 (58.49/41.51)	0.0001
PTEN rs2735343 G>C	C	GG/GC/CC	169/355/97 (27.21/57.17/15.62)	217/280/127 (34.78/44.87/20.35)	0.0001
		G/C	693/549 (55.80/44.20)	714/534 (57.21/42.79)	0.52

Note. *P* is the significance of group differences in allele and genotype frequencies (sample χ^2 homogeneity test).

Table 2. Significant association of the studied PI3K/AKT/mTOR signaling pathway and sirtuins genes loci with COPD

Gene, SNP	Rare allele	*N*	Genotype/model	OR_{adj} (CI 95 %)	P_{adj}	$P_{cor\text{-}FDR}$
SIRT1 rs3818292 A>G	G	1245	AA AG+GG dominant	1.00 1.40 (1.10–1.78)	0.0066	0.0093
			AA+GG AG	1.00 1.51 (1.18–1.94)	0.001	0.0024
			Log-additive	1.21 (0.99–1.49)	0.063	0.068
			G	1.19 (0.99–1.44)	0.059	0.062
SIRT3 rs3782116 G>A	A	1245	GG GA+AA dominant	1.00 0.69 (0.53–0.90)	0.0055	0.008
			AA+GG AG	1.00 0.69 (0.53–1.90)	0.0052	0.008
			Log-additive	0.84 (0.7–1.00)	0.055	0.061
			A	0.82 (0.69–0.96)	0.02	0.03
SIRT3 rs536715 G>A	A	1245	GG GA+AA dominant	1.00 0.50 (0.39–0.65)	0.00001	3.85e-05
			AA+GG AG	1.00 0.48 (0.36–0.62)	0.00001	3.85e-05
			Log-additive	0.66 (0.55–0.80)	0.00001	3.85e-05
			A	0.63 (0.52–0.76)	0.00001	3.85e-05
SIRT6 rs107251 C>T	T	1245	CC CT+TT dominant	1.00 0.65 (0.51–0.83)	0.0005	0.0015
			CC+TT CT	1.00 0.55 (0.42–0.71)	0.00001	3.85e-05
			Log-additive	0.84 (0.70–1.00)	0.051	0.059
			T	0.82 (0.68–0.97)	0.032	0.039
PIK3R1 rs10515070 T>A	A	1245	TT TA+AA dominant	1.00 1.47 (1.13–1.92)	0.0042	0.007
			TT+AA TA	1.00 1.47 (1.15–1.88)	0.0023	0.0044
			Log-additive	1.17 (0.98–1.41)	0.087	0.09
			A	1.15 (0.98–1.36)	0077	0.088
PIK3R1 rs831125 A>G	G	1245	AA AG+GG dominant	1.00 2.61 (1.99–3.42)	0.00001	3.85e-05
			AA+GG AG	1.00 2.28 (1.73–3.00)	0.00001	3.85e-05
			Log-additive	2.19 (1.74–2.75)	0.00001	3.85e-05
			G	2.16 (1.78–2.62)	0.00001	3.85e-05
PIK3R1 rs3730089 G>A	G	1245	AA AG+GG dominant	1.00 1.30 (1.02–1.66)	0.037	0.045
			AA+AG GG recessive	1.00 1.73 (1.26–2.39)	0.0007	0.0018
			Log-additive G	1.31 (1.11–1.55) 1.35 (1.15–1.58)	0.0014 0.0001	0.003 0.00033
PTEN rs701848 T>C	C	1245	TT TC+CC dominant	1.00 1.52 (1.15–2.02)	0.0035	0.006
			TT+TC CC recessive	1.00 1.44 (1.04–1.99)	0.028	0.036
			Log-additive	1.35 (1.12–1.63)	0.0015	0.003
			C	1.34 (1.14–1.57)	0.0001	0.00033
PTEN rs2735343 G>C	C	1245	GG GC+CC dominant	1.00 1.42 (1.09–1.87)	0.01	0.013
			GG+CC GC	1.00 1.64 (1.28–2.12)	0.0001	0.00033
			Log-additive	1.06 (0.88–1.27)	0.53	0.53
			C	1.06 (0.9–1.24)	0.52	0.522

