Combined analysis of polymorphisms in glutathione S-transferase M1 and microsomal epoxide hydrolase in lung cancer patients

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Many genes involved in the metabolism of carcinogens have been found to be polymorphic in the human population, and specific alleles are associated with increased risk of cancer at various sites. The etiology of most commonly occurring cancers cannot be explained by allelic variability at a single locus. A combined analysis of two polymorphic enzymes, glutathione S-transferase M1 (GSTM1), microsomal epoxide hydrolase (EPHX1)) and their implication as lung cancer risk factors was performed in a case-control study of non small cell lung cancer. Polymerase chain reaction (PCR) or PCR-RFLP-based methods were used to detect variant genotypes of GSTM1 and EPHX1 (113Tyr→113His in exon 3 and 139His→139Arg in exon 4) in 150 controls and group of lung cancer patients (n=121). The slow 113His EPHX1 allele tended to be more frequent among the patients (frequency 0.587) than among the controls (0.320) (Fisher's exact test, p=0.33). The combined EPHX1 homozygous genotype His113/His139 (predicted very slow activity) versus all other genotype combination was associated with an increased risk of lung cancer (OR=2.29; 95% C.I.: 0.94–5.82), particularly in non-smokers (OR=11.23; 95% C.I.: 1.48–88.41). Polymorphism in GSTM1 had no statistically significant impact on lung cancer risk alone (OR=1.09; 95% C.I.: 0.65–1.82). However, obtained the results revealed that combinations GSTM1 null with homozygous His113/His139 genotype (predicted very slow activity EPHX1) significantly increased lung cancer risk (OR=3.65; 95% C.I.: 1.04–16.07). No overall relationship between genotype combinations predicted high EPHX1 activity and lung cancer risk was confirmed in all followed respects. However, the number of investigated individuals in our study was relatively small, therefore these findings should be judged with circumspectness.

Key words: susceptibility, lung carcinoma, polymorphism, EPHX1, GSTM1

Many genes involved in the metabolism of carcinogens have been found to be polymorphic in the human population and specific alleles are associated with increased risk of cancer at various sites. The etiology of most commonly occurring cancers cannot be explained by allelic variability at a single locus. A combined analysis of polymorphic enzymes, glutathione S-transferase M1 and microsomal epoxide hydrolase (EPHX1) and their implication as lung cancer risk factors was performed in a case-control study.

The glutathione S-transferases (GSTs) play a central role in the detoxification of carcinogens and consist of a super family of enzymes involved in the conjugation of a wide range of electrophilic substrates with glutathione (GSH). This reaction facilitates direct excretion of compounds into urine or bile, or more often through further metabolism by transpeptidases, N-acetylases and then excretion. The detoxification pathway helps protect cellular components from the toxic effects of many exogenous and endogenous electrophilic compounds [11]. Four classes of cytosolic GST and one microsomal form have so far been characterized in humans. The glutathione S-transferase M1 from class μ has been studied recurrently for a well-defined phenotypic polymorphism [4]. The model, based on three alleles, GSTM1*0, GSTM1*A and GSTM1*B, has been confirmed by many studies. GSTM1*0 is deleted and homozygotes (GSTM1 null genotype) express no GSTM1 protein. GSTM1*A and GSTM1*B differ by only asingle base in exon 7 and encode enzyme homo- and heterodimeric enzymes with similar catalytic effectiveness [20].

