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GSTM1 and GSTT1 copy numbers and mRNA expression in lung cancer

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Abstract

Large fractions of the human population do not express GSTM1 and GSTT1 (GSTM1/T1) enzymes because of deletions in these genes. These variations affect xenobiotic metabolism and have been evaluated in relation to lung cancer risk, mostly based on null/present gene models. We measured GSTM1/T1 heterozygous deletions, not tested in genome-wide association studies, in 2120 controls and 2100 cases from the Environment And Genetics in Lung cancer Etiology (EAGLE) study. We evaluated their effect on mRNA expression on lung tissue and peripheral blood samples and their association with lung cancer risk overall and by histology types. We tested the null/present, dominant and additive models using logistic regression. Cigarette smoking and gender were studied as possible modifiers. Gene expression from blood and lung tissue cells was strongly down-regulated in subjects carrying GSTM1/T1 deletions by both trend and dominant models ($p < 0.001$). In contrast to the null/present model, analyses distinguishing subjects with 0, 1 or 2 GSTM1/T1 deletions revealed several associations. There was a decreased lung cancer risk in never-smokers ($OR = 0.44$; $95\% CI = 0.23-0.82$; $p = 0.01$) and women ($OR = 0.50$; $95\% CI = 0.28-0.90$; $p = 0.02$) carrying 1 or 2 GSTM1 deletions. Analogously, male smokers had an increased risk ($OR = 1.13$; $95\% CI = 1.0-1.28$; $p = 0.05$) and women a decreased risk ($OR = 0.78$; $95\% CI = 0.63-0.97$; $p = 0.02$) for increasing GSTT1 deletions. The corresponding gene-smoking and gene-gender interactions were significant ($p < 0.05$). Our results suggest that decreased activity of GSTM1/T1 enzymes elevates lung cancer risk in male smokers, likely due to impaired carcinogens' detoxification. A protective effect of the same mutations may be operative in never-smokers and women, possibly because of reduced activity of other genotoxic chemicals.

Keywords

GST; copy numbers; gene expression; lung cancer; smoking and gender differences

Introduction

Glutathione S-transferase M1 (GSTM1) and T1 (GSTT1) belong to a family of enzymes that metabolize glutathione and play different physiologic functions depending on their specific substrate specificities. GSTM1 and GSTT1 enzymes are primarily known for promoting the

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excretion of carcinogenic products (e.g., *trans*-stilbene oxide, a potent epoxide mutagen) including smoking derived carcinogens (1, 2), which are the primary cause of lung cancer (3). On the other hand, there is increasing evidence that these enzymes can also bioactivate xenobiotics to form genotoxic products (4–6). GSTM1 and GSTT1 enzymes are also induced by isothiocyanates (7) that are present in cruciferous vegetables (8) and are potentially protective for lung cancer (9, 10). In addition, GSTM1 and GSTT1 metabolize estradiol (11) and deactivate estrogen-derived metabolites (12), a role that could contribute to gender differences in cancer susceptibility. Thus, polymorphisms in GSTM1 and GSTT1 genes can impact cancer risk, in particular lung cancer, by altering the metabolism of several cancer-related components.

