International Journal of Occupational Medicine and Environmental Health, Vol. 14, No. 2, 99—113, 2001

SIGNIFICANCE OF GENETIC POLYMORPHISMS IN GLUTATHIONE S-TRANSFERASE MULTIGENE FAMILY AND LUNG CANCER RISK

EDYTA RESZKA and WOJCIECH WĄSOWICZ

Department of Toxicology and Carcinogenesis
Nofer Institute of Occupational Medicine
Łódź, Poland

Abstract. A vast number of studies are focused on investigating genetic polymorphism in order to estimate genetic contribution to the development of cancer. Possible cancer susceptibility genes have been sought among oncogenes, tumor suppressor genes, DNA repair genes and genes encoding phase I and phase II enzymes. Large individual differences in the biotransformation of xenobiotics have been explained on the basis of genetic polymorphisms in some detoxifying enzymes, regardless of environmental and occupational exposure. Among these enzymes, glutathione S-transferases (GST) constitute a large multigene family of phase II enzymes involved in detoxification of potentially genotoxic chemicals. Five genetic polymorphisms of GST have been well documented. Total or partial deletions and (or) single nucleotide polymorphisms in alleles encoding GSTM1, GSTM3, GSTP1, GSTT1, GSTZ1 are associated with reduction of enzymatic activity toward several substrates of different GST isoenzymes. In addition, molecular epidemiology studies indicate that a single genetic polymorphism of glutathione S-transferase appears to be a moderate lung cancer risk factor. However, the risk is higher when interactions with more GST polymorphisms and other risk factors (e.g. cigarette smoking) occur. Individuals with decreased rate of detoxification, with "high risk" glutathione S-transferase genotypes have a slightly higher level of carcinogen-DNA adducts and more cytogenetic damages.

Key words:
Glutathione S-tranferase, Lung cancer, Susceptibility, Polymorphism, Epidemiology, Xenobiotics

INTRODUCTION

People living in industrialised countries are at risk of developing cancer, asthma, diabetes and cardiovascular diseases due to extensive exposure to environmental and occupational pollutants, which contribute to genetic material damage due to DNA mutations and DNA adduct formation. Mutations in oncogenes and tumor-suppressor genes result in uncontrolled cell growth and cancer. However, owing to individual susceptibility or resistance to carcinogens not everybody develops cancer. Therefore, the study of cancer risk must be evaluated with respect to genetic predisposition, and environmental and occupational exposure to carcinogens. The genetic predisposition results from differences in metabolism of genotoxic compounds and DNA repairing mechanisms. In fact, genetic differences in expression and activity of the xenobiotic metabolizing enzymes are due to the existence of polymorphic alleles encoding these enzymes. The influence of genetic polymorphism on proper or altered enzyme activity has been well documented for several enzymes engaged in activating (phase I), as well as in conjugating (phase II) exo- and endogenous xenobiotics [1].

Glutathione S-transferases (GST), a large group of dimeric enzymes play a critical role in the defence

Address reprint requests to Ass. Prof. W. Wąsowicz, Department of Toxicology and Carcinogenesis, Nofer Institute of Occupational Medicine, P.O. Box 199, 90-950 Łódź, Poland.

against oxidative stress products and various electrophilic compounds. GST, as phase II metabolic enzymes, catalyse the conjugation of reduced glutathione with potential genotoxic substances, especially those from tobacco smoke. Many studies have demonstrated that individual differences in GST activity are the result of genetic polymorphism of these enzymes. Indeed, glutahione S-transferases are products of the gene superfamily, some of them polymorphic due to deleted or mutated alleles. Based on sequence homology and chromosomal location, glutathione S-transferases present in the human cytosolic fraction have been divided into several classes: alpha (GSTA), mu (GSTM), pi (GSTP), theta (GSTT), sigma (GSTS), kappa (GSTK) and zeta (GSTZ) [2,3]. Within the GSTs superfamily, five allelic variants in autosomal gene locus have been well documented: *GSTM1*, *GSTM3*, *GSTP1*, *GSTT1* and *GSTZ1*. Table 1 presents major alleles and chromosomal location of polymorphic glutathione S-transferases.

GENETIC POLYMORPHISM IN GST GENES

Genes encoding GSTM isoforms were localised in 1p13.3 chromosome in the following sequence: 5'-*GSTM4-GSTM2-GSTM1-GSTM5-GSTM3*-3' [4]. GSTM1 isoform demonstrates genetic polymorphism through the existence of 3 enzyme encoding alleles: *GSTM1*0* with partial or total gene sequence deletion, *GSTM1*A* and *GSTM1*B*, which differ in single base at 534 position (C→G substitution). Catalytically active enzyme is encoded by the *GSTM1*A* and *GSTM1*B* genes and the absence of GSTM1 enzyme activity is caused by deletion of both copies of the *GSTM1* gene. Four possible phenotypes have been distinguished, resulting from homo- and heterozygotic combination of the *GSTM1*0*, *GSTM1*A* and *GSTM1*B* alleles encoding homo- and heterodimeric enzymes: GSTM1 A, GSTM1 B, GSTM1 A,B, GSTM1 null [5,6]. Most studies are based on the assumption that two positive alleles: *GSTM1*A* and *GSTM1*B* are equally protective against genotoxic compounds [1,7,8]. However, Perret et al. [9] found greater frequency of

Table1. Genetic polymorphism of glutathione S-transferases

Gene	Known alleles	Nucleotide change	Amino acid change	Chromosome location
GSTM1	*GSTM1*0*	gene deletion in intron 6		1p13.3
	*GSTM1*A*	C (exon 7, 534), wild type	Lys (codon 172)	
	*GSTM1*B*	G (exon 7, 534)	Asn (codon 172)	
GSTP1	*GSTP1*A*	A (exon 5, 313), wild type C (exon 6, 341)	Ile (codon 105) Ala (codon 114)	11q13.3
	*GSTP1*B*	G (exon 5, 313) C (exon 6, 341)	Val (codon 105) Ala (codon 114)	
	*GSTP1*C*	G (exon 5, 313) T (exon 6, 341)	Val (codon 105) Val (codon 114)	
	*GSTP1*D*	A (exon 5, 313) T (exon 6, 341)	Ile (codon 105) Val (codon 114)	
GSTZ1	*GSTZ1*A*	A (exon 3, 94) A (exon 3, 124)	Lys (codon 32) Arg (codon 42)	14q24.3
	*GSTZ1*B*	A (exon 3, 94) G (exon 3, 124)	Lys (codon 32) Gly (codon 42)	
	*GSTZ1*C*	G (exon 3, 94) G (exon 3, 124)	Glu (codon 32) Gly (codon 42)	
GSTM3	*GSTM3*A*	wild type		1p13.3
	*GSTM3*B*	3 bp deletion in intron 6		
GSTT1	*GSTT1*0*	gene deletion		22q11.2
	GSTT1(positive)*			

GSTM1 A genotype among patients with pituitary tumors from the United Kingdom (34%) than in the control group (27%) which indicates a stronger protective role of *GSTM1*B* gene than *GSTM1*A*. A similar prevalence in frequency of *GSTM1 A* genotype in lung cancer individuals compared with the control group of North-West Mediterraneans may suggest a different catalytic activity of GSTM1 isoenzyme encoded by these two alleles. It is also considered that GSTM3 genetic polymorphism due to deletion of a 3 base-pair fragment in intron 6 and the presence of a recognition motif for transcription factor YY1 in *GSTM3*B*, appears to be strictly connected with *GSTM1* gene. To-Figueras et al. [10] found prevalence of individuals with both *GSTM1*A* and *GSTM3*B* alleles, suggesting association of *GSTM1*A* with an increased frequency of *GSTM3*B* and linkage disequilibrium between both GSTM polymorphic genes. *GSTM1* and *GSTM3* genes are located in chromosome 1 cluster and it has been suggested that this disequilibrium linkage is also due to the low probability of recombination between both alleles. Another data showed that individuals with different GSTM1 genotypes vary in GSTM3 expression in lungs. To date, subjects with *GSTM1*A* and *GSTM3*B* genes seem to express more GSTM3 than those with *GSTM1*0*/*GSTM3*A* or *GSTM1*B*/*GSTM3*A* combined alleles, because *GSTM3*A* sequence is not recognized by transcription factor YY1 [11].

