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Interaction of the *CYP1A1* gene polymorphism and smoking in non-small cell lung cancer susceptibility

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ABSTRACT. Many studies have shown that genetic factors, environmental factors, and bad living habits, especially smoking, are risk factors for lung cancer. However, not all smokers develop lung cancer, which may be related to different genetic backgrounds. Currently, most research has investigated the *GSTM1*, *XRCC1*, *XRCC3*, *CYP2D6*, and *C188T* genes. Little research has been done on the cytochrome P450 (*CYP*) *1A1* gene, and results have varied. In addition, no results have been reported on the interactive effects of smoking and the *CYP1A1* gene on lung cancer development. We used polymerase chain reaction restriction fragment length polymorphism to detect the *CYP1A1* genotype, and investigate the effects of the *CYP1A1* gene deletion and smoking alone, and in combination, on non-small cell lung cancer susceptibility. We enrolled 150 non-small cell lung cancer patients and 150 healthy control subjects. Subjects' smoking habits and *CYP1A1* gene polymorphism were analyzed to investigate their role in the occurrence of lung cancer. The *CYP1A1* gene deletion was found in 73.3% of non-small cell lung cancer patients and 20.0% of healthy subjects. The OR value was 2.28 ($P < 0.05$). Among smoking subjects,

77.8% exhibited non-small cell lung cancer, significantly higher than the 27.3% in non-smokers ($P < 0.05$). The OR value for the interaction of smoking and *CYP1A1* gene deletion was 5.60, larger than the product of their individual OR values. The *CYP1A1* gene deletion is a lung cancer risk factor, and interacts with smoking in non-small cell lung cancer development.

Key words: CYP1 A1; Gene Polymorphism; Smoking; Non-small cell lung cancer

INTRODUCTION

Lung cancer has the highest incidence rate among malignant tumors. It can be classified into non-small cell lung cancer and small cell lung cancer according to its pathology, with non-small cell lung cancer accounting for most cases. Lung cancer is a serious disease with a poor prognosis, as most lung cancers have already metastasized when diagnosed. Early prevention plays an important role in the treatment of lung cancer; thus, finding the risk factors for lung cancer is currently one of the most important tasks in lung cancer prevention (Shi et al., 2008; Cornfield et al., 2009; Jin et al., 2011). Smoking is one of the risk factors for lung cancer. However, not all smokers develop lung cancer, which may be related to different individual genetic factors. It has been reported that the lung cancer risk increases significantly when certain environmental and genetic factors are combined (Honma et al., 2008; Crosbie et al., 2009; Cabral et al., 2010). The roles of *GSTM1*, *XRCC1*, *XRCC3*, *CYP2D6*, and *C188T* genes have been studied thoroughly. However, the cytochrome P450 (*CYP*) *1A1* gene has not been studied extensively, and the results so far are confusing (Aldawsari et al., 2014; Wohak et al., 2014). Several studies (Lee et al., 2010; Arinc et al., 2014; Henderson et al., 2014; Wang et al., 2014; Gaspar-Ramirez et al., 2015) have shown that patients with *CYP1A1* deletion cannot produce the active enzyme, so that carcinogenic substances cannot be metabolized and excreted. The long-term presence of carcinogenic substances coupled with smoking increased the susceptibility to lung cancer. We used a case-control method to study the relationship between the susceptibility to non-small cell lung cancer and the *CYP1A1* gene deletion alone as well as the combined effect of *CYP1A1* deletion and smoking.

MATERIAL AND METHODS

Main instruments and reagents

The blood genomic DNA extraction kit was bought from Sunshine Biotechnology Co., Ltd. (Nanjing, China).

Patients

The study protocol was approved by the Research Ethics Committee of The First People's Hospital of Jingmen City, Hubei, China, and all patients gave their informed consent before study commencement. The blood samples of 150 hospitalized patients diagnosed with non-small cell lung cancer between April 2013 and October 2014 were collected. There were

80 cases of lung adenocarcinoma and 70 cases of lung squamous cell carcinoma. Among the patients, 95 were males and 65 were females, with an average age of 50 ± 4.9 years.

For the healthy control group, 150 healthy subjects were enrolled. Lung disease was ruled out by chest X-ray or chest CT. Among the healthy subjects, 75 were males and 75 were females, with an average age of 52 ± 3.5 years.

There were no significant difference of gender and age between the two groups ($P > 0.05$).

Blood sample collection and DNA extraction

Venous blood (3 mL) was collected with anticoagulants and stored in a -70°C freezer until further use. DNA was extracted according to the blood genomic DNA extraction kit instructions and stored in a -20°C freezer until further use.

