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ORIGINAL ARTICLE

Correlation of GSTP1 gene variants of male Iraqi waterpipe (Hookah) tobacco smokers and the risk of lung cancer

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Abstract
Glutathione-*S*-transferases (GSTs) play a role in the detoxification of environmental chemicals and mutagens, such as those inhaled during tobacco smoking. There have been conflicting reports concerning GST polymorphisms as risk factors in the development of lung cancer. No studies focused on Arab populations exposed to Waterpipe (WP) tobacco smoke have been undertaken. Here Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) and gene sequencing were applied to analyze allelic variations in *GSTP1*-rs1695 and -rs1138272 amongst 123 lung cancer patients and 129 controls. The data suggest that WP smoking raised the risk of lung cancer more than three-fold (OR 3.6; 95% CI 2.1–6.0; $p < 0.0001$). However, there was no significant association between individual *GSTP1* polymorphisms and the risk of lung cancer. In contrast, analysis of the rs1695 and rs1138272 combination suggested that the risk of lung cancer was raised more than two-fold for carriers of the *GSTP1*-rs1695 (G) allele (OR 2.5; 95% CI 1.0–6.4; $p < 0.05$), however, the presence of the *GSTP1*-rs1138272 (T) allele, in addition to *GSTP1*-rs1695, did not significantly change the risk ratio (OR 2.8; 95% CI 1.4–5.7; $p < 0.004$). WP tobacco smokers who carried the *GSTP1*-rs1695, but not *GSTP1*-rs1138272, allele were similarly susceptible to lung cancer (OR 2.4; 95% CI 1.1–5.3; $p < 0.03$). Hence, the results suggest that smoking WP tobacco and carrying *GSTP1*-rs1695 polymorphisms are risk factors for lung cancer in Arab Iraqi males.

Keywords Lung cancer · Glutathione-*S*-transferase · *GSTP1* · Waterpipe tobacco · rs1695 · rs1138272

Introduction

In 2018, lung cancer accounted for 18.4% of global cancer deaths and tobacco smoking is the most important risk factor for cancers [1, 2]. Polycyclic aromatic hydrocarbons (PAHs) are one of the main carcinogens present in tobacco smoke. PAHs can form covalent adducts with DNA, causing mutations that can trigger cancer if they affect the functions of tumor suppressor genes [3].

Waterpipe (WP) smoking is a type of tobacco smoking that has been prevalent in the Middle East, parts of Asia, and Africa for centuries. Since the 1990s WP usage has increased in western countries [4]. WP tobacco smoking is associated with prolonged exposure to tobacco smoke; one session of WP smoking may last for one hour [5]. This means that WP smokers are exposed to mainstream smoke and to second-hand (sidestream) smoke exhaled by nearby smokers or emitted from used charcoal for extended periods. Indeed, urine sample analysis of long-term WP smokers revealed that their exposure to nitrosamines is ~ tenfold greater than that of cigarette smokers [6]. Several other carcinogens, such as benzene, volatile aldehydes, heavy metals (lead, chromium, arsenic), nitric oxide, as well as PAHs present additional risks [7]. Biomarker studies of toxicants in blood and urine revealed that WP smokers absorb high doses of these carcinogens [8].

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Glutathione *S*-transferase (GST) superfamily members are enzymes that operate in Phase II metabolism and are crucial in detoxification processes. They are small proteins (200 to 250 amino acid residues) that are expressed upon exposure to a variety of toxins and/or in response to oxidative damage [9]. GSTs catalyze the conjugation of many environmental carcinogens to the reduced form of glutathione, permitting conversion to less toxic and excretable products. Based on their biochemical properties, human GSTs are classified into seven families, the four main classes are; α, μ, π, and θ. Each family has multiple members, except GSTπ, which consists of a single gene *GSTP1*. *GSTP1* expression in the lung epithelium and lymphocytes is greater than other genes of the GST family [10, 11]. GSTπ has a complex influence on the development of respiratory diseases, playing essential roles as an anti-apoptotic and anti-oxidative agent, in addition to its detoxification role [12].

