ASSOCIATION OF FUNCTIONAL VARIANTS OF PHASE I AND II GENES WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE IN A SERBIAN POPULATION

UDRUŽENOST FUNKCIONALNIH VARIJANTI GENA FAZE I I II SA HRONIČNOM OPSTRUKTIVNOM BOLEŠĆU PLUĆA U SRPSKOJ POPULACIJI

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Summary

Background: Chronic obstructive pulmonary disease (COPD) is a complex disorder characterized by increased oxidative stress. Functional genetic variants of phase I and II genes are implicated in oxidants–antioxidants imbalance and may be involved in COPD development. In this study, we aimed to investigate the role of cytochrome P450 (CYP), glutathione S-transferase (GST) and microsomal epoxide hydrolase (mEH) functional variants in the pathogenesis of COPD in a Serbian population.

Methods: The genotypes of 122 COPD patients and 100 controls with normal lung function were determined for CYP1A1 *1A/*2A, CYP2E1 *1A/*5B, GSTM1 null, GSTT1 null GSTP1 Ile105Val, mEH Tyr113His and mEH His139Arg gene variants.

Results: Results obtained showed that GSTM1 null variant was significantly more represented in COPD patients than in controls (61.5% vs. 47.0%; OR=1.80; p=0.042). Also, a significant difference was observed for combinations of GSTM1 null and GSTP1 105Val/(Val) (38.5% vs. 24.0%; OR=1.98; p=0.029), as well as for CYP1A1 *1A/*2A,

Kratak sadržaj

Uvod: Hronična opstruktivna bolest pluća (HOBP) jeste složeno oboljenje koje karakteriše povišen oksidativni stres. Funkcionalne varijante gena faze I i II ksenobiotičkog metabolizma mogu uticati na ravnotežu oksidanti–antioksidanti i mogu dovesti do razvoja HOBP. Cilj ove studije je bio ispitivanje uloge funkcionalnih genskih varijanti u genima za citohrom P450 (CYP), glutation S-transferazu (GST) i mikrozomalnu epoksidnu hidrolazu (mEH) u patogenezi HOBP u srpskoj populaciji.

Metode: U ovoj studiji analizirane su genske varijante CYP1A1 *1A/*2A, CYP2E1 *1A/*5B, GSTM1 null, GSTT1 null, GSTP1 Ile105Val, mEH Tyr113His i mEH His139Arg u grupi obolelih od HOBP koja je obuhvatala 122 ispitanika i kontrolnoj grupi koja je obuhvatala 100 ispitanika sa normalnom funkcijom pluća.

Rezultati: Dobijeni rezultati su pokazali da je GSTM1 null varijanta statistički značajno povišena u grupi obolelih od HOBP u poređenju sa kontrolnom grupom (61,5% i 47,0%; OR=1,80; p=0,042). Takođe, uočena je značajna razlika u zastupljenosti kombinacije genotipova GSTM1

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List of abbreviations: COPD, chronic obstructive pulmonary disease; CYP, cytochrome P450; GST, glutathione S-transferase; mEH, microsomal epoxide hydrolase; PAH, polycyclic aromatic hydrocarbons; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; SABA, short-acting β2 agonist; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; OR, odds ratio; CI, confidence interval; SPSS, statistical package for the social sciences; NS, nonsignificant; ROS, reactive oxygen species.

GSTM1 null and mEH 113His/(His) genotypes (7.4% vs. 1.0%; OR=7.88; p=0.025).

Conclusions: These are the first data concerning the analysis of the variants of phase I and II genes in the pathogenesis of COPD in a Serbian population. Results obtained in this study open up the possibility for thorough analyses of the role of genetic factors in COPD on larger cohorts. Also, they implicate the importance of previously described genetic associations with COPD in our population, as well as reveal a new one, not reported so far.

Keywords: COPD, candidate gene association study, genetic variation, oxidative stress

null i GSTP1 105Val/(Val) (38,5% i 24,0%; OR=1,98; p=0,029), kao i kombinacije CYP1A1 *1A/*2A, GSTM1 null i mEH 113His/(His) (7,4% i 1,0%; OR=7,88; p=0,025).

