



PULMONOLOGY

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ORIGINAL ARTICLE

Synonymous mutations in *TLR2* and *TLR9* genes decrease COPD susceptibility in the Chinese Han population

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Received 14 July 2022; received in revised form 6 September 2022; accepted 26 September 2022

Available online xxx

KEYWORDS

COPD;
TLR9;
TLR2;
Synonymous mutation

Abstract

Introduction: Previous studies have found associations between polymorphisms in some candidate genes and chronic obstructive pulmonary disease (COPD) risk. However, the association between *TLR2* and *TLR9* polymorphisms and COPD risk remains uncertain.

Methods: Four variants (rs352140, rs3804099, rs3804100, and rs5743705) of the *TLR2* and *TLR9* genes in 540 COPD patients and 507 healthy controls were genotyped using the Agena MassARRAY system. Odds ratio (OR) and 95% confidence interval (CI) were calculated to assess the association of *TLR2* and *TLR9* polymorphisms with COPD risk by logistic regression analysis.

Results: *TLR9*-rs352140, *TLR2*-rs3804100, and *TLR2*-rs5743705 were related to a lower risk of COPD among Chinese people and the significance still existed after Bonferroni correction. Additionally, rs3804099, rs3804100, and rs352140 were found to be associated with COPD development in different subgroups (males, age ≤ 68 years, smokers, BMI < 24 kg/m², and acute exacerbation).

Conclusions: Our findings indicated that *TLR9* and *TLR2* polymorphisms had protective effects on the development of COPD among Chinese people.

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Abbreviations: COPD, chronic obstructive pulmonary disease; SNP, single nucleotide polymorphism; *TLR2*, Toll-like receptor 2; *TLR9*, Toll-like receptor 9; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; HWE, Hardy-Weinberg equilibrium.

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<https://doi.org/10.1016/j.pulmoe.2022.09.010>

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Please cite this article in press as: X. Ding, Q. Lin, J. Zhao et al., Synonymous mutations in *TLR2* and *TLR9* genes decrease COPD susceptibility in the Chinese Han population, Pulmonology (2022), <https://doi.org/10.1016/j.pulmoe.2022.09.010>

Introduction

Chronic obstructive pulmonary disease (COPD) is chronic bronchitis and/or emphysema characterized by airflow obstruction, eventually leading to chronic respiratory failure. It has been reported that the prevalence of COPD in adults over 40 years old worldwide ranges from 5% to 10%.¹ Furthermore, COPD is currently the third leading cause of death globally.² In 2015, the overall incidence of COPD in China was 13.6%, and men were more significantly affected than women.³ In addition, COPD poses enormous challenges to health care systems worldwide because of its high prevalence, morbidity, and mortality.⁴ Therefore, it is necessary to explore the pathogenesis and etiology of COPD.

To the best of our knowledge, cigarette smoking, air pollution, and biomass fuels are important causative factors for COPD development.⁵⁻⁷ Moreover, genome-wide association studies (GWAS) have shown that single-nucleotide polymorphisms (SNPs) are significantly associated with COPD susceptibility and are involved in multiple aspects of COPD pathogenesis.^{8,9} Deng et al. have found that *SERPINA1*-rs8004738 could augment the risk of COPD in Chinese people.¹⁰ A meta-analysis conducted by Zhang et al. also indicated that the *TNF- α* -308 G/A polymorphism increases the susceptibility to COPD among Asians.¹¹ Taken together, these results suggest an important role of SNPs of some candidate genes in the development of COPD.

Toll-like receptor 2 (*TLR2*) and Toll-like receptor 9 (*TLR9*) belong to the Toll-like receptor (*TLR*) family which plays an essential role in chronic respiratory diseases.¹²⁻¹⁴ A previous study has revealed that *TLR4* participates in the pathogenesis of asthma and COPD.¹⁵ *TLR5* could inhibit COPD exacerbation by mediating lung immune stimulation.¹⁶ Moreover, there is increasing evidence indicating that *TLR2* is elevated in patients with COPD, asthma, and bronchiectasis.^{17,18} Besides, *TLR9* deletion improves smoke-induced loss of lung function and inflammation in mice, and *TLR9* is required for emphysema development.¹⁹ Meanwhile, Nadigel et al. have recognized that *TLR9* is abnormally expressed in lung CD8⁺ T cells in patients with COPD.²⁰ These findings demonstrate the critical roles of *TLR2* and *TLR9* in COPD and other chronic respiratory diseases.

Therefore, we conducted a case-control study of 1047 subjects and investigated the correlation of *TLR2* and *TLR9* gene polymorphisms with COPD risk to further elucidate their roles in COPD development.

