Original Article

Cytochrome P450 2E1 RsaI/PstI and DraI Polymorphisms Are Risk Factors for Lung Cancer in Mongolian and Han Population in Inner Mongolia

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ABSTRACT

Objective: To explore the relationship between cytochrome P450 2E1 (CYP2E1) RsaI/PstI and DraI polymorphism and lung cancer susceptibility in Mongolian and Han population in Inner Mongolia of China.

Methods: CYP2E1 RsaI/PstI and DraI polymorphisms were detected by polymerase chain reaction-restriction fragment length polymorphism in 64 lung cancer patients, 150 healthy Mongolian and 150 healthy Han individuals. The distribution of genotype and allele frequencies of CYP2E1 RsaI/PstI and DraI polymorphisms were studied.

Results: The risk of lung cancer was increased in individuals with CYP2E1 (cl/cl) and CYP2E1 (DD) with OR values of 2.431 (95%CI=1.082-5.460) and 2.778 (95%CI=1.358-5.683) respectively (P<0.05). When CYP2E1 RsaI/PstI and DraI polymorphisms were combined, the risk of lung cancer was reduced in individuals with CYP2E1 (cl/c2+c2/c2 and DD+CC) with OR values of 0.233 (95%CI=0.088-0.615, P<0.05). In smokers, the susceptibility to lung cancer was higher in the individuals with CYP2E1 (c1/c1) and CYP2E1 (DD) than in the individuals with c2 and C allele (P<0.05, OR =2.643 and 4.308 respectively). There was no significant difference in distribution of CYP2E1 genotype frequency between healthy Mongolian, Han population and lung cancer patients, healthy controls in Inner Mongolia.

Conclusion: CYP2E1 (c1/c1) and CYP2E1 (DD) are predisposing factors of lung cancer in population in Inner Mongolia. CYP2E1 (c2 + C) co-mutation may decrease the risk of lung cancer. Smoking exerts synergetic effect with CYP2E1 (c1/c1) and CYP2E1 (DD) on the occurrence of lung cancer.

Key words: Cytochrome p450 2E1; Gene polymorphism; Lung cancer; Susceptivity

INTRODUCTION

Great improvement has been made in the prevention and treatment of lung cancer, however, the incidence of lung cancer is still high and prognosis is poor. Lung cancer is still the first cause of cancer-related death worldwide[1]. Smoking and environmental carcinogens is one of the triggers for lung cancer, but only 10% of smokers eventually suffer from lung cancer, indicating that there is difference in individual susceptibility to lung cancer.

Of all the main enzymes involved in metabolism of carcinogens, cytochrome P450 superfamily plays an important role in detoxification of reactive intermediates of chemicals[2]. Cytochrome P450 2E1 (CYP2E1) is a main member of CYP450 family. CYP2E1 RsaI/PstI (c2) polymorphism has G→C substitution at 1293bp in 5′-untranslated region of CYP2E1 gene and introduces PstI restriction enzyme site, CTGCA‖G[3] which causes a decrease in enzymatic activity. CYP2E1 DraI (C) polymorphism has a T→A substitution at 7632bp in sixth intron and destroys the DraI restriction enzyme site[3]. The RsaI/PstI and DraI mutations of CYP2E1 can reduce effects on the metabolism of precarcinogens in tobacco, showing a protective effect on the body.

It has been reported that CYP2E1 polymorphism is involved in several types of diseases[4-7], including smoking-induced lung carcinogenesis, and it is related to genetic susceptibility of lung cancer[8-10]. Therefore, studying the relationship between CYP2E1 polymorphism and lung cancer susceptibility may provide information for pathogenesis and early diagnosis of lung cancer. However, the findings about the relationship between CYP2E1 polymorphism and lung cancer susceptibility are different[11-12], and research about the relationship between CYP2E1 polymorphism and lung cancer susceptibility in Inner Mongolia has not been done. In present study, we detected CYP2E1 polymorphism and

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explored the relationship between CYP2E1 polymorphism and lung cancer susceptibility in Inner Mongolia of China.