Zaključak: Ovo su prvi podaci o ulozi genskih varijanti gena faze I i II u patogenezi HOBP u srpskoj populaciji. Rezultati dobijeni u ovoj studiji otvaraju mogućnost za detaljniju analizu uloge genetičkih faktora u HOBP na većim grupama ispitanika. Pored toga, podaci dobijeni u našoj studiji potvrđuju važnost genetičkih determinanti povezanih sa HOBP u prethodnim studijama, ali takođe otkrivaju nove genetičke faktore, koji nisu objavljeni do sada.

Ključne reči: HOBP, asocijativna studija gena kandidata, genska varijanta, oksidativni stres

Introduction

Chronic obstructive pulmonary disease (COPD) is a common preventable and treatable disease, characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammation response in the airways and the lung to noxious particles or gases, as defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD, 2011). COPD manifests in two major forms: chronic bronchitis and emphysema, that commonly coexist in the same individual (1).

Although cigarette smoking is the main risk factor for COPD, the role of genetic determinants is emphasized by the evidence that only 25% of continuous smokers develop this disease (2). Additionally, family and twin studies suggested genetic influence on pulmonary function and correlation of the rate of airflow obstruction in COPD relatives (3). Severe α1-antitrypsin deficiency demonstrates the influence of genetic determinants in the pathogenesis of COPD (3). However, only a small fraction of COPD cases could be explained by the deficiency of α1-antitrypsin, which is in accordance with the data for a Serbian population (4). Furthermore, exposure to indoor and outdoor air pollutants, infectious and noninfectious agents during childhood, as well as socioeconomic status might play an important role in COPD development (5). Altogether, COPD is a complex disease influenced by multiple genetic and environmental factors, including their interactions.

Foreign chemical compounds inhaled into the lung are rapidly absorbed and processed in the metabolism of xenobiotics composed of phase I and II enzyme systems. Phase I enzymes include the cytochrome P450 system (CYP) involved in the bioactivation, while phase II enzymes, glutathione S-transferase (GST) and microsomal epoxide hydrolase (mEH), are involved in the detoxification (6).

The CYP1A1 enables metabolic activation of procarcinogenic substances from tobacco smoke, including polycyclic aromatic hydrocarbons (PAH) and aromatic amines. T3801C (CYP1A1*2A) variant, in the 3' flanking region of the gene, is associated with two-fold higher enzyme activity (7). The CYP2E1 is implicated in the activation of nitrosamines from cigarette smoke. Two variants in the 5' flanking region of the CYP2E1 gene, in complete linkage disequilibrium, form the alleles CYP2E1 *1A (PstI – and RsaI +) and CYP2E1 *5B (PstI + and RsaI –). CYP2E1 *5B allele is linked with reduced enzyme activity and inducibility (8–10). The GSTs supergene family encodes isoenzymes that catalyse the conjugation of reduced glutathione with electrophilic compounds and metabolize toxic substrates in tobacco smoke, such as PAHs, methyl-halogens and diol-epoxides (11). In the GSTM1 and GSTT1 genes, a homozygous deletion variant (GSTM1 null and GSTT1 null) results in complete lack of enzymes activity. In the GSTP1 gene, transition at A+313G nucleotide leads to the Ile105Val amino acid substitution and altered catalytic activity (12). The mEH catalyzes the hydrolysis of arenes and aliphatic epoxides to dihydrodiols. Two functional variants in the mEH gene are Tyr113His, denoted as the slow allele and connected with 39% decreased enzyme activity, and His139Arg, denoted as the fast allele and connected with 25% increased enzyme activity (13).

Oxidative stress is recognized as a major predisposing factor in the COPD pathogenesis (14). Since genetic variants of phase I and II genes may be involved in the oxidants–antioxidants imbalance, their role in COPD pathogenesis was extensively studied. Nevertheless, data concerning the possible contribution of phase I and II gene variants in the development of COPD in the Serbian population is still lacking.

In this study, we investigated the role of the functional gene variants of CYP1A1, CYP2E1, GSTM1, GSTT1, GSTP1 and mEH genes in COPD in a Serbian population. The single and combined genotypes were analyzed in a group of COPD patients and in controls with normal lung function.