GSTM1 and GSTT1 genes have been studied extensively in association with lung cancer due to their purported role as a detoxifier of tobacco-related carcinogens and to their high polymorphic deletions frequency (13, 14). Although copy number-based studies for these genes have shown that their enzymatic activity is directly proportional to the number of copies (15, 16), until recently, available technologies could not correctly distinguish individual carrying two copies from those carrying only one copy (17, 18). Moreover, arrays used in the current genome-wide association studies did not interrogate these alleles. Consequently, most studies to date have only evaluated lung cancer risk differences between individuals with GSTM1 and GSTT1 homozygous deletions (null genotype) and individuals with one or two copies of these genes (present genotype) (19). The earliest studies reported increased risk of smoking-induced lung cancer (20, 21). While mechanistic studies demonstrated evidence that the GSTM1 null genotype was also associated with increased PAH adducts, sister chromatid exchange, urine mutagenicity and increased specific somatic mutations (summarized in review by Rebbeck (22)), much larger summary meta-analyses showed an overall null association between lung cancer and GSTM1 or GSTT1 null/present genotype (19, 23–26). Since these studies did not characterize the associations of GSTM1 and GSTT1 with lung cancer risk on a *per allele* basis, further investigations that distinguish subjects with heterozygous alleles are important. Two studies evaluated the heterozygous genotype status for GSTM1 and GSTT1 deletions in relation to lung cancer (27, 28). Sorensen *et al.* (430 cases and 767 controls) found that fruit and vegetables intakes conferred a protective effect on lung cancer only among individuals carrying at least one GSTM1 copy (28). Lam *et al.* did not find significant overall results, possibly due to the small sample size (143 cases and 447 controls), although their data were suggestive of an increasing risk for lung cancer with number of GSTM1 deletions among subjects who smoked less than 20 cigarettes per day (27). Both authors recommended follow up in larger population-based studies.

The purpose of our study was to evaluate the association between lung cancer and GSTM1 and GSTT1 copy numbers in a large (2100 cases and 2210 controls) and well defined population-based case-control study, the Environment And Genetics in Lung cancer Etiology (EAGLE) study (29). We first functionally validated our copy number data by measuring the effect of GSTM1 and GSTT1 deletions on gene expression derived from whole peripheral blood and lung tissue samples in a subgroup of the same subjects. We then studied the association between lung cancer risk (overall and by histology type) and GSTM1 and GSTT1 deletions using the null/present model as well as additive and dominant models. Gene-environment interactions were evaluated for smoking history and sex.

Methods

Study population

The EAGLE study was described in detail previously (29). Briefly, between 2002 and 2005, 2100 consecutive primary lung cancer cases were enrolled from the major hospitals in the

Lombardy region of Italy and 2120 controls were enrolled from the surrounding municipalities and frequency matched to cases by gender, age (5 years categories), and 5 residential areas. The enrolled individuals were all of Caucasian ethnicity. Patients' diagnosis was ascertained by pathologists at the local hospitals in Italy and by a specialist at the National Institutes of Health in the USA. Detailed demographic and exposure characteristics were collected using computerized and self reported questionnaires. Blood samples were collected for cases and controls at time of enrollment and lung tissue samples were collected for cases at time of surgery. All study participants signed an informed consent. The study was approved by the involved hospitals and institutions in Italy and by the National Cancer Institute Review Board in the USA.

GSTM1 and GSTT1 copy numbers and gene expression determination

GSTM1 and GSTT1 copy numbers were measured at the Core Genotyping Facility of the NCI using the Applied Biosystems TaqManR Gene Copy Number Assays (<https://products.appliedbiosystems.com/ab/en/US/adirect/ab?cmd=catNavigate2&catID=606182&tab=Overview>). The assay was performed as a quantitative duplex PCR assay on the target gene along with a reference gene (RNaseP). Samples were run in duplicate on the same plate. Data were collected and analyzed using the Roche Light Cycler 480 Instrument and Software. Analysis was performed in duplicate using the advanced relative quantitation method, based on a calibrator sample on every plate. This method compares the levels of two different target sequences in a single sample and expresses the final result as a ratio of these targets. All samples passed pre-defined Sample Handling and Pre-Genotyping QC criteria before being genotyped. PicoGreenR required twenty-six nanograms of DNA. Samples that did not contain enough DNA to complete all Copy Number assays in the project were not genotyped. Six percent of the subjects were included in duplicates for quality checks. Copy number results were initially reported as number of gene copies. We converted the gene copies into numbers of gene deletions for analysis purposes (i.e. 0 copies = 2 deletions, 1 copy = 1 deletion, 2 copies = 0 deletions).

