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ORIGINAL ARTICLE

The correlation of combined *OGG1*, *CYP1A1* and *GSTP1* gene variants and risk of lung cancer of male Iraqi waterpipe tobacco smokers

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Abstract

Genetic polymorphisms of genes whose products are responsible for activities, such as xenobiotic metabolism, mutagen detoxification and DNA-repair, have been predicted to be associated with the risk of developing lung cancer (LC). The association of LC with tobacco smoking has been extensively investigated, but no studies have focused on the Arab ethnicity. Previously, we examined the association between genetic polymorphisms among Phase I and Phase II metabolism genes and the risk of LC. Here, we extend the data by examining the correlation of *OGG1* Ser326Cys combined with *CYP1A1* (Ile462Val and MspI) and *GSTP1* (Ile105Val and Ala103Val) polymorphisms with the risk of LC. Polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) and gene sequencing were carried out for genotyping the *OGG1* polymorphisms of 123 LC patients and 129 controls. No significant differences in the frequencies of *the OGG1* mutant allele between patients and controls were found. The distributions of heterozygous Ser/Cys or Cys/Cys genotypes of *OGG1* were not associated with the risk of LC either according to the histological types of LC or based on waterpipe tobacco (WP) smoking status. In contrast, the combined effect of *OGG1* variants with *CYP1A1* and *GSTP1* variants revealed a significant correlation with the *OGG1* Ser326Cys—*CYP1A1* MspI variants pairing. This association was significant ($p=0.001$) in individuals who carried homozygous or heterozygous variant type genotypes of both genes in a reference with carriers of both wild-type genotypes (wt/wt – wt/wt). The odds ratios were 2.99 (95% CI 1.67–5.36), 2.68 (95% CI 1.08–6.62), and 2.80 (95% CI 1.18–6.69) for those who carried (wt/wt – wt/vt + vt/vt), (wt/vt + vt/vt – wt/wt), and (wt/vt + vt/vt – wt/vt + vt/vt), respectively. The study suggests a limited correlation is present between carrying OGG1 Ser326Cys polymorphism and the risk of developing LC in Arab populations.

Keywords Lung cancer · 8-oxoguanine DNA glycosylase · *OGG1* · rs1052133 · *CYP1A1* · *GSTP1* · Waterpipe tobacco

Introduction

Oxidative stress is an important factor in carcinogenesis, and lung cancer (LC) is a well-known example. Several gaseous oxidants in addition to volatile particles that trigger oxidative stress are emitted from cigarette tobacco smoke [1, 2]. Waterpipe (WP) tobacco smokers, however, are exposed to remarkably higher concentrations [3, 4]. Among carcinogens that are inhaled from smoky coal are carbon monoxide (CO) and polycyclic aromatic hydrocarbons (PAHs) [5–7]. It is estimated that the level of CO sidestream emissions produced from the charcoal in a single session of WP smoking (30–90 min) is 35 times higher than the emissions produced from smoking a single cigarette and the direct CO exposure is equivalent to that produced from ten cigarettes. The total emitted level of CO in the case of WP smoking is greater than the emitted CO from smoking 1 cigarette pack per day

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[8, 9]. As with CO, emissions of PAHs from a session of WP smoking is equivalent to smoking 50 cigarettes. WP charcoal is responsible for elevating the PAHs concentration as replacing the charcoal with an electrical heater source was found to reduce the emissions dramatically [6, 10]. Oxidants threaten genome stability because of their ability to induce DNA mutations, which may influence the activity of tumor suppressor enzymes which can activate oncogenes and/or carcinogenesis [11, 12].

Single nucleotide polymorphisms (SNPs) located in genes that are required for processes such as growth, development, and differentiation, cell cycle regulation, DNA mutation repair pathways, immunity, and metabolism, are linked with the genetic predisposition of individuals to various cancers [13–15]. Understanding the mechanisms by which SNPs affect the activity of proteins is critical to understand cancer pathogenesis. SNPs may change the structure–function properties of proteins or alter gene expression [16–18]. The effect of SNPs depends on their location, which can be in coding and non-coding regions of the genome such as 5′ and 3′ UTR, promoters, introns, and exons [19–22].