Materials and methods

Study population

In the present study, we used G*power software (version 3.1.9.7) to calculate the minimum required sample size based on the probability of a type I error of $\alpha = 5\%$, and type II error of $\beta = 15\%$ (power = 85%). This calculation generated a sample of at least 450 cases and 450 controls. Accordingly, this study recruited 540 COPD patients and 507 healthy controls, which was larger than the sample size required for G*power software. All participants were recruited from Hainan General Hospital. Patients were assessed by pulmonary function examination according to

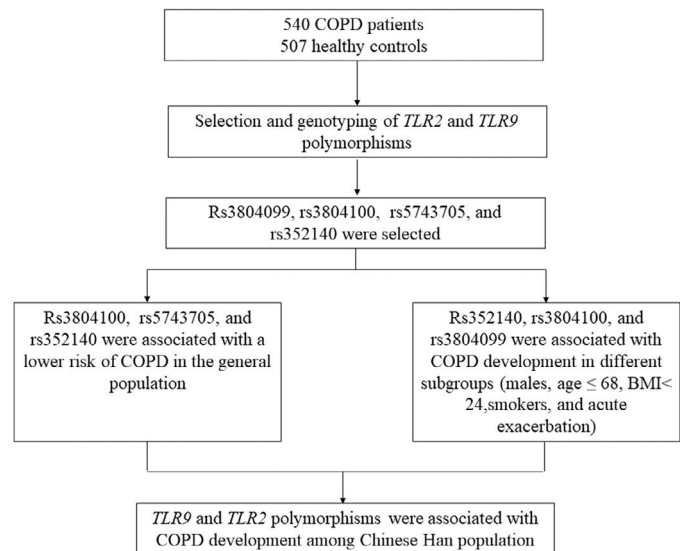


Fig. 1 The schematic representation of work-flow.

the Global Initiative for Chronic Obstructive Lung Disease.²¹ Individuals with forced expiratory volume in 1 second (FEV1) / forced vital capacity (FVC) < 70% and predicted FEV1 < 80% after inhaling bronchodilators were included in this study. Patients with lung cancer, bronchitis, pulmonary fibrosis, tuberculosis, asthma, and other respiratory diseases were excluded. The healthy controls came from the physical examination center of the same hospital. Healthy subjects who had no lung dysfunction, no lung-related disease, no other chronic diseases and disorders, and no history of cancers were included in the control group. The schematic representation of work-flow is shown in Fig. 1.

This study was approved by the hospital ethics committee. And all experimental procedures followed the Declaration of Helsinki. At the same time, informed consent was obtained from each participant.

Selection and genotyping of SNPs

Three variants (rs3804099, rs3804100, and rs5743705) in *TLR2* and one variant (rs352140) in *TLR9* were selected from the dbSNP database. All SNPs had minor allele frequencies (MAFs) larger than 1% in the 1000 Genomes Project. Total DNA was isolated from whole blood using the DNA extraction kit (GoldMag Co., Ltd., Xi'an, China). DNA concentration was measured by NanoDrop 2000 spectrophotometer (ND2000; Thermo Scientific, Waltham, MA, USA). Genotyping of *TLR2* and *TLR9* polymorphisms was performed using the Agena MassARRAY analyzer 4 (Agena Bioscience, San Diego, CA, USA). The primers of four SNPs are presented in Supplementary Table 1.

Data analysis

Student's t-test and Pearson's χ^2 test were applied to analyze the distribution of continuous variables (age and BMI) and categorical variables (sex and smoking) in the two groups, respectively. The Hardy-Weinberg equilibrium (HWE) for the control group was detected by the χ^2 test.

Table 1 Characteristics of COPD patients and healthy controls.

Variables	COPD patients (n=540)	Controls (n=507)	<i>p</i>
Age (years)	70.65±10.22	66.08±5.22	< 0.001 ^a
Sex (male/female)	350/190	329/178	0.979 ^b
Smoking status (yes/no)	235/305	213/294	0.622 ^b
Body mass index	20.21±3.13	24.19±3.19	< 0.001 ^a
Disease stage			
Acute exacerbation	276 (51.1%)		
Stable stage	237(43.9%)		
Missing	27 (5.0%)		
Smoking time (years)			
≥40	139 (25.7%)		
<40	122 (22.6%)		
Missing	279 (51.7%)		

COPD: chronic obstructive pulmonary disease.

^a and ^b values were calculated by t-test and χ^2 test, respectively.

Logistic regression analysis was performed to estimate the correlation between *TLR2* and *TLR9* variants and COPD susceptibility. $P < 0.05$ represented statistical significance. Bonferroni correction was used to correct for multiple testing.

Results

Basic information about subjects and candidate SNPs

The characteristics of the study population (including 540 COPD patients and 507 healthy controls) are summarized in Table 1. The average ages of COPD patients and controls were 70.65 ± 10.22 years and 66.08 ± 5.22 years, respectively. In addition, the distributions of age ($p < 0.001$) and BMI ($p < 0.001$) were significantly different between COPD patients and healthy controls. However, there was no significant difference in sex ($p = 0.979$) and smoking ($p = 0.622$) distributions between the two groups.

Table 2 displays the main information about candidate SNPs in *TLR9* and *TLR2* genes. We found that these four SNPs (rs352140, rs3804099, rs3804100, and rs5743705) were synonymous variants and met HWE ($p > 0.05$). According to the prediction from the HaploReg database

(<https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php>), rs352140, rs3804099, rs3804100, and rs5743705 were associated with the regulation of promoter histone marks, enhancer histone marks, GRASP QTL hits, selected eQTL hits, SiPhycons, and motif changes.

