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LETTER TO THE EDITOR

Combinations of the Variant Genotypes of *CYP1A1*, *GSTM1* and *GSTT1* are Associated with an Increased Lung Cancer Risk in North Indian Population: a Case-Control Study

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To the Editor

Genetic variability along with smoking determines an individual's susceptibility towards lung cancer. A person's inherent detoxification system is responsible for metabolising xenobiotic compounds present in tobacco smoke. *CYP1A1* gene, belonging to the cytochrome P450 family which codes for enzyme aryl hydrocarbon hydrolase (AHH) plays an important role in Phase I biotransformation leading to the activation of pro-carcinogens. These pro-carcinogens further bind to DNA forming DNA adducts causing mutations [1]. Whereas in Phase II biotransformation mediated by Glutathione-S-Transferases (GSTs) eliminate carcinogens by rendering them water soluble by conjugation reactions [2]. Therefore the toxicity effects of carcinogens, its absorption and removal are delicately mediated by the tandem coordinated balance between the phase-I and phase-II enzymes. It is likely that genetic polymorphisms within the two xenobiotic metabolic systems might play an important role in the determining individual's susceptibility to lung cancer. Amongst the four allelic variants of *CYP1A1* gene, m1 and m2 are found to play a role in lung carcinogenesis. The m1 polymorphism in the 3′ non-coding region (3′-UTR) of the *CYP1A1* gene results in elevated induction of the enzyme, and thus, increased levels of activated intermediates. The m2 polymorphism located in heme binding region results in an increase in microsomal enzyme activity [3]. In case of phase –II detoxification both the GSTM1 and GSTT1 gene deletions in the populations render the enzymes inactivated thereby hindering the detoxification mechanism [4].

Various studies have been done so far to observe the significance of single and combined effect of genotypic variations of CYP1A1 and GST polymorphism on the development of lung cancer in Asian population particularly amongst Indian, Chinese and Japanese [5]. In pooled analysis on Asian population suggested that there was a significant association between the genotype of CYP and GST polymorphism with development of the lung cancer [5]; whereas some other studies observed no association between them. Keeping the contradictory data available so far from different and same populations the primary objective of the study was to evaluate the influence of genetic polymorphisms in genes coding for xenobiotic-metabolizing enzymes like *CYP1A1* Msp1, *CYP1A1* Ile^{462}Val, and *GSTM1* and *GSTT1* on lung cancer risk overall and on basis of histological sub-types with a large sample size. Very few studies have been conducted so far in Indian population to observe the combined effects of CYP and GST polymorphism towards susceptibility of lung cancer. Another important objective was to the test the hypothesis that whether lung cancer risk is increased in patients carrying rare combinations of phase I and phase II variant genotypes.

Peripheral blood from each of the 320 lung cancer patients and 320 controls was collected from the Department of Pulmonary Medicine, Post Graduate Institute of Medical Education and Research (PGIMER) Chandigarh and its DNA was extracted. This study has been reviewed and approved by the Institute ethics committee of PGIMER. Informed written consent was obtained from all participants and their representatives. There was no age, gender, smoking, histological or tumour node metastasis (TNM) stage restrictions. The control group of the study consisted of unrelated volunteers having no lung cancer history at the time of blood

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collection who were pair-matched for sex, age (±10 years) and smoking parameters in order to avoid any sampling bias. Genotyping of *CYP1A1 m1* and *m2* polymorphisms was carried out by PCR-RFLP technique which was earlier reported by Cascorbi et al., [6]. Similarly genotyping of *GSTM1* and *GSTT1* was done using multiplex PCR where the presence or absence of GSTM1 and GSTT1 was detected [4]. To assess the risk for lung cancer in *CYP* and *GST* as single and combine polymorphisms adjusted Odds Ratio (ORs) along with 95 % Confidence Intervals (CI) were calculated using logistic regression analysis with adjustment for possible confounders (age and pack-years of smoking as continuous variables; and gender as a nominal variable. All statistical analysis were evaluated using statistical software Medcalc 15.5.5 (Medcalc software, Ostend, Belgium) and SPSS version 20.0. (Chicago, IL, USA) software.