Genetic polymorphism of GSTP1 (11q13.3 chromosomal location) results from transition of G to A in exon 5, which causes isoleucine (Ile) to valine (Val) replacement at codon 105. Point mutation at exon 5 strongly affected GSTP1 enzyme efficiency because this substitution is located in the hydrophobic substrate-binding site. The isoforms encoded by *GSTP1*A* and *GSTP1*B* alleles show different catalytic activity toward carcinogenic electrophiles and Ile to Val replacement can cause reduction of activity of GSTP1 isoenzyme, which elevates cancer risk [3,12]. Exon 5 GSTP1 genetic polymorphism occurs in 3 existing genotypes: *GSTP1*A*/*A* (Ile/Ile), *GSTP1*A*/*B* (Ile/Val) and *GSTP1*B*/*B* (Val/Val). Another well-known genetic polymorphism of GSTP1 is connected with Ala to Val change at 114 codon [13-14]. On this basis, another nomenclature system for GSTP1 genetic polymorphism is suggested (Table 1) [16].

Genetic polymorphism of GSTT1 (chromosome location – 22q11.2) results from gene deletion. Therefore, in human population there are two distinct genotypes: *GSTT1 positive* with proper activity and *GSTT1 null* with lack of expression and elevated cancer risk [13,17].

Two polymorphic sites for glutathione S-transferase Z1 have been established within exon 3 of *GSTZ1* gene, due to A to G transitions at 94 and 124 nucleotides. On this basis, three genotypes of GSTZ1 have been identified: *GSTZ1*A* ($A^{94}A^{124}$), *GSTZ1*B* ($A^{94}G^{124}$), *GSTZ1*C* ($G^{94}G^{124}$). These A to G point mutations at the encoding region of *GSTZ1* cause changes in the amino acids sequence in codons 32 (Lys → Glu) and 42 (Arg → Gly), respectively. The analysis of the activity of recombinant GSTZ1 proteins towards different α-haloacids displayed that detoxification of dichloroacetate and R-2-chloropropionate by GSTZ1*A is higher than by GSTZ1*B and GSTZ1*C [2].

INDIVIDUAL AND ETHNIC VARIATIONS IN GST GENOTYPES

Glutathione S-transferases are widely distributed and expressed in many mammalian tissues in a tissue- and cell type-specific manner. Many studies have shown evidence of individual variations in GST expression in different tissues. The *GSTM1*0* alleles are strictly connected with the lack of activity of GSTM1 isoenzyme due to total or partial deletion of these genes. In this respect, a correlation between genotype and phenotype of GSTM1 has been confirmed, because phenotyping assays showed lower activity of the enzyme in leukocytes of subjects with lacking genes of GSTM1, as measured with trans-stilbene oxide (tSBO) as a substrate [18–21]. Genotype-derived differences have been observed also for GSTP1. Genotypes with mutated genes, namely: *GSTP1*A*/*B* (Ile/Val) and *GSTP1*B*/*B* (Val/Val) are considered to be strongly associated with susceptibility to different diseases, mainly to cancer, which is closely related to lower protection against carcinogenic compounds provided by enzymes. The individuals with *GSTP1*B* mutated alleles

have distinctly lower activity of GSTP1 in lung tissue than those with homozygotes *GSTP1*A/*A* (Ile/Ile) [22].

The influence of GSTM1 genetic polymorphism on lung cancer risk probably depends on detoxification of carcinogens in the liver, where this form of glutathione S-transferase and GSTT1 isoenzyme is very abundant [8,23]. In people with *GSTM1 null* genotype, high concentration of bioactivated compounds may influence the lung through the bloodstream, after phase I detoxification enzymes have acted. Due to very low expression of GSTM1 isoform in the lung, the GSTM1 genotype has probably no substantial role in xenobiotic detoxification in this tissue. GSTM3 isoform, which is very common in the lung, seems to play the most critical role in the determination of lung cancer risk [20]. GSTP1 is also abundant in peripheral parts of the lung, mainly in alveoli, alveolar macrophages, respiratory bronchioles [3], human endometrium [24] and pancreas [25]. This isoenzyme is widely distributed in gastrointestinal tract, but less than GSTT1 [26].

Genetic polymorphism of GST strongly influences the activity and expression of the dimeric cytosol enzymes, which show distinct interindividual differences. A study on normal epithelial cells from subjects with or without bronchogenic carcinoma showed different expression levels of GST. The levels of GSTP1, GSTM3, GSTM1,2,4,5 and GSTT1 were significantly lower in normal bronchial epithelial cells from individuals with bronchogenic carcinoma, although only in the case of GSTP1 and GSTM3, the mRNA level was significantly different (6.11 mRNA/10^3 β-actin mRNA vs. 26.0 mRNA/10^3 β-actin mRNA and 0.09 mRNA/10^3 β-actin mRNA vs. 0.29 mRNA/10^3 β-actin mRNA, respectively) [27].

Glutathione S-transferases have a wide activity range towards different substrates, and usually GST isoenzymes have distinct but overlapping substrate specificity and catalytic efficiency. Because GST genetic polymorphism seems to be associated with increased risk for tobacco-related cancers, particularly lung [12] and bladder [28], several researchers have tried to explain the mechanisms of such relationships using *in vivo* biotransformation assays. Compounds, which are particularly biotransformated by GST, derive mainly from tobacco smoke, such as polycyclic aromatic hydrocarbons (PAHs) and nitrosamines. The detoxification role of GSTM1 is mainly focused on arene oxides, including the ultimate carcinogenic form of benzo[a]pyrene (BP), BP-epoxide (7,8-epoxide) or BP-diol epoxide (7,8-diol-9,10-epoxide), the metabolites, which are derived from the activity of P450 monooxygenases and epoxide hydrolase. Glutathione S-transferase M1 also metabolises other carcinogenic epoxides, aflatoxin B1 7,8-epoxide and tSBO, by conjugation with reduced glutathione [1,8,29–32]. GSTP1 and GSTM1 have a similar catalytic activity toward BP-epoxide and BP-diol epoxide, which is also detoxified by GSTA1 isoenzyme [29]. GSTT1 isoenzyme is of high toxicological interest as it catalyses the conjugation of a number of low-molecular-weight industrial chemicals: methyl, ethyl, propyl halomethanes, ethylene oxide [33], methylene dihalides [34], and benzene [35]. *GSTT1 null* individuals have lower GST activity toward cumene hydroperoxide, but with non-significant differences in peroxidation-dependent changes (MDA formation) in *GSTT1 null*, as well as *GSTT1 positive* individuals [36]. Combined nulled genotypes of GSTM1 and GSTT1 may cause individual susceptibility to a potent anticholinesterase inhibitor, tacrine hydrochloride, a drug used in the treatment of Alzheimer's disease [32].

Genetically determined individual differences in metabolising different hazardous exogenous and endogenous compounds make people more or less likely to develop cancer. It is well known that three major human races (Caucasian, Mongoloid and Negroid) differ genetically in frequencies of alleles encoding polymorphic phase I and phase II enzymes. As ethnic differences in reactions to xenobiotics, and therefore, in the distribution of GSTM1 genotypes can also affect the risk of lung cancer, ethnic homogeneity of the studied populations appears to be very important in investigations. *GSTM1 null* genotype has the highest prevalence in Mongoloids, the lowest in Negroids, while *GSTM1*0* Caucasian homozygotes have 50% of distribution. Similarly, polymorphisms in exon 5 GSTP1 and GSTM3 have quite different distribution patterns in the three ethnic groups. Table 2 shows distribution of GST genotypes in the three major races.