DNA repair systems are required to avoid the effects of reactive oxygen species (ROS) on genome integrity [23]. Base excision repair (BER) is required to eliminate defective bases caused by oxidative stress. Through this system, the damaged base is first recognized and removed by DNA glycosylases, which leaves an abasic site (AP). Then, removal of the apurinic/apyrimidinic AP site is accomplished by AP endonuclease; the subsequent DNA ends are processed, and the resulting nucleotide gap is filled by DNA polymerase ß; where the repair process is completed by DNA ligase [23]. Among human BER protein members is 8-oxoguanine DNA glycosylase 1 (hOGG1 or OGG1) which is involved in the process of repairing DNA mutagenetic lesions. OGG1 is a small protein (345 amino acid residues) encoded by the *OGG1* gene (17638 bp) located on chromosome 3p26 [24]. In mammalian cells, OGG1 is responsible for excising 8-hydroxy guanine (8-OH-G) from dsDNA through cleaving the glycosylic bond between the sugar moiety and the modified base [23]. If the activity of OGG1 is disrupted through mutagenesis, DNA repair through BER could be affected leading to further mutations in the genome. Indeed, several SNPs located at the human *OGG1* locus (Arg46Gln, Arg154His, Arg229Gln, A7143G, and A11657G) have been reported [25–28]. One common SNP found in OGG1, C1245G resulting in Ser326Cys, rs1052133, has been widely studied and shown to have reduced activity [29]. Studying the association between OGG1 gene variants, particularly the Ser326Cys, and the risk of LC has been investigated widely [30–32], however, little is known regarding this association in Arab ethnicity populations. It is worth mentioning that WP tobacco smoking is very popular among Iraqi youth, especially in rural populations. Generally, these populations face in addition several barriers to get treatment, thus they experience a higher likelihood of LC diagnosis and death [33]. Previously, we conducted studies that highlighted the correlation of SNPs located in Phase I (CYP1A1-Ile462Val and CYP1A1-MspI) and Phase II (*GSTP1*- rs1695 and GSTP1-Ile105Val) metabolism genes of male Iraqi WP tobacco smokers and risk of LC [34, 35]. Here, we have extended the study by investigating the correlation of OGG1 Ser326Cys polymorphism individually and in combination with CYP1A1 and GSTP1 gene variants with the risk of LC.

Materials and methods

Study subjects

All subjects (123 cases and 129 controls), whose genomic DNA was analyzed by evaluation the *CYP1A1* and *GSTP1* gene variants association with LC in our previous studies [34, 35], have been studied here as well by evaluating the correlation with *OGG1* Ser326Cys gene variants. All subjects were Arab Iraqi men from the Middle Euphrates region. Other Iraqi ethnicities such as Turkmen, Kurds, Assyrians, Yazidis, Afro Iraqis, and Shabaks, who live in the middle and north regions of Iraq, were not enrolled. The experimental smoker groups contained smokers who regularly smoke WP tobacco (> 5 sessions a week), subjects who smoked cigarettes as well were excluded. LC cases were diagnosed by specialists as mentioned previously [34, 35]. Controls were healthy volunteers recruited from a clinic (Mahdi Al-Attar) located in Najaf province close to WP cafes. Controls had no any history of diagnosed malignant or benign tumors. Matching was based on ethnicity, age, and smoking habits. Controls was selected based on their responses to a questionnaire; after that, a 30 min interview was undertaken to gather detailed information. Peripheral blood samples were drawn after the interview using heparinized tubes. The scientific committee at the Faculty of Science at the University of Kufa has ethically approved the study.

