Genetic polymorphisms of *CYP1A1, GSTM1* and *GSTT1* genes and lung cancer risk

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Abstract. Genetic polymorphisms of the genes encoding for the xenobiotic metabolizing enzymes result in individual variations in the efficiency of detoxification of environmental carcinogens, and have been extensively associated with variable risk for lung neoplasms in different ethnic and environmental backgrounds. In this study, using PCR-RFLP based assays, we investigated the distribution of genetic polymorphisms in *CYP1A1, GSTM1* and *GSTT1* genes in Greek lung cancer patients (N=122) and healthy controls (N=178). The frequency of *CYP1A1* m1 homozygous genotype was 0.04 in patients and 0.02 in controls (detected in 4.10% of patients and in 1.69% of controls, respectively), that of *GSTM1* null genotype was 0.52 in patients and 0.54 in controls, whereas those of *GSTT1* null genotype was 0.17 and 0.11, in patients and controls, respectively. The *GSTM1* null genotype was more frequent in adenocarcinoma, as well as in lung cancer patients with history of chronic obstructive pulmonary disease (COPD). The *GSTT1* null genotype correlated with advanced age of the patients at the time of diagnosis. Three combinations of rare genotypes - in subjects carrying simultaneously deviations from the common genotype in more than one gene - were over-represented in lung cancer patients, compared to control population, and were furthermore significantly associated with history of heavy tobacco consumption in lung cancer patients. The results imply involvement of specific genotype combinations of *CYP1A1, GSTM1* and *GSTT1* alleles in the development of lung cancer in heavy smokers.

Introduction

Incidence and mortality from lung cancer in Greece have increased significantly during the last decades (1). Changes in environmental factors and in lifestyle, mainly in smoking habits, have been associated with increased lung cancer risk (2). The environment-gene interaction for cancer development is largely attributed to the action of xenobiotic metabolizing enzymes (XME). Individual differences in the bioactivation of procarcinogens and in detoxification of carcinogens, arising from alterations in XME genes, may explain varying susceptibilities to lung cancer (3,4).

CYP1A1 enzyme is involved in the activation of benzo(*a*)pyrene, other polycyclic aromatic hydrocarbons (PAHs) and aromatic amines, all of them major classes of tobacco procarcinogens. The m1 polymorphism in the 3' non-coding region (3'-UTR) of the *CYP1A1* gene arises from a T→C transition, thus the m1 allele bears a *Msp*I cleavage site, in contrast with the wild-type variant (5). The *CYP1A1* m1 polymorphism has been associated with alterations in regulation and transcript half-life, which result in elevated induction of the enzyme, and thus, increased levels of activated intermediates (6). The m1 homozygous genotype has been associated with increased risk of lung cancer among Asian populations (7,8), but this association has not been established in Caucasian or Afro-American populations (9,10).

The cytosolic glutathione S-transferase (GST) enzyme family consists of at least 20 isoenzymes. GSTM1 is involved in degradation of active metabolites of polycyclic aromatic hydrocarbons (PAH) (11), whereas GSTT1 participates in detoxification of small hydrocarbons of tobacco smoke (such as monohalomethanes and ethylene oxide) (12). Lack of enzyme detoxification activity is due to inherited complete deletion of the respective genes (13,14). Ethnic variations have been reported for polymorphisms of *GST* genes, but generally the homozygous *GSTM1* null genotype is more frequent than the *GSTT1* null genotype (15,16). Homozygous deletions of *GST* genes, alone or in combinations, have been associated with increased risk of lung cancer among Caucasians (17,18).

In this study, we investigated the distribution of genotypes of *CYP1A1, GSTM1* and *GSTT1*, as well as their combinations, among Greek lung cancer patients and healthy controls. Furthermore, we tested for associations of the polymorphisms with the epidemiologic parameters, exposure to established risk factors for lung cancer, and the clinicopathological data of cancer cases, in order to evaluate the role of polymorphisms of those XME genes in lung cancer susceptibility.

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Materials and methods

Specimens and data collection. During the 18-month study period (April 2001-September 2002) peripheral blood was collected from 122 Greek patients (105 male and 17 female), newly diagnosed with lung cancer, as well as from 178 Greek healthy controls (148 male and 30 female), at the 'Sotiria' University Hospital, Athens, Greece. Samples were stored at 4°C. Histological type and degree of differentiation of all cancer cases was assessed by histological examination of surgery or bronchoscopy specimen. All non-small cell lung cancer cases were staged on initial diagnosis according to the TNM International Staging System, while small cell lung cancer cases were grouped as limited-stage or extensive-stage disease (19). Furthermore, data on patients were collected upon the time of blood sampling, through the completion of a questionnaire. This included sociodemographic characteristics - gender, age, lifetime occupational history (including exposure to known carcinogens, such as asbestos, aniline, ortho-toluidine, etc.), area of origin, residence setting (rural-urban), family history of cancer in first-degree relatives - as well as smoking status (expressed as pack/years), medication history, and the pre-existence of respiratory disease (i.e. COPD) or history of other lung disease (i.e. pulmonary fibrosis, tuberculosis). The survival data for the study period, as well as the treatment modalities were available for all patients in the study. Patients gave written informed consent prior to blood and data collection. All control blood samples were derived from apparently healthy individuals, without previous history of any cancer type, and without family history of lung cancer among first-degree relatives. The ethics Committee of the University of Crete approved this study.