Gene expression data from tumor (n=49) and non-involved (n=42) lung tissue samples of adenocarcinoma patients and from peripheral whole blood of adenocarcinoma patients (n=68) and controls (n=79), with GSTM1 and GSTT1 genotype information, were available from previous experiments based on Affymetrix HG U133A Chip (30, 31). Inclusion criteria for the gene expression study were based on availability of fresh frozen lung tissue samples and balanced distribution by smoking, sex, and age-based categories.

Statistical analysis

GSTM1 and GSTT1 genotype were modeled as: (1) a continuous variable for the additive model or trend test (i.e., 0, 1, and 2 deletions), (2) a dichotomous variable for the dominant model (i.e., 1 and 2 deletions *versus* 0 deletions), (3) a dichotomous variable for the null/present model [i.e., 2 deletions *versus* present (0 and 1 deletions)]. In addition, we evaluated a model based on the total number of deletions in GSTM1 and GSTT1 combined (i.e., 0, 1, 2, 3 or 4 deletions).

We evaluated the correlation between the number of deletions and mRNA expression in GSTM1 and GSTT1 using linear models (i.e., $\log_2 \text{expression} = \alpha + \beta \times \text{GST status}$) and computing fold changes ($\text{FC} = 2^\beta$) of expression between individuals with different copy number variants.

The association between lung cancer incidence and GSTM1 and GSTT1 genotype was assessed using logistic regression models and by estimating the Odds Ratios (OR), 95%

confidence intervals (CI), and Wald test based p-values. Main effect analyses were adjusted for the study matching variables (age, sex, and residence area) and for cigarette smoking exposure (intensity, cumulative pack-years, and years since quit smoking). We tested these models in the entire population overall and in stratified analyses by cigarettes smoking status (never, ever), number of cigarettes per day (4 categories), cumulative pack-years (4 categories), and sex. Genotype-exposure interaction effects were evaluated by means of likelihood ratio test (LH). When sample size allowed, analyses were repeated separately for lung adenocarcinoma (AD), squamous cell carcinoma (SQ), small cell carcinoma (SC), and large cell carcinoma (LC).

Results

Subjects characteristics and genotype frequencies

Descriptive characteristics of the study populations have been published (29, 32, 33). Among the EAGLE study participants, 2006 controls and 1967 cases had enough DNA from blood or buccal samples available for genotyping. Of these, 1981 controls and 1938 cases passed pre-genotyping quality checks. The 246 pairs of duplicates showed concordant data and the genotype completion rate was above 99%. The resulting data for GSTM1 and GSTT1 copy numbers passed the test for Hardy-Weinberg equilibrium genotype proportions among controls (p-value = 0.1). The copy numbers frequencies among controls were as following: 7%, 41%, and 52% for 2, 1, and 0 copies of GSTM1, respectively; 34%, 47%, and 19% for 2, 1, and 0 copies of GSTT1, respectively.

Gene expression

For a subgroup of the population, we studied the impact of GSTM1 and GSTT1 deletions on gene expression from blood of cases and controls and from tumor and non-involved lung tissues of cases. Available Affymetrix probes 204550_x_at and 215333_x_at for GSTM1 and 203815_at for GSTT1 were used in the analysis. Gene expression levels from blood of controls, blood of lung adenocarcinoma cases, non-involved lung tissue cells and tumor cells of lung adenocarcinoma cases were consistently strongly down-regulated in subjects carrying one and/or two deletions compared to subjects with both copies of GSTM1 or GSTT1 (Table I). The trend by number of deletions was also significant in all sample types tested for both GSTM1 and GSTT1 probes (Table I).

Gene-lung cancer association using the null/present model

We then studied the association between GSTM1 and GSTT1 genotype and lung cancer incidence. The analyses based on the null/present model, where 0 and 1 gene deletions are combined, did not show any significant association (Supplemental Table I). There was a reduced lung cancer risk among female ever smokers who carried the present GSTT1 genotype, but the likelihood ratio test for gene-smoking interaction among women for the null/present model was non-significant ($p_{LH} = 0.27$).

