Correlation between metabolic enzyme GSTP1 polymorphisms and susceptibility to lung cancer

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Abstract. The aim of the present study was to determine the frequency distribution and characteristics of polymorphic alleles and genotypes in glutathione *S*-transferase π 1 (GSTP1) exon 5, and to explore the correlation between GSTP1 exon 5 polymorphisms and susceptibility to lung cancer using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. Patients were diagnosed with lung cancer from May 2006 to October 2008 by postoperative pathological examination. A total of 150 patients, including 115 males and 35 females, aged 31-76 years (mean, 57.1 years) were enrolled. The control group consisted of 152 healthy volunteers who received physical examination at outpatient clinics. Genomic DNA was extracted from the peripheral venous blood of the 302 subjects, and the GSTP1 genotype was determined by PCR-RFLP and restricted enzyme digestion of PCR products. GSTP1 polymorphisms were analyzed in the 302 subjects. The C and G allele frequencies of GSTP1 in the control and lung cancer groups showed no significant difference (P=0.135); the frequencies of three different genotypes, A/A, A/G and G/G, of GSTP1 in the control and lung cancer groups exhibited no significant differences between the two groups (P=0.223). GSTP1 genotype frequencies in the study population fitted the Hardy-Weinberg equilibrium, demonstrating that the genotype results of this study conform to this genetic law. Overall, 50.7% of the subjects in the lung cancer group carried the non-A/A genotype of GSTP1, which was higher than the 43.4% of the control group. The risk of lung cancer in subjects with the non-A/A genotype was 1.43-fold higher than that in those with the A/A genotype, but no statistical significance was found (P=0.138). GSTP1 exon 5 polymorphisms were demonstrated to be associated with lung cancer susceptibility on the whole. However, stratified analysis suggested the correlation of GSTP1 exon 5 polymorphisms with lung squamous cell carcinoma risk, and that exon 5 polymorphisms might increase the risk of lung squamous cell carcinoma. Exon 5 GSTP1 polymorphisms were not found to be a strong influencing factor in lung cancer risk, but may play a certain role.

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Key words: GSTP1, polymorphisms, lung cancer, Chinese Han population

Introduction

Human cytoplasmic glutathione *S*-transferases (GSTs) are a group of phase II metabolic enzymes, which are exogenous compounds, and are a supergene family. Glutathione *S*-transferase π 1 (GSTP1) has been identified as a member of the GSTP family. As GSTP1 is present at the highest level in lung tissue among all GST isoenzymes, it may have an important role in the detoxification of inhaled carcinogens, such as benzo(a)pyrene and tobacco carcinogens. Polymorphisms have been detected in exons 5 and 6 of the GSTP1 gene. GSTP1 enzymes with a Val allele in exon 5 have a significantly decreased enzymatic activity, while those with a Val allele in exon 6 have no significant reduction in activity. Moreover, GSTP1 exon 5 polymorphisms have been found to be associated with lung cancer risk, whereas GSTP1 exon 6 polymorphisms have not been found to be correlated (1,2).

Although GSTP1 exon 5 polymorphisms have been demonstrated to be associated with lung cancer risk, the findings have not been consistent across studies (2). Moreover, few studies on GSTP1 exon 5 polymorphisms in the Chinese Han population have been reported. Therefore, the present study attempted to determine the frequency distribution and characteristics of polymorphic alleles and genotypes in GSTP1 exon 5 in a Chinese Han population, and to explore the correlation between GSTP1 exon 5 polymorphisms and susceptibility to lung cancer using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique in a case-control study.

Materials and methods

Subjects

Lung cancer group. Hospitalized patients with primary lung cancer who received surgical treatment in the Department of Thoracic Surgery of the Affiliated Hospital of Inner Mongolia

Medical University (Hohhot, China) from May 2006 to October 2008 were diagnosed by postoperative pathological examination. A total of 150 patients with lung cancer, including 115 male patients and 35 female patients, aged 31-76 years (mean, 57.1 years) were enrolled. Of them, 70 patients had squamous cell carcinoma, 61 had adenocarcinoma and 19 had other types of lung cancer.

