Glutathione S-transferase M1 and P1 metabolic polymorphism and lung cancer predisposition*

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Individual susceptibility to different environmental agents is expected to be associated with alterations in metabolism of xenobiotics. Thus, genetic polymorphism of glutathione S-transferase (GST) can be recognized as a potential risk modifier in lung cancer development. The distribution of GSTM1 and GSTP1 genotypes was studied in a group of 138 diagnosed lung cancer patients and in 165 controls living in central Poland and RFLP-PCR technique was applied. The frequency of *GSTM1 null* genotype and *GSTP1* Val single and duplicated alleles was similar among patients and controls. *GSTM1* homozygous deletion was most prevalent in small-cell carcinoma groups (adjusted odds ratio (OR): 2.32, 95% confidence interval (CI): 0.98–5.52). In patients and controls, *GSTM1 A* genotype was most frequent (34.1% vs. 37.0%). The estimated lung cancer risk for *GSTM1 null*, *GSTP1* Ile/Val and *GSTP1* Val/Val combined genotype was 1.44 (95% CI: 0.73–2.83), suggesting the absence of modifying effect of defective *GSTM1* and *GSTP1* alleles on lung cancer predisposition.

Key words: Metabolic polymorphism, glutathione S-transferase, GSTM1, GSTP1, lung cancer, individual susceptibility.

Over dozens of years, growing incidence and mortality from lung cancer have been observed across the world. Of the 1.2 million cases registered in 2000, 380.000 incidences occurred in Europe, and it is estimated, that this figure will increase to 520.000 cases in 2050 [21]. The highest incidence rates are observed in Europe, especially in the male population. In Poland, lung cancer occupies the first place among causes of deaths from neoplasms in males, and second (after breast cancer) in females [30].

Tobacco smoking is the major cause of lung cancer, however, it develops only in a part of smokers. It is thought that individual susceptibility, due to variant alleles of xenobiotic metabolizing enzymes, may be a second host factor of the cancer development. Glutathione S-transferase, a key phase II enzyme, plays an important protecting role against electrophilic xenobiotics and products of oxidative stress [5]. The observed distinct individual variances of GST expression, activity and substrate affinity are mainly due to genetic polymorphism. Two of five polymorphic GSTs, GSTP1 and GSTM1, are found in lung tissue [20, 28], mainly in alveolar macrophages and bronchial epithelial cells [29]. GSTM1 and GSTP1 may regulate the entry of inhaled xenobiotics, because of catalyzed conjugation of tobacco smoke and occupationally derived epoxides with reduced glutathione, including N-nitrozamines and benzo[a]pyrene (B[a]P) [9]. Apart from the main role in detoxification of geno- and cytotoxic compounds, the increased level of GSTP1 is supposed to be a tumor marker. Moreover, this isoform is associated with the resistance of malignant cells to anticancer drugs, thus it reduces the concentration of chemotherapeutic drugs by increasing detoxification [10, 17].

Although there is no clear association between malignant diseases and glutathione S-transferase genotype, the frequency of defective alleles among ethnically different populations, *inter alia* European Caucasians, suggests that affected individuals are at increased risk of cancer at a number of sites, e.g. bladder [24], breast [8, 19] or larynx [4]. In the study group of Finnish lung cancer patients, significantly

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higher distribution of *GSTM1 null* genotype, compared with controls, allowed to estimate the lung cancer risk with odds ratio (OR) at 1.5 (95% confidence interval (CI): 0.9–2.3) [12]. In the Norwegian study of lung cancer patients, more individuals with both copies of mutated gene (*GSTP1* Val/Val genotype) were found in the cancer group than in controls (OR: 1.9, 95% CI: 1.04–3.47) [23]. However, it is believed that genetic polymorphism, when analyzed individually, confer only moderate risk of lung cancer. In meta-analysis of several case-control studies among Caucasians and Mongoloids with *GSTM1 null* genotype, OR value was estimated at about 1.17 (95% CI: 0.98–1.40) [18]. Another meta-analysis of 23 case-control studies also showed a minor effect of GSTM1 genetic polymorphism on lung cancer risk with OR: 1.13 (95% CI: 1.04–1.25) [14].

The aim of the study was to investigate a potential role of GSTM1 and GSTP1 genotypes in susceptibility to lung cancer in Polish study populations.

