RESEARCH ARTICLE

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The Relation between Polymorphisms in Exon 5 and Exon 6 of *GSTP1* Gene and the Risk of Lung Cancer in Iranian People

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Abstract

Objective: The *GSTP1* gene, which is located on chromosome 11q13, consists of 7 exons and 6 introns. There are two polymorphisms in *GSTP1* that have been exposed to a transposition for codon 105 (Ile/Val) and 114 (Ala/Val) in exons 5 and 6, which have been studied previously in relation to lung cancer. Since the level of *GSTP1* expression in lung tissues and other human epithelial tissues is high, *GSTP1Val-105* polymorphism is recognized as a sensitive factor for tobacco-related cancers, especially lung cancer. **Methods:** One hundred and twenty tissue block samples of patients with lung cancers and 120 peripheral blood samples of the control group were obtained from two referral cancer centers in Tehran, Iran, from 2011 to 2016. Genomic DNA was extracted from tissue blocks and buffy coat of study cases to detect SNP of *GSTP1* gene using Tetra-primer ARMS-PCR. **Results:** There was a notable correlation between the incidence of lung cancer and variant Val105 (P-value=0.001; OR=2/6; 95% CI=1.49-4.53) and Ile105 (P-value=0.003; OR=0.41; 95% CI=0.23-0.73). The odds ratio for lung cancer in the homozygous Ile105/Ile105 genotype was 3.56 times higher than that of individual with heterozygous Ile105/Val105 (P-value<0.001; OR=3/56; 95% CI=1.826-6.934) genotype, that was statistically significant. Furthermore, the results showed that there was no significant correlation between Ala114/Val114 genotypes and lung cancer. The BC (P-value=0.007; OR=0.16; 95% CI=0.04-0.61) and AA (P=0.001) genotypes were statistically significant (P-value <0.05); and for those who had AA genotype, the odds ratio was almost six times higher than those with BC genotype. **Conclusions:** The study of *GSTP1* polymorphisms indicated that unlike the polymorphism in exon 5, the *GSTP1* exon 6 polymorphism correlated with the lung cancer risk in the select group of Iranian people. Likewise, the potential use of this genetic polymorphism as a lung cancer predictor is confirmed.

Keywords: Glutathione S-Transferase pi- Lung Neoplasms- Single-Nucleotide Polymorphism (SNP)- ARMS-PCR

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Introduction

Environmental contamination with polycyclic aromatic hydrocarbons (PAHs) has become one of the major health concerns. It has been distinguished as a major cause of life threating diseases such as lung cancer. Studying the world's population exposed to PAHs inhalation has indicated that those who were exposed to these bioavailable sources were more susceptible to lung cancer than others (Rengarajan et al., 2015; Abdel-Shafy and Mansour, 2016).

As a developing country, Iran is profoundly exposed to environmental pollution caused by PAHs. Studies in Tehran (Karyab et al., 2013; Naddafi et al., 2017) and other large cities of Iran (Lübeck et al., 2016; Goudarzi et al., 2017) have confirmed that PAHs, which are abundant in smoke, contaminated air, and smoke from fossil fuels, are environmental pollutants. The human body has two protective mechanisms (i.e., the first and the second phases of antioxidant defense) to protect against these threatening compounds. In the first phase, the antioxidant defense is performed by the cytochrome P450 (CYP) enzymes. Furthermore, the defense in the second phase is done by the glutathione S-transferase (GSTs) enzymes and through conjugation of PAHs with glutathione GSH, which is the first stage of the mercapturic acid synthesis reaction that can prevent the damage in macromolecules (Mota et al., 2015; Peddireddy et al., 2016). GSTs (EC 2.5.1.18) have been identified as a superfamily of dopaminergic enzymes, which combine endogenous products derived from oxidative stress and electrophilic xenobiotics such as carcinogens, pesticides, drugs and bio-contaminating

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materials targeted (Hayes et al., 2005).