Genetic polymorphisms determination

The procedures of Genomic DNA extraction, PCR–RFLP, and DNA sequencing were carried out as mentioned previously [34, 35]. DNA oligos of forward primer 5′ CACTGT CACTAGTCTCACCAGCC 3′ and reverse primer 5′ CAT CCTTAGCGCTGTCTCCCTC 3′ were used for PCR reactions. For *OGG1* genotype determination through RFLP assay, the 359 bp PCR product was cleaved using Fnu4HI restriction enzyme according to the manufacturer's instructions (NEB). When analyzed by agarose gel electrophoresis, the RFLP generates bands of 359 bp for homozygous (CC), 101 and 258 bp for homozygous (GG) and 359, 258 and

101 bp for heterozygous (GC) *OGG1* genotypes. The RFLP-PCR results of *CY1P1A, GSTP1, and OGG1* are as shown in Fig. 1. The sequence of *OGG1* exon 7, the position of rs1052133 SNP, the position of DNA sequence recognized by the Fnu4HI restriction enzyme, and the annealing sites of DNA primers are as shown in Fig. S1.

Statistical analysis

Statistical analysis of this study was carried out as mentioned previously [35].

Results

Baseline characterizations of LC patients and controls

No significant demographic or clinical differences between LC cases and controls were found, except that significantly more LC cases were observed in the WP smoking group (Fig. 2), where the ratio was 69.9% (Fig. 2a, b) [34, 35]. WP smoking is only popular among men in Iraq;