The enzyme EPHX1 is an important biotransformation

system that catalyses the hydrolysis of a wide variety of xenobiotic epoxides, resulting in the formation of corresponding trans-dihydrodiol derivates [9, 12]. Substrates for enzyme include epoxide derivates of certain pharmaceuticals, such as metabolites of phenytion and other antiepileptic medications [5, 13] and epoxides of environmental toxins, such as the carcinogenic PAHs, aromatic amines and benzene [7, 12]. The metabolism of epoxide-containing compounds by EPHX1 results in the production of inherently less reactive and less toxic intermediates [9]. However, in certain instances, notably in concern with oxidative metabolism by the cytochrome P450s hydrolysis of particular PAH-epoxide by EPHX1 can lead to the formation of highly electrophilic and mutagenic diol-epoxides [12]. The EPHX1 gene is located on the long arm of chromosome 1 and composed of 9 exons. Two single-base pair polymorphisms in coding region EPHX1 gene that result in amino acid substitutions have been reported. In one polymorphism seen in exon 3 of the EPHX1 gene, tyrosine replaces histidine at residue 113 because a C has been substituted for a T. This allelic conversion has been referred to as the "slow" allele and this change results in a 40–50% decrease in the enzyme activity. The change of His 139→Arg exon 4, because a G has been substituted for an A, results in a 25% increase in activity and thus this allele has been called the "fast" allele [10]. The result of EPHX1 polymorphisms is therefore to produce metabolic phenotypes in the population – dependent on the specific genotype combination.

A case-control study was conducted to investigate the relationship between two polymorphic xenobiotic-metabolizing enzymes and lung cancer development. Because both enzymes metabolized environmental and tobacco-related substrates corresponding to lung carcinogenesis we also tested whether combined effect of GSTM1 and mEPHX1 polymorphisms may also modify lung cancer risk.

Material and methods

Blood samples were collected from 121 patients with histologically proven diagnosis of non-small cell carcinoma recruited in the Clinic of Tuberculosis and Respiratory Diseases at the Teaching Hospital Košice. The case group was classified according to the tumor histology into four subgroups: squamous cell carcinoma (59.50%), adenocarcinoma (15.70%), large cell carcinoma (4.96%) and other cancers (19.83%). Into category other cancers were included not differentiated carcinoma or carcinoma with unclear tumor histology. Controls under 65 years of age were healthy blood donors from the Clinic of Hematology and Blood Transfusion, those over 65 years of age from the Geriatric Center, both at the Teaching Hospital Košice. Controls and cases were interviewed and asked about histories of cancer, chronic diseases, occupation and smoking status. Only individuals without history of cancer and chronic respiratory disease were eligible to participate as controls. The main medical diagnoses in the control over 65 years were rheumatological or cardiovascular diseases. All cases and controls were Slovak (Caucasians) from the general population of Eastern Slovakia. Participants were given an explanation of the nature of the study and informed consent was obtained. Case and control groups were not specifically matched, although both mean ages and proportion of male/female were similar. The studied population is described in Table 1.

Table 1. Basic characteristics of subjects

		Controls	Patients
Total		150	121
Gender	Male	114 (76.00%)	103 (85.10%)
	Female	36 (24.00%)	18 (14.90%)
Age	Mean ± SD	55.66 ± 20.79	61.45 ± 10.80
	< 65 years	94 (62.67%)	72 (59.50%)
	≥ 65 years	56 (37.33%)	49 (40.50%)
Smoking status	Non-smoker	62 (41.33%)	11 (9.10%)
	Smoker	88 (58.67%)	110 (90.90%)
Histology	Squamous cell carcinoma		72 (59.50%)
	Adenocarcinoma		19 (15.70%)
	Large cell carcinoma		6 (4.96%)
	Other cancer		24 (19.83%)

SD – standard deviation

Table 2. Genotype and predicted EPHX1 activity

Phenotype (EPHX1 activity)	Genotype	
	Exon 3	Exon 4
Intermediate	*Tyr113-Tyr113*	*His139-His139*
	Tyr113-Tyr113	*His139-Arg139*
	Tyr113-His113	*His139-His139*
	Tyr113-His113	*His139-Arg139*
High	*Tyr113-Tyr113*	*Arg139-Arg139*
	Tyr113-His113	*Arg139-Arg139*
Slow	*His113-His113*	*His139-Arg139*
	His113-His113	*Arg139-Arg139*
Very slow	*His113-His113*	*His139-His139*

Genomic DNA was prepared from peripheral blood leukocytes by salted method [18].

