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NLRP3 rs1539019 is significantly associated with chronic obstructive pulmonary disease in a Chinese Han population: a case-control study

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Abstract. – OBJECTIVE: COPD is a complex respiratory disease characterized by chronic airway inflammation and the airflow limitations are not fully reversible due to the combination of genetic and environmental factors. Genetic factors such as polymorphisms, may affect the susceptibility of COPD. In the present study, we examined the association between the polymorphisms of three genes and COPD risk in a Chinese Han population.

PATIENTS AND METHODS: A total of 375 COPD patients and 284 control subjects were recruited from November 2018 to June 2021. Data on demographic basic information, smoking status, history of coal dust exposure, and peripheral blood were collected from subjects of two groups. Three polymorphisms (NLRP3 rs1539019, LAMB1 rs4320486, IL-6 rs1800796) were analyzed. Logistic analysis was used to evaluate the genetic contribution of selected SNPs to COPD susceptibility.

RESULTS: The AC genotype of NLRP3 rs1539019 significantly decreased COPD risk compared with CC genotype (adjusted OR = 0.508, 95% CI 0.336-0.767). In the stratification analyses, the AC genotype significantly decreased the risk of COPD in subjects aged 60 and over (p=0.005; adjusted OR = 0.553; 95% CI 0.366-0.835) with current smoking status (p=0.002; adjusted OR = 0.419; 95% CI 0.240-0.732) when compared with AA+CC genotype. Moreover, a significantly decreased risk for GOLD III COPD was found in genotype AC of NLRP3 rs1539019 (p=0.006; adjusted OR = 0.502; 95% CI 0.306-0.822).

CONCLUSIONS: Our present study revealed that the genotype AC of NLRP3 rs1539019 is related to a decreased risk of COPD in a Chinese Han population, a large-sample, multi-center, multi-ethnic study is needed to further confirm our study.

Key Words:
COPD, Polymorphism, NLRP3 rs1539019, Susceptibility.

Introduction

Chronic obstructive pulmonary disease (COPD), which includes chronic obstructive bronchitis and emphysema, is a disease of the respiratory system. It is characterized by chronic airway inflammation and incomplete reversible airflow limitations, and further will develop into pulmonary heart disease and respiratory failure[1,2]. The principal clinical symptoms of COPD are cough, sputum, shortness of breath, or dyspnea. This disease affects 210 million people worldwide and causes more than 4 million deaths each year, 90% of which occur in low- and middle-income countries[3]. According to the World Health Organization (WHO), COPD will be the fourth most common cause of death and the fourth most prevalent disease by 2035[4]. The onset of COPD is a complex pathological process and the specific pathogenesis is not fully understood, mainly including inflammatory response, protease/protease imbalance, oxidative stress, environmental and genetic factors[5]. Although a history of smoking is considered to be the most important risk factor for COPD, which may trigger production of other reactive oxygen species, lipid peroxidation and subsequent pulmonary inflammation, not all smokers develop COPD[6,7]. Interestingly, life-long smokers have only 10%-20% lifetime incidence of this disease[8], which indicates that host genetics play an irreplaceable role in the onset of COPD besides of environmental factors. Therefore, we try to find some genes that contribute to COPD risk in genetic polymorphisms, which will be helpful for the early diagnosis, ear-

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ly prevention, early treatment so as to improve the life quality of patients.

Pathogen recognizing receptors (PRRs) which mainly include nod-like receptors (NLRs), toll-like receptors (TLRs), retinoic acid inducible gene (RIG)-I-like receptors, C-type lectin receptors and absent in melanoma 2(AIM2)-like receptors, are significant for the recognition of pathogen-associated molecular patterns (PAMPs)[9]. The NLR family is thought to be a component of intracellular PRRs and plays an essential role in the initiation of immune inflammatory response[10]. Recently, more than 20 NLRs have been discovered, among which pyrin domain containing 3 (NLRP3) is the one most studied[10]. When pathogens invade the body, NLRP3 rapidly forms a cytoplasmic complex with ASC (apoptosis-associated speck-like protein) and the cysteine protease caspase 1, known as the NLRP3 inflammasome, which has been shown to play an important role in regulating the maturation and secretion of pro-inflammatory cytokines IL-1B[10,11]. Increasing evidence[12] shows that genetic variants of NLRP3 might be a significant determinant of immune inflammatory responses and disease susceptibility. The rs1539019 TT genotype of NLRP3 was associated with a significantly increased risk of coal workers' pneumoconiosis (CWP) in a Chinese population[13]. The NLRP3 rs10754558 GG genotype was found to be closely associated with Gout arthritis risk compared to the healthy controls[14]. The C allele of NLRP3 rs1539019 had a significant correlation with multiplicative and additive interactions for the risk of renal cell carcinoma[15]. However, the functionality of NLRP3 rs1539019 SNP in COPD has not yet been reported.

