Title page

Title: Polymorphisms of CYP1A1 I462V and GSTM1 genotypes and lung cancer susceptibility in Mongolian

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Project supported by the National Natural Science Foundation of China (No. 30661021), Natural Science Foundation of Inner Mongolia Autonomous region (No. 200607010907) and Doctor Initializing Fund Application of Inner Mongolia medical college(No.2005BQ001).

Abstract **Aim** To study the genotype of cytochrome P450 1A1(CYP1A1) I462V and glutathions S-transferase M1(GSTM1) with the susceptibility of lung cancer in Mongolia of China. **Methods** Allele-specific and a multiplex PCR were employed to identify the genotypes of them in a case-control study of 210 lung cancer patients and 210 matched controls. **Results** The CYP1A1(Val/Val) and GSTM1(-) were the risk factor of lung cancer, the OR was 2.56 and 1.89 respectively. The relative risk increased to 2.6 – fold when the patients carried with two valine alleles in cases of SCC. GSTM1(-) was the risk factor of SCC (OR=2.39) and AC(OR=2.16). The light smokers were the risk factor for lung cancer. **Conclusion** The valine allele of CYP1A1 was the risk factor of lung cancer especially for SCC and GSTM1(-) was the risk factor of lung cancer and especially for SCC and AC of Mongolian, China. There may be a synergetic interaction between CYP1A1 valine allele and GSTM1(-) genotypes on the elevated susceptibility of lung cancer. So do those genotypes with light smokers.

Key words polymorphism; genotype; lung cancer; cytochrome P450；glutathione S-transferase

Abbreviations: SCC, squamous cell carcinoma; AC, adenocarcinoma; SCLC, small cell lung cancer; LCLC, large cell lung cancer

Cytochrome P4501A1 (CYP1A1) metabolizes a number of suspected procarcinogens, especially, polycyclic aromatic hydrocarbons(PAHs), into highly reactive intemediates. These compounds are capable of binding to DNA to form adducts, which, if not repaired, may initiate or promote carcinogenesis. Although PAHs are ubiquitous in the environment, exposure sources of particular concern include smoking, air pollution, diet and certain occupations[1]. A A4889G substitution resulting in a Ile462Val exchange in the heme binding region of exon7 was first described in 1991 by Hayashi et al. [2]. This polymorphism has been show to correlate with inducibility of aryl hydrocarbon hydrolase activity[3]. Lung cancer is one of the commonest human neoplasms . Epidemiological studies have shown that squamous cell carcinoma is associated with tobacco and alcohol consumption, and is commonest in regions where these products are most often consumed. Chemical compounds present in tobacco and alcohol undergo metabolic activation by phase 1 enzymes, usually the cytochrome P450 enzymes(CYP). CYP catalyse reactions that result in the creation of functional groups on their substrates, thus generating the ultimate carcinogen. These carcinogens form DNA adducts, leading to genetic damage. The glutathione S-transferases consist of a superfamily of enzymes that catalyse the reduction by glutathione of several electrophilic substrates, facilitating the excretion of these compounds. These compounds are often products of CYP mediated metabolism[4]. Tobacco and ethanol, similarly to most environmental toxins, requires metabolic activation and subsequent detoxification by a series of enzymes. Glutathione S-transferases (GSTs) are phase II xenobiotic metabolizing enzymes that catalyze the conjugation of electrophilic compounds with reduced glutathione to produce less toxic or readily excretable metabolites.

Individual variations in the metabolic activation and detoxification of chemical carcinogens and genotoxins, such as tobacco and ethanol, 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 most important role in this context. An increasing number of xenobiotic metabolizing enzymes, such as GSTs and CYPs , have been shown to be polymorphic[5].

A relatively large number of studies have been published on the association of CYP1A1 and GSTM1 polymorphisms and lung cancer susceptibility and the findings have been inconsistent. Some of the studies have found that there are relationship between the polymorphisms of GSTM1 and CYP1A1 Ile/Val genotype[6-8]. Other

results are opposite [9;3].Although there are some of Chinese populations data[10], but there are few data on Mongolian populations.