MATERIALS AND METHODS

Subjects

Sixty four patients with primary lung cancer diagnosed by histopathological examination were enrolled in the study. Of the 64 patients, 47 were male and 17 were female, with a mean age of 58.28±9.596 (range 34-78 years). The 64 patients with lung cancer were from our hospital and Baotou Central Hospital, and did not undergo anti-cancer treatment. All the patients did not have history of familial lung cancer and exposure to carcinogens. Sixty four healthy individuals who matched the age, sex of the lung cancer patients were selected from 300 healthy people (150 Mongolian and 150 Han people, respectively). The healthy controls did not have either tumors, tumor related diseases detected by physical examination, or history of familial lung cancer, exposure to carcinogens. The following data were recorded for each subject: name, age, gender, ethnicity, occupation, smoking, history of familial tumor and exposure to carcinogens. There was no significant difference in age and sex between the two groups. All the subjects gave informed consent.

DNA Extraction and PCR-RFLP

Two milliliter peripheral venous blood was drawn from overnight fast subjects into sodium citrate solution, and then stored at -80oC. Genomic DNA was extracted by kit (blood DNAzol Reagent kit, TaKaRa). The polymorphism of CYP2E1 gene was analyzed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). PCR was performed in a volume of 25 μl including 8 μl of ddH2O, 12.5 μl of Go Taq Green Master Mix (Promege), 0.25 μl (5 μmol/L) of primer RasI/PstI (sense 5′-TCTCCACCCTGATGCTTCC T-3′ and antisense 5′-TCTGTCTTCTAACTGGCAATA-3′) and primer DraI (sense 5′-AGTCGACATGTGATGGAT CCA-3′ and antisense 5′-GACAGGGTTTCATCATGTT GG-3′) respectively, and 4μl of template DNA. The reaction conditions were as follows: initial denaturation at 95°C for 10min, followed by 45 cycles of degeneration at 94°C for 30s, reannealing at 60°C (primer DraI at 62°C) for 30s, and extension at 72°C for 45s; with a final extension at 72°C for 10 min. PCR products were digested with PstI and DraI (TaKaRa), respectively, in 37°C water bath for 2 h. Digestion products (10μl) were electrophoresed using 3% agarose gel under 80 V for 30 min and analyzed by Gel-Pro imaging instrument.

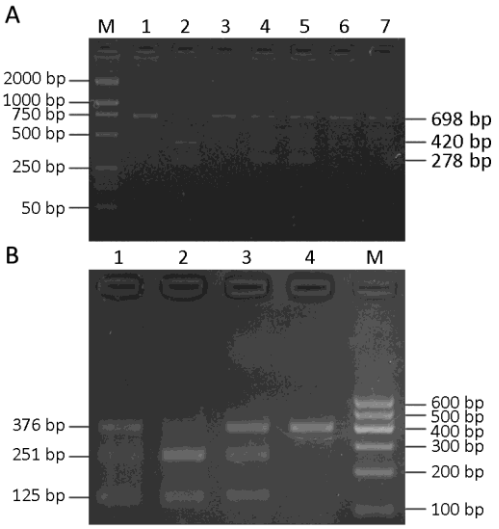
Statistical Analysis

Statistical analysis was performed with SPSS13.0 software. Chi square test was used to compare frequencies of genotype and allele between each group and to identify whether individual variants accord with Hardy-Weinberg equilibrium. Measurement data were expressed as $\bar{x} \pm s$ deviation. Logistic regression was used to investigate the correlations of CYP2E1 RsaI/PstI and DraI polymorphism with disease. Statistical significance was established at $P<0.05$.