Several studies investigated the polymorphism of this family of genes confirm that the combination of *GSTT1*, *GSTM1*, and *GSTP1* genes increases the susceptibility to Glaucoma (Kazemi Safa et al., 2014; Safa et al., 2015) and various cancers such as lung (Sharma et al., 2015; Adibhesami et al., 2018), bladder (Yu et al., 2016), colorectal (Hezova et al., 2012), and head/neck cancers (Russo et al., 2013). *GSTπ*, also known as *GSTP1*, belongs to GSTs family with high expression in the lung, and it is positioned on chromosome 11q13. It is 2.8 kilobytes and consists of 7 exons and 6 introns (Sies, 1999; Hammond et al., 2001). There are two single-nucleotide polymorphisms (SNPs) in *GSTP1* derived from amino acid substitutions, which are replaced by Ile105 to Val105 in exon 5 and in codon 114 as substitution of Ala114 to Val114 in exon 6 in codon 105, contributing to lung cancer (Ketterer et al., 1992; Zatorska et al., 2003). SNPs changes in *GSTP1* have been found in four different alleles, including *GSTP1*A*, *GSTP1*B*, *GSTP1*C*, and *GSTP1*D* ones (Board et al., 1989).

The most abundant *GSTP1*A* allele is wild type of this allele, which appears from the substitution of Adenine (A) with Guanine (G) in the nucleotide of the 313+ alleles *(Ile105-Val114) GSTP1*B* and *GSTP1*C (Val105-Ala114)*, and displacement of Cytosine (C) by Thymine (T) in the nucleotide position of the *341+ GSTP1*D* type allele (*Ile105-Val114*) (Hemmingsen et al., 2001; Sharma et al., 2014).

Although there are various studies on the frequency of *GSTP1* genotype and its relation to lung cancer, this research tried to address this issue in developing countries such as Iran with increasing cumulative hazard of PAHs due to air pollution in many of its large cities. These findings can expand the horizon of our knowledge concerning the role of *GSTP1* polymorphism through studying *105 (Ile/ Val)* and *114 (Ala/ Val)* codon transposition in exon 5 and exon 6 and evaluating the risk of lung cancer in a selected group of Iranian people.

Materials and Methods

Patients and Tissue Specimens

In this study, 120 samples of paraffin tissue blocks (FFPE) were prepared from two centers of National Bank of Iran in Imam Khomeini Hospital and Dr. Masih Daneshvari Hospital located in Tehran, Iran. Histopathologically being confirmed by at least two pathologists, the tissue block samples consisted of various types of lung cancer including non-small cell lung cancer (NSCLC), lung squamous cell carcinoma (LSCC), and lung adenocarcinoma (LAC). Moreover, there were other lung cancers, namely large cell carcinoma (LCC), mesothelioma, and bronchial carcinoids, which have been addressed in Table 2.

Regarding the clinical data which were obtained from the medical history of patients, therapeutic regimen had not been prescribed for any of the patients in the study. Exclusion criteria included suffering from acute or chronic inflammatory diseases in the last six months or having any other malignant cancers. In addition, 120 blood samples of healthy people without any metabolic or chronic disease were considered as the control group. Verbal or written informed consent was obtained from each of the participants before entering the study. The Ethics Committee of Lorestan University of Medical Sciences reviewed and approved the study protocol (Lums. rec.1394.1). This study was administered in accordance with the Declaration of Helsinki and its consequent revisions (Carlson et al., 2004).

DNA Extraction and PCR Methodology

The DNA extraction of tumorous and normal lung tissue samples, and the DNA extraction of the control group were performed based on the GeneAll® kit and the Cinna Gene DNA extraction kit protocol , respectively. The ARMS-PCR method was used for the diagnosis of *Ile105Val* in the *GSTP1* gene and recognition of techno-specific SNPs (Sharma et al., 2014). The primary ARMS-PCR was used in order to describe the *Ala114→Val114* genotype (Table 1) .

Two PCRs were performed for each DNA sample. PCRs were performed in 25µl reaction volume containing 50–200 ng of 1 mM genomic DNA, 0.5 mM $MgCl_2$, 8 mM H_2O, 12.5 µM of PCR Master Mix each (Thermo Fisher Scientific), and forward primer and reverse primers A or B (1.5 µM each). PCR was performed with initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 62°C for 1 min, elongation at 72°C for 2 min, and a final extension at 72°C for 7 min. A 998 bp fragment was amplified.

After PCR, the product was electrophoresed on 1% gel and the PCR products were observed with *Ala114* and Val114 bands, which were kept inoculated by the *Alw26I* enzyme at 37°C for 16 hours and then electrophoresed on 2% gel (Figures 1 and 2) .