We have studied the distribution of CYP1A1 MspI and GSTM1 genotype between the Monglolian and Han nationality of Inner Mongolia. We have not found difference between them on the distribution of CYP1A1 Msp I and GSTM1 [11]. We also have studied the relationship of polymorphisms of CYP1A1 MspI and GSTM1 genotype with the susceptibility of lung cancer on the population of Inner Mongolia. We have found the CYP1A1 MspI mutation homozygote and GSTM1 null genotype is the risk factor of lung cancer on Inner Mongolia population[12] . In the present study we analyzed the polymorphisms of CYP1A1 I462V and GSTM1 genotypes and lung cancer susceptibility on Mongolian. We also considered possible effect modification by cigarette smoking and alcohol.

Subjects and Methods

Study subjects and epidemiology information This case-control study consisted of 210 patients suffering from lung cancer and 210 general population-selected healthy controls. Lung cancer cases were recruited from patients undergoing bronchoscopy at the first affiliated hospital, Inner Mongolia Medical College, China. The ages of patients range from 43 to 86, averaging 43±13; and controls from 36 to 83, averaging 67 ± 11. At recruitment, each participant was personally interviewed to obtain a detailed information about smoking, dietary habits, alcohol consumption and demographic characteristics. Controls were individually matched to the patients with respect to gender and age, who were selected from healthy individuals free of malignancy. 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, and the study was approved by the Ethics Committee of the Inner Mongolia Medical College. Each person donated 5 ml of whole blood in haparinized tubes, stored in freezer at -80 centigrade.

CYP1A1 I462V genotyping

Genomic DNA was isolated from peripheral blood samples of cases and controls according to the protocol of Nakachi et al[13]. To detect CYP1A1 I462V polymorphism, an allele-specific PCR procedure was developed using two sets of primers[14;2]. Each of primers (1A1A ; 5`- GAAGTGTATCGGTGAGACCA-3` and 1A1G; 5`-GAAGTGTATCGGTGAGACCG-3`), was used for PCR amplification together with another strand of primer (C53; 5`-GTAGACAGAGTCTAGGCCTCA-3`). Initial denaturation was carried out at 94 centigrade for 10 min, followed by 30 cycles of denaturation at 95 centigrade for 1 min, annealing at 65 centigrade for 1 min and extension at 72centigrade for 1 min and final extension at 72 centigrade for 10 min in thermal cycler （GeneAmp PCR System 2400, PERKIN ELMER, Singapore）. The two resulting PCR products were subjected to electrophoreses in a 2% agarose gel and stained with ethidium bromide and observed under the ultraviolet light. The products were all 210pb fragment for the two sets of primers. If the product was shown only when the primer 1A1A and C53 were used, not when the primer 1A1G and C53 were used, the subject was identified as a homozygote of Ile (Ile/Ile). On the other hand,

if the product was got only when the primer 1A1G and C53 were used, not when the primer 1A1A and C53 were used the subject was identified as a homozygote of Val (Val/Val). When the products present in both primers (1A1A , C53 and 1A1G, C53) indicated the heterozygotes of Ile/Val[7].

GSTM1 genotyping

The GSTM1 genotypes were analyzed by a multiplex PCR according to the protocol of Arand et al[15]. Briefly genomic DNA was amplified by using four sets of primers GSTM1 (F) 5`- GAACTCCCTGAAAAGCTAAAGC, GSTM1(R) 5`-GTTGGGCTCAAATATACGGTGG, Beta-globin (F)5`-CAACTTCATCCACGTTCACC , ( R)5`-GAAGAGCCAAGGACAGGTAC, Initial denaturation was carried out at 95 centigrade for 5 min , followed by 35 cycles under the following conditions: denaturation at 95 centigrade for 1 min ,annealing at 57 centigrade for 1 min , extension at 72 centigrade for 50 sec and final extension at 72 centigrade for 10 min in thermal cycler (GeneAmp PCR System 2400, PERKIN ELMER, Singapore). The PCR products were then subjected to electrophoresis on a 2.5% agarose gel. The presence of a 268 (Beta-globin) and 215(GSTM1) bps was indicative of the GSTM1 genotypes; whereas the absence of the product of GSTM1 indicated the GSTM1(-) genotype. Beta-globin was used as an internal control.

Statistical analysis SPSS 11.5 was used to do the statistics and the Odds Ratio; their 95% confidence interval (CI) and p value for the data were calculated. All data were considered significant difference when $p < 0.05$.

