RESEARCH ARTICLE

Susceptibility of Lung Cancer with Polymorphisms of CYP1A1, GSTM1, GSTM3, GSTT1 and GSTP1 Genotypes in the Population of Inner Mongolia Region

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Abstract

Background: To study the relationship of susceptibility to lung cancer with the gene polymorphisms of CYP1A1, GSTM1, GSTM3, GSTT1, GSTP1 and smoking status in Han and Mongolian populations of Inner Mongolia, an autonomous region of China. Materials and Methods: PCR-RFLP, allele-specific and multiplex PCR were employed to identify the genotypes of CYP1A1, GSTM1, GSTM3, GSTT1 and GSTP1 in a case-control study of 322 lung cancer patients diagnosed by bronchoscopy and 456 controls free of malignancy. Results: There is a significant difference in genotypic frequency of GSTT1 of healthy Mongolian and Han subjects. A statistically prominent association was found between CYP1A1 Msp1 (vt/vt) (OR=4.055, 95%CI:2.107-7.578, *p*=0.000), GSTM1 (-) (OR=2.290, 95%CI:1.467-3.573, *p*=0.000) and lung cancer in Mongolians. Similarly, in the Han population, CYP1A1 Msp1 (vt/vt) (OR=3.194, 95%CI:1.893-5.390, *p*=0.000) and GSTM1 (-) (OR=1.884, 95%CI:1.284-2.762, *p*=0.001) carriers also had an elevated risk of lung cancer. The smokers were more susceptible to lung cancer 2.144 fold and 1.631 fold than non-smokers in Mongolian and Han populations, respectively. The smokers who carried with CYP1A1 Msp1 (wt/vt+vt/vt), exon7 (Val/Val+Ile /Val), GSTM1 (-), GSTM3 (AB+BB), and GSTT1 (-) respectively were found all to have a high risk of lung cancer. Conclusions: CYP1A1 Msp1 (vt/vt) and GSTM1 (-) are risk factors of lung cancer in Han and Mongolian population in the Inner Mongolia region. The smokers with CYP1A1 Msp1 (wt/vt+vt/vt), CYP1A1 exon7 (Val/Val+Ile /Val), GSTM1 (-), GSTM3 (AB+BB), and GSTT1 (-) genotypes, respectively, are at elevated risk of lung cancer.

Keywords: Lung cancer - Mongolians - Han population - cytochrome P450 - glutathione S-transferase SNPs

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Introduction

Epidemiological studies have shown that lung cancer is associated with tobacco and alcohol use, and is common in regions where these products are often consumed. Similar to other environmental toxins, tobacco requires metabolic activation and subsequent detoxification by a series of enzymes such as phase I enzymes i-e the cytochrome P450 enzymes (CYP) and phase II xenobiotic metabolizing enzymes i-e the glutathione S-transferases (GSTs). An association between CYP1A1 polymorphisms and lung cancer was first reported by Kawajri and co-workers in 1990 in an asian population study (Kawajiri et al., 1990). Cytochrome P4501A1 (CYP1A1) metabolizes a number of suspected procarcinogens, especially, polycyclic aromatic hydrocarbons (PAHs) into highly reactive intermediates. These compounds are capable to bind with DNA to form adducts, which, if not repaired, may initiate or propagate carcinogenesis. Although PAHs are ubiquitous in nature and exposed with sources of particular concern including smoking, air pollution, diet and certain occupations (Veglia et al., 2007). GSTs consist of a super family of enzymes that catalyze the reduction by glutathione of several electrophilic substrates, facilitating the excretion of these compounds, which are often by-products of CYP mediated metabolism (Shin et al., 2010). GSTs are involved in detoxification of reactive metabolites of carcinogens, therefore, could be potentially important in susceptibility to cancer.

Variations in the metabolic activation as well as detoxification of chemical carcinogens and genotoxins, such as tobacco, these are likely to be one of the major determinants of inter-individual differences in susceptibility to environmentally induced cancers. The genetic constitution seems to play the key role in this context with an increased number of xenobiotic metabolizing enzymes, such as GSTs and CYPs, have been shown to be polymorphic (Chow et al., 2010; Malik

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et al., 2010).

We studied the distribution of CYP1A1 Msp1, exon7, GSTM1, GSTM3, GSTT1 and GSTP1 genotype polymorphisms between the Mongolian and Han population in Inner Mongolia region. We did not find differences between these populations on the distribution of CYP1A1 Msp1, exon7, GSTM1, GSTM3, and GSTP1, in addition to the GSTT1 (Chang et al., 2006; Bai et al., 2011). In the present study, we analyzed the polymorphisms of CYP1A1 Msp1, exon7, and GSTM1, GSTM3, GSTT1 and GSTP1 genotypes and lung cancer susceptibility on Mongolian and Han population with possible modifications by cigarette smoking.