The allelic and genotypic frequencies of *CYP1A1 m1* and *CYP1A1 m2* were found to follow the Hardy Weinberg equilibrium. As shown in Table 1 it was observed that subjects having mutant genotype of *CYP1A1 m1* were at two fold higher risk of lung cancer which was found to be significant (OR = 2.31;95%CI = 1.2–4.3;p = 0.008). Also for SQCC histological sub-type, the association was found to be stronger and statistically significant (OR = 3.37; 95 % CI = 1.6–7.1; p = 0.001) in comparison to ADCC or SCLC. In case of *CYP1A1 m2*, patients with heterozygous genotype (*Ile*/*Val*) a 2-fold increased risk towards lung cancer was observed (OR = 1.96 95%CI = 1.3–2.8; p = 0.0004). Individuals with *GSTM1* null genotype were at a significant risk for developing lung cancer as compared to the subjects who had presence of *GSTM1* gene (OR = 1.68; 95%CI = 1.2–2.3, p = 0.001). When stratified according to histology, it was observed that subjects having *GSTM1* null genotype had risk towards ADCC (OR = 1.79; 95%CI = 1.1–1.1-2.8; p = 0.01) and SCLC (OR = 1.77; 95%CI = 1.0–3.00, p = 0.03) which was found to be statistically significant. The findings in the present study are in line with previous study conducted on an Indian population [7]. However Kumar et al. observed that *CYP1A1 m1* polymorphism show no such significance in North Indian population of Delhi [8]. Similarly, studies conducted in various other ethnic Asian populations like Chinese [9] have also confirmed an association of the *CYP1A1 m1* polymorphism with lung cancer development. The results presented in the current study are inconsistent with those reported in the study done on Caucasians [3]. Our study has also observed a strong and significant association for lung cancer susceptibility in those group of individuals having heterozygous genotype (Ile/Val) of the *CYP1A1 m2* gene (p = 0.0004), furthermore both SQCC and ADCC sub-types were also associated with this genotype. The data is consistent with other Indian studies and some other Asian studies who also have reported an association for the heterozygous genotype of *CYP1A1 m2* gene towards risk for lung and. It has been reported that the frequency of the mutant (*Val*/*Val*) genotype is highly represented in Japanese and Chinese as compared to Indian population [10]. However, Sobti et al. reported a high frequency of *m2* mutant genotype in a North Indian study as compare to our current study [11]. The present study has also revealed that absence of *GSTM1* gene might be a risk factor of acquiring lung cancer in North Indian population. Similar studies done on Chinese, Korean, Japanese and Caucasian [12] populations have shown consistent results with this study. These studies have reported 50–55 % subjects lack *GSTM1* gene in population which is in agreement with our data. However, our data is inconsistent with studies done in North-East population of India [7]. Furthermore *GSTT1* null genotype and lung cancer risk has also been studied in varied ethnicity with conflicting results. The current study is consistent with a study done in Tunisian [13] population who have also reported a higher risk for ADCC as compared to SQCC with a similar genotype. However many previous studies have reported no significant association of *GSTT1* with either ADCC OR SQCC [14]. These differences might be due to either intra or inter-ethnic differences that exist in Indian populations. Thus our results show the impact of ethnicity on the overall distribution of genotypes for the GST gene.

