Association between *IL18*-607C/A and -137G/C polymorphisms and susceptibility to non-small cell lung cancer in a Chinese population

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ABSTRACT. Lung cancer is one of the main causes of cancer-related mortality in males and females worldwide. A pleiotropic effect has been observed in the interleukin 18 gene (*IL18*); its effects include the activation of natural killer cell cytotoxicity and the promotion of the Th1 immune response through the alteration of the expression of interferon-γ and TNF-α in humans. IL18 is therefore involved in the elimination of tumor cells in the human body. We recruited 357 patients with non-small cell lung cancer (NSCLC) and 414 controls to evaluate the correlation between two genetic variations (*IL18*-607C/A and *IL18*-137G/C) and the pathogenesis of NSCLC. We used polymerase chain reaction-restriction fragment length polymorphism to genotype *IL18*-607C/A and *IL18*-137G/C. Statistical analysis revealed that individuals harboring the AA genotype of *IL18*-607C/A had an increased risk of NSCLC compared to those harboring the CC genotype (OR = 2.20, 95%CI = 1.30-3.74). Individuals expressing the A allele of *IL18*-

607C/A had an elevated risk of developing NSCLC compared to those expressing the C allele (OR = 1.31, 95%CI = 1.06-1.62). In summary, our analysis shows that the *IL18*-607C/A genetic variation is related to the risk of NSCLC, whereas the *IL18*-137G/C variation is not. Therefore, the *IL18*-607C/A variation is related to the pathogenesis of NSCLC in the Chinese population studied.

Key words: Non-small cell lung cancer; IL18; *IL18*-607C/A; *IL18*-137G/C; Polymorphism

INTRODUCTION

Lung cancer is the one of the main cause of cancer-related mortality in both males and females worldwide (IARC, 2012). It is estimated that there were 1.8 million new lung cancer cases in 2012 and approximately 58% of those cases occurred in less developed regions (IARC, 2012). In China, it is estimated that 193,347 new cases of lung cancer occurred in 2012, resulting in 175,487 deaths from the disease (IARC, 2012). The incidence and mortality rates of lung cancer in China are approximately $23.1/10^5$ and $19.7/10^5$, respectively. Non-small cell lung cancer (NSCLC) patients make up more than 80% of all lung cancer patients. The pathogenesis of NSCLC is poorly understood. It is well known that the etiology and pathogenesis of NSCLC involves multiple factors, such as long-term tobacco smoking, low intake of fresh fruit and vegetables, low body mass index (BMI), respiratory diseases, and long-term exposure to cooking oil fumes (Zhong et al., 1999a,b; Herbst et al., 2008; O'Callaghan et al., 2010; Goeckenjan et al., 2011; Yano et al., 2011). Moreover, certain hereditary factors are involved in its pathogenesis. Such factors include the genes that encode XPA-binding protein 2, vascular endothelial growth factor, stromal-derived factor-1, tankyrase 1, epidermal growth factor, TNF-related apoptosis-inducing ligand, and chemokine (C-C motif) ligand 2 (Krupnova et al., 2015; Luo et al., 2015; Masroor et al., 2015; Pei et al., 2015; Wang et al., 2015; Xu et al., 2015; Li et al., 2016b).

It has been reported that interleukin-18 (IL18) has a role in both anticancer and pro-cancer (Dwivedi et al., 2015a) activity. IL18 is involved in eliminating tumor cells in the human body (Kalina et al., 2000; Marshall et al., 2006). The *IL18* gene has the chromosomal locus 11q22.2-q22.3, and comprises six exons and five introns. *IL18*-607C/A and -137G/C are two important polymorphisms of this gene; they are located upstream of exon one and their genetic variations can affect the function of the protein (Kalina et al., 2000; Marshall et al., 2006). To date, no one has investigated the relationship between the *IL18* -137G/C polymorphism and the risk of cancer. Therefore, in this study, we recruited 357 NSCLC patients and 414 controls with the aim of evaluating the role of *IL18*-607C/A (rs1946518) and -137G/C (rs187238) polymorphisms in the development of NSCLC.