Control group. The control group consisted of 152 healthy volunteers who received a physical examination in the outpatient clinics of the Affiliated Hospital of Inner Mongolia Medical University during the same period, including 117 males and 35 females, aged 33-78 years (mean, 54.7 years). The present study was approved by the ethics committee of Inner Mongolia Medical University. All patients provided written informed consent .

Main reagents. The main reagents were GoldView™ DNA dye, 1 ml/tube, purchased from SBS Genetech (Beijing, China); an UltraPure™ rapid genomic DNA extraction kit, 50 extractions/kit, purchased from SBS Genetech; and DNA Marker I, 200 reactions/tube, purchased from Tiangen Biotech (Beijing, China). In addition, 10 mM dNTP mix, 0.2 ml/tube; 5 U/μl Taq DNA polymerase, 500 U/tube; 10X PCR reaction buffer, 1 ml/tube; 25 mM $MgCl_2$, 1 ml/tube; and Restriction endonuclease *Alw*26I, 1,000 U/tube were purchased from Fermentas (Pittsburgh, PA, USA).

Key instruments. The key instruments used were an electronic balance (BP310S; Sartorius AG, Göttingen, Germany); a heated shaking water bath and fast digital thermostatic tank (HZS-H; Donglian Electronic & Technology Development Co., Ltd., Harbin, China); a microwave oven (Guangdong Galanz Microwave Oven and Electrical Appliances Manufacturing Co., Ltd., Guangdong, China); a constant-voltage electrophoresis system (Vokam 2541; Bio-Rad, Hercules, CA, USA); a horizontal electrophoresis tank (Liuyi Instrument Factory, Beijing, China); a gel scanning image analysis system (Bio-Rad); a UV spectrophotometer (Smart Spec 3000; Bio-Rad); a 96-well gradient PCR machine (Hybaid, Thermo Fisher Scientific, Inc., Carlsbad, CA, USA); a vortexing shaker (WH-851; KOLED, Beijing, China); and a UV transilluminator (ZF-90; Gucun Electro-optical Instrument Factory, Shanghai, China).

Genomic DNA extraction. Genomic DNA was extracted from the peripheral venous blood of the 302 subjects. The blood, which was stored as frozen EDTA-anticoagulated samples, was thawed at room temperature and mixed. DNA extraction was performed using 0.5 ml whole blood in strict accordance with the instructions of the DNA extraction kit. Then, 5 μl extracted genomic DNA was electrophoresed on 0.8% agarose gel to determine its purity and yield. In addition, the purity of the extracted genomic DNA was determined by UV spectrophotometry, and optical density (OD) values were measured at 260 and 280 nm to calculate $OD_{260:280\ nm}$.

Determination of GSTP1 genotype by PCR-RFLP

Design and synthesis of primers. A pair of primers for exon 5 of the GSTP1 gene were designed according to the method described by Ryberg *et al* (3) (Table I) and synthesized by SBS Genetech.

PCR amplification. i) PCR system: A 25-μl reaction system consisting of the following components was used: 2.5 μl 10X PCR reaction buffer, 2 μl 25 mM $MgCl_2$, 2.5 μl 2 mM 4X dNTP, 0.5 μl 20 μM primer 1, 0.5 μl 20 μM primer 2, 3 μl genomic DNA and 0.3 μl 5 U/μl Taq DNA polymerase. The solution was made up with double-distilled water to a total volume of 25 μl. The components were placed in a tube immersed in an ice water bath, well mixed in a short time in a vortexing shaker, and centrifuged briefly prior to being placed into the PCR machine.

ii) PCR conditions: The mixture was amplified under the following cycling conditions: Initial denaturation at 94˚C for 3 min for 1 cycle; denaturation at 94˚C for 45 sec and annealing at 60˚C for 1 min for 35 cycles; extension at 72˚C for 1 min and a final extension at 72˚C for 6 min for 1 cycle

iii) Detection of PCR products: The amplified fragment length was 433 bp. A 3 μl quantity of PCR products was electrophoresed on 2% agarose gel (stained with GoldView™ DNA dye, 0.5 μl/10 ml) under 120 V and 60 mA for 20 min, and observed in a UV transilluminator to determine whether a target gene fragment of 433 bp was obtained.