Furthermore analysis was conducted to elucidate whether the genotypic combinations between *CYP1A1*, *GSTM1* and *GSTT1* genes play an important role towards susceptibility for lung cancer as shown in Table 2. For the combination of *CYP1A1* and *GSTM1* taking the wild type genotype (*TT*) of *CYP1A1* gene along with presence of *GSTM1* genotype as a reference group, it was observed that subjects carrying the mutant form of the gene along with *GSTM1* null genotype had 2.47 fold increased risk for lung cancer. Furthermore when stratified on basis of histological sub-types the high risk genotypic combination of mutant *CYP1A1 m1* and null *GSTM1* gene was found to be strongly associated with SQCC (OR = 3.35;95%CI = 1.28–9.8; p = 0.01). Similarly, the combined role of *CYP1A1 m2* (*Ile*/*Vail*) and *GSTM1* genes to alter the risk for lung cancer was evaluated. It was observed that the individuals with *CYP1A1 m2* (*Ile*/*Val*) and *GSTM1 null* genotypic combination were at three fold increased risk for overall lung cancer (OR = 2.80; 95%CI = 1.6–4.8; p = 0.0003) and this risk was found to be more elevated for ADCC (OR = 3.31; 95%CI = 1.6–6.7; p = 0.001). In case of combined genotype of *CYP1A1 m1* and *GSTT1* gene, it was observed that subjects carrying the genotypic combination of mutant *CYP1A1 m1* (CC) allele and *GSTT1* null genotype were at four times risk towards lung cancer. On the other hand in case of combined genotype of *CYP1A1 m2* polymorphism with *GSTT1* gene, the presence of a copy of *CYP1A1 m2* heterozygous variant allele and null genotype of *GSTT1* showed a two-fold increased risk for overall lung cancer, however this risk was found to be significantly elevated in case of ADCC (OR = 3.95; 95%CI = 1.6–9.3; p = 0.001).

Table 1 Adjusted ORs for lung cancer overall and for the main histologic subtypes in relation to genotypes

	Controls, (%) $N = 320$	Cases, (%) $N = 320$	OR[a] (95 % CI)	*p*-value	Squamous cell Carcinoma			Adenocarcinoma			Small cell lung carcinoma		
					$N = 132$ (%)	OR[a] (95 % CI)	*p*-value	$N = 106$ (%)	OR[a] (95 % CI)	*p*-value	$N = 76$ (%)	OR[a] (95 % CI)	*p*-value
CYP1A1 m1													
TT	158 (49.4)	145 (45.3)	1.00(Ref.)	Ref.	58 (43.9)	1.00(Ref.)	Ref.	53 (50)	1.00(Ref.)	Ref.	31 (40.8)	1.00(Ref.)	Ref.
TC	144 (45)	140 (43.8)	1.07 (0.7–1.4)	0.67	54 (40.9)	1.01 (0.6–1.5)	0.93	43 (40.6)	0.89 (0.5–1.4)	0.63	40 (52.6)	1.45 (0.8–2.4)	0.18
CC	18 (5.6)	35 (10.9)	2.31 (1.2–4.3)	**0.008**	20 (15.2)	3.37 (1.6–7.1)	**0.001**	10 (9.4)	1.60 (0.6–3.8)	0.29	5 (6.6)	1.71 (0.5–5.3)	0.35
TC + CC	162 (50.6)	175 (54.7)	1.21 (0.8–1.6)	0.23	74 (56.1)	1.25 (0.8–1.9)	0.28	53 (50)	0.97 (0.6–1.5)	0.90	45 (59.2)	1.46 (0.8–2.4)	0.15
T	230	215											
C	90	105											
MAF	0.28	0.32											
CYP1A1 m2													
AA	253 (79.1)	217 (67.8)	1.00(Ref.)	Ref.	88 (66.6)	1.00(Ref.)	Ref.	68 (64.2)	1.00(Ref.)	Ref.	56 (73.7)	1.00(Ref.)	Ref.
AG	60 (18.7)	99 (30.9)	1.96 (1.3–2.8)	**0.0004**	41 (31.1)	2.10 (1.2–3.4)	**0.002**	37 (34.9)	2.19 (1.3–3.6)	**0.002**	20 (26.3)	1.71 (0.9–3.1)	0.08
GG	7 (2.2)	4 (1.3)	0.75 (0.2–2.6)	0.65	3 (2.3)	1.73 (0.4–7.5)	0.45	1 (0.9)	0.50 (0.05–4.4)	0.53	0 (0)	0.0 (0.0–0.0)	0.99
AG + GG	67 (20.9)	103 (32.2)	1.85 (1.2–2.6)	**0.001**	44 (33.4)	2.07 (1.2–3.3)	0.002	38 (35.8)	2.03 (1.2–3.3)	**0.005**	20 (26.3)	1.55 (0.8–2.8)	0.15
A	283	266											
G	37	54											
MAF	0.11	0.16											
GSTM1													
Positive	198 (61.9)	160 (50)	1.00 (Ref.)	Ref.	69 (52.3)	1.00 (Ref.)	Ref.	51 (48.1)	1.00 (Ref.)	Ref.	35 (46.1)	1.00 (Ref.)	Ref.
Null	122 (38.1)	160 (50)	1.68 (1.2–2.3)	**0.001**	63 (47.7)	1.47 (0.9–2.2)	0.07	55 (51.9)	1.79 (1.1–2.8)	**0.01**	41 (53.9)	1.77 (1.0–3.0)	0.03
GSTT1													
Positive	263 (82.2)	258 (80.6)	1.00 (Ref.)	Ref.	116 (87.9)	1.00 (Ref.)	Ref.	78 (73.6)	1.00 (Ref.)	Ref.	61 (80.3)	1.00 (Ref.)	Ref.
Null	57 (17.8)	62 (19.4)	1.18 (0.7–1.7)	0.41	16 (12.1)	0.71 (0.3–1.3)	0.29	28 (26.4)	1.76 (1.0–3.0)	0.04	15 (19.7)	1.19 (0.6–2.3)	0.61