Note. N is the number of individuals included in the analysis; P_{adj} – significance in the likelihood ratio test for the regression model adjusted for age, sex, smoking status and pack-years; OR_{adj} – adjusted odds ratio and CI 95 % – confidence interval; $P_{cor\text{-}FDR}$ – significance after the FDR correction; in the log-additive model per rare allele dosage, the rare allele dosage increases in the following order: homozygote for the common allele (0) – heterozygote, (1) – homozygote for the rare allele (2).

Table 3. Association of the PI3K/AKT/mTOR signaling pathway and sirtuins gene loci with lung function parameters and smoking index

Gene, SNP	Genotypes	M ± SE	*P*	beta (CI 95 %)
FVC				
MTOR rs2536 T>C	TT CT	53.76 (1.03) 64.79 (4.02)	0.013	0.00 11.03 (2.38–19.67)
PIK3R1 rs3730089 G>A	AA+GG AG	56.5 (1.31) 51.4 (1.51)	0.013	0.00 –5.10 (–9.09…–1.11)
SIRT3 rs3782116 G>A	GG+AG AA	53.31 (1.1) 62.59 (3.06)	0.0015	0.00 9.28 (3.61–14.95)
FEV1				
SIRT1 rs3818292 A>G	AA AG+GG	56.06 (1.45) 51.06 (1.51)	0.017	0.00 –5.00 (–9.10…–0.90)
	AA+GG AG	55.52 (1.39) 51.35 (1.59)	0.04	0.00 –4.17 (–8.31…–0.03)
MTOR rs2295080 T>G	TT+GG GT	57.42 (1.61) 52.09 (1.75)	0.025	0.00 –5.34 (–9.99…–0.68)
PIK3R1 rs3730089 G>A	AA+AG GG	53.15 (1.13) 61.18 (2.57)	0.0026	0.00 8.03 (2.83–13.23)
SIRT3 rs3782116 G>A	GG+AG AA	54.6 (1.2) 63.95 (3.23)	0.0028	0.00 9.36 (3.27–5.45)
	GG+AA AG	58.28 (1.5) 52.61 (1.73)	0.016	0.00 –5.67 (–10.24…–1.10)
Smoking index in pack-years				
PIK3R1 rs10515070 T>A	TT+AA AT	31.61 (1.3) 31.00 (1.11)	0.025	0.00 –3.30 (–6.17…–0.42)
PIK3R1 rs831125 A>G	AA+GG AG	30.85 (1.08) 32.48 (1.61)	0.0082	0.00 –4.37 (–7.60…–1.14)
SIRT1 rs3818292 A>G	AA AG+GG	32.00 (1.13) 31.03 (1.25)	0.036	0.00 –3.04 (–5.88…–0.20)

Note. M ± SE is the mean ± standard error of the mean; *P* is the significance level for the regression equation; beta (95 % CI) is the regression coefficient (95 % confidence interval of the coefficient).

value (see Table 3). The carriers of the heterozygous genotype of *PIK3R1 (*rs831125) (*P* = 0.0082), the AA genotype of *SIRT1* (rs3818292) (*P* = 0.036) had a significantly higher smoking index.

Analysis of gene-gene interactions

Using the APSampler algorithm, we have identified gene-gene combinations significantly associated with chronic obstructive pulmonary disease. In order to identify significant interactions of functionally related sirtuins genes, *SIRT2* (rs10410544) was included in the analysis (Korytina et al., 2019). We obtained 2324 patterns associated with chronic obstructive pulmonary disease. Table 4 shows the results of the most significant gene-gene combinations with P_{FDR} less than 0.05 and OR more than 2.0 for risk combinations) or less than 0.35 for protective combinations. A total of 19 gene-gene combinations fulfilled the above-mentioned criteria. Nine patterns were associated with an increased risk of COPD; ten were protective. Allele G and genotype GG of *PIK3R1* (rs831125) contributed to the most significant combinations associated with COPD risk (four patters).