Gene-lung cancer association using heterozygous-based models

When we applied models that consider 0 and 1 gene deletions separately, we observed multiple significant lung cancer associations (Table II). Analysis by histology groups are reported in Supplemental Table II. The most relevant results are graphically summarized in Supplemental Figure I.

GSTM1 gene—We found a significant decrease in lung cancer risk among never smokers who carried 1 or 2 GSTM1 deletions (OR=0.44, 95%CI=0.23–0.82, $p=0.01$), with a significant likelihood ratio test for gene-smoking interaction ($p_{LH} = 0.04$). In addition, we

observed a reduced risk among women who carried 1 or 2 GSTM1 deletions (OR=0.50, 95% CI=0.28–0.90, $p=0.02$), and a suggestion of a gene-sex interaction effect ($p_{LH}=0.10$).

We further explored the identified GSTM1-smoking interaction and estimated lung cancer risk by increasing pack-years of cigarette smoke at fixed ranges of cigarettes smoked per day (CPD) among men carrying two GSTM1 copies and among men carrying one or two GSTM1 deletions (blue and red curves, respectively, in Figure I). For men consuming between 0 and 24 cigarettes per day, lung cancer risk was consistently higher among carriers of one or two GSTM1 deletions than in carriers of two GSTM1 copies (<15 CPD: $p_{LH}=0.08$, 15–19 CPD: $p_{LH}=0.11$, 20–24 CPD: $p_{LH}=0.13$). At higher consumption of cigarette per day, the observed difference in lung cancer risks was attenuated (25–100 CPD: $p_{LH}=0.41$).

GSTT1 gene—Using the additive model, we observed a significant increase in lung cancer risk among men who smoked (OR=1.13, 95% CI=1.00–1.28, $p=0.05$) and in squamous cell carcinoma risk (OR=1.18, 1.00–1.39, $p=0.05$), particularly among men (OR=1.25, 95% CI=1.04–1.50, $p=0.01$). Conversely, among women, we found a significant decrease in lung cancer risk by both additive and dominant models for GSTT1 deletions (OR=0.78, 95% CI=0.63–0.97, $p=0.02$; OR=0.72, 95% CI=0.52–0.90, $p=0.04$; respectively), with similar results among women who smoked (see Table II). The likelihood ratio test for GSTT1-sex interaction was highly significant ($p_{LH}=0.003$ and 0.005, for the additive and dominant models, respectively).

In summary, as shown in Table II, we observed 8 significant (i.e., with p -value ≤ 0.05) gene lung cancer associations over 36 tested single-gene heterozygous-based models (i.e., 2 gene variants by dominant and additive models, smoking status and gender), which are more than the 1.8 expected by chance (overall p -value < 0.001) based on the z -statistic.

GSTM1 and GSTT1—By evaluating the association between lung cancer risk and the cumulative number of deletions of GSTM1 and GSTT1 genes combined (i.e. 0, 1, 2, 3, and 4 deletions, Table III), we observed a significant trend of increased risk of squamous cell carcinoma by increasing number of deletions in GSTM1/GSTT1 combined (OR=1.16, 95% CI=1.02–1.32, $p=0.02$), particularly among men (OR=1.23, 95% CI=1.07–1.41, $p=0.004$). There was suggestive linear trend for men (OR=1.08, 95% CI=0.99–1.18, $p=0.09$) and among men who smoked (OR=1.09, 95% CI=1.00–1.20, $p=0.06$). Conversely among women, we found a significant decrease in lung cancer risk by numbers of deletions in GSTT1/GSTM1 combined (OR=0.83, 95% CI=0.70–0.98, $p=0.03$). The likelihood ratio test for interaction between sex and the number of deletions in GSTM1/GSTT1 combined was highly significant ($p_{LH}=0.004$).

Discussion

In the largest study of GSTM1 and GSTT1 copy numbers and lung cancer to date, we showed that 1 and 2 deletions of the genes correspond to linear decrease of gene expression levels in blood and in lung tissue cells and that copy number deletions are associated with lung cancer susceptibility in male smokers, never smokers, and women. These findings suggest that results from the previously used present/null model may be diluted due to the pooling of individuals with 1 or 2 copies of GST genes.