[a] Adjusted odds ratio, 95 % confidence intervals and their corresponding *p*-values were calculated by unconditional logistic analysis after adjusting for age, gender, smoking status and histological subtypes

Bold numbers in tables show association with disease

Table 2 Analysis of double gene-gene interactions of *CYP1A1 MspI*, *CYP1A1 Ile*^{462}*Val*, *GSTM1* present/null and *GSTT1* present/null polymorphisms in lung cancer risk

CYP1A1 m1 vs *GSTM1*	Controls, (%) *N* = 166	Cases, (%) *N* = 157	OR[a] (95 % CI)	*p*-value	Squamous cell Carcinoma			Adenocarcinoma			Small cell lung carcinoma		
					N = 69 (%)	OR[a] (95 % CI)	*p*-value	*N* = 46 (%)	OR[a] (95 % CI)	*p*-value	*N* = 40 (%)	OR[a] (95 % CI)	*p*-value
TT/present	101 (60.8)	71 (45.2)	1.00 (Ref.)	Ref.	32(46.4)	1.00 (Ref.)	Ref.	22 (47.8)	1.00 (Ref.)	Ref.	15 (37.5)	1.00(Ref)	Ref.
TC/null	56 (33.8)	71 (45.2)	1.86 (1.15–2.98)	0.01	27 (39.1)	1.49 (0.8–2.78)	0.20	21 (46.6)	1.75 (0.8–3.5)	0.11	23 (57.5)	2.69 (1.2–5.7)	0.01
CC/null	9 (5.4)	15 (9.5)	2.47 (1.0–6.08)	0.04	10 (14.5)	3.56 (1.28–9.8)	0.01	3 (6.5)	1.44 (0.3–5.9)	0.61	2 (5)	1.55 (0.2–8.6)	0.61
TC + CC/null	65 (39.2)	86 (54.7)	1.95 (1.24–3.07)	0.003	37 (53.6)	1.78 (1.0–3.19)	0.04	24 (52.2)	1.71 (0.8–3.3)	0.11	25 (62.5)	2.51 (1.2–5.2)	0.01
CYP1A1 m2 vs *GSTM1*	Controls, (%) *N* = 185	Cases, (%) *N* = 149	OR[a] (95 % CI)	*p*-value	Squamous cell Carcinoma			Adenocarcinoma			Small cell lung carcinoma		
					N = 63 (%)	OR[a] (95 % CI)	*p*-value	*N* = 51 (%)	OR[a] (95 % CI)	*p*-value	*N* = 31 (%)	OR[a] (95 % CI)	*p*-value
AA/present	158 (85.4)	103 (69.1)	1.00(Ref.)	Ref.	44 (69.8)	1.00(Ref.)	Ref.	32 (62.7)	1.00(Ref.)	Ref.	23 (74.2)	1.00(Ref.)	Ref.
AG/null	23 (12.4)	44 (29.5)	3.07 (1.72–5.4)	0.0001	18 (28.6)	2.85 (1.3–5.9)	0.004	18 (35.3)	3.67 (1.7–7.7)	0.0006	8 (25.8)	2.62 (1.0–6.8)	0.04