DNA extraction. Genomic DNA was extracted from whole blood using proteinase K, followed by phenol extraction and ethanol precipitation according to standard procedures (20). DNA was resuspended in 50 µl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Working stocks were prepared by 10-fold dilution in double distilled H_2O.

Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). PCR assays were performed by introducing 100 ng of genomic DNA in a PCR reaction mixture containing 1X PCR buffer, 200 µM dNTPs, 2.0 mM $MgCl_2$ and 0.35 U *Taq* DNA polymerase (Life Technologies Ltd., UK) to a 15 µl total reaction volume. For the *CYP1A1* polymorphism, a 340 bp DNA fragment was amplified by PCR with primers (used to a final concentration of 0.3 µM) and amplification parameters as previously described (21). RFLPs were performed by complete digestion of the PCR product with *Msp*I. Wild-type (wt) genotype was characterized by an approximately 340 bp fragment, while the presence of 134, 206 and 340 bp fragments signified heterozygosity (wt/m1 genotype), and the homozygous m1 genotype was recorded when only the 134 and 206 fragments were observed. The oligonucleotide primer sequences (used to a final concentration of 0.3 µM) for the co-amplification of either *GSTM1* or *GSTT1* with ß-globin as an internal positive control, as well as the PCR conditions have been previously described (22).

Electrophoresis. PCR and RFLP products were analysed by 8% polyacrylamide gel electrophoresis (29:1 ratio acrylamide/bis-acrylamide) and silver stained. Gels were scanned on an Agfa SnapScan 1212u (Agfa-Gevaert N.V., Belgium).

Statistical analysis. To analyze the polymorphism data against the risk factors and the clinicopathological parameters, Chi-square test with estimation of odds ratio or Fisher's exact test were used, as appropriate, for comparison of categorical variables, while one-way ANOVA was used for comparison of continuous variables. In addition, correlation coefficient analysis and non-parametric procedures were applied, where appropriate. Statistical significance was accepted for p-values of <0.05. Analysis was performed using the SPSS (version 11.0) software (SPSS Inc., USA).

Results

The present study involved 122 lung cancer patients and 178 apparently healthy controls, all of them being of Greek ethnicity. The main characteristics of the patient study group are shown in Table I. Additionally, among smoker patients the mean cigarette exposure (estimated in pack/years) was 65.06 (median 60, SD 33.67, range 10-160). Sixteen patients had a positive history of lung cancer among first-degree relatives. Fifty-eight and 64 patients lived in urban and rural settings, respectively. Thirty-nine patients declared definitive occupational exposure to known carcinogens, while 48 patients had no history of occupational risk exposure and 19 patients declared probable exposure for a limited period of time. For the remaining 16 patients, detailed occupational history could not be recalled with accuracy. Chemotherapy was offered to 67 patients, 30 patients underwent operative treatment, while for 36 patients the treatment schedule contained radiotherapy. Twenty-nine patients had died at the end of the study period.

The control group consisted of 148 males and 30 females. Gender distrubution did not differ between cases and controls (Pearson χ^2 p=0.494), the mean age, however, of controls was slightly lower than that of the patient group (58.81 vs 64.64, respectively, one-way ANOVA p=0.004).

The PCR-RFLP analysis for the *CYP1A1* polymorphism is presented in Fig. 1. The multiplex PCR analysis for the *GSTM1* and *GSTT1* polymorphisms, with *ß-globin* as positive internal control, are shown in Figs. 2 and 3. The distribution of individual genotypes for *CYP1A1, GSTM1* and *GSTT1* in the study population is shown in Table II. The frequency of homozygous individuals for m1 *CYP1A1* polymorphism was 0.04 in lung cancer cases and 0.02 in controls (odds ratio: 0.40, 95% CIs: 0.09-1.71). The frequency of *GSTM1* null(-) genotype was 0.52 in cases and 0.54 in controls (OR: 0.91, 95% CIs: 0.58-1.45), while that of *GSTT1* null(-) genotype was 0.17 and 0.11 in cases and controls, respectively (OR: 1.64, 95% CIs: 0.85-3.18). Statistical analysis did not reveal any significant differences in genotype prevalence between the two study groups (patients, controls), for all three genes examined.

The *CYP1A1* m1 allele was encountered more frequently in adenocarcinoma (10 out of 30 cases) compared to squamous cell carcinoma (2 out of 22 cases), and the difference marginally failed to reach statistical significance (Fisher's exact test, p=0.051, OR: 5.00, 95% CIs: 0.97-25.77). On the

Table I. Characteristics of the patient study group.

	Cases	(%)
Total no.	122	
Mean age	64.6	
Gender		
Male	105	(86)
Female	17	(14)
Histological type		
Small cell carcinoma	21	(17.2)
Squamous cell carcinoma	22	(18)
Adenocarcinoma	30	(24.6)
Other (bronchoalveolar, large-cell)	2	(1.7)
NSCLC (poorly differentiated or non-specified)	47	(38.5)
Previous medical history		
COPD	21	(17.2)
Other lung disease (i.e. Tb, bronchiectasis etc.)	10	(8.2)