Association of *TLR9* and *TLR2* polymorphisms with COPD risk

Table 3 exhibits the association of *TLR9* and *TLR2* polymorphisms with COPD risk. *TLR9*-rs352140 was found to be associated with a decreased risk of COPD in the allele (OR = 0.70, 95% CI = 0.58-0.83, $p = 6.70E-05$), homozygote (OR = 0.51, 95% CI = 0.34-0.75, $p = 0.001$), heterozygote (OR = 0.67, 95% CI = 0.51-0.88, $p = 0.005$), dominant (OR = 0.63, 95% CI = 0.48-0.81, $p = 0.0004$), recessive (OR = 0.63, 95% CI = 0.43-0.90, $p = 0.012$), and additive (OR = 0.70, 95% CI = 0.58-0.84, $p = 0.0002$) models. Meanwhile, *TLR2*-rs3804100 and *TLR2*-rs5743705 were also related to a reduced risk of COPD in the allele (rs3804100: OR = 0.76, 95% CI = 0.63-0.93, $p = 0.006$; rs5743705: OR = 0.49, 95% CI = 0.32-0.74, $p = 0.0007$), heterozygote (rs3804100: OR = 0.65, 95% CI = 0.49-0.84, $p = 0.001$; rs5743705: OR = 0.46, 95% CI = 0.29-0.72, $p = 0.001$), dominant (rs3804100: OR = 0.66, 95%

Table 2 Basic information about *TLR9* and *TLR2* polymorphisms.

SNP	Gene	Chromosome	Allele (minor/major)	Location	MAF Control	Case	HWE <i>p</i> -value	HaploReg
rs352140	<i>TLR9</i>	3p21.2	T/C	Synonymous	0.408	0.324	0.780	Promoter histone marks; Enhancer histone marks; GRASP QTL hits; Selected eQTL hits
rs3804099	<i>TLR2</i>	4q31.3	C/T	Synonymous	0.338	0.305	0.770	GRASP QTL hits; Selected eQTL hits
rs3804100	<i>TLR2</i>	4q31.3	C/T	Synonymous	0.296	0.243	0.520	Enhancer histone marks
rs5743705	<i>TLR2</i>	4q31.3	C/T	Synonymous	0.064	0.032	0.250	SiPhycons; Enhancer histone marks; Motifs changed

SNP: single nucleotide polymorphism; MAF: minor allele frequency; HWE: Hardy-Weinberg equilibrium. p values were calculated by χ^2 test.

Table 3 Logistic regression analysis of associations between *TLR9* and *TLR2* polymorphisms and COPD risk.

SNP	Gene	Model	Genotype	Case	Control	OR (95% CI)	<i>p</i>	<i>p_c</i>
rs352140	<i>TLR9</i>	Allele	C	726 (67.6%)	599 (59.2%)	1.00	6.70E-05	0.0003*
			T	348 (32.4%)	413 (40.8%)	0.70 (0.58-0.83)		
		Codominant	CC	179 (35.4%)	250 (46.5%)	1.00	0.005	0.018*
			TC	241 (47.6%)	226 (42.1%)	0.67 (0.51-0.88)		
			TT	86 (17%)	61 (11.4%)	0.51 (0.34-0.75)		
		Dominant	CC	179 (35.4%)	250 (46.5%)	1.00	0.0004	0.002*
			TT-TC	327 (64.6%)	287 (53.5%)	0.63 (0.48-0.81)		
		Recessive	TC-CC	420 (83%)	476 (88.6%)	1.00	0.012	0.048*
			TT	86 (17%)	61 (11.4%)	0.63 (0.43-0.90)		
		Additive	—	—	—	0.70 (0.58-0.84)	0.0002	0.001*
rs3804099	<i>TLR2</i>	Allele	T	751 (69.5%)	670 (66.2%)	1.00	0.103	0.411
			C	329 (30.5%)	342 (33.8%)	0.86 (0.71-1.03)		
		Codominant	TT	220 (43.5%)	269 (49.8%)	1.00	0.066	0.266
			CT	230 (45.5%)	213 (39.4%)	0.78 (0.60-1.02)		
			CC	56 (11.1%)	58 (10.7%)	0.86 (0.57-1.32)		
		Dominant	TT	220 (43.5%)	269 (49.8%)	1.00	0.075	0.300
			CT-CC	286 (56.5%)	271 (50.2%)	0.79 (0.62-1.02)		
		Recessive	TT-CT	450 (88.9%)	482 (89.3%)	1.00	0.890	1.000
			CC	56 (11.1%)	58 (10.7%)	0.97 (0.65-1.45)		
		Additive	—	—	—	0.88 (0.73-1.06)	0.165	0.640
rs3804100	<i>TLR2</i>	Allele	T	818 (75.7%)	714 (70.4%)	1.00	0.006	0.024*
			C	262 (24.3%)	300 (29.6%)	0.76 (0.63-0.93)		
		Codominant	TT	248 (48.9%)	318 (58.9%)	1.00	0.001	0.005*
			CT	218 (43%)	182 (33.7%)	0.65 (0.49-0.84)		
			CC	41 (8.1%)	40 (7.4%)	0.76 (0.47-1.24)		
		Dominant	TT	248 (48.9%)	318 (58.9%)	1.00	0.002	0.006*
			CT-CC	259 (51.1%)	222 (41.1%)	0.66 (0.52-0.86)		
		Recessive	TT-CT	466 (91.9%)	500 (92.6%)	1.00	0.710	1.000
			CC	41 (8.1%)	40 (7.4%)	0.91 (0.57-1.47)		
		Additive	—	—	—	0.76 (0.63-0.93)	0.008	0.033*
rs5743705	<i>TLR2</i>	Allele	T	1045 (96.8%)	947 (93.6%)	1.00	0.0007	0.003*
			C	35 (3.2%)	65 (6.4%)	0.49 (0.32-0.74)		
		Codominant	TT	441 (87.2%)	506 (93.7%)	1.00	0.001	0.003*
			CT	65 (12.8%)	33 (6.1%)	0.46 (0.29-0.72)		
			CC	0 (0%)	1 (0.2%)	/		
		Dominant	TT	441 (87.2%)	506 (93.7%)	1.00	0.001	0.003*
			CT-CC	65 (12.8%)	34 (6.3%)	0.47 (0.30-0.73)		
		Recessive	TT-CT	506 (100%)	539 (99.8%)	1.00	/	/
			CC	0 (0%)	1 (0.2%)	/		
		Additive	—	—	—	0.49 (0.32-0.75)	0.001	0.004*