The simultaneous amplification of GSTM1 and additional albumin genomic fragments (served as an internal positive control for success of amplification reaction) was used in the same reaction [1]. The fragments were separated on 2% agarose gel.

Two separate PCR reactions were used to detect the two mutations in the EPHX1 gene [21] with small modification. The primer pairs
EPO1 (5'-GATCGATAAGTTCCGTTTCACC-3') and
EPO2 (5'-ATCCTTAGTCTTGAAGTGAGGAT-3')

Table 3. Distribution of individuals according to EPHX1 predicted activity and genotypes frequences of GSTM1 polymorphism

	GSTM1 positive		GSTM1 null		EPHX1 activity							
					High		Intermediate		Slow		Very slow	
	Controls	Patients	Controls	Patients	Controls	Patients	Controls	Patients	Controls	Patients	Controls	Patients
Age												
< 65 years	42(44.68%)	30(41.67%)	52(55.32%)	42(58.33%)	19(20.21%)	12(16.67%)	41(43.62%)	29(40.28%)	29(30.85%)	22(30.55%)	5(5.32%)	9(12.50%)
≥ 65 years	27(48.21%)	23(46.94%)	29(51.79%)	26(53.06%)	11(19.64%)	4(8.17%)	26(46.43%)	19(38.77%)	14(25.00%)	18(36.73%)	5(8.93%)	8(16.33%)
Gender												
Female	16(44.44%)	9(50.00%)	20(55.56%)	9(50.00%)	7(19.44%)	4(22.22%)	17(47.23%)	5(27.78%)	10(27.78%)	5(27.78%)	2(5.55%)	4(22.22%)
Male	53(46.49%)	44(42.72%)	61(53.51%)	59(57.28%)	23(20.17%)	12(11.65%)	50(43.86%)	43 (41.75%)	33(28.95%)	35(33.98%)	8(7.02%)	13(12.62%)
Smoking status												
Non-smokers	29(46.77%)	6(54.55%)	33(53.23%)	5(45.45%)	10(16.12%)	2(18.19%)	32(51.62%)	4(36.36%)	17(27.42%)	1(9.09%)	3(4.84%)	4(36.36%)
Smokers	40(45.45%)	47(42.73%)	48(54.55%)	63(57.27%)	20(22.73%)	14(12.73%)	35(39.77%)	44(40.00%)	26(29.55%)	39(35.45%)	7(7.95%)	13(11.82%)
Total	69(46.00%)	53(43.80%)	81(54.00%)	68(56.20%)	30(20.00%)	16(13.22%)	67(44.66%)	48(39.67%)	43(28.67%)	40(33.06%)	10(6.67%)	17(14.05%)

