Original Article
Association of CYP1A1 gene polymorphism and susceptibility to lung cancer: a case-control study

Hao Bai, Liwen Xiong

Department of Respiratory Medicine, Shanghai Chest Hospital, Shanghai Jiaotong University, No. 241, West Huaihai Rd, Shanghai 200030, China

Abstract: Objectives: To investigate the relationship between the metabolic activation enzyme cytochrome P4501A1 gene (CYP1A1) polymorphism and lung cancer in the Chinese population. Methods: Case-control study was used to investigate the distribution frequencies of the CYP1A1 polymorphic genotypes in 98 lung cancer patients and 98 healthy subjects and analyze the relationship between gene polymorphism and susceptibility to lung cancer. Results: Significant differences were observed (x^2=13.57, P<0.05) between the case group and the control group in terms of the gene frequency distributions of the three CYP1A1 genotypes (the mutant, the heterozygous genotype and the wild type). For CYP1A1 gene, a lower risk of developing lung cancer was observed in subjects carrying Ile/Ile genotype than subjects carrying Ile/Val or Val/Val genotype [OR: 0.37 (95% CI 0.20-0.70, x^2=9.67, P<0.05); 0.27 (95% CI 0.11-0.65, x^2=9.03, P<0.05)], which probably indicated that Ile/Ile genotype was a protective factor against lung cancer. In the smoking group, the odds ratio (OR) for susceptibility of Ile/Val or Val/Val subjects to lung cancer was 3.1 compared with Ile/Ile subjects, which was statistically significant. Conclusions: A relationship is found between CYP1A1 gene polymorphism and lung cancer in the Chinese population. The mutant Val allele is probably the susceptible genotype for lung cancer in the Chinese population. Val allele mutation presumably causes lung cancer in coordination with smoking.

Keywords: Lung cancer, metabolic activation enzyme cytochrome P4501A1 gene

Introduction

Lung cancer is one of the most common malignancies globally with high mortality, seriously threatening human health. It is reported that the lung cancer mortality of the Chinese population has grown at an annual rate of 4.5% on average since the beginning of the 1990s. Moreover, heavy haze in China, has increased the risk of developing lung cancer in recent years [1, 2]. In the light of the malignancy cases structure in China over the past decade, lung cancer accounts for 22.7% of all malignancies as the overriding cause of death on the list of malignancies. WHO estimates that China will have become the home to the most lung cancer cases in the world by 2025. The prevention and control of lung cancer has been one of the most pressing issues globally at present. The prevention and treatment against lung cancer should be conducted earlier, and early screening and detection should be emphasized when healthy

lifestyle education is delivered to healthy population due to its insidious onset, late clinical detection, low 5-year survival rate of 10%~15% and high treatment costs. Therefore, seeking effective screening and early diagnosis indicators of lung cancer is a critical step to effectively prevent and control lung cancer.

Risk factors leading to lung cancer are complicated and it is generally believed to be the result of the interaction between environmental and genetic factors [3]. With the development of molecular biotechnology, greater importance has been attached to the genetic susceptibility to lung cancer with more focus on the gene polymorphism of cytochrome P450 and glutathione S-transferases, such as CYP1A1, CYPZE1, GSTM1, etc. However, the results of various studies are not consistent. In this research, we adopted matched case-control study, and investigated CYP1A1 genotypes of cases and controls, with the confirmed cases of
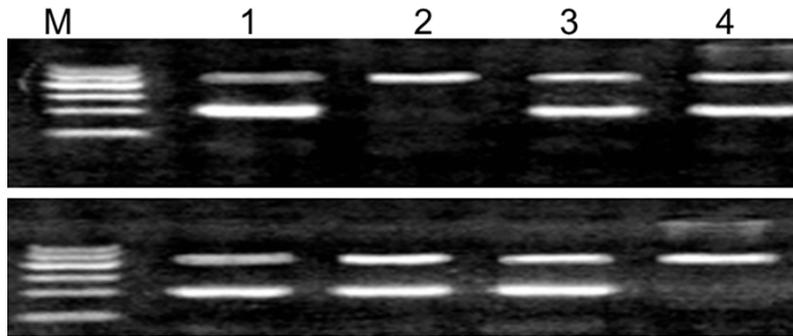
Figure 1. Investigation of CYP1A1 Exon 7 gene polymorphism. Note: M: DNA marker; 1, 3: heterozygous genotype (Ile/Val); 2: homozygous genotype (Val/Val); 4: wild type (Ile/Ile).

Table 1. Basic information about the age and gender distribution and the smoking status of the case groupand the control group

Basic information	Lung cancer group	Control group	P
N	98	98	
Age	32.2±10.4	30.5±10.7	0.065
Sex (male/female)	48/50	49/49	0.87
Smoking status			
Smoking	32	64	0.00
Non-smoking	66	34	

lung cancer and physical examination individuals in a certain hospital of Shanghai Chest Hospital, as the subjects, in an effort to explore the relationship between CYP1A1 gene and the susceptibility of the Chinese population to lung cancer, and then to seek suitable screening indicators of lung cancer.

