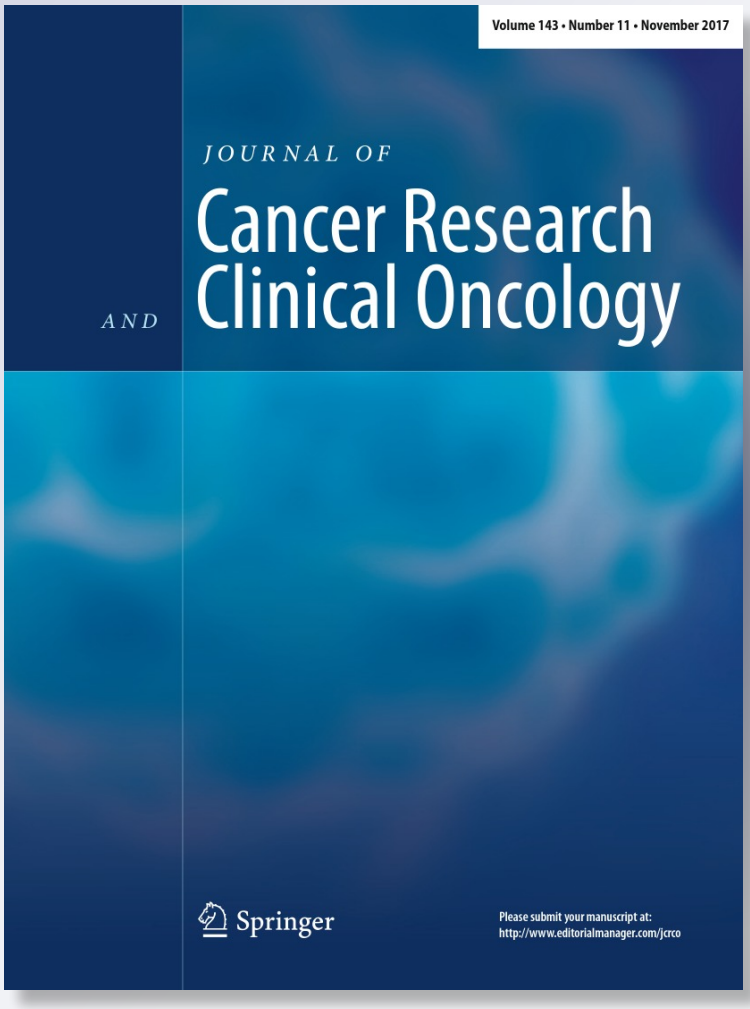
Polymorphisms of GSTM1, GSTT1, GSTP1 *genes and chromosomal aberrations in lung cancer patients*

Varvara I. Minina, Olga A. Soboleva, Andrey N. Glushkov, Elena N. Voronina, Ekaterina A. Sokolova, Marina L. Bakanova, et al.

Journal of Cancer Research and Clinical Oncology

ISSN 0171-5216
Volume 143
Number 11

J Cancer Res Clin Oncol (2017)
143:2235-2243
DOI 10.1007/s00432-017-2486-3



Springer

J Cancer Res Clin Oncol (2017) 143:2235–2243
DOI 10.1007/s00432-017-2486-3

ORIGINAL ARTICLE – CANCER RESEARCH

Polymorphisms of *GSTM1*, *GSTT1*, *GSTP1* genes and chromosomal aberrations in lung cancer patients

Varvara I. Minina[1,2] · Olga A. Soboleva[1] · Andrey N. Glushkov[1] · Elena N. Voronina[3] · Ekaterina A. Sokolova[3,4] · Marina L. Bakanova[1] · Yana A. Savchenko[1] · Anastasia V. Ryzhkova[1] · Ruslan A. Titov[1] · Vladimir G. Druzhinin[1,2] · Maxim Yu. Sinitsky[1,5] · Maxim A. Asanov[1]

Received: 10 February 2017 / Accepted: 27 July 2017 / Published online: 2 August 2017

Abstract
Purpose To study the potential links between genetic polymorphisms in the *GSTT1*, *GSTM1, GSTP1* genes and the frequency of chromosomal aberrations (CAs) in lung cancer patients and healthy residents in Russian Federation.
Methods 200 cells in well-spread metaphase with 46 chromosomes were examined for 353 newly diagnosed lung cancer patients (males) who received medical treatment in the Kemerovo Regional Oncology Center (Kemerovo, Russian Federation), and 300 healthy males from Kemerovo, Russian Federation. The polymorphisms of the *GSTM1* del and *GSTT1* del genes were analysed by multiplex PCR. Genotyping of the polymorphic variants in the *GSTP1* (*A313G, T341C*) gene was performed using Real-time PCR with competing TaqMan probes complementary to the polymorphic DNA sites. The data analysis was performed using software STATISTICA 8.0 (StatSoft Inc., USA).
Results We discovered that a *GSTM1* del polymorphism increases the frequency of chromosomal damage in smoking patients with lung cancer, a general group of lung cancer patients, donors with non-small cell lung cancer and patients in the latest stages of the malignant process. The synergetic effects of occupational exposure and the malignant process can induce some modifications in the cytogenetic status in lung cancer patients harbouring the *GSTM1* del polymorphism.
Conclusions CAs in peripheral blood lymphocytes can be used as biomarkers of the early biological effects of exposure to genotoxic carcinogens and may predict future cancer incidence in several epidemiologic studies. Genetic changes in genes encoding phase II detoxification enzymes are linked to decreases in the metabolic detoxification of environmentally derived genotoxic carcinogens.

Keywords Chromosomal aberrations · Lung cancer · Carcinogenesis · Xenobiotic biotransformation · Gene polymorphisms · GST supergene family

Electronic supplementary material The online version of this article (doi:10.1007/s00432-017-2486-3) contains supplementary material, which is available to authorized users.