Table 2. Frequency of glutathione S-transferase genotypes in three major ethnic groups

Population	Frequency (range)				References
	GSTM1 A	*GSTM1 B*	*GSTM1 A,B*	*GSTM1 null*	
Caucasians	0.30–0.16	0.30–0.07	0.18–0.01	0.54–0.46	[9,10,37–39]
Mongoloids				0.44–0.66	[40,41]
Negroids				0.16–0.27	[42,]43
	Exon 5				
	*GSTP1*A/*A*	*GSTP1*A/*B*	*GSTP1*B*/B*		
Caucasians	0.38–0.54	0.34–0.51	0.05–0.16		[22,44–46]
Mongoloids	0.61–0.72	0.25–0.44	0.03–0.04		[3,14,47]
Negroids	0.35	0.46	0.19		[22]
	Exon 6				
	*GSTP1*A/*A*	*GSTP1*A/*B*	*GSTP1*B/*B*		
Caucasians	0.82–0.75	0.25–0.18	0		[22,44]
Mongoloids	1	0	0		[3]
Negroids	0.95	0.05	0		[22]
	*GSTM3*A/*A*	*GSTM3*A/*B*	*GSTM3*B/*B*		
Caucasians	0.65–0.58	0.37–0.31	0.05–0.04		[10,43]
Negroids	0.11	0.41	0.48		[43]
				GSTT1 null	
Caucasians				0.12–0.44	[17,48]
Mongoloids				0.39–0.62	[47,49]
Negroids				0.22	[17]

EVIDENCE OF GST AS A CANCER SUSCEPTIBILITY GENES

Genetic polymorphism of GST has been investigated in many molecular epidemiological studies as a factor of susceptibility to cancer development in different types of malignancies. It has been suggested that individuals with GST deleted genes: *GSTM1*0*, *GSTT1*0* and mutated genes: *GSTP1*B* (Ile^{105}Val), *GSTM3*A*, leading to changes in GST catalytic activity, are more susceptible to genotoxic chemicals. Although there is no clear correlation between the development of cancer and hereditary GST genotype, the frequency of deleted or mutated alleles among ethnically different populations suggests that individuals carrying these defective genes have an increased risk of cancer at a number of sites, oral [16,50],

Table 3. Case-control studies involving glutathione S-transferase genotypes and lung cancer risk

Population	Lung cancer risk associations (OR, 95% CI)	References
Finnish	*GSTM1 null* 1.45 (0.90–2.32)	[54]
US Caucasians	*GSTM1 null* 1.10 (0.8–1.4)	[55]
US Mexican-Americans	*GSTT1 null* 1.50 (0.7–3.5)	[17]
US African-Americans	*GSTT1 null* 1.20 (0.7–2.2)	[17]
European Caucasians	*GSTP1*B/*B* 1.90 (1.04–3.47)	[12]
Mediterranean Caucasians	*GSTP1*B/*B* 1.18 (0.67–2.07)	[15]
US Mexican-Americans	*GSTM1 null* and *GSTT1 null* 3.41 (1.0–12.9)	[17]
US African-Americans	*GSTM1 null* and *GSTT1 null* 1.4 (0.5–3.8)	[17]
Japanese	*GSTM1 null* and *GSTP1*B/*B* 2.47 (1.15–5.32)	[14]
Mediterranean Caucasians	*GSTM1 null* and *GSTM3*A/*A* 2.14 (1.08–4.25)	[10]

gastric [51], colorectal [48,52], bladder [28], breast [53], and lung. However, single polymorphisms in GST genes have occurred to be a minor lung cancer risk factor, as presented in Table 3. In multianalysis of several case-control studies among Caucasians and Mongoloids with *GSTM1 null* genotype the odds ratio (OR) value was estimated at about 1.17 (95% confidence interval (CI): 0.98–1.40) [56], the result similar to that obtained in case-control studies performed in the USA, where *GSTM1 null* or *GSTT1 null* individuals were at a moderate risk of lung cancer [17,42]. However, when lung cancer patients from Finland were investigated, significantly higher distribution of *GSTM1*0/*0* genotype compared with controls allowed estimating the risk of lung cancer at 1.5 (95% CI: 0.9–2.3) [54]. On the other hand, point mutations at 105 codon of GSTP1 gene appear to be strongly associated with the development of lung cancer. In an investigation of Norwegian lung cancer patients more individuals with both copies of mutated genes (*GSTP1*B/*B* genotype) were found in the group with cancer than in the control group, with OR of 1.9 (95% CI: 1.04–3.47) [12]. A single polymorphism, even if it modifies, may have little influence on general individual genetic susceptibility, which probably occurs as a result of allelism of several GST loci. This has been confirmed by studies concerning different GST genetic polymorphisms in potential co-operation in protection against carcinogens. Kelsey et al. [17] estimated adjusted odds ratio (OR = 1.1, 95% CI: 0.7–1.6) for *GSTM1 null* lung cancer patients from Texas. However, when the data were stratified by ethnicity, no relationship was found between homozygous deletion of *GSTM1* and lung cancer status for African-Americans with OR = 1.0 (95% CI: 0.5–1.8), while among Mexican-Americans the risk was twice as high (OR = 1.8, 95% CI: 1.0–3.3). A similar prevalence of *GSTT1 null* genotype was observed for the two ethnic groups with lung cancer. Although OR was 1.4 (95% CI: 0.9–2.3), no association was shown between the *GSTT1 null* genotype and overall lung cancer. The comparison of genetic polymorphism of two enzymes engaged in detoxification of PAHs, led to very interesting results. The risk of lung cancer was almost 3-fold higher (95% CI: 1.1–1.7) for individuals with both homozygous deletions, while in the patients with only one possible null genotype it was similar to that in the control group. As GSTM3 has been found to be polymorphic in humans, its frequency was also investigated. The distribution of GSTM3 genotypes showed no significant differences between controls and Catalonia patients with diagnosed cancer. However, GSTM3 genetic polymorphism analysed together with GSTM1 combined genotype (*GSTM1 null* and *GSTM3*A/*A*) appeared to increase the risk of lung cancer because of significant overrepresentation of lung cancer individuals with these alleles and with OR = 2.14 (95%CI: 1.08–4.25) [10]. Investigations of Japanese smokers with lung cancer showed no significant differences in GSTP1 genotype in comparison with the control group. When high cancer risk genotypes, *GSTM1 null*, *GSTP1*A/*B* or *GSTP1*B/*B*, were compiled, the risk of lung cancer was 2.58 (95% CI: 1.26–5.30) in male smokers, while among wild-type homozygous (*GSTP1*A/*A*) lung cancer patients with *GSTM1 null* genotype, it was about 1.17 (95% CI: 0.77–1.79) [14]. Lung cancer patients of Caucasian origin from Catalonia showed a similar distribution of GSTP1 genotypes to that found in the general population and in healthy smokers without any significant differences. To date, no association has been found between lung cancer risk and a combination of at-risk genotypes: *GSTM1 null*, *GSTP1*B/*B* and *GSTT1 null*, although the results of this study seem to confirm the hypothesis that *GSTM1 null* genotype has a slight, albeit consistent influence on the development of lung cancer with OR about 1.4 [15].

Lung cancer risk can be evaluated if additional factors, which strongly influence heterogeneity of the investigated individuals, are taken into account, such as: ethnic diversity, age, gender, histopathological type of cancer and occupational and environmental exposure, including the history of tobacco smoking.

The association between lung cancer susceptibility and deletion of both copies of GSTM1 genes was found to be similar in two investigated populations from Los Angeles: African-Americans (OR = 1.20, 95% CI: 0.72–2.00) and Caucasians (OR = 1.37, 95% CI: 0.91–2.06) in spite of a significantly lower frequency of *GSTM1 null* genotype in

the former group (0.27 versus 0.52) [42]. In another study the risk of lung cancer in Mexican-Americans with this genotype was almost twice as high as in Caucasians [17].

In several studies, the age and gender of the investigated subjects were also taken into consideration in order to estimate the risk of lung cancer. In most of them, the frequency of nulled or mutated GST alleles was more frequent among younger individuals, probably due to elimination of the older ones. Lung cancer risk for *GSTM1 null* in French male patients estimated by the crude OR was 1.3 (95% CI: 0.9–1.8). In addition, lung cancer risk was higher in younger subjects, under 60 years of age (OR = 1.6, 95% CI: 0.6–1.7). This age-related dependence showed similar tendency for each separate histological type of cancer [57]. Alexandrie et al. [18] have also found a much higher risk of lung cancer among Swedish lung cancer patients below 66. When the Norwegian patients were stratified according to age, the highest frequency of those with mutated Val allele (*GSTP1*A/*B* and *GSTP1*B/*B* genotypes) was found among patients below 50 years of age. Similar results of greater frequency of *GSTM1 null* genotype were obtained for patients under 56 years of age [12]. The percentage of *GSTM1 null* genotype was higher, although without statistical significance, among Chinese lung cancer females (70%) than in males (58%) and a greater frequency of this genotype was found in patients under 55 than in older ones (≥55 years old) (70% vs. 60%) [41].