Materials and Methods

Study subjects and epidemiological information

This case-control study consisted of 322 patients suffering from lung cancer and 456 general population-selected healthy controls. Of them, 446 (180 cases and 266 controls) were Han subjects and 332 (142 cases and 190 controls) were Mongolian subjects. Lung cancer cases were recruited from patients undergoing bronchoscopy at the first affiliated hospital, Inner Mongolia Medical University, China. The ages of patients range from 43 to 86, and controls from 36 to 83 (Table 1). At recruitment, each participant was personally interviewed to obtain a detailed information about smoking, dietary habits and demographic characteristics. Controls that were selected from healthy individuals free of malignancy were individually matched to the patients with respect to gender and age, they were interviewed using the same questionnaire at the time of their admission for the study. All of the patients and controls gave their informed consent. The smoking habits of the patients were investigated during personal interviews before test. The patients were divided into current non-smokers, including patients who had never smoked, as well as those who had stopped smoking at least one week earlier and current smokers. The study was approved by the Ethics Committee of the Inner Mongolia Medical University, China. Each person donated 5ml of whole blood in heparinized tubes, stored in freezer at -80 centigrade. DNA was extracted by the phenol - chloroform method.

PCR Analysis

CYP1A1 genotyping: The A4889G (Ile-Val) polymorphism at exon7 of the CYP1A1 gene was assessed by allele specific PCR (Hayashi et al., 1991). For this, genomic DNA was amplified with primers (Ile primer:5'-GAAGTGTATCGGTGAGACCA-3', Val primer:5'-GAAGTGTATCGGTGAGACCG-3'), and (universal primer:5'-GTAGACAGAGTCTAGGCCTCA-3'). PCR was carried out in a total volume of 25μl, containing 4μl genomic DNA; 12.5μl PCR MasterMix (Zymo Research, Orange, CA, USA), 1μl of Val primer or Ile primer and 1μl of universal primer, 6.5μl ddH_2O. Initial denaturation cycling was carried out at 94 centigrade for 10min, followed by 30cycles of denaturation at 95 centigrade for 1 min. Then annealing at 65centigrade for 1min and extension at 72 centigrade for 1 min along with this final extension at 72 centigrade for 10 min in thermal cycler (Applied Biosystems, Foster City, CA, USA). The PCR products were then subjected to electrophoresis on a 1.8% agarose gel and stained with ethidium bromide and observed under the ultraviolet light (UVP, Upland, USA). The PCR analysis resulted in a 210bp fragment with the classification wild type allele (Ile/Ile), heterozygous allele (Ile/Val), and variant mutant allele (Val/Val).

The CYP1A1 Msp1 restriction sites genotypes were analyzed by PCR-RFLP. Primers designed according to the protocol of Shou M G, et al (Shou et al., 1996; Shimada et al., 1996). Briefly genomic DNA was amplified by using two sets of primers CYP1A1 Msp1 forward primer (P1)5'-TAGGAGTCTTGTCTCATGCCT-3', reverse primer (P2)5'-CAGTGAAGAGGTGTAGCCGCT-3', PCR was carried out in a total volume of 25 μl, containing 4 μl genomic DNA, 12.5μl PCR MasterMix (Zymo Research, Orange, CA, USA);1μl of each primer, 6.5μl ddH_2O. Initial denaturation was carried out at 94 centigrade for 5 min followed by 30 cycles under the following conditions: denaturation at 94 centigrade for 1 min annealing at 63 centigrade for 1 min extension at 72 centigrade for 1 min and final extension at 72 centigrade for 10 min in thermal cycler (Applied Biosystems, Foster City, CA, USA). The PCR products were digested with Msp1 restriction enzyme at 37 centigrade for 4 h, and then subjected to electrophoresis on a 1.8% agarose gel and stained with ethidium bromide and observed under the ultraviolet light (UVP, Upland, USA). The wild-type allele (wt/wt) revealed a single band of 340bp.The variant mutant allele (vt/vt) resulted in two fragments of 200bp and 140bp, whereas the heterozygous allele (wt/vt) showed three bands of 340, 200 and 140bp.