CYP1A1 genotype detection

Polymerase chain reaction (PCR) restriction fragment length polymorphism was used to detect the *CYP1A1* genotype. The primers were designed according to the sequence in GenBank (No. 012540.2): forward primer 5-CAGTGAAGAGGTGTAGCCGCT-3, reverse primer 5-TAGGAGTCTTGTCTCATGCCT-3. Each PCR (20 µL final volume) contained 2 µL template DNA and 0.5 µL of each primer. Samples were denatured at 95°C for 1 min, annealed and extended for 1 min each, and cycled 10 times. Samples were then denatured for 50 s, annealed for 1 min at 62°C, extended for 1 min at 72°C, cycled 30 times, and extended for 10 min at 72°C. The products were visualized and separated by agarose gel electrophoresis. *Msp*I restriction enzyme was used for digestion. After electrophoresis on an agarose gel, the digested products were stained with ethidium bromide and imaged.

Statistical analysis

All statistical analyses were performed using the SPSS18.0 software (Chicago, IL, USA). The χ^2 test and the Fisher exact test were used for enumeration data analysis. Logistic regression analysis was applied for genetic factors alone, smoking alone and the combined effect of the two. P values <0.05 were considered to be statistically significant.

RESULTS

CYP1A1 gene agarose gel electrophoresis

The *CYP1A1* (-) genotype showed only one electrophoresis band, while the *CYP1A1* (+) genotype showed two electrophoresis bands. Lanes 1 and 3 show samples from healthy control subjects; lanes 5, 6, and 8 show samples from non-small cell lung cancer patients (Figure 1).

CYP1A1 genotype distribution

The *CYP1A1* gene was deleted in 73.3% of non-small cell lung cancer patients, whereas the deletion was found in 20.0% of healthy control subjects. The OR value was 2.28 ($P < 0.05$). On the other hand, non-deletion of the *CYP1A1* gene was 80% in healthy controls, whereas it was 26.7% in non-small cell lung cancer patients. The OR value was 2.40 ($P < 0.05$) (Table 1).

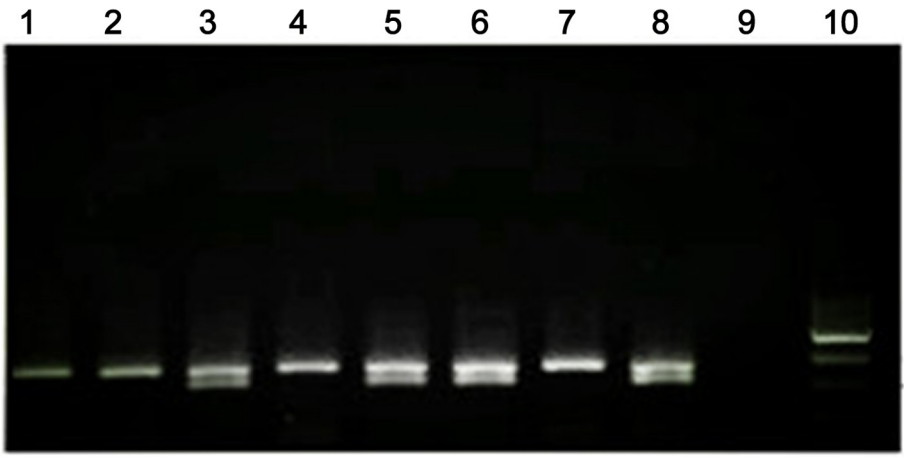

Figure 1. *CYP1A1* gene electrophoresis. *Lanes 3*, *5*, *6*, and *8* show samples from patients with a *CYP1A1* (+) genotype; *lanes 1*, *2*, *4*, and *7* show samples from patients with a *CYP1A1* (-) genotype; *lane 9* is the negative control and *lane 10* contains the marker.

Table 1. *CYP1A1* genotype distribution (%).

Group	Cases	*CYP1A1* gene deletion	*CYP1A1* gene non-deletion
Non-small cell lung cancer	150	73.3 (110/150)	26.7 (40/150)
Healthy control	150	20.0 (30/150)	80.0 (120/150)

$P < 0.05$.

Relationship between smoking and lung cancer

A total of 135 subjects were smokers among the 300 enrolled people. Of the 135 smokers, 105 had developed lung cancer, which indicates a prevalence of lung cancer of 77.8%. Of the 165 non-smokers, 45 had developed lung cancer, which indicates a prevalence of lung cancer of 27.3%. Smokers exhibited a significantly higher incidence rate than non-smokers ($P < 0.05$; Table 2).