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 ability to activate or detoxify carcinogens, such as PAHs present in cigarette smoke. In this context, many studies have tried to analyse in detail a hypothesis on the influence of tobacco smoking on lung cancer risk with association with polymorphism of GST. Some investigators have reported a stronger association between lung cancer risk and *GSTM1 null*, *GSTP1*A/*B* or *GSTP1*B/*B* genotype among heavy smokers [41,54]. However, several studies showed contradictory results of stronger association for low or moderate doses of smoking [12,55]. When the analysis of lung cancer susceptibility in relation to *GSTM1 null* genotype was restricted to smoking history in a population in Los Angeles, there was no significant difference in lung cancer risk between former and current smokers. Frequency of homozygous deletion of *GSTM1* allele among smoking patients was significantly different between smokers with a history of less than 40 pack-years and with greater lifetime smoking (OR = 1.77, 95% CI: 1.11–2.82 vs. OR = 0.90, 95% CI: 0.56–1.44). The association between lung cancer and *GSTM1 null* among lighter smokers increased considerably in the case of squamous cell carcinoma patients with the value of OR = 4.06 (95% CI: 1.77–9.31) [42]. Similar results concerning association of GSTM1 genotype with the dose of smoking were obtained in a study of Chinese lung cancer patients. Lighter smokers (<37 pack-years) with lack of *GSTM1* gene appeared to be more susceptible to lung cancer development (OR = 3.97, 95% CI: 0.95–16.52) than heavier smokers (≥37 pack-years) and only for the first group the frequency of GSTM1 genotypes was significantly different from that in hospital control individuals [58]. Another Chinese study showed prevalence of *GSTM1 null* lung cancer patients, who smoked heavier (>25 pack-years) over those with less smoking dose (≤25 pack-years), namely 73% vs. 56% without statistical significance [41]. To date, investigations among patients of Polish origin showed lack of GSTM1 and GSTT1 genotypes association with susceptibility to another smoking-related larynx cancer, because of prevalence of *GSTM1* and *GSTT1* nulled controls in comparison with cancer patients (57.7% vs. 49.1%, and 21.7% vs. 17.5%) [59]. Studies of French male lung cancer subjects also showed statistically insignificant dependence between smoking category and homozygous absence of GSTM1 allele. According to the histological type, slight differences were observed in the distribution of this genotype. The highest frequency was noted in patients with adenocarcinoma, which increased with the pack-years category, although only significantly higher OR was found for small cell carcinoma affecting the medium smoking category from 25 to 40 pack-years (OR = 7.2, 95% CI: 1.3–39.4) [57]. A study of Finnish lung cancer patients showed distinct but not statistically significant association between duration

of exposure to tobacco smoke and the risk of cancer. Smoking cancer patients were divided into two groups, depending on the smoking history, less than 40 pack-years and 40 or more pack-years, a higher prevalence of *GSTM1(-)* genotype was found in heavier smokers with an OR of 3.96 (95% CI: 0.74–21.3) than in all lung cancer smokers (OR = 1.38, 95% CI: 0.58–3.32) (24). Another study of Finnish male smokers showed that the risk of lung cancer significantly increased with longer period of smoking, manifesting a stronger association among *GSTM1 null* individuals, but the relation between GSTM1 genotype and smoking was not statistically significant. The association between smoking duration and *GSTM1 null* genotype was weaker among patients supplemented with 50 mg/day of α-tocopherol for 5–8 years than in those without α-tocopherol supplementation. Furthermore, supplementation of 20 mg β-carotene a day for the same period did not influence the relation between smoking period and lung cancer risk [60]. Investigations on a US population from Massachusetts have shown an increased risk of lung cancer only for light and medium smokers with *GSTM1 null*, while for *GSTM1 positive* individuals the risk of lung cancer rose with the increased number of pack-years. This suggests a negative relationship between the dose of smoking and lung cancer development among *GSTM1 null* subjects [55]. Another study of Norwegian male lung cancer patients showed significant increase in the frequency of potential risk of cancer for genotypes: *GSTP1*A/*B* and *GSTP1*B/*B* and *GSTM1 null* among individuals with short lifetime smoking or lower pack-years index [12].

Several investigators have tried to find a stronger association between GST genetic polymorphism and lung cancer risk, taking into account histopathological type of lung cancer. However, several studies showed a similar risk of lung cancer regardless of the histopathological type. When investigating two lung cancer patient groups: African-American and Caucasian from Los Angeles, the strongest association between *GSTM1 null* genotype and lung cancer risk was found for squamous cell carcinoma (OR = 1.57, 95% CI: 0.93–2.63) regardless of other types differentiated by histology (adenocarcinoma, small-cell carcinoma, other types combined) [42]. An investigation referring to GSTM1 polymorphism in Norwegian lung cancer patients showed similar over-representation of *GSTM1 null* genotype among squamous cell carcinoma (OR = 1.7, 95% CI: 1.1–2.7) with increasing frequency for non-operable patients (OR = 3.9, 95% CI: 1.1–13.5) [30]. Another study on Norwegian male patients indicated an increased prevalence of *GSTM1 null* or *GSTP1*A/*B* and *GSTP1*B/*B* in the group with squamous cell carcinoma, but not in adenocarcinoma group, when the patients were analysed according to the major histological tumor types [12]. Small, but statistically significant distribution of *GSTM1 null* genotype was also observed among the UK individuals with squamous cell carcinoma, comparing with those with adenocarcinoma [21]. In Finnish individuals with this type of lung cancer, a statistically significant increase of *GSTM1*0/*0* genotype was detected, with OR of 2.1 (95% CI: 1.2–3.8). Furthermore, among patients with adenocarcinoma, there was no statistically significant association between the lack of GSTM1 allele and lung cancer risk [13]. These results are opposite to those obtained in studies of French smoking lung cancer male patients, in which no relationship was observed between *GSTM1 null* allele and squamous cell carcinoma. The frequency of homozygous deletion for the other two histopathological types of lung cancer was higher, with OR = 1.7 (95% CI: 0.9–3.2) for small cell carcinoma and OR = 2.0 (95% CI: 1.1–3.6) for adenocarcinoma. However, the differences in the distribution of *GSTM1 null* genotype in all sub-groups of subjects based on histopathological diagnoses, were not statistically significant [57]. Similar over-representation of *GSTM1(-)* genotypes was observed in Swedish individuals with adenocarcinoma and small cell carcinoma, while this deficient genotype was common among 72% of female squamous cell carcinoma (OR = 3.3, 95% CI: 1.2–9.7) [18]. The case-control study of Chinese lung cancer patients showed the greater frequency of *GSTM1 null* genotype in the adenocarcinoma group than in controls (76.9% vs. 49.2%), with OR for this type of cancer 3.42 (95% CI: 1.23–9.51) [58]. When Japanese male smokers with lung cancer were investigated, the significantly elevated number of smoking patients with squamous cell carcinoma, carrying two

copies of *GSTP1*B* allele, was detected (7.2%) compared with smoking controls (1.6%). It should be noted that a slightly higher adjusted OR value for those with squamous cell carcinoma and with mutated or deleted genotypes: *GSTM1 null*, *GSTP1*A*/**B* or *GSTP1*B*/**B* was obtained (2.67, 95% CI: 1.09–6.55) [14].