GSTM1, GSTT1 genotyping: The GSTM1 and GSTT1 genotypes were analyzed by a multiplex PCR. Primers designed according to Chen S, et al (Houlston et al., 1999; Chen et al., 2001; Benhamou et al., 2002; Chan-Yeung et al., 2004). Briefly genomic DNA was amplified by using six sets of primersGSTM1 (F)5'-GAACTCCCTGAAAAGCTAAAGC-3', GSTM1 (R)5'-GTTGGGCTCAAATATACGGTGG-3', GSTT1 (F)5'-TTCCTTACTGGTCCTCACATCTC-3', GSTT1 (R) 5'-TCACCGGATCATGGCCAGCA-3', β-globin (F)5'-CAACTTCATCCACGTTCACC-3' and β-globin (R) 5'-GAAGAGCCAAGGACAGGTAC-3' in a total volume of 25 μl, each systems contain 2μl GSTM1, GSTT1 primers respectively and 2μl β-globin primers; 4 μl genomic DNA; 12.5μl PCR MasterMix (Zymo Research, Orange, CA, USA); 4.5μl ddH_2O. The procedure followed for PCR was: primary denaturation at 95 centigrade for 5min, denaturation at 95 centigrade for 1min, annealing at 57 centigrade (GSTT1 at 59 centigrade) for 1min, extension at 72 centigrade for 1min, 30 cycles were conducted, final extension was at 72 centigrade for 10min. The PCR products were then subjected to electrophoresis on a 1.8% agarose gel, stained with ethidium bromide and observed under the ultraviolet light (UVP, Upland, USA). The presence of a 215 and 480bps was indicative of the GSTM1 and GSTT1 genotypes; whereas the absence of the product indicated the null genotype. β-globin was used as a internal control.

Briefly genomic DNA was amplified by using three sets of primers of GSTP1: Ile primer (P1)5'-GTTTCTGACCTCCGCTGCAAATACA-3', Val primer (P2) 5'-GTTTCTCTTGACCTCCGCTGCAAATACG-3', Universal primer (P3)5'-GTTTCTCAGCCCAAGCCACC TGA-3', Primers designed according to Soo Joong Kim, et al (Soo Joong Kim et al., 2008). PCR was carried out in a total volume of 25μl, containing 4μl genomic DNA; 12.5μl PCR MasterMix (Zymo Research, Orange, CA, USA), 1μl of Ile primer or Val primer and 1μl of universal primer , 6.5μl ddH_2O.Initial denaturation was carried out at 94 centigrade for 5 min, followed by 32 cycles under the following conditions: denaturation at 94 centigrade for 1 min, annealing at 64 centigrade for 50 sec, extension at 72 centigrade for 50 sec and final extension at 72 centigrade for 6 min in thermal cycler (Applied Biosystems, Foster City, CA, USA). The PCR products were then subjected to electrophoresis on a 1.8% agarose gel and stained with ethidium bromide and observed under the ultraviolet light (UVP, Upland, USA). Wild genotype AA: There is 150bp amplification strip in PCR system consisted of universal primers and Ile primer. There is no 150bp amplification strip in PCR system consisted of universal primers and Val primer. Heterozygous mutation AG: there appears the 150bp amplification of strip in the two PCR systems respectively consisted of universal primer and the Ile primer or Val primer. Homozygote (Val/Val): There is no 150bp amplification strip in PCR system consisted of universal primers and Ile primer.There is 150bp amplification strip in PCR system consisted of universal primers and Val primer.

To detect GSTM3 polymorphism, the PCR-RFLP was used. Each of primers was (P1:5'- CCTCAGTACTTGGA AGAGCT-3' and P2:5'-CACATGAAAGCCTTCAGGT T-3'). Primers designed according to Inskip A , et al (Inskip et al., 1995; Pearson et al., 1993). PCR was carried out in a total volume of 25 μl, containing 4 μl genomic DNA; 1μl each of primers P1 and P2; 12.5μl PCR MasterMix (Zymo Research, Orange, CA, USA); 6.5μl ddH_2O. Initial denaturation was carried out at 94 centigrade for 5 min, followed by 35 cycles of denaturation at 94 centigrade for 45 sec, annealing at 72 centigrade for 45 sec and extension at 72 centigrade for 5 min in thermal cycler. The PCR products (273bp) were digested with MnlIat 37 centigrade for 4 h, then subjected to electrophoresis on a 20% polyacrylamide sequencing gel, stained with ethidium bromide and observed under the ultraviolet light (UVP, Upland, USA). Wild genotype AA: 11bp, 50bp, 87bp, 125bp locus enzyme electrophoresis bands. Heterozygotes AB: In 11bp, 50bp, 87bp, 125bp, 134bp sites appears digestion electrophoretic bands. Mutations BB: In 11bp, 125bp, 134bp sites appears digestion electrophoretic bands.

