Glutathione S-transferase gene polymorphism and lung cancer in Indian population: a meta-analysis of case-control studies

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Among the respiratory diseases, lung cancer is one the most critical in terms of mortality. In India, more than half a million people die every year because of lung cancer. Tobacco smoke along with polymorphism of glutathione S-transferase gene is considered to be one of the most important factors for lung cancer. Here a review has been carried out to find the association of gene polymorphism and lung cancer, especially in the Indian population. This article deals with reviews on the effect of *GSTT1* and *GSTM1* polymorphism on lung cancer specifically from case-control studies of the Indian population. In the overall population, from the pooled odds ratio, a significant association of *GSTM1* (null versus present) was found with lung cancer, whereas the association of *GSTT1* (null versus present) genotype was found to be statistically insignificant. From the null test (two-tailed), *GSTM1* (null versus present genotype; *P* value = 0.035, I^2 = 40.58%) was found to be significantly associated with lung cancer. Begg's funnel plot analysis showed no publication bias for both gene polymorphisms. Thus this review shows that *GSTM1* polymorphism is a significant risk factor for lung cancer in the Indian population.

Keywords: Gene polymorphism, glutathione S-transferase, lung cancer, meta-analysis.

LUNG cancer is one of the leading causes of death due to disease worldwide. Males compared to females are more prone to death due to lung cancer. According to the World Health Organization (WHO) World Cancer Report 2014 (ref. 1), about 20% of total cancer deaths that occurred worldwide during 2012 was due to lung cancer.

The most vital factor considered for 70% of global lung cancer deaths is the use of tobacco products[1]. Along with the tobacco smoke, there are also other several factors which are directly or indirectly associated with the development of lung cancer. These include viruses, UV radiation or adverse occupational environment. The malfunctioning of the enzymes that are involved in the detoxification process of the xenobiotic compounds seems to be an important phenomenon linked to the occurrence of lung cancer due to tobacco smoke and other related exposures.

Glutathione S-transferase (GST) enzymes are the phase-II metabolic enzymes. The main function of these enzymes is to conjugate the non-polar xenobiotic compounds with the highly charged glutathione (antioxidant) to make it polar, so that those compounds can easily be excreted out from the body with the help of phase-III metabolic enzymes[2,3]. The GST genes are a superfamily arising from three different origins such as cytosolic, mitochondrial and membrane-bound microsomal[4]. The phase-I and phase-II metabolic enzymes are found to be associated with different disorders related to the lungs[4–8].

The genes coding for phase-II metabolic enzymes such *GSTT1* and *GSTM1* were found to be associated with lung cancer in different ethnic groups[9–12]. However, there are also some studies where no significant relation between the *GSTT1*/*GSTM1* and lung cancer risk was observed[13,14]. Considering the fact that using the results of a single study to draw a conclusive statistical analysis has certain limitations, a meta-analysis is needed to come to an effective conclusion. Despite India having the second largest population in the world, there is no meta-analysis report related to GST and lung cancer risk in the Indian population. Though there are only a few studies related to the respective genes reported as case- control studies on the Indian population, we have done an extensive statistical analysis to draw an effective conclusion from those studies in the form of a meta-analysis. The current meta-analysis contains 16 studies considered independently for both *GSTT1* and *GSTM1* in combination to investigate the susceptibility to lung cancer risk due to polymorphism of *GSTT1* and *GSTM1* genes[12,15–21].

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Methods

Search strategies

A comprehensive web search was performed on PubMed, *Web of Science* (*WoS*) and HuGENet databases using the terms 'GSTT1 polymorphism and lung cancer', 'GSTM1 polymorphism and lung cancer', 'lung cancer in India' and 'lung cancer case-control studies'. The criteria for selection of suitable reported studies were set as follows: (i) the study should be entirely on *GSTT1* polymorphism and lung cancer; (ii) it should be entirely on *GSTM1* polymorphism and lung cancer risk; (iii) it should include male and female subjects from the Indian population. The mere published 'abstracts and review articles' were not included in this meta-analysis. We have also gone through the cross references of suitable articles to get the entire relevant articles based on the above criteria.