Restriction enzyme digestion of PCR products. i) Digestion system: A 15 μl quantity of PCR products was digested with *Alw*26I in a 20-μl reaction system comprising the following components: 15 μl PCR products, 2 μl 10X buffer and 1 μl 10 U/μl *Alw*26Ia, made up with double-distilled water to a total volume of 20 μl. The components were placed in a tube immersed in an ice water bath, well mixed in a vortexing shaker, centrifuged briefly, and placed into a 37˚C water bath.

ii) Enzyme digestion conditions: The mixtures were placed in a 37˚C water bath for 3 h.

iii) Determination of digestion results: A 10 μl quantity of the digestion products was electrophoresed on 2% agarose gel (stained with GoldView™ DNA dye, 0.5 μl/10 ml) under 120 V and 60 mA for 20 min. The digestion results were observed with a UV transilluminator and recorded.

iv) Determination of GSTP1 genotypes according to digestion results: No *Alw*26I restriction site was present at nucleotide 1578 of the wild-type allele. However, the A→G mutation created a restriction site at nucleotide 1578. The presence of one restriction site indicated A/A, and the presence of two and three restriction sites indicated A/G and G/G, respectively. Genotypes were determined as follows: A/A: 328 and 105 bp, wild-type homozygote; A/G: 328, 222, 106 and 105 bp, mutant heterozygote; G/G: 222, 106 and 105 bp; mutant homozygote.

v) *Alw*26I restriction sites are: 5'…G T C T C (N) ↓…3' and 3'…C A G A G (N)↑ …5'.

Statistical analysis. Data were analyzed using SPSS software, version 10.0 (SPSS, Inc., Chicago, IL, USA). Allele and genotype frequencies were compared between lung cancer and control groups by Chi-square test. Lung cancer risk assessment was presented as the odds ratio (OR) and 95% confidence interval (CI). Logistic regression analysis was performed to calculate OR and 95% CI. In addition, OR was adjusted by gender, age, and smoking status based on the actual statistical data.

Results

PCR amplification of DNA and digestion of PCR products

PCR amplification of genomic DNA. The GSTP1 gene from all study samples was successfully amplified by PCR. An electrophoretogram of PCR products is shown in Fig. 1. A target fragment of 433 bp was observed, which was consistent with the theoretical length from the primer design. There was a high yield of PCR products. No non-specific bands were observed.

Digestion of PCR products. An electrophoretogram of *Alw*26I-digested PCR products is shown in Fig. 2, indicating effective digestion. As it is sometimes difficult to distinguish 105 and 106 bp during electrophoresis, they appeared as a single band. However, this did not affect the determination of GSTP1 genotypes. Three target fragments with lengths of 328, 222 and 105-106 bp, respectively, were obtained following the digestion of PCR products, and three different genotypes, A/A, A/G and G/G, were observed.

Allele and genotype frequencies of GSTP1

Allele frequencies of GSTP1 in the control and lung cancer groups. In this study, GSTP1 polymorphisms were analyzed in 302 subjects, including 150 healthy control subjects and 152 lung cancer patients. The C and G allele frequencies of GSTP1 in the control and lung cancer groups are shown in Table II. No significant difference was found between the two groups (P=0.135).

Genotype frequencies of GSTP1 in the control and lung cancer groups. GSTP1 polymorphisms were analyzed in the 302 subjects. The frequencies of three different genotypes, A/A, A/G and G/G, of GSTP1 in the control and lung cancer groups are shown in Table III. No significant difference was observed between the two groups (P=0.223).

Goodness of fit of GSTP1 genotype distribution. All subjects in the lung cancer and control groups were genotyped for GSTP1 exon 5. The goodness of fit of the GSTP1 genotype distribution in the healthy control group to Hardy-Weinberg equilibrium was tested. The actual and expected numbers of healthy controls with the three genotypes are shown in Table IV. No significant difference was observed between the actual and expected distributions (P=0.372). This indicated that GSTP1 genotype frequencies in the study population fitted the Hardy-Weinberg equilibrium, demonstrating that the genotype results of this study conform to this genetic law.

Correlation between GSTP1 polymorphisms and lung cancer risk. Since the activity of GST is significantly lower with the A/G and G/G genotypes than with the A/A genotype, the former has a decreased ability to detoxify activated carcinogens, and their carriers thus have an increased susceptibility to lung cancer (4). Therefore, the A/G and G/G genotypes were placed in the same category when analyzing the correlation between GSTP1 polymorphisms and lung cancer risk. Specifically, the A/G and G/G genotypes represented the non-A/A genotype. Subjects with the A/A genotype were regarded as a reference group to evaluate the risk of lung cancer.