To our knowledge, this is the first study to have shown the effect of GSTM1 and GSTT1 deletions on gene expression from the same individuals across cell types and cancer status. Our results show that the absence of even one copy of GSTM1 and GSTT1 decreases their

gene expression and further buttressed our confidence on the observed associations between lung cancer risk and GSTM1 and GSTT1 copy numbers.

We observed gene-environment interaction effects for smoking (GSTM1) and sex (stronger for GSTT1) with increased risk in male smoker deletion carriers in comparison to female or never smokers.

The involvement of GSTM1 and GSTT1 in metabolic processing of carcinogens in tobacco is well documented (34, 35). Glutathione S-transferase enzymes detoxify potential tobacco-related carcinogens through a conjugation with reduced glutathione into excretable metabolites. GSTM1 specifically targets large hydrophobic electrophiles, such as polycyclic aromatic hydrocarbons derived epoxides (PAH) and benzo[a]pyrene (34, 35), whereas GSTT1 is involved in the metabolism of a more restricted class of compounds, such as monohalomethane and ethylene oxide (36). We found a significant gene-smoking interaction for GSTM1 ($p_{LH}=0.04$) but not for GSTT1. This is consistent with the narrower range of carcinogen substrates for GSTT1 which for example, is not rate-limiting in the metabolism of PAH (22). Nevertheless, we observed an increased lung cancer risk among male smokers with GSTT1, particularly for squamous cell carcinoma of the lung. We were limited in sample size of never smoker squamous cell carcinoma cases and did not evaluate the gene-smoking interaction for GSTT1 among squamous cell carcinoma. Of note, our results for GSTM1 (Figure I) were in agreement with those of Lam *et al.*, who also observed a higher smoking-related lung cancer risk among carriers of GSTM1 deletions at low dose of cigarettes smoked per day (27). The reduced lung cancer risk in never smokers carrying GSTM1 variant alleles might be due to genotoxic components that are activated by GSTM1, although we cannot exclude a chance finding.

The analysis by histology types showed different results for deletions in GSTM1 (protection in adenocarcinoma never smokers) and in GSTT1 (increased risk in squamous cell carcinoma). These differences by histology can be related to the gender/smoking differences since adenocarcinoma cases include the majority of females and never smokers, while squamous cell carcinoma cases are mostly male smokers.

GSTM1 and GSTT1 have been hypothesized to play a role in estradiol metabolism (11) and in the deactivation/elimination of estrogen-derived metabolites (12). This is a possible explanation for the sex differential effects of GSTM1 and GSTT1 variants on lung cancer risk. Deactivation of estradiol and estrogen-derived metabolites, inducible by GSTM1 and GSTT1, has been linked to cancer risk (37). It is possible that in women who are less exposed to estrogens (as postmenopausal women), concentration of genotoxic intermediates due to exposure to other chemicals that are activated rather than deactivated by GSTM1 and particularly by GSTT1 becomes more prominent (5). This is evident in two recent publications, which reported a reduced risk of endometrial cancer and of breast cancer among women null for GSTM1 and GSTT1 (38, 39).

Moreover, the observed lung cancer protection by GSTM1 and GSTT1 deletions among women and never smoker men could be explained by lifestyle factors such as dietary habits. Intakes of fruit and vegetables and cruciferous vegetables, rich in isothiocyanates, have been shown to be inversely associated with lung cancer (9, 10). Components of fruits and vegetables, such as isothiocyanates, are metabolized by GSTM1 and GSTT1 (7). A previous study showed that individuals with GSTM1 deletions who consumed higher intake of fruits and vegetables were more protected against lung cancer compared to those who were GSTM1 present (28). In our population, the women tended to consume higher intakes of fruit and vegetables than men (33). Nevertheless, we did not find an interaction effect between fruit/vegetables or isothiocyanates and GSTM1 or GSTT1 in lung cancer (data not

shown). This may be due to the low cruciferous-specific intakes that characterize our population and subsequent limited power to detect effects of this dietary component. In addition, individuals carrying GSTM1 and GSTT1 deletions may have other GST active enzymes, such as GST-alpha (40) and GSTP1 which metabolize fruit and vegetables derived components. A pathways analysis including all GST genes could shed some light into this complex problem, but it would require larger sample sizes.