SNP: single nucleotide polymorphism; OR: odds ratio; CI: confidence interval.

p values were calculated by logistic regression analysis adjusted by age, sex, and smoking.*p_c* values were calculated after Bonferroni correction.Bold values indicate statistical significance (*p* < 0.05) and * indicates significance after Bonferroni correction.

CI = 0.52-0.86, $p = 0.002$; rs5743705: OR = 0.47, 95% CI = 0.30-0.73, $p = 0.001$), and additive (rs3804100: OR = 0.76, 95% CI = 0.63-0.93, $p = 0.008$; rs5743705: OR = 0.49, 95% CI = 0.32-0.75, $p = 0.001$) models. Moreover, all of these genetic models of *TLR9*-rs352140, *TLR2*-rs3804100, and *TLR2*-rs5743705 remained statistically significant even after Bonferroni correction ($p_c < 0.05$).

Next, we performed stratified analysis based on age, sex, smoking, BMI, and disease stage. According to sex-stratified analysis (Table 4), *TLR9*-rs352140 decreased the susceptibility to COPD in males in the allele (OR = 0.65, 95% CI = 0.52-0.82, $p = 0.0002$), homozygote (OR = 0.48, 95% CI = 0.29-0.81, $p = 0.006$), heterozygote (OR = 0.59, 95% CI = 0.42-0.84, $p = 0.004$), dominant (OR = 0.57, 95% CI = 0.41-0.79, $p = 0.001$), and additive (OR = 0.66, 95% CI = 0.52-0.84, $p = 0.001$) models, and the significance existed after Bonferroni correction.

In age-stratified analysis (Table 4), we found that *TLR2*-rs3804099 reduced the likelihood of developing COPD in individuals younger than 68 years in the allele (OR = 0.76, 95% CI = 0.59-0.99, $p = 0.039$), heterozygote (OR = 0.65, 95% CI = 0.44-0.95, $p = 0.028$), dominant (OR = 0.64, 95% CI = 0.45-0.93, $p = 0.017$), and additive (OR = 0.73, 95% CI = 0.56-0.97, $p = 0.029$) models. However, the significance was lost after Bonferroni correction ($p_c > 0.05$). Likewise, rs3804100 was also correlated with a lower risk of COPD in the allele (OR = 0.64, 95% CI = 0.48-0.85, $p = 0.002$), heterozygote (OR = 0.55, 95% CI = 0.37-0.81, $p = 0.002$), dominant (OR = 0.55, 95% CI = 0.38-0.79, $p = 0.001$), and additive (OR = 0.64, 95% CI = 0.48-0.86, $p = 0.003$) models, and the association remained significant after Bonferroni correction ($p_c < 0.05$).

In Table 5, the CT (OR = 0.59, 95% CI = 0.38-0.90, $p = 0.015$) and CT-CC (OR = 0.64, 95% CI = 0.43-0.96, $p = 0.030$) genotypes of rs3804100 decreased the occurrence of COPD among smokers, but there was no significant difference when Bonferroni correction was performed ($p_c > 0.05$). When stratified by BMI (Table 5), rs352140 decreased the susceptibility to COPD in individuals with BMI < 24 kg/m² under the allele (OR = 0.68, 95% CI = 0.53-0.89, $p = 0.005$), homozygote (OR = 0.44, 95% CI = 0.25-0.76, $p = 0.003$), dominant (OR = 0.68, 95% CI = 0.46-1.00, $p = 0.049$), recessive (OR = 0.50, 95% CI = 0.30-0.82, $p = 0.006$), and additive (OR = 0.69, 95% CI = 0.53-0.90, $p = 0.006$) models. However, after Bonferroni correction ($p_c < 0.05$), the significance persisted under all genetic models except the dominant model. Meanwhile, the CT genotype (OR = 0.61, 95% CI = 0.41-0.90, $p = 0.012$) of rs3804100 was found to be correlated with a lower risk of COPD and its association remained significant when Bonferroni correction was conducted ($p_c < 0.05$). Rs3804100 under the dominant model (OR = 0.63, 95% CI = 0.43-0.92, $p = 0.016$) significantly decreased the risk of COPD, and the significance was lost after Bonferroni correction ($p_c < 0.05$).

When stratified by disease stage (Table 6), the C allele of *TLR2*-rs3804100 was associated with an increased risk of COPD in patients with acute exacerbation of COPD compared with the T allele (OR = 1.36, 95% CI = 1.02-1.81, $p = 0.037$). However, *TLR2*-rs3804100 failed to retain its significance after Bonferroni correction ($p_c < 0.05$).

Discussion

Numerous studies have illustrated that *TLR2* and *TLR9* genes participate in the development and progression of COPD.^{18,20} At the same time, we found *TLR9*-rs352140, *TLR2*-rs3804100, and *TLR2*-rs5743705 had protective effects on the development of COPD among Chinese people and the significance remained after Bonferroni correction. Additionally, rs352140, rs3804100, and rs3804099 were correlated with COPD susceptibility in different subgroups. Our results further confirmed the importance of *TLR2* and *TLR9* genes in COPD development. Furthermore, the study of these polymorphisms of *TLR2* and *TLR9* may provide new insights into the prevention and treatment of COPD.