Correlation between GSTP1 polymorphisms and overall lung cancer risk. Overall, 50.7% of the subjects in the lung cancer group carried the non-A/A genotype of GSTP1, which was higher than the 43.4% of the control group. The risk of lung cancer in the subjects with the non-A/A genotype was 1.43-fold higher that that of the subjects with the A/A genotype; however, no statistical significance was identified (P=0.138; Table V).

Table I. Sequences of the primers for use in the analysis of GSTP1 by polymerase chain reaction.

Direction	Primer sequence	Product size (bp)
Forward	5'-GTAGTTTGCCCAAGGTCAAG-3'	433
Reverse	5'-AGCCACCTGAGGGGTAAG-3'	

GSTP1, glutathione *S*-transferase π 1.

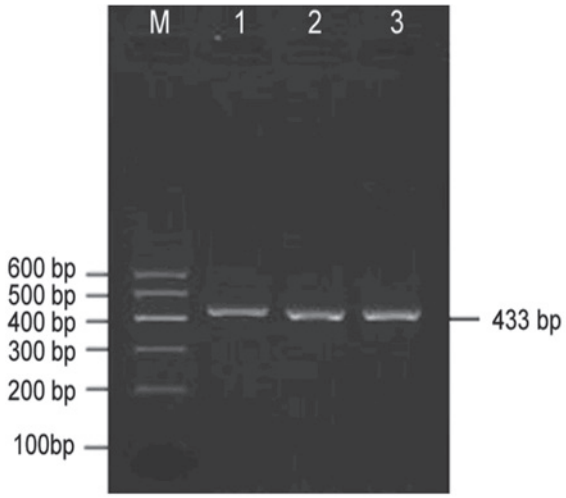

Figure 1. Electrophoresis of PCR products of the GSTP1 gene. Lane M, 100 bp DNA Marker; lanes 1-3: PCR products. PCR, polymerase chain reaction; GSTP1, glutathione *S*-transferase π 1.

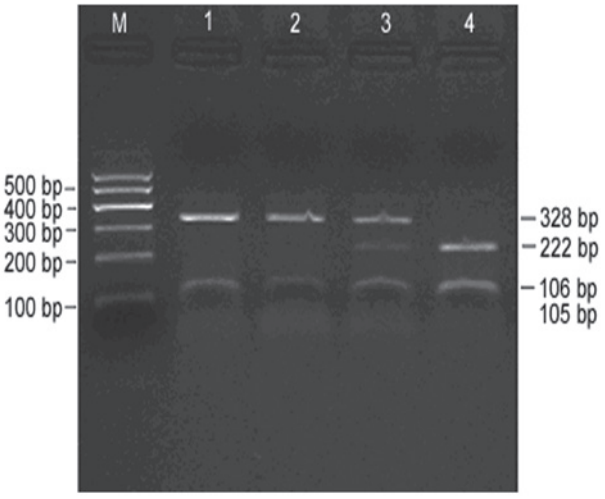

Figure 2. Electrophoresis of GSTP1 PCR products digested with *Alw*26I restriction enzyme. Lane M, 100 bp DNA Marker; lanes 1 and 2, homozygous for the wild-type GSTP1 gene; lane 3, heterozygous for the mutated GSTP1 gene; lane 4, homozygous for the mutated GSTP1 gene. PCR, polymerase chain reaction; GSTP1, glutathione *S*-transferase π 1.

Correlation between GSTP1 polymorphisms and risk of different histological types of lung cancer. The lung cancer group was stratified by different histological types of lung cancer.

Table II. Distribution of GSTP1 alleles in the control and lung cancer groups.

		Alleles of GSTP1, n (%)			
Group	No. of alleles	A	G	χ^2	P-value
Control	304	234 (77.0)	70 (23.0)	2.229	0.135
Lung cancer	300	215 (71.7)	85 (28.3)		

GSTP1, glutathione *S*-transferase π 1.

Table III. Distribution of GSTP1 genotypes in the control and lung cancer groups.