RESULTS

The DNA fragment of the CYP2E1 gene was 698bp after PCR amplification with primer RasI/PstI. The expected sizes of the products after digestion with RasI/PstI were the following: homozygous wild-type (c1/c1) without a restriction site and an electrophoresis band of 698bp; mutant homozygote (c2/c2) with a restriction site in each DNA chain, resulting in electrophoresis bands of 420bp and 278bp; and heterozygote (c1/c2) with restriction sites in one of the DNA chains, resulting in 3 electrophoresis bands at 698bp, 420bp, and 278bp (Figure 1a).

The DNA fragment of the CYP2E1 gene was 376bp after PCR amplification with primer DraI. Homozygous wild type (DD) containing restriction site in each DNA chain become into two fragments of 251bp and 125bp fragment after being digested with DraI. Homozygotic mutant (CC) without a restriction site only had a fragment of 376bp. Heterozygote (DC) containing restriction sites in one of the DNA chains become into 3 fragments of 376bp, 251bp and 125bp (Figure 1b).



70×78mm (300×300DPI)

Figure 1. Agarose gel electrophoresis of PCR-RFLP products of CYP2E1 RasI/PstI and DraI polymorphisms. A: PCR-RFLP products of CYP2E1 RasI/PstI polymorphism. M: Marker. Lane 1, 3, 6 and 7: homozygous wild-type (cl/cl); Lane 2: mutant homosygote (c2/c2); Lane 4 and 5: heterozygote (c1/c2). B: PCR-RFLP products of CYP2E1 DraI polymorphisms. M: Marker. Lane 2: homozygous wild type (DD); Lane 4: homozygotic mutant (CC); Lane1 and 3: heterozygote (DC).

We observed that the RsaI/PstI and DraI polymorphism genotype distribution was in accordance with Hardy-Weinberg expectations in Mongolian and Han population in Inner Mongolia ($P>0.05$). It showed

that the study groups were representative. There was no significant difference in distribution of CYP2E1 genotype frequency between healthy Mongolian and Han population in Inner Mongolia (Table 1-2).

Table 1. Frequencies of genotype and allele distribution of CYP2E1 RsaI/PstI Polymorphism in healthy Mongolian and Han population

Group	Case	Genotype			x^2	P	Alleles		x^2	P
		c1/c1 n(%)	c2/c2 n(%)	c1/c2 n(%)			c1 n(%)	c2 n(%)		
Group	150	100(66.67)	5(3.33)	45(30.00)			245(81.67)	55(18.33)		
mongolian	150	102(68.00)	5(3.33)	43(28.67)	0.065	0.968	247(82.33)	53(17.67)	0.045	0.832

Compared with Han population, *P*>0.05

Table 2. Frequencies of genotype and allle distribution of CYP2E1 DraI Polymorphism in healthy Mongolian and han population

Group	Case	Genotype			x^2	P	Alleles		x^2	P
		DD n(%)	CC n(%)	DC n(%)			D n(%)	C n(%)		
Han	150	76(50.67)	9(6.00)	65(43.33)			217(72.33)	83(27.67)		
mongolian	150	59(39.33)	11(7.34)	80(53.33)	3.892	0.143	198(66.00)	102(34.00)	2.821	0.093

Compared with Han population, *P*>0.05

The CYP2E1 gene c1/c1, c1/c2, and c2/c2 genotype distributions were 72.66%, 25.00%, and 2.34%, respectively. The c1 allele frequency in our study population was 85.16% and the c2 allele frequency was 14.84%. There was no significant difference in frequencies of CYP2E1 RasI/PstI genotype and allele between lung cancer patients and healthy controls (Table 3). Lung cancer risk was higher in individuals with c1/c1 genotype than in individuals with c2 allele (c1/c2 and c2/c2; OR=2.431, 95%CI=1.082-5.460, P=0.029; Table 4). $\chi 2$ test showed that lung cancer susceptibility was greater in smokers with c1/c1 genotype than in smokers with mutant genotype (OR=2.643, 95%CI=0.991-7.045, P=0.048; Table 5). While the CYP2E1 gene DD, DC, and CC genotype distributions were 50.00%, 45.31%, and 4.69%, respectively. The D allele frequency in our study population was 72.66% and the C allele frequency was 27.34%. There was no significant difference in frequencies of CYP2E1 DraI genotype and allele between lung cancer patients and healthy controls (Table 6). The lung cancer risk in individuals with DD genotype was significantly increased (OR=2.778, 95%CI=1.358-5.683, =0.005; Table 7). Lung cancer susceptibility was greater in smokers with DD genotype than in smokers with C allele (OR=4.308, 95%CI=1.768-10.494, P=0.001; Table 8). The lung cancer risk was significantly lower in individuals with c1/c2+c2/c2 and DC+CC than in individuals with c1/c1 and DD (OR=0.233, 95%CI=0.088-0.615, *P*=0.002; Table 9).