✉ Varvara I. Minina
vminina@mail.ru

1 Federal State Budget Scientific Institution, The Federal Research Center of Coal and Coal Chemistry of Siberian Branch of the Russian Academy of Sciences, Sovetskiy Ave 18, Kemerovo 650065, Russian Federation

2 Department of Genetics, Biology Faculty, Kemerovo State University, Krasnaya St 6, Kemerovo 650043, Russian Federation

3 Institute of Chemical Biology and Fundamental Medicine of SB RAS, Lavrentiev Ave 8, Novosibirsk 630090, Russian Federation

4 Novosibirsk State University, Pirogova St 2, Novosibirsk 630090, Russian Federation

5 Laboratory of Genome Medicine, Research Institute for Complex Issues of Cardiovascular Diseases, Sosnovy Blvd 6, Kemerovo 650002, Russian Federation

Introduction

Structural chromosomal aberrations (CAs) in peripheral blood lymphocytes are used as biomarkers of the early biological effects of exposure to genotoxic carcinogens. Generally, CAs are divided into the following two main classes: chromosome-type aberrations (CSAs), which are primarily associated with exposure to ionising radiation (or radiomimetic chemicals), and chromatid-type aberrations (CTAs), which are mostly produced by chemical genotoxicants (Norppa 2004). CAs have been found to

predict future cancer incidence in several epidemiologic studies (Hagmar et al. 2004; Boffetta et al. 2007; Bonassi et al. 2008; Rossner et al. 2005). Other studies have shown that genetic polymorphisms of xenobiotic-metabolising enzymes may modify the frequency of CAs (Skjelbred et al. 2011; Hoyos-Giraldo et al. 2013; Hemminki et al. 2015).

Genetic changes in the genes encoding Phase II detoxification enzymes are linked to decreases in the metabolic detoxification of environmentally derived genotoxic carcinogens. Phase II enzymes (e.g., the *GST* supergene family) play a central role in the detoxification of toxic and carcinogenic electrophilic compounds. *GSTM1* metabolises large hydrophobic electrophiles, such as polycyclic aromatic hydrocarbon-derived epoxides (Hayes and Strange 2000). *GSTT1*, in contrast, is involved in the metabolism of smaller compounds, such as monohalomethane and ethylene oxide (Landi 2000). *GSTP1* plays an important role in a conjugation of reduced glutathione to a wide number of exogenous and endogenous hydrophobic electrophiles and negatively regulates CDK5 activity via a p25/p35 translocation (Sun et al. 2011). Certain genetic variants in the *GSTM1* and *GSTT1* del polymorphisms are prevalent among 50 and 20% of Caucasians, respectively (Garte et al. 2001), and these genetic variants result in a lack of an active enzyme (Rebbeck 1997). Carriers of the *GSTM1* del or *GSTT1* del genotypes have a slightly higher risk of developing lung cancer compared to carriers of at least one functional allele (Benhamou et al. 2002; Rotunno et al. 2012; Pliarchopoulou et al. 2012). The *GSTP1* gene A313G (rs1695) and T341C (rs1138272) polymorphisms are amongst the most studied variants. It was shown that the carriers of minor alleles in the *GSTP1* gene A313G (rs1695) polymorphism are characterised by a fourfold increased risk of asthma (Palmer et al. 2006; Tamer et al. 2004) and breast cancer (Lee et al. 2008). An investigation of the T341C (rs1138272) polymorphism showed that this substitution can be a predictor of lung volume reduction surgery risk (Hersh et al. 2007), various cancer incidences (Anantharaman et al. 2011; Oskina et al. 2014), and asthma (MacIntyre et al. 2014). Numerous studies have examined the association between one or more genetic polymorphisms of the *GST* supergene family and CAs (Hemminki et al. 2015; Skjelbred et al. 2011; Mušák et al. 2009; Kadioglu et al. 2012; Kumar et al. 2011). However, the results are contradictory. The inconsistent results may be due to the specific features of the mutagenic factors and the confounding influence of the polymorphisms on each other.

In the present study, we explored the potential links between genetic polymorphisms in the *GSTT1*, *GSTM1,* and *GSTP1* genes and the frequency of CAs in lung cancer patients and healthy residents in Western Siberia (Russian Federation).

Materials and methods

Subjects

In total, 353 newly diagnosed lung cancer patients (only males, mean age = 59.80 ± 7.30 years) who received medical treatment in the Kemerovo Regional Oncology Center (Kemerovo, Russian Federation), and 300 healthy males from Kemerovo, Russian Federation, (mean age = 50.30 ± 11.70 years) were investigated in this study. For this study, blood samples from lung cancer patients were obtained prior to all diagnostic or therapeutic procedures. All patients were examined by doctors in the Kemerovo Regional Oncology Center using a full complex of diagnostic techniques. The analysis of the histological material allowed for the determination of the exact pathomorphological diagnosis of each donor (96.0%—non-small cell lung cancer, 4.0%—small cell lung cancer). For each patient, the type of disease based on the TNM (tumour, nodus и metastasis) classification was determined according to the recommendation by Goldstraw (Goldstraw 2013). A full description of this group is presented in Table 1.

All healthy donors included in the control group did not have any chronic diseases, take any drugs with known mutagenic effects or underwent any radiographic procedures for at least 3 months prior to participation in the study. The full information of the control group is presented in Table 1.

The assessment of smoking status in both groups showed that 323 and 150 donors from the studied and the control groups, respectively, were smokers. The full description of smoking status is provided in Table 1.

Table 1 Characteristics of studied cohorts

Variable	Lung cancer patients (case)	Healthy Kemerovo residents (control)
Age, years (mean ± SD)	59.8 ± 7.3	50.3 ± 11.7
Number of donors		
Total	353	300
Smoking		
Smokers	323	150
Non-smokers	30	150
Pack–years of smoking (%)	40.4	25.0
Occupational hazard		
Occupational hazard (yes/no)	233/120	159/141
Length of service	21.9	17.7
Cancer type		
Small cell lung cancer	14 (4.0%)	
Non-small cell lung cancer	339 (96.0%)	
pTNM		
0, I, II	185 (52.4%)	
III, IV	168 (47.6%)	

For each person, informed consent for participation in the study was obtained. The study has been performed in accordance with the World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects with amendments in 2000 and the « Rules of clinical practice in the Russian Federation, which was approved by the Ministry of Health of the Russian Federation in 19.06.2003.

Blood sampling

Lymphocytes were obtained from venous blood samples supplemented with the anticoagulant heparin for CAs determination and ethylenediaminetetraacetic acid for genotyping. CAs were assessed immediately. Blood samples for genotyping were stored at −20 °C until use.