GG/null	4 (2.2)	2 (1.4)	1.01 (0.17–5.8)	0.98	1 (1.6)	1.33 (0.1–13.6)	0.81	1 (2.0)	1.25 (0.1–12.0)	0.84	0 (0)	0.0 (0.0–0.0)	0.99
AG + GG/null	27 (14.6)	46 (30.9)	2.80 (1.6–4.8)	0.0003	19 (30.2)	2.68 (1.3–5.4)	0.006	19 (37.3)	3.31 (1.6–6.7)	0.001	8 (25.8)	2.33 (0.9–6.0)	0.07
CYP1A1 m1 vs *GSTT1*	Controls, (%) *N* = 155	Cases, (%) *N* = 151	OR[a] (95 % CI)	*p*-value	Squamous cell Carcinoma			Adenocarcinoma			Small cell lung carcinoma		
					N = 58 (%)	OR[a] (95 % CI)	*p*-value	*N* = 61 (%)	OR[a] (95 % CI)	*p*-value	*N* = 30 (%)	OR[a] (95 % CI)	*p*-value
TT/present	128 (82.5)	117 (77.5)	1.00(Ref.)	Ref.	50 (86.2)	1.00(Ref.)	Ref.	43 (70.5)	1.00(Ref.)	Ref.	23 (76.7)	1.00(Ref.)	Ref.
TC/null	24 (15.5)	25 (16.5)	1.25 (0.6–2.3)	0.48	4 (6.9)	0.50 (0.1–1.5)	0.23	14 (23.0)	1.93 (0.8–4.1)	0.09	6 (20)	1.99 (0.6–5.9)	0.21
CC/null	3 (1.9)	9 (6.0)	4.06 (1.0–15.9)	0.04	4 (6.9)	4.96 (0.9–25.6)	0.05	4 (6.5)	4.13 (0.8–20.9)	0.08	1 (3.3)	2.88 (0.1–50.6)	0.46
TC + CC/null	27 (17.4)	34 (22.5)	1.57 (0.8–2.8)	0.12	8 (13.8)	0.92 (0.3–2.2)	0.87	18 (29.5)	2.21 (1.0–4.5)	0.03	7 (23.3)	2.03 (0.7–5.9)	0.19
CYP1A1 m2 vs *GSTT1*	Controls, (%) *N* = 228	Cases, (%) *N* = 203	OR[a] (95 % CI)	*p*-value	Squamous cell Carcinoma			Adenocarcinoma			Small cell lung carcinoma		
					N = 88 (%)	OR[a] (95 % CI)	*p*-value	*N* = 64 (%)	OR[a] (95 % CI)	*p*-value	*N* = 47 (%)	OR[a] (95 % CI)	*p*-value
AA/present	212 (93.0)	179 (88.2)	1.00(Ref.)	Ref.	80 (90.9)	1.00(Ref.)	Ref.	52 (81.3)	1.00(Ref.)	Ref.	44 (93.6)	1.00(Ref.)	Ref.
AG/null	13 (5.7)	24 (11.8)	2.29 (1.1–4.6)	0.02	8 (9.1)	1.81 (0.7–4.6)	0.21	12 (18.7)	3.95 (1.6–9.3)	0.001	3 (6.4)	1.24 (0.3–4.8)	0.75
GG/null	3 (1.3)	0 (0)	0.0 (0.0–0.0)	0.99	0 (0)	0.0 (0.0–0.0)	0.99	0 (0)	0.0 (0.0–0.0)	0.99	0 (0)	0.0 (0.0–0.0)	0.99
AG + GG/null	16 (7.0)	24 (11.8)	1.93 (0.9–3.8)	0.05	8 (9.1)	1.57 (0.6–3.9)	0.33	12 (18.7)	3.30 (1.4–7.5)	0.004	3 (6.4)	1.07 (0.2–4.1)	0.91