Statistical analysis

SPSS 13.0 was used to do the statistical analysis. The chi-square test was used to study differences in genotype distributions. Relative risk was estimated with odds ratio (OR), 95% confidence interval (95%CI) and *p* value. All data were considered significant when $p<0.05$.

Results

CYP1A1 Msp1, exon7, GSTM1, GSTM3, GSTT1 and GSTP1 gene polymorphism in normal Mongolian and Han Nationality

The distribution of CYP1A1 Msp1, exon7, GSTM1, GSTM3, GSTT1 and GSTP1 in the study populations is shown in Table 2. The frequency of GSTT1 in healthy Mongolian and Han subjects in Inner Mongolia was GSTT1 (+) 46.32%, GSTT1 (-) 53.68% and GSTT1 (+) 55.64%, GSTT1 (-) 43.98%. The Chi-square tests showed the significant difference just in the distribution of GSTT1 between the two groups ($p<0.05$).

The relationship of CYP1A1 Msp1, exon7, GSTM1, GSTM3, GSTT1 and GSTP1 genotyping polymorphisms with the susceptibility of lung cancer in Mongolian population

As Table 3 shows, a statistically significant association was found between CYP1A1 Msp1 (vt/vt) and lung cancer (OR=4.055, 95%CI:2.107-7.578, $p=0.000$) in Mongolian population. And a risk of lung cancer (OR=2.290, 95%CI:

Table 1. Frequency Distribution of Demographic Variables for Patients and Controls

Variables	Patients (n=322) (%)	Controls (n=456) (%)
Gender		
Male	270(83.85)	382(83.77)
Female	52(16.15)	74(16.23)
Age(years)		
Less than 50	29(9.01)	63(13.82)
During 50 to 65	156(48.45)	243(53.29)
Older than 65	137(42.55)	150(32.89)
Smoking Status		
Never	122(37.88)	245(53.73)
Light Smokers a	136(42.24)	186(40.79)
Chain Smokers b	64(19.88)	25(5.48)

Table 2. Frequency Distribution of CYP1A1, GSTM1, GSTM3, GSTT1 and GSTP1 Genotype of Mongolian and Han Populations in Inner Mongolia Region

Genotypes		Han (N=266)		Mongolian population) (N=190)	
		n	%	n	%
CYP1A1 Msp1	wt/wt	82	30.83	72	37.89
	wt/vt	135	50.75	93	48.95
	vt/vt	49	18.42	25	13.16
CYP1A1 exon7	Ile/Ile	58	21.8	42	22.11
	Val/Val	183	68.8	125	65.79
	Ile /Val	25	9.4	23	12.1
GSTM1	GSTM1(+)	142	53.38	118	62.11
	GSTM1(-)	124	46.62	72	37.89
GSTM3	GSTM3(AA)	138	51.88	90	47.37
	GSTM3(AB)	118	44.36	95	50
	GSTM3(BB)	10	3.76	5	2.63
GSTT1	GSTT1(+)	148	55.64	88	46.32*
	GSTT1(-)	117	43.98	102	53.68*
GSTP1	GSTP1(AA)	138	51.88	90	47.37
	GSTP1(AB)	117	43.98	95	50
	GSTP1(BB)	11	4.14	5	2.63

*$p<0.05$

1.467-3.573, p=0.000) was seen for the GSTM1 (-) carriers compared with the GSTM1 (+) carriers. It showed the genotypes of the CYP1A1 exon7, GSTM3, GSTT1, GSTP1 polymorphisms with no significant difference among the lung cancer patients and controls (p>0.05) in Mongolian population.

The relationship of CYP1A1 Msp1, exon7, GSTM1, GSTM3, GSTT1 and GSTP1 genotyping polymorphisms with the susceptibility of lung cancer in Han population

The distribution of genotypes of CYP1A1 Msp1, exon7, GSTM1, GSTM3, GSTT1 and GSTP1 in cases and controls in Han population are shown in Table 4. The frequency of GSTM1 (-) was notably high in lung cancer patients than GSTM1 (+). There was significant difference between the two groups (OR=1.884, 95%CI:1.284-2.762, p=0.001)and considerable difference in the frequency of Msp1 (OR=3.194, 95%CI:1.893-5.390, p=0.000) in Han population.