Cytogenetic analysis

Heparinised peripheral blood samples were cultured in an RPMI-1640 medium (PanEco, Moscow, Russian Federation) supplemented with 20% foetal calf serum (PanEco, Moscow, Russian Federation), 2% phytohaemagglutinin (PanEco, Moscow, Russian Federation), and L-glutamine for 48 h at 37 °C. Colchicine (PanEco, Moscow, Russian Federation) (0.05 mg/mL) was added at 46 h. After hypotonic treatment with 0.075 M KCl, the cells were fixed in methanol/acetic acid (3:1), spread on wet slides, and stained with 5% Giemsa for 5 min. In total, 200 cells in well-spread metaphase with 46 chromosomes were examined for each subject. The different types of CAs (without gaps) were recorded using the scoring criteria described by Brogger and Savage (Brogger et al. 1990; Savage 1976). The fixed cells and slides were stored for all subjects. The assessment of CAs was performed by two independent researchers using enciphered slides.

DNA preparation

DNA was isolated from venous blood using a standard procedure. Briefly, the blood cells were isolated and lysed, and the protein contents of the cells were hydrolysed by proteinase K (SibEnzyme, Novosibirsk, Russian Federation); the DNA was then purified by the extraction of impurities with phenol–chloroform and precipitation of DNA with ethanol (Sambrook et al. 1989).

Genotype analysis

The polymorphisms of the *GSTM1* del and *GSTT1* del genes were analysed by multiplex PCR. Each sample was amplified using the following pair of specific primers for *GSTM1*: 5′GGTCAAGGACATCATAGACGAGAA3′ (forward) and 5′CTCAGGAGAAACTGAAGCCAAA3′ (reverse). The following primers were used for *GSTT1*: 5′GCTAGTTGCTGAAGTCCTGCTTA3′ (forward) and 5′CTTGGCCTTCAGAATGACCT3′ (reverse). The following primers were used for the internal positive control, which was a fusible A/T-rich non-coding genomic fragment conventionally referred to as LTM (low temperature melting): 5′TGGGTGCTAGAGGTATAATCG3′ (forward) and 5′TTAGAGGAAGCTGGGTAAGAG3′ (reverse). The amplified fragment sizes in base pairs (bp) were as follows: GSTM1-229; GSTT1-287 and LTM-127. The calculated annealing temperature of the primers ranged from 64–66 °C. The melting points of the expected amplification products were as follows: GSTM1-86.5 °C; GSTT1-92.5 °C and LTM- 78.5 °C. The total reaction volume was 25 µl. The mixture contained the following: 40–100 ng of DNA; 65 mM Tris–HCl (pH 8.9); 0.05% Tween 20; 16 mM $(NH4)_2SO4$; 2.4 mM $MgCl_2$; 0.2 mM dNTP; 0.3 µM oligonucleotide primer solution; 0.8X SYBR Green I (SibDNA, Novosibirsk, Russian Federation) and 0.5 ed.ak. thermostable Taq-polymerase (SibDNA, Novosibirsk, Russian Federation). Amplification was performed using the thermocycler iCycler iQ5 (Bio-Rad, Hercules, California, USA). The absence of a fluorescent signal indicated an individual homozygous deletion of the gene (phenotype del), whereas the presence of the signal indicated a homozygous deletion, resulting in the genotypes positive/del and positive/positive (phenotype positive).

Genotyping of the polymorphic variants in the *GSTP1* (A313G, T341C) gene was performed using Real-time PCR with competing TaqMan probes complementary to the polymorphic DNA sites. Each sample was amplified using a pair of specific primers and two probes carrying "quencher" at the 3′-end (BHQ) and fluorescence dyes (FAM and R6G) at the 5′-end (Table 2). The volume of the reaction mixture was 25 µl and contained 40–100 ng of DNA, 300 nM of primers, 100–200 nM of the TaqMan probes (SibDNA, Novosibirsk, Russian Federation) conjugated with FAM or R6G, 200 µM of dNTP (SibDNA, Novosibirsk, Russian Federation), amplification buffer, and thermostable Taq-polymerase (SibDNA, Novosibirsk, Russian Federation)—0.5 ed.ak./reaction. The amplification was performed using amplifier CFX (Bio-Rad, USA). The results were interpreted based on an analysis of graphs of the fluorescence accumulation using the software Bio-Rad CFX Manager V1.6.541.1028.

Genotyping was performed in the Laboratory of Cytogenetics, The Federal Research Center of Coal and Coal Chemistry. Verification of the results was conducted in the Department of Genetics, Kemerovo State University by repeating the genotyping (laboratory assistants did not know the results of the previous investigation). The distribution of the genotypes in the studied groups is presented in Table 3.

Table 2 Characteristics of the primers and probes used for the assessment of the *GSTP1* loci

Loci	Primer	Probe
A313G (rs1695)	5′-GATGCTCACATAGTTGGTGTAG-3′ (forward)	5′-FAM-CTGCAAATACATCTCCCTCAT-BHQ-3′ (forward)
	5′- GGTGGACATGGTGAATGAC-3′ (reverse)	5′-R6G-CGCAAATACGTCTCCCTCAT-BHQ-3′ (reverse)
T341C (rs1138272)	5′-GGAGCAAGCAGAGGAGAATC-3′ (forward)	5′-FAM-CCTTGCCCGCCTCCTGC-BHQ-3′ (forward)
	5′-CAGCAGGGTCTCAAAAGGC-3′ (reverse)	5′-R6G-CTTGCCCACCTCCTGC-BHQ-3′ (reverse)

Table 3 Distribution of the genotypes in xenobiotic biotransformation genes in the studied groups

Loci	Genotype	Lung cancer patients, % (*N*)	Healthy donors, % (*N*)	χ^2, *p*	OR (CI 95%)
GSTM1	Del	40.5 (143)	42.7 (128)	0.23, 0.6328	0.92 (0.66–1.27)
	Positive	59.5 (210)	57.3 (172)		
GSTT1	Del	24.4 (86)	22.3 (67)	0.27, 0.6049	1.12 (0.77–1.64)
	Positive	75.6 (267)	77.7 (233)		
GSTP1 A313G rs1695	A/A	38.2 (135)	42.3 (127)	0.97, 0.3267	0.84 (0.61–1.17)
	A/G	51.0 (180)	47.0 (141)	0.88, 0.3490	1.17 (0.85–1.62)
	G/G	10.8 (38)	10.7 (32)	0.0005, 1.0005	1.01 (0.60–1.71)
GSTP1 T341C rs1138272	T/T	1.4 (5)	1.6 (5)	0.0005, 1.0005	0.85 (0.21–3.41)
	T/C	17.6 (62)	18.7 (56)	0.07, 0.7930	0.93 (0.61–1.41)
	C/C	81.0 (286)	79.7 (239)	0.11, 0.7377	1.09 (0.73–1.63)

Statistical analysis

The data analysis was performed using software STATISTICA 8.0 (StatSoft Inc., USA). Mann–Whitney's *U* test was employed to test for an association between CAs and genotype. All statistical tests were two-sided. A *p* value <0.05 was considered statistically significant. Adjusted relative ratios (RR) with 95% confidence intervals were calculated to compare the mean CAs frequencies in the different genotypes using a log-linear Poisson regression as described previously (Bonassi et al. 1994).

