# Genetic Polymorphism of GSTP1 Gene and Lung Cancer Risk in Northern Romania

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**Background:** Glutathione S transferase P1 – an important member of the xenobiotic encoding enzymes, might contribute to the variability in individual susceptibility to lung cancer and may be important in exposure to carcinogens and therefore lung cancer development in smokers.
**Objectives:** This is a cross-sectional, randomized, case-control study for the evaluation of the frequency of GSTP1 alleles among patients with lung cancer.
**Subjects and methods:** The study included 108 cases of lung cancer diagnosed patients (histopathological examination), and 123 healthy unrelated controls. GSTP1 genotyping was carried out using PCR amplification of relevant gene fragment, followed by restriction enzyme digestion. Detection of GSTP1 alleles was determined by analysis of resulting restriction fragment length polymorphism (RFLP), followed by gel electrophoresis.
**Results:** Molecular analysis revealed an increased frequency of GSTP1 mutant genotype in the study group compared to the control group ($X^2$ = 0.133, p = 0.049, OR = 1.726, CI = 1–2.977). It appears that the effect of the GSTP1 mutant allele may vary according to histological subtype. The polymorphic I105V allele of GSTP1 gene was associated with an increased risk of lung adenocarcinoma.
**Conclusions:** GSTP1 polymorphism may be associated with increased risk to lung cancer and the homozygous Ile105Val genotype was found at a significantly higher frequency in the adenocarcinoma group.

**Keywords:** GSTP1, genetic polymorphism, lung cancer

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## Introduction

Incidence and mortality from lung cancer in Romania have increased significantly during the last two decades and lung cancer remains the major cause of cancer related death in Romania, with more than 10,000 new cases diagnosed each year [1].

Although tobacco smoking is the major cause of lung cancer, individual genetic susceptibility modulates the risk of smoking-related lung cancer [2,3]. An interesting approach for evaluating the role of genetic influences of lung cancer is genetic polymorphism study, which could provide new evidence and explanations, and therefore answer the following question: "Why only some smokers develop lung cancer?" [4].

Several polymorphisms of enzymes involved in xenobiotic metabolism have been found to be associated with lung cancer risk development, but information regarding the role of GSTP1 genetic polymorphism and lung carcinogenesis is still incompletely understood [5,6].

Glutathione S-transferase P1 (GSTPi1) is a member of the GST phase II enzyme superfamily, with an important role in detoxification and metabolism of cytotoxic drugs and cigar smoke [7], given the fact that GSTP1 isoforms are mostly abundant in the lungs; it can be hypothesized to have a particular importance in the detoxification of inhaled carcinogens, especially polycyclic aromatic hydrocarbons [8]. GSTP1 is responsible for more than 90% of the GST activity within the adult human lung epithelial cell population, the product encoded by GSTP1 mutant allele exhibits different activity, affinity and thermostability according to the metabolized substrates [9].

GSTP1 has two polymorphic sites: one on exon 5, A1578G, which encodes for I105V, and another one on exon 6C2508T, encoding for A114V. Polymorphic allele A114 V has been reported to increase lung cancer risk among smokers [10], but in the case of A1578G polymorphic variant association with lung cancer risk has no adequate scientific support in the literature [11,12,13].

In the present study, we wish to evaluate the hypothesis whether the genetic polymorphism Ile105Val of GSTP1 gene is a marker for lung cancer susceptibility in the Northern Romanian population.

## Material and method

### 1. Study population

This is a cross-sectional, randomized case control study on 108 patients with lung cancer and 123 cancer free controls. Lung cancer patients were recruited at the Leon Danielo Pneumology Hospital from Cluj-Napoca, Romania. Randomly selected controls were recruited from Medical Clinic I, Emergency Hospital Cluj.

All of the subjects signed a written informed consent and each participant was personally interviewed by specialist physicians to obtain detailed information on general lifestyle and family history, associated pathology and, of course, tobacco use.