Data extraction

Three of the authors of the present study independently reviewed the literature. The information extracted from the suitable reported studies was as follows: first author, publication year, Indian population, age, gender, smoking history, genotyping methods, total number of case and control subjects, and genotyping population size.

Statistical analysis

The association of *GSTT1* and *GSTM1* polymorphism with lung cancer was assessed with 95% confidence interval (CI) and odds ratio (OR). In this meta-analysis null variant was compared with wild type (null versus present). Pooled OR was estimated by both fixed-effects model as well as random-effects model. Heterogeneity was estimated through Q-test and considered statistically significant if $P < 0.05$. Publication bias was analysed using Begg's funnel plot and Egger's test. All statistical analyses were performed using comprehensive meta-analysis 2.0 (CMA) (Biostat Inc, USA) and GraphPad Prism 5 (GraphPad Software, Inc, USA) software.

Results

Characteristics of included studies

After performing a comprehensive search on PubMed, *WoS* and HuGENet, 181 research articles relevant to the search criteria were selected, out of which 165 studies were excluded for being not relevant to *GSTT1* and *GSTM1* polymorphism in the Indian population. Gene polymorphism analysis of both *GSTT1* and *GSTM1* was carried out independently in the 16 articles selected. The studies included in this meta-analysis have been performed in different geographical locations of India (Table 1).

Meta-analysis of lung cancer association with GSTT1 and GSTM1 gene polymorphism

In the present meta-analysis, after pooling all the data together, both random-effects model as well as fixed-effects model were studied depending on the significance of heterogeneity. A summary of *GSTT1* and *GSTM1* gene polymorphism with lung cancer in the Indian population is given in Figure 1.

Analysis of the overall population

Eight studies with a total population of 1347 cases and 1855 control subjects were included in the *GSTT1* polymorphism study. Similarly, eight studies with a total

Table 1. Comparison of polymorphism of glutathione S-transferase genes reported in various studies in different geographic locations of India

	Genes studied for glutathione S-transferase gene polymorphism								
	GSTM1 (null/present)		Percentage of *GSTM1* null genotype	*GSTT1* (null/present)		Percentage of *GSTT1* null genotype			
Mean age (case)	Case	Control		Case	Control		Method	Geographic location	Reference
55.50 ± 11.30	62/38	52/24	64.77	82/18	65/11	83.52	Multiplex PCR	North India	15
58.17 ± 10.95	46/100	39/107	29.11	32/114	13/133	15.41	Multiplex PCR	South India	16
42.60 ± 6.30	44/49	99/154	41.33	24/69	56/197	23.12	Multiplex PCR	North India	18
60.41 ± 10.56	66/122	113/177	37.45	33/155	73/217	22.17	Multiplex PCR	North East India	20
56.14 ± 11.91	82/136	58/180	30.70	84/134	90/148	38.16	Multiplex PCR	North India	17
56.14 ± 11.91	84/134	90/148	38.16	82/136	58/180	30.70	PCR	North India	19
59.16 ± 9.95	57/97	66/88	39.94	22/132	42/112	20.78	Real-Time PCR	North East India	12
59.02 ± 13.02	116/114	168/292	41.16	113/117	193/267	44.35	Multiplex PCR	North East India	21

*Total population = Case + control. Percentage of GSTM1 (null genotype) in overall population is 40.33. Percentage of GSTT1 (null genotype) in overall population is 34.78.
% Null genotypes of both *GSTT1* and *GSTM1* genes is lowest among South Indian population compared to the rest of India.