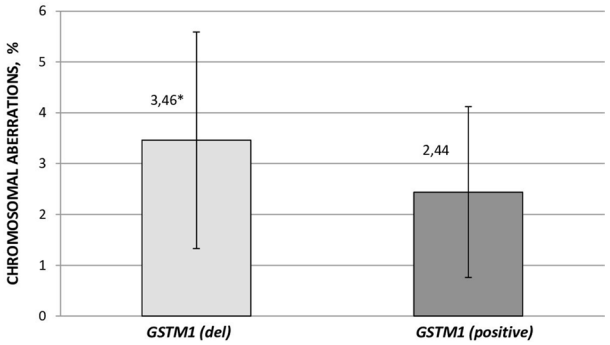


Fig. 1 CAs frequency in the groups of lung cancer patients harbouring different genotypes of GSTM1. *Asterisk* significant differences in comparison with *GSTM1* (positive), $p = 0{,}000012$; *p* value = 0.000001; RRadj = 1.20 (1.13–2.17)

Results

Various genotypes of the *GST* family genes and CAs in lung cancer patients and healthy subjects

In our previous article, we discovered a significant increase in the frequency of cytogenetic damage in lung cancer patients compared to that in the control group (CAs frequency was 3.14 and 2.01%; CTAs frequency was 2.26 and 1.60%; CSAs frequency was 0.96 and 0.42%, respectively). In addition, the increase in the frequency of CSAs was shown in smoking donors, and heavy smokers were characterised by the largest differences in all studied indicators in comparison with the control (Minina et al. 2016). The frequency of CAs in healthy donors has not exceeded the average regional values (Druzhinin 2003).

Based on our previous results, in this article, we studied the influence of various genotypes of the *GST* family genes on CAs in lung cancer patients and healthy residents in the Kemerovo Region. The mean frequencies of CAs in lung cancer patients harbouring the different genotypes are shown in Fig. 1. In the group of lung cancer patients, the mean frequency of CAs in *GSTM1* del subjects was significantly higher than that in *GSTM* positive subjects (3.46 ± 2.13 vs. 2.44 ± 1.68%, $p = 0.000001$, OR = 1.20). The mean frequency of CTAs and CSAs in *GSTM1* del subjects was significantly higher than that in *GSTM* positive subjects (2.61 ± 1.65 vs. 1.83 ± 1.42%, $p = 0.00001$; 0.93 ± 1.32 vs.

0.62 ± 0.89%, $p = 0.013$). We have discovered no significant associations between the CAs level and the other studied polymorphisms ($p > 0.05$) (Supplementary Table S1).

We have discovered no significant associations between the level of DNA damage and the genotypes in healthy carriers of various polymorphisms in the xenobiotic biotransformation genes (Supplementary Table S2).

Various genotypes of the *GST* family genes and CAs in smokers and non-smokers

We subdivided the groups into smokers and non-smokers to investigate the modifying influence of smoking on the level of cytogenetic damage in the carriers of various allele variants of the xenobiotic biotransformation genes. In smoking lung cancer patients (Fig. 2), a significant increase in the frequency of CTAs and CSAs and the overall frequency of CAs in the genotype *GSTM1* del compared to the *GSTM1* positive genotype was detected (2.62 ± 1.66 vs. 1.81 ± 1.37%; 0.94 ± 1.34 vs. 0.61 ± 0.89% and 3.47 ± 2.15 vs. 2.42 ± 1.65%, respectively). We found no significant differences between the non-smoking lung cancer patients and the healthy donors (Supplementary Table S3).

Patients with various types of lung cancer

Next, we accessed the level of CAs in patients with various types of lung cancer. In small cell lung cancer patients, we found no significant differences in the level of cytogenetic damage in the carriers of the *GST* family gene polymorphisms. Non-small cell lung cancer patients were characterised by an increased frequency of all studied markers in the carriers of the *GSTM1* del genotype (Fig. 3; Supplementary Table S4).

As shown in Fig. 4, carriers of the *GSTM1* del genotype are characterised by increased level of DNA damage (CAs and CTAs frequency) in both subgroups of donors with

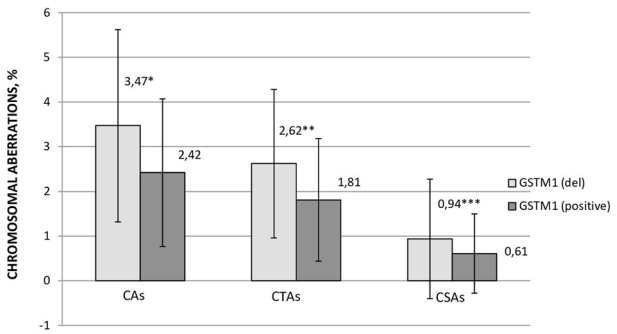


Fig. 2 Effect of genetic polymorphisms on chromosomal aberrations among smoking lung cancer patient. Significant differences in comparison with *GSTM1* (positive): *$p = 0.000015$; **$p = 0.000017$; ***$p = 0.009513$

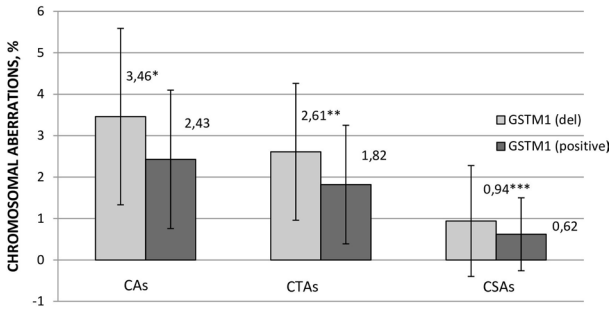


Fig. 3 Effect of genetic polymorphisms on chromosomal aberrations among nonsmall cell lung cancer patient. Significant differences in comparison with *GSTM1* (positive): *$p = 0.000001$; **$p = 0.000012$; ***$p = 0.015764$

(3.70 ± 2.22 and 2.81 ± 1.65%) and without metastases (3.15 ± 1.99 and 2.35 ± 1.61%). We found no significant differences in the association of the other studied polymorphisms with the degree of DNA damage (Supplementary Table S5).