A previous study[16] showed that LABM1 rs4320486 polymorphism was significantly associated with the risk of CWP. The functional LAMB1 rs4320486 polymorphism decreased the risk of CWP, possibly due to the reduced transcriptional activity of LAMB1. The expression of LAMB1 increased during pulmonary fibrosis, suggesting that LAMB1 may influence the progression of CWP[16]. A meta-analysis[17] suggested that IL-6 rs1800796 polymorphism was correlated to the risk of rheumatoid arthritis. And the GG genotype might be the key contributor in increasing susceptibility to rheumatoid arthritis. IL-6 rs1800796 polymorphism was associated with the risk of tuberculosis, and the CC genotype was the main contributor in decreasing susceptibility to tuberculosis[18].

In this study, we assessed three single nucleotide polymorphisms of different genes (NLRP3 rs1539019, LAMB1 rs4320486, IL-6 rs1800796) in patients with COPD and healthy control individuals and investigated whether these SNPs would be associated with the susceptibility to COPD in a Chinese Han population.

Patients and Methods

Study Population

This study compared two groups of subjects from the same area. All participants were Han from China. A total of 375 male Chinese Han COPD patients diagnosed by the Sinopharm Tongmei General Hospital in Shanxi Province were recruited from November 2018 to June 2021. The diagnostic criteria of COPD are based on WHO Global initiative for chronic Obstructive Lung Disease (GOLD). The diagnostic criteria for COPD were as follows: post bronchodilator FEV_1 <80% of the predicted value, FEV_1/FVC <70%, and FEV_1 reversibility after inhalation of 200 mg salbutamol <12% of the pre-bronchodilator FEV_1. The severity of COPD was classified by the guidelines of the GOLD in terms of the percentage predicted FEV_1: mild (>80%), moderate (50-80%), severe (30-50%) or very severe (<30%). The exclusion criteria for COPD patients were as follows: 1) The patient could not be tested for lung function; 2) The patient had other significant respiratory diseases such as asthma, lung cancer, congestive heart failure, tuberculosis, and cystic fibrosis. 3) The patients had previous history of chemotherapy, radiotherapy or other cancers. Participants were chosen without restrictions of age, smoking status, or diseases stage. A total of 284 male control subjects were recruited during the same period, who visited the outpatient or health check-up center of Sinopharm Tongmei General Hospital in Shanxi Province. Individuals with any severe disease were excluded by clinical examination, chest computed tomography (CT) examination, and laboratory examination. Trained interviewers completed detailed questionnaires for each participant. After the interview, 4-6 ml of venous blood were collected from each participant. All participants in this study signed informed consent. This study was approved by the Ethics Committee of Sinopharm Tongmei General Hospital (No. 201902) and by the Helsinki declaration.

Genotyping

Genomic DNA was obtained from venous blood using proteinase K digestion, phenol and

Table I. Demographic and selected variables among the COPD cases and control subjects.

Variables	COPD(N=375)		Control (284)		*P*
	N	%	N	%	
Age, year (mean ± SD)	69.75±10.41		62.00±10.91		< 0.001
Smoking status					< 0.001
Never	39	10.4	72	25.4	
Former	215	57.3	99	34.8	
Current	121	32.3	113	39.8	
FEV_1 observed (L)	1.47±0.69		2.74±0.66		
FVC observed (L)	2.61±0.89		3.58±0.81		
FEV_1% predicted	54.29±22.71		89.29±16.07		
FEV_1/FVC (%)	54.27±11.10		76.53±4.60		

Notes: Data are presented as mean ±SD.
Abbreviations: N: number of subjects; FEV1: forced expiratory volume in one second; FVC: forced vital capacity; COPD: chronic obstructive pulmonary disease.

chloroform extraction. Isolated DNA samples from two groups were randomly segmented into 96-well plates. According to the manufacturer's instructions (Applied Biosystem, Foster City, MA, USA), the TaqMan method and the ABI 7900HT Real Time PCR system were to genotype. To ensure quality control, genotyping was performed by two laboratory staff in a double-blind manner. Due to DNA quality problems, a few samples failed genotyping and were excluded in subsequent analysis. For NLRP3 rs1539019, 1 control's DNA sample failed genotyping.

