

# Interleukin-33 gene polymorphisms and chronic obstructive pulmonary disease in the Chinese Han population

Journal of International Medical Research

48(12) 1–7

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DOI: 10.1177/0300060520962340

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## Abstract

**Objective:** To investigate the relationship between interleukin (IL)-33 gene polymorphisms rs928413 and rs7044343 with chronic obstructive pulmonary disease (COPD) in the Chinese Han population.

**Method:** We assessed IL-33 rs928413 and rs7044343 polymorphisms by Sanger sequencing of PCR products amplified from the genomic DNA of 160 COPD patients and 123 healthy controls.

**Results:** There was no significant difference in the distribution of rs928413 AA, AG, or AA genotypes or rs7044343 CC, CT, or TT genotypes between the two groups. However, COPD patients had a significantly higher frequency of the rs928413 G allele G (14.1% vs 7.3%, respectively). This allele was significantly associated with susceptibility to COPD (odds ratio [OR]: 2.04, 95% confidence interval [CI]: 1.12–3.57). The rs928413 dominant inheritance model was associated with COPD susceptibility (OR: 2.08, 95%CI: 1.09–4.04).

**Conclusion:** The G allele of rs928413 and the rs928413 dominant inheritance model were associated with susceptibility to COPD in the Chinese Han population.

## Keywords

Chronic obstructive pulmonary disease, interleukin-33, polymorphism, Chinese Han population, allele, genotype

Date received: 19 April 2020; accepted: 8 September 2020

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## Background

Chronic obstructive pulmonary disease (COPD) is one of the most common respiratory diseases and the third leading cause of death worldwide. It is characterized by an abnormal inflammatory response of the lung to noxious particles or gases.<sup>1</sup> Persistent airway inflammation results in airway destruction and remodeling, leading to progressive airway stenosis, airflow restriction, and lung function impairment. Although noxious particles and gases have been recognized as key risk factors, only approximately 25% of smokers develop COPD by the age of 80 years.<sup>2</sup> Moreover, COPD shows familial aggregation, suggesting the involvement of genetic factors as well as environmental factors.

Interleukin (IL)-33 is one of the most recently discovered members of the IL-1 family. The IL-33 gene is located on chromosome 9p24.1, and is expressed by endothelial and epithelial cells, macrophages, and fibroblasts in both healthy and inflammatory tissues. It plays a critical role in various inflammatory and immunological responses as a ligand for ST2. The contribution of IL-33 to chronic respiratory disorders, such as asthma, COPD, and obstructive sleep apnea, has recently been reviewed.<sup>3</sup> IL-33 promotes the development of inflammatory airway diseases by enhancing production of inflammatory cytokines such as IL-4, IL-6, IL-8, and IL-13.<sup>4</sup> IL-33 expression levels were reported to be elevated in patients with asthma and correlate with asthma severity,<sup>5,6</sup> while high IL-33 expression in peripheral blood and lung tissues was also detected in patients with COPD.<sup>7,8</sup> Indeed, IL-33 levels in induced sputum, exhaled breath condensate, and bronchial mucosa are similar in COPD and asthma patients.<sup>9</sup> Moreover, exposure to cigarette smoke and lipopolysaccharide can increase the expression of IL-33 by airway epithelial cells, endothelial cells,

peripheral blood mononuclear cells, and lymphocytes.<sup>6,10</sup>

rs928413 (NG\_047209.1:g.3239G>A, HGSV nomenclature) and rs7044343 (NM\_033439.4:c.521-254C>T, HGSV nomenclature) single nucleotide polymorphisms of the IL-33 gene have been shown to affect the expression of IL-33 mRNA and protein. Additionally, the G allele of rs928413 was identified as a susceptible variant for asthma with increased IL-33 levels.<sup>9,11</sup> However, no association was reported between rs7044343 and asthma in pediatric Tunisian asthma patients.<sup>12</sup>

The relationship of IL-33 gene variants with COPD is unclear. Given the importance of IL-33 in inflammatory airway diseases and its elevation in COPD patients, the present study investigated whether the rs928413 and rs7044343 polymorphisms were associated with susceptibility to COPD in the Chinese Han population which comprises the majority of the population in China.

## Methods

### Study populations

A cohort of 160 unrelated COPD patients (45 women and 115 men) diagnosed according to the Global Initiative for Chronic Obstruction Pulmonary Disease at Shandong University Qilu Hospital was studied as the COPD group.<sup>1</sup> Patients were excluded if they had other diseases that could influence lung function, such as asthma, bronchiectasis, and interstitial lung disease. The control group (43 women and 80 men) consisted of 123 healthy, unrelated, age-matched subjects with a smoking index (cigarette packs/day  $\times$  number of smoking years) over 10 pack-years. The ages and smoking indexes of all participants were collected. The Medical Ethics Committee of Shandong University approved the

study, and participants provided their informed written consent.