In patients with III stage of lung cancer, we found an increased frequency of CAs (3.79 ± 1.85 vs. 2.57 ± 1.78%; $p = 0.000874$), CTAs (2.96 ± 1.49 vs. 2.11 ± 1.63%; $p = 0.003348$); and in patients with IV stage of lung cancer, we found an increased frequency of CAs (3.87 ± 2.71 vs. 2.26 ± 1.71%; $p = 0.034358$), CTAs (3.00 ± 2.16 vs. 1.39 ± 1.16%; $p = 0.007018$) in the carriers of deletions in the *GSTM1* gene compared to that in the carriers of the *GSTM1* positive polymorphism (Fig. 5; Supplementary Table S6).

Occupational hazards exposure

Finally, we divided all studied groups according to the presence of occupational hazards exposure. We discovered that the genotype *GSTM1* del modified the level of all detected

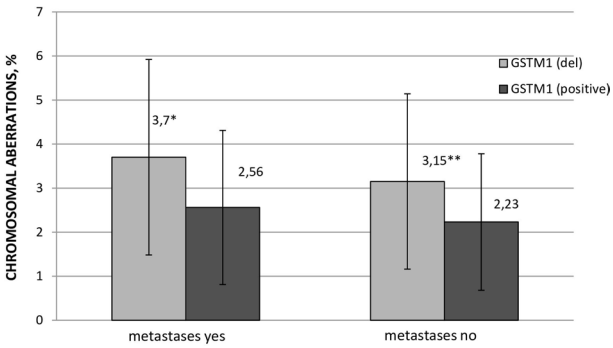


Fig. 4 Effect of genetic polymorphisms on chromosomal aberrations among lung cancer patients stratified by metastases. Significant differences in comparison with *GSTM1* (positive): *$p = 0.000427$; **$p = 0.005001$

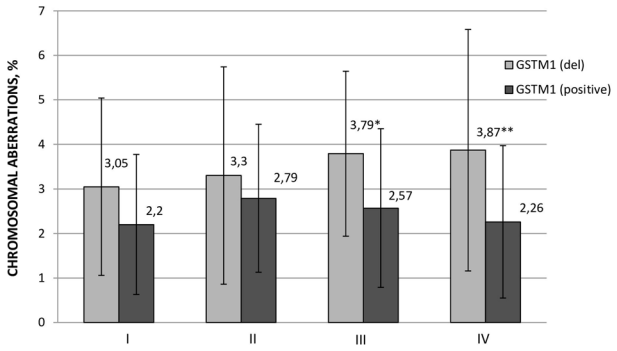


Fig. 5 Effect of genetic polymorphisms on chromosomal aberrations among patient stratified by lung cancer stage. Significant differences in comparison with *GSTM1* (positive): $*p = 0.000874$; $**p = 0.034358$

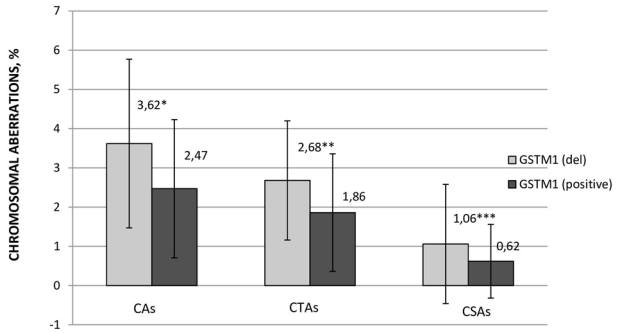


Fig. 6 Effect of genetic polymorphisms on chromosomal aberrations of lung cancer patient exposure to clastogenic/carcinogenic agents at work. Significant differences in comparison with *GSTM1* (positive): $*p = 0.000103$; $**p = 0.000077$; $***p = 0.006864$

indicators in lung cancer patients exposed to occupational hazards (Fig. 6). In a subgroup of healthy donors, we have discovered no significant results (Supplementary Table S7).

Discussion

CAs are useful biomarkers for the functional characterisation of individuals with various genotypes. The frequency of CAs in lymphocytes has been associated with a higher risk of cancer development. Patients with various types of cancers show increased frequencies of CAs (Vodicka et al. 2010; Vodenkova et al. 2015). Our results also showed a significant increase in the frequency of CAs in newly diagnosed lung cancer patients (Druzhinin et al. 2016; Minina et al. 2016). There are articles in which the association between the metabolic genotype and other cytogenetic endpoints (micronuclei, sister chromatid exchanges, and DNA damage evaluated by the comet assay) has been confirmed (Dusinska et al. 2012; Mušák et al. 2009; Mielzynska-Svach et al. 2013).

There is some evidence that the *GSTM1* and *GSTT1* genes are associated with lung cancer risk. According to Pan et al. (2014), the null genotypes of both polymorphisms resulted in an increased lung cancer risk with an odds ratio (OR) of 1.57 (95% confidence interval (CI) 1.23–2.00) for *GSTT1* and OR 1.87 (95% CI 1.46–2.39) for *GSTM1* ($p < 0.01$). Significant associations remained after stratification by histopathology ($p < 0.01$) and smoking status ($p < 0.05$). In a research study by Pan et al., it was found that the deletion of the *GSTT1* gene showed an increased risk among males (adjusted OR 2.95, 95% CI 2.07–4.20; $p < 0.01$), while the deletion of the *GSTM1* gene showed an increased risk among females (adjusted OR 2.95, 95% CI 2.07–4.20; $p < 0.01$) (Pan et al. 2014). At the same time, in a study performed in an Indian population, only the association of the *GSTM1* null genotype with increased lung cancer risk was detected (Sharma et al. 2015). These data demonstrate that *GSTM1* and *GSTT1* not only participate in the formation of a response to xenobiotic exposure but can also be factors in the increased risk of lung cancer. As shown in Fig. 1, lung cancer patients harbouring a *GSTM1* del are characterised by increased levels of CAs compared to carriers of the *GSTM1* positive genotype. The findings by Rossi et al. (2009) suggest that the association between CA and cancer is not modified by the *GSTM1* and *GSTT1* polymorphisms (Rossi et al. 2009). However, Cajas-Salazar et al. found that the *GSTM1*-null genotype was associated with a significant increase in CAs in lung cancer patients (Cajas-Salazar et al. 2003). The CAs were studied in a subgroup of 79 patients and 69 matched controls, and the patients had significantly more CAs than the controls. After stratifying by smoking history and genotypes, the patients still had significantly more CAs than the respective controls in most genotype categories.