The results of the initial PCR determined the site of SNP position 114 of two alleles *Ala114* and *Val114*, and the end of the Alw26I enzyme after PCR product revealed SNP position 105 of two *Val105* and *Ile105* alleles . Results on the observed fracture PCR products electrophoresis with 4 bands at positions 73, 260, 322, and 343, the single-nucleotide mutant defines the Ile105, the probability of each of the (*GSTP1*A or GSTP1*D*) alleles and in the presence of 5 bands at positions 73, 93, 250, 260, and 322 pairs represent the single-nucleotide mutation Val105, which was the probability of each of the (*GSTP1*B or GSTP1*C*) alleles. In the 6-band observation at positions 73, 93, 250, 260, 322, and 343, the pair showed the status of the heterozygote *Ile105/Val105*.

Statistical Analysis

Data analyses were performed using SPSS (version 16; SPSS Inc., Chicago). Analyzing the appropriate ratios and indices and appropriate dispersion, Chi-square and independent t-test and multivariate logistic regression were used. Data were expressed as the mean ± standard deviation (SD) and the differences were considered statistically significant if p-values were <0.05. This study was conducted using Hardy-Weinberg equilibrium that in this population showed no significant association with $P > 0.05$.

Results

The demographic information of patients and that of the control group have been indicated in Table 2. The results revealed that unlike gender (P-value =0.19), two groups differed significantly in terms of mean age (P-value =0.013) (Table 2) .

GSTP1 Ala/Val114 genotype and allele frequency have been indicated in Table 3. For those with *Ala114/Ala114* genotype, the odds ratio of lung cancer was 34% higher than that for the control group with *Ala114/Val114* heterozygote genotype (P-value =0.515; OR=1/34; 95% CI=0.556-3.22), although it was not statistically significant. The results also showed that for those who had the *Ala114/Ala114* genotype, the odds ratio for lung cancer was 65% higher than that for control group with homozygous *Val114/Val114* genotype (P-value =0.089; OR=0.61; 95% CI=0.34-1.08) (Table 3).

The odds ratio of lung cancer for variant *Val105* allele was 2.6 higher than that for the control group (P-value =0.001; OR=2/6; 95% CI=1.49-4.53). The frequency of an Ile105 allele in lung cancer was approximately 2.45-fold higher than that for control group (P-value =0.003; OR=0.41; 95% CI=0.23-0.73) (Table 4) .

Those presenting the *Ile105/Val105* genotype with lung cancer had 3.56 times higher odds than the control group with heterozygous *Ile105/Val105* genotype (P-value <0.001; OR=3/56; 95% CI=1.83-6.93). Individuals with the genotype *Ile105/Ile105* with lung cancer showed 1.75 times higher odds than the control group with heterozygous *Val105/Val105* genotype (P-value =0.86; OR=1.75; 95% CI=0.92-3.34), that was not statistically significant (Table 5).

The *GSTP1 BC* genotype was statistically significant considering its p-value (0.007); and for those who had AA genotype, the odds ratio was almost six times higher than that of their *GSTP1 BC* genotype (P-value =0.007; OR=0.16; 95% CI=0.04-0.61). The *GSTP1 AA* genotype was statistically significant regarding p-value (0.001) (Table 6).

In the next step, we used the multivariate logistic regression model to evaluate the effects of *GSTP1* genotype on lung cancer while adjusting age and sex (Table 7). Assuming that other variables are stable, the odds of having *Val105-* allele was 2.22 times higher than those with *Val105+* allele, which was statistically significant (P-value=0.015; OR=2.22; 95% CI=1.16-4.23). Furthermore, the odds of lung cancer in women were 2 times higher than that of men. This increase was statistically significant (P-value =0.02, OR=0.5; 95% CI=0.28-0.9). Considering the effect of age and sex using logistic regression model, there was no significant difference in odds ratios of Ile105 with the risk of lung cancer (Table 7).