Single nucleotide polymorphisms (SNPs) may modulate gene expression or change the structure–function characteristics of proteins [13, 14]. SNPs can increase the risks of developing cancer if they occur in genes required for carcinogen detoxification, cell cycle regulation, or DNA mismatch repair systems [13, 14]. Understanding the relationships between SNPs and cancer susceptibility has the potential to shed light on cancer pathogenesis. SNPs in promoters can alter gene expression by dysregulating the binding of transcription factors, DNA methylation processes, or histone modifications [15]– [17]. In introns, SNPs may lead to splice variants, or they may impair or enhance the binding activity of long non-coding RNAs (IncRNAs). Suppression of gene transcription and translation can also occur if the SNPs are present in exons [18, 19]. SNPs in 5′- and 3′-UTRs can alter translation, and microRNA binding respectively, and even remotely located SNPs can affect transcription of specific genes via long-range *cis* influences [20–22].

Two common SNPs in *GSTP1* were found to alter the enzyme's activity; rs1695 is an A1578G substitution located in the exon 5 that leads to an exchange of isoleucine to valine (Ile105Val) in GSTπ. This variant decreases the GSTπ activity and is associated with the presence of high levels of hydrophobic PAH-DNA adducts in lung tissue and lymphocytes [10, 23, 24]. Another SNP, rs1138272, is a C2508T transition located in exon 6, resulting in an exchange of alanine to valine (Ala114Val) in GSTπ. Previous studies on the correlation between these polymorphisms and developing lung cancer produced conflicting data [25]– [32]. A single study to investigate the association between genetic polymorphisms of phase I and phase II metabolism genes, WP smoking, and consumption of salted tea as risk factors for esophageal cancer was conducted in Kashmir (India) [33]. However, to the best of our knowledge, no study has been performed to investigate genetic polymorphisms in exons 5 and 6 of *GSTP1*, WP smoking and lung cancer.

Materials and methods

Study subjects

This study involved 123 lung cancer patients and 129 controls of the Middle Euphrates region of Iraq. Patient diagnosis was done at the Middle Euphrates Cancer Center (MECC) in Najaf city between December 2017 and June 2019. All study subjects were male, amongst whom were smokers who previously smoked WP tobacco > 5 times a week. Clinical laboratory examination and histopathological or cytological laboratory tests of tumor biopsies were done by specialists for diagnosis of lung cancer. Smoking and medical history of patients were carefully checked. Written informed consent was obtained from all study subjects, and the study was ethically approved by the committee of Faculty of Science at the University of Kufa. This study excluded all subjects who smoked both cigarette and WP tobacco. Healthy volunteers were recruited from the Mahdi Al-Attar clinic (Najaf province), which serves a population close to cafes providing WP tobacco smoking services. The control group consisted of individuals who did not have a previous diagnosis of any benign or malignant tumors. Controls were matched with patients based on age, ethnicity, and WP smoking habits. Only individuals born in Iraq and of Arab ethnicity were selected. Other Iraqi ethnicities such as Kurds, Chald-Assyrians, Turkmen, Afro Iraqis, Yazidis, and Shabaks were not enrolled. Participants were required to complete a questionnaire, and based on the responses a follow up interview/information session was undertaken at which a peripheral blood sample was drawn into a heparinized tube.

Genetic polymorphisms determination

Peripheral blood samples (5 ml) were collected from study subjects for genomic DNA extraction using DNA Mini kit (Qiagen). *GSTP1* alleles and genotypes were determined using Polymerase Chain Reaction combined with Restriction Fragment Length Polymorphism (PCR-RFLP). The DNA primers, restriction enzymes, and the length of digested and undigested amplicons based on gene polymorphisms are provided in Table 1.

The sequence of exons 5 and 6 of *GSTP1*, positions and type of SNPs, primer annealing sites and sequences recognized by restriction enzymes are shown in Figure S1. Q5 DNA polymerase was used for DNA amplification according to the manufacturer's instructions (NEB). The total reaction volume was 20 μl, where each PCR tube contained: genomic DNA (> 50 ng), 2X Q5 master mix (10 μl), forward and reverse primers (0.2 μM of

Table 1 DNA primers and restriction enzymes used for RFLP-PCR assay to determine the GSTP1 polymorphisms