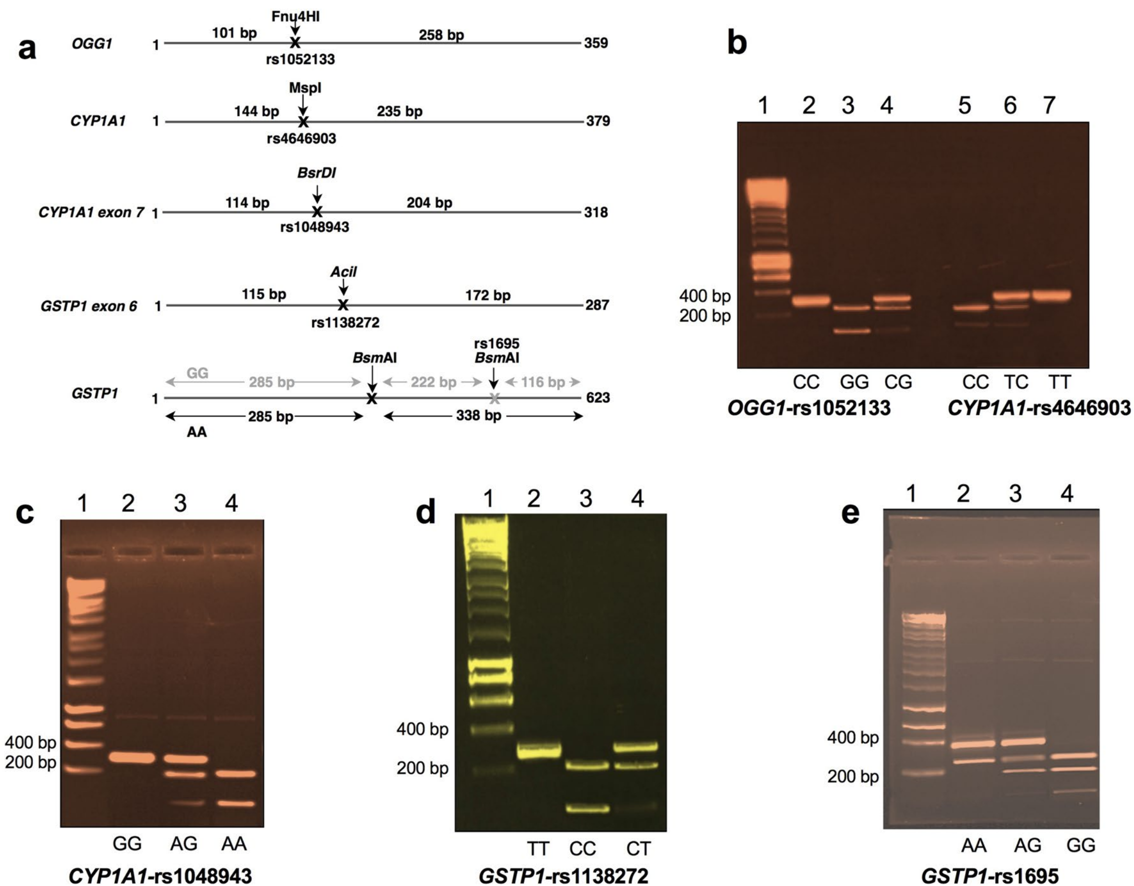

Fig. 1 Genotyping of *CYP1A1, GSTP1*, and *OGG1* by PCR–RFLP. **a** Schematic diagram shows the size of each amplified gene region containing the indicated SNPs, the restriction enzymes used for RFLP assay, and the length of cleaved fragments. **b** The PCR amplificon of *OGG1*(359 bp) and *CYP1A1 MspI* (379 bp) are digested with *Fnu4HI* and *MspI* respectively. In the case of *OGG1* C > G variant, two fragments of 101- and 258 bp are generated; *CYP1A1* T > C variant is also cleaved to two fragments (144- and 235 bp). The presence of wild type allele of both will abolish the recognition sequence and produce single fragment. Lane 1, DNA marker; lanes 2, 3, and 4, are homozygous major CC, homozygous minor GG, and heterozygote CG respectively of *OGG1- rs1052133*; lanes 5, 6, and 7 represent homozygous minor CC, heterozygote TC, and homozygous major TT respectively of *CYP1A1-rs4646903*. **c, d** Amplicons of *CYP1A1* exon 7 (318 bp) and *GSTP1* exon 6 (287 bp) are cleaved by *BsrDI* and *AciI* respectively. Cleavage site is present in *CYP1A1* wildtype*;* thus, two fragments are generated (114- and 204 bp). Similarly, *GSTP1* generates two fragments after digestion (115- and 172 bp), unlike the variant alleles of *CYP1A1* A > G and GSTP1 C > T which prevent digestion leaving the PCR amplicon intact. **c** Of *CYP1A1-rs1048943* genotyping, lane 1, DNA marker; lanes 2, 3, and 4 are homozygous minor GG, heterozygote AG, and homozygous major AA respectively. **d** Of *GSTP1-rs1138272* genotyping, lane 1, DNA marker; lanes 2, 3, and 4 are homozygous minor TT, homozygous major CC, and heterozygote CT respectively of. **e** Amplicon of GSTP1 (623 bp) with rs1695 SNP is digested with *BsmAI* generating two fragments (285- and 338 bp) when wildtype allele is present, and three fragments (285-, 222-, and 116 bp) when *GSTP1* contains the variant alleles (A > G). Lane 1, DNA marker; lanes 2, 3, and 4 are homozygous major AA, heterozygote AG, and homozygous major GG respectively

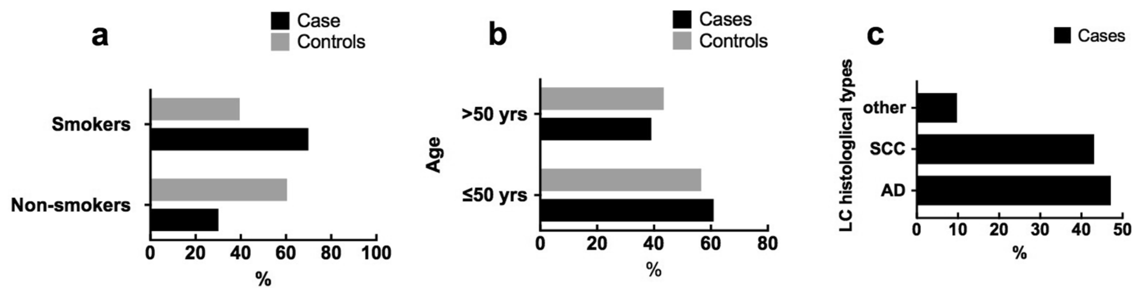

Fig. 2 Demographic and clinical characteristics of LC patients and controls. Cases are represented as black bars while the gray bars represent the controls. Individuals of each subject groups are plotted against the percentage of distribution

thus, this study did not include women. Stratifying the data based on histological types, the dominant types of LC were adenocarcinoma (AD) and squamous cell carcinoma (SCC) with ratios of 47.1% and 43.1% respectively (Fig. 2c).