The *TLR2* gene is located on human chromosome 4q31.3 and has been reported to be involved in COPD development.²² Some research has reported that several SNPs of *TLR2* could influence the occurrence of chronic respiratory diseases, including COPD.^{23,24} A meta-analysis has also discovered that *TLR2*-rs4696480 is related to an increased risk of asthma and is a risk factor for asthma.²⁵ Rs1898830 and rs11938228 of *TLR2* have been proved to participate in FEV1 decline.²⁴ In our study, the correlation of rs3804099, rs3804100, and rs5743705 with COPD susceptibility was investigated among the Chinese population. We found that these three SNPs reduced the risk of COPD in the overall group and different subgroups. Rs3804099 decreased the susceptibility to COPD in individuals aged ≤ 68 years, while it increased the risk of asthma in a mixed-ancestry cohort.²⁶ In addition, some studies have demonstrated that rs3804100 is correlated with an increased risk and severity of tuberculosis,^{27,28} which is inconsistent with our results. The reasons for this contradiction are likely due to the heterogeneity of the disease and racial differences. In a word, these data underscore the vital role of *TLR2* polymorphisms in COPD development.

The *TLR9* gene is found on chromosome 3p21.2 and plays a crucial role in the pathogenesis of COPD.^{19,20} The *TLR9*-rs187084 polymorphism is associated with diminished FEV1% predicted and affects the progression of COPD among the European population.²⁹ In addition, rs352140 of *TLR9* has been reported to be correlated with many diseases, including bacterial meningitis,³⁰ tuberculosis,³¹ malaria,³² and inflammatory bowel disease.³³ Nevertheless, rs352140 has not been studied in COPD. As far as we know, this research is the first to investigate the association between rs352140 and COPD risk and suggests that rs352140 has a protective effect on COPD development.

Synonymous mutations can interrupt the formation of correct mRNA secondary structures, reduce the accuracy and speed of translation, and even alter the start of transcription.³⁴ Moreover, some research has demonstrated that synonymous mutations could alter disease susceptibility by affecting the expression of mRNA and protein of candidate genes.^{35,36} Therefore, we speculated that synonymous mutations (rs352140, rs3804099, rs3804100, and rs5743705) could alter disease susceptibility by influencing the expression of *TLR2* and *TLR9*. We will perform functional experiments to verify our hypothesis in follow-up studies.

Admittedly, there are some limitations in our study. Firstly, only one SNP in *TLR9* and three SNPs in *TLR2* were analyzed, and more polymorphisms of these two genes

Table 4 Age- and sex-stratified analysis of the association between *TLR9* and *TLR2* polymorphisms and COPD risk.

Sex				Male			Female		
Gene	SNP	Model	Genotype	OR (95% CI)	p^a	p_c	OR (95% CI)	p^a	p_c
TLR9	rs352140	Allele	C	1.00			1.00		
			T	0.65 (0.52–0.82)	0.0002	0.001*	0.77 (0.57-1.03)	0.082	0.327
		Codominant	CC	1.00			1.00		
			TC	0.59 (0.42-0.84)	0.004	0.015*	0.82 (0.51-1.32)	0.418	1.000
			TT	0.48 (0.29-0.81)	0.006	0.022*	0.57 (0.30-1.07)	0.082	0.245
		Dominant	CC	1.00			1.00		
			TT-TC	0.57 (0.41-0.79)	0.001	0.003*	0.75 (0.47-1.17)	0.201	0.800
		Recessive	TC-CC	1.00			1.00		
			TT	0.62 (0.38-1.02)	0.058	0.224	0.63 (0.35-1.13)	0.122	0.360
		Additive	–	0.66 (0.52-0.84)	0.001	0.003*	0.76 (0.56-1.04)	0.089	0.352
Age				> 68 years			≤ 68 years		
Gene	SNP	Model	Genotype	OR (95% CI)	p^b	p_c	OR (95% CI)	p^b	p_c
TLR2	rs3804099	Allele	T	1.00			1.00		
			C	1.04 (0.76-1.42)	0.802	1.000	0.76 (0.59-0.99)	0.039	0.154
		Codominant	TT	1.00			1.00		
			CT	0.90 (0.57-1.42)	0.658	1.000	0.65 (0.44-0.95)	0.028	0.083
			CC	1.35 (0.64-2.84)	0.436	1.000	0.62 (0.33-1.16)	0.134	0.402
		Dominant	TT	1.00			1.00		
			CT-CC	0.98 (0.64-1.51)	0.927	1.000	0.64 (0.45-0.93)	0.017	0.068
		Recessive	TT-CT	1.00			1.00		
			CC	1.41 (0.69-2.89)	0.347	1.000	0.77 (0.42-1.39)	0.377	1.000
		Additive	–	1.06 (0.77-1.46)	0.709	1.000	0.73 (0.56-0.97)	0.029	0.108
TLR2	rs3804100	Allele	T	1.00			1.00		
			C	0.98 (0.71-1.36)	0.893	1.000	0.64 (0.48-0.85)	0.002	0.007*
		Codominant	TT	1.00			1.00		
			CT	0.85 (0.54-1.34)	0.471	1.000	0.55 (0.37-0.81)	0.002	0.007*
			CC	1.13 (0.48-2.65)	0.779	1.000	0.54 (0.26-1.11)	0.095	0.284
		Dominant	TT	1.00			1.00		
			CT-CC	0.89 (0.58-1.37)	0.591	1.000	0.55 (0.38-0.79)	0.001	0.005*
		Recessive	TT-CT	1.00			1.00		
			CC	1.21 (0.53-2.77)	0.657	1.000	0.69 (0.34-1.40)	0.307	0.900
		Additive	–	0.96 (0.69-1.35)	0.816	1.000	0.64 (0.48-0.86)	0.003	0.011*