Gene (SNPs)	Primers (5′ to 3′)	Restriction enzyme	Alleles (resulted fragment)
GSTP1 (rs1695)	Sense: GAGACCTCACCCTGTACCAGTC	BsmAI	A = Digested fragment (285 and 338 bp)
	Antisense: GTCAGCCCAAGCCACCTGAG		G = Digested fragment (285, 222, and 116 bp)
GSTP1 (rs1138272)	Sense: GGAGCAAGCAGAGGAGAATCTGG	Acil	C = Digested fragment (115 and 172 bp)
	Antisense: GGCTCACACCTGTGTCCATCTG		T = Intact fragment (287 bp)

each) and nuclease-free water. The annealing temperature of the primers was adjusted to 58 °C. The procedure of DNA digestion by restriction enzymes was according to the manufacturer's instructions (NEB). Resolving digested DNA amplicons was achieved on 1% agarose gel containing GelRed stain (Biotium) (Fig. 1). Gel Extraction kits (Qiagen) were used to extract the intact and digested amplicons, and the isolated DNA fragments were sequenced (Macrogen) to verify the PCR-RFLP results.

Statistical analysis

Baseline characteristics of age comparison between controls and lung cancer patients were initially performed using Pearson's Chi-square (χ^2) test. To evaluate the strength of correlation between WP smoking, gene polymorphisms, and lung cancer risk, conditional logistic regression was carried out to calculate the odds ratio (ORs) with 95% confidence intervals (CIs). Mantel–Haenszel method was used to estimate the univariate ORs with 95% CIs. Hardy–Weinberg equilibrium test was used for determining the genotype frequencies in both control and patient groups, the significance of any deviation among the observed and expected

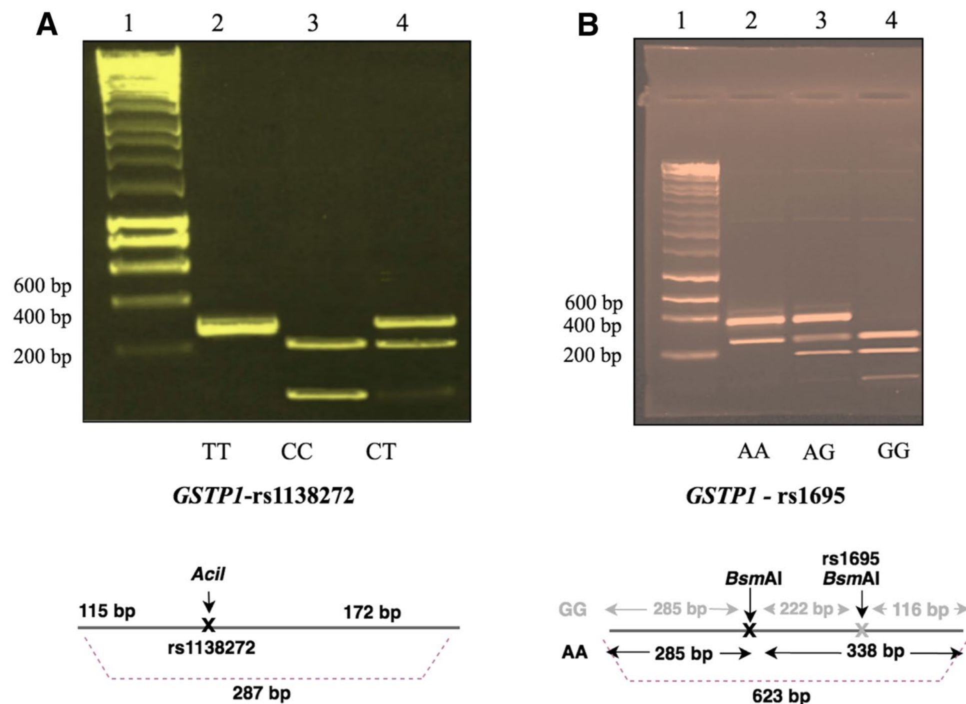

Fig. 1 PCR–RFLP assay to determine the *GSTP1* SNPs in exon 5 and 6. The amplicons of *GSTP1* exon 6 (287 bp) were digested with *Aci*II. The cleavage site is present in the wild type (C) allele generating two fragments (115 and 172 bp), unlike the variant (T) allele, which gives a single species due to the abolition of the *Aci*II recognition sequence. In **a** lane 1, DNA marker; lanes 2, 3, and 4 are homozygous minor (TT), homozygous major (CC), and heterozygote (CT), respectively of *GSTP1*-rs1138272. **b** The amplicon of *GSTP1* (623 bp) that contains the rs1695 SNP is digested with *Bsm*AI generates two fragments (285 and 338 bp) in the case of the wild type (A) allele and three fragments (285, 222, and 116 bp) in the case of the variant (G) allele. Lane 1, DNA marker; lanes 2, 3, and 4 are homozygous major (AA), heterozygote (AG), and homozygous major (GG), respectively. The diagram beneath each electrophoresis panel describes the cleavage positions of each variant by the aforementioned restriction endonucleases