Table 2. Relationship between smoking and lung cancer.

Group	Cases	Non-small cell lung cancer
Smoker	135	77.8 (105/125)
Non-smoker	165	27.3 (45/165)

$P < 0.05$.

Relationship between the CYP1A1 genotype and smoking history in non-small cell lung cancer

The OR value for the *CYP1A1* non-deletion genotype with non-smoking was 1.00; the OR value for the *CYP1A1* non-deletion genotype with smoking in non-small cell lung cancer patients was 2.25. The OR value for the *CYP1A1* deletion genotype with non-smoking in non-

small cell lung cancer patients was 1.89. The OR value for the *CYP1A1* deletion genotype with smoking in non-small cell lung cancer patients was 5.60, which was significantly higher than the product of the OR values for smoking and *CYP1A1* gene deletion alone. This suggested that the lung cancer risk increased significantly when individuals with the *CYP1A1* deletion genotype were exposed to additional environmental risk factors (smoking) (Table 3).

Table 3. Relationship between the *CYP1A1* genotype and smoking history in non-small cell lung cancer patients.

CYP1A1	Smoking history	Non-small cell lung cancer	Healthy control	β	OR value	P value
Non-deletion	Non-smoker	15	41	-	1.00	-
Non-deletion	Smoker	25	20	0.407	2.25	0.030
Deletion	Non-smoker	30	79	0.324	1.89	0.089
Deletion	Smoker	80	10	0.870	5.60	0.001

DISCUSSION

Lung cancer has become one of the most common types of cancers, and men exhibit a higher mortality rate than women. However, following the increase of smokers in women in recent years, the lung cancer-related mortality rate has reached 25%, which is nearly equal to that of breast cancer (Shi et al., 2008; Dogan et al., 2009; Cabral et al., 2010; Krishnan et al., 2010). Although experts worldwide have been working to improve the diagnosis and treatment of lung cancer, the 5-year survival rate of lung cancer, which was only about 12.8%, did not improve much (Shibayama et al., 2001; Winton et al., 2005; Askari et al., 2005; Cagle et al., 2011). Early detection of lung cancer can significantly improve the prognosis and prolong the survival time. On the other hand, patients with different pathological types of lung cancer have a different prognosis. Lung cancer can be classified into non-small cell lung cancer and small cell lung cancer according to the pathology. Non-small cell lung cancer, which is further divided into squamous carcinoma, adenocarcinoma, anaplastic large cell carcinoma, and non-differentiated large cell carcinoma, accounts for about 80% of the total number of lung cancer cases, and is thus enormously harmful to human health (Molina et al., 2005). Various factors, including *GSTM1*, *XRCC1*, *XRCC3*, *CYP2D6*, and *C188T* genotypes, contribute to lung cancer development (Mitsudomi et al., 2010). The role of the *CYP1A1* gene in lung cancer occurrence has not been studied extensively yet.

CYP1A1 is a type of metabolic enzyme that can activate polycyclic aromatic hydrocarbons and participates in the estrogen metabolism. Several related studies have shown that there is a significant relationship between *CYP1A1* deletion and the occurrence of endometrial carcinoma. However, its role in lung cancer is still under debate (Zhang et al., 2010; Brevet et al., 2011; Licznerska et al., 2015). Japanese researchers found that Japanese patients with a *CYP1A1* deletion genotype showed a seven times higher lung cancer risk than other individuals (Krais et al., 2014). In contrast, Norwegian researchers reported that Nordic patients with a *CYP1A1* deletion genotype showed no difference in the risk of lung cancer compared to other individuals (Zakiullah et al., 2014). Our results showed that the *CYP1A1* gene was deleted in 73.3% of non-small cell lung cancer patients, whereas only 20.0% of healthy control subjects had the deletion. Our results are consistent with the results from the Japanese study, which may also indicate ethnic differences in the role of the *CYP1A1* deletion.

A previous study found that smoking is one of the risk factors for lung cancer, but

not all smokers are lung cancer patients. We found that the lung cancer incidence in smokers (77.8%) was significantly higher than in non-smokers (22.3%).

We found that the lung cancer risk increased significantly when individuals with the *CYP1A1* deletion genotype were exposed to environmental factors (smoking) compared to individuals who had only one of the two risk factors (smoking or *CYP1A1* deletion genotype).

In conclusion, smoking and *CYP1A1* gene deletion are both risk factors for non-small cell lung cancer, and they showed interaction in non-small cell lung cancer. This research provides a new path for the investigation of lung cancer susceptible genes.

Conflicts of interest

The authors declare no conflict of interest.

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