GENE-CARCINOGEN INTERACTIONS

Glutathione S-transferases play a very important role in the protection against different hazardous agents, which may be responsible for the development of environmentally induced cancers. Since GSTM1 catalyses a large number of potential genotoxic compounds, several investigations have been undertaken to look into the effect of GSTM1 genetic polymorphism on the metabolism and toxicity of xenobiotics, in which DNA-adducts generation, cytogenetic damages and susceptibility to cancer development were observed. The individuals without false metabolizing enzyme expression are probably more susceptible to the development of different malignancies, especially after environmental and occupational exposure. Several investigators have focused on the correlation between cytogenetic damage and genetic polymorphism of phase I and phase II enzymes engaged in xenobiotics metabolism. As it is suggested that GST participate in the scavenging of PAH derivatives, it seemed important to find out whether GST polymorphic isoforms exerts any effect on DNA adduct levels, chromosomal aberration (CA) and changes in micronuclei (MN) caused by electrophilic substances [1]. A number of studies show significantly higher PAH-DNA adducts and higher levels of cytogenetic changes among lung cancer patients with deleted or mutated GST genes than among those with properly expressed enzymes. Norwegian lung cancer male patients with the lowest level of hydrophobic DNA adducts had a similar GSTP1 genotype frequency pattern as healthy controls. Significantly higher mean of DNA adducts was found in patients with one or two GSTP1 mutated allele: *GSTP1*A*/**B* or *GSTP1*B*/**B* contrary to those with wild type genotype of GSTP1. Moreover, the highest level of adducts was found among *GSTP1*B*/**B* individuals, though without statistically significant difference in the *GSTP1*A*/**B* group. Lung cancer patients with homozygous deletion of GSTM1 gene also showed the highest level of PAH-DNA adducts. The analysis of the combined effect of two polymorphisms indicated significantly higher level of DNA damages in individuals with *GSTM1 null* and *GSTP1*A*/**B* or *GSTP1*B*/**B* genotypes than in those with all other genotype combinations. In conclusion, the wild type genotypes might therefore play a protective role against the damage of DNA by reactive PAH derivatives [12]. A study of PAH-DNA adducts in the US smokers in relation to the levels of antioxidant vitamins (E, C, A, β-carotene) and genetic polymorphism of GSTM1 showed no significant difference in PAH-DNA adducts in individuals with homozygous *GSTM1* deletion compared to those with *GSTM1 positive* genotype. A negative association between PAH-DNA adducts and α-tocopherol and ascorbic acid levels measured in serum was found only among *GSTM1 null* persons, which may suggest a protective role of these two antioxidants against formation of PAH-DNA adducts in *GSTM1 null* individuals [61]. Matsui et al. [62] estimated the level of oxidative DNA damage in human breast cancer tissue by measuring 8-hydroxy-5'-deoxyguanosine (8-OhdG) as a result of hydroxyl radical interaction with DNA. Individuals with *GSTP1*A*/**A* genotype had significantly higher level of 8-OhdG and of DNA damages in breast tissue, when compared to *GSTP1*A*/**B* and *GSTP1*B*/**B* genotypes (1.91 ± 0.79 vs. 2.43 ± 1.19).

Cytogenetic tests: CA, MN and sister chromatid exchanges (SCE), are often applied in exploring genetic polymorphisms of xenobiotic metabolising enzymes. Deleted or mutated encoding enzyme alleles are suspected genetic factors causing cytogenetic damages. When one of the genotoxins, diepoxybutane (DEB), a reactive metabolite of butadiene (BD), was studied, a significant increase in SCE frequency was observed among *GSTT1 null* individuals as compared to positive ones after DEB treatment, while no statistically significant changes in this test were obtained for *GSTM1 null* persons. There were no differences between *GSTT1 null* and *GSTT1 positive* individuals in chromosome aberrations test. Similar results were observed for two

GSTM1 genotypes [63]. However, *in vivo* 4-(methylnitrosoamino)-1-(3-bipirydyl)-1-butanone induced genotoxicity in cultured human lymphocytes showed that cells with *GSTM1 null* genotype had significantly increased CA compared with *GSTM1 positive* cells [31].

Hemoglobin adducts in acrylonitryle workers were investigated with regard to polymorphism of glutathione S-transferase M1 and T1. Biomonitoring of N-(cyanoethyl)valine (CEV), N-(hydroxyethyl)valine (HEV) and N-(methyl))valine (MV) showed no influence of this polymorphism on the adduct level in either *GSTM1 positive* or *GSTM1 negative* workers. *GSTT1 null* individuals have MV and HEV adduct levels, while there was no consistent effect of the GSTM1 and GSTT1 genotypes on CEV adduct levels induced by acrylonitryle exposure [33].

When genetic GST polymorphism, carcinogen-DNA adducts and the level of cytogenetic damages were investigated, occupational risk was also often taken into account. A study on the influence of genetic polymorphism of GSTM1 on anti-BPDE-DNA adduct levels in mononuclear white blood cells showed a higher risk of adduct formation in *GSTM1 null* workers of Caucasian origin exposed to high level of polycyclic aromatic hydrocarbons. In the investigated coke-oven workers, the majority consisted of exposed workers with adduct levels exceeding the 95th percentile control value (4.4 adducts/10^8 nucleotides), which was significantly higher in *GSTM1 null* individuals with OR = 17.3 (95% CI: 3.78–78.6) [64]. A similar increase in PAH-DNA adducts in peripheral blood mononuclear cells among *GSTM1 null* coke-oven workers from Italy was found especially in smoking individuals with high PAH exposure. Surprisingly, the elevated level of PAH adducts was observed among *GSTT1 positive* workers in comparison with *GSTT1 null* subjects [65]. Another Italian investigation demonstrated that a higher occupational PAH dose exerted a greater effect of the polymorphic "high risk" GSTM1 genotype on the PAH-DNA adduct levels among coke-oven workers [66]. The formation of BPDE-DNA adducts as a result of B[a]P exposure was investigated in leukocytes of French male coke-oven workers. No significant difference in BPDE-DNA adduct levels was found between individuals with *GSTT1 positive* and *GSTT1 null* genotypes. In contrast, about 93% of *GSTM1 null* coke-oven workers had the level of BPDE adducts with a median of 1.6 adducts/10^8 nucleotides, while all of *GSTM1 positive* individuals did not show detectable BDPE-DNA adducts, namely <0.2 adducts/10^8 nucleotides [67]. A study of Swedish chimney sweeps showed no relationship between DNA adduct level and GSTT1 genetic polymorphism irrespective of their smoking status [68]. Investigations carried out in an aluminium plant in Sweden used 1-hydroxypyrene (1-OHP) as a biomarker of PAH exposure measured in urine. Individuals from the exposed and control groups had the highest 1-OHP levels, when carrying *GSTM1 null* and *CYP1A1 Ile/Val* genotypes. A possible impact of GSTM1 and GSTT1 genetic polymorphisms on 1-OHP urinary excretion was enhanced by smoking habit [69]. Aromatic DNA adduct levels were found to be slightly higher among Chinese coke-oven workers with GSTP1*B/*B genotype, compared with individuals carrying *GSTP1*A/*A* and *GSTP1*A/*B* genotypes, although it was not statistically significant [70].

When cytogenetic damages among workers under different occupational exposure were investigated, contradictory results were obtained. A cytogenetic study performed among workers from central Taiwan exposed to chromium compounds showed no influence of genetic polymorphism of GSTT1 and GSTM1 on SCE between the exposed and reference groups. Although a slightly increased frequency of SCE (7.1%) was found among chromium (Cr) workers with both deleted *GSTM1* genes, a similar result was seen in the control group. To the contrary, mean SCE levels were slightly higher among *GSTT1 positive* workers and controls with a decrease of 3.5% in SCE among *GSTT1 null* workers and of 2.5% among controls with the same genotype. Among Cr exposed workers, the prevalence of nulled genotypes of GSTM1 and GSTT1 was higher than in the controls. The results showed that the frequency of *GSTM1 null* genotype was 77.1% in Cr workers and 60% in controls, while the distribution of double nulled GSTT1 was 62.9% for exposed individuals and 42.9% among controls [71]. *GSTM1*0/*0* genotype was found to be associated with an increased level of chromosome damage in

investigated bus drivers from Copenhagen, but not in the postal workers [72]. No correlation between genetic polymorphism of GSTM1 and CA was found in Brazilian agricultural workers exposed to pesticides, probably due to very low frequencies of *GSTM1 null* genotype (33%) in this ethnic group [73]. Lucero et al. [74] observed no significant associations when analysing MN frequency in GSTM1 and GSTT1 polymorphisms of another group of agricultural workers from Spain. However, the *GSTT1 null* subject exposed to pesticides had higher proliferative indexes when compared to the control group.