frequencies was calculated according to the Chi-square test. The SPSS software (Version 23.0) was used for all analysis. All statistical tests were accomplished as two-sided analysis with p value < 0.05 as an indicator level of the significance. SNP-SNP interaction was evaluated to reveal the correlation between *GSTP1* polymorphisms and the risk of lung cancer. The genotypes of *GSTP1*-rs1695 and -rs1138272 were combined and classified into four classes; no risk alleles (CC: AA) for both *GSTP1* polymorphisms, no risk allele for the *GSTP1*-rs1138272 and any risk allele for the *GSTP1*-rs1695 (CC: AG + GG), any risk allele for the *GSTP1*-rs1138272 and no risk allele for *GSTP1*-rs1695 (CT + TT: AA), and the presence of two risk alleles (CT + TT: AG + GG). The alleles T and G were categorized as the principal risk alleles of *GSTP1*-rs1138272 and *GSTP1*-rs1695, respectively.

Table 2 Demographic characterizations of study subjects

	Case (n = 123)	Control (n = 129)	p*
Age group			0.48
≤ 50 years	75 (61.1)	73 (56.6)	
> 50 years	48 (38.9)	56 (43.4)	
Gender			
Male	123 (100)	129 (100)	
WP smoking[a]			< 0.001
Non-smoker	37 (30.1)	78 (60.5)	
Smoker	86 (69.9)	51 (39.5)	
Histology			
Adenocarcinoma	58 (47.1)		
Squamous cell carcinoma	53 (43.1)		
Other	12 (9.8)		

*Chi-squared p value

[a]OR 3.6; 95% CI 2.1–6.0; with the reference of non-smoker

Results

Demographic and clinical characteristics of lung cancer patients and controls

Baseline characterization indicated that no significant age difference was present between the two groups (cancer cases and controls); however, there was a significant difference regarding smoking status. This study did not include women, as the smoking of WP is not popular among women in the Iraqi population. Histological diagnosis revealed that adenocarcinoma (AD) and squamous cell carcinoma (SCC) were the dominant lung cancer types among patients with frequencies of 47.1% and 43.1% respectively (Table 2).

Distribution analysis of GSTP1 polymorphisms in cases and controls

The distribution of alleles and genotypes of both *GSTP1*-rs1695 and -rs1138272 were determined by using the Hardy–Weinberg equilibrium test. The frequencies of A wild type (wt) and G variant (vt) alleles of *GSTP1*-rs1695 were 73.98% and 26.02% in patients and 79.84% and 20.16% in controls, respectively. Similarly, the frequency of the wild type (C) allele of *GSTP1*-rs1138272 was higher than the variant (T) allele for both patients (79.67% vs 20.33%) and controls (84.11% vs 15.89%). However, there was no

Table 3 Distribution of *GSTP1* polymorphisms among cases and controls

	Case (n = 123) n (%)	Control (n = 129) n (%)	OR (95% CI)	p*
GSTP1-rs1695				
AA (Ile/Ile)	74 (60.16)	89 (69)	Reference	
AG (Ile/Val)	34 (27.64)	28 (21.70)	1.46 (0.81–2.63)	0.21
GG (Val/Val)	15 (12.20)	12 (9.30)	1.50 (0.66–3.41)	0.33
AG + GG	49 (39.84)	40 (31)	1.47 (0.88–2.48)	0.14
A carrier	182 (73.98)	206 (79.84)	Reference	
G carrier	64 (26.02)	52 (20.16)	1.39 (0.91–2.11)	0.12
GSTP1-rs1138272				
CC (Ala/Ala)	80 (65.04)	96 (74.42)	Reference	
CT (Ala/Val)	36 (29.27)	25 (19.38)	1.73 (0.96–3.12)	0.07
TT (Val/Val)	7 (5.69)	8 (6.20)	1.05 (0.36–3.02)	0.97
CT + TT	43 (34.96)	33 (25.58)	1.56 (0.91–2.69)	0.11
C carrier	196 (79.67)	217 (84.11)	Reference	
T carrier	50 (20.33)	41 (15.89)	1.35 (0.86–2.13)	0.19

*Chi-squared p value

statistical difference between patients and controls for both *GSTP1* SNPs (Table 3).