SNP: single nucleotide polymorphism; OR: odds ratio; CI: confidence interval.

 p^a values were calculated by logistic regression analysis adjusted by age and smoking. p^b values were calculated by logistic regression analysis adjusted by age, sex, and smoking. p_c values were calculated after Bonferroni correction.Bold values indicate statistical significance ($p < 0.05$) and * indicates significance after Bonferroni correction.

Table 5 Correlation between *TLR9* and *TLR2* polymorphisms and COPD risk stratified by smoking and BMI.

Smoking				Smoking			Non-smoking		
Gene	SNP	Model	Genotype	OR (95% CI)	p^a	p_c	OR (95% CI)	p^a	p_c
<i>TLR2</i>	rs3804100	Allele	T	1.00			1.00		
			C	0.81 (0.60-1.08)	0.149	0.596	0.73 (0.56-1.04)	0.055	0.201
		Codominant	TT	1.00			1.00		
			CT	0.59 (0.38-0.90)	0.015	0.045*	0.73 (0.51-1.04)	0.077	0.307
			CC	0.91 (0.44-1.89)	0.809	1.000	0.67 (0.34-1.33)	0.253	1.000
		Dominant	TT	1.00			1.00		
			CT-CC	0.64 (0.43-0.96)	0.030	0.120	0.72 (0.51-1.01)	0.054	0.216
		Recessive	TT-CT	1.00			1.00		
			CC	1.15 (0.57-2.31)	0.706	1.000	0.76 (0.39-1.50)	0.433	1.000
		Additive	—	0.79 (0.58-1.08)	0.137	0.560	0.77 (0.59-1.01)	0.063	0.248
BMI				$\geq 24 \text{ kg/m}^2$			$< 24 \text{ kg/m}^2$		
Gene	SNP	Model	Genotype	OR (95% CI)	p^b	p_c	OR (95% CI)	p^b	p_c
<i>TLR9</i>	rs352140	Allele	C	1.00			1.00		
			T	0.70 (0.44-1.11)	0.126	0.505	0.68 (0.53-0.89)	0.005	0.020*
		Codominant	CC	1.00			1.00		
			TC	0.65 (0.32-1.31)	0.224	0.673	0.79 (0.52-1.19)	0.263	1.000
			TT	0.58 (0.20-1.62)	0.297	0.891	0.44 (0.25-0.76)	0.003	0.013*
		Dominant	CC	1.00			1.00		
			TT-TC	0.63 (0.32-1.21)	0.165	0.640	0.68 (0.46-1.00)	0.049	0.188
		Recessive	TC-CC	1.00			1.00		
			TT	0.71 (0.27-1.90)	0.497	1.000	0.50 (0.30-0.82)	0.006	0.030*
		Additive	—	0.72 (0.45-1.17)	0.188	0.720	0.69 (0.53-0.90)	0.006	0.022*
<i>TLR2</i>	rs3804100	Allele	T	1.00			1.00		
			C	0.95 (0.59-1.55)	0.851	1.000	0.76 (0.57-1.01)	0.059	0.235
		Codominant	TT	1.00			1.00		
			CT	0.63 (0.30-1.29)	0.205	0.616	0.61 (0.41-0.90)	0.012	0.048*
			CC	2.45 (0.75-8.01)	0.138	0.413	0.78 (0.37-1.65)	0.514	1.000
		Dominant	TT	1.00			1.00		
			CT-CC	0.81 (0.42-1.58)	0.542	1.000	0.63 (0.43-0.92)	0.016	0.060
		Recessive	TT-CT	1.00			1.00		
			CC	2.96 (0.93-9.44)	0.067	0.216	0.97 (0.47-2.00)	0.925	1.000
		Additive	—	1.08 (0.64-1.83)	0.773	1.000	0.75 (0.56-1.00)	0.050	0.204

SNP: single nucleotide polymorphism; OR: odds ratio; CI: confidence interval.

 p^a values were calculated by logistic regression analysis adjusted by age and smoking. p^b values were calculated by logistic regression analysis adjusted by age, sex, and smoking. p_c values were calculated after Bonferroni correction.Bold values indicate statistical significance ($p < 0.05$) and * indicates significance after Bonferroni correction.

Table 6 Relationship between disease stage and *TLR2* rs3804100 in COPD patients.