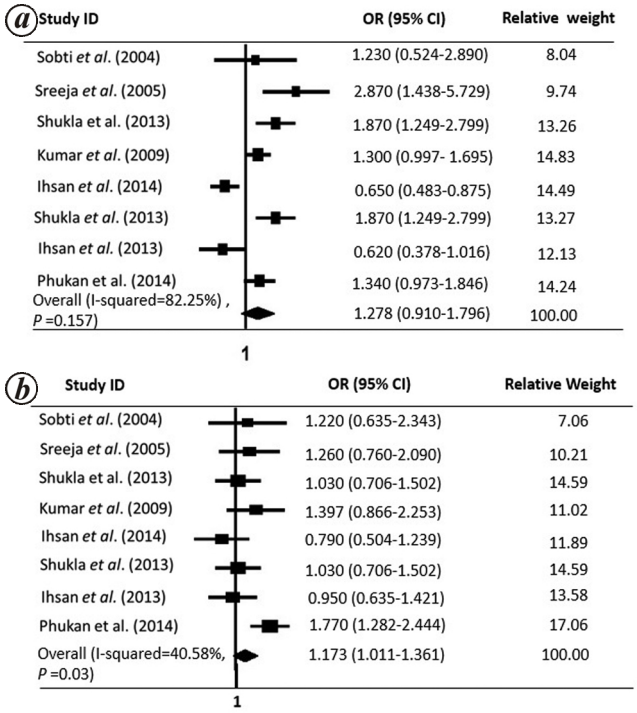

Figure 1. Comparison of odds ratio (OR) and relative weight of *GSTT1* and *GSTM1* gene polymorphism and their susceptibility to lung cancer reported in different studies. ***a***, Forest plot analysis of *GSTT1* gene polymorphism and lung cancer, where relative weights are measured by random effects analysis ($P > 0.05$). ***b***, Forest plot analysis of *GSTM1* gene polymorphism and lung cancer, where relative weights are measured by random effects analysis ($P < 0.05$). OR, Odds ratio; 95%CI, 95% confidence interval.

population of 1347 cases and 1855 control subjects were included for *GSTM1* polymorphism in this meta-analysis. The pooled results showed a significant association of *GSTM1* polymorphism (null versus present, OR = 1.173; 95% CI: 1.011–1.361; $P = 0.03$; $I^2 = 40.58\%$) with incidence of lung cancer in the Indian population. However, the *GSTT1* gene polymorphism (null versus present, OR = 1.278; 95% CI: 0.914–1.796; $P = 0.157$; $I^2 = 82.25\%$) was found to be not significantly associated with lung cancer in the Indian population.

Publication bias

Publication bias was evaluated using Begg's funnel plot and Egger's test. It can be observed from the test results shown in Figure 2 that there is no significant publication bias for both *GSTM1* ($t = 0.68$, $P = 0.52$) polymorphism as well as *GSTT1* polymorphism ($t = 0.67$, $P = 0.53$) in the published literatures.

Discussion

In this meta-analysis, an extensive study was done on the data collected from 16 studies based on *GSTT1* and *GSTM1* gene polymorphism to find their association with the occurrence of lung cancer among the Indian population. In the present meta-analysis study, a significant association of *GSTM1* (homologous deletion) polymorphism with the elevated risk of lung cancer has been observed. The study involved more than 2500 cases and control subjects to find a strong correlation of candidate genes with the risk of lung cancer. However, there was no significant association in the case of *GSTT1* (homologous deletion) gene polymorphism with elevated risk of lung cancer.

The association of GST genes with increase in the risk of lung cancer has long been studied, but the results are inconsistent. Saarikoski *et al.*[22] did not find any significant association of *GSTT1* and *GSTM1* genes with lung cancer. The results of some studies[13,14,23,24] were found to be consistent with those of Saarikoski *et al.*[22]. However, results of the present study are similar to those of Matakova *et al.*[11] and Nazar-Stewart *et al.*[25], who found significant association of *GSTM1* (null genotype) with elevated risk of lung cancer. Results of the present study also predict that *GSTT1* (null genotype) is not significantly associated with lung cancer, similar to the results of Malats *et al.*[26].

Comparing the gene polymorphism and susceptibility to lung cancer across the world population, it was found that association of *GSTM1* gene polymorphism and lung cancer is highly prevalent in most of the Asian population[27]. However, results of the present study are in contrast with those of Yang *et al.*[28], who reported significant correlation of *GSTT1* (null genotype) (OR = 1.28, 95% CI = 1.10–1.49; heterogeneity, $P < 0.001$ and $I^2 = 62.0\%$) with lung cancer in the Asian population.