Association between cigarette smoking and lung cancer in Mongolian and Han population

The proportion of smokers in the controls and the patients with lung cancer were 46.3% and 64.8% in Table 5. In comparison, the smokers are susceptible to lung cancer 2.143 fold (95%CI=1.452-3.167, p=0.000) than non-smokers in Mongolian population and 1.631 fold (95%CI=1.051-2.525, p=0.029) in Han population. There was significant difference between the two groups (p<0.05)in Mongolian and Han subjects.

The relationship of the CYP1A1 Msp1, exon7, GSTM1, GSTM3, GSTT1 and GSTP1, genotyping polymorphisms combined with smoking status with the susceptibility of lung cancer

Table 6 shows the risk of developing lung cancer in relation to CYP1A1, GSTM1, GSTM3, GSTT1 and GSTP1. By the analysis of CYP1A1 combined with smoking status, we found that the smokers who were

Table 3. Distribution of CYP1A1, GSTM1, GSTM3, GSTT1 and GSTP1 Genotypes among Controls and Patients with Lung Cancer in Mongolian population

Genotypes		Controls (N=190) n (%)	Lung cancer (N=142) n (%)	OR(95%CI)	X^2	p-value
CYP1A1 Msp1	wt/wt	64(33.7)	28(19.7)	1		
	wt/vt	95(50.0)	59(41.6)	1.420(0.819-2.461)	1.563	0.211
	vt/vt	31(16.3)	55(38.7)	4.055(2.107-7.578)	20.067	0.000*
CYP1A1 exon7	Ile/Ile	28(14.7)	18(12.7)	1		
	Ile /Val	136(71.6)	101(71.1)	1.155(0.606-2.203)	0.192	0.661
	Val/Val	26(13.7)	23(16.2)	1.376(0.609-3.111)	0.59	0.433
GSTM1	GSTM1(+)	111(58.4)	54(38.0)	1		
	GSTM1(-)	79(41.6)	88(62.0)	2.290(1.467-3.573)	13.519	0.000*
GSTM3	GSTM3(AA)	99(52.1)	78(54.9)	1		
	GSTM3(AB)	83(43.7)	61(43.0)	0.933(0.598-1.454)	0.094	0.759
	GSTM3(BB)	8(4.2)	3(2.1)	0.476(0.122-1.854)	1.191	0.275
GSTT1	GSTT1(+)	105(55.3)	80(56.3)	1		
	GSTT1(-)	85(44.7)	62(43.7)	0.957(0.618-1.483)	0.038	0.845
GSTP1	GSTP1(AA)	96(50.5)	78(54.9)	1		
	GSTP1(AB)	79(41.6)	53(37.3)	0.826(0.522-1.306)	0.67	0.413
	GSTP1(BB)	15(7.9)	11(7.8)	0.903(0.392-2.077)	0.058	0.809

*p<0.05

Table 4. Distribution of CYP1A1, GSTM1, GSTM3, GSTT1 and GSTP1Genotypes among Controls and Patients with Lung Cancer in Han Population

Genotypes		Controls (N=260) n (%)	Lung cancer (N=180) n (%)	OR(95%CI)	X^2	p-value
CYP1A1 Msp1	wt/wt	87(32.70)	37(20.56)	1		
	wt/vt	126(47.37)	71(39.44)	1.325(0.818-2.146)	1.311	0.252
	vt/vt	53(19.93)	72(40)	3.194(1.893-5.390)	19.492	0.000*
CYP1A1 exon7	Ile/Ile	57(21.43)	42(23.33)	1		
	Ile /Val	183(68.80)	112(62.22)	0.831(0.523-1.319)	0.619	0.432
	Val/Val	26(9.77)	26(14.44)	1.357(0.692-2.663)	0.79	0.374
GSTM1	GSTM1(+)	157(59.02)	78(43.33)	1		
	GSTM1(-)	109(40.98)	102(56.67)	1.884(1.284-2.762)	10.601	0.001*
GSTM3	GSTM3(AA)	138(51.88)	96(53.33)	1		
	GSTM3(AB)	117(43.98)	80(44.44)	0.983(0.668-1.445)	0.008	0.93
	GSTM3(BB)	11(4.14)	4(2.22)	0.523(0.162-1.690)	1.209	0.271
GSTT1	GSTT1(+)	148(55.64)	95(52.78)	1		
	GSTT1(-)	118(44.36)	85(47.22)	1.122(0.768-1.640)	0.354	0.552
GSTP1	GSTP1(AA)	138(51.88)	96(53.33)	1		
	GSTP1(AB)	117(43.98)	79(43.89)	0.971(0.660-1.428)	0.023	0.88
	GSTP1(BB)	11(4.14)	5(2.78)	0.653(0.220-1.941)	0.594	0.441