Using available GWAS data from EAGLE (41), we checked whether GSTM1 and GSTT1 copy numbers were tagged by other SNPs that could then be used for independent validation. We found that GSTM1 and GSTT1 copy numbers were not well tagged in the referenced GWAS platform, with maximum r^2 values of 0.1 and 0.5 for the two genes, respectively. This finding underscores the importance of obtaining GSTM1 and GSTT1 copy number information through direct genotyping.

In conclusion, in a high quality, large population-base study including copy number and gene expression analyses, we found that a decreased activity of GSTM1 and GSTT1 enzymes is positively associated with lung cancer risk among smokers, but inversely associated in nonsmokers and women. This study provides a new level of understanding on the role of the GST family of enzymes, and calls for further research in the precise mechanisms underlying the observed differences.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure I. Association between lung cancer and cumulative pack-years of cigarette smoking at fixed range of cigarettes smoked per day (CPD) by GSTM1 status among males
 The difference between the subjects carrying 0 deletions (blue symbols) and subjects carrying 1 or 2 deletions (red symbols) are quantified by the p-value for likelihood ratio test (p_{LH}). Colored lines are exponential interpolations of the corresponding OR values.

Table 1
Association between mRNA expression from blood and lung tissue and deletions in GSTM1 and GSTT1.

Genotype	Blood of Controls			Blood of Cases			Lung Non-Involved Tissue			Lung Tumor Tissue		
	Samples	-1/FC	P-value	Samples	-1/FC	P-value	Samples	-1/FC	P-value	Samples	-1/FC	P-value
GSTM1 [*]												
+/+	3	Ref	-	12	Ref	-	7	Ref	-	10	Ref	-
+/-	38	-1.33	0.0322	22	-1.29	0.0005	15	-1.13	0.0972	14	-1.24	0.0219
-/-	38	-1.97	<0.0001	34	-2.04	<0.0001	20	-1.68	<0.0001	25	-1.65	<0.0001
+/-, -/-	76	-1.62	0.0063	56	-1.71	<0.0001	35	-1.42	0.0011	39	-1.49	<0.0001
Trend		-1.45	<0.0001		-1.46	<0.0001		-1.34	<0.0001		-1.29	<0.0001
GSTM1 [†]												
+/+	3	Ref	-	12	Ref	-	7	Ref	-	10	Ref	-
+/-	38	-1.16	0.2448	22	-1.33	0.0002	15	-1.09	0.3077	14	-1.21	0.0346
-/-	38	-1.79	<0.0001	34	-1.98	<0.0001	20	-1.61	<0.0001	25	-1.57	<0.0001
+/-, -/-	76	-1.44	0.0423	56	-1.70	<0.0001	35	-1.36	0.0055	39	-1.43	0.0001
Trend		-1.47	<0.0001		-1.42	<0.0001		-1.31	<0.0001		-1.26	<0.0001
GSTT1 [‡]												
+/+	27	Ref	-	27	Ref	-	15	Ref	-	19	Ref	-
+/-	40	-1.41	<0.0001	32	-1.31	<0.0001	19	-1.59	<0.0001	25	-1.64	0.0003
-/-	12	-1.84	<0.0001	9	-1.74	<0.0001	8	-7.72	<0.0001	4	-5.81	<0.0001
+/-, -/-	52	-1.50	<0.0001	41	-1.40	<0.0001	27	-2.54	0.0001	29	-1.95	0.0001
Trend		-1.37	<0.0001		-1.32	<0.0001		-2.56	<0.0001		-2.04	<0.0001

P-values based on Wald test. Trend test was used for testing the additive model.