Patients and methods

Study population. Two study populations were recruited among people living in central Poland. They had to be residents of a given area for at least one year. There were 138 lung cancer patients of clinical and pathological departments and 165 non-malignant patients treated in the Lodz hospitals. Controls were matched by gender and age (± 3 years). Each participant was interviewed within three months following the diagnosis or selection and blood collection. All individuals (cases and controls) completed a questionnaire that provided information on demographic characteristics (age, gender, etc.), as well as complete residential, smoking and job histories. Occupational exposure associated with elevated cancer risk was classified according to Boffetta et al [2]. Eligible cases included all patients with initial diagnosis of lung cancer confirmed histologically within three months. Histological diagnosis of lung cancer comprised squamous cell carcinoma (SqCC), small cell carcinoma (SCC), non-small cell carcinoma (NSCC), and adenocarcinoma (AC). The Regional Ethics Committee for Scientific Research approved the study protocol and a written consent was obtained from each participant of the study. Pack-years (PY) were calculated according to daily cigarette consumption and duration of smoking (1 PY = daily consumption of 20 cigarettes/ year). All volunteers were informed about the purpose of the investigations. Blood samples from each participant were collected and stored at –70 °C before DNA isolation.

Genotype analyses. Genomic DNA was extracted from whole venous blood using the procedures of Qiagen Kit (Syngen). GSTM1 and GSTP1 genetic polymorphism was determined by applying RFLP-PCR. GSTM1 genotyping was conducted with intron 6 specific primer GSTM1I6 and exon 6 specific primers GSTM1E7A, as well as with GSTM1E7B and β-globin primers thoroughly described elsewhere [6]. Allele-specific PCR primers to intron 6 and exon 7 were used to introduce a restriction site into *GSTM1* A,* and to distinguish between *GSTM1 A*, *GSTM1 B* and *GSTM1 A,B* genotypes by restriction endonuclease *Hae*II (Promega, Symbios). Genomic DNA was amplified with *Taq* Polymerase (Qiagen, Syngen) on a PTC-200 DNA Engine (MJ Research, Syngen). After amplification, PCR products were electrophoresed on 1% agarose with ethidium bromide (EtBr), along with 50 bp ladder to determine positive GSTM1 samples. Then, after digestion (37 °C/5 h), positive probes were again electrophoresed on 2.5% agarose with BrEt, along with 50 bp ladder. Each sample, GSTM1 positive and negative, showed 268 bp band for β-globin amplification, while *GSTM1 B* samples (homozygotes or heterozygotes) showed additionally 132 bp band, *GSTM1 A* (homozygotes or heterozygotes), 112 bp digested band and *GSTM1 A,B* either 132 bp or 112 bp bands. Samples with ambiguous results were re-tested and 10% of all samples were repeated.

Genetic polymorphism of GSTP1 was identified in exon 5 of *GSTP1* gene and resulted in $Ile^{105}Val$ amino acid change, according to Watson et al [28] and Kihara et al [15], with a slight modification of the protocol. DNA amplification with primers complementary to 2306 bp and 2721 bp was followed by overnight digestion with *Alw*26I restriction endonuclease (Fermentas, ABO). RFLP-PCR products were electrophoresed on 4% agarose with BrEt, along with 20 bp ladder and then visualized and analyzed. *GSTP*1 Ile/Ile wild-type homozygotes demonstrated 329 and 104 bp fragments, while *GSTP1* Val/Val mutated homozygotes demonstrated 222, 107 and 104 bp bands. The *GSTP1* Ile/Val pattern gave four products: 329, 222, 107 and 104 bp. Samples with ambiguous results were re-tested and 10% of all samples were repeated.

Statistical analyses. To compare the frequency distribution of *GSTM1* and *GSTP1* genotypes between lung cancer patients and controls, the Pearson chi-square analysis in contingency tables was used. If at least one genotype frequency value was under 5, exact Fisher's test was included. The influence of GSTM1 and GSTP1 genotypes on lung cancer risk was determined by the logistic regression model with 95% confidence intervals. Adjusted ORs ratios for each value were calculated, including potential variables: age, gender, and PY. In the statistical analysis, BMDP Statistical Software Manual (University of California Press) was used.

The *GSTM1 null* genotype and *GSTP1* Val (one and both copies) were considered as high-risk genotypes [25, 28]. When estimating odds ratio, positive GSTM1 genotypes: *GSTM1 A*, *GSTM1 B*, and *GSTM1 A,B* were assumed as well as *GSTP1* Ile/Val and *GSTP1* Val/Val.