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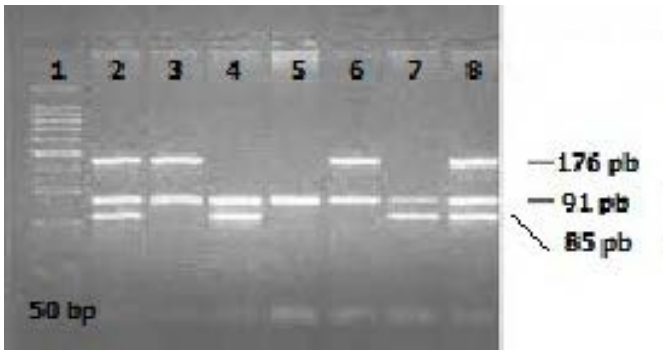


Fig. 1. Electrophotetic analysis of GSTP1 gene Ile[105]Val polymorphism. Lane 1: 50bp DNA marker; lane 2, 8: RQ genotype; lane 3, 6: RR genotype; lane 4,7: QQ genotype

Table I. Lung cancer risk evaluation for GSTP1 gene, Ile[105]Val polymorphism

*Fisher test, dominant model*

| Genotype | $X^2$ | p | Odds ratio | 95% CI | |
|---|---|---|---|---|---|
| | | | | Inferior limit | Superior limit |
| GSTP1 Ile[105]Val | 0.133 | 0.049* | 1.726 | 1.000 | 2.977 |

*Fisher test, recessive model*

| Genotype | $X^2$ | p | Odds ratio | 95% CI | |
|---|---|---|---|---|---|
| | | | | Inferior limit | Superior limit |
| GSTP1 Ile[105]Val | 0.030 | 0.654 | 0.883 | 0.512 | 1.524 |

Primary lung cancer was confirmed in all cases by pathological examination of a lung tissue sample. We must specify that all patients included in the study are active smokers for more than 10 years. A sample of 2 ml of venous blood was collected from all patients.

### 2. Genotyping [14]

DNA was extracted from 300 µl peripheral blood samples using Wizard Genomic DNA Purification Kit (Promega, Madison, USA).

The GSTP1 Ile105Val polymorphism was detected using a PCR-RFLP method. The PCR primers were synthesized by Eurogentec (Belgium):

- Forward primer:
  5`-ACCCCAGGGCTCTATGGGAA-3';
- Reverse primer:
  5'-TGAGGGCACAAGAAGCCCCT-3'.

A total amount of 100 ng genomic DNA was amplified in a total volume of 25 µl reaction mixture, containing reaction buffer of 1.5 nM $MgCl_2$, 20 pmol of each primer, 200 µm of each dNTPs and 0.5 units of Taq polymerase. Thermocycling was carried out according to the following conditions: initial denaturation at 95°C for 5 minutes, 30 cycles of denaturation at 94°C for 30 seconds, primer annealing at 55°C for 30 seconds, primer extension at 72°C for 30 seconds and then a final extension at 72°C for 5 minutes.

The amplification products were digested with 5 units of BsmAI enzyme (Fermentas) for 12 hours and separated on a 3% agarose gel. The undigested 176bp fragment corresponding to wild type homozygous genotype (RR), complete digestion to 91 and 85bp fragment for homozygous genotypes (QQ), while the presence of all three fragments (176, 91 85 bp) defines a distinct banding pattern, identifying heterozygous genotypes (RQ).

For statistical analysis we used SPSS 18.0 for Windows (SPSS, Inc, Chicago, Il).

## Results

There was no statistically significant difference in mean age and gender distribution between the cases and controls.

Molecular analysis of GSTP1 Ile[105]Val using the Fisher test by autosomal recessive model (mutant homozygous vs. heterozygous and normal homozygous), reveals no statistically significant differences between the study group compared to the control group ($X^2$ = 0.030, p = 0.654, OR = 0.883, CI = 0.512–1.524). However, the autosomal dominant model (mutant homozygous and heterozygous vs. normal homozygous) shows a statistically increased frequency of the mutant allele in the study group compared with controls ($X^2$ = 0.133, p = 0.049, OR = 1.726, CI = 1–2.977) (Table I).