		Genotypes of GSTP1, n (%)				
Group	n	A/A	A/G	G/G	χ^2	P-value
Control	152	86 (56.6)	62 (40.8)	4 (2.6)	3.004	0.223
Lung cancer	150	74 (49.3)	67 (44.7)	9 (6.0)		

GSTP1, glutathione *S*-transferase π 1.

Table IV. Actual and expected numbers of GSTP1 genotypes in the control group.

	Genotypes of GSTP1				
Item	A/A	A/G	G/G	χ^2	P-value
Expected	90	54	8	1.976	0.372
Actual	86	62	4		

GSTP1, glutathione *S*-transferase π 1.

Table V. Correlation between genetic polymorphisms of GSTP1 and susceptibility to lung cancer and histology of lung cancer.

		Genotypes of GSTP1, n (%)				
Group	n	A/A	Non-A/A	OR[a]	95% CI	P-value
Control	152	86 (56.6)	66 (43.4)	1.00		
Lung cancer	150	74 (49.3)	76 (50.7)	1.43	0.890-2.306	0.138
SQ	70	27 (38.6)	43 (61.4)	2.31	1.180-4.512	0.015
AD	61	34 (55.7)	27 (44.3)	1.12	0.579-2.180	0.730
Others	19	9 (47.4)	10 (52.6)	1.71	0.636-4.617	0.286

[a]adjusted by gender, age and smoking status. GSTP1, glutathione *S*-transferase π 1; OR, odds ratio; CI, confidence interval; SQ, squamous cell carcinoma; AD, adenocarcinoma.

Comparison with the control group suggested that the non-A/A genotype was significantly associated with an increased risk of lung squamous cell carcinoma. The risk of squamous cell lung carcinoma in subjects with the non-A/A genotype was 2.31-fold higher than that in those with the A/A genotype (95% CI: 1.180-4.512, P=0.015). This correlation was not observed in adenocarcinoma or other types of lung cancer (Table V).

Correlation between GSTP1 polymorphisms and lung cancer risk in populations with different smoking statuses. The lung cancer and control groups were stratified by smoking

Table VI. Correlation between genetic polymorphisms of GSTP1 and susceptibility to lung cancer according to smoking status.

Item	Control, n (%)	Lung cancer, n (%)	OR[a]	95% CI	P-value
Total subjects	152	150			
Non-smokers	100	52			0.102
A/A	55 (55.0)	25 (48.1)	1.00		
Non-A/A	45 (45.0)	27 (51.9)	1.85	0.884-3.878	
Ex-smokers	4	12			0.756
A/A	1 (25.0)	4 (33.3)	1.00		
Non-A/A	3 (75.0)	8 (66.7)	0.67	0.051-8.639	
Current smokers	48	86			0.415
A/A	30 (62.5)	45 (52.3)	1.00		
Non-A/A	18 (37.5)	41 (47.7)	1.36	0.649-2.853	
PY<30	33	41			0.347
A/A	22 (66.7)	31 (52.1)	1.00		
Non-A/A	11 (33.3)	18 (43.9)	1.61	0.598-4.318	
PY≥30	15	45			0.766
A/A	8 (53.3)	22 (48.9)	1.00		
Non-A/A	7 (46.7)	23 (51.1)	1.19	0.371-3.852	

[a]Adjusted by gender and age. GSTP1, glutathione *S*-transferase π 1; OR, odds ratio; CI, confidence interval; PY, pack-year.

status and intensity and analyzed. In terms of smoking status, GSTP1 polymorphisms were found to have no correlation with susceptibility to lung cancer in never, former or current smokers (all P>0.05). In terms of smoking intensity, GSTP1 polymorphisms were found to have no association with susceptibility to lung cancer in mild and heavy smokers (Table VI).