Material and Methods

Subjects and study design

The patient group consisted of 122 patients with COPD (66 with chronic bronchitis and 56 with

emphysema) recruited from the Clinical Center of Serbia and Zvezdara University Medical Center from 2002 to 2010. The diagnosis was established based on medical history, physical examination, pulmonary function tests, blood gas analyses and chest radiography, according to GOLD. The inclusion criteria for COPD were as follows: forced expiratory volume in one second (FEV_1)/forced vital capacity (FVC) of <70%, postbronchodilator FEV_1 of <80% of the predicted value for age and height, and short-acting β2 agonist (SABA) reversibility of <12% and 200 mL of prebronchodilator FEV_1. The control group encompassed 100 unrelated subjects from the same geographic area, without clinical evidence of COPD, with normal pulmonary function and parameters FEV1/FVC>70%, as well as FEV1 >80%.

The study was approved by the local Ethics Committee, and informed consent was obtained from each participant.

Determination of genotypes

Genomic DNA was extracted from whole blood using a GFX Genomic Blood DNA Purification Kit (Amersham Biosciences).

Detection of CYP1A1 *1A/*2A, CYP2E1 *1A/*5B, GSTP1 Ile105Val, mEH Tyr113His and mEH His139Arg variants was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), while the presence of GSTM1 and GSTT1 deletions was analyzed by a multiplex PCR method, as previously described (15–19). Alleles were separated on agarose gel electrophoresis and visualized with ethidium-bromide staining and UV transillumination. Genotypes were scored without knowledge of the sample phenotypes by two independent observers.

Statistical analysis

The age of patients, representing the age when COPD was diagnosed, age of control subjects at the time of medical check-up for the study, and pulmonary function data were expressed as mean ± standard deviation. Clinical data of the patients and controls were compared using χ^2-test or Student's t-test, as appropriate. Deviations of genotype distributions from the Hardy–Weinberg equilibrium were assessed by χ^2-test for each cohort. The association of alleles, single and combined genotypes with the COPD status was tested by Fisher's exact test. Odds Ratios (ORs) and 95% Confidence Intervals (95% CIs) were calculated. A p-value of less than 0.05 was considered significant. Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences).

Results

Clinical characteristics

The main characteristics and clinical parameters of patients with COPD and control subjects are shown in *Table I*. The mean age of control subjects was higher and males were more frequent in the patient group. The mean FEV_1 and FEV_1/FVC were significantly decreased in the patient group.

The role of single and combined genotypes in COPD

All the genotype distributions were in the Hardy–Weinberg equilibrium, with the exception of CYP2E1 *1A/*5B variant in the control group. Since the frequency of CYP2E1 *5B allele was 2.5%, the homozygous carrier in the control group was not expected ($\chi^2=14.79$, df=1, p<0.05). The genotype and allele frequency distributions of analyzed variants in COPD and the control group are given in *Table II*.

The frequency of GSTM1 null genotype was significantly increased in patients in comparison to controls (61.5% vs. 47.0%; p=0.042; *Table II*). The allele and genotype frequencies of CYP1A1 *1A/*2A, CYP2E1 *1A/*5B, GSTT1 null, GSTP1 Ile105Val, mEH Tyr113His and mEH His139Arg did not show significant differences between the groups (*Table II*).

In order to examine the role of the cumulative impact of genetic variants in the pathogenesis of COPD, the distributions of combined genotypes including: CYP1A1 *2A, GSTM1 null, GSTT1 null, GSTP1 105Val and mEH 113His alleles were tested among the groups. According to the analysis, two combined genotypes with significantly different distributions among the patient and control groups were identified in this study. The frequency of subjects with GSTM1 null and GSTP1 Val/(Val) combination was higher in patients in comparison to controls (38.5% vs. 24.0%), and carriers of these genotypes had two-fold increased risk for COPD (OR=1.98; p=0.029). The incidence of the combination with CYP1A1 *1A/*2A, GSTM1 null and mEH 113His/(113His) alleles was overrepresented in the patient group (7.4% vs. 1.0%;

Table I The main characteristics and clinical parameters of patients and controls.

	COPD	Controls
Subjects (n)	122	100
Age (years)	44.96±16.71[a]	50.9±13.66
Sex, male (%)	70[a]	35
Smokers (%)	70	50
FEV_1 (% pred)	45.3±24.74[a]	110.1±16.69
FEV_1/FVC	62.4±21.65[a]	98.3±8.57

[a]comparison among COPD and control group, p≤0.05.