Table 4. Distributions of combined GSTM1 genotype and EPHX1 predicted activity

		GSTM1 positive							
		High		Intermediate		Slow		Very slow	
		Controls	Patients	Controls	Patients	Controls	Patients	Controls	Patients
Age	< 65 years	9(9.57%)	3(4.17%)	18(19.15%)	13(18.06%)	12(12.77%)	12(16.67%)	3(3.19%)	2(2.78%)
	≥65years	5(8.93%)	3(6.12%)	14(25.00%)	5(10.20%)	5(8.93%)	11(22.45%)	3(5.36%)	4(8.16%)
Gender	Female	4(11.11%)	3(16.66%)	8(22.22%)	2(11.11%)	4(11.11%)	3(16.67%)	0(0.00%)	1(5.56%)
	Male	10(8.77%)	3(2.91%)	24(21.05%)	16(15.53%)	13(11.40%)	20(19.42%)	6(5.26%)	5(48.54%)
Smoking status	Non-smoker	5(8.06%)	2(18.18%)	15(24.19%)	1(9.09%)	8(12.90%)	1(9.09%)	1(1.61%)	2(18.18%)
	Smoker	9(10.23%)	4(3.64%)	17(19.32%)	17(15.45%)	9(10.23%)	22(20.00%)	5(5.68%)	4(3.64%)
Total		14(9.33%)	6(4.96%)	32(21.33%)	18(14.89%)	17(11.33%)	23(19.00%)	6(4.00%)	6(4.96%)
		GSTM1 null							
		High		Intermediate		Slow		Very slow	
		Controls	Patients	Controls	Patients	Controls	Patients	Controls	Patients
Age	< 65 years	10(10.64%)	9(12.50%)	23(24.47%)	16(22.21%)	17(18.09%)	10(13.89%)	2(2.13%)	7(9.72%)
	≥65 years	6(10.71%)	1(2.04%)	12(21.43%)	14(28.58%)	9(16.07%)	7(14.29%)	2(3.57%)	4(8.16%)
Gender	Female	3(8.33%)	1(5.56%)	9(25.00%)	3(16.66%)	6(16.67%)	2(11.11%)	2(5.56%)	3(16.67%)
	Male	13(11.40%)	9(8.74%)	26(22.81%)	27(26.21%)	20(17.54%)	15(14.56%)	2(1.75%)	8(7.77%)
Smoking status	Non-smoker	5(8.06%)	0(0.00%)	17(27.42%)	3(27.27%)	9(14.52%)	0(0.00%)	2(3.23%)	2(18.18%)
	Smoker	11(12.50%)	10(9.09%)	18(20.45%)	27(24.54%)	17(19.32%)	17(15.46%)	2(2.27%)	9(8.18%)
Total		16(10.67%)	10(8.26%)	35(23.33%)	30(24.79%)	26(17.33%)	17(14.05%)	4(2.67%)	11(9.09%)

were used for the exon-3 variants. The primer pairs EPO3 (5'-ACATCCACTTCATCCACGT-3') and EPO4 (5'-ATGCCTCTGAGAAGCCAT-3') were used to test mutation in exon 4. The PCR was carried out in a MJ Research thermal cycler; after 3 min pre-treatment to 94 ˚C the reaction mixture was subjected to 38 cycles of 94 ˚C for 30 s, 56 ˚C for 1 min., and 72 ˚C for 1 min. This was followed by a final step at 72 ˚C for 10 min. The resulting DNA fragments were digested with EcorV (exon 3) and RsaI (exon 4) (37 ˚C, overnight). The fragments after digestion were separated by electrophoresis through a non-denaturing 10% polyacrylamide gel. The gels were stained with ethidium bromide and transilluminated with UV light. Because both polymorphisms should be altered EPHX1 function and because stratification of data on one polymorphism by another results in small numbers, it is suitable to combine the data on both polymorphisms to afford an index predicted activity (Tab. 2). Indices rise on the assumption that exon 3 Tyr113 (wild-type or wt) allele confers normal activity, while the His 113 allele is slow allele. For the exon 4 polymorphism, His139 allele is considered the normal (wild-type) activity and the Arg139 allele is fast.

Statistical analysis was performed by Fisher's exact test. The relative associations between cases and controls were assessed by calculating crude Gart's odds ratio (OR) and 95% confidence intervals (CI). All computations were undertaken using statistical software Arcus Quickstat Biomedical ver. 1.1.

Results

A total of 121 cancer patients and 150 controls were included in this study. Data on the overall proportions of the GSTM1 and EPHX1 genotypes or combined genotypes in the case and control groups are presented in Table 3 and 4, ORs are described in Table 5.