We evaluated the risk for each of three major lung cancer subtypes: squamous cell carcinoma (79 cases, 73.14%), small cell carcinoma (15 cases, 13.88%) and adenocarcinoma (14 cases, 12.96 %). The mutant variant of GSTP1 Ile[105]Val polymorphism was associated with an increased risk of lung adenocarcinoma (p = 0.0063), as compared to other histological types of cancer.

Another finding is that heterozygous RQ status is associated with an increased risk for squamous cell carcinoma and small cell carcinoma as compared to the adenocarcinoma histological subtype.

## Discussions

Functional polymorphisms of GSTP1 have been studied as risk factors for lung cancer in the last two decades, but still there is insufficient data regarding the interaction between GSTP1 polymorphisms and cumulative exposure to smoking and lung cancer risk.

The results of our study suggest that GSTP1 (Ile[105]Val) polymorphism is associated with lung cancer risk. Current scientific evidence regarding the effects of the GSTP1 polymorphisms on enzyme function and therefore the relationship with lung cancer risk in smokers is quite contradicting; other studies involving xenobiotic GSTP polymorphisms showed no relevant modifying effect on lung cancer risk [15]. Another recent study also highlighted that there is no correlation of the GSTP1 polymorphism and GSTM1 null genotype and the risk of lung cancer in a Brazilian population [16]. However, our results are in agreement with other studies that showed that there is

a correlation between GSTP1 polymorphic variants and lung cancer risk [17,18,19,20].

Our main finding is that the mutant allele of GSTP1 ($Ile^{105}Val$) seems to be associated with an increased risk of lung adenocarcinoma as compared to other histological subtypes. Few studies associated GSTP1 ($Ile^{105}Val$) with certain lung cancer histological subtypes; a study on an Asian population highlighted an increased risk for lung squamous carcinoma for Q mutant allele carriers [21], while an other European study showed an increased risk for lung adenocarcinoma [22].

## Conclusions

Our results suggest that GSTP1 105 ($Ile^{105}Val$) polymorphism could influence the risk of lung cancer in Romanian patients and also the presence of the mutant allele increases the risk for lung cancer adenocarcinoma. Further studies including polymorphism of genes involved in the metabolic activation or detoxification of cigarette smoke carcinogens are anticipated to improve the ability of identifying individual genetic factors contributing to lung cancer susceptibility.