Statistical Analysis

Microsoft Excel and SPSS 26.0 software (IBM, Armonk, NY, USA) were used for statistical analyses. The Student's *t*-test for continuous variable and Pearson's chi-square test for categorical variable were used to assess differences in the distribution of demographic characteristics between the case subjects and the control subjects. Frequency of COPD cases and controls genotypes was calculated for Hardy-Weinberg equilibrium by goodness-of-fit chi-square test (χ^2 test). Logistic regression was used to calculate crude odds ratios (ORs), adjusted ORs and 95% confidence interval (CI) for COPD risk and SNPs. Adjustment of multivariate logistic regression model to age and smoking status were done. The significance of all statistical tests was two-tailed and set at $p < 0.05$.

Results

Demographic Characteristics

The participants consisted of 375 male cases and 284 male controls. As shown in Table I, case subjects on average were older than control subjects (aged 69.75 years versus 62.00 years, $p<0.001$). There was a remarkable difference in the smoking status between the cases and the controls ($p<0.001$), with more former smokers in the case groups (57.3%) than in the control groups (34.8%), with fewer never and current smokers (10.4% and 32.3% respectively) in the case subjects than in the control subjects (25.4% and 39.8% respectively). Because of the study design, the COPD cases had worse pulmonary function (FEV_1/FVC) than the control subjects.

Allelic Frequencies and Genotype Distributions of three genes Polymorphism

The detailed information of candidate SNPs in three genes is showed in Table II. The distribution of all genotypes in both COPD case subjects and control subjects followed Hardy-Weinberg equilibrium ($p>0.05$). The minor allele frequency (MAF) for all four SNPs was consistent with the reported in the HapMap database.

The Association Between NLRP3 rs1539019 Polymorphism and COPD Risk

As observed in Table III, the genotype frequency of NLRP3 rs1539019 polymorphism was significantly different between the COPD cases and the controls ($p=0.007$), which indicated that the polymorphism of NLRP3 rs1539019 was associated with the onset of COPD, while there was no significant difference between C allele and A allele ($p=0.196$). Multivariate logistic regression demonstrated that the AC genotype of NLRP3 rs1539019 significantly decreased COPD risk compared with CC genotype (adjusted OR =

Table II. Primary information of genotyped SNPs.

Gene	SNP	Chromosomal position	Base	MAF		HWE P
				Cases	Controls	
NLRP3	rs1539019	chr1:247436999	A>C	0.531	0.495	0.509
LAMB1	rs4320486	chr7:108003532	C>T	0.248	0.222	0.301
IL-6	rs1800796	chr7:22726627	G>C	0.317	0.319	0.999

Abbreviations: HWE: Hardy-Weinberg equilibrium; SNP: single nucleotide polymorphism; MAF: minor allele frequency.

0.508, 95% CI 0.336-0.767). However, other polymorphisms (LAMB1 rs4320486, IL-6 rs1800796) detected in this study were not strongly connected to the susceptibility of COPD.

Stratification Analyses of NLRP3 rs1539019 Genotype and COPD Risk

The stratification analyses of NLRP3 rs1539019 polymorphism by age are shown in Table IV. When using the genotype AA+CC as a reference, we found that the AC genotype was associated with a decreased risk of COPD (p=0.007; adjusted OR = 0.629; 95% CI 0.449-0.883). In addition, AC genotype considerably decreased the risk of COPD in subjects who older than 60 years (p=0.005; adjusted OR = 0.553; 95% CI 0.366-0.835). Analyses of the genotypes of NLRP3 rs1539019 polymorphism and COPD risk stratified by smoking status are observed in Table V. The AC genotype was associated with a decreased COPD risk in subjects with current smoking status (p=0.002; adjusted OR = 0.419; 95% CI 0.240-0.732). Moreover, a markedly decreased risk for GOLD III COPD was found in genotype AC of NLRP3 rs1539019 (p=0.006; adjusted OR = 0.502; 95% CI 0.306-0.822), but not for GOLD I, II or IV (Table VI).

Discussion

To understand the influence factors of COPD risk in a Chinese Han population, we examined three candidate genetic polymorphisms in our study. A remarkable result was shown that the AC genotype of NLRP3 rs1539019 significantly

Table III. Distributions of genotypes of different genes their associations with risk of COPD.