Results

Study lung cancer cases and hospital patients did not differ in their age, gender and occupational exposure. The differences between the investigated groups were found in genotype frequencies when stratified by the smoking status (Tab. 1). In addition, a very small number of non-smoking lung cancer cases (4.3%), compared with controls (24.4%), were observed.

The distribution of *GSTM1* and *GSTP1* genotypes (Tab. 2) did not show statistically significant differences between lung cancer cases and controls. Nor were there statistically significant differences in the distribution of *GSTM1* positive genotypes between both groups investigated (P = 0.78), but distinct over-representation of *GSTM1 A* genotype in patients (34.1%) and controls (37.0%) was found when compared to *GSTM1 B* and *GSTM1 A,B* genotypes. The frequency of *GSTP1* Ile/Ile, *GSTP1* Ile/Val and *GSTP1* Val/Val was according to Hardy-Weinberg equilibrium in controls and lung cancer patients.

The present study indicated, that homozygous *GSTM1* deletion and *GSTP1* variant alleles (Ile/Val and Val/Val), analysed separately, influenced neither lung cancer risk, nor histological sub-types risk (Tab. 3). However, when cancer cases were divided according to histological diagnoses, the highest risk associated with *GSTM1 null* was observed for SCC (OR: 2.32, 95% CI: 0.98–5.52). In SCC group, *GSTP1* Val homozygotes and heterozygotes made up only 37.1%.

All patients and controls with identified *GSTM1* and *GSTP1* genes were divided into three sub-groups according to GST status (Tab. 4). An elevated lung cancer risk was found for *GSTM1 null*/*GSTP1* Ile/Val + *GSTP1* Val/Val

Table 1. Basic characteristics of lung cancer patients and controls

	Cases N=138 age (mean) 59.7 N(%)	Controls N=165 age (mean) 59.6 N(%)
Female	32 (23.2)	37 (22.4)
Male	106 (76.8)	128 (77.6)
Smokers	72 (52.2)	53 (32.1)
Non-smokers	6 (4.3)	40 (24.4)
Ex-smokers <1 year	38 (27.5)	71 (43.0)
Ex-smokers ≤1 year	22 (15.9)	1 (0.6)
Occupational exposure	93 (67.4)	103 (62.4)
Non-occupational exposure	45 (32.6)	62 (37.6)
Squamous cell carcinoma (SqCC)	61 (44.2)	
Small cell carcinoma (SCC)	35 (25.4)	
Non-small cell carcinoma (NSCC)	24 (17.4)	
Adenocarcinoma (AC)	12 (8.7)	
Others[a]	6 (4.3)	

[a]Mixed or missing histological type.

Table 2. Distribution of GSTM1 and GSTP1 genotypes in lung cancer patients and controls

	N(%)				P
	GSTM1A	*GSTM1B*	*GSTM1 A,B*	*GSTM1 null*	
Controls	61(37.0)	36(21.8)	4(2.4)	64(38.8)	0.918[a]
Cases	47(34.1)	34(24.6)	4(2.9)	53(38.4)	
	GSTP1 Ile/Ile		*GSTP1* Ile/Val	*GSTP1* Val/Val	
Controls	83(50.3)		77(46.7)	5(3.0)	
Cases	73(52.9)		60(43.5)	5(3.6)	0.841[b]

[a]Pearson chi-square, d.f.=3, [b]Pearson chi-square, d.f.=2.

Table 3. GSTM1 and GSTP1 genotypes and lung cancer risk

	GSTM1 null N	(%)	OR[a](95% CI)	P[b]	*GSTP1* Ile/Val + Val/Val N	(%)	OR[a](95% CI)	P[b]
Controls	64	38.8	1.00 (reference)		82	49.7	1.00 (reference)	
Cases	53	38.4	1.02 (0.59–1.75)	0.94	65	47.1	1.06 (0.62–1.82)	0.65
SqCC	21	34.4	0.75 (0.37–1.54)	0.55	28	45.9	0.69 (0.34–1.39)	0.61
SCC	18	51.4	2.32 (0.98–5.52)	0.17	13	37.1	0.68 (0.29–1.61)	0.18
NSCC	6	25.0	0.57 (0.20–1.59)	0.19	12	50.0	1.10 (0.43–2.82)	0.97
AC	5	41.7	1.17 (0.33–4.22)	0.84	6	50.0	0.89 (0.25–3.17)	0.98

[a]Adjusted OR estimated by gender, age, smoking status, occupational exposure; [b]Pearson chi-square, d.f.=1.