Results

The gender , age , histological types, smoking and alcohol status data were summarized on Table 1. 42.86% of patients are light smokers who smoked less than 30 packs per year and 26.19% of patients are heavy smokers who smoked more than 30 packs per year. Both light smokers and heavy smokers have significant difference between the group of control and patient ($p<0.001$). The light drinkers are those drank alcohol liquid (Containing alcohol less than 42%) less than 36 litres per year and the heavy drinkers are those drank alcohol liquid more than 36 litres per year. There are no significant difference between the control and patients on the gender, age, alcohol status.

Table 2 and 3 show the overall distribution of patients and controls by genotypes for the CYP1A1 (Ile/Val) and GSTM1 polymorphism. The mutant homozygous CYP1A1(Val/Val) of lung cancer was 15.24% and was two time more than the CYP1A1(Val/Val) of control(7.4%). The CYP1A1(Val/Val) genotype of the SCC was higher than that of AC and others, it was 22.77 , 7.69 and 9.09%, respectively. The proportion of GSTM1(-) of lung cancer was also higher than that of control, it was 56.67 and 40.95%, respectively. The frequency of the GSTM1(-) of the SCC was also higher than that of AC and others, it was 62.38, 60.0 and 38.64%, respectively. The one who carried with CYP1A1 (Val/Val) genotype, the risk of lung cancer was increased , the OR was 2.56 (95%CI: 1.32~4.93). The statistics show significant difference ($p=0.004$). Carried with CYP1A1(Ile/Val), the risk of lung cancer was increased, the OR is 1.42(95%CI: 0.89~2.26), but the statistics show no significant difference($p=0.142$). When stratified according to histology, there was a strong association for SCC with an OR of 4.45(95%CI:2.16~9.17) that the patients who carried with CYP1A1(Val/Val) genotype. The risk of AC and others who carried with CYP1A1 (Val/Val) genotype were increased, the OR was 1.14 and 1.34 , respectively, but the statistics show no significant difference($p=0.805$ and 0.621). From the table 3, the presence of at least one Val allele of CYP1A1, the risk of lung cancer was increased , the OR was all larger than 1.0. There were no statistics significant difference of them. The risk of lung cancer who carried two Val alleles of CYP1A1 was 1.8-fold of the one who carried with one Val allele of CYP1A1. At the same condition, the risk of SCC was 2.6-fold. The one who carried with GSTM1(-) genotype, the risk of lung cancer was increased , the OR was 1.89(95% CI: 1.28~2.78)

and statistics show significant difference (p=0.001).When stratified according to histology, there was a strong association for SCC and AC, the OR was 2.39(95%CI: 1.47~3.89, p=0.000) and 2.16(95%CI: 1.23~3.81, p=0.007), but not others, the OR was 0.91(95%CI: 0.45~1.77, p=0.776).

The combined effects of CYP1A1(Ile/Val) and GSTM1 genotypes for all lung cancer were also analyzed (table 4). The presence of at least one Val allele of CYP1A1 and GSTM1(-), the risk of lung cancer was increased, the OR was 4.15(95%CI: 2.02~8.51) for one Val allele and GSTM1(-) and 2.67(95%CI:1.22~5.84) for two Val alleles and GSTM1(-), the statistics show significant difference (p=0.000 and 0.012). When there was one Val allele of CYP1A1 and combined another GSTM1(-) genotype, the risk of lung cancer was increased more.

The risk of developing lung cancer in relation to CYP1A1(Ile/Val) and GSTM1 genotypes and age , smoking and alcohol drinking was shown in Tab. 5. Mongolian has a habit of drinking and most people like to smoke especially for men. Since the Val allele of CYP1A1 was very important in the susceptibility of lung cancer not one or two, we combined the heterozygous (Ile/Val) and mutant (Val/Val) alleles as a single genotype. Individuals who were lighter smokers and carrying the CYP1A1(Ile/Val and Val/Val) or GSTM(-) genotype, the risk of lung cancer was increased , the OR was 2.04(95%CI: 1.06~3.94) and 2.0(95%CI:1.03~3.89). The statistics show significant difference, the p value is 0.045 and 0.04, respectively. There was no associations between CYP1A1 and GSTM1 genotypes and increased risk in heavy smokers.

When stratified the association of risk of lung cancer and the age , CYP1A1(Ile/Val) and GSTM1(-) genotypes, an interesting results were found, the individuals who age were less than 50 and carrying the CYP1A1(Ile/Val and Val/Val) genotypes, the risk of lung cancer was increased, the OR was 6.64(95%CI: 1.97~22.36, p=0.001), but the individuals who age during the 51 to 65 and carrying the GSTM1(-) genotypes, the risk of lung cancer was increased, the OR is 2.42(95%CI:1.34~4.4, p=0.003).