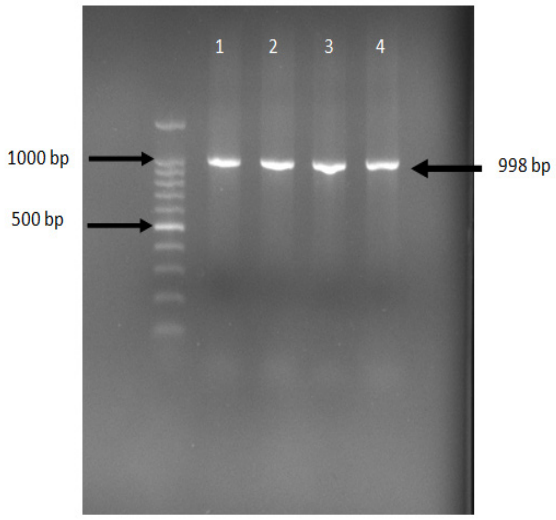

Figure 1. Electrophoresis of the GSTP1 Genotype Ala114 and Val114. Lines 1 and 2 are associated with genotype Ala114 and lines 3 and 4 correlated to genotype Val114 that are observed in all specimens

Discussion

GSTP1 plays an important role in the detoxification process , which protecting cells form inhaled carcinogens such as benzo(a)pyrene and PAHs. The results of the present study indicated a significant difference in the frequency of *GSTP1 Ile/Val* genotypes in exon 5; whereas, there was no significant difference in *GSTP1 Ala/Val* genotypes in exon 6 with the risk of lung cancer. Moreover, we found that *GSTP1 BC* and *AA* increased in lung cancer. Studying *GSTP1* polymorphism also showed a significant

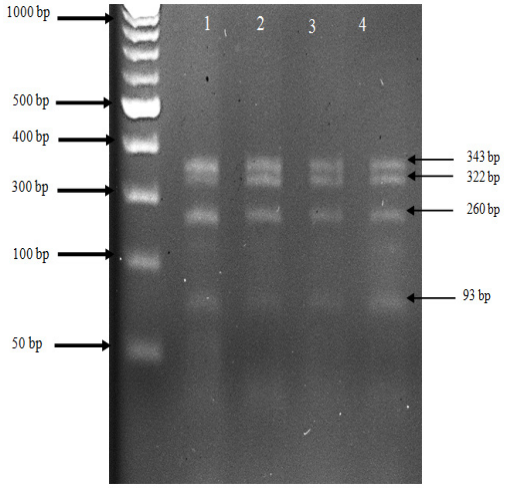

Figure 2. Electrophoresis Results after Incubation with the Alw26I Enzyme. In the presence of 4 bands at positions 73, 260, 322, and 343 pairs the single-nucleotide mutant defines the Ile105, the probability of each of the (GSTP1*A or GSTP1*D) alleles (Lines 1, 2, 3, and 4).

Table 1. Primer Sequences

Polymorphism	Primer	Annealing	Product PCR	Reference
GSTP1 forward	5'- ACC CCA GGG CTC TAT GGG AA-3'		998 bp	(Sharma et al., 2014)
GSTP Ala reverse	5'- TCA CAT CAT CCT TGC CGG-3'	62 C		
GSTP Val reverse	5'- TCA CAT CAT CCT TGC CGA-3'	60C		

Table 2. Demographic Data of the Study Population

Study group	Control group	Lung cancer group	P-value
Number of subjects	120	120	-
Sex			
Men, n (%)	90 (75.0%)	72 (60.0%)	0.019
Women, n (%)	30 (25.0%)	48 (40.0%)	
Lung Cancer Type			
Non-Small Cell Lung Cancer (NSCLC)		19 (15.8%)	
Lung Adenocarcinoma (LAC)		39 (32.5%)	
Lung Squamous Cell Carcinoma (LSCC)		36 (30%)	
Other Types		26 (21.7%)	
Age (Mean year ± SD)	55.2167 ± 12.63660	54.4583 ± 9.98730	0.13

Table 3. Genotype Distribution and Risk Lung Cancer Associated with *GSTP1 Ala/Val114* genotype and Allele Frequency

Genotype	Control Group	Lung Cancer Group	OR	95% CI	P-Value
Ala114/Ala114	11 (9.2%)	19 (15.8%)	Reference	-	0.071
Ala114/Val114	78 (65%)	61 (50.8%)	1.34	0.556-3.22	0.515
Val114/Val114	31 (25.8%)	40 (33.3%)	0.61	0.34-1.08	0.089
Total	120 (100%)	120 (100%)	-		