Table 3. Frequencies of genotype and allele distribution of CYP2E1 RsaI/PstI Polymorphism in healthy individuals and patients with lung cancer

Group	Case	Genotype			Alleles	
		c1/c1 n(%)	c2/c2 n(%)	c1/c2 n(%)	c1 n(%)	c2 n(%)
Healthy	64	41(64.06)	1(1.56)	22(34.38)	104(81.25)	24(18.75)
Lung cancer	64	52(81.25)	2(3.12)	10(15.63)	114(89.06)	14(10.94)
Total	128	93(72.66)	3(2.34)	32(25.00)	218(85.16)	38(14.84)

Table 4. Association between CYP2E1 RsaI/PstI Polymorphism and lung cancer susceptibility

Genotype	Control (n=64) n(%)	Lung cancer (n=64) n(%)	OR(95%CI)	X^2	P
c1/c1	41(64.06)	52(81.25)	1		
c2/c2+c1/c2	23(35.94)	12(18.75)	0.525(0.273-1.011)	4.758	0.029*
c2/c2+c1/c2	23(35.94)	12(18.75)	1		
c1/c1	41(64.06)	52(81.25)	2.43(1.082-5.460)	4.758	0.029*

**P*<0.05; 95%CI: 95% confidence interval

Table 5. Associatioin between CYP2E1 RsaI/PstI Polymorphism, smoking and lung cancer susceptibility

Genotype	Non-smoker				Smoker			
	Control n(%)	lung cancer n(%)	OR (95%CI)	*P*	Control n(%)	lung cancer n(%)	OR (95%CI)	*P*
c2/c2+c1/c2	7(35.0)	4(21.1)	1		16(36.4)	8(17.8)	1	
c1/c1	13(65.0)	15(78.9)	2.019 (0.481-8.485)	0.333	28(63.6)	37(82.2)	2.643 (0.991-7.045)	0.048*

**P*<0.05

Table 6. Frequencies of genotype and allele distribution of CYP2E1 DraI Polymorphism in healthy individuals and patients with lung cancer

Group	Case	Genotype			Alleles	
		DD n(%)	CC n(%)	DC n(%)	D n(%)	C n(%)
Healthy	64	24(37.5)	3(4.69)	37(57.81)	85(66.4)	43(33.59)
Lung cancer	64	40(62.50)	3(4.69)	21(32.81)	101(78.91)	27(21.09)
Total	128	64(50.00)	6(4.69)	58(45.31)	186(72.66)	70(27.34)

Table 7. Association between CYP2E1 DraI Polymorphism and lung cancer susceptibility

Genotype	Control(n=64) n(%)	Lung cancer(n=64) n(%)	OR(95%CI)	x^2	*P*
DD	27(37.50)	40(62.50)	1		
CC+DC	40(62.50)	24(37.50)	0.360(0.176-0.736)	8.000	0.005*
CC+DC	40(62.50)	24(37.50)	1		
DD	24(37.50)	40(62.50)	2.778(1.358-5.683)	8.000	0.005*

**P*<0.01

Table 8. Association between CYP2E1 DraI Polymorphism, smoking and lung cancer susceptibility