Discussion

The GSTP1 gene is located on chromosome 11q13 and consists of 7 exons and 6 introns, with a length of ~2.8 kb. Board *et al* (5) isolated cDNA clones of the human GSTP1 gene in 1989, and were the first to report the polymorphisms of the human GSTP1 gene in exons 5 (Ile^{105}→Val) and 6 (Ala^{114}→Val). Exon 5 polymorphism is mutation from A to G at position 1578 of exon 5 of the GSTP1 gene, turning isoleucine (Ile) into valine (Val) at the 105th amino acid at the protein level. This segment is important for the biological function of proteins. The substitution of the 105th amino acid decreases the thermal stability and catalytic activity of the enzyme (2). GSTP1 exon 5 polymorphism has two alleles, A and G, and has three genotypes, that is, wild-type homozygote A/A (Ile/Ile), mutant heterozygote A/G (Ile/Val) and mutant homozygote G/G (Val/Val). Exon 6 polymorphism is mutation from C to T at position 2293 of exon 6 of the GSTP1 gene, turning alanine (Ala) into valine (Val) at the 313th amino acid at the protein level. This segment has no significant impact on the biological function of the protein. The substitution of the 313th amino acid does not affect the thermal stability and catalytic activity of the enzyme (2). GSTP1 exon 6 polymorphism also has two alleles, C and T, and has three genotypes, that is, wild-type homozygote C/C (Ala/Ala), heterozygote C/T (Ala/Val) and mutant homozygote T/T (Val/Val). In the present study, the exon 6 GSTP1 gene polymorphism and its correlation with susceptibility to lung cancer were not investigated in light of the facts that: i) Exon 6 polymorphism does not affect the activity of GSTP1; ii) the majority of studies have found the exon 6 polymorphism to have no association with the risk of lung cancer (6,7); iii) the mutant T allele was not found to be present in exon 6 in a study on GSTP1 polymorphism in the Japanese population (8); and iv) Zhang *et al* also did not find the mutant T allele in exon 6 in a population from Shanghai (9).

A number of studies have reported the distribution of GSTP1 exon 5 polymorphisms in different populations (4,10-12). The results of the present study indicated that the mutant G allele of the GSTP1 gene occurred at a frequency of 23.0% in a Chinese population, and that the A/A, A/G, and G/G genotypes of the GSTP1 gene occurred at frequencies of 56.6, 40.8 and 2.6%, respectively. The frequencies of the GSTP1 exon 5 polymorphism genotypes and the mutant T allele in populations from different regions reported by relevant studies are listed in Table VII. As shown in Table VII, the mutant G allele is most frequently observed in African Americans (42%), and occurs least frequently in Chinese (23%); while the frequency has been found to be 33-36% in European Americans, British and other Caucasians, and 26% in a Japanese population (4,10-12). Although these samples may not be truly representative of their respective race, these results at least suggest the difference in the distribution of GSTP1 alleles and genotypes among Chinese, African Americans and Europeans.

Human GSTs detoxify toxins by catalyzing the binding of toxins to glutathione, thus protecting intracellular macromolecules from injury induced by cytotoxins and carcinogens (13). The main human GSTs are GSTA, GSTM, GSTT and GSTP (or α, μ, θ and π in the old nomenclature). GSTP1 is an important member of the phase II metabolic enzymes GSTs, which

Table VII. GSTP1 genotypes and allele frequencies in different populations.

Population	n	Genotypes of GSTP1, n (%)			G allele	Study
		A/A	A/G	G/G		
Chinese	151	86 (56.6)	62 (40.8)	4 (2.6)	0.23	Present study
Japanese	50	26 (52.0)	22 (44.0)	2 (4.0)	0.26	Ishii (8)
African American	137	48 (35.0)	63 (46.0)	26 (19.0)	0.42	Watson (9)
European American	287	119 (42.0)	147 (51.0)	21 (7.0)	0.33	Watson (9)
English	151	64 (42.4)	74 (49.0)	13 (8.6)	0.33	Lewis (10)[a]
Caucasian	151	61 (40.4)	72 (47.7)	18 (11.9)	0.36	Wenzlaff (11)

[a]Adjusted by gender and age. GSTP1, glutathione *S*-transferase π 1.