Materials and methods

Subjects

Case group: Patients of lung cancer receiving treatment at the Shanghai Chest Hospital from April, 2013 to April, 2015 were enrolled. Inclusion criteria: Han nationality; primary lung cancer confirmed by pathologic histology; without radiation treatment; without a family history of lung cancer and occupational exposure. All patients were voluntary to participate in this study on verbal consent.

Control group: Healthy people receiving check-ups at the physical examination center of the Shanghai Chest Hospital from April, 2013 to April, 2015, all of which had Han nationality

and were volunteers in this study.

Research methods

DNA extraction

EDTA anticoagulative tubes were used to collect venous blood (2 mL) from each subject and Qiagen kit (Germany) was used to extract DNA according to instructions.

Investigation of CYP1A1 Exon 7 gene polymorphism

Primers design referred to literature [4]. The universal primer was 5'-GAACTGCCACTTCA GCTGTCT-3'. The primer of Ile was 5'-AAGA CCTCCCA GCGGGCAAT-3'. The primer of Val was 5'-A AGA CCTCCCA GCGGGCA AC-3'. The primers were synthesized by Shanghai Sangon Biotech, and RT-PCR was used in this study. The total volume of the PCR reaction system was 25 μL containing template DNA 100 ng, each primer 0.5 μmol/L, dNTP 0.2 mmol/L, $MgCl_2$ 1.5 mmol/L and TaqDNA polymerase 1.5 U. The same DNA template was amplified in two parallel reaction tubes respectively using the two pairs of primers formed by the universal primer and the primer of Ile or Val. PCR reaction conditions: predenaturation at 95°C for 3 min; denaturation at 94°C for 45 s, 61°C for 1 min, 72°C for 1 min, 35 circles; final extension at 72°C for 10 min.

Statistics

The data were typed into Excel, processed and analyzed with SPSS16.0. The differences of the genotypes and the allele frequencies between groups were analyzed by Chi-square test.

Results

Results of CYP1A1 Exon7 investigation

The genotyping test results are shown in **Figure 1**.

Compare of the general demographic information between cases and controls

A total of 98 patients were included in this study comprising 48 males and 50 females, aged 18 to 65 years. A total of 98 controls were included comprising 49 males and 49 females,

Table 2. CYP1A1 Exon 7 genotypes and the allele frequency distributions of the case group and the control group

Group	Subjects	Allele (%)		Genotype (%)		
		Ile	Val	Ile/Ile	Ile/Val	Val/Val
Lung cancer group	98	103 (52.5)	93 (47.5)	29 (29.6)	49 (50.0)	20 (20.4)
Control group	98	142 (72.4)	54 (27.6)	54 (55.1)	34 (34.7)	10 (10.2)
P		0.016		<0.001		

Table 3. Relations between CYP1A1 Exon 7 genotypes as well as smoking and susceptibility to lung cancer

Genotype	Smoking		OR	P	Non-smoking		OR	P
	Lung cancer	Control			Lung cancer	Control		
Ile/Ile	12	8	1*	0.02	9	17	1*	0.87
Ile/Val+Val/Val	26	54	3.1		23	47	1.08	

*: reference value.

Table 4. Logistic analysis results

Variables	Beta value	X^2	OR	95% CI	P
CYP1A1 polymorphism	-0.49	6.25	3.55	1.87-8.44	0.015
Smoking	0.22	4.23	2.09	1.06-4.29	0.032
Gender	0.98	2.11	1.04	0.89-2.33	0.294
Age	0.35	1.09	0.98	0.79-1.89	0.587

aged 18 to 63 years. No statistically significant difference was found in terms of age or gender between cases and controls, revealed by t test and Chi-square test (**Table 1**).

Hardy-Weinberg genetic equilibrium test

In the case group, the genotype frequency was 29.6% for Ile/Ile, 50.0% for Ile/Val and 20.4% for Val/Val. And in the control group, the genotype frequency was 55.1% for Ile/Ile, 34.7% for Ile/Val and 10.2% for Val/Val. The Hardy-Weinberg genetic equilibrium goodness of fitting test between the two groups was conducted by Chi-square goodness of fitting test. No statistically significant difference was found between the rs5569 gene distribution in the case group and the theoretical distribution of Hardy-Weinberg genetic equilibrium (x^2=0.043, P>0.05), and no statistically significant difference was found between the observations of the rs5569 gene distribution in the control group and the theoretical distribution (x^2= 5.5, P>0.05). It indicated that the included subjects were all representative of each population.

Distributions of CYP1A1 Exon 7 gene polymorphism in the case group and the control group

The results of the study on the Exon gene polymorphism of included subjects are as follows: in the case group, the genotype frequency was 29.6% for Ile/Ile, 50.0% for Ile/Val and 20.4% for Val/Val; in the control group, the allele frequency was 55.1% for Ile/Ile, 34.7% for Ile/Val and 10.2% for Val/Val. The differences of the gene frequencies or the allele frequency distributions between the case group and the control group were statistically significant (x^2=13.57, P<0.05) (See **Table 2**).