Distribution analysis of *OGG1* rs1052133 SNP in cases and controls

Distribution of allelic and genotypic frequencies of the *OGG1* rs1052133 variant was analyzed using the Hardy–Weinberg equilibrium test. The frequencies of C wild type (wt) and G variant (vt) alleles were 84.96% and 15.4% in cases and 89.15 and 10.85% in controls, respectively (Table 1).

Genotypic frequency of CG + GG (wt/vt + vt/vt) in the LC patient group was higher in comparison to the control group with an odds ratio of 1.48 (95% CI 0.81–2.74), however, the statistical analysis indicated that the difference was not significant.

Distribution of *OGG1* variants based on histological types

The distribution of heterozygous (CG) and homozygous (GG) variant type genotypes of *OGG1* rs1052133 SNP in both AD and SCC were higher in LC patient group than in control group, however, no statistical association was found (Table 2).

Association of *OGG1* polymorphisms and smoking of WP tobacco in the risk of LC

Results indicated that no association was found between the *OGG1* gene variants and the risk of LC among WP tobacco smokers. The distribution frequency of *OGG1* variant type (CG + GG) genotypes was not significantly higher than the WP smokers who were carriers of wild-type (CC) genotype (OR = 1.33, 95% CI 0.58–3.02, $p = 0.05$). Furthermore, no significant difference was found among non-smokers (Table 3).

Association of gene–gene interaction with the risk of LC

A combination of variant genotypes showed a significant association between the presence of variant pairing of *OGG1* (rs1052133) + *CYP1A1* (rs4646903) and the risk of LC. The SNP-SNP interaction revealed that the carriers of any variant genotypes of *OGG1*-rs1052133 and *CYP1A1*-rs4646903 have a predisposition to developing LC three-fold higher than those who are carriers of only wild genotypes. Several other genotype combinations of variant alleles had increased

Table 1 Distribution of *OGG1*-rs1052133 polymorphism among cases and controls

	Case (n = 123) n (%)	Control (n = 129) n (%)	OR (95% CI)	*p**
OGG1-rs1052133				
CC (Ser/Ser)	93 (75.61)	106 (82.17)	Reference	
CG (Ser/Cys)	23 (18.70)	18 (13.95)	1.46 (0.74–2.86)	0.27
GG (Cys/Cys)	7 (5.69)	5 (3.88)	1.59 (0.49–5.19)	0.43
CG + GG	30 (24.39)	23 (17.83)	1.48 (0.81–2.74)	0.20
C carrier	209 (84.96)	230 (89.15)	Reference	
G carrier	37 (15.04)	28 (10.85)	1.45 (0.86–2.46)	0.16

*Chi-squared *p*-value

Table 2 Distribution analysis of *OGG1*-rs1052133 polymorphism among lung cancer patients according to the histological types of cancer

	Control (n = 129) n (%)	AD[1] (n = 58) n (%)	OR (95% CI)	*p**	SCC[2] n (%)	OR (95% CI)	*p**	Other (n = 12) n (%)	OR (95% CI)	*p*[†]
OGG1-rs1052133		087			0.12			–		
CC (Ser/Ser)	106 (82.17)	46 (79.31)	Reference		37 (69.81)	Reference		10 (83.33)	Reference	
CG (Ser/Cys)	18 (13.95)	9 (15.52)	1.15 (0.48–2.76)		12 (22.64)	1.91 (0.84–4.34)		2 (16.67)	1.18 (0.24–5.82)	0.84[†]
GG (Cys/Cys)	5 (3.88)	3 (5.17)	1.38 (0.37–6.03)		4 (7.55)	2.23 (0.57–8.75)		0	–	
CG + GG (Ser/Cys + Cys/Cys)	23 (17.83)	12 (20.69)	1.2 (0.55–2.62)	0.64	16 (30.19)	1.99 (0.95–4.18)	0.07	2 (16.67)	0.92 (0.19–4.49)	0.92