MATERIAL AND METHODS

Subjects

A total of 357 patients with NSCLC and 414 controls were recruited to the current study. Between October 2013 and July 2015, the NSCLC patients were recruited from the

Zhumadian City Center Hospital. The diagnosis of NSCLC was confirmed by pathological biopsy. The patients did not receive any chemotherapy or radiotherapy treatment prior to enrollment in the study. Patients with a history of secondary or recurrent malignant tumors and malnutrition were excluded from the study.

Between December 2013 and May 2015, 414 individuals were recruited from the outpatient clinics and health examination centers of the Zhumadian City Center Hospital. All the patients were confirmed to be free of malignant tumors, respiratory system diseases, or end-stage kidney or liver diseases.

Data on exposure to potential risk factors for NSCLC, such as gender, age, tobacco smoking, years of tobacco smoking, BMI, exposure to cooking oil fumes, and years of exposure to cooking oil fumes, were collected using an ad hoc questionnaire or from medical records. The TNM stage of the NSCLC patients was retrieved from their medical records. Tobacco use was defined as smoking at least one pack of cigarettes per week for six months. Informed consent was obtained from all NSCLC patients and controls before enrollment. The protocol for our study received approval from the ethics committee of the Zhumadian City Center Hospital. The ethical standards adopted for this study were based on the Declaration of Helsinki.

The mean ages of the NSCLC patients and controls were 64.32 ± 7.50 and 64.10 ± 7.37 years, respectively. There were 113 (31.65%) female and 244 (68.35%) male NSCLC patients, and 142 (34.30%) female and 272 (65.70%) male controls. Of the patients with NSCLC, 125 (35.01%) were at stage I-II, and 232 were at stage III-IV.

Genotyping

Peripheral venous blood samples (5 mL) were collected from all participants and stored in tubes with 3.2% ethylenediaminetetraacetic acid. The blood samples were stored in a refrigerator at 4°C. Extraction of genomic DNA was carried out using a TIANamp Blood DNA Kit (Tiangen, Beijing, China). Genotyping of *IL18*-607C/A and -137G/C was carried out by polymerase chain reaction -restriction fragment length polymorphism (PCR-RFLP). The primers, restriction enzymes, and enzyme-digested products were provided by Sangon Biotech Company and these are presented in Table 1 (all reagents were provided by Sangon Biotech Inc., Shanghai, China). The PCR regimen was as follows: one cycle of initial denaturation at 94°C for 5 min; 42 cycles of 94°C for 30 s, 53°C for 30 s, and 72°C for 45 s; and a final extension at 72°C for 10 min. The PCR products of *IL18*-607C/A and -137G/C were digested with *Mse*I and *Bfu*CI restriction enzymes at 37°C for 4 h. The PCR amplification and enzyme digestion was observed on 2% agarose gel.

Table 1. Primers, restriction enzymes, and enzyme-digested products for *IL18*-607C/A and -137G/C.

Genotypes	Primers (5'-3')	Restriction enzymes	Enzyme-digested products
IL18 -607C/A	TTGTAACATTGTAGGAATTACC ATGTAATATCACTATTTTCATGAGA	*Mse*I	137, 91 and 46bp
IL18 -137G/C	ATGCTTCTAATGGACTAAGGA GTAATATCACTATTTTCATGAATT	*Bfu*CI	256, 229 and 27bp

Statistical analysis

The comparison of lifestyle variables and genotype frequencies between the two investigated groups was analyzed using the chi-square test or the Student *t*-test. Using the

Pearson chi-square test, deviation of *IL18*-607C/A and -137G/C from the Hardy-Weinberg equilibrium (HWE) was determined by comparing the actual with the predicted values. The association between the *IL18*-607C/A and -137G/C polymorphisms and the risk of NSCLC was examined by logistic regression analysis. The odds ratios (ORs) and 95% confidence intervals (95%CIs) were calculated to express the association. The IBM SPSS Statistics for Windows, Version 20.0. platform (IBM Corp., Armonk, NY, USA) was used to analyze the data.