Table II The allelic and genotype distributions of analyzed variants in COPD and control group.

Gene	Genotype, allele	COPD n=122 (%)	Controls n=100 (%)	OR (95% CI)	p
CYP1A1	*1A/*1A	97 (79.5)	83 (83.0)	1	1
	*1A/*2A	24 (19.7)	17 (17.0)	1.21 (0.61–2.40)	NS
	*2A/*2A	1 (0.8)	0 (0.0)		NS
	*2A	26 (10.7)	17 (8.5)	1.28 (0.68–2.44)	NS
CYP2E1	*1A/*1A	115 (94.3)	96 (96.0)	1	1
	*1A/*5B	7 (5.7)	3 (3.0)	1.95 (0.49–7.74)	NS
	*5B/*5B	0 (0.0)	1 (1.0)		NS
	*5B	7 (2.9)	5 (2.5)	1.15 (0.36–3.69)	NS
GSTM1	+	47 (38.5)	53 (53.0)	1	1
	null	75 (61.5)	47 (47.0)	1.80 (1.05–3.07)	0.042
GSTT1	+	95 (77.9)	76 (76.0)	1	1
	null	27 (22.1)	24 (24.0)	0.90 (0.48–1.68)	NS
GSTP1	Ile/Ile	48 (39.3)	45 (45.0)	1	1
	Ile/Val	59 (48.4)	46 (46.0)	1.20 (0.69–2.11)	NS
	Val/Val	15 (12.3)	9 (9.0)	1.56 (0.62–3.92)	NS
	Val	89 (36.5)	64 (32.0)	1.22 (0.82–1.81)	NS
mEH	Tyr/Tyr	58 (47.5)	48 (48.0)	1	1
(Tyr113His)	Tyr/His	48 (39.4)	40 (40.0)	0.99 (0.56–1.75)	NS
	His/ His	16 (13.1)	12 (12.0)	1.10 (0.48–2.56)	NS
	113His	80 (32.8)	64 (32.0)	1.22 (0.82–1.81)	NS
mEH	His/His	82 (67.2)	66 (66.0)	1	1
(His139Arg)	His/Arg	38 (31.1)	29 (29.0)	1.05 (0.59–1.89)	NS
	Arg/Arg	2 (1.7)	5 (5.0)	0.32 (0.06–1.71)	NS
	139Arg	42 (17.2)	39 (19.5)	0.86 (0.53–1.39)	NS

Table III The distributions of combined genotypes.

Combination of genes	Genotypes	COPD n=122 (%)	Controls n=100 (%)	OR (95% CI)	p
GSTM1-GSTP1	null-Val/(Val)	47 (38.5)	24 (24.0)	1.98 (1.10–3.57)	0.029
	other	75 (61.5)	76 (76.0)		
CYP1A1-GSTM1-mEH	*1A/*2A-null-113His/(His)	9 (7.4)	1 (1.0)	7.88 (0.98–63.34)	0.025
	other	113 (92.6)	99 (99.0)		

p=0.025), while carriers of these genotypes had eight-fold higher risk of developing COPD (OR=7.88) (*Table III*).

Discussion

In this study, the distribution of the CYP1A1 *1A/*2A, CYP2E1 *1A/*5B, GSTM1 null, GSTT1 null, GSTP1 Ile105Val, mEH Tyr113His and mEH His139Arg alleles, single and combined genotypes were compared in a group of 122 COPD patients and 100 control subjects with normal pulmonary function. The impetus to perform an association study of candidate genes was the lack of data concerning the role of genetic variants of the genes involved in xeno-biotic metabolism in COPD pathogenesis for the Serbian population, since there might be an ethnicity-specific genetic basis associated with the disease. The data regarding single and combined genotypes comparisons were presented without correction for multi-