*p<0.05

carrier of CYP1A1 exon7 (Val/Val+Ile/Val) had a 2.569 fold increased risk of lung cancer than the non-smokers who were carrier of CYP1A1 exon7 (Ile/IIe) (95%CI=1.465-4.507). There was significant difference between two groups (p=0.001); smokers who were carrier of CYP1A1 Msp1 (wt/vt+vt/vt) genotype, the risk of lung cancer was approximately 4.866 fold than the non-smokers and carrier of CYP1A1 (wt/wt)genotype. Similarly, the risk was 0.977 fold for the combined CYP1A1 (wt/vt+vt/vt) genotype and non-smokers.

The combined CYP1A1 (wt/vt+vt/vt) genotype and smoking were found to be a major risk factor of lung cancer (p<0.05). By the analysis of lung cancer susceptibility combined with smoking status, we found that the smokers with GSTM1 (+) carrier and the non-smokers with GSTM1 (-) carrier had increased risk of lung cancer than the nonsmokers with GSTM1 (+) carrier, OR values were 1.531 (95%CI=0.977-2.400) and 1.155 (95%CI=0.735-1.815), but there was no significant difference between the groups (p>0.05). The smokers with GSTM1 (-) carrier had increased risk of lung cancer, and there was significant difference between the two groups (OR=5.453, 95%CI:3.542-8.395, p=0.000). The smokers with GSTM3 (AB+BB) carrier had increased risk of lung cancer than the non-smokers with GSTM3 (AA) carrier, OR values were 1.600 (95%CI=1.034-2.475), and the Chi-square tests showed there was significant difference (p<0.05). The smokers who carried with GSTT1 (-) had increased risk of lung cancer than the non-smokers who GSTT1 (+) carrier , OR values were 1.574 (95%CI=1.044-2.372), and the Chi-square tests showed the significant difference (p<0.05)in both. The smokers who GSTP1 (AA) carrier or GSTP1 (AG+GG) had increased risk of lung cancer than the non-smokers who GSTP1 (AA) carrier, OR values were 0.824 (95%CI=0.405-1.678)and 1.338 (95%CI=0.800-2.236), and the Chi-square tests showed there was no significant difference (p>0.05).

Discussion

Some studies found that the genetic alteration was associated with the risk of lung cancer (Liu et al., 2010; Guan et al., 2011). CYP1A1 plays an important role in the metabolism of PAHs, an important group of lung carcinogens. This gene has several polymorphic forms (Song et al., 2001). Two functionally important non synonymous polymorphisms have been described for the CYP1A1gene.These include an A→G transition in

Table 5. Associations between Cigarette Smoking and Lung Cancer in Mongolian Nationality and Han Population

Genotypes		Controls n (%)	Lung cancer n (%)	OR(95%CI)	X^2	p-value
Mongolian population	Non-smoker	143 (53.8)	63 (35.0)	1		
	Smokers	123 (46.2)	117 (65.0)	2.144 (1.452-3.167)	14.902	0.000*
Han population	Non-smoker	102 (53.7)	59 (41.6)	1		
	Smokers	88 (46.3)	83 (58.4)	1.631 (1.051-2.525)	4.791	0.029*

*p<0.05

Table 6. Interaction of the CYP1A1, GSTM1, GSTM3, GSTT1 and GSTP1 Polymorphism and Smoking Status on Lung Cancer Risk