The distribution frequency of wt/vt + vt/vt genotypes in the patient group was higher than in the control group for both *GSTP1* SNPs, but the difference was not statistically significant (Table 3).

Polymorphisms of GSTP1 stratified by histological types

Adenocarcinoma and SCC were the main histological types in the patient group. However, no association was found between the occurrence of *GSTP1*-rs1695 and -rs1138272 variants and the histological typing (Table 4).

Association of SNP-SNP interaction with the risk of lung cancer

Results of SNP-SNP interaction indicated that the presence of any risk allele of *GSTP1*-rs1695 is correlated with the risk of lung cancer regardless of the presence of the risk allele of *GSTP1*-rs1138272. The risk of developing cancer was OR 2.54, 95% CI 1.00–6.42, $p < 0.05$ for CC:AG + GG carriers, however, a highly significant association was found for carriers of both *GSTP1* risk alleles (OR 2.80, 95% CI 1.39–5.67, $p = 0.004$) (Table 5).

Interaction of GSTP1 polymorphisms and WP tobacco smoking in the risk of lung cancer

The distribution frequency of the *GSTP1*-rs1695 allele among WP smokers was more than two-fold higher than the smokers who were carriers of wild-type (AA) genotypes (OR 2.38, 95% CI 1.07–5.27, $p = 0.03$). Moreover, WP smokers who were the carriers of the variant genotype had more than a six-fold increased risk of developing lung cancer in comparison with the non- smokers who carried wild-type genotypes. The distribution of the *GSTP1*-rs1138272 allele among WP smokers was higher than in non-smokers (43.02% vs 32.43%), however, no significant difference was found when comparing WP smokers in the patient and control groups ($p = 0.18$). Furthermore, amongst the non-smokers, there was no significant difference in the occurrence of variant genotypes of both *GSTP1* polymorphisms in patient and control groups compared to wt genotype (Table 6).

Discussion

Cigarette tobacco smoking is the leading cause of lung cancer, yet despite this little is known of the relationship between WP tobacco smoking and lung cancer. However, WP smokers were found to be exposed to some mutagens,

Table 4 Distribution analysis of *GSTP1* polymorphisms among lung cancer patients according to the histological types of cancer

	Control (n = 129)	AD[a] (n = 58)	OR (95% CI)	p*	SCC[b] (n = 53)	OR (95% CI)	p*	Other (n = 12)	OR (95% CI)	p*
	n (%)	n (%)			n (%)			n (%)		
GSTP1-rs1695				0.48			0.43			0.65
AA (Ile/Ile)	89 (69)	35 (60.34)	Reference		32 (60.38)	Reference		7 (58.33)	Reference	
AG (Ile/Val)	28 (21.70)	17 (29.31)	1.54 (0.75–3.17)		13 (24.53)	1.29 (0.60- 2.79)		4 (33.33)	1.82 (0.50–6.66)	
GG (Val/Val)	12 (9.30)	6 (10.34)	1.27 (0.44- 3.65)		8 (15.09)	1.85 (0.69- 4.95)		1 (8.33)	1.06 (0.12–9.38)	
GSTP1-rs1138272				0.24			0.36			0.73†
CC (Ala/Ala)	96 (74.42)	38 (65.52)	Reference		34 (64.15)	Reference		9 (75.0)	Reference	
CT (Ala/Val)	25 (19.38)	18 (31.03)	1.82 (0.89–3.71)		15 (28.30)	1.69 (0.80- 3.59)		3 (25.0)	1.28 (0.32- 5.08)	
TT (Val/Val)	8 (6.20)	3 (5.17)	0.95 (0.24–3.76)		4 (7.55)	1.41 (0.40-4.98)		0	–	