*Chi-squared *p*-value

[†]Logistic regression *p*-value

[1]Adenocarcinoma

[2]Squamous cell carcinoma

odds of developing LC; however, these combinations were not statistically significant (Table 4).

Discussion

It is well understood that individual predisposition to cancer varies regardless of the exposure to environmental risk factor(s). Therefore, genetic variation in the human genome, resulting from SNPs, maybe directly involved in carcinogenesis [13]. DNA repair genes are responsible for maintaining the integrity and stability of DNA molecules. BER repair system is critical for repairing genomic DNA lesions generated by ROS [23]. Thus, mutations in BER enzymes, such as *OGG1,* would be expected to alter the protein activity. Indeed, several SNPs in the coding region of *OGG1* have been reported, but few of them have been extensively studied for their correlation with cancer susceptibility, particularly with LC risk. Here, we conducted this study to evaluate the individual and the combined OGG1 association with CYP1A1 or GSTP1 polymorphisms and LC risk among Arab men who smoke WP tobacco. Results indicated that no statistical evidence was found for the association between OGG1 Ser326Cys polymorphisms and the occurrence of LC, neither among those who were carriers of homozygous (Cys/Cys) nor in the carriers of heterozygous (Ser/Cys) genotypes. There was also no association when the data stratified by LC histological types or according to the WP smoking status.

HOGG1 Ser326Cys polymorphism was firstly identified by Kohno et al. who indicated that this SNP reduced the OGG1 repair activity of excising 8-OH-G paired [36]. In the Japanese population, it was found that the carriers of Cys/Cys genotype were at a 2–3 times higher risk of developing LC than those who were carriers of other genotypes [37]. Similarly, a study composed of 298 LC patients among the ethnic group of Japanese, Caucasian, and Hawaiians showed that the distribution of homozygous (Cys/Cys) genotype in cases was significantly two-fold higher than homozygous (Ser/Ser) wildtype genotype. This study suggested the presence of a strong association between carrying the homozygous variant genotype of *OGG1*, but not the heterozygous (Ser/Cys) genotypes, and the risk of developing LC [38]. The risk among Caucasians was also found to be significantly raised by about two-fold in the case of carrying heterozygous (Ser/Cys) genotype, and it increased about four-fold among the carriers of (Cys/Cys) genotypes [39]. However, the main limitation of these studies is the small number of their study subjects and the ethnic heterogeneity of their population. On the other hand, when the association between urinary excretion of 8-oxodG, considered as a biomarker of oxidative stress reflecting the rate of DNA damage, and risk of LC was examined in a large population

Table 3 Distribution analysis of *OGG1*-rs1052133 polymorphism 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*
OGG1-rs1052133			0.63			0.50
CC	28 (75.68) / 66 (84.62)	Reference		65 (75.58) / 40 (78.43)	Reference	
CG + GG	9 (24.32) / 12 (15.38)	1.28 (0.46–3.58)		21 (24.42) / 11 (21.57)	1.33 (0.58–3.02)	

*Chi-squared *p*-value

Table 4 Analysis of interaction between *OGG1, CYP1A1, and GSTP1* polymorphisms in risk of lung cancer