Analysis of the relationship between CYP1A1 Exon 7 gene polymorphism and lung cancer

Study showed that Ile/Ile was always a protective factor among CYP1A1 Exon genotypes. With Ile/Ile as a reference, the odds ratios of Ile/Val and Val/Val relative to Ile/Ile were calculated, and the OR values were 0.37 (95% CI 0.20-0.70, x^2=9.67, P<0.05); 0.27 (95% CI 0.11-0.65, x^2=9.03, P<0.05).

Relations between CYP1A1 Exon 7 genotypes as well as smoking and susceptibility to lung cancer

Stratified analysis of smoking factor showed that in the non-smoking group, the subjects

carrying Ile/Val or Val/Val were more susceptible to lung cancer (OR=1.08, **Table 3**) than the subjects carrying Ile/Ile, although the value of OR was not statistically significant. And in the smoking group with an obviously increasing trend, the odds ratio (OR) of the susceptibility of the subjects carrying Ile/Val or Val/Val to lung cancer was 3.1 compared with the subjects carrying Ile/Ile, which was statistically significant. After adjustments of age, gender, and smoking, the CYP1A1 polymorphism remained association with lung cancer risk (OR=3.55; 95% CI: 1.87-8.44; P=0.015, **Table 4**).

Discussion

CYP1A1 is an extrahepatic enzyme mainly distributed in the lung. It is primarily located on chromosome 15 and the whole gene contains 6311 base pairs. Study shows that CYP1A1 relates to the metabolism of polycyclic aromatic hydrocarbons (PAHs) [5]. Exon7 comes from mutation at the seventh exon of CYP1A1 gene, with the mutation of Ile into Val at the heme-binding site of the catalytic domain of trypsin [6]. CYP1A1 and its adjacent m^2 are both C-A mutation. The existing study shows that m^2 mutation significantly correlates to heightened risk of lung cancer, especially pulmonary squamous cell carcinoma [7-9]. In addition, it is now universally believed that tumor is the result of the interaction between gene and environment. Among environmental factors such as smoking, pollution, drugs, etc., smoking is universally acknowledged as a risk factor of lung cancer. Study shows that 70~80% of lung cancer cases occur in individuals who have a smoking history, or in other words, correlate to smoking exposure [10, 11]. By case-control study, this research aims at verifying the relationship between CYP1A1 Exon 7 gene polymorphism and susceptibility to lung cancer and meanwhile, together with the smoking history, exploring the relationship between smoking status and the level of the susceptibility to lung cancer in subjects carrying each CYP1A1 Exon 7 genotype.

Exon 7 gene polymorphism distributions are different among various races. For instance, Val allele is rare in African population but common in Asian population [12]. In this study, the Val allele frequencies of the case group and the control group were 47.5% and 27.6% in the Chinese population, respectively, showing a significantly lower than the mutant frequencies (78.6% and 72%) reported by Li*et al.* and Zhou *et al.* [12, 13], which might be related to multiple factors, such as heredity or diet, environment, etc. The subjects carrying Ile/Val genotype were more susceptible to lung cancer than the subjects carrying Ile/Ile, and the difference was statistically significant (OR=0.37, 95% CI 0.20-0.70, x^2=9.67, P<0.05). The odds ratio of Val/Val was also higher than that of Ile/Ile, showing a statistically significant difference (OR=0.27, 95% CI 0.11-0.65, x^2=9.03, P<0.05). It was indicated that the mutant Val was a risk factor of susceptibility to lung cancer.

Besides the association between CYP1A1 Exon 7 gene polymorphism and susceptibility to lung cancer, environmental factors such as smoking significantly increase the risk of developing lung cancer, which may be related to the metabolism of PAHs induced by smoking and CYP1A1 [14, 15]. This study also investigated the association between smoking status and susceptibility to lung cancer in subjects carrying each CYP1A1 Exon 7 genotype, and the results showed that in the non-smoking group, the subjects carrying Ile/Val or Val/Val were more susceptible to lung cancer (with the OR 1.08 times that of Ile/Ile) than the subjects carrying Ile/Ile. And in the smoking group with an obviously increasing trend, the odds ratio (OR) of the susceptibility of the subjects carrying Ile/Val or Val/Val to lung cancer was 3.1 compared with the subjects carrying Ile/Ile, which was statistically significant. The results indicated that smoking and Val gene mutation presumably caused lung cancer in a coordinative way.

Based on the results of this study, we believe that CYP1A1 Exon 7 gene polymorphism correlates to susceptibility to lung cancer, and mutant Val can increase the risk of developing lung cancer individually. Meanwhile, smoking and Val gene mutation presumably cause lung cancer in a coordinative way. The mutant Val can be used as an early screening indicator of lung cancer.

Disclosure of conflict of interest

None.

Address correspondence to: Liwen Xiong, Department of Respiratory Medicine, Shanghai Chest Hospital, Shanghai Jiaotong University, No. 241, West Huaihai Rd, Shanghai 200030, China. Tel:

+86-02162821990; Fax: +86-02162821990; E-mail: xlwen786@163.com

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