	Genotypes	Smoking Status	Controls (N=456) n (%)	Lung cancer (N=322) n (%)	OR(95%CI)	X^2	p-value
CYP1A1 Msp1	wt/wt	Non-smoker	116(25.4)	48(14.9)	1		
		Smokers	125(27.4)	68(21.1)	1.315(0.840-2.057)	1.438	0.23
	wt/vt+vt/vt	Non-smoker	141(30.9)	57(17.7)	0.977(0.619-1.541)	0.01	0.92
		Smokers	74(16.2)	149(46.3)	4.866(3.144-7.532)	53.31	0.000*
CYP1A1 exon7	Ile/Ile	Non-smoker	48(10.5)	20(6.3)	1		
		Smokers	45(9.9)	28(8.6)	1.493(0.739-3.017)	1.254	0.263
	Val/Val+Ile /Val	Non-smoker	193(42.3)	92(28.7)	1.144(0.642-2.039)	0.209	0.648
		Smokers	170(37.3)	182(56.4)	2.569(1.465-4.507)	11.345	0.001*
GSTM1	GSTM1(+)	Non-smoker	130(28.5)	48(14.8)	1		
		Smokers	115(25.2)	65(20.3)	1.531(0.977-2.400)	3.465	0.063
	GSTM1(-)	Non-smoker	136(29.9)	58(18)	1.155(0.735-1.815)	0.391	0.532
		Smokers	75(16.4)	151(46.9)	5.453(3.542-8.395)	63.258	0.000*
GSTM3	GSTM3(AA)	Non-smoker	100(21.9)	55(17.1)	1		
		Smokers	139(30.5)	100(31.1)	1.308(0.862-1.986)	1.592	0.207
	GSTM3(AB+BB)	Non-smoker	117(25.7)	79(24.5)	1.228(0.794-1.898)	0.853	0.356
		Smokers	100(21.9)	88(27.3)	1.600(1.034-2.475)	4.482	0.034*
GSTT1	GSTT1(+)	Non-smoker	120(26.3)	73(22.7)	1		
		Smokers	131(28.7)	109(33.9)	1.368(0.93-2.013)	2.531	0.112
	GSTT1(-)	Non-smoker	111(24.3)	50(15.5)	0.740(0.476-1.153)	1.773	0.183
		Smokers	94(20.6)	90(28.0)	1.574(1.044-2.372)	4.72	0.030*
GSTP1	GSTP1(AA)	Non-smoker	51(11.1)	28(8.7)	1		
		Smokers	42(9.3)	19(5.9)	0.824(0.405-1.678)	0.285	0.594
	GSTP1(AB+BB)	Non-smoker	186(40.7)	144(44.7)	1.410(0.847-2.348)	1.756	0.185
		Smokers	177(38.9)	131(40.7)	1.338(0.800-2.236)	1.238	0.266

*p<0.05

exon7 of CYP1A1 locus results in the substitution of isoleucine to valine in the heme-binding region which increases the microsomal activitation (Sobti et al., 2004). A A4889G substitution resulting in a Ile462Val exchange in the heme-binding region of exon7 was first described in 1991 by Hayashi et al. (1991). The second polymorphism is a T→C transition in the 3' non-coding region (Msp1 polymorphism). This polymorphism has been shown to correlate with inducible aryl hydrocarbon hydrolase activity (Hecht et al., 2006). CYP1A1 Msp1 and exon7 polymorphisms are associated with the smoking related lung cancer risk in Kashmiri population (Sheikh et al., 2009).

In recent years, GSTM1 and GSTT1 have been studied widely. Shukla R et al found a significant difference in the GSTT1 null deletion frequency in northern Indian population when compared with other populations. However, GSTM1 null genotype was found associated with lung cancer in the non-smoking subgroup (Shukla et al., 2013). In the Korean population, the GSTM1 and GSTT1 null genotypes are risk factors for lung cancer in men; the GSTT1 null genotype has a more prominent effect on lung cancer risk in younger people (age 55 years and under) than in older individuals (Jin-Mei et al., 2013). Both GSTM1 and GSTT1 gene exhibit an inherited homozygous deletion polymorphism (null genotype), which has been associated with the loss of enzymatic activity and increased vulnerability to cytogenetic damage (Norppa, 2004). As a result of decreased efficiency in protection against carcinogens, the individuals with homozygous deletion polymorphism are considered to be at an increased risk for malignancies (Hayes et al., 2005; Mcllwain et al., 2006; Singh et al., 2010; Jin et al., 2010; Ihsan et al., 2011). Many studies have been published on the association of CYP1A1 and GSTM1 polymorphisms and lung cancer susceptibility with inconsistent findings. Some of the studies showed the relationship between the polymorphisms of GSTM1 and CYP1A1 Ile/Val genotype (Singh et al., 2010; Jin et al., 2010; Ihsan et al., 2011) but other results are contradictory (Le Marchand et al., 1998; London et al., 2000). However, some data of Chinese Han population (Shi et al., 2008) and little data on Mongolian population are still available.