Our results are consistent with the data in the literature. Therefore, smoking lung cancer patients with a *GSTM1* del genotype are characterised by an increased frequency of all studied indicators compared to carriers of the *GSTM1* positive genotype (Fig. 1). This fact is of particular interest because these enzymes play a central role in the detoxification of the major classes of tobacco carcinogens. Genetic variations within *GSTM1* may have an influence on the CAs frequency following exposure to the carcinogen compounds found in cigarette smoke and decrease the ability to detoxify these compounds.

In the group of healthy subjects, we have not found any significant differences. In healthy donors Skjelbred, Ada, Santovito et al. showed no significant effects of *GSTM1* polymorphisms on CAs (Skjelbred et al. 2011; Ada et al. 2013; Santovito et al. 2015). A modifying effect of the genetic polymorphisms of *GSTT1* on the mean frequencies of the different types of CAs in healthy donors has been demonstrated (Skjelbred et al. 2011; Kadioglu et al. 2012). Hemminki tested whether variants in metabolic genes and a

cyclin D1 splice site variant associate with CAs in healthy volunteers (Hemminki et al. 2014, 2015). Functional polymorphisms in the following six genes were selected for the analysis: *CYP1B1, EPHX1, NAD(P)H:*quinone oxidoreductase *1 (NQO1), GSTP1, GSTM1,* and *GSTT1*. Only *EPHX1* was individually associated with CA. In all genotype combinations with significant odds ratios for CAs, a *GST* variant was involved. Highly significant interactions for CA were found for *GSTM1* with *CYP1B1/453* (Hemminki et al. 2015). In our study, a modifying effect of the *GSTP1*, *GSTT1* and *GSTM1* genetic polymorphisms on CAs in healthy subjects was not found (Supplementary Table S2).

Scarpato R. reported that cigarette smokers with a homozygous deletion of the *GSTM1* gene had higher baseline CAs frequencies than those with at least one copy of the gene (Scarpato et al. 1997). This effect was mainly due to an excess of CTAs. It should be noted that the cohort studied by Scarpato R. was very different from cohort in our research. Scarpato R. et al. discovered association between homozygous deletion of the *GSTM1* gene and CAs in healthy—pesticide-exposed Italian greenhouse floriculturists and bank clerks (age 39.5 years; 34 males and 28 females; 18 smokers and 44 non-smokers). In our research were included only men (age 50.3 years; 150 non-smokers, 150 smokers; 53% have occupational hazard). Obtained results are consistent with the data of Skjelbred (Skjelbred et al. 2011). Skjelbred C.F. in the population consisted of 651 healthy Norwegian males of Caucasian descent (mean age of the subjects was 41 years; 51% smokers) have not discovered associations between *GSTM1* polymorphism and CAs frequency both in overall group and smokers.

The *GSTM1* del polymorphic variant has modified the CAs level in non-small cell lung cancer patients (Fig. 3), but we could not find any significant differences in the group of small cell lung cancer patients (Supplementary Table S4). Most likely, this result can be explained by the small number of such patients (14 donors) included in this study. There are some data in the literature regarding the associations between the *GSTM1* null и *GSTT1* null genotypes and the increased frequency of oxidative DNA damage in non-small lung cancer patients (Zhang et al. 2014).

The overall frequency of CAs and CTAs showed an increase in carriers of the deletion genotype on the *GSTM1* gene in both groups that were stratified according to the presence of metastases (metastases yes/no) (Supplementary Table S5); therefore, we can suggest that the activity of the GSTM1 enzyme equally influences the level of DNA damage in lung cancer patients regardless of the presence of metastases.

In lung cancer patients with III–IV stages of the malignant process, the increased DNA damage in carriers of the *GSTM1* del genotype was described, whereas in the group of donors with I-II stages of the disease, we have found no significant associations (Fig. 5). This fact can be explained by the observation that in the late stages of the malignant process, cytogenetic damages are accumulated, and the role of xenobiotic biotransformation under such conditions became more important.

Bus drivers (non-smoking) from Copenhagen and its suburbs with the *GSTM1* null and slow acetylator *NAT2* genotype had an increased frequency of cells with chromosomal aberrations. The effect of the *GSTM1* genotype, which was observed only in the bus drivers, appears to be associated with air pollution (Knudsen et al. 1999). Norppa suggests that *GSTM1* affects the frequency of CA, especially in smokers and individuals exposed to polyaromatic hydrocarbons or polluted air, while *NAT2* and, possibly, *GSTT1* could affect the baseline level of CAs (Norppa 1997). Higher frequencies of total CAs and CA constituent types (chromatid-type aberrations (CTAs) and chromosome-type aberrations (CSAs)) were detected in workers occupationally exposed to cytostatics or anaesthetics (Mušák et al. 2009), coaltar workers (Kumar et al. 2011), and workers employed in the heat and power industry (Minina et al. 2011) who had the del genotype for the *GSTM1* and *GSTT1* genes. No positive associations were detected between the frequency of CA and the genotypes *GSTM1, GSTP1,* and *GSTT1* in workers employed in tire plants (Vodicka et al. 2004). In a population exposed to organic solvents, the *GSTM1-null* genotype is associated with an increase in CA frequencies (Hoyos-Giraldo et al. 2013).

Because the Kemerovo Region is a developmental industry area and the habitants of this region are characterised by a high level of occupational exposure, we assessed the modifying influence of such exposure on the level of CAs in lung cancer patients and healthy donors (Supplementary Table S7). We discovered that the *GSTM1* del polymorphism was associated with an increased frequency of all studied indicators in lung cancer patients (Fig. 6), while we have discovered no significant differences in the group of healthy donors. This result can be explained by the modifying influence of occupational genotoxic factors on the level of DNA damage in lung cancer patients. The simultaneous lack of any significant differences in the healthy inhabitants in the Kemerovo Region exposed to occupational hazards can be explained by the decrease in the functional activity of xenobiotic biotransformation enzymes in lung cancer patients, which is probably associated with a decrease in adaptive capacity, and the synergetic effects of occupational exposure and malignant process that can induce some modifications in the cytogenetic status in donors.