RESULTS

General information about the NSCLC patients and controls is given in Table 2. The chi-square test revealed that the NSCLC patients had lower BMIs ($\chi^2 = 4.93$, $P = 0.03$), were more likely to smoke ($\chi^2 = 11.07$, $P = 0.001$), and had smoked for longer durations ($\chi^2 = 68.99$, $P < 0.001$) than the controls. However, no significant differences were observed in age ($\chi^2 = 0.54$, $P = 0.46$) or sex ($\chi^2 = 61$, $P = 0.44$).

Table 2. General information about NSCLC patients and controls.

Variables	Patients (N = 357)	%	Controls (N = 414)	%	χ^2 value	P value
Age, years						
<60	122	34.17	152	36.71		
≥60	235	65.83	262	63.29	0.54	0.46
Gender						
Female	113	31.65	142	34.30		
Male	244	68.35	272	65.70	0.61	0.44
BMI, kg/m^2						
<24	248	69.47	256	61.84		
≥24	109	30.53	158	38.16	4.93	0.03
Smoking status						
No	241	67.51	231	55.80		
Yes	116	32.49	183	44.20	11.07	0.001
Years of smoking						
<10	258	72.27	176	42.51		
≥10	99	27.73	238	57.49	68.99	<0.001
TNM stage						
I-II	125	35.01				
III-IV	232	64.99				

NSCLC = non-small cell lung cancer; BMI = body mass index.

The frequencies of the CC, CA, and AA genotypes of *IL18*-607C/A were significantly different between the NSCLC patients and the controls ($\chi^2 = 10.04$, $P = 0.01$), whereas the frequencies of the GG, GC, and CC genotypes of *IL18*-137G/C did not differ significantly between the two investigated groups ($\chi^2 = 1.14$, $P = 0.56$) (Table 3). The Pearson chi-square test revealed that the genotype frequencies of *IL18*-137G/C were in agreement with the HWE in both patients ($\chi^2 = 0.18$, $P = 0.67$) and controls ($\chi^2 = 3.29$, $P = 0.07$). However, the genotype frequencies of *IL18*-607C/A were in agreement with the HWE in the patients ($\chi^2 = 2.71$, $P = 0.10$) but not in the controls ($\chi^2 = 13.71$, $P < 0.001$).

Logistic regression analysis revealed that individuals with the AA genotype of *IL18*-607C/A showed an increased risk of NSCLC compared to those carrying the CC genotype (OR = 2.20, 95%CI = 1.30-3.74) (Table 4). Moreover, individuals expressing the A allele of *IL18*-607C/A had a higher risk of developing NSCLC than those with the C allele (OR = 1.31, 95%CI = 1.06-1.62). However, we found no significant association between the *IL18*-137G/C genetic variation and the risk of NSCLC.

Table 3. Genotype distributions of *IL18*-607C/A and -137G/C in investigated controls.

IL18	Patients (N = 357)	%	Controls (N = 414)	%	χ^2 value	P value	HWE in patients		HWE in controls	
-607C/A							χ^2 value	P for HWE	χ^2 value	P for HWE
CC	116	32.49	159	38.41						
CA	188	52.66	222	53.62						
AA	53	14.85	33	7.97	10.04	0.01	2.71	0.10	13.71	<0.001
-137G/C										
GG	174	48.74	215	51.93						
GC	148	41.46	156	37.68						
CC	35	9.80	43	10.39	1.14	0.56	0.18	0.67	3.29	0.07

HWE = Hardy-Weinberg equilibrium.

Table 4. Association between *IL18*-607C/A and -137G/C polymorphisms and risk of NSCLC.