Genotype	Non-smoker				Smoker			
	Control n(%)	lung cancer n(%)	OR (95%CI)	*P*	Control n(%)	lung cancer n(%)	OR (95%CI)	*P*
CC+DC	12(60.0)	11(57.9)	1		28(56.2)	13(33.3)	1	
DD	8(42.1)	8(42.1)	1.091 (0.304-3.910)	0.894	16(43.8)	32(66.7)	4.308 (1.768-10494)	0.001*

**P*<0.01; ORL Odds ratio; 95%CI: 95% confidence interval

Table 9. Association between CYP2E1 RsaI/PstI and DraI Polymorphism and lung cancer

Genotype		Control(n=4) n(%)	Lung cancer (n=64) n(%)	OR(95(%CI)	x^2	*P*
RsaI/PstI	DraI					
C1/c1	DD	22(34.38)	36(56.25)	1		
C1/c1	DC+CC	19(29.69)	16(25.00)	0.515(0.220-1.205)	2.369	0.124
C1/c2+c2/c2	DD	2(3.13)	4(6.25)	1.222(0.206-7.235)	0.000	1.000
C1/c2+c2/c2	DC+CC	21(32.80)	8(12.50)	0.233(0.088-0.615)	9.197	0.002*

**P*<0.01; 95%CI: 95% confidence interval

DISCUSSION

Distribution of CYP2E1 RasI/PstI alleles (c1, c2) and DraI alleles (D, C) varies much in different races[3,13]. Frequencies of allele c2 and C are 4% and 11% in European-Americans, 1% and 8% in African-Americans, 28% and 24% in Taiwanese[13], 1.94% and 8.25% in Turkish population, respectively[3]. Our study finds that frequencies of allele c2 and C are 18% and 34% in Mongolian population, 18% and 28% in Han population, which are similar to the allele frequencies of Taiwanese. Our study shows that there is no difference in CYP2E1 RsaI/PstI and DraI polymorphism between Mongolian and Han population in Inner Mongolia, which may due

to the small sample size. Therefore, larger sample size will be needed to further confirm the result of our study.

Our study shows that CYP2E1 (c1/c1) and CYP2E1 (DD) are predisposing factors of lung cancer in population in Inner Mongolia. Smoking exerts synergetic effect with CYP2E1 (c1/c1) and CYP2E1 (DD) on the occurrence of lung cancer. Previous studies also show that individuals with CYP2E1 c1/c1 or DD genotype have more risk to developing lung cancer compared to those with CYP2E1 c1/c2+c2/c2 or CC+CD genotype in Chinese and Indian population[14-17], CYP2E1 (c1/c1) or CYP2E1 (DD) increased the risk of lung cancer in smokers[14,17]. However, Le Marchand et al. find that CYP2E1 RsaI/PstI and DraI polymorphism did not related with lung squamous cell carcinoma but lung adenocarcinoma in Caucasian, Japanese and Hawaiian originated population in Hawaii[18], there is no risk in relation to CYP2E1 and lung cancer in north Indian population[19]. Mechanism of genetic susceptibility to lung cancer is very complex. Different genetic background and environmental exposure may lead to significant differences in susceptibility to lung cancer. Therefore, regional and ethnic differences should be considered when a specific gene is served as a genetic marker. Additionally the small sample size of lung cancer patients in our current study has limitation in representing the population. Large sample size will be needed to further confirm the result of our study.

In summary, CYP2E1 RsaI/PstI and DraI polymorphism is associated with the risk of lung cancer among population in Inner Mongolia. CYP2E1 DD and cl/cl genotype may be susceptibility gene and exert a synergetic effect with smoking in the occurrence of lung cancer. However, there was no significant difference in CYP2E1 RsaI/PstI and DraI polymorphism between Mongolian and Han population living in Inner Mongolia, indicating that there may be no regional and race difference in distribution of CYP2E1 genotype in Chinese population. In order to obtain more accurate and valuable genetic markers, large sample sizes will be needed in further study.

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