Studies on individuals of Polish origin did not show statistically significant differences in PAH-DNA adducts in mononuclear white blood cells in adduct levels, regardless of genetic polymorphism of two GST isoforms. When the combined GSTP1 and GSTM1 genotypes among smokers, carriers of mutated *GSTP1* alleles: *GSTP1*A/*B* or *GSTP1*B/*B* and *GSTM1 null* genotype were investigated, a significantly higher level of PAH adducts in summer were found as compared with *GSTP1*A/*B* or *GSTP1*B/*B* and *GSTM1 positive* individuals ($5.60/10^8$ nucleotides vs. $1.82/10^8$ nucleotides). People with another combination of GST genes, *GSTP1*A/*A* and *GSTM1 null* genotype, have also significantly higher adducts ($4.13/10^8$ nucleotides) [75]. Another data, according to the investigation of polymorphic xenobiotic metabolising enzymes, and their possible effect on DNA aromatic adduct levels in healthy Polish female population, showed a lack of statistically significant differences between individuals with various genotypes. However, the highest level of DNA adducts was found among carriers of *GSTM1 null/CYP1A1 Ile/Val* genotypes ($8.00/10^8$ nucleotides) and individuals with *GSTP1*A/*A/CYP1 Ile/Val* genotypes ($7.00/10^8$ nucleotides), both measured in summer samples [76].

Certain isoenzymes of glutathione S-transferase may play a protective role as a part of the repair system after free-radical-induced lipid peroxidation, a possible mechanism of asbestos-related carcinogenesis. There have been a number of investigations on the role of GSTM1, GSTT1 and N-acetyltransferase (NAT2) in asbestos-associated pulmonary disorders. The risk for all (malignant and non-malignant) pulmonary disorders among patients with homozygous deletion of GSTM1 gene was at a moderate level (OR = 1.8, 95% CI: 0.3–3.4), which was lower for malignant diseases (OR = 2.3; 95% CI: 0.8–7.1), but frequencies observed for GSTM1 and GSTT1 genotypes were not significantly different. On the contrary, the distribution of *NAT2 slow-acetylator* genotypes was statistically higher in workers with malignant or non-malignant asbestos-induced diseases than in the control group; OR value for malignant mesothelioma was 3.8 (95% CI: 1.2–14.3). In addition, carrying a few potential risk genotypes increases the risk of malignant asbestos-associated pulmonary disorders, as the risk of mesothelioma in the investigated asbestos workers with this disease, i.e. *NAT2 slow acetylators* lacking the *GSTM1* allele, was almost eightfold higher (OR = 7.8, 95% CI: 1.4–78.7) [77].

The complex interplay between the genes and environment has been in focus for several years, being a great challenge to scientists who try to understand and reduce the development of diseases in humans. There is substantial evidence that GST, strongly involved in occupational- and environmental-origin xenobiotic detoxification, are associated with lung cancer. Lack or altered enzymatic activity as a result of carrying deleted or mutated GST alleles due to DNA and cytogenetic damages, can lead to cancer development. Although GSTM1, GSTM3, GSTP1, GSTT1 genetic polymorphisms may contribute to the lung cancer risk, especially if more than single GST polymorphism and other risk factors (e.g. occupational exposure, smoking habit) occur, the role of GST in cancer susceptibility remains unclear. The clarification of inconsistent results will require carefully designed studies with sufficient sample sizes and consideration of multifunctional and complex etiology of cancer developing in the general population. The conducted investigations will be a crucial contribution to understanding of the potential role of polymorphic glutathione S-transferases in cancer prevention.

REFERENCES

1. Autrup H. *Genetic polymorphisms in human xenobiotica metabolizing enzymes as susceptibility factors in toxic response.* Mutat Res 2000; 464: 65–76.
2. Blackburn AC, Tzeng HF, Anders MW, Board PG. *Discovery of a functional polymorphism in human glutathione transferase zeta by*

expressed sequence tag database analysis. Pharmacogenetics 2000; 1: 49–57.

3. Ishii T, Matsuse T, Teramoto S, Matsui H, Miyao M, Hosoi T, et al. *Glutathione S-transferase P1 (GSTP1) polymorphism in patients with chronic obstructive pulmonary disease.* Thorax 1999; 54: 693–6.
4. Kerb R, Brockmöller J, Sachse C, Roots I. *Detection of the GSTM1*0 allele by long polymerase chain reaction.* Pharmacogenetics 1999; 9: 89–94.
5. Fryer A, Zhao L, Alldersea J, Pearson WR, Strange RC. *Use of site-directed mutagenesis of allele-specific PCR primers to identify the GSTM1 A, GSTM1 B, GSTM1 A,B and GSTM1 null polymorphisms at the glutathione S-transferase, GSTM1 locus.* Biochem J 1993; 295: 313–5.
6. Fryer AA, Zhao L, Alldersea J. *The glutathione S-transferases: polymerase chain reaction studies on the frequency of the GSTM1 0 genotype in patients with pituitary adenomas.* Carcinogenesis 1993;14: 563–6,
7. Lemos MC, Cabrita FJ, Silva HA, Vivan M, Plácido F, Regateiro FJ. *Genetic polymorphism of CYP2D6, GSTM1 and NAT2 and susceptibility to hematological neoplasias.* Carcinogenesis 1999; 20: 1225–9.
8. Liu YH, Taylor J, Linko P, Lucier GW, Thompson CL, et al. *Glutathione S-transferase in human lymphocyte and liver: role in modulating formation of carcinogen-derived DNA adducts*. Carcinogenesis 1991; 12: 2269–75.
9. Perret CW, Clayton RN, Pistorello M, Boscaro M, Scanarini M, Bates A, et al. *GSTM1 and CYP2D6 genotype frequencies in patients with pituitary tumours: effects on P53, ras and gsp*. Carcinogenesis 1995; 16: 1643–5.
10. To-Figueras J, Gené M, Gómez-Catalán J, Piqué E, Borrego N, Marfany G, et al. *Polymorphism of glutathione S-transferase M3: interaction with glutathione S-transferase M1 and lung cancer susceptibility.* Biomarkers 2000; 5: 73–80.
11. Strange RC, Fryer AA. *The glutathione S-transferase: influence of polymorphism on cancer susceptibility*. In: Vineis P, Malats N, Lang M, d'Errico A, Caporaso N, Cuzick J, et al., editors. *Metabolic Polymorphisms and Susceptibility to Cancer.* Lyon: IARC Scientific Publications No. 148; 1999. p. 231–49.
12. Ryberg D, Skaug V, Hewer A, Phillips DH, Harries LW, Wolf CR, et al. *Genotypes of glutathione transferase M1 and P1 and their significance for lung DNA adduct levels and cancer risk.* Carcinogenesis 1997, 12: 1285–9.
13. Hirvonen A. *Polymorphisms of xenobiotic-metabolising enzymes and susceptibility to cancer*. Environ Health Perspect 1999; 107, Suppl. 1: 37-47.
14. Kihara M, Kihara M, 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 Lett. 1999; 137: 53-60.
15. To-Figueras J, Gene M, Gomez-Catalan J, Piqué E, Borrego N, Carrasco JL, et al. *Genetic polymorphism of glutathione S-transferase P1 and lung cancer risk*. Cancer Causes Control 1999; 10: 65-70.
16. Park JY, Schantz SP, Stern JC, Kaur T, Lazarus P. *Association between glutathione S-transferase π genetic polymorphisms and oral cancer risk.* Pharmacogenetics 1999; 9: 497–504.
17. Kelsey KT, Spitz MR, Zuo Z-F, Wiencke JK. *Polymorphisms in the glutathione*
S-transferase class mu and theta genes interact and increase susceptibility to lung cancer in minority populations (Texas, United States). Cancer Causes Control 1997; 8: 554–9.
18. Alexandrie AK, Ingelman Sundgerg M, Seidegĺrd J, Tornling G, Rannug A. *Genetic susceptibility to lung cancer with special emphasis on CYP1A1 and GSTM1: a study on host factors in relation to age at onset, gender and histological cancer types*. Carcinogenesis 1994;15: 1785–90.
19. Stewart RK, Smith GBJ, Donnelly PJ, Reid KR, Petsikas D, Conlan AA, et al. *Glutathione S-transferase-catalyzed conjugation of bioactivated aflatoxin B_1 in human lung: differential cellular distribution and lack of significance of the GSTM1 genetic polymorphism*. Carcinogenesis 1999; 20: 1971–7.
20. Wormhoudt LW, Commandeur JNM, Vermeulen NPE. *Genetic polymorphisms of human N-acetyltransferase, cytochrome P450, glutathione S-transferase, and epoxide hydrolase enzymes: relevance to xenobiotic metabolism and toxicity.* Crit Rev Toxicol 1999; 29: 59–124.
21. Zhong S, Forbes Howie A, Ketterer B, Taylor J, Hayes JD, Beckett GJ, et al. *Glutathione S-transferase mu locus: use of genotyping and phenotyping assays to assess association with lung cancer susceptibility.* Carcinogenesis 1991; 12: 1533–7.
22. Watson MA, Stewart RK, Smith GBJ, Massey TE, Bell DA. *Human glutathione S-transferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution.* Carcinogenesis 1998; 19: 275–80.
23. Seidegård J, Ekström G. *The role of human glutathione transferases and epoxide hydrolases in the metabolism of xenobiotics.* Environ Health Perspect 1997; 105, Suppl. 4: 791-7.
24. Barnette KG, Sarkar MA, Glover DD, Li P, Boyd C, Lalka D. *Glutathione S-transferase in human endometrium: quantitation and interindividual variability in isoform content.* Gynecol Obste Invest 1999; 47: 114–9.