Although the OR was all larger than 1.0 of the individuals who was light drinkers or heavy drinkers and carrying CYP1A1(Ile/Val and Val/Val) and GSTM1(-), we could not find the association of lung cancer and the drinkers (light or heavy) with CYP1A1(Ile/Val and Val/Val) genotypes.

Discussion

In this population –based case-control study of genetic susceptibility to lung cancer, the presence of at least one copy of the Val allele of CYP1A1, the risk of lung cancer was increased. When combined with the GSTM(-) genotypes ,the risk increased more, from 1.42 of OR for CYP1A1(Ile/Val) to 4.15 of OR for CYP1A1(Ile/Val) and GSTM1(-). CYP1A1(Val/Val) and GSTM1(-) was a factor of susceptibility of lung cancer of Mongolian. When considered the histological types, the CYP1A1(Val/Val) was association with the SCC and GSTM1(-) was association with SCC and AC. No association was found between CYP1A1(Val/Val) and GSTM1(-) with other types of lung cancer.

Cigarette smoke contains hundreds of constituents that may play a role in carcinogenesis. However, only a fraction of smokers will develop lung cancer. Individual susceptibility to chemically induced cancer may be partly explained by genetic difference in the activation and detoxification of procarcinogen[7]. Marchand et al [1]. made a pooled analysis of the CYP1A1 exon 7 polymorphism and lung cancer and found that the CYP1A1 exon 7 polymorphism may confer an increased risk of lung cancer, particularly of SCC, and especially in never – smokers and in women. Our result was similar part of theirs. Shi et al [10] summarized the data from 46 studies on polymorphism of Msp I and exon 7- Val of CYP1A1 and GSTM1 and lung cancer risk in Chinese population and found evidence of an association between the CYP1A1 variant and GSTM1 null genotypes and increased risk of lung cancer, the results were also similar with us. We have not found the report about the Mongolian CYP1A1 and GSTM1 polymorphisms and the relationship with the susceptibility of lung cancer. From our research we found that the risk for mutant homozygote (Val/Val) and heterozygote (Ile/Val) of CYP1A1 exon 7 was 2.56-fold and 1.42-fold, respectively, compared with the wild homozygote of CYP1A1 exon 7 (Ile/Ile), and that the risk for the GSTM1(-) genotype was 1.89-fold , compared with the GSTM1 (+) genotype. No statistics significant difference was found between the Ile/Val genotype and Ile/Ile genotype (p=0.142). When considered the histological types, the risk of SCC increased 1.72-fold when the individuals with Ile/Val genotype of CYP1A1 and 4.45 –fold when the ones with Val/Val genotype of CYP1A1, compared with Ile/Ile genotype of CYP1A1. Also no significant difference was found between the Ile/Val genotype and Ile/Ile genotype (p=0.066). The risk of SCC increased 2.39-fold the individual carrying GSTM1(-) genotype, compared with the individual carrying GSTM1(+) genotype, the statistics show significant difference

(p=0.000). The reason why CYP1A1(Val/Val) genotype has a high risk of lung cancer was not known, it may be connect with activity of enzyme of CYP1A1. CYP1A1 is an enzyme with AHH activity that exhibits a three –way distribution in human populations with its higher activity shown to be associated with an elevated risk of lung cancer. GSTM1 acts to protect the organism from bioactivated carcinogens, and its absence would facilitate the potential carcinogenicity of such agents. The intermediates activated by AHH, if not detoxificated by GSTM1 enzyme could accumulate the carcinogen and increase the risk of lung cancer[7]. Presenting at least one Val allele, the risk of lung cancer was increased. Individual carrying GSTM1(-) and at the same time when they took the Ile/Val genotypes, the risk of lung cancer was 1.6-fold than that of individual carrying Val/Val genotypes. Those results indicated that change of metabolic balance including the activation of procarcinogens by CYP1A1 and the detoxification by GSTM1 might have multiplicative effects on lung cancer.