[a] Adjusted odds ratio, 95 % confidence intervals and their corresponding *p*-values were calculated by unconditional logistic analysis after adjusting for age, gender, smoking status and histological subtypes

Table 3 Analysis of triple gene-gene interactions of *CYP1A1 MspI CYP1A1 Ile*^{462}*Val*, *GSTM1* present/null and *GSTT1* present/null polymorphisms in lung cancer risk

CYP1A1 vs *GSTM1*	Controls, (%) *N* = 113	Cases, (%) *N* = 82	OR[a] (95 % CI)	*p*-value	Squamous cell Carcinoma			Adenocarcinoma			Small cell lung carcinoma		
					N = 31 (%)	OR[a] (95 % CI)	*p*-value	*N* = 28 (%)	OR[a] (95 % CI)	*p*-value	*N* = 21 (%)	OR[a] (95 % CI)	*p*-value
0*	94 (83.2)	55 (67.1)	1.00(Ref.)	Ref.	24 (77.4)	1.00(Ref.)	Ref	16 (57.2)	1.00(Ref.)	Ref.	13 (61.9)	1.00(Ref.)	Ref.
1*	16 (14.2)	26 (31.7)	3.21 (1.5–6.6)	0.001	6 (19.4)	1.50 (0.5–4.4)	0.45	12 (42.8)	4.28 (1.6–11.0)	0.002	8 (38.1)	4.56 (1.5–13.8)	0.007
2*	3 (2.6)	1 (1.2)	0.72 (0.06–7.5)	0.78	1 (3.2)	2.03 (0.2–23.4)	0.56	0 (0)	0.0 (0.0–0.0)	0.99	0 (0)	0.0 (0.0–0.0)	0.99
3*	19 (16.8)	27 (32.9)	2.78 (1.4–5.6)	0.004	7 (22.6)	1.57 (0.6–4.3)	0.38	12 (42.8)	3.76 (1.5–9.6)	0.005	8 (38.1)	4.06 (1.3–12.2)	0.012
CYP1A1	Controls, (%)	Cases, (%)	OR[a] (95 % CI)	*p*-value	Squamous cell Carcinoma			Adenocarcinoma			Small cell lung carcinoma		
vs *GSTT1*	*N* = 131	*N* = 108			*N* = 41 (%)	OR[a] (95 % CI)	*p*-value	*N* = 41 (%)	OR[a] (95 % CI)	*p*-value	*N* = 24 (%)	OR[a] (95 % CI)	*p*-value
4#	122 (93.1)	95 (88.0)	1.00(Ref.)	Ref	39 (95.1)	1.00(Ref.)	Ref.	34 (82.9)	1.00(Ref.)	Ref.	21 (87.5)	1.00(Ref.)	Ref.
5#	8 (6.1)	13 (12.0)	2.30 (0.9–5.9)	0.08	2 (4.9)	1.12 (0.2–5.8)	0.88	7 (17.1)	3.63 (1.2–11.2)	0.02	3 (12.5)	3.22 (0.6–15.8)	0.15
6#	1 (0.8)	0 (0)	0.0 (0.0–0.0)	0.99	0 (0)	0.0 (0.0–0.0)	0.99	0 (0)	0.0 (0.0–0.0)	0.99	0 (0)	0.0 (0.0–0.0)	0.99
7#	9 (6.9)	13 (12.0)	2.11 (0.8–5.3)	0.11	2 (4.9)	1.09 (0.2–5.6)	0.91	7 (17.1)	3.24 (1.1–9.7)	0.03	3 (12.5)	3.09 (0.6–15.1)	0.16

[a] Adjusted odds ratio, 95 % confidence intervals and their corresponding *p*-values were calculated by unconditional logistic analysis after adjusting for age, gender, smoking status and histological subtypes