FC is the fold change derived from linear modeling of gene expression changes ($\log_2 \text{expression} = \alpha + \beta \times \text{SNP}; \text{FC} = 2\beta$).

+/+, two genes copies or zero deletions; +/-, 1 gene copy or one deletion; -/-, zero gene copies or two deletions.

^{*} Affymetrix probe 204550_x_at.

[†] Affymetrix probe 215333_x_at.

Table 2

Association between lung cancer and GSTM1 and GSTT1 deletions overall and by histology, gender, and smoking status, based on the additive and dominant models.

Group	Genotype	All						Never Smoker						Ever Smoker						LH P-val
		Control	Case	OR	CI1	CI2	P-val	Control	Case	OR	CI1	CI2	P-val	Control	Case	OR	CI1	CI2	P-val	
GSTM1																				
All	+/+	144	166	1.00	-	-	-	40	16	1.00	-	-	-	104	150	1.00	-	-	-	-
	+/-	807	740	0.77	0.58	1.03	0.081	275	51	0.39	0.20	0.77	0.007	529	683	0.88	0.64	1.21	0.433	G×Smoke Dominant
	-/-	1017	1028	0.87	0.66	1.16	0.352	311	71	0.47	0.25	0.91	0.026	704	951	0.99	0.73	1.34	0.935	0.037
	+/-, -/-	1824	1768	0.83	0.63	1.09	0.183	586	122	0.44	0.23	0.82	0.010	1233	1634	0.94	0.70	1.27	0.691	Additive 0.256
	Trend			1.02	0.90	1.14	0.788			0.86	0.64	1.17	0.343			1.05	0.92	1.19	0.493	
Male	+/+	119	127	1.00	-	-	-	28	5	1.00	-	-	-	91	122	1.00	-	-	-	-
	+/-	617	593	0.86	0.62	1.20	0.374	160	10	0.31	0.10	0.98	0.045	454	579	0.93	0.66	1.30	0.666	
	-/-	767	809	0.97	0.70	1.35	0.859	179	16	0.45	0.15	1.32	0.144	587	787	1.03	0.74	1.44	0.846	
	+/-, -/-	1384	1402	0.92	0.67	1.26	0.610	339	26	0.38	0.14	1.07	0.068	1041	1366	0.99	0.71	1.36	0.933	G×Sex Dominant 0.098
	Trend			1.04	0.91	1.20	0.527			0.84	0.48	1.50	0.563			1.06	0.92	1.21	0.431	Additive 0.320
Female	+/+	25	39	1.00	-	-	-	12	11	1.00	-	-	-	13	28	1.00	-	-	-	-
	+/-	190	147	0.45	0.24	0.84	0.013	115	41	0.36	0.14	0.90	0.028	75	104	0.55	0.24	1.24	0.150	
	-/-	250	219	0.53	0.29	0.96	0.038	132	55	0.42	0.17	1.03	0.057	117	164	0.63	0.29	1.40	0.258	
	+/-, -/-	440	366	0.50	0.28	0.90	0.020	247	96	0.39	0.16	0.93	0.035	192	268	0.60	0.28	1.30	0.196	
	Trend			0.90	0.70	1.14	0.384			0.85	0.59	1.23	0.393			0.93	0.68	1.29	0.677	
GSTT1																				
All	+/+	658	616	1.00	-	-	-	202	47	1.00	-	-	-	454	566	1.00	-	-	-	-
	+/-	922	936	1.04	0.88	1.23	0.640	291	67	0.98	0.64	1.49	0.914	630	863	1.05	0.88	1.27	0.579	G×Smoke Dominant 0.521
	-/-	378	371	1.03	0.83	1.28	0.767	131	23	0.79	0.45	1.38	0.405	245	346	1.08	0.86	1.37	0.496	Additive 0.326
	+/-, -/-	1300	1307	1.04	0.89	1.22	0.638	422	90	0.92	0.62	1.37	0.686	875	1209	1.06	0.89	1.26	0.493	
	Trend			1.02	0.92	1.13	0.718			0.90	0.69	1.18	0.449			1.04	0.93	1.17	0.471	

Group	Genotype	All										Never Smoker					Ever Smoker					LH P-val																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																								
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Analyses adjusted for age, residential area, sex (if not stratified by sex), cigarette per day, cumulative pack-years and years since quitting smoking (not in never smoker). Bolded results have p-value 0.05; italicized results have p-value 0.1. Trend test was used for testing the additive model. Likelihood Ratio Test P-values (LH P-val) are reported for Gene-Smoking (G×Smoke) and Gene-Gender (G×Sex) interactions.