*Chi-squared p value, †Logistic regression p value

[a] Adenocarcinoma

[b] Squamous cell carcinoma

Table 5 Analysis of interaction between *GSTP1*-rs1138272 and rs1695 polymorphisms in risk of lung cancer

GSTP1-rs1138272	*GSTP1*-rs1695	Ca (n:123)/Co (n:129) n (%)/n (%)	OR (95% CI)	*p*
0	0	60 (48.78)/87 (67.44)	Reference	
0	1	14 (11.38)/8 (6.20)	2.54 (1.00- 6.42)	**0.049**
1	0	20 (16.26)/19 (14.73)	1.53 (0.75–3.10)	0.24
1	1	29 (23.58)/15 (11.63)	2.80 (1.39- 5.67)	**0.004**

Values in bold indicate statistically significant results

0:0 = CC: AA, 0:1 = CC: AG + GG, 1:0 = CT + TT: AA, 1:1 = CT + TT: AG + GG

such as PAHs, at higher levels than those experienced by cigarette smokers [7]. In a meta-analysis study, it was estimated that a session of WP smoking exposes individuals to nicotine equivalent to 2 cigarettes, CO equivalent to 11 cigarettes, and tar equivalent to 25 cigarettes [34].

Genes encoding GSTs, including *GSTP1*, have numerous polymorphic loci, suggesting that these polymorphisms could impair the ability to detoxify carcinogens leading to susceptibility to cancer [35]. *GSTP1* is expressed in human epithelial tissues and is the dominant GST isoform of the respiratory system [36]. Therefore, an association analysis between *GSTP1* allelic variants and the risk of developing lung cancer could be of value in establishing *GSTP1* polymorphisms as lung cancer risk factors. Previous studies indicated that SNPs of *GSTP1* particularly rs1695 and rs1138272 impair GSTπ activity. Reduction of GSTπ activity could lead to lower carcinogen detoxification and increase the risk of cancer [27, 37].

This study suggests that the *GSTP1*-rs1695 variant is possibly associated with the risk of lung cancer, and that a synergistic interaction with variant genotypes of *GSTP1* in exon 6 may increase the risk of lung cancer. The correlation analysis of WP smoking and *GSTP1* polymorphisms revealed that WP smokers who are carriers of *GSTP1*-rs1695 have a significantly higher tendency to develop lung cancer than WP smokers who have a wt *GSTP1*. Our findings are consistent with those reported by Ryberg et al. [23] who showed a strong association between the *GSTP1*-rs1695 variant and the risk of lung cancer among Norwegian men. In addition, cigarette tobacco smokers who are carriers of *GSTP1*-rs1695 (GG) genotypes showed significantly higher levels of hydrophobic DNA-adducts in lung tissue than lung cancer patients with a homozygous (AA) wild-type genotype. Moreover, a significant difference was found between patients and control when a combination analysis of *GSTμ* null and the presence of any risk allele of *GSTP1* in exon 5, which was higher than other genotype combinations. Similar results were found in a study conducted among the Caucasian population of the United States, where an association was found between the combined genotypes of *GSTμ* null mutant and the *GSTP1* (GG) allele and lung cancer, with the association being higher among smokers. In contrast, aromatic/hydrophobic–DNA adducts in white blood cells were significantly higher among smokers who were carriers of *GSTμ* non-null/*GSTP1* (AA) genotype [38]. Another study indicated that African Americans carrying any risk allele of *GSTP1*-rs1695 were about three-fold more likely

Table 6 Distribution analysis of *GSTP1*-rs1138272 and -rs1695 polymorphisms and WP tobacco smoking in lung cancer risk

	Non-smokers			Smokers		
	Ca (n:37)/Co (n:78) n (%)/n (%)	OR (95% CI)	*p**	Ca (n:86)/Co (n:51) n (%)/n (%)	OR (95% CI)	*p**
GSTP1-rs1138272			0.86			0.18
CC	25 (67.57)/54 (69.23)	Reference		49 (56.98)/35 (68.63)	Reference	
CT + TT	12 (32.43)/24 (30.77)	1.08 (0.47–2.50)		37 (43.02)/16 (31.37)	1.65 (0.80–3.43)	
GSTP1-rs1695						
AA	28 (75.68)/56 (71.80)	Reference	0.66	52 (60.47)/40 (78.43)	Reference	**0.03**
AG + GG	9 (24.32)/22 (28.20)	0.82 (0.33–2.01)		34 (39.53)/11 (21.57)	2.38 (1.07–5.27)	
GSTP1-rs1695	Non-smokers	AA	28 (22.76%)[a]/56 (43.41%)[a]	Reference		
	Smokers	AG + GG	34 (27.64%)[a]/11 (8.52%)[a]	OR = 6.12, 95% CI = 2.7–14.0, P < 0.0001		