The highest risk of COPD was conferred by the combination of these variants of *PIK3R1* (rs831125) with the GG genotype of *SIRT3* (rs536715) (OR = 3.45); and with the C allele of *PTEN* (rs2735343) (OR = 3.06) and their combination: genotype GA of *PIK3R1* (rs831125) together with the G allele of *SIRT3* (rs536715) and the C allele of *PTEN* (rs2735343) (OR = 2.86). The analysis on the gene-gene interaction of the studied gene loci established an association of the T allele of *MTOR* (rs2536) only in combinations with the G allele of *PIK3R1* (rs831125) (OR = 2.71). Three of the identified patterns included the G allele of *PIK3R1* (rs3730089) in

Table 4. Gene-gene combinations of the PI3K/AKT/mTOR signaling pathway and sirtuins gene loci associated with COPD

Pattern	COPD patients	Healthy individuals	*P*	P_{FDR}	OR	CI (95 %)
Risk patterns						
PIK3R1 (rs831125) G + *SIRT3* (rs536715) GG	0.36	0.12	1.06e-14	2.45e-11	3.45	2.49–4.78
PIK3R1 (rs831125) G + *MTOR* (rs2536) T	0.53	0.29	7.65e-13	1.77e-09	2.71	2.05–3.57
PIK3R1 (rs831125) G + *PTEN* (rs2735343) C	0.39	0.17	7.7e-13	1.79e-09	3.06	2.23–4.18
PIK3R1 (rs831125) GA + *SIRT3* (rs536715) G + *PTEN* (rs2735343) C	0.31	0.14	9.07e-10	2.10e-06	2.86	2.02–4.04
SIRT3 (rs536715) GG + *PTEN* (rs2735343) CG	0.61	0.42	2.98e-09	6.92e-06	2.17	1.67–2.83
PIK3R1 (rs3730089) G + *SIRT3* (rs536715) GG	0.46	0.29	1.14e-08	2.64e-05	2.09	1.62–2.72
PIK3R1 (rs3730089) G + *SIRT6* (rs107251) CC	0.45	0.29	4.13e-08	9.60e-05	2.01	1.56–2.59
PIK3R1 (rs10515070) A + *PTEN* (rs701848) C + *PTEN* (rs2735343) C	0.39	0.23	1.11e-06	0.0025	2.10	1.54–2.85
Protective patterns						
PIK3R1 (rs831125) AA + *PIK3R1* (rs3730089) A	0.34	0.61	1.11e-16	2.58e-13	0.32	0.24–0.42
PIK3R1 (rs831125) A + *PIK3R1* (rs3730089) A + *SIRT3* (rs3782116) A + *SIRT3* (rs536715) A	0.03	0.22	2.7e-15	6.27e-12	0.13	0.07–0.24
PIK3R1 (rs831125) A + *SIRT3* (rs3782116) A + *SIRT3* (rs536715) A	0.06	0.25	1.07e-14	2.49e-11	0.18	0.11–0.29
PIK3R1 (rs831125) A + *PIK3R1* (rs3730089) A + *SIRT3* (rs3782116) AG + *SIRT3* (rs536715) A	0.02	0.17	3.82e-14	8.87e-11	0.09	0.04–0.20
PIK3R1 (rs3730089) A + *SIRT3* (rs3782116) A + *SIRT3* (rs536715) A	0.06	0.22	2.3e-13	5.36e-10	0.21	0.13–0.32
SIRT3 (rs3782116) A + *SIRT3* (rs536715) A	0.08	0.26	6.52e-13	1.52e-09	0.25	0.17–0.38
SIRT3 (rs3782116) AG + *SIRT3* (rs536715) GA	0.04	0.18	4.0e-11	9.29e-08	0.20	0.12–0.35
SIRT3 (rs536715) A + *SIRT6* (rs107251) T	0.10	0.25	8.12e-11	1.89e-07	0.33	0.23–0.47
SIRT3 (rs536715) A + *PTEN* (rs2735343) GG	0.06	0.18	4.73e-09	1.09e-05	0.27	0.17–0.44
SIRT3 (rs536715) GA + *SIRT6* (rs107251) TC	0.06	0.17	9.75e-09	2.26e-05	0.30	0.19–0.47