Table 5. Odds ratio (OR) of lung cancer according to GSTM1 and EPHX1 activity

		Controls (%)	Patients (%)	OR[a](95% CI)
Total		150	121	
GSTM1				
Positive		69(46.00%)	53(43.80%)	
Null		81(54.00%)	68(56.20%)	1.09(0.65–1.82)
EPHX1 activity				
High		30(20.00%)	16(13.22%)	0.61(0.29–1.23)
Intermediate		67(44.67%)	48(39.67%)	0.81(0.48–1.36)
Slow		43(28.67%)	40(33.06%)	1.23(0.70–2.13)
Very slow		10(6.67%)	17(14.05%)	2.29(0.94-5.82)
Very slow non-smokers		3(4.83%)	4(36.36%)	11.23(1.48–88.41)
Combined analysis				
GSTM1 null/EPHX1	High	16(10.67%)	10(8.26%)	0.75(0.29–1.85)
GSTM1 null/EPHX1	Intermediate	35(23.33%)	30(24.79%)	1.08(0.59–1.96)
GSTM1 null/EPHX1	Slow	26(17.33%)	17(14.05%)	0.78(0.38–1.59)
GSTM1 null/EPHX1	Very Slow	4(2.67%)	11(9.09%)	3.65(1.04–16.07)

[a]Crude OR computed between selected genotype versus all others genotypes in corresponding group.

GSTM1 genotype (Tab. 3 and 5). The prevalence of the GSTM1 null genotype in lung cancer patients (56.20%) was similar to that found in the population controls (54.00%). The GSTM1 gene deletion among control group is in close agreement with the GSTM1 null frequency among Caucasians (53%) reported by international collaborative study (GSEC) [6]. Although the GSTM1 null genotype was slightly over-represented in the group of adenocarcinoma patients (57.89%) the differences were not statistically significant. The odds ratio for lung cancer risk in individual with GSTM1 null genotype was 1.09 (95% C.I.: 0.66–1.82).

The frequencies of alleles and predicted activity of EPHX1 (Tab. 3 and 5).The frequencies obtained for the exon 3 EPHX1 113Tyr and 113 His alleles in control group were 0.680 and 0.320, respectively, and the genotype frequencies fitted the HW equilibrium (Fisher's exact test; $p<0.05$). The frequencies for the exon 4 EPHX1 polymorphisms were 0.790 and 0.210 for 139His and 139Arg, and also fitted HW equilibrium (Fisher's exact test; $p<0.05$). The frequencies obtained for both rare alleles in healthy controls are in close agreement with the frequency in the GSEC report (0.316 and 0.215) [6]. The 113His EPHX1 allele tended to be more frequent among the patients (frequency 0.587) than among the controls (0.320) (Fisher's exact test $p=0.33$). Exon 4 genotype distribution did not differ significantly between lung cancer patients and controls. The frequencies for the exon 4 EPHX1 polymorphisms were 0.826 and 0.174 for 139His and 139Arg in lung cancer group, and fitted the HW equilibrium (Fisher's exact test; $p<0.05$). However there was none patient with homozygote mutation in exon 4. No overall relationship between exon 4 EPHX1 and lung cancer risk was confirmed.

When the data were analyzed according to combinations of exon 3 and exon 4 genotypes there was found an increased frequency of His113-His113/His139-His139 genotype (predicted very slow EPHX1 activity) in lung cancer group (OR=2.29; 95% C.I.: 0.94–5.82) compared to all other combinations. This combination statistically significantly increased risk of lung cancer particularly in non-smokers (OR=11.23; 95% C.I.: 1.48–88.41). No overall relationship between genotype combination that predicted high EPHX1 activity and lung cancer risk was confirmed (OR=0.61; 95% C.I.: 0.29–1.23).

Analysis of combined EPHX1 activity and GSTM1 genotypes (Tab. 4 and 5). Thereafter, we investigated possible interactions between EPHX1 and GSTM1 polymorphisms. Initially we studied genotype combinations that had been previously addressed and reported to be possibly associated with cancer risk. A significant interactive effect between EPHX1 polymorphisms and GSTM1 null genotype was found. The frequency of individuals with homozygote His113/His139 EPHX1 genotype (very slow activity) and GSTM1 null genotype was over-represented in the lung cancer group (OR=3.65; 95% C.I.: 1.04–16.07). The small counts of EPHX1 genotype combination that predicted high EPHX1 activity with GSTM1 polymorphism in control and patients group precluded an analysis stratified by age, gender, smoking. The interaction between a combination of high EPHX1 activity with GSTM1 null genotype and overall lung cancer risk was not confirmed (OR=0.75; 95% C.I.: 0.29–1.85).