SNP	Model	Genotype	Acute exacerbation	Stable stage	OR (95% CI)	<i>p</i>	<i>p_c</i>
rs3804100	Allele	T	401 (72.6%)	371 (78.3%)	1.00		
		C	151 (27.4%)	103 (21.7%)	1.36 (1.02-1.81)	0.037	0.150
	Codominant	TT	152 (55.1%)	147 (62%)	1.00		
		CT	97 (35.1%)	77 (32.5%)	1.21 (0.82-1.78)	0.340	1.000
		CC	27 (9.8%)	13 (5.5%)	1.60 (0.78-3.28)	0.199	0.796
	Dominant	TT	152 (55.1%)	147 (62%)	1.00		
		CT-CC	124 (44.9%)	90 (38%)	1.27 (0.88-1.82)	0.200	0.800
	Recessive	TT-CT	249 (90.2%)	224 (94.5%)	1.00		
		CC	27 (9.8%)	13 (5.5%)	1.49 (0.74-3.02)	0.263	1.000
	Additive	—	—	—	1.24 (0.93-1.65)	0.143	0.560

SNP: single nucleotide polymorphism; OR: odds ratio; CI: confidence interval.

p values were calculated by logistic regression analysis adjusted by age, sex, and smoking.

p_c values were calculated after Bonferroni correction.

Bold values indicate statistical significance (*p* < 0.05).

should be investigated. Secondly, all subjects were Han Chinese from the same hospital, so selection bias was inevitable. Thirdly, the molecular mechanism by which *TLR2* and *TLR9* polymorphisms affect COPD susceptibility remains unclear, which should be further explored in subsequent studies.

Conclusions

To sum up, we are the first to reveal that *TLR9* and *TLR2* polymorphisms have protective effects on the development of COPD among Chinese people. The investigation into these synonymous mutations may shed new light on the prevention and treatment of COPD.

Statement

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Hainan General Hospital and we obtained written informed consent from all individual participants.

Availability of data and materials

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

Funding

This paper was supported by the National Natural Science Foundation of China (No. 81860015 and No. 82160011), and National Key Research and Development Program of China (2018YFC2002304).

Consent for publication

Not applicable.

Authors' contributions

XD and QL completed genotyping and wrote the manuscript. JZ, YF, and YZ participated in data management, statistical analysis and manuscript revision. RM, LZ, BZ, and JC collected samples. TX, HW, and YD designed the study, co-supervised the work, and modified the manuscript. All authors have approved the final manuscript.

Conflicts of interest

The authors declare that they have no competing interests.

Acknowledgements

We thank the individuals who participated in this study.

Supplementary materials

Supplementary materials associated with this article can be found in the online version at [doi:10.1016/j.pulmoe.2022.09.010](https://doi.org/10.1016/j.pulmoe.2022.09.010).

References

- Vijayan VK. Chronic obstructive pulmonary disease. *Indian J Med Res.* 2013;137:251–69.
- Austin V, Miller A, Vlahos R. Prior cigarette smoke exposure does not affect acute post-stroke outcomes in mice. *PLoS One.* 2019;14:e0214246.
- Fang L, Gao P, Bao H, Tang X, Wang B, Feng Y, et al. Chronic obstructive pulmonary disease in China: a nationwide prevalence study. *Lancet Respir Med.* 2018;6:421–30.
- López-Campos JL, Tan W, Soriano JB. Global burden of COPD. *Respirology.* 2016;21:14–23.
- Hou W, Hu S, Li C, Ma H, Wang Q, Meng G, et al. Cigarette smoke induced lung barrier dysfunction, EMT, and tissue remodeling: a possible link between COPD and lung cancer. *Biomed Res Int.* 2019;2019:2025636.