The susceptibility of lung cancer may be association to age. There were few articles about that [16] and in our result we found an interesting phenomena, the risk of lung cancer for the combined variant genotypes(Ile/Val and Val/Val) was 6.64 – fold , compared with Ile/Ile genotype when the age was less than 50. The statistics show significant difference (p=0.001). When the age was during the 51~65, the risk of lung cancer for the GSTM1(-) genotype was 2.24-fold, compared with GSTM1(+) genotype. The statistics also show significant difference (p=0.003).

The smoke tar can regulate up the expression of aromatic hydrocarbon receptor (AHR) and CYP1A1 gene at a certain dosage and time. The regulation of smoke tar for the expression of AHR was earlier than that of CYP1A1[17]. The action of the smoking on the CYP1A1 , the reports were different. Some of the authors reported that there were association between light smokers who carried with CYP1A1(Val/Val) genotypes and lung cancer[18]. The present data clearly indicates that the one who carried with CYP1A1(Val/Val) genotype and were light smokers had a high risk of lung cancer(OR=2.14, 95% CI: 1.06 ~3.94, p=0.045). Others of them reported that individuals who were heavy smokers and had CYP1A1(Val/Val) genotypes had a high risk of lung cancer[8;19]. GSTM1(-) , smokers and the association with the risk of lung cancer also had a controversial reports. A slight elevation in risk of lung cancer in more casual smokers(BI > 400) was observed[8;19] whereas other studies found a strong association with a low smoking exposure[18]. The present data had

shown association the risk of lung cancer who carried with GSTM1(-) and light smokers, the OR was 2.04(95%CI: 1.03 ~ 3.89,p=0.04). There were a strong association the smoking and lung cancer, some of gene polymorphism may play a more important role, such as CYP1A1(Ile/ Val and Val/Val), others may act as a secondary role, such as GSTM1(-). The risk of lung cancer was increased the individuals carried with CYP1A1(Ile/Val and Val/Val) and were light drinkers or heavy drinkers, the OR were all larger than 1, especially for heavy drinkers and carrying CYP1A1(Ile/Val and Val/Val) genotypes(OR=1.79, 95%CI: 0.52~6.02), but the statistics show no significance($p > 0.05$).

In this study, a strong association was found between the CYP1A1 valine allele and GSTM1 (-) with lung cancer in the Mongolian population. Combined genotyping of susceptible CYP1A1 valine allele and GSTM1(-) genotypes revealed a higher risk than that ascribed to a single susceptible genotype, for example, CYP1A1(Ile/Val, Val/Val) and GSTM1(-). Further these data, as given, provide additional evidence that some of polymorphic genes are an important determinant in susceptibility to smoking induced lung cancer especially light smokers, such as CYP1A1(Ile/Val and Val/Val). Drinking alcohol might slightly increase the risk of lung cancer when individuals carried with CYP1A1(Ile/Val and Val/Val) and GSTM1(-). These data may also support the hypothesis that susceptibility to certain cancer may depend upon ethnic-specific gene polymorphisms. On the susceptibility to the lung cancer with the CYP1A1 and GSTM1 genotypes depending on the different age may be biased by the relatively small number of subjects in the various subgroup. There are many different reports about the association of lung cancer and CYP1A1 and GSTM1 gene polymorphism, the inconsistency between our results and other Asian populations, such as Japanese[9], other parts of Chinese[19], might be ascribed to different environmental factors and the number of cases. The study suggested that CYP1A1 (Ile/Val, Val/Val) and GSTM1(-) could be regard as the susceptible factors of lung cancer of the Mongolian population.

Acknowledgement: We think Prof. Tingmao Hu of Inner Mongolia University for technical guidance and Teacher Lei Fan of Inner Mongolia Medical College for technical assistance. The Project was supported by the National Natural Science Foundation of China (No. 30661021), Natural Science Foundation of Inner Mongolia Autonomous region (No. 200607010907) and Doctor Initializing Fund Application of Inner Mongolia medical college(No.2005BQ001).