Value in bold indicate statistically significant result

*Chi-squared *p* value

[a]Calculated percentages in respect with the total number of cases (Ca) or controls (Co)

to develop lung cancer than those with wild type genotypes [39]. Increased risk of developing lung cancer was also found in the case of exposure to environmental tobacco (sidestream) smoke for those carrying the *GSTP1* (GG) genotype among the New England population of the USA [40]. Our results are inconsistent with a study conducted in a Brazilian population, which indicated no association between genetic polymorphisms of *GSTP1*-rs1695 alone or in a combination with other polymorphisms of *GST* genes and the risk of lung cancer, even when the risk factor of tobacco smoking was applied in both cases [25]. However, it is worth mentioning that the Brazilian study included both genders (34.5% women) and adjusting the results based on gender was not taken into consideration [25], while, in our study we did not include women. Gender-related differences have been described in animal models for many metabolic enzymes, including P450 family members and males have been associated with elevated concentrations of DNA adducts [41]–[43]. Several other studies among different populations regarding the *GSTP1*-rs1138272 polymorphisms as a risk factor for various cancers and even lung cancer, provide inconsistent results. For example, in the Caucasian population of the United States and the Norwegian population, the *GSTP1*-rs1138272 polymorphism was found to be associated with non-small cell lung cancer (NSCLC) [26, 27]. In addition, a meta-analysis conducted in 2013 in an Asian population, which included 28 case control studies, revealed that *GSTP1*-rs1138272 was associated with an increased risk of lung cancer [28]. Another meta-analysis included 43 studies with more than 15,000 cases and more than 17,000 controls, revealed that the *GSTP1*-rs1138272 (TT) genotype was more likely to be associated with cancers in Asian and African populations [29]. While the *GSTP1*-rs1138272 (CT) genotype was possibly related to lung cancer susceptibility in Caucasians [29]. No significant association between the *GSTP1*-rs1695: rs1138272 combinations (AA:CC), (GG:CC) and (GG:TT) and the risk of lung cancer was found in a Finnish population [30]. A study of Caucasians in the United States showed that *GSTP1*-rs1138272, but not *GSTP1*-rs1695, polymorphisms were associated with a risk of lung cancer, which was especially evident in young men and smokers [27]. In contrast, other studies revealed no association between carriers of these SNPs and the risk of lung cancer in Denmark, and even amongst Russian cigarette smokers [31, 32], which is consistent with our results.

The occurrence of tumors due to exposure to an environmental carcinogen is not well known. The presence of genetic factors determines an individual's predisposition to develop malignancy. The presence of familial clustering of specific tumors revealed the importance of genetic factors regardless of the presence of external factors [44]. This combination of environmental and genetic factors could account for some of the variation observed among different populations in spite of their exposure to the same environmental risk factor(s). Tobacco smoking is the main carcinogen that induces lung cancers, however, several heavy smokers did not develop lung cancers, clearly suggesting that the association between environmental and genetic factors can determine the tendency of specific individuals/populations to develop cancer.

In conclusion, our results suggest polymorphisms in *GSTP1* exon 5, but not in exon 6, combined with an environmental risk factor (WP tobacco smoking) are associated with higher risk of lung cancer among Arab Iraqi males. However, limitations in the sample size prevents further subgrouping, such as the distribution of *GSTP1* polymorphisms based on WP smoking in addition to the histological type of cancer. Thus, a larger study may provide further insights.

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Author contributions BKK, NNA, and IJL, carried out the experiments and analyzed data. NNA also contributed by doing the statistical analysis and designing the schematic figures of this study. BKK conceived the study, supervised the experiments, and wrote the paper. AT-K provided extra supervision.

Compliance with ethical standards

Conflict of interest None of the authors have any non-financial conflict of interest. The authors also declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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