The GST enzymes are well known for their detoxifying properties. Most of the carcinogenic chemicals of cigarette smoke that enter the human body are metabolised by the GST enzymes. The polymorphism of μ-class (Mu) and θ-class (theta) of GST enzymes is the most studied enzymes for different types of respiratory diseases. Polymorphism of these two genes has been considered as the potentially important marker for environmentally induced lung cancer[22]. Although there are considerable number of studies relating polymorphism of these genes with lung cancer among different ethnicities across the world, there are only a few reported studies on the Indian population. Therefore, the present meta-analysis of these reported studies was done specifically on the Indian population. Results of the present study indicate that *GSTM1* gene (null versus present) (OR = 1.173; 95% CI: 1.011–1.361) is associated with elevated risk of lung cancer in the overall population.

There have been certain limitations in the present study. The number of published results is less, which limits a comprehensive meta-analysis on the Indian population. The data required for stratified analysis of gene–smoking, gene–ethnicity, gene–age interactions are not

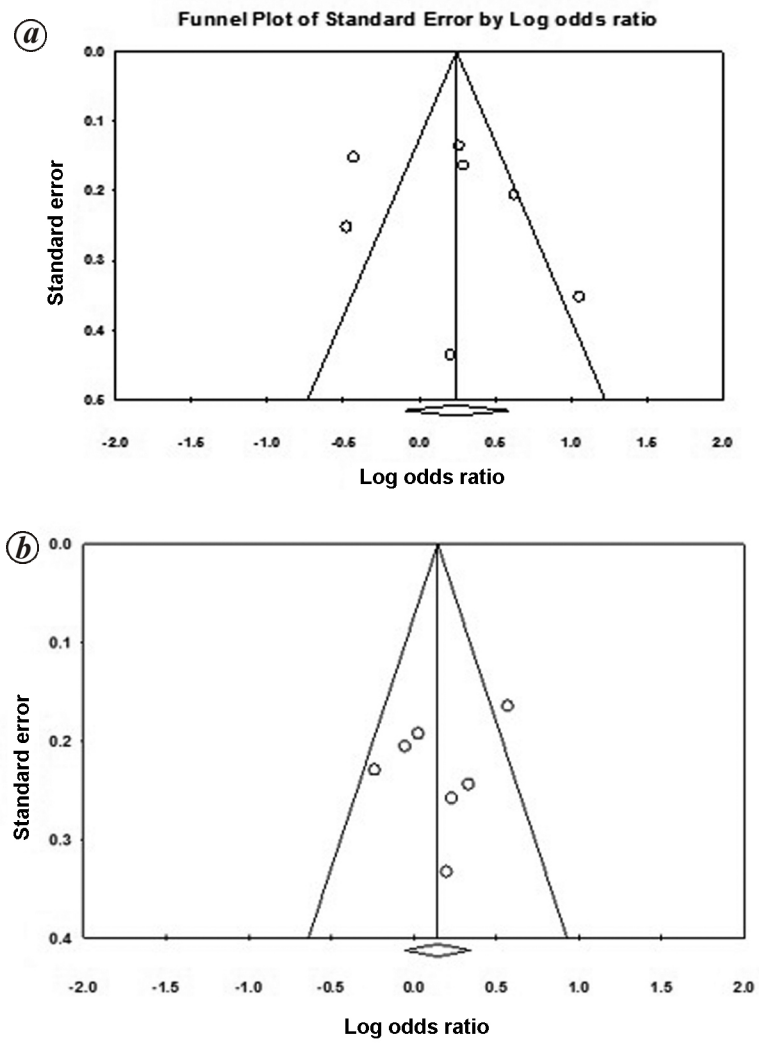

Figure 2. Analysis of data bias from different studies reported on *GSTT1*/*GSTM1* gene polymorphism and lung cancer using funnel plot. ***a***, Begg's funnel plot analysis of publication bias for *GSTT1* polymorphism and lung cancer ($t = 0.67$, $P = 0.53$). ***b***, Begg's funnel plot analysis of publication bias for *GSTM1* polymorphism and lung cancer ($t = 0.68$, $P = 0.52$).

sufficient. Although all the relevant publications were taken into consideration in the present study, the number of reported studies is less; hence, publication bias may exist.