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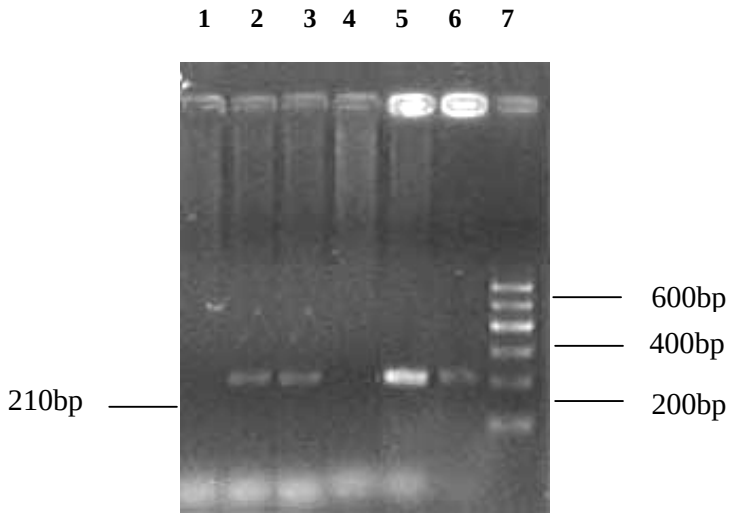

Fig 1. Analysis of electrophoretogram of PCR products of CYP1A1 exon 7 gene
1 and 2 , Val/Val homozygote; 3 and 4, Ile/Ile homozygote; 5 and 6, Ile/Val heterozygote; 7, Size markers

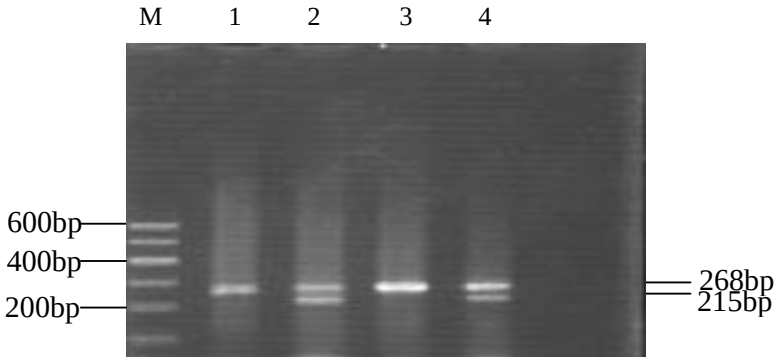

Fig.2 The electrophoretogram of the products of PCR of GSTM1 gene and Beta-globin

M：Markers　1，3：GSTM1（-）　2，4：GSTM1（+）

Table 1: Frequency distribution of demographic variables for patients and controls

Variables	Patients (n=210)(%)	Controls (n=210)(%)
Gender		
Male	176(83.81)	176(83.81)
Female	34(16.19)	34(16.19)
Age(years)		
Less than 50	19(9.04)	29(13.81)
During 50 to 65	102(48.57)	112(53.33)
Older than 65	89(42.38)	69(32.86)
Smoking Status		
Never	65(30.95)	130(61.91)
Light Smokers [a]	90(42.86) **	65(30.95)
Heavy Smokers [b]	55(26.19) **	15(7.14)
Drinking Alcohol		
Never	31(14.76)	38(18.09)
Light Drinkers [c]	156(74.79)	153(72.86)
Heavy Drnkers [d]	23(10.95)	19(9.05)
Histological type		
Squamous cell carcinoma	101(48.10)	
Adenocarcinoma	65(30.95)	
Other	44(20.95)	

[a] less than 30 pack-year, [b] 30 or more pack-year, [c] less than 36 litres of alcohol(<42%) – year,

[d] 36 or more litres of alcohol(<42%) – year .

Two – sided X^2 –test, $^{**}p<0.001$

Table 2 Distribution of CYP1A1 and GSTM1 genotypes of lung cancer and controls

Genotypes	CYP1A1 (Ile/Val)			GSTM1	
	Ile/Ile	Ile/Val	Val/Val	GSTM1 (+)	GSTM1(-)
Control	151(71.9)	44(20.95)	15(7.4)	124(59.05)	86(40.95)
Lung cancer	126(60.0)	52(24.76)	32(15.24)	91(43.33)	119(56.67)
SCC	52(51.49)	26(25.74)	23(22.77)	38(37.62)	63(62.38)
AC	44(67.69)	16(24.62)	5(7.69)	26(40.0)	39(60.0)
Others	30(68.18)	10(22.73)	4(9.09)	27(61.36)	17(38.64)