Notes. *P*-value is the significance level for Fisher's test, P_{FDR} – is the FDR value after FDR correction; OR is odds ratio, 95 % CI is the 95 % confidence interval for the OR.

combination with the GG genotype of *SIRT3* (rs536715) (OR = 2.09), or with the CC genotype of *SIRT6* (rs107251) (OR = 2.01).

The most significant protective patterns included the A allele or the AA genotype of *PIK3R1* (rs831125) and the A allele of *PIK3R1* (rs3730089) in combination with the A allele or the AG genotype of *SIRT3* (rs3782116) and the A allele of *SIRT3* (rs536715) (see Table 4). Thus, the *PIK3R1* (rs831125), *PIK3R1* (rs3730089), and *SIRT3* (rs536715) loci exhibited an allele-specific effect, when the G allele of *PIK3R1* (rs831125), the G allele of *PIK3R1* (rs3730089), and the G allele and the GG genotype of *SIRT3* (rs536715) were part of risk patterns, and alternative alleles of the same polymorphic loci were present in protective patterns.

Discussion

As a result our study, significant associations between the *SIRT1* (rs3818292), *SIRT3* (rs3782116, rs536715), *SIRT6* (rs107251) polymorphic variants and COPD were found. SIRT1 is the most studied member of the mammalian sirtuin family. It has been shown that SIRT1 plays an important role in signaling pathways involved in cellular senescence and cell death (Finkel et al., 2009). SIRT1 deacetylates many key regulatory proteins and transcription factors involved in DNA repair, inflammation, expression of antioxidant genes, and cellular senescence, including the PI3K/AKT/mTOR signaling pathway genes, transcription factor FOXO3a, p21, p16, Klotho proteins (Cao et al., 2013).

Increased expression of SIRT1 inhibits the TGF-β1/SMAD3 signaling pathway and impairs epithelial-mesenchymal transformation, which leads to a decrease in COPD-associated airway remodeling (Zhang et al., 2022). SIRT1 levels are reduced in peripheral pulmonary and peripheral blood mononuclear cells of patients with COPD (Rajendrasozhan et al., 2008).

We found that the COPD risk is higher in heterozygous carriers of *SIRT1* (rs3818292). Moreover, this polymorphic variant demonstrates an association with a decrease in FVC1,

which reflects the progression of the disease. Functional analysis showed that *SIRT1* (rs3818292) is in linkage with a SNP in the 5′-untranslated DNA region (rs3740051) that changes the NFKB transcription factor binding site. We did not associate *SIRT1* (rs3758391) with COPD. The results of our study are in agreement with the previously published data reported for the Han Chinese population (Gao et al., 2018). According to functional analysis, rs3758391 is located in the promoter region of the *SIRT1* gene, and the C variant disrupts binding sites for several transcription factors and regulatory proteins, affecting gene expression. The role of rs3758391 in the development of age-associated diseases is well known (Wu et al., 2022).

Mitochondrial dysfunction in respiratory epithelial cells plays an important role in the pathogenesis of COPD (Zhang et al., 2022). SIRT3 is the main mitochondrial deacetylase regulating many enzymes involved in energy metabolism, respiratory chain components, the tricarboxylic acid cycle, ketogenesis, and fatty acid beta-oxidation (Wu et al., 2022).