## References


1. Grupul de Lucru pentru Cancer Pulmonar al Societății Române de Pneumologie. Ghid pentru managementul clinic al cancerului pulmonar. MediaMedPublicis. 2012;6:8.
2. Li D, Wang M, Cheng L, Spitz MR, Hittelman WN and Wei Q. In vitro induction of benzo(a)pyrene diol epoxide-DNA adducts in peripheral lymphocytes as a susceptibility marker for human lung cancer. Cancer Res. 1996; 56:3638-3641.
3. Sellers TA, Potter JD, Bailey-Wilson SS, Rich H, Rotschild RC, Elston RC. Lung cancer detection and prevention: evidence for an interaction between smoking and genetic predisposition. Cancer Res.1992;52:2694s-2697s.
4. Shields PG. Molecular epidemiology of lung cancer. Annals of Oncology. 1990;10(Suppl. 5):S7-S1.
5. Kiyohara C, Shirakawa T, Hopkin J. Genetic polymorphisms of enzymes involved in xenobiotic metabolism and risk of lung cancer. Environ Health Prev Med., 2002;7(2):47-59.
6. Kamataki T. Metabolism of xenobiotics. In: Omura T, Ishimura Y, Fuijii-Kuriyama Y, editors. Cytochrome P-450. 2nd Edition. Tokyo, Kodansha Ltd. 1993:141-158.
7. Ketterer B, Harris JM, Talaska G et al. The human glutathione S-transferase supergene family, its polymorphism, and its effects on susceptibility to lung cancer. Environ Health Perspect. 1992;98:87-94.
8. Antilla S, Hirvonen A, Vainio H, Husgafvel-Pursiainien K, Hayes JD, Kettere B. Immunohistochemical localisation of glutathione S-transferases in human lung. Cancer Res.1993;53:5643-5648.
9. Kim SU, Lee KM, Park SK et al. Genetic Polymorphism of Glutathione S-transferase P1 and Breast Cancer Risk. Journal of Biochemistry and Molecular Biology. 2004;37(5):582-585.
10. McWilliams JE, Sanderson BJS, Harris EL, Richert-Boe KE and Henner WD. Glutathione S-transferase M1 (GSTM1) deficiency and lung cancer risk. Cancer Epidemiol. Biomarkers Prev. 1995;4:589-594.
11. Kiyohara C, Otsu A, Shirakawa T, Fukuda S and Hopkin JM. Genetic polymorphisms and lung cancer susceptibility: a review. Lung Cancer. 2002;37:241-256.
12. Miller DP, Neuberg D, De Vivo I, Wain JC, Lynch TJ, Su L and Christiani DC. Smoking and the risk of lung cancer: susceptibility with GSTP1 polymorphisms. Epidemiology. 2003;14:545-551.
13. Kiyohara C, Shirakawa T and Hopkin JM. Genetic polymorphism of enzymes involved in xenobiotic metabolism and the risk of lung cancer. Environ. Health Prev. Med. 2002;7:47-59.
14. Hohaus S, Di Ruscio A, Di Febo A, Massini G, D'Alo' F, Guidi F, Mansueto G, Voso MT, Leone G. Glutathione S-transferase P1 genotype and prognosis in Hodgkin's lymphoma Clin. Cancer Res. 2005;11(6):2175-2179.
15. Schneider J, Bernges U, Monika Philipp, H.J Woitowitz. GSTM1, GSTT1, and GSTP1 polymorphism and lung cancer risk in relation to tobacco smoking. Cancer Letters, 2004;208:1, 65-74.
16. Honma HN, De Capitani EM, Perroud Jr. MW et al. Influence of p53 codon 72 exon 4, GSTM1, GSTT1 and GSTP1*B polymorphisms in lung cancer risk in a Brazilian population. Lung Cancer. 2008;61:152-162.
17. Ritchie KJ, Henderson CJ, Wang XJ et al. Glutathione Transferase P Plays a Critical Role in the Development of Lung Carcinogenesis following Exposure to Tobacco-Related Carcinogens and Urethane. Cancer Res. 2007;67(19):9248-9257.
18. Jourenkova-Mironova N, Wikman H, Bouchardy C et al. Role of glutathione S-transferase GSTM1, GSTM3, GSTP1 and GSTT1 genotypes in modulating susceptibility to smoking related lung cancer. Pharmacogenetics. 1998;8:495-502m.
19. Kihara M and Noda K. Lung cancer risk of the GSTM1 null genotype is enhanced in the presence of the GSTP1 mutated genotype in male Japanese smokers. Cancer Letter. 1999;137:53-60.
20. Miller DP, Neuberg D, de Vivo I, Wain JC, Lynch TJ, Su L, Christiani DC, Smoking and the risk of lung cancer: susceptibility with GSTP1 polymorphisms. Epidemiology. 2003;14(5):545-551.
21. Lin P, Hsueh YM, Ko JL, Liang YF, Tsai KJ, Chen CY. Analysis of NQO1, GSTP1, and MnSOD genetic polymorphisms on lung cancer risk in Taiwan. Lung Cancer. 2003;40(2):123-129.
22. Dzian A, Halasova E, Matakova T, Kavcova E, Smolar M, Dobrota D, Hamzik J, Mistuna D. Lung Adenocarcinoma and Squamous Cell Carcinoma in association with Genetic Polymorphisms of GSTs in Slovak Population. Neoplasma. 2012;59(2):160-167.