Variables	COPD		Controls		P1	COR (95%CI)	P2	AOR (95% CI)
	N	%	N	%				
NLRP3 rs1539019	375		283					
CC	116	30.9	61	21.6	0.007	1.00	0.006	1.00
AC	166	44.3	158	55.8	0.002	0.552 (0.378-0.807)	0.001	0.508 (0.336-0.767)
AA	93	24.8	64	22.6	0.235	0.764 (0.490-1.192)	0.065	0.634 (0.391-1.029)
C allele	398	53.1	280	49.5	0.196	1.00		
A allele	352	46.9	286	50.5		0.886 (0.696-1.077)		
LAMB1 rs4320486	375		284					
CC	209	55.7	166	58.5	0.340	1.00	0.320	1.00
CT	147	39.2	110	38.7	0.715	1.061 (0.771-1.462)	0.264	1.220 (0.860-1.731)
TT	19	5.1	8	2.8	0.144	1.886 (0.806-4.417)	0.244	1.740 (0.685-4.416)
C allele	565	75.2	442	77.8	0.274	1.00		
T allele	186	24.8	126	22.2		1.155 (0.892-1.495)		
IL-6 rs1800796	375		284					
GG	46	12.3	29	10.2	0.464	1.00	0.550	1.00
CG	146	38.9	123	43.3	0.277	0.748 (0.444-1.262)	0.696	0.893 (0.506-1.575)
CC	183	48.8	132	46.5	0.609	0.874 (0.522-1.464)	0.765	1.089 (0.622-1.906)
G allele	238	31.7	181	31.9	0.959	1.00		
C allele	512	68.3	387	68.1		1.006 (0.796-1.272)		

Notes: P1-values were calculated by unconditional logistic regression without adjusted. P2-values were calculated by unconditional logistic regression adjusted for age, smoking status.
Abbreviations: COPD: chronic obstructive pulmonary disease; COR: crude odds ratio; AOR: adjusted odds ratio; CI: confidence interval; N: number of subjects.

Table IV. Analyses the genotypes of NLRP3 rs1539019 polymorphism and COPD risk stratified by age.

Variables	Cases/Controls	Genotypes(cases/controls)				*p*	AOR (95% CI)
		AA+CC		AC			
		N	%	N	%		
Total	375/283	209/125	55.7/44.2	166/158	44.3/55.8	0.007	0.629 (0.449-0.883)
Age							
<60	69/130	32/56	46.4/43.1	37/74	53.6/56.9	0.621	0.858 (0.448-1.574)
≥60	306/153	177/69	57.8/45.1	129/84	42.2/54.9	0.005	0.553 (0.366-0.835)

Notes: p-values were calculated by unconditional logistic regression adjusted for age, smoking status. *p*: Statistically significant difference between AA+CC and AC;
Abbreviations: AOR: adjusted odds ratio; CI: confidence interval; N: number of subjects.

decreased COPD risk, and the relationship was more pronounced in older patients with current smoking status. Moreover, statistical evidence for AC genotype and GOLD III was found. However, no significant association with COPD was identified for the other polymorphisms genotyped in our study.

NLRP3 inflammasome participates in the process of sterile inflammation, and they are activated by exogenous or endogenous damage-associated molecular patterns (DAMPs), which consist of NLRP3, ASC and caspase-1 proteins[19]. NLRP3 contains three domains: C-terminal leucine-rich repeats (LRR), N-terminal terminal effector domain, and a central nucleotide domain named the NACHT domain. ASC contains two domains: N-terminal PYD and C-terminal caspase recruitment domain (CARD). Caspase-1 has conservative domains for hemophilic interaction, and it also contains CARD and catalytic domains[20]. The activated inflammasome contributes to the release of mature cytokines which lead to the formation of the innate immune response[21]. Various exogenous and endogenous danger signals, including bacterial pathogens[22], fungal pathogens[23], viral pathogens[24], parasites[25], monosodium urate (MSU) crystal, extracellular adenosine triphosphate (ATP), are known to activate NLRP3 inflammasome[26,27]. Although the exact molecular mechanism of NLRP3 recognition of DAMPs remains unclear, three possible mechanisms have been proposed. The first one is potassium efflux through the purinergic P2X7 receptor and other ion channels and pore-forming toxins; a second possible reason is that mitochondrial reactive oxygen species (ROS) are associated with NLRP3 activation[19,28]; the third theory suggests that the instability of the phagosomes and the cytoplasmic release of lysosomal cathepsin contribute to NLRP3 activation. It is now clear that Nlrp3 inflammasome activation requires two signals. First of all, an NF-kB-activating stimulus contributes to the expression of pro-IL-1B and NLRP3[29]. NLRP3 deubiquitination is the second signal necessary for the assembly and activation of inflammasome[30,31]. An accumulating body of evidence[14,32] shows that genetic polymorphism of NLRP3 rs1539019 might be a crucial determinant of several disease susceptibility. To date, there is no report on the relationship between the polymorphisms of NLRP3 rs1539019 and COPD risk.