6. Hansel NN, McCormack MC, Kim V. The effects of air pollution and temperature on COPD. *COPD*. 2016;13:372–9.
7. Sana A, Somda SMA, Meda N, Bouland C. Chronic obstructive pulmonary disease associated with biomass fuel use in women: a systematic review and meta-analysis. *BMJ Open Respir Res*. 2018;5:e000246.
8. Zhou H, Yang J, Li D, Xiao J, Wang B, Wang L, et al. Association of IREB2 and CHRNA3/5 polymorphisms with COPD and COPD-related phenotypes in a Chinese Han population. *J Hum Genet*. 2012;57:738–46.
9. Yuan C, Chang D, Lu G, Deng X. Genetic polymorphism and chronic obstructive pulmonary disease. *Int J Chron Obstruct Pulmon Dis*. 2017;12:1385–93.
10. Deng X, Yuan CH, Chang D. Interactions between single nucleotide polymorphism of SERPINA1 gene and smoking in association with COPD: a case-control study. *Int J Chron Obstruct Pulmon Dis*. 2017;12:259–65.
11. Zhang L, Gu H, Gu Y, Zeng X. Association between TNF- α -308 G/A polymorphism and COPD susceptibility: a meta-analysis update. *Int J Chron Obstruct Pulmon Dis*. 2016;11:1367–79.
12. Kosamo S, Hisert KB, Dmyterko V, Nguyen C, Black RA, Holden TD, et al. Strong toll-like receptor responses in cystic fibrosis patients are associated with higher lung function. *J Cyst Fibros*. 2020;19:608–13.
13. Farkas D, Thompson AAR, Bhagwani AR, Hultman S, Ji H, Kotha N, et al. Toll-like receptor 3 is a therapeutic target for pulmonary hypertension. *Am J Respir Crit Care Med*. 2019;199:199–210.
14. Drake MG, Kaufman EH, Fryer AD, Jacoby DB. The therapeutic potential of toll-like receptor 7 stimulation in asthma. *Inflamm Allergy Drug Targets*. 2012;11:484–91.
15. Zuo L, Lucas K, Fortuna CA, Chuang CC, Best TM. Molecular regulation of toll-like receptors in asthma and COPD. *Front Physiol*. 2015;6:312.
16. Pérez-Cruz M, Koné B, Porte R, Carnoy C, Tabareau J, Gosset P, et al. The toll-like receptor 5 agonist flagellin prevents non-typeable *Haemophilus influenzae*-induced infection in cigarette smoke-exposed mice. *PLoS One*. 2021;16:e0236216.
17. Simpson JL, Grissell TV, Douwes J, Scott RJ, Boyle MJ, Gibson PG. Innate immune activation in neutrophilic asthma and bronchiectasis. *Thorax*. 2007;62:211–8.
18. Haw TJ, Starkey MR, Pavlidis S, Fricker M, Arthurs AL, Nair PM, et al. Toll-like receptor 2 and 4 have opposing roles in the pathogenesis of cigarette smoke-induced chronic obstructive pulmonary disease. *Am J Physiol Lung Cell Mol Physiol*. 2018;314:L298–317.
19. Foronjy RF, Salathe MA, Dabo AJ, Baumlín N, Cummins N, Eden E, et al. TLR9 expression is required for the development of cigarette smoke-induced emphysema in mice. *Am J Physiol Lung Cell Mol Physiol*. 2016;311:L154–66.
20. Nadigel J, Préfontaine D, Bagloli CJ, Maltais F, Bourbeau J, Eidelman DH, et al. Cigarette smoke increases TLR4 and TLR9 expression and induces cytokine production from CD8(+) T cells in chronic obstructive pulmonary disease. *Respir Res*. 2011;12:149.
21. Vogelmeier CF, Criner GJ, Martinez FJ, Anzueto A, Barnes PJ, Bourbeau J, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive lung disease 2017 report. GOLD executive summary. *Am J Respir Crit Care Med*. 2017;195:557–82.
22. Sidletskaia K, Vitkina T, Denisenko Y. The role of toll-like receptors 2 and 4 in the pathogenesis of chronic obstructive pulmonary disease. *Int J Chron Obstruct Pulmon Dis*. 2020;15:1481–93.
23. Apostolou A, Kerenidi T, Michopoulos A, Gourgoulis KI, Noutsias M, Germeis AE, et al. Association between TLR2/TLR4 gene polymorphisms and COPD phenotype in a Greek cohort. *Herz*. 2017;42:752–7.
24. Budulac SE, Boezen HM, Hiemstra PS, Lapperre TS, Vonk JM, Timens W, et al. Toll-like receptor (TLR2 and TLR4) polymorphisms and chronic obstructive pulmonary disease. *PLoS One*. 2012;7:e43124.
25. Gao Y, Xiao H, Wang Y, Xu F. Association of single-nucleotide polymorphisms in toll-like receptor 2 gene with asthma susceptibility: a meta-analysis. *Medicine*. 2017;96:e6822.
26. Zhao J, Shang H, Cao X, Huang Y, Fang X, Zhang S, et al. Association of polymorphisms in TLR2 and TLR4 with asthma risk: an update meta-analysis. *Medicine*. 2017;96:e7909.
27. Soedarsono S, Amin M, Tokunaga K, Yuliwulandari R, Suameitria Dewi DNS, Mertaniasih NM. Association of disease severity with toll-like receptor polymorphisms in multidrug-resistant tuberculosis patients. *Int J Mycobacteriol*. 2020;9:380–90.
28. Schurz H, Daya M, Möller M, Hoal EG, Salie M. TLR1, 2, 4, 6 and 9 variants associated with tuberculosis susceptibility: a systematic review and meta-analysis. *PLoS One*. 2015;10:e0139711.
29. Berenson CS, Kruzel RL, Wrona CT, Mammen MJ, Sethi S. Impaired innate COPD alveolar macrophage responses and toll-like receptor-9 polymorphisms. *PLoS One*. 2015;10:e0134209.
30. Xue H, Peng H, Li J, Li M, Lu S. TLR9 Rs352140 polymorphism contributes to a decreased risk of bacterial meningitis: evidence from a meta-analysis. *Epidemiol Infect*. 2020;148:e294.
31. Mittal M, Biswas SK, Singh V, Arela N, Katoch VM, Das R, et al. Association of Toll like receptor 2 and 9 gene variants with pulmonary tuberculosis: exploration in a northern Indian population. *Mol Biol Rep*. 2018;45:469–76.
32. Mario-Vásquez JE, Naranjo-González CA, Montiel J, Zuluaga LM, Vásquez AM, Tobón-Castaño A, et al. Association of variants in IL1B, TLR9, TREM1, IL10RA, and CD3G and Native American ancestry on malaria susceptibility in Colombian populations. *Infect Genet Evol*. 2021;87:104675.
33. Wang H, Zhou S, Zhang J, Lei S, Zhou J. Correlations between TLR polymorphisms and inflammatory bowel disease: a meta-analysis of 49 case-control studies. *Immunol Res*. 2019;67:142–50.
34. Im EH, Choi SS. Synonymous codon usage controls various molecular aspects. *Genomics Inform*. 2017;15:123–7.
35. Simhadri VL, Hamasaki-Katagiri N, Lin BC, Hunt R, Jha S, Tseng SC, et al. Single synonymous mutation in factor IX alters protein properties and underlies haemophilia B. *J Med Genet*. 2017;54:338–45.
36. Wang SY, Cheng YY, Liu SC, Xu YX, Gao Y, Wang CL, et al. A synonymous mutation in IGF-1 impacts the transcription and translation process of gene expression. *Mol Ther Nucleic Acids*. 2021;26:1446–65.