25. Coles BF, Anderson KE, Doerge DR, Churchwell MI, Lang NP, Kadlubar FF. *Quantitative analysis of interindividual variation of glutathione S-transferase expression in human pancreas and the ambiguity of correlating genotype with phenotype*. Cancer Res 2000; 60: 573–9.
26. de Bruin WCC, Wagenmans MJM, Peters WHM. *Expression of glutathione S-transferase α, P1-1 and T1-1 in the human gastrointestinal tract.* Jpn J Cancer Res 2000; 91: 310–6.
27. Crawford EL, Khuder SA, Durham SJ, Frampton M, Utell M, Thilly WG, et al. *Normal bronchial epithelial cell expression of glutathione transferase P1, glutathione transferase M3, and glutathione peroxidase is low in subjects with bronchogenic carcinoma.* Cancer Res 2000; 60: 1609–18.
28. Šalagovič J, Kalina I, Habalová V, Hrivňák M, Valanský L, Biroš E. *The role of human glutathione S-transferases M1 and T1 in individual susceptibility to bladder cancer*. Physiol Res 1999; 48: 465–71.
29. Hecht SS. *Tobacco smoke carcinogens and lung cancer.* J Natl Cancer Inst 1999; 91: 1194–210.
30. Hou SM, Ryberg D, Fält S, Deverill A, Tefre T, Børresen AL, et al. *GSTM1 and NAT2 polymorphisms in operable and non-operable lung cancer patients*. Carcinogenesis 2000; 21: 49–54.
31. Salama SA, Abdel-Rahman SZ, Sierra-Torres CH, Hamada FA, Au WW. *Role of polymorphic GSTM1 and GSTT1 genotypes on NNK-induced genotoxicity*. Pharmacogenetics 1999; 9: 735–43.
32. Simon T, Becquemont L, Mary-Krause M, de Waziers I, Beaune P, Funck-Brentano C, et al. *Combined glutathione-S-transferase M1 and T1 genetic polymorphism and tacrine hepatotoxicity.* Clin Pharmacol Ther 2000; 67: 432–7.
33. Their R, Lewalter J, Kempkes M, Selinski S, Brüning T, Bolt HM. *Haemoglobin adducts of acrylonitrile and ethylene oxide in acrylonitrile workers, dependent on polymorphisms of the glutathione transferases GSTT1 and GSTM1*. Arch Toxic ol 1999; 73: 197–202.
34. Guengerich FP, Thier R, Persmark M, Taylor JB, Pemble SE, Ketterer B. *Conjugation of carcinogenes by Θ class glutathione S-transferases: mechanisms and relevance to variations in human risk*. Pharmacogenetics 1995; 5: S103–7.
35. Rossi AM, Guarnieri C, Rovesti S, Gobba F, Ghittori S, Vivoli G, et al. *Genetic polymorphisms influence variability in benzene metabolism in humans*. Pharmacogenetics 1999; 9: 445–51.
36. Onaran I, Özaydin A. *Are individuals with glutathione S-transferase GSTT1 null genotype more susceptible to in vitro oxidative damage?* J Toxicol Environ Health Part A 2000; 59: 15-26.
37. Baranova H, Bothorishvilli R, Canis M, Albuisson E, Perriot S, Glowaczower E, et al. *Glutathione S-transferase M1 gene polymorphism and susceptibility to endometriosis in French population.* Mol Hum Rep 1997; 9: 775–80.
38. Mattsson P, Bjelfman C, Lundberg PO, Rane A. *Cytochrome P450 2D6 and glutathione S-transferase M1 genotypes and migraine*. Europ J Clin Invest 2000; 30: 367–71.
39. Stroombergen MCMJ, Waring RH. *Determination of glutathione S-transferase and polymorphisms in neurological disease.* Hum Exp Toxicol 1999; 18: 141–5.
40. Katoh T, Nagata N, Kuroda Y, Itoh H, Kawahara A, Kuroki N, et al. *Glutathione S-transferase M1 (GSTM1) and T1 (GSTT1) genetic polymorphism and susceptibility to gastric and colorectal adenocarcinoma*. Carcinogenesis 1996; 17: 1855–9.
41. Persson I, Johansson I, Lou YC, Yue QY, Duan LS, Bertilsson L, et al. *Genetic polymorphism of xenobiotic metabolizing enzymes among Chinese lung cancer patients*. Int J Cancer 1999; 81: 325–9.
42. London SJ, Daly AK, Cooper J, Navidi WC, Carpenter CL, Idle JR. *Polymorphism of glutathione S-transferase M1 and lung cancer risk among African-Americans and Caucasians in Los Angeles County, California.* J Natl Cancer Inst 1995; 87: 1853-6.
43. Park JY, Muscat JE, Kaur T, Schantz SP, Stern JC, Richie Jr JP, et al. *Comparison of GSTM polymorphisms and risk for oral cancer between African-Americans and Caucasians.* Pharmacogenetics 2000; 10: 123–31.
44. Stanulla M, Schrappe M, Müller Brechlin A, Zimmermann M, Welte K. *Polymorphisms within glutathione S-transferase genes (GSTM1, GSTT1, GSTP1) and risk of relapse in childhood B-cell precursor acute lymphoblastic leukemia: a case-control study*. Blood 2000; 95: 1222-8.
45. Wilson MH, Grant PJ, Hardie LJ, Wild CP. *Glutathione S-transferase M1 null genotype is associated with a decreased risk of myocardial infarction.* FASEB J 2000; 14: 791-6.
46. Zusterzeel PLM, Nelen WLDM, Roelofs HMJ, Peters WHM, Blom HJ, Steegers EAP. *Polymorphisms in biotransformation enzymes and the risk for recurrent pregnancy loss.* Mol Hum Reprod 2000; 5: 474–8.
47. Tan W, Song N, Wang GQ, Liu Q, Tang HJ, Kadlubar FF, et al. *Impact of genetic polymorphisms in cytochrome P450 2E1 and glutathione S-transferases M1, T1, and P1 on susceptibility to esophageal cancer among high-risk individuals in China*. Cancer Epidemiol Biomark Prev 2000; 9: 551-6.
48. Zhang H, Ahmadi A, Arbman G, Zdolsek J, Carstensen J, Nordenskjöld B, et al. *Glutathione S-transferase T1 and M1 genotypes in normal mucosa, transitional mucosa and colorectal adenocarcinoma.* Int J Cancer 1999; 84: 135–8.