ple tests (Bonferroni correction) due to several reasons. The aim of this study was to evaluate the role of functional variants of candidate genes for COPD pathogenesis in Serbians, since their contribution to the disease differs in various populations, while Bonferroni adjustment is suggested to be used in examinations without preestablished hypotheses (20). Additionally, application of Bonferroni adjustment allows different interpretation of the results depending on the number of tests performed, which from a biological standpoint might not be reasonable when well-studied polymorphisms suspected in the disorder are considered. Successful elimination of xenobiotics depends on the activity of both phases of reactions of the xenobiotic metabolism pathway, while genetic variants analyzed in this study may influence the kinetics of these reactions and generate highly reactive intermediates, that can exert toxicity *in situ* (6). Taking this into account, coincidental presence of functional variants linked to increased activity of phase I and decreased activity of phase II represents a physiologically relevant reason for assessment of the risk in carriers of combined genotypes for this complex disease, while introducing Bonferroni correction may lead to type II error and consequently negligence of significant results (20).

We would also like to highlight that the patients enrolled in this study were strictly selected according to the GOLD criteria, and subjects with asthma, malignant disease, bronchiectasis and other severe concomitant nonpulmonary diseases were excluded from the analysis. Apart from this, the mean age of controls was higher in comparison to the mean age of diagnosed COPD in the patient group, which should not affect the results since control subjects did not suffer pulmonary diseases in their lifetime. Also, females were more frequent in our control group, which may represent a confounding factor in our study. In regard to this, we would like to emphasize that women may be characterized by increased susceptibility, faster rate of FEV_1 decline, more hospitalisation, more dyspnea and more deaths from COPD than men, suggesting our control group may be more susceptible to COPD (21). Accordingly, it is important to stress that all our control subjects had normal lung function and had no evidence of any lung disease previously, a condition set up in order to ensure the quality of chosen participants and validity of results obtained. However, since a difference in gender distribution between the groups was observed, caution in the interpretation of our findings is recommended.

According to the results of our study, the distribution of heterozygotes for CYP1A1 *1A/*2A variant in patients (19.7%) and the control group (17.0%) was similar. In studies of Japanese and Indian populations, *2A variant was associated with centriacinar emphysema in patients with lung cancer and COPD, while other studies conducted on Caucasians and the Japanese failed to confirm its role in COPD and lung cancer (22–25).

The enzyme CYP2E1 is involved in the metabolism of nitrosamines from cigarette smoke. The frequency of CYP2E1 *5B allele in our controls was 2.5%, which is in concordance with the results obtained for Caucasians (16). In total, three heterozygotes and one homozygote with *5B allele were detected, resulting in absence of the Hardy–Weinberg equilibrium in our control group. Since the lack of Hardy–Weinberg equilibrium might result from genotyping errors, inbreeding, genetic drift or population substructure, our results were regenotyped, the subjects were not related and uniformly originated from the whole Serbian territory (26). Besides, absence of the Hardy–Weinberg equilibrium may implicate a connection between the genetic variant examined and the selected group, with obligatory normal lung function and no evidence of any lung disease previously in our controls. The *5B allele is related to reduced enzyme activity and inducibility, and is characterized by reactive oxygen species (ROS) production even in the absence of substrate, which might explain its connection with the normal lung function of control subjects (8–10). Our results showed no significant differences in the distribution of *5B variant in patients (2.9%) and controls (2.5%), while the data published for other populations were controversial (24, 27, 28).

GST enzymes have a key position in biological detoxification processes. In Caucasians, the frequency of GSTM1 null is approximately 50%, and for GSTT1 null about 24%, which is in concordance with the results in our controls, 47% and 24% respectively. Results of our study showed significantly higher frequency of GSTM1 null in patients than in controls (61.5% vs. 47.0%; p=0.042, respectively), while the frequency of GSTT1 null was similar among the groups (22.1% vs. 24.0%) (*Table II*). Considering the role of GSTM1 null and GSTT1 null variants in COPD pathogenesis, studies conducted in France, Taiwan, Brazil, Korea, Turkey, Switzerland and Tunisia gave controversial results that might be explained by the different genetic disease associations in different populations (24, 29–34).

Among the various isoforms, GSTP1 is expressed more abundantly in alveoli, alveolar macrophages and respiratory bronchioles, and may play an important role in detoxification in the lung. It has been suggested that GSTP1 105Val has a higher catalytic efficiency to the metabolism of carcinogenic aromatic epoxides (18). In a study on Turkish and Japanese populations, 105Ile was connected with COPD, while in the research on Indian, Tunisian and Russian populations, 105Val allele represented a significant risk factor for COPD (18, 32, 35–37). However, numerous studies did not confirm the role of this polymorphism in COPD pathogenesis, which is in concordance with the results of our study (24, 28, 30).