Discussion

Susceptibility *to cancer* due to chemical exposure is likely to be determined by an individual's phenotype for a number of enzymes, both activating and detoxifying, relevant to that of single carcinogens or mixture of carcinogens. Many studies have considered only single-locus associations with cancer. Because the obvious complexity in cancer etiology exists, it is unlikely that asingle gene such as GSTM1 or EPHX1 will be sufficient to explain most cancer susceptibility in the absence of knowledge about other susceptibility genotypes or specific exposures. In our study we evaluated the effect of a combination of GSTM1 and EPHX1 genotypes on lung cancer susceptibility.

Immediately after identification of GST polymorphisms in 1981, BOARD [4] pointed out that GSTM1 null genotype might influence susceptibility to cancer. Since then there have been many studies on lung cancer, though conflicting data have been obtained and the influence of GSTM1 null on risk remains unclear (for review see [22]). Our introductory results indicate that GSTM1 null genotype has low penetrance as a risk factor for lung cancer alone. This observed role of GSTM1 agreed with a meta-analysis by MCWILLIAMS et al [17], where GSTM1 deficiency was concluded to be only a moderate risk factor (OR=1.2; 95% CI: 0.98–1.4) for Caucasian population.

As relate to EPHX1, the literature shows relatively inconsistent findings in association of susceptibility to lung cancer and EPHX1 polymorphism. The high EPHX1 activity was observed as associated with increased lung cancer risk among French Caucasians smokers with squamous and small cell carcinoma, however an association between lung cancer risk and the homozygous or heterozygous genotypes of the exon 3 and 4 variant alleles was not found [2]. ZHOU et al [25] suggest that cumulative cigarette smoking exposures play pivotal roles in the association between EPHX1 polymorphisms and lung cancer risk. The combined genotype His113/His139 of both EPHX1 exons was termed as a risk factor in non-smokers, to a protective factor in heavy smokers observed the decreasing the ORs for homozygote His113/His139 genotype versus those other genotypes with cumulative smoking. Two others studies [16, 21] found no overall association for EPHX1 polymorphism and lung cancer risk among Caucasians. The quantitative review did not find a consistent modification of the carcinogenic effect of smoking according to EPHX1 polymorphism, although the risk of lung cancer decreased among never smokers with high EPHX1 activity and among heavy smokers with the exon 3 His113-His113 genotype in pooled analysis. The protective effect of exon 3 polymorphism was seen stronger for adenocarcinoma than other histological types [15]. The hypothesis that genetically reduced EPHX1 activity may be protective against lung cancer, especially for adenocarcinoma, was supported in an Austrian study [8]. In our preliminary study, homozygosity for the His113/His 139 genotype combination significantly increased lung cancer risk in the non-smokers (OR=11.23; 95% C.I.: 1.48–88.41). These results indirectly confirmed the hypothesis that high EPHX1 activity is protective in non-smokers or smokers with low cumulative dose. However, our group of patients was relatively small, such these findings should be accepted with circumspectness. No relationship between the exon 4 polymorphism and lung cancer risk was confirmed in all followed respects. The presented data show that the involvement of EPHX1 polymorphism in lung cancer susceptibility is still an issue difficult to make out. It should be noted that EPHX1 has been implicated in both protection against and potention of the effects of chemical carcinogens. Either slow or high EPHX1 metabolizers should be seriously considered for their ability to simultaneously decrease or increase the bioactivation of specific compounds. In non-smokers, environmental pollutants or professional exposure may play a role in the development of lung cancer. Examples of these chemicals include alkene, arene, or reactive epoxide intermediates, which are detoxified by EPHX1 and should be explained why genotype combination His113/His139 (predicted very slow activity) increases the risk of lung cancer.It is possible, that additional factors such as ethnic differences in distributions of alleles, dietary pattern [24], genetic polymorphism in 5'-flanking region [19] or post-transcriptional processing mechanisms [14] might contribute to the contradiction in results published until now. Differences in exposure to carcinogens in cigarette smoke and appropriate considering of the smoking habits might also affect the relation between EPHX1 genotypes and lung cancer risk.