Thus in conclusion, it can be summarized that *GSTM1* (null genotype) gene polymorphism is significantly associated with elevated risk of lung cancer in the Indian population. Further, case-control studies on larger populations are required to elucidate the effect of gene polymorphism on lung cancer in the Indian population. Studies relating the GST enzymes profile and their interaction with cigarette smoke compounds will be useful.

1. Stewart, B. W. and Wild, C. P. (eds), WHO: World Cancer Report 2014, IARC Publishers, France, 2014.
2. Hayes, J. D., Flanagan, J. U. and Jowsey, I. R., Glutathione transferases. *Annu. Rev. Pharmacol. Toxicol.*, 2005, **45**, 51–88; doi: 10.1146/annurev.pharmtox.45.120403.095857.
3. Josephy, P. D., Genetic variations in human glutathione transferase enzymes: significance for pharmacology and toxicology. *Hum. Genom. Proteom.*, 2010, 876940; doi:10.4061/2010/876940.
4. Liang, S. *et al*., Significant association between asthma risk and the GSTM1 and GSTT1 deletion polymorphisms: an updated meta-analysis of case-control studies. *Respirology*, 2013, **18**(5), 774–783; doi:10.1111/resp.12097.
5. Dey, T. *et al*., Role of glutathione S transferase polymorphism in COPD with special reference to peoples living in the vicinity of the open cast coal mine of Assam. *PLoS ONE*, 2014, **9**(5), e96739; doi:10.1371/journal.pone.0096739.
6. Iorio, A., Polimanti, R., Piacentini, S., Liumbruno, G. M., Manfellotto, D. and Fuciarelli, M., Deletion polymorphism of *GSTT1* gene as protective marker for allergic rhinitis. *Clin. Respir. J.*, 2014; doi:10.1111/crj.12170.
7. Marson, F. A., Bertuzzo, C. S., Ribeiro, A. F. and Ribeiro, J. D., Polymorphisms in the glutathione pathway modulate cystic fibrosis severity: a cross-sectional study. *BMC Med. Genet.*, 2014, **4**, 15–27; doi:10.1186/1471-2350-15-27.
8. Ma, Z., Guo, W., Gong, T., Niu, H. J., Wang, R. W. and Jiang, Y. G., CYP1A2 rs762551 polymorphism contributes to risk of lung