Table 4. Genotype Distribution and Risk Lung Cancer Associated with *GSTP1 Ile105* and *Val105* Allele Frequency

Allele	Control group (n=120)	Lung cancer group (n=120)	OR	95% CI	P-value
Val105			2.6	1.49-4.53	0.001
Present, n (%)	92 (76.7%)	67 (55.8%)			
n (%)	28 (23.3%)	53 (44.2%)			
Ile105			0.41	0.23-0.73	0.003
Present, n (%)	73 (60.8%)	95 (79.2%)			
n (%)	47 (39.2%)	25 (20.8%)			

relationship between *GSTP1 Ile/Val* genotype and lung cancer in exon 5; while, no significant relation was found between *GSTP1 Ala/Val* genotypes with exon 6 and lung cancer. Considering the different types of alleles, this polymorphism in *GSTP1 BC* and *AA* showed a significant relation with lung cancer.

Epidemiological studies have confirmed that those who express different types of *GSTP1* alleles are less susceptible to various types of carcinogenic agents (Josephy, 2010; Andreoli and Sprovieri, 2017). Recent studies have reported the relation between *GSTP1 Val105* polymorphism and other types of cancers, including prostate (Wei et al., 2013), breast (Louie et al., 2016), and bladder cancers (Yu et al., 2016).

We found no relationship between the homozygote genotype Ala114/Ala114 (P-value=0.071), *Ala114/Val114* (P-value =0.515), and *Val114/Val114* (P-value =0.089) and the risk of lung cancer. In a nutshell, no significant difference was observed in *GSTP1 Ala /Val* genotype in exon 6 with the risk of lung cancer. On the other hand, a significant correlation between SNP genotype exon 6 *Ala114/Val114* and the risk of lung cancer was reported previously (Yan et al., 2016). Our findings; however, were in line with those reported by Wang et al., (2003), Lakhdar et al.,(2010), and Yang et al., (2004).

In this study, those who had the Val105 allele with lung cancer had a 2.6-fold higher odds than healthy individuals, which was statistically significant (P-value=0.001). Furthermore, there was a significant correlation between the frequency of the alleles *Ile105* (P-value=0.003) in lung cancer compared to the control group.

The lowest proportion of the *Val105* allele exists in a

Table 5. Genotype Distribution and Risk Lung Cancer Associated with *GSTP1 Ile/Val105* Genotype Frequency

Genotype	Control Group	Lung Cancer Group	OR	95% CI	P-Value
Ile105/Ile105	28 (23.3%)	53 (42.2%)	Reference	-	0.001
Ile105/Val105	45 (37.5%)	42 (35%)	3.56	1.83-6.93	< 0.001
Val105/Val105	47 (39.2%)	25 (20.8%)	1.75	0.92-3.34	0.86
Total	120 (100%)	120 (100%)	-		

Table 6. Genotype Distribution and Risk Lung Cancer Associated with *GSTP1* Genotype and Allele Frequency

Allele	Control Group	Lung Cancer Group	OR	95% CI	P-Value
AA	5 (4.2%)	8 (6.7%)	Reference	-	0.001
AB	5 (4.2%)	5 (4.2%)	0.63	0.12-3.32	0.56
AC	19 (15.8%)	20 (16.7%)	0.66	0.18-2.37	0.52
AD	20 (16.7%)	27 (22.5%)	0.84	0.24-2.97	0.79
BB	2 (1.7%)	6 (5%)	1.88	0.27-13.2	0.53
BC	38 (31.7%)	10 (8.3%)	0.16	0.04-0.61	0.007
BD	1 (0.8%)	3 (2.5%)	1.88	0.15-23.37	0.63
CC	7 (5.8%)	9 (7.5%)	0.81	0.18-3.57	0.77
CD	20 (16.7%)	13 (10.8%)	0.41	0.11-1.52	0.18
DD	3 (2.5%)	19 (15.8%)	3.96	0.76-20.67	0.103
Total	120 (100%)	120 (100%)	-		

Table 7. The Effect of *Val 105* Allele, *Ile105* Allele, Adjusting Age and Sex Genotypes, on Lung Cancer Using Multivariate Logistic Regression Model

Variable	Reference	OR	95% CI	P-Value
Age		0.99	0.97-1.018	0.609
Sex				
Men	Reference	0.5	0.28-0.9	0.02
Women				
Val105 +	Reference	2.22	1.16-4.23	0.015
Val105 -				
Ile105 +	Reference	0.61	0.31-1.19	0.15
Ile105 -				

number of Asians in the Caucasus. The complete deletion of homozygous mutations of the *Val105/Val105* genotype was also stated in a Japanese study (Kiyohara et al., 2002; Bull et al., 2009). Like this study, Garte et al., found that the Val/Val genotype was seldom showdown and there were less than 5% among the Caucasus (Garte et al., 2001).