According to the spectrum of chromosomal damage in patients with lung cancer resident in the Kemerovo region, there was close contact with chemical mutagens (tobacco smoke, industrial toxicants) and radiation (most likely radon, because other radiological indicators in the region

were within norms) (Minina et al. 2016). At the same time, the most sensitive to mutagenic exposure were carriers of *GSTM1* del genotype. In lung cancer patients, the ability to effectively repair DNA damage has been reduced (Spitz et al. 2003), which in conditions of inefficient biotransformation of xenobiotics can lead to an increase in the frequency of cytogenetic damage.

Conclusions

The frequency of chromosomal aberrations is dependent on complex environmental, occupational, and genetic factors. The results of this work found that a *GSTM1* del polymorphism increases the frequency of chromosomal damage in smoking patients with lung cancer, a general group of lung cancer patients, donors with non-small cell lung cancer and patients in the latest stages of the malignant process. The synergetic effects of occupational exposure and the malignant process can induce some modifications in the cytogenetic status in lung cancer patients harbouring the *GSTM1* del polymorphism.

Acknowledgements We thank the many people who participated in these studies over the years as researchers, collaborators and donors. This study was funded by Russian Science Foundation Grant No. 16-15-00034.

Compliance with ethical standards

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

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional 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.

References

Ada A, Demiroglu C, Yilmazer M et al (2013) Cytogenetic damage in Turkish coke oven workers exposed to polycyclic aromatic hydrocarbons: association with CYP1A1, CYP1B1, EPHX1, GSTM1, GSTT1, and GSTP1 gene polymorphisms. Arh Hig Rada Toksikol 64(3):359–369

Anantharaman D, Samant TA, Sen S, Mahimkar MB (2011) Polymorphisms in tobacco metabolism and DNA repair genes modulate oral precancer and cancer risk. Oral Oncol 47:866–872

Benhamou S, Lee WJ, Alexandrie AK et al (2002) Meta- and pooled analyses of the effects of glutathione S-transferase M1 polymorphisms and smoking on lung cancer risk. Carcinogenesis 23:1343–1350

Boffetta P, van der Hel O, Norppa H et al (2007) Chromosomal aberrations and cancer risk: results of a cohort study from Central Europe. Am J Epidemiol 165:36–43

Bonassi S, Ceppi M, Fontana V, Merlo F (1994) Multiple regression analysis of cytogenetic human data. Mutat Res 313:69–80

Bonassi S, Norppa H, Ceppi M et al (2008) Chromosomal aberration frequency in lymphocytes predicts the risk of cancer: results from a pooled cohort study of 22,358 subjects in 11 countries. Carcinogenesis 29:1178–1183

Brogger A, Hagmar L, Hansteen IL et al (1990) An inter-Nordic prospective study on cytogenetic endpoints and cancer risk. Nordic Study Group on the Health Risk of Chromosome Damage. Cancer Genet Cytogenet 45:85–92

Cajas-Salazar N, Sierra-Torres CH, Salama S et al (2003) Combined effect of MPO, GSTM1 and GSTT1 polymorphisms on chromosome aberrations and lung cancer risk. Int J Hyg Environ Health 206:473–483

Druzhinin VG (2003) Quantitative characteristics of the frequency of chromosomal aberrations in a group of residents of a large western Siberian industrial region. Russ J Genet 39:1161–1167

Druzhinin V, Bakanova M, Fucic A, Golovina T, Savchenko Y, Sinitsky M, Volobaev V (2016) Lymphocytes with multiple chromosomal damages in a large cohort of West Siberia residents: results of long-term monitoring. Mutat Res 784–785:1–7

Dusinska M, Staruchova M, Horska A, Smolkova B, Collins A, Bonassi S, Volkovova K (2012) Are glutathione S transferases involved in DNA damage signalling? Interactions with DNA damage and repair revealed from molecular epidemiology studies. Mutat Res 736:130–137

Garte S, Gaspari L, Alexandrie AK et al (2001) Metabolic gene polymorphism frequencies in control populations. Cancer Epidemiol Biomarkers Prev 10:1239–1248

Goldstraw P (2013) New staging system: how does it affect our practice? J Clin Oncol 31:984–991

Hagmar L, Stromberg U, Bonassi S et al (2004) Impact of types of lymphocyte chromosomal aberrations on human cancer risk: results from Nordic and Italian cohorts. Cancer Res 64:2258–2263

Hayes JD, Strange RC (2000) Glutathione S-transferase polymorphisms and their biological consequences. Pharmacology 61:154–166

Hemminki K, Musak L, Vymetalkova V et al (2014) Cyclin D1 splice site variant triggers chromosomal aberrations in healthy humans. Leukemia 28:721–722

Hemminki K, Frank C, Försti A et al (2015) Metabolic gene variants associated with chromosomal aberrations in healthy humans. Genes Chromosomes Cancer 54:260–266

Hersh CP, DeMeo DL, Reilly JJ, Silverman EK (2007) Xenobiotic metabolizing enzyme gene polymorphisms predict response to lung volume reduction surgery. Respir Res 8:59

Hoyos-Giraldo LS, Escobar-Hoyos LF, Reyes-Carvajal I et al (2013) The effect of genetic admixture in an association study: genetic polymorphisms and chromosome aberrations in a Colombian population exposed to organic solvents. Ann Hum Genet 77:308–320

Kadioglu E, Kocabas NA, Demircigil GC et al (2012) Assessment of individual susceptibility to baseline DNA and cytogenetic damage in a healthy Turkish population: evaluation with lifestyle factors. Genet Test Mol Biomarkers 16:1157–1164

Knudsen LE, Norppa H, Gamborg MO et al (1999) Chromosomal aberrations in humans induced by urban air pollution: influence of DNA repair and polymorphisms of glutathione S-transferase M1 and *N*-acetyltransferase 2. Cancer Epidemiol Biomark Prev 8:303–310

Kumar A, Yadav A, Giri SK, Dev K, Gautam SK, Gupta R, Aggarwal N (2011) Effect of genetic polymorphism of GSTM1 and GSTT1 genotypes on cytogenetic biomarkers among coaltar workers. Environ Toxicol Pharmacol 32:128–135