SIRT3 can directly control the production of reactive oxygen species (ROS) by deacetylating manganese superoxide dismutase (SOD2), the main mitochondrial antioxidant enzyme (Dikalova et al., 2017). SIRT3 is involved in regulating the activity of the DNA repair enzyme OGG1, which leads to increased damage to mtDNA and apoptosis of alveolar epithelial cells (Sun et al., 2018). A number of studies have shown the SIRT3 association with various complex diseases (Wu et al., 2022).

We studied the association between two *SIRT3* gene functional polymorphisms (rs3782116 and rs536715) and chronic obstructive pulmonary disease. Functional analysis showed that rs3782116 is located in the region of binding sites for hsa-miR-328; polymorphic loci rs3782116 and rs536715 are located in DNA regions that bind regulatory proteins. Both polymorphic loci were associated with COPD in our cohort; the disease development risk was associated with the G alleles of rs3782116 and rs536715. It should be noted that the carriers of the homozygous rare allele A of rs3782116 of the *SIRT3* gene had higher values of VC and FVC1. The contribution of *SIRT3* variants to COPD has not been studied previously. At the same time, effects of the *SIRT3* gene SNPs have been fairly extensively investigated in age-associated diseases in which oxidative stress and cellular senescence play a key role (Song et al., 2022).

We obtained significant associations of *SIRT6* (rs107251) with COPD; the frequent C allele is associated with COPD risk, while the heterozygous genotype has a protective effect on the disease development. rs107251 is located in the DNA region that binds to the SOX8 regulatory protein and it is in close linkage with rs350846, localized in the 3-non-translational region of the *SIRT6* gene – a binding site for several miRNAs (hsa-miR-1207-5p, hsa-miR-24, hsa-miR-34a, hsa-miR-644, hsa-miR-940). SIRT6 participates in the regulation of genome stability, NF-kB signaling, glucose homeostasis, exhibits ADP-ribosyltransferase and histone deacetylase activity, plays a role in DNA repair and maintenance of telomeric chromatin integrity (Kugel, Mostoslavsky, 2014). In the study by N. Takasaka et al. (2014), a decrease in SIRT6 levels was shown in respiratory epithelial cells of COPD patients due to cigarette smoke exposure, leading to cellular senescence and disruption of autophagy processes. Association of the *SIRT6* gene SNPs with COPD has not been studied previously, but there is evidence of their association with cardiovascular diseases, which are often comorbid pathologies in COPD (Song et al., 2022).

The *PIK3R1* gene encodes regulatory subunit 1 of phosphoinositide 3-kinase, a key element of the PI3K/AKT/mTOR signaling cascade (Ersahin et al., 2015). We investigated three *PIK3R1* gene polymorphic loci (rs10515070, rs831125, and rs3730089), which showed a significant association with COPD in our studied group. Carriers of rare alleles of these polymorphic loci had a high risk of COPD. In addition, we investigated the relationship between rs3730089 genotypes and VC and FVC1 values; thus, heterozygotes have lower values, and these results are in agreement with association analysis. Functional analysis showed that an intronic variant rs831125 is located in a binding site for regulatory proteins; rs3730089 is a missense variant with a "benign" effect according to the PolyPhen-2 database (http://genetics.bwh.harvard.edu/pph2/), located in a binding site for regulatory proteins and affecting splice sites. SNPs of the *PIK3R1* gene have not been evaluated for COPD before. Previously, an association has been observed between rs3730089 and type 2 diabetes (Karadoğan et al., 2018).

The phosphatase PTEN regulates the activity of phosphoinositide-3 kinase (PI3K) (Worby Dixon, 2014). Smoking as a major risk factor for COPD provokes oxidative stress, which, in turn, affects *PTEN* expression (Cai et al., 2022). We investigated two *PTEN* gene functional polymorphic loci; rs70184 is located in *PTEN* gene 3′ region and changes binding sites for hsa-miR-1252 and hsa-miR-1304 miRNA; rs2735343, located in the intronic region, affects binding sites for several regulatory proteins.