Moreover, for those who had the heterozygous *Val105/Val105* genotype (CI=0.92-3.34), considering the P-value of 0.86, this increase did not statistically affect the risk of lung cancer. Individuals with *Val105/Val105* polymorphism genotype of hepatocellular cancer had the chance for a better life (Li et al., 2012). However, this genotype is barely related to essential basal cell epitheliums (Ramachandran et al., 2000) and breast cancer (Zhang et al., 2011).

In this study, the results indicated that the Ile105/Ile105 homozygote genotype implied a statistically significant effect (P-value=0.001) for the risk of lung cancer. In addition, we observed a significant relation between *GSTP1 Ile/Val* genotype in exon 5 and the risk of lung cancer. Moreover, those with the heterozygous *Ile105/Val105* genotype, the odds ratio for lung cancer was 3.36 times higher than that for the control group in this study. Another study revealed that the genotype of the *Ile105/Ile105* and *Ile105/Val105* genotypes, which have a certain variance in Asians but not in Africans, Europeans, and Indians, were presented (Mishra et al., 2004; Polimanti et al., 2011). The stability of this form of the *GSTP1* enzyme is 2-3 times less than that of the *Ile105* polymorphism form (Hengstler et al., 1998) and may be due to high level of additional DNA combinations (Ramachandran et al., 2000). In another study by Li et al., (2015) in China, a significant correlation between *GSTP1* polymorphism and lung cancer in exon 5 in the Ile105/*Val105* and *Val105/Val105* genotypes was reported. In a study carried out by Uddin et al., (2014) all three *GSTP1 Ile105Val* genotypes were recognized as risk factors for lung cancer.

We found that *GSTP1 BC* (P=0.007) decreased in patients with lung cancer. In another study conducted by Anja Hemmingsen et al. with a similar method concerning the link between *GSTP1* alleles and asthma phenotypes, the researchers designated that ARMS assay, atopic asthmatic and Nonatopic non-asthmatic persons had the *GSTP1 BD*, *CD*, or *DD* genotypes. *GSTP1 BC* was significantly related to the reduction of the risk for atopy (P-value=0.031) (Hammond et al., 2001).

Abdel-Alim et al., (2007) studied fifty asthmatic children and identified *GSTP1* genotypes indicated that *GSTP1 BB* accounts for practically a threefold lower risk of asthma than did *GSTP1 AA*. Anita Sharma et al., (2014) examined the occurrences of two polymorphisms in exon 5 and exon 6 of *GSTP1* gene in 500 healthy individuals since Delhi was investigated by PCR-RFLP.

This study suffers from some restrictions, including the fact that the evaluation of *GSTP1* polymorphism has been accompanied with other cancer-associated GST polymorphisms. With regard to the impact of these polymorphisms, they can be evaluated either alone or in combination with other polymorphisms. Regarding the effect of PAHs compounds on lung cancer, a geographical assessment of the presence of this substance in target population should be considered. This polymorphism can be studied in other ways, i.e. measuring the expression of selected genes, measuring its performance against an antioxidant agent, and comparing various cancer-causing compounds.

In conclusion, investigating two gene polymorphisms related to *GSTP1*, This study confirmed that there was no significant relation between the risk of lung cancer and selected SNPs in exon 5; wherein, there was a significant relation between lung cancer and selected alleles, including *Ile105* and *Val105* as well as *Ile105/Val105* and *Ile105/Ile105* genotypes. Considering all the diverse

alleles of *GSTP1*, the *GSTP1 * B* allele only displayed a significant relation with lung cancer, which is probably due to the influence of SNP on reduction of the activity of this enzyme.

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Statement conflict of Interest

All authors of this article have no conflict of interest with regard to the present research and its results.

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