### *Clinical evaluation*

Lung function tests were performed for each participant using a MasterScreen™ computerized spirometer (Jaeger Corp., Hoechberg, Germany) according to American Thoracic Society and European Respiratory Society recommendations.<sup>13</sup> The forced expiratory volume in the first second/predicted normal value (%) (FEV<sub>1</sub>%), forced vital capacity/predicted normal value (FVC%), and FEV<sub>1</sub>/FVC were recorded.

### *DNA extraction and genotyping*

Peripheral venous blood samples (3 mL) were collected in tubes containing 1.8 mg/mL ethylenediaminetetra-acetic acid. Genomic DNA was isolated using the DNA extraction Kit (Tiangen Biochemical Technology Co., Beijing, China) according to the manufacturer's instructions and stored at -20°C before use in genotyping.

The sequences of sense and antisense primers to amplify a 472 bp fragment of rs928413 were 5'-ATTATACGATTACTAGAATAAAGT-3' and 5'-TGC GTAATTCGGCAAGTGT TTTTGA-3', respectively. Sense and antisense primers to amplify a 335 bp fragment of rs7044343 were 5'-GGGTACAACCTAGAAAGTTGCACCT-3' and 5'-TAAGATGTTACATAAAATAATTAC-3', respectively. PCR conditions were initial denaturation at 96°C for 1 minute, then 25 cycles of 96°C for 1 s, 50°C for 5 s, and 60°C for 4 minutes. PCR products were sequenced using Sanger sequencing on an ABI 3730xl DNA analyzer (Life Technologies Corp., Carlsbad, CA, USA) according to the manufacturer's instructions. Sanger sequencing trace files were analyzed using Sequencher software (Gene Codes Corp., Ann Arbor, MI, USA).

### *Statistical analysis*

Statistical analysis was performed using SPSS software version 19.0 (SPSS, Chicago, IL, USA). The Kolmogorov–Smirnov test was used to analyze whether the data followed a normal distribution. Normal data were described by means ± standard deviations. The Student's t-test was used to determine the significance of quantitative data. Non-normal data were described by medians (P25, P75) and tested by the non-parametric Kruskal–Wallis test. Hardy–Weinberg equilibrium was assessed using chi-square tests to evaluate the deviation of genotype distribution. The chi-square test was also used to compare differences in genotype or allele frequencies between patients and controls. The Bonferroni correction was used for multiple comparisons, and a P value <0.025 was considered statistically significant.

The association between susceptibility to COPD and genotypes in recessive, dominant, and overdominant models or alleles was analyzed using logistic regression analysis. These were adjusted for sex, age, and smoking index. A P value <0.05 was considered statistically significant. SHEsis online haplotype analysis software was used for haplotype estimation.<sup>14</sup>

### **Results**

Clinical and demographic characteristics of subjects are shown in Table 1. There was no significant difference in sex, age, or smoking index between COPD patients and the control group. However, FEV<sub>1</sub>% predicted, FVC% predicted, and FEV<sub>1</sub>/FVC were significantly different between the two groups (P < 0.01).

IL-33 gene polymorphisms rs928413 and rs7044343 were successfully genotyped for all subjects. Patients and controls were all found to be in Hardy–Weinberg equilibrium and none deviated significantly.

**Table 1.** Characteristics of the subjects.

	COPD group (n = 160)	Control group (n = 123)	P
Men/women	115/45	80/43	0.22
Age (years)	64.9 ± 9.7	64.0 ± 9.3	0.43
Smoking index (pack-years)	37.5 (20, 50)	30.0 (20, 45)	0.10
FEV1 % predicted	51.9 ± 18.2	94.5 ± 11.6	<0.01
FVC% predicted	78.45 ± 16.96	97.89 ± 10.46	<0.01
FEV1/FVC	56.07 ± 10.58	81.19 ± 6.04	<0.01

Normal values are expressed as means ± SD. Non-normal data are expressed as medians (P25, P50).

COPD, chronic obstructive pulmonary disease; FEV, forced expiratory volume; FVC, forced vital capacity.

**Table 2** Genotype frequencies of IL-33 rs928413 and rs7044343 polymorphisms.

Group	rs928413			rs7044343		
	A/A	A/G	G/G	C/C	C/T	T/T
COPD	121 (75.6%)	33 (20.6%)	6 (3.7%)	52 (32.5%)	80 (50.0%)	28 (17.5%)
Control	107 (87.0%)	14 (11.3%)	2 (1.6%)	41 (33.3%)	64 (52.0%)	18 (14.6%)
	$\chi^2 = 5.80$	P = 0.06		$\chi^2 = 0.42$	P = 0.81	

Values are shown as n (%).

IL, interleukin; COPD, chronic obstructive pulmonary disease.