In our study, we found significant difference of the frequency of GSTT1, but there was no difference in CYP1A1, GSTM1, GSTM3, and GSTP1 genotypes distribution between the healthy Mongolian and Han population in Inner Mongolian region. Genetic polymorphism in drug metabolizing enzymes has been found to be a factor in an individual's susceptibility to cancer. Among several candidates of a high risk allele for lung cancer, cytochrome 450 has been investigated most extensively because of its potential involvement in chemical carcinogenesis. Thus, CYP1A1 Msp1 polymorphism and polymorphism of its exon7 catalytic site have been reported in connection to the lung cancer risk in at least one study (Danie et al., 2005; Mcllwain et al., 2006). Although many studies carried out in different populations have not yielded consistent results. In recent study, our results indicate no difference in the genotypic frequencies of CYP1A1 exon7 polymorphism and a considerable difference in CYP1A1 Msp1 polymorphism between controls and lung cancer patient groups in Mongolian population. The results of this study support the CYP1A1 Msp1 variant mutant as lung cancer risk factors similar to most of the existing reports. In the analysis of the relationship with susceptibility to lung cancer, we found that Mongolian subjects with GSTM1 (-) carrier genotype had a 2.290 fold increased risk of lung cancer than GSTM1 (+) carrier genotype (95%CI =1.467-3.573). The Chi-square tests showed the significant difference between the two groups ($p<0.05$). The result indicated that the genotypes of the CYP1A1 exon7, GSTM3, GSTT1, GSTP1 polymorphisms of Mongolian population had no significant difference among the lung cancer group and controls ($p>0.05$).

By the analysis of relationship between CYP1A1, GSTM1, GSTM3, GSTT1, GSTP1 gene polymorphism and lung cancer susceptibility of Han population in Inner Mongolia region, the Chi-square tests showed the significant difference between the two groups of GSTM1, CYP1A1 Msp1 gene ($p<0.05$) but no major difference between the two groups of other genes ($p>0.05$).

Epidemiological studies indicated that most lung cancer can be dependent on external environmental or behavioural factors. Our study discovered that the smokers had a 2.144 fold increased risk of lung cancer than non-smokers (95%CI:1.452-3.167) in Mongolian population and 1.631 fold (95%CI:1.051-2.525) in Han population with significant differences ($p<0.05$).

In individuals with the combined effects of cigarette smoking and the CYP1A1 (Ile/Ile)or (Ile/Val+Val/Val) genotype, the risk of lung cancer was approximately 1.493 or 2.569 fold than the persons, both CYP1A1 (Ile/Ile)genotype carrier and without smoking habits. Similarly, the risk was 1.144 fold for the combined CYP1A1 (Ile/Val +Val/Val) genotype and non-smokers. The combined CYP1A1 (Ile/Val+Val/Val) genotype and smoking were found to be a significant risk factor of lung cancer ($p<0.05$). In the analysis of CYP1A1 Msp1gene polymorphism joined with smoking status and susceptibility to lung cancer, we found the significant difference between the two groups of the smokers with CYP1A1 (wt/vt+vt/vt) carrier ($p<0.05$). We found that the non-smokers with GSTM1 (-) carrier and the smokers with GSTM1 (+) carrier had increased risk of lung cancer than the non-smokers with GSTM1 (+) carrier, OR values were 1.155 (95%CI=0.735-1.815) and 1.531 (95%CI =0.977-2.400), but there was no major difference between the groups ($p>0.05$). The smokers with GSTM1 (-) carrier had increased risk of lung cancer, and there was significant difference between the two groups ($p<0.05$). The smokers who carried with GSTM3 (AB+BB) had increased risk of lung cancer compared with the nonsmokers who carried with GSTM3 (AA), OR values were 1.600 (95%CI=1.034-2.475), and the Chi-square tests showed the significant difference ($p<0.05$). The smokers with GSTT1 (-) carrier had increased risk of lung cancer than the non-smokers with GSTT1 (+) carrier, OR values were 1.574 (95%CI:1.044-2.372), and the Chi-square tests showed the significant difference ($p<0.05$). The smokers with GSTP1 (AG+GG) carrier had increased risk of lung cancer than

the non-smokers who carried with GSTP1 (AA) carrier, OR values were 1.338 (95%CI:0.800-2.236), and the Chi-square tests showed no significant difference (p>0.05).

In conclusion, the results of this study indicated that the CYP1A1 (vt/vt) and GSTM1 (-) genotypes were doubtful risk factors of lung cancer in Mongolian and Han population but smoking was the susceptibility factor of lung cancer. Moreover, combination of CYP1A1 (wt/vt+vt/vt), CYP1A1 (Ile/Val+Val/Val), GSTM1 (-), GSTM3 (AB+BB), GSTT1 (-) genotypes with smoking can increase the risk of lung cancer. These illustrations showed the synergetic effects between CYP1A1 (wt/vt+vt/vt), CYP1A1 (Ile/Val +Val/Val), GSTM1 (-), GSTM3 (AB+BB), GSTT1 (-) and smoking in the manifestation of lung cancer.

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