IL18	Patients (N = 357)	%	Controls (N = 414)	%	Adjusted OR (95%CI)[1]	P value
-607C/A						
CC	116	32.49	159	38.41	Reference	-
CA	188	52.66	222	53.62	1.16 (0.84-1.60)	0.34
AA	53	14.85	33	7.97	2.20 (1.30-3.74)	0.002
Allele						
C	420	58.82	540	65.22	Reference	-
A	294	41.18	288	34.78	1.31 (1.06-1.62)	0.01
-137G/C						
GG	174	48.74	215	51.93	Reference	-
GC	148	41.46	156	37.68	1.17 (0.86-1.60)	0.30
CC	35	9.80	43	10.39	1.01 (0.60-1.69)	0.98
Allele						
G	496	69.47	586	70.77	Reference	-
C	218	30.53	242	29.23	1.06 (0.85-1.33)	0.58

[1]Adjusted for body mass index (BMI), smoking status, and years of smoking. NSCLC = non-small cell lung cancer.

DISCUSSION

In the present study, we found that individuals carrying the AA genotype and the A allele of *IL18* had an increased risk of developing NSCLC compared with those carrying the wide-type genotype. The expression of *IL18*, which encodes an important inflammatory factor, has a critical inflammatory effect on various physiological processes in many cancers (Bao et al., 2015; Dwivedi et al., 2015b; Lu et al., 2015; Ko et al., 2016; Li et al., 2016a). Genetic polymorphisms can change the structure and quantity of the gene product, ultimately affecting its function. Genetic polymorphisms in *IL18* may determine the expression and function of the protein it encodes, thereby influencing cancer susceptibility in the subjects.

Previous studies have indicated an association between *IL18*-607C/A and -137G/C polymorphisms and the risk of many malignant tumors, such as esophageal cancer, gastrointestinal cancer, papillary thyroid cancer, hepatocellular carcinoma, prostate carcinoma, and oral squamous cell carcinoma (Liu et al., 2013; Singh et al., 2014; Bao et al., 2015; Chung et al., 2015; Dwivedi et al., 2015b; Li et al., 2015; Yao et al., 2015; Zhu et al., 2016). Singh et al. (2014) performed a study on 272 patients with oral squamous cell carcinoma and 185 controls, and reported that the *IL18*-137G/C genetic variation was correlated with the progression of this cancer, but the -607C/A polymorphism was not. The authors of a study on 153 hepatocellular carcinoma patients and 165 healthy controls reported that the *IL18*-137G/C polymorphism was negatively associated with the risk of hepatocellular carcinoma, but the -607C/A polymorphism was not (Bao et al., 2015). Chung et al. (2015) reported that the *IL18* rs549908, rs360717, and rs187238 SNPs were positively correlated with the development

of papillary thyroid cancer. Dwivedi et al. (2015b) found that individuals harboring the GG genotype of *IL18* could be diagnosed at an earlier stage than those with the CC genotype. Yao et al. (2015) examined five studies comprising 1618 patients and 1155 healthy controls, and reported that the *IL18*-607C/A polymorphism was associated with a higher risk of esophageal cancer.

To date, only one research group has investigated the role of the *IL18*-607C/A genetic variation in the pathogenesis of NSCLC; Jia et al. (2016) performed a study on a Chinese population including 500 patients and 500 healthy controls, and observed a significant association between the *IL18*-607C/A polymorphism and the risk of NSCLC. In our study, we also observed a significant positive relationship between the *IL18*-607C/A genetic variation and the development of NSCLC, but no association between the *IL18*-137G/C polymorphism and the risk of this cancer. Further studies with large-scale sample sizes are required to confirm our results. One important limitation of our study should be noted. The NSCLC patients and the controls were recruited from only one hospital in China, and *IL18*-607C/A did not conform with the HWE in the controls. Therefore, some selection bias could not be avoided in our study.

In conclusion, we observed that the *IL18*-607C/A polymorphism contributes to an elevated risk of NSCLC, whereas the *IL18*-137G/C polymorphism does not. Therefore, the *IL18*-607C/A polymorphism could be a risk factor for NSCLC.

Conflicts of interest

The authors declare no conflict of interest.

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