Significant associations with COPD in our sample were found with *PTEN* gene loci; thus, homozygous carriers of the rare C allele of rs701848 and heterozygous carriers of rs2735343 had a significant risk of COPD development.

Published data have demonstrated an association between *PTEN* (rs701848) and COPD; the risk was significantly reduced for homozygous T allele carriers, which is consistent with the data obtained for our sample (Hosgood et al., 2009). PTEN participates in the regulation of various biological processes, including cell proliferation, apoptosis, inflammatory reactions, transcription, and genomic stability (Cai et al., 2022). Decreased levels of PTEN lead to activation of PI3K signaling and increased cell senescence in COPD (Barnes et al., 2019). It has been shown that decreased PTEN activity in COPD increases the activity of matrix metalloprotease MMP9 in bronchial epithelial cells, which consequently contributes to the progression of inflammation and extracellular matrix degradation (Vannitamby et al., 2017).

The analysis of gene-gene interactions revealed significant synergy between polymorphic loci of genes encoding phosphoinositide-3-kinase (PIK3R1) and mitochondrial deacetylase (SIRT3), which were present in most significant combinations associated with an increased risk of chronic obstructive pulmonary disease. The C allele in the *PTEN* (rs2735343) was part of four informative combinations associated with a high risk of chronic obstructive pulmonary disease.

The results of polygenic analysis indicate the interaction of genes encoding sirtuins *SIRT3*, *SIRT2*, *SIRT6* and *PI3KR1*, *PTEN*, *MTOR* and confirm the functional relationship between sirtuins and the PI3K/AKT/mTOR signaling pathway.

Conclusion

The obtained results of single locus and polygenic analysis indicate the contribution of *SIRT3* (rs3782116, rs536715), *SIRT6* (rs107251) and *PIK3R1* (rs10515070, rs831125, rs3730089) polymorphisms to COPD and interaction of genes encoding the key components of the PI3K/AKT/mTOR signalling pathway and sirtuins, and confirm the involvement of cellular senescence mechanisms with COPD development.

References

Barnes P.J., Baker J., Donnelly L.E. Cellular senescence as a mechanism and target in chronic lung diseases. *Am. J. Respir. Crit. Care Med.* 2019;200(5):556-564. DOI 10.1164/rccm.201810-1975TR.

Cai B., Yang L., Do Jung Y., Zhang Y., Liu X., Zhao P., Li J. PTEN: An emerging potential target for therapeutic intervention in respiratory diseases. *Oxid. Med. Cell Longev*. 2022;2022:4512503. DOI 10.1155/2022/4512503.

Cao L., Liu C., Wang F., Wang H. SIRT1 negatively regulates amyloid-beta-induced inflammation via the NF-κB pathway. *Braz. J. Med. Biol. Res.* 2013;46(8):659-669. DOI 10.1590/1414-431X20132903.

Chuchalin A.G., Avdeev S.N., Aisanov Z.R., Belevskiy A.S., Leshchenko I.V., Ovcharenko S.I., Shmelev E.I. Federal guidelines on diagnosis and treatment of chronic obstructive pulmonary disease. *Pul'monologiya = Pulmonology*. 2022;32(3):356-392. DOI 10.18093/0869-0189-2022-32-3-356-392. (in Russian)

Dikalova A.E., Itani H.A., Nazarewicz R.R., McMaster W.G., Flynn C.R., Uzhachenko R., Fessel J.P., Gamboa J.L., Harrison D.G., Dikalov S.I. Sirt3 impairment and SOD2 hyperacetylation in vascular oxidative stress and hypertension. *Circ. Res*. 2017;121(5):564-574. DOI 10.1161/CIRCRESAHA.117.310933.

Ersahin T., Tuncbag N., Cetin-Atalay R. The PI3K/AKT/mTOR interactive pathway. *Mol. Biosyst*. 2015;11(7):1946-1954. DOI 10.1039/c5mb00101c.