Table 3 OR and 95% CI for lung cancer by CYP1A1 and GSTM1 genotypes

	All (219/230)	SCC	AC	Other
	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
CYP1A1				
Ile/Ile	1	1	1	1
Ile/Val	1.42(0.89~2.26)	1.72(0.96~3.06)	1.25(0.64~2.42)	1.14(0.52~2.52)
	p=0.142	p=0.066	p=0.512	p=0.739
Val/Val	2.56(1.32~4.93)	4.45(2.16~9.17)	1.14(0.39~3.32)	1.34(0.42~4.33)
	p=0.004*	p=0.000* *	p=0.805	p=0.621
GSTM1				
GSTM1(+)	1	1	1	1
GSTM1(-)	1.89(1.28~2.78)	2.39(1.47~3.89)	2.16(1.23~3.81)	0.91(0.45~1.77)
	p=0.001*	p=0.000**	p=0.007 *	p=0.776

Table 4 Combined analysis of genotypes of CYP1A1 and GSTM1 in relation to incidence of lung cancer

GSTM1	CYP1A1	Lung cancer n (%)	Control n (%)	OR (95% CI)	p-value
GSTM1(+)	Ile/Ile	88(41.9)	69(32.86)	1	
GSTM1(+)	Ile/Val	32(15.24)	13(4.76)	0.52(0.25~1.06)	0.07
GSTM1(+)	Val/Val	4(1.9)	9(5.71)	2.87(0.85~9.71)	0.079
GSTM1(-)	Ile/Ile	63(30.0)	57(27.14)	1.15(0.72~1.86)	0.556
GSTM1(-)	Ile/Val	12(5.71)	39(20.0)	4.15(2.02~8.51)	0.000**
GSTM1(-)	Val/Val	11(5.24)	23(9.52)	2.67(1.22~5.84)	0.012*

Table 5 Stratification analysis of CYP1A1 and GSTM1 polymorphism in relation to lung cancer by age and smoking

Age; Smoking and Drinking status	Lung cancer n (%)	Control n (%)	OR(95%CI)	p- value
Less than 50				
Ile/Ile	7(3.33)	31(14.76)	1	
Ile/Val	12(5.71)	8(3.81)	6.64(1.97~22.36)	0.001
GSTM1(+)	8(3.81)	22(10.48)	1	
GSTM1(-)	11(5.24)	17(8.1)	1.78(0.59~5.39)	0.306
During the 51~65				
Ile/Ile	68(32.38)	52(24.76)	1	
Ile/Val	34(16.19)	15(7.14)	1.73(0.86~3.51)	0.125
GSTM1(+)	43(20.48)	53(25.24)	1	
GSTM1(-)	59(28.1)	30(14.28)	2.42(1.34~4.4)	0.003*
Older than 65				
Ile/Ile	51(24.28)	68(32.38)	1	
Ile/Val	38(18.1)	36(17.14)	1.41(0.79~2.52)	0.25
GSTM1(+)	40(19.05)	49(23.33)	1	
GSTM1(-)	49(23.33)	39(18.57)	1.54(0.85~2.78)	0.153

Light smokers				
Ile/Ile	29(13.81)	32(15.24)	1	
Ile/Val	61(20.05)	33(15.71)	2.04(1.06~3.94)	0.045*
GSTM1(+)	27(12.86)	30(14.28)	1	
GSTM1(-)	63(30.0)	35(16.67)	2.0(1.03~3.89)	0.04*
Heavy smokers				
Ile/Ile	34(16.19)	7(3.33)	1	
Ile/Val	21(10.0)	8(3.81)	0.54(0.17~1.71)	0.291
GSTM1(+)	32(15.24)	9(4.28)	1	
GSTM1(-)	23(10.95)	6(2.86)	1.08(0.34~3.45)	0.899
Light Drinkers				
Ile/Ile	76(36.19)	79(37.62)	1	
Ile/Val	80(38.09)	74(35.24)	1.12(0.72~1.76)	0.608
GSTM1(+)	73(34.76)	81(38.57)	1	
GSTM1(-)	83(39.52)	72(34.28)	1.28(0.82~2.0)	0.28
Heavy Drinkers				
Ile/Ile	10(4.76)	11(5.24)	1	
Ile/Val	13(6.19)	8(3.81)	1.79(0.52~6.02)	0.352
GSTM1(+)	11(5.24)	10(4.76)	1	
GSTM1(-)	12(5.71)	9(4.28)	1.21(0.36~4.09)	0.757

Light smokers: less than 30 pack-year

Heavy smokers: 30 or more pack-year

Light drinkers: less than 36 litres of alcohol(<42%) – year

Heavy drinkers: 36 or more litres of alcohol(<42%) – year

Ile/Val=Ile/Val +Val/Val