49. Jim JJ, Park GY, Lee CT, Kim YW, Han SK, Shim YS, et al. *Genetic susceptibility to chronic obstructive pulmonary disease in Koreans: combined analysis of polymorphic genotypes for microsomal epoxide hydrolase and glutathione S-transferase M1 and T1.* Thorax 2000; 55: 121-2.

50. Nair UJ, Nair J, Mathew B, Bartsch H. *Glutathione S-transferase M1 and T1 null genotypes as risk factors for oral leukoplakia in ethnic Indian betel quid/tobacco chewers*. Carcinogenesis 1999; 20: 743-8.

51. Setiawan VW, Zhang ZF, Yu GP, Li YL, Lu ML, Tsai CJ, et al. *GSTT1 and GSTM1 null genotypes and the risk of gastric cancer: a case-control study in Chinese population.* Cancer Epidemiol Biomarkers Prev 2000; 9: 73-80.

52. Chenevix–Trench G, Young J, Coggan M, Board P. *Glutathione S-transferase M1 and T1 polymorphisms: susceptibility to colon cancer and age of onset.* Carcinogenesis 1995;16: 1655-7.

53. Helzlsouer K, Selmin O, Huang HY, Strickland PT, Hoffman S, Alberg AJ, et al. *Association between glutathione S-transferase M1, P1, and T1, genetic polymorphisms and development of breast cancer*. J Natl Cancer Inst 1998; 90: 512-8.

54. Hirvonen A, Husgafvel-Pursiainen K, Anttila S, Vainio H. T*he GSTM1 null genotype as a potential risk modifier for squamous cell carcinoma of the lung.* Carcinogenesis 1993; 1: 1479-81.

55. Garcia-Closas M, Kelsey KT, Wiencke JK, Xu X, Wain JC, Christiani DC. *A case-control study of cytochrome P450 1A1, glutathione S-transferase M1, cigarette smoking and lung cancer susceptibility (Massachusetts, United States).* Cancer Causes Contro 1997; 8: 544–53.

56. McWilliams JE, Sanderson BJS, Harris EL, Richert-Boe KE, Henner WD. *Glutathione S-transferase M1 (GSTM1) deficiency and lung cancer risk.* Cancer Epidemiol Biomarkers Prev 1997; 4: 589–94.

57. Stücker I, de Waziers I, Cenée S, Bignon J, Depierre A, Milleron B, et al. *GSTM1, smoking and lung cancer: a case-control study.* Int J Epidemiol 1999; 28: 829–35.

58. Gao Y, Zhang Q. *Polymorphisms of the GSTM1 and CYP2D6 genes associated with susceptibility to lung cancer in Chinese*. Mutation Res 1999; 444: 441–9.

59. Jaskula-Sztul R, Reinikainen M, Husgafvel-Pursiainen K, Szmeja Z, Szyfter W, Szyfter K, et al. *Glutathione S-transferase M1 and T1 genotypes and susceptibility to smoking related larynx cancer*. Biomarkers 1998; 3: 149–55.

60. Woodson K, Stewart C, Barrett M, Bhat NK, Virtamo J, Taylor PR, et al. *Effect of vitamin intervention on the relationship between GSTM1, smoking, and lung cancer risk among male smokers*. Cancer Epidemiol Biomarkers Prev 1999; 8: 965-70.

61. Grinberg-Funes R, Singh VN, Perera FP, Bell DA, Young TL, Dickey C, et al. *Polycyclic aromatic hydrocarbon-DNA adducts in smokers and their relationship to micronutrient levels and the glutathione S-transferase M1 genotype*. Carcinogenesis 1994; 15: 2449–54.

62. Matsui A, Ikeda T, Enomoto K, Hosoda K, Nakashima H, Omae K, et al. *Increased formation of oxidative DNA damage, 8-hydroxy-2'-deoxyguanosine, in human breast cancer tissue and its relationship to GSTP1 and COMT genotypes.* Cancer Lett 2000; 151: 87–95.

63. Schlade-Bartusiak K, Sasiadek M, Kozlowska J. *The influence of GSTM1 and GSTP1 genotypes on the function of sister chromatid exchanges and chromosome aberrations by 1,2:3,4-diepoxybutane.* Mutation Res 2000; 465: 69-75.

64. Pavanello S, Gabbani G, Mastrangelo G, Brugnone F, Maccacaro G, Clonfero E. *Influence of GSTM1 genotypes on anti-BPDE-DNA adduct levels in mononuclear white blood cells of human exposed to PAH.* Int Arch Occup Environ Health 1999; 72: 238–46.

65. Viezzer C, Norppa H, Clonfero E, Gabbani G, Mastrangelo G, Hirvonen A, et al. *Influence of GSTM1, GSTT1, GSTP1, and EPHX polymorphisms on DNA adduct level and HPRT mutant frequency in coke-oven workers.* Mutat Res 1999; 431: 259–69.

66. Brescia G, Celotti L, Clonfero E, Neumann HG, Forni A, Foá V, et al. *The influence of cytochrome P450 1A1 and glutathione S-transferase M1 genotypes on biomarker levels in coke-oven workers*. Arch Toxicol 1999; 73: 431–9.

67. Rojas M, Cascorbi I, Alexandrov K, Kriek E, Auburtin G, Mayer L, et al. *Modulation of benzo[a]pyrene diolepoxide-DNA adduct levels in human white blood cells by CYP1A1, GSTM1 and GSTT1 polymorphism.* Carcinogenesis 2000; 21: 35–41.

68. Ichiba M, Hagmar L, Rannug A, Högstedt B, Alexandrie AK, Carstensen U, et al. *Aromatic DNA adducts, micronuclei and genetic polymorphism for CYP1A1 and GSTT1 in chimney sweeps.* Carcinogenesis 1994; 15: 1347–52.

69. Alexandrie AK, Warholm M, Carstensen U, Axmon A, Hagmar L, Levin JO, et al. *CYP1A1 and GSTM1 polymorphisms affect urinary 1-hydroxypyrene levels after PAH exposure.* Carcinogenesis 2000; 2: 669–76.

70. Zhang J, Ichiba M, Feng Y, Pan G, Hanaoka T, Yamano Y ,et al. *Aromatic DNA adducts in coke-oven workers, in relation to exposure, lifestyle and genetic polymorphism of metabolic enzymes.* Int Arch Occup Environ Health 2000; 73: 127–35.

71. Wu FY, Tsai FJ, Kuo HW, Tsai CH, Wu WY, Wang RY, et al. *Cytogenetic study of workers exposed to chromium compounds.* Mutation Res 2000; 464: 289–96.

72. Knudsen LB, Norppa H, Gamborg MO, Nielsen PS, Okkels H, Soll-Johanning H, et al. *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 Biomarkers Prev 1999; 8: 303–10.

73. Grégo D'Arce LP, de Syllos Cólus IM. *Cytogenetic and molecular biomonitoring of agricultural workers exposed to pesticides in Brazil.* Teratogen. Carcinog. Mutagen 2000; 20: 161–70.

74. Lucero L, Pastor S, Suárez S, Durbán R, Gómez C, Parrón T, et al. *Cytogenetic biomonitoring of Spanish greenhouse workers exposed to pesticides and buccal epithelial cells.* Mutation Res 2000; 464: 255–62.

75. Butkiewicz D, Grzybowska E, Philips DH, Hemminki K, Chorąży M. *Polymorphisms of the GSTP1 and GSTM1 genes and PAH-DNA adducts in human mononuclear white blood cells.* Environ Mol Mutagen 2000; 35: 99–105.

76. Grzybowska E, Butkiewicz D, Motykiewicz G, Chorazy M. *The effect of the genetic polymorphisms of CYP1A1, CYP2D6, GSTM1 and GSTP1 on aromatic DNA adduct levels in the population of healthy women*. Mutat Res 2000; 469: 271–7.

77. Hirvonen A, Saarikoski ST, Linnainmaa K, Koskinen K, Husgafvel-Pursiainen K, Mattson K, et al. *Glutathione S-transferase and N-acetyltransferase genotypes and asbestos-associated pulmonary disorders*. J Natl Cancer Inst 1996; 88: 1853–6.

Received for publication: November 3, 2000
Approved for publication: April 30, 2001