Given the number and variability in expression of carcinogen-metabolizing enzymes now identified and the complexity of chemical exposure, assessment of a single polymorphic enzyme or genotype may not be sufficient. The risk of lung cancer determined by the GSTM1 null genotype is in small magnitude. However, this risk grows when the interaction of the GSTM1 genotype with other genotypes is considered. Our preliminary data outlined significant increasing of lung cancer risk when combination of both GSTM1 and EPHX1 potentially risk genotypes was considered. Individuals with GSTM1 null and homozygous His113/His139 EPHX1 genotype combination had an approximately 3.65 significantly increased risk of lung cancer.

Our findings can be compared only with results of one report because we failed to find corresponding references in literature concerning associations between combination of GSTM1 and EPHX1 polymorphism and lung cancer. Significant overrepresentation of cancer patients with a combination of exon 3 Tyr 113-Tyr 113 EPHX1 and exon 5 105Ile/105Ile GSTP1was found, but no association between EPHX1, GSTM1 polymorphisms and lung cancer was observed [23].

In summary, it is evident that polymorphisms of individual enzymes involved in the metabolism of xenobiotics may alter the susceptibility to environmentally induced lung cancer but relevancy of evaluation of cancer risk is considerably increased when combination of corresponding genotypes of polymorphic enzymes is considered. Larger epidemiological studies are needed to confirm the results we observed and to assess the role of genes that encode additional enzymes participating in the same metabolic pathways in relationship to histology, diet and different smoking exposure. Our study indicates that polymorphism of the EPHX1 gene taken together with the polymorphism of GSTM1 may be modifying factors in lung carcinogenesis.

References

[1] ARAND M, MÜHLBAUER R, HENGSTLER J, JÄGER E, FUCHS J et al. A multiplex polymerase chain reaction protocol for the simultaneous analysis of the glutathione S-transferase GSTM1 and GSTT1 polymorphisms. Anal Biochem 1996; 236: 184–186.

[2] BENHAMOU S, REINIKAINEN M, BOUCHARDY CH, DAYER P, HIRVONEN A. Association between lung cancer and microsomal epoxide hydrolase genotypes. Cancer Res 1998; 58: 5291–5293.

[3] BOARD PG. Biochemical genetics of glutathione S-transferase in man. Am J Hum Genet 1981; 33: 36–43.

[4] BOARD PG, COGGAN M, JOHNSTON P, ROSS V, SUZUKI T, WEBB G. Genetic heterogeneity of the human glutathione transferase: a complex of gene families. Pharmacol Ther 1990; 48: 357–369.

[5] BUEHLER BA, DELIMONT D, VANWAES M, FINNEL RH. Prenatal prediction of risk of the fetal hydantion syndrome. N Engl J Med 1990; 322: 1567–1572.

[6] GARTE S, GASPARI L, ALEXANDRIE AK, AMBROSONE CH et al. Metabolic gene polymorphism frequencies in control populations. Cancer Epidemiol Biomarkers Prev 2001; 10: 1239–1248.

[7] GONZALES FJ, SAMORE M, MC QUIDDY P, KASPER CB. Effects of 2-acetylaminoflourene and N-hydroxy-2-acetylaminoflourene on the cellular levels of epoxide hydratase, cytochromeP-450b, Pand NADPH-cytochrome c (P-450) oxidoreductase messenger ribonucleic acids. J Biol Chem 1982; 257: 11032–11036.

[8] GSUR A, ZIDEK T, SHNATTINGER K, FEIK E, HAIDINGER G et al. Association of microsomal epoxide hydrolase polymorphisms and lung cancer risk. BJ Cancer 2003; 89: 702–706.