cancer: a meta-analysis. *Tumor Biol*., 2014, **35**(3), 2253–2257; doi:10.1007/s13277-013-1298-6.
9. Taningher, M., Malacarne, D., Izzotti, A., Ugolini, D. and Parodi, S., Drug metabolism polymorphisms as modulators of cancer susceptibility. *Mutat. Res*., 1999, **436**, 227–261; doi:10.1016/S1383-5742(99)00005-8.
10. Reszka, E., Wasowicz, W. and Gromadzinska, J., Antioxidant defence markers modulated by glutathione S-transferase genetic polymorphism: results of lung cancer case-control study. *Genes Nutr*., 2007, **2**, 287–294; doi:10.1007/s12263-007-0057-y.
11. Matakova, T. *et al*., Gene polymorphisms of biotransforming enzymes (gsts) and their association with lung cancer in the Slovakian population. *Eur. J. Med. Res*. (*Suppl. IV*), 2009, **14**, 275–279.
12. Ihsan, R. *et al*., Copy number polymorphism of glutathione-S-transferase genes (*GSTM1* & *GSTT1*) in susceptibility to lung cancer in a high-risk population from North-East India. *Indian J. Med. Res*., 2014, **139**(5), 720–729.
13. Demir, A. *et al*., The role of *GSTM1* gene polymorphisms in lung cancer development in Turkish population. *J. Carcinogen*., 2007, **6**, 13; doi:10.1186/1477-3163-6-13.
14. Lopez-Cima, M. F., Alvarez-Avellon, S. M., Pascual, T., Fernandez-Somoano, A. and Tardon, A., Genetic polymorphisms in *CYP1A1*, *GSTM1*, *GSTP1* and *GSTT1* metabolic genes and risk of lung cancer in Asturias. *BMC Cancer*, 2012, **12**, 433; doi:10.1186/1471-2407-12-433.
15. Sobti, R. C., Sharma, S., Joshi, A., Jindal, S. K. and Janmeja, A., Genetic polymorphism of the *CYP1A1*, *CYP2E1*, *GSTM1* and *GSTT1* genes and lung cancer susceptibility in a north Indian population. *Mol. Cell. Biochem*., 2004, **266**, 1–9.
16. Sreeja, L., Syamala, V., Hariharan, S., Madhavan, J., Devan, S. C. and Ankathil, R., Possible risk modification by *CYP1A1*, *GSTM1* and *GSTT1* gene polymorphisms in lung cancer susceptibility in a South Indian population. *J. Hum. Genet*., 2005, **50**, 618–627; doi:10.1007/s10038-005-0303-3.
17. Shukla, R. K. *et al*., Associations of *CYP1A1*, *GSTM1* and *GSTT1* polymorphisms with lung cancer susceptibility in a Northern Indian population. *Asian Pac. J. Cancer Prev*., 2013, **14**(5), 3345–3349; doi:http://dx.doi.org/10.7314/APJCP.2013.14.5.3345.
18. Kumar, M., Agarwal, S. K. and Goel, S. K., Lung cancer risk in north Indian population: role of genetic polymorphisms and smoking. *Mol. Cell. Biochem*., 2009, **322**, 73–79; doi:10.1007/s11010-008-9941-z.
19. Shukla, R. K., Kant, S., Mittal, B. and Bhattacharya, S., Comparative study of GST polymorphism in relation to age in COPD and lung cancer. *Tuberk Toraks*, 2013, **61**(4), 275–282; doi:10.5578/tt.6252.
20. Ihsan, R. *et al*., Multiple analytical approaches reveal distinct gene–environment interactions in smokers and non smokers in lung cancer. *PLoS ONE*, 2011, **6**(12), e29431; doi:10.1371/journal.pone.0029431.
21. Phukan, R. K., Saikia, B. J., Borah, P. K., Zomawia, E., Sekhon, G. S. and Mahanta, J., Role of household exposure, dietary habits and glutathione S-transferases M1, T1 polymorphisms in susceptibility to lung cancer among women in Mizoram, India. *Asian Pac. J. Cancer Prev*., 2014, **15**(7), 3253–3260; doi:http://dx.doi.org/10.7314/APJCP.2014.15.7.3253.
22. Saarikoski, S. T. *et al*., Combined effect of polymorphic *gst* genes on individual susceptibility to lung cancer. *Int. J. Cancer*, 1998, **77**, 516–521.
23. Brockmoller, J., Kerb, R., Drakoulis, N., Nitz, M. and Roots, I., Genotype and phenotype of glutathione S-transferase class μ isoenzymes μ and ψ in lung cancer patients and controls. *Cancer Res*., 1993, **53**, 1004–1011.
24. To-Figueras, J. *et al*., Glutathione S-transferase M1 (*GSTM1*) and T1 (*GSTT1*) polymorphisms and lung cancer risk among northwestern Mediterraneans. *Carcinogenesis*, 1997, **18**(8), 1529–1533.
25. Nazar-Stewart, V. *et al*., The glutathione S-transferase polymorphism as a marker for susceptibility to lung carcinoma. *Cancer Res*., 1993, **53**, 2313–2318.
26. Malats, N. *et al*., Lung cancer risk in nonsmokers and *GSTM1* and *GSTT1* genetic polymorphism. *Cancer Epidem. Biomar*., 2000, **9**, 827–833.
27. Liu, X. *et al*., Meta-analysis of *GSTM1* null genotype and lung cancer risk in Asians. *Med. Sci. Monit*., 2014, **20**, 1239–1245; doi:10.12659/MSM.890490.
28. Yang, X. *et al*., *GSTT1* null genotype contributes to lung cancer risk in Asian populations: a meta-analysis of 23 studies. *PLoS ONE*, 2013, **8**(4), e62181; doi:10.1371/journal.pone.0062181.

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