Table 4. GSTM1 and GSTP1 combined genotypes and lung cancer risk

	Neither defective[a] N	(%)	Either defective N	(%)	Both defective N	(%)	OR[b](95%CI)	P[c]
Controls	47	28.5	90	54.5	28	17.0	1	
Cases	48	34.8	62	44.9	28	20.3	1.44 (0.73–2.83)	0.248
SqCC	27	44.3	19	31.3	15	24.6	1.48 (0.64–3.42)	0.007
SCC	10	28.6	19	54.3	6	17.1	1.57 (0.53–4.61)	0.990
NSCC	9	37.5	12	50.0	3	12.5	0.82 (0.21–3.20)	0.634
AC	2	16.7	9	75.0	1	8.3	0.47 (0.05–4.13)	0.385

[a]Dfective genotypes: *GSTM1 null* and *GSTP1* Ile/Val + *GSTP1* Val/Val; [b]Adjusted OR estimated by gender, age, smoking index, occupational exposure; [c]Pearson chi-square, d.f.=2.

Table 5. GSTM1 A and GSTM1 B genotypes in lung cancer patients

	GSTM1 A N	(%)	OR[a](95% CI)	*GSTM1 B* N	(%)	OR[b](95% CI)
Cases	47	34.1	1.09 (0.64–1.85)	34	24.6	0.89 (0.49–1.61)
SqCC	22	36.1	0.92 (0.46–1.85)	16	26.2	0.75 (0.35–1.62)
SCC	7	20.0	2.49 (0.97–6.38)	9	25.7	1.14 (0.47–2.81)
NSCC	11	45.8	0.53 (0.18–1.52)	6	25.0	0.57 (0.17–1.50)
AC	4	33.3	1.21 (0.31–4.72)	3	25.0	0.95 (0.21–4.22)

[a]Crude odds ratio, calculated with comparison to *GSTM1 A* genotype; [b]Crude odds ratio, calculated with comparison to *GSTM1* genotype.

defective genotype (OR: 1.44, 95% CI: 0.73–2.83). The most frequent "high risk" combined genotype was found in the SqCC group (24.6%). Moreover, significant differences in *GST* combined genotypes distribution in SqCC, compared with controls, were observed.

The data analyses with regard to smoking history or age did not show significant differences in the observed genotype distribution. Stratification by occupational exposure did not influence the estimation of lung cancer risk (data not shown).

Discussion

The role of glutathione S-transferase in cancer susceptibility remains unclear. Glutathione S-transferase comprises many isoenzymes, and some of them were found to be polymorphic in individual response to environmental and occupational xenobiotics. The impaired action of one of GST isoforms, resulting from variant alleles, do not decrease detoxificant and/or antioxidant potential of the body due to broad overlapping tissue and substrate specificity of GSTs. Therefore, the GST variant alleles may contribute to lung cancer risk, especially if more than a single polymorphism of GST and other risk factors are simultaneously investigated (e.g. occupational exposure, smoking habit).

The current data suggest the lack of association between GSTM1 and GSTP1 metabolic polymorphism and lung cancer predisposition. Some epidemiological studies of polymorphic GST in different European populations also indicate insignificantly increased risk of single GSTM1 and GSTP1 at-risk genotypes for lung cancer [7]. Hirvonen et al [11] summarized their own investigations on the role of different GST genotypes in the predisposition to cancer in Finnish and French populations. Genetic polymorphism of GSTM1, GSTP1, GSTM3 and GSTT1 was not strongly associated with lung cancer risk when evaluated separately. However, an analysis of the combined potential effect of GST showed weaker effect than expected. High risk GST genotype combinations (including GSTM1 and GSTP1) do not appear to play a significant role in lung cancer susceptibility in both investigated populations. In our study, there were no differences between lung cancer patients and controls in the distribution of *GSTM1* and *GSTP1* genotypes with adjusted odds ratio at about reference value for *GSTM1 null* and *GSTP1* Ile/Val + *GSTP1* Val/Val genotypes, respectively. But, having analyzed the interaction between "high risk" genotypes, we found their statistically insignificant over-representation in the lung cancer group with OR: 1.44; 95% CI: 0.73–2.83.