In this study, the most important finding was the association between NLRP3 rs1539019 and COPD risk. Our result first demonstrated that the

Table V. Analyses the genotypes of NLRP3 rs1539019 polymorphism and COPD risk stratified by smoking status.

Variables	Cases/Controls	Genotypes (cases/controls)				*p*	AOR (95% CI)
		AA+CC		AC			
		N	%	N	%		
Total	375/283	209/125	55.7/44.2	166/158	44.3/55.8	0.007	0.629 (0.449-0.883)
Smoking status							
Never	39/72	25/36	64.1/50.0	14/36	35.9/50.0	0.632	0.807 (0.335-1.945)
Former	215/98	110/44	51.2/44.9	105/54	48.8/55.1	0.430	0.820 (0.500-1.343)
Current	121/113	74/45	61.2/39.8	47/68	38.8/60.2	0.002	0.419 (0.240-0.732)

Notes: p-values were calculated by unconditional logistic regression adjusted for age, smoking status. *p*: Statistically significant difference between AA+CC and AC;
Abbreviations: AOR: adjusted odds ratio; CI: confidence interval; N: number of subjects.

Table VI. Analyses the genotypes of NLRP3 rs1539019 polymorphism and COPD risk stratified by severity.

Variables	Cases	Genotypes (cases/controls)				p	AOR (95% CI)
		AA+CC		AC			
		N	%	N	%		
Total	375	209/125	55.7/44.2	166/158	44.3/55.8	0.007	0.629 (0.449-0.883)
severity							
GOLD I	66	34/125	51.5/44.2	32/158	48.5/55.8	0.327	0.755 (0.430-1.325)
GOLD II	139	76/125	54.7/44.2	63/158	45.3/55.8	0.079	0.675 (0.435-1.046)
GOLD III	107	65/125	60.7/44.2	42/158	39.3/55.8	0.006	0.502 (0.306-0.822)
GOLD IV	63	34/125	54.0/44.2	29/158	46.0/55.8	0.140	0.648 (0.365-1.152)

Notes: p-values were calculated by unconditional logistic regression adjusted for age, smoking status. *p*: Statistically significant difference between AA+CC and AC;
Abbreviations: AOR: adjusted odds ratio; CI: confidence interval; N: number of subjects.

genotype frequency of NLRP3 rs1539019 polymorphism was significantly different between the cases and the controls, indicating that the polymorphism of NLRP3 rs1539019 was correlated to the onset of COPD. When compared with CC genotype, the AC genotype of NLRP3 rs1539019 significantly decreased COPD risk, which suggests that genetic polymorphisms may play an important role in COPD susceptibility. There was no significant difference in the AA genotype compared with the CC genotype. More interestingly, when comparing with AA+CC genotype, we revealed that the protective effects of AC genotype were more pronounced in older patients with current smoking status. However, there was no significant difference in patients who were younger than 60 years old with never smoking status or former smoking status. Moreover, a significant decreased risk for GOLD III COPD was found in genotype AC but not GOLD I, GOLD II and GOLD IV COPD, which suggests that there is a complex relationship between pulmonary ventilation dysfunction and genotype, and the exact mechanism needs further study.

Despite the novelty of this study, there are some limitations. First, the study was a case-control study, and the possibility of subject selection bias was inevitable. Second, the cross-sectional study design was not able to assess the effect of time on disease. As a result, we were unable to evaluate dynamic changes in lung function, which may lead to bias in COPD grading diagnosis in some subjects. Third, although this study showed that NLRP3 rs1539019 polymorphism was associated with COPD risk, more studies on pathophysiological mechanisms and functional experiments are needed to explain this result. Finally, the sample size of this experiment was relatively small and only male participants were included, which may lead to other differences between groups that cannot be well reflected and the subjects are not fully representative of the general population. Therefore, it is necessary to further expand the sample size and functional experiments to verify the results of our study.

Conclusions

Our present study suggests that the genotype AC of NLRP3 rs1539019 is associated with a decreased risk of COPD in a Chinese Han population, which may provide evidence for the susceptibility and genetic polymorphism of COPD. Therefore, a multi-center, multi-ethnic, large-sample study is needed to further confirm our study.

Conflict of Interest

All authors declare that they have no conflict of interest.

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Authors' Contribution

ZF Hou conceived of the idea, performed the literature search, collected the data, and drafted the entire article. ZH Yuan, K Chang and ZF Hou analyzed the data. YH Cao, FX Guan and Y Gao collected the data and sample. Y Gao and ZF Hou revised the manuscript. All authors contributed to the article and approved the final version of the manuscript.

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Data Availability Statement

Data supporting the finding of this study are available from the corresponding author upon reasonable request.

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