Finkel T., Deng C.X., Mostoslavsky R. Recent progress in the biology and physiology of sirtuins. *Nature.* 2009;460(7255):587-591. DOI 10.1038/nature08197.

Gao S.L., Wang Y.H., Li C.Y., Yang L.W., Zou B.A., Chen Z.G., Yao W.M., Song Z.Q., Cheng J.F., Lin Z.Y., Liu G. A highly significant association between Cathepsin S gene polymorphisms rs12068264 and chronic obstructive pulmonary disease susceptibility in Han Chinese population. *Biosci. Rep.* 2018;38(4):BSR20180410. DOI 10.1042/BSR20180410.

Hosgood H.D., Menashe I., He X., Chanock S., Lan Q. *PTEN* identified as important risk factor of chronic obstructive pulmonary disease. *Respir. Med*. 2009;103(12):1866-1870. DOI 10.1016/j.rmed.2009. 06.016.

Ito K., Barnes P.J. COPD as a disease of accelerated lung aging. *Chest.* 2009;135(1):173-180. DOI 10.1378/chest.08-1419.

Karadoğan A.H., Arikoglu H., Göktürk F., İşçioğlu F., İpekçi S.H. PIK3R1 gene polymorphisms are associated with type 2 diabetes and related features in the Turkish population. *Adv. Clin. Exp. Med.* 2018;27(7):921-927. DOI 10.17219/acem/68985.

Kirkham P.A., Barnes P.J. Oxidative stress in COPD. *Chest.* 2013; 144(1):266-273. DOI 10.1378/chest.12-2664.

Korytina G.F., Akhmadishina L.Z., Aznabaeva Y.G., Kochetova O.V., Zagidullin N.S., Kzhyshkowska J.G., Zagidullin S.Z., Viktorova T.V. Associations of the NRF2/KEAP1 pathway and antioxidant defense gene polymorphisms with chronic obstructive pulmonary disease. *Gene.* 2019;692:102-112. DOI 10.1016/j.gene.2018.12.061.

Kugel S., Mostoslavsky R. Chromatin and beyond: the multitasking roles for SIRT6. *Trends Biochem. Sci*. 2014;39(2):72-81. DOI 10.1016/j.tibs.2013.12.002.

Purcell S., Neale B., Todd-Brown K., Thomas L., Ferreira M.A., Bender D., Maller J., Sklar P., de Bakker P.I., Daly M.J., Sham P.C. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet*. 2007;81(3):559-575. DOI 10.1086/519795.

Ragland M.F., Benway C.J., Lutz S.M., Bowler R.P., Hecker J., Hokanson J.E., Crapo J.D., Castaldi P.J., DeMeo D.L., Hersh C.P., Hobbs B.D., Lange C., Beaty T.H., Cho M.H., Silverman E.K. Genetic advances in chronic obstructive pulmonary disease. Insights from COPDGene. *Am J. Respir. Crit. Care. Med*. 2019;200(6):677-690. DOI 10.1164/rccm.201808-1455SO.

Rajendrasozhan S., Yang S.R., Kinnula V.L., Rahman I. SIRT1, an antiinflammatory and antiaging protein, is decreased in lungs of patients with chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 2008;177(8):861-870. DOI 10.1164/rccm.200708-1269OC.

Ryter S.W., Rosas I.O., Owen C.A., Martinez F.J., Choi M.E., Lee C.G., Elias J.A., Choi A.M.K. Mitochondrial dysfunction as a pathogenic mediator of chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis. *Ann. Am. Thorac. Soc*. 2018;15(Suppl. 4):S266-S272. DOI 10.1513/AnnalsATS.201808-585MG.

Song X., Wang H., Wang C., Ji G., Jiang P., Liang D., Wang X. Association of sirtuin gene polymorphisms with susceptibility to coronary artery disease in a North Chinese population. *Biomed. Res. Int.* 2022;2022:4294008. DOI 10.1155/2022/4294008.