Landi S (2000) Mammalian class theta GST and differential susceptibility to carcinogens: a review. Mutat Res 463:247–283

Lee SA, Fowke JH, Lu W et al (2008) Cruciferous vegetables, the GSTP1 Ile105Val genetic polymorphism, and breast cancer risk. Am J Clin Nutr 87:753–760

MacIntyre EA, Brauer M, Melén E et al (2014) GSTP1 and TNF gene variants and associations between air pollution and incident childhood asthma: the traffic, asthma and genetics (TAG) study. Environ Health Perspect 122:418–424

Mielzynska-Svach D, Blaszczyk E, Butkiewicz D, Durzynska J, Rydzanicz M (2013) Influence of genetic polymorphisms on biomarkers of exposure and effects in children living in Upper Silesia. Mutagenesis 28:591–599

Minina VI, Savchenko IA, Bakanova ML et al (2011) Contribution of genetic polymorphism to the formation of individual genome sensitivity in workers engaged in heat and power industry. Gig Sanit 5:30–32

Minina VI, Sinitsky MY, Druzhinin VG et al (2016) Chromosome aberrations in peripheral blood lymphocytes of lung cancer patients exposed to radon and air pollution. Eur J Cancer Prev. doi:10.1097/CEJ.0000000000000270

Mušák L, Halašová E, Matáková T et al (2009) Comparison of chromosomal aberrations frequency and polymorphism of GSTs genes in workers occupationally exposed to cytostatics or anaesthetics. Interdiscip Toxicol 2:190–194

Norppa H (1997) Cytogenetic markers of susceptibility: influence of polymorphic carcinogenme- tabolizing enzymes. Env Health Pers 105:829–835

Norppa H (2004) Cytogenetic biomarkers and genetic polymorphisms. Toxicol Lett 149:309–334

Oskina NA, Ermolenko NA, Boyarskih UA et al (2014) Associations between SNPs within antioxidant genes and the risk of prostate cancer in the Siberian region of Russia. Pathol Oncol Res 20:635–640

Palmer CN, Doney AS, Lee SP, Murrie I, Ismail T, Macgregor DF, Mukhopadhyay S (2006) Glutathione S-transferase M1 and P1 genotype, passive smoking, and peak expiratory flow in asthma. Pediatrics 118:710–716

Pan C, Zhu G, Yan Z, Zhou Y, Liu Z (2014) Glutathione S-transferase T1 and M1 polymorphisms are associated with lung cancer risk in a gender-specific manner. Oncol Res Treat 37:164–169

Pliarchopoulou K, Voutsinas G, Papaxoinis G et al (2012) Correlation of CYP1A1, GSTP1 and GSTM1 gene polymorphisms and lung cancer risk among smokers. Oncol Lett 3:1301–1306

Rebbeck TR (1997) Molecular epidemiology of the human glutathione S-transferase genotypes GSTM1 and GSTT1 in cancer susceptibility. Cancer Epidemiol Biomarkers Prev 6:733–743

Rossi AM, Hansteen IL, Skjelbred CF et al (2009) Association between frequency of chromosomal aberrations and cancer risk is not influenced by genetic polymorphisms in GSTM1 and GSTT1. Environ Health Perspect 117:203–238

Rossner P, Boffetta P, Ceppi M et al (2005) Chromosomal aberration in lymphocytes of healthy subjects and risk of cancer. Environ Health Perspect 113:517–520

Rotunno M, Lam TK, Vogt A et al (2012) GSTM1 and GSTT1 copy numbers and mRNA expression in lung cancer. Mol Carcinog 51:E142–E150

Sambrook J, Fritsch E, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor

Santovito A, Cervella P, Delpero M (2015) Baseline frequency of chromosomal aberrations and sister chromatid exchanges in peripheral blood lymphocytes of healthy individuals living in Turin (North-Western Italy): assessment of the effects of age, sex and GSTs gene polymorphisms on the levels of genomic damage. Ann Hum Biol 24:1–10

Savage JR (1976) Classification and relationships of induced chromosomal structural changes. J Med Genet 13:103–122

Scarpato R, Hirvonen A, Migliore L, Falck G, Norppa H (1997) Influence of GSTM1 and GSTT1 polymorphisms on the frequency of chromosome aberrations in lymphocytes of smokers and pesticide-exposed greenhouse workers. Mutat Res 389:227–235

Sharma N, Singh A, Singh N, Behera D, Sharma S (2015) Genetic polymorphisms in GSTM1, GSTT1 and GSTP1 genes and risk of lung cancer in a North Indian population. Cancer Epidemiol 39:947–955

Skjelbred C, Svendsen M, Haugan V et al (2011) Influence of GSTM1, GSTT1, GSTP1, NAT1, NAT2, EPHX1, MTR and MTHFR polymorphism on chromosomal aberration frequencies in human lymphocytes. Carcinogenesis 32:399–405

Spitz MR, Wei Q, Dong Q, Amos CI, Wu X (2003) Genetic susceptibility to lung cancer: the role of DNAdamage and repair. Cancer Epidemiol Biomarkers Prev 12:689–698

Sun KH, Chang KH, Clawson S et al (2011) Glutathione-S-transferase P1 is a critical regulator of Cdk5 kinase activity. J Neurochem 118:902–914

Tamer L, Calikoğlu M, Ates NA et al (2004) Glutathione-S-transferase gene polymorphisms (GSTT1, GSTM1, GSTP1) as increased risk factors for asthma. Respirology 9:493–498

Vodenkova S, Polivkova Z, Musak L et al (2015) Structural chromosomal aberrations as potential risk markers in incident cancer patients. Mutagenesis 30:557–563

Vodicka P, Kumar R, Stetina R et al (2004) Markers of individual susceptibility and DNA repair rate in workers exposed to xenobiotics in a tire plant. Environ Mol Mutagen 44:283–292

Vodicka P, Polivkova Z, Sytarova S et al (2010) Chromosomal damage in peripheral blood lymphocytes of newly diagnosed cancer patients and healthy controls. Carcinogenesis 31:1238–1241

Zhang H, Wu X, Xiao Y, Chen M, Li Z, Wei X, Tang K (2014) Genetic polymorphisms of glutathione S-transferase M1 and T1, and evaluation of oxidative stress in patients with non-small cell lung cancer. Eur J Med Res 19:67