Most studies are based on the assumption that two positive alleles, *GSTM1*A* and *GSTM1*B,* are equally protective against genotoxic compounds [16, 25]. However, Perret et al [22] found a higher frequency of *GSTM1 A* genotype among the U.K. patients with pituitary tumors, which indicated a stronger protective role of *GSTM1*B* gene than that of *GSTM1*A*. On the contrary, the studies in a German population showed a protective role of *GSTM1*A* alleles in bladder cancer susceptibility [3]. In the present study, despite a higher frequency of *GSTM1 A* genotype in lung cancer patients and controls (34.1% vs. 37%) there were no differences in frequency of *GSTM1* positive genotypes in both investigated groups, which may show no lung cancer modification. These data are in agreement with To-Figueras et al [27] who found similar prevalence of *GSTM1 A* genotype in lung cancer individuals and controls from North-western Mediterraneans. Moreover, this study indicated the differences in *GSTM1 A* genotype, but not in *GSTM1 B* frequency between different histologic sub-types (Tab. 5).

After adjusting for age, gender, PY and occupational exposure, the prevalence of *GSTM1* homozygous deletion in the SCC group made it possible to estimate over a two-fold risk of this type of cancer (OR: 2.32, 95% CI: 0.98–5.52), however it was statistically insignificant. These results are in agreement with the studies in a Spanish population, which indicated slight over-representation of *GSTM1 null* in the SCC group (OR: 1.40, 95% CI: 0.74–2.61) [27]. The investigations on the French smoking lung cancer male patients showed elevated risk not only for SCC at OR: 1.7 (95% CI: 0.9–3.2), but also for AC at OR = 2.0 (95% CI: 1.1–3.6) [28]. Similar over-representation of *GSTM1 null* genotypes was found in Swedish individuals with AC and SCC, while this deficient genotype was common in 72% of SqCC females (OR = 3.3, 95% CI: 1.2–9.7) [1]. We observed only slight, statistically insignificant increase in risk of AC in investigated patients (adjusted OR: 1.17; 95% CI: 0.33–4.22). The study of Norwegian male patients showed over-representation of *GSTM1 null* or *GSTP1* Ile/Val and *GSTP1* Val/Val in the SqCC group, having analyzed the patients according to the major histological tumor types [23]. In the present study, in none sub-groups of the subjects, the differences in the distribution of *GSTP1* genotypes, based on histopathological diagnoses, were found. A study of Japanese male smokers showed a significantly elevated number of smoking patients with SCC, carrying two copies of *GSTP1* Val allele (7.2%) compared with smoking controls (1.6%. When genetic polymorphism of GSTM1 and GSTP1 were analyzed jointly, SCC risk was highest for at-risk genotypes, *GSTM1 null*, *GSTP1* Ile/Val or *GSTP1* Val/Val with the adjusted OR value (2.67, 95% CI: 1.09–6.55) [15]. The present data also show the statistically significant difference in the distribution of three combined *GSTM1* and *GSTP1* genotypes (both, either, neither defective) in the SqCC group compared to controls. However, the distribution of *GSTM1 null* and *GSTP1* Ile/Val + *GSTP1* Val/Val in controls and cases was similar (17.0% vs. 24.6%), and the statistical differences were probably due to the distribu-

tion of positive genotypes in the groups under study (28.5% vs. 44.3%).

Tobacco exposure is clearly associated with the development of lung cancer, and individual susceptibility to this type of cancer has been investigated in relation to the ability to activate or detoxify carcinogens, such as B[a]P present in cigarette smoke. In this context, many studies have attempted to analyze thoroughly a hypothesis on the influence of tobacco smoking on lung cancer risk in association with polymorphism of GSTs [13, 28]. However, the present analyses of smoking habit (current, ex- and non-smokers) in all sub-groups of controls and lung cancer patients showed only statistically significant modifying role of smoking in the development of lung cancer.

Neither the association between two genetic polymorphisms of glutathione S-transferase GSTM1 and GSTP1, and lung cancer development in the population of Polish origin, nor an independent or interactive role of "high risk" *GSTM1* and *GSTP1* genotypes in different histological types of cancer has been proved in our study. We have only observed non-statistical over-representation of *GSTM1 null* genotypes among SCC individuals. Nevertheless, we believe that polymorphism merits further studies. A constantly increasing proportion of ex-smokers in the population, and the change in cigarette composition expressed by a lower content of nicotine and tar, but an increasing amount of nitrate that enhances the of N-nitrozamines level [21], arise the need to develop a good research model of gene-environment interaction with special respect to a history of tobacco smoking. Moreover, to clarify contradictory results, the development of a carefully designed study with sufficient sample sizes and consideration of multifunctional and complex etiology of cancer in the general population is required.

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