[9] GUENGERICH FP. Epoxide hydrolase. Properties and metabolic roles. Rev Biochem Toxicol 1982; 4: 5–30.

[10] HASSETT C, AICHER L, SIDHU JS, OMIECINSKI CJ. Human microsomal epoxide hydrolase: genetic polymorphism and functional expression in vitro of amino acid variants. Hum Mol Genet 1994; 3: 421–428.

[11] HAYES JD, PULFORD DJ. The glutathione S-transferase supergene family regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit Rev Biochem Mol Biol 1995; 30: 445–600.

[12] JERINA DM. Metabolism of aromatic hydrocarbons by cytochrome P-450 system and epoxide hydrolase. Drug Metab Dispos 1983; 11: 1–4.

[13] KANEKO S. Antiepileptic drug therapy and reproductive consequences: Functional and morphological effects. Reprod Toxicol 1991; 5: 179–198.

[14] LAURENZANA FM, HASSETT C, OMIECINSKI CJ. Postranscriptional regulation of human microsomal epoxide hydrolase. Pharmacogenetics 1998; 8: 157–167.

[15] LEE WJ, BRENNAN P, BOFFETTA P, LONDON SJ, BENHAMOU S et al. Microsomal epoxide polymorphisms and lung cancer risk: a quantitative review. Biomarkers 2002; 7: 230–241.

[16] LONDON SJ, SMART J, DALY AK. Lung cancer risk in relation to genetic polymorphisms of microsomal epoxide hydrolase among African-Americans and Caucasians in Los Angeles County. Lung cancer 2000; 28: 147–155.

[17] MC WILLIAMS JE, SANDERSON BJS, HARRIS EL, RICHERT-BOE KE, HENNER WD. Glutathione S-transferase M1 (GSTM1) deficiency and lung cancer risk. Cancer Epidemiol Biomarkers Prev 1995; 4: 589–594.

[18] MILLER SA, DYKES DD, POLESKY HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16: 1215.

[19] RAAKA S, HASSETT C, OMIECINSKI CJ. Human microsomal epoxide hydrolase: 5'-flanking region genetic polymorphisms. Carcinogenesis 1998; 19: 387–393.

[20] SEIDEGARD J, VORACHEK WR, PERO RW, PEARSON WR. Hereditary difference in the expression of the human glutathione transferase active on trans-stilbene oxide are due to a gene deletion. Proc Natl Acad SCI USA 1988; 85: 7203–7207.

[21] SMITH CHAD, HARRISON DJ. Association between polymorphism in gene for microsomal epoxide hydrolase and susceptibility to emphysema. The Lancet 1997; 350: 630–633.

[22] STRANGE RC, FRYER AA. The glutathione S-transferases: influence of polymorphism on cancer susceptibility. In: Vineis P, Malatas N, Lang M, d'Errico A, Caporaso N, Cuzik J, Boffetta P, editors. Metabolic Polymorphisms and Susceptibility to Cancer. Lyon: IARC, 1999: 231–250.

[23] TO-FIGUERAS J, GENE M, GOMEZ-CATALAN J, PIQUE E, BORREGO N, CORBELLA J. Lung cancer susceptibility in relation to combined polymorphisms of microsomal epoxide hydrolase and glutathione S-transferase P1. Cancer Lett 2001; 173: 155–162.

[24] TSAI YY, MCGLYNN KA, HU Y, CASSIDY AB, ARNOLD J et al. Gentic susceptibility and dietary patterns in lung cancer. Lung Cancer 2003; 41: 269–281.

[25] ZHOU W, THURSTON SW, LIU G, XU LL, MILLER D et al. The interaction between microsomal epoxide hydrolase polymorphisms and cumulative cigarette smoking in different histological subtypes of lung cancer. Cancer Epidemiol Biomarkers Prev 2001; 10: 461–466.