Sun W., Liu C., Chen Q., Liu N., Yan Y., Liu B. SIRT3: A new regulator of cardiovascular diseases. *Oxid. Med. Cell Longev*. 2018;2018: 7293861. DOI 10.1155/2018/7293861.

Takasaka N., Araya J., Hara H., Ito S., Kobayashi K., Kurita Y., Wakui H., Yoshii Y., Yumino Y., Fujii S., Minagawa S., Tsurushige C., Kojima J., Numata T., Shimizu K., Kawaishi M., Kaneko Y., Kamiya N., Hirano J., Odaka M., Morikawa T., Nishimura S.L., Nakayama K., Kuwano K. Autophagy induction by SIRT6 through attenuation of insulin-like growth factor signaling is involved in the regulation of human bronchial epithelial cell senescence. *J. Immunol.* 2014;192(3):958-968. DOI 10.4049/jimmunol.1302341.

Vannitamby A., Seow H.J., Anderson G., Vlahos R., Thompson M., Steinfort D., Irving L.B., Bozinovski S. Tumour-associated neutrophils and loss of epithelial PTEN can promote corticosteroid-insensitive MMP-9 expression in the chronically inflamed lung microenvironment. *Thorax.* 2017;72(12):1140-1143. DOI 10.1136/thoraxjnl-2016-209389.

Wang C.H., Wu S.B., Wu Y.T., Wei Y.H. Oxidative stress response elicited by mitochondrial dysfunction: implica-

tion in the pathophysiology of aging. *Exp. Biol. Med.* 2013;238(5):450-460. DOI 10.1177/1535370213493069.

Ward L.D., Kellis M. HaploReg v4: systematic mining of putative causal variants, cell types, regulators and target genes for human complex traits and disease. *Nucleic Acids Res*. 2016;44(D1):D877-D881. DOI 10.1093/nar/gkv1340.

Worby C.A., Dixon J.E. PTEN. *Annu. Rev. Biochem*. 2014;83:641-669. DOI 10.1146/annurev-biochem-082411-113907.

Wu Q.J., Zhang T.N., Chen H.H., Yu X.F., Lv J.L., Liu Y.Y., Liu Y.S., Zheng G., Zhao J.Q., Wei Y.F., Guo J.Y., Liu F.H., Chang Q., Zhang Y.X., Liu C.G., Zhao Y.H. The sirtuin family in health and disease. *Signal Transduct. Target. Ther*. 2022;7(1):402. DOI 10.1038/s41392-022-01257-8.

Zhang X.Y., Li W., Zhang J.R., Li C.Y., Zhang J., Lv X.J. Roles of sirtuin family members in chronic obstructive pulmonary disease. *Respir. Res*. 2022; 23(1):66. DOI 10.1186/s12931-022-01986-y.

ORCID ID

G.F. Korytina orcid.org/0000-0002-1695-5173
L.Z. Akhmadishina orcid.org/0000-0003-0043-5090
V.A. Markelov orcid.org/0000-0002-0663-7219
Y.G. Aznabaeva orcid.org/0000-0002-1518-774X
O.V. Kochetova orcid.org/0000-0002-2944-4428
T.R. Nasibullin orcid.org/0000-0001-8823-8678
A.P. Larkina orcid.org/0009-0003-0710-6705
N.N. Khusnutdinova orcid.org/0000-0003-4127-078X
N.Sh. Zagidullin orcid.org/0000-0003-2386-6707
T.V. Victorova orcid.org/0000-0001-8900-2480

Acknowledgements. The study was performed with the support of the grant of Russian Science Foundation 23-25-00019 (http://rscf.ru/project/23-25-00019/). The work was performed using the equipment of the Centre for Collective Use "Biomika" and the unique KODINK research facility (Institute of Biochemistry and Genetics, Ufa Federal Research Centre, Russian Academy of Sciences).

Conflict of interest. The authors declare no conflict of interest.

Received March 20, 2023. Revised April 19, 2023. Accepted April 19, 2023.