Gene–gene interaction	Case (n = 123) n (%)	Control (n = 129) n (%)	OR (95% CI)	p*
OGG1 (rs1052133) – *CYP1A1* (rs4646903)				0.001
CC – TT	29 (23.58)	61 (47.29)	Reference	
CC – TC + CC	64 (52.03)	45 (34.88)	2.99 (1.67–5.36)	
CG + GG – TT	14 (11.38)	11 (8.53)	2.68 (1.08–6.62)	
CG + GG – TC + CC	16 (13.01)	12 (9.30)	2.80 (1.18–6.69)	
OGG1 (rs1052133) – CYP1A1 (rs1048943)				–
CC – AA	41 (33.33)	56 (43.41)	Reference	
CC – AG + GG	52 (42.28)	50 (38.76)	1.42 (0.81–2.49)	0.22[a]
CG + GG – AA	14 (11.38)	23 (17.83)	0.83 (0.38–1.81)	0.64[a]
CG + GG – AG + GG	16 (13.01)	0	–	–
OGG1 (rs1052133) – GSTP1 (rs1695)				0.30
CC – AA	58 (47.15)	76 (58.91)	Reference	
CC – AG + GG	35 (28.46)	30 (23.26)	1.53 (0.84–2.77)	
CG + GG – AA	16 (13.01)	13 (10.08)	1.61 (0.72–3.62)	
CG + GG – AG + GC	14 (11.38)	10 (7.75)	1.80 (0.76–4.43)	
OGG1 (rs1052133) – GSTP1 (rs1138272)				0.25
CC – CC	62 (50.41)	81 (62.79)	Reference	
CC – CT + TT	31 (25.20)	25 (19.38)	1.62 (0.87–3.02)	
CG + GG – CC	18 (14.63)	15 (11.63)	1.57 (0.73–3.36)	
CG + GG – CT + TT	12 (9.76)	8 (6.20)	1.96 (0.76–5.08)	

*Chi-squared *p*-value

[a]Logistic regression *p*-value

case-cohort study composed of 25,717 men and 27,972 women, who were Caucasian, results showed that no association among smokers was found. In addition, the OGG1 Ser326Cys polymorphism did not alter the excretion levels of 8-oxodG [40]. Our findings are consistent with those indicated by Li et al. [41] who showed, in a meta-analysis study included 6375 cases and 6406 controls from 17 case–control studies, that no statistical evidence of the association was found between the *OGG1* Ser326Cys variants and risk of LC neither in the recessive (Cys/Cys versus Ser/Cys + Ser/Ser) nor the dominant model (Cys/Cys + Ser/Cys versus Ser/Ser). However, when the data were analyzed based on ethnicity, significant association in the case of the dominant model was found among Asian subjects, but not among Caucasians. Results of another larger meta-analysis composed of 9663 cases and 11,348 controls from 27 publications showed similar results for both models in addition to the model of the heterozygous codominant model of Ser/Cys versus Cys/Cys genotypes. Moreover, when further stratification by ethnicity (Caucasian and Asian) was done, no significant association was found between *OGG1* Ser326Cys variants and risk of LC [30].

Several LC reports studied the association with *OGG1* variant stratified by histological types of LC, however, the direct association is still under debate. Our findings are consistent with those reported by Ito et al., and Liang et al., who found a limited association of *OGG1* Ser326Cys variants for risk of LC adenocarcinoma [42, 43]. On the contrary, Sugimura et al., who analyzed the distribution of OGG1 Ser326Cys variants among 241 cases and 197 controls in

a study conducted in Okinawa, found that the homozygous variant type genotype (Cys/Cys) raised the risk of SCC more than three-fold, but there was no statistical association in the case of AD [37]. Additionally, in disagreement with our findings, a strong association of Cyc/Cys genotype was found for SCC, and the association was also proved for AD, and small cell lung cancer (SCLC) in a case–control study conducted among Caucasian, Japanese, and Hawaii. This association was confirmed after adjusting the Odd ratios for ethnicity, gender, age, and smoking status. However, in a meta-analysis study, a significant association of *OGG1* Ser326Cys variants was found with the risk of AD, but not with other histological types such as SCC, SCLC, and large cell carcinoma (LCC) [30]. The inconsistency of the association between the *OGG1* Ser326Cys variants and the risk of LC may reflect the various prevalence of Cys allele among various ethnic populations, it seems that Cys allele among Caucasians is less prevalent than Asians.