* 0–TT + AA/present (Wild genotype CYP1A1 *m1* & *m2* & *GSTM1 present*), 1–TC + AG (Heterozygous genotype CYP1A1 *m1* & *m2* & *GSTM1 null*), 2–CC + GG (Mutant genotype, CYP1A1 *m1* & *m2* & *GSTM1 null*), 3-TC + CC + AG + GG (Heterozygous genotype combined with mutant genotype of CYP1A1 *m1* & *m2* & *GSTM1 null*)

4–TT + AA/present (Wild genotype CYP1A1 *m1* & *m2* & *GSTT1 present*), 5–TC + AG (Heterozygous genotype CYP1A1 *m1* & *m2* & *GSTT1 null*), 6–CC + GG (Mutant genotype, CYP1A1 *m1* & *m2* & *GSTT1 null*),73-TC + CC + AG + GG (Heterozygous genotype combined with mutant genotype of CYP1A1 *m1* & *m2* & *GSTT1 null*)

Furthermore as shown in Table 3, it was evaluated whether lung cancer risk is modified by rare genotypic combinations of all the three genes i.e. *CYP1A1*, *GSTM1* and *GSTT1* when analyzed as a single genotype. It was noteworthy that the combination of *CYP1A1 m1* & *m2* heterozygous (TC + AG) along with null *GSTM1* gene (OR = 3.21, 95 % CI = 1.5–6.6, $p = 0.001$), was significantly associated with lung cancer. When stratified on basis of histological subtypes, patients having the genotypic combination as mentioned above had a four-fold high risk for ADCC and SCLC. These rare triple combinations have also been reported in South Asian population [16].

Very few studies have been conducted so far to evaluate the joint modifying effects of the *CYP1A1* and GST genes together towards susceptibility for lung cancer. Since the tandem cooperative action of both phase I and phase II enzymes are involved in the removal of chemical carcinogens, a metabolic imbalance created due to the polymorphic nature of both the pathways might lead to accumulation of carcinogens which then may bind to DNA form adducts and which might lead to mutations in either tumour suppressor genes or proto-oncogenes and hence resulting in lung carcinogenesis and cancer. It has been hypothesized that null *GSTM1* genotype deletion is a moderate susceptibility factor for lung cancer but it might become a dominant risk factor in the presence of gene-gene combinations [4]. Data from our study suggests a strong gene-gene interaction between the *CYP1A1 m2* (*Ile*^{462}*Val*) variant and *GSTM1* null genotype and this association was highly significant for ADCC ($p = 0.0006$) and SQCC ($p = 0.004$). The consistency of our result was also seen in Chilean population [15]. It has been proven that the enzyme expressed from the *Val*/*Val* type has shown to have higher enzyme activity and hence mutagenicity towards benzo (a) pyrene than that corresponding to the *Ile*/*Ile* type [15]. Thus it is plausible that individuals with the *Ile*/*Val* and null *GSTM1* genotype have the metabolic capacity to increase and/or activate pro-carcinogens into carcinogens and hence have elevated risk for lung cancer. Thus our data implies a synergy of susceptible genotypes of *CYP1A1Ile*/*Val* and *GSTM1* null gene to enhance individual susceptibility to lung cancer. Similarly we have also observed that the individuals' carrying *CYP1A1* mutant *m1* genotype and having null *GSTM1* gene were found to be significantly associated with lung cancer development. However unlike the *m2* polymorphism, subjects with such a combination were at a three-fold risk to develop SQCC ($p = 0.01$) and not ADCC. Study from North Indian population by Sobti et al. reported a 2-fold elevated risk for lung cancer in individuals with a single copy of the variant *CYP1A1* and null *GSTM1* [11].

In summary, our results suggest that the polymorphic variants in the *CYP1A1* gene along with *GSTM1* and *GSTT1* do act as a genetic modifier for lung cancer susceptibility and are strongly associated with lung cancer risk in population of

North Indians. Furthermore, the positive results in the gene-gene interactions analysis seem to indicate that these interactions play an important role with lung cancer development.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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