In the mEH gene two variants were detected, Tyr113His and His139Arg. In this study, allelic and

genotype distribution of these variants did not show association with COPD pathogenesis. Although, in a previous study, 113His allele was associated with COPD, emphysema, decrease in FEV_1 and severe disease, while 139Arg allele showed a protective role, the conclusions were not consistent (19, 34, 37, 38). However, the results of a recently performed comprehensive meta-analysis showed association of the 113His homozygous genotype with COPD in Caucasians, while the 139Arg heterozygote was connected with a decreased risk for COPD in Asians (39).

Since COPD is a complex disease, a single gene might have a small effect, while coexistence of several pathological variants might be more important in the pathogenesis of this disorder. Our results showed association of the GSTM1 null and GSTP1 Val/(Val) combination with COPD in a Serbian population and two-fold higher risk in its carriers (38.5% vs. 24.0%; OR=1.98). Connection of GSTM1 null and GSTP1 Val/Val with COPD was reported in the study on a Tunisian population that confirms the importance of this combination in COPD development and the validity of results obtained in our study (40). Another combination, including CYP1A1 *1A/*2A, GSTM1 null and mEH 113His/(His) genotypes, was found with higher frequency in patients (7.4% vs. 1%). The carriers of this genotype combination have an eightfold higher risk for COPD (OR=7.88, p=0.025). Oxidant–antioxidant balance is influenced not only by xenobiotics from cigarette smoke, but also by environmental and occupational toxic compounds. Genetic variants of the genes involved in phase I and II of xenobiotic metabolism might change the enzymatic activity and the kinetics of reactions of activation and detoxification of numerous toxic compounds causing increased oxidative stress (6). The genotype combinations revealed in this study may enable the realization of toxic effects. The presence of CYP1A1 *2A allele is associated with two-fold increased enzyme activity, and consequently increased concentration of reactive PAH intermediaries. On the other hand, deficiency of the GSTM1 enzyme, caused by a null genotype, as well as slower mEH activity in the presence of 113His allele, decrease phase II efficacy, and might lead to increased oxidative stress.

To our knowledge, this is the first study in which the interaction of variants connected with increased activity of phase I, CYP1A1 *2A, and deficiency or decreased activity of phase II enzymes, GSTM1 null and mEH 113His, was associated with COPD pathogenesis. In a study on Taiwanese, a combination of GSTM1 null, GSTP1 Ile/Ile and mEH 113His/(His) with a 6.8 risk for COPD development was identified (30). In another study, combination of GSTM1 null, GSTT1 null and GSTP1 Ile/Ile with a risk of 2.83 for COPD and a rapid decline in lung function was revealed (41).

Bearing in mind that COPD is a heterogeneous disease, identification of patients genetically susceptible to increased oxidative stress may help in the prevention and control of disease symptoms by antioxidant therapy, since it was found that antioxidant supplements reduce the risk of chronic lung disease development (14).

In conclusion, these are the first data concerning the analysis of functional genetic variants of genes involved in the metabolism of xenobiotics in the COPD pathogenesis in Serbians. Although our small sample size represents a limitation of the study, these data may be useful for planning future investigations, meta-analyses or more thorough research concerning antioxidant therapy and COPD. Results of our study revealed a significant correlation of one single genotype, GSTM1 null, and two combined genotypes of GSTM1 and GSTP1, as well as CYP1A1, GSTM1 and mEH, with the development of COPD. The genotype combination identified for the first time in our study, CYP1A1 *1A/*2A, GSTM1 null and mEH 113His/(His), in association with smoking, occupational toxins and community air pollution, might have a prominent role in the generation of oxidative stress, inflammation and destruction of the lungs. Although our results should be confirmed on a larger cohort, they implicate the importance of genetic associations with COPD previously described in different ethnic backgrounds, and also reveal a new one, not reported in other populations tested so far.

Conflict of interest statement

The authors stated that have no conflicts of interest regarding the publication of this article.

The views expressed herein represent those of the authors and do not necessarily represent the views or practices of the authors' employers or any other party.

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