are exogenous compounds, and is the only identified member of the GSTP family so far. GST genes, including the GSTP1 gene, have polymorphisms, and the enzymatic activity varies among different genotypes. The GSTP1 gene is located on human chromosome 11q13, and is widely expressed in human epithelial tissues, such as lung, kidney, and prostate, but is not expressed or expressed at low levels in the liver (14). GSTP1 is expressed at the highest level in the lung tissue among all GST isozymes, accounting for 83% of all GST isozymes (15). Therefore, GSTP1 plays an important role in the detoxification of inhaled carcinogens, such as activated polycyclic aromatic hydrocarbons (PAHs), benzo(a)pyrene and tobacco carcinogens (16). It has even been suggested to be the most important GST influencing lung cancer risk (17). The GSTP1 gene has polymorphisms in exon 5. Watson *et al* (4) reported the association between GSTP1 activity and GSTP1 exon 5 polymorphisms in 1998, and determined the GSTP1 activity and GSTP1 exon 5 polymorphism genotypes in lung tissue of 34 subjects. Their results indicated that GSTP1 activity was significantly higher in subjects with the A/A genotype than in those with the A/G or G/G genotype (P=0.014 and P=0.05, respectively), demonstrating that the presence of the mutant G allele in exon 5 significantly reduced the enzyme activity. Related studies have also confirmed this finding. It has been demonstrated that GSTP1 exon 5 Val allele decreases the GSTP activity, and that higher levels of hydrophobic DNA adducts are present in the lung tissue in individuals with low activity alleles (17), in addition to increased levels of PAH-DNA adducts in peripheral blood lymphocytes (18). The GSTP1 exon 5 polymorphisms change the structure, function and expression level of GSTP1, alter the body's ability to detoxify carcinogens, and affect individual susceptibility to lung cancer.

Previous studies have demonstrated that GSTP1 polymorphisms are associated with many tumors, such as rectal cancer, bladder cancer, prostate cancer, head and neck cancer, and breast cancer. Although GSTP1 is expressed at a very high level in human lung tissue, the correlation between GSTP1 polymorphisms and lung cancer risk has not yet been widely investigated. Case-control studies found GSTP1 exon 5 polymorphisms to be associated with lung cancer risk (2). However, other research results suggested that GSTP1 polymorphisms were not statistically associated with lung cancer risk (15,17). In the present study, the non-A/A genotype of GSTP1 occurred at frequencies of 43.4 and 50.7% in the control and lung cancer groups, respectively. Although a higher frequency of non-A/A genotype was observed in the lung cancer group than in the control group, there was no significant difference between the two groups (P=0.138). This indicates that GSTP1 exon 5 polymorphisms were not significantly associated with lung cancer susceptibility. The stratified analysis by histological type of lung cancer revealed a significant difference in the frequency of the susceptible GSTP1 genotype, non-A/A, between the control group and lung squamous cell carcinoma group; and that subjects with the GSTP1 mutant allele had a higher risk of lung squamous cell carcinoma (OR=2.31, 95% CI: 1.180-4.512, P=0.015), which is consistent with the findings of Ryberg *et al* (3). As for lung adenocarcinoma, GSTP1 exon 5 polymorphisms were not found to be associated with it in both a previous study (1) and the present study. It is known that lung squamous cell carcinoma mostly originates from the large bronchi, while lung adenocarcinoma mostly originates from peripheral lung tissue. A higher expression level of GSTs has been observed in bronchial epithelium than in terminal small airway epithelium (19). The distribution characteristics of GSTs in the lung account for the association of GSTP1 polymorphisms with lung squamous cell carcinoma, and its non-association with lung adenocarcinoma. Furthermore, stratified analysis by smoking status and intensity in the lung cancer and control groups indicated non-association of GSTP1 exon 5 polymorphisms with lung cancer risk in nonsmokers and smokers, and in mild and heavy smokers. This result may be explained by the findings of Yang and Xian (20), that the GSTP1 levels were not associated with gender, age or smoking status, and that certain GSTP1 inducers, such as PAHs and diet may influence the expression of GSTP1.

In summary, the present study has demonstrated that GSTP1 exon 5 polymorphisms are associated with lung cancer susceptibility. However, stratified analysis suggested the correlation of GSTP1 exon 5 polymorphisms with lung squamous cell carcinoma risk, and that exon 5 polymorphisms might increase the risk of lung squamous cell carcinoma. These results suggest that exon 5 GSTP1 polymorphisms are not a strong influencing factor in lung cancer risk, but may have a certain effect. These are only preliminary findings, which can be evaluated in future in-depth studies; for example, the

correlation between GSTP1 polymorphisms and lung cancer susceptibility can be investigated by measuring DNA adduct levels on the basis of the present study.

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