Because high levels of 8-OH-G have been found in lung tissue and leukocytes of tobacco smokers, it has been hypothesized that a possible interaction between *OGG1* variants and LC risk exists [44]. In our study, the interaction between *OGG1* Ser326Cys variants and WP tobacco smoking in the development of LC was not detected. Our results are consistent with the results of studies conducted in the Chinese and Japanese population who indicated that *OGG1* Ser326Cys variants were not found to be risk factors of LC among cigarette smokers [42, 43]. Similarly, Loft et al., indicated that the urinary excretion of 8-oxodG was higher in smokers, however, the OGG1 Ser326Cys polymorphism did not correlate with LC [40].

Very little has been reported regarding the combined association of *OGG1* Ser326Cys variants and other gene variants with the risk of LC. An interesting study conducted among northern Thai women by Klinchid et al. [45], showed no significant association when *OGG1* variants were analyzed individually. However, significant associations were observed in the case of any combinations between *OGG1* variants genotypes and any gene variants of tumor protein p53 (*TP53*), myeloperoxidase (*MPO*), or matrix metallopeptidase 1 (*MMP1*), additionally, the combination of the null mutant of glutathione *S*-transferase Mu1 (*GSTM1*) and *OGG1* variants was also significant. To the best of our knowledge, the correlation of gene variant pairing of *OGG1* and *GSTP1* or *CYP1A1* and the risk of LC among tobacco smokers has not been examined. Our results showed that variant allele combinations of *OGG1* Ser326Cys and *CYP1A1*-MspI raised the risk of LC to three-fold, but the other variant pairing was not statistically associated with LC occurrence.

Polymorphisms in different other genes are also found to increase the risk of developing LC. Perhaps, one of the known genes is Epidermal Growth Factor Receptor (*EGFR*), which is found to be overexpressed in non-small lung cancer [46]. In normal expression, *EGFR* is required to trigger essential processes such as cell growth, development, and differentiation. However, some SNPs located at the promoter or at the exon regions of EGFR are found to alter its expression which leads to alter several signaling pathways that cause uncontrolled proliferation [47, 48]. Interestingly, *EGFR* variants are found to occur more frequently in Asian populations, the association with smoking status are also reported [47, 48]. Other somatic mutations in genes such as Mouse Double Minute 2 (MDM2) and TP53 are also common in LC patients [49, 50]. LC is a multifactorial disease, and an individual SNP may be insufficient to alter a proteins activity or be solely responsible for developing LC. Therefore, ethnic difference may overlap with the effect of other factors such as gene–gene interaction and/or gene-environment interaction.

Despite the fact of the inconsistent and controversial data produced from individual studies, which is partly because of small size and heterogenicity of subjects that may affect the statistical power required to explore the real correlation, meta-analysis can solve this problem and produce a more precise conclusion. In this work, the limitation of sample size prevents further analysis stratified by multifactor such as the distribution based on histological types of LC in addition to the smoking status and the analysis based on more than two genes association. However, no such studies have been conducted neither among Arab populations nor among Iraqis, thus, our data can be used as the preliminary data for larger future meta-analysis studies. In conclusion, this study suggests that OGG1 Ser326Cys polymorphism is not a potential risk factor for developing LC. Nevertheless, the association of *OGG1* Ser326Cys variants and other gene variants could play roles in developing the risk of LC. Larger studies may lead to producing a better comprehensive understanding of the association between SNPs and the occurrence of cancers.

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Author contributions B.K.K., N.N.A., and K.S.A., carried out the experiments and analyzed data. N.N.A. also contributed by doing the statistical analysis. B.K.K conceived the study, supervised the experiments, and wrote the manuscript. I.J.L and A.T-K provided extra supervision.

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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.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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