The genotype and allele frequencies of rs928413 and rs7044343 are shown in Table 2 and Table 3. There was no significant difference in the genotype distribution of either polymorphism between the two groups (Table 2). COPD patients were found to have a significantly higher frequency of the G allele of rs928413 compared with controls (14.1% vs 7.3%, respectively,  $P = 0.01$ ) but there was no significant difference in C or T allele frequency of rs7044343 between the two groups (Table 3). The low linkage disequilibrium between rs928413 and rs7044343 ( $D' = 0.094$ ,  $r^2 = 0.002$ ) necessitated the genotyping of both polymorphisms.

Logistic regression analysis, adjusted for age, sex, and smoking index, showed that the minor allele (G allele) of rs928413 was associated with significant susceptibility to COPD ( $P = 0.02$ , odds ratio [OR]: 2.04, 95% confidence interval [CI]: 1.12–3.57).

rs7044343 alleles did not show an association with COPD. The rs928413 dominant inheritance model was significantly negatively associated with susceptibility to COPD ( $P = 0.03$ , OR: 2.08, 95% CI: 1.09–4.04), but there was no significant association under the rs928413 recessive inheritance model. Additionally, there was no significant association between COPD susceptibility and the control group under rs7044343 dominant, recessive, or overdominant inheritance models (Table 4).

## Discussion

Cigarette smoke exposure is the most important risk factor for COPD, but the observed variation in its development among individuals with similar exposure histories also suggests the partial influence of genetic factors.

**Table 3.** Allele frequencies of IL-33 rs928413 and rs7044343 polymorphisms.

Group	rs928413		rs7044343	
	A	G	C	T
COPD	275 (85.9%)	45 (14.1%)	184 (57.5%)	136 (42.5%)
Control	228 (92.7%)	18 (7.3%)	146 (59.5%)	100 (41.5%)
	$\chi^2 = 6.40$	$P = 0.01$	$\chi^2 = 0.196$	$P = 0.66$

Values are shown as n (%).

IL, interleukin; COPD, chronic obstructive pulmonary disease.

**Table 4.** Logistic regression analysis for COPD susceptibility adjusted for sex, age, and smoking index.

Variable	Odds ratio	95% CI	P
rs928413 A	1.00		
G	2.04	1.12–3.57	0.02*
A/A	1.00		
G/G	2.56	0.49–12.5	0.26
A/G	2.01	1.10–4.03	0.04*
A/G+G/G versus A/A (Dominant)	2.08	1.09–4.04	0.03*
G/G versus A/A + A/G (Recessive)	2.33	0.46–11.7	0.31
A/A + G/G versus A/G (Overdominant)	1.95	0.98–3.88	0.06
rs7044343 C	1.00		
T	0.88	0.63–1.24	0.47
C/C	1.00		
T/T	0.76	0.48–1.27	0.29
C/T	0.90	0.62–1.30	0.58
CT+T/T versus C/C (Dominant)	0.86	0.52–1.42	0.56
T/T versus C/T+C/C (Recessive)	1.23	0.65–2.36	0.53
T/T+C/C versus C/T (Overdominant)	0.98	0.61–1.58	0.93

COPD, chronic obstructive pulmonary disease; CI, confidence interval.

Nano-organic carbon particles and combustion-generated ultrafine particles in cigarette smoke can induce the release of IL-33 from peripheral blood mononuclear cells of exacerbated COPD patients.<sup>15</sup> IL-33

has not only been shown to promote COPD airway inflammation by inducing IL-6 and IL-8 expression in bronchial epithelial cells and mononuclear cells,<sup>3</sup> but also to induce autoantibodies against lung tissue. Additionally, plasma IL-33 levels positively correlate with the peripheral eosinophil count in COPD patients.<sup>16</sup>

In the present study the rs928413 G allele and A/G genotype were associated with susceptibility to COPD. This is in accordance with the observed elevated IL-33 expression in COPD patients because the rs928413 G allele was found to increase activity of the IL-33 promoter through binding the transcription factor cAMP response element-binding protein 1.<sup>17</sup>

The rs7044343 polymorphism affects binding of transcription factors SC35 and SF/ASF, which has possible consequences for the expression of IL-33.<sup>18</sup> Although the rs7044343 CC genotype is associated with higher levels of IL-33 expression than CT and TT genotypes,<sup>18</sup> we observed no significant difference in genotype or allele distribution of rs7044343 between COPD and control groups.

A limitation of the present study is that we did not evaluate IL-33 expression in participants; however, rs928413 and rs7044343 have previously been shown to affect IL-33 expression levels.<sup>10,11,18</sup>

In conclusion, the IL-33 gene rs928413 polymorphism may contribute to the development of COPD in the Chinese

Han population. Further investigations of the impact of IL-33 genetic variants on the inflammatory process of COPD are required.

### Declaration of conflicting interest

The authors declare that there is no conflict of interest.

### Funding

This research is supported by the Key Research and Development project of Shandong Province (2019GSF108114).

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