Table 3

Association between lung cancer and the cumulative number of deletions in GSTM1 and GSTT1 combined, overall and by smoking status, by histology and by gender.

		All Smoking Categories					Never Smokers					Ever Smokers				
#		0, 1, 2, 3, 4 deletions	OR	CI1	CI2	P-val	0, 1, 2, 3, 4 deletions	OR	CI1	CI2	P-val	0, 1, 2, 3, 4 deletions	OR	CI1	CI2	P-val
All	Co	45, 338, 745, 636, 189	1.02	0.94	1.10	0.6804	14, 107, 227, 216, 58	0.87	0.70	1.07	0.1859	31, 229, 518, 418, 130	1.04	0.96	1.14	0.3351
	Ca	61, 291, 725, 655, 184					8, 21, 51, 47, 10					53, 269, 668, 606, 172				
AD	Co	45, 338, 745, 636, 189	0.99	0.90	1.09	0.8477	14, 107, 227, 216, 58	0.82	0.64	1.05	0.1106	31, 229, 518, 418, 130	1.02	0.92	1.14	0.6647
	Ca	29, 112, 307, 267, 69					5, 17, 36, 33, 6					24, 95, 268, 233, 63				
SQ	Co	45, 338, 745, 636, 189	1.16	1.02	1.32	0.0203	14, 107, 227, 216, 58	NA	NA	NA	NA	31, 229, 518, 418, 130	1.15	1.01	1.31	0.0356
	Ca	12, 67, 185, 169, 53					0, 0, 5, 5, 2					12, 66, 179, 164, 51				
SC	Co	45, 338, 745, 636, 189	1.10	0.92	1.32	0.2843	14, 107, 227, 216, 58	NA	NA	NA	NA	31, 229, 518, 418, 130	1.10	0.92	1.32	0.3016
	Ca	5, 26, 70, 66, 23					0, 0, 2, 2, 0					5, 26, 68, 64, 22				
LC	Co	45, 338, 745, 636, 189	0.96	0.75	1.23	0.7606	14, 107, 227, 216, 58	NA	NA	NA	NA	31, 229, 518, 418, 130	1.01	0.78	1.29	0.9634
	Ca	5, 9, 42, 24, 9					1, 0, 1, 0, 0					4, 9, 41, 24, 9				
M	Co	42, 265, 569, 478, 138	1.08	0.99	1.18	0.0913	13, 62, 128, 127, 35	0.90	0.61	1.32	0.5778	29, 201, 441, 350, 102	1.09	1.00	1.20	0.0603
	Ca	46, 232, 565, 527, 147					2, 5, 11, 11, 2					44, 226, 549, 514, 143				
F	Co	3, 73, 176, 158, 51	0.83	0.70	0.98	0.0259	1, 45, 99, 89, 23	0.86	0.66	1.11	0.2387	2, 28, 77, 68, 28	0.81	0.65	1.00	0.0530
	Ca	15, 59, 160, 128, 37					6, 16, 40, 36, 8					9, 43, 119, 92, 29				

Analyses adjusted for age, residential area, sex (if not stratified by sex), cigarette per day, cumulative pack-years and years since quitting smoking.

AD, adenocarcinoma; SQ, squamous cell carcinoma; SC, small cell carcinoma; LC, large cell carcinoma; M, male; F, female; Co, controls; Ca, Cases.

Bolded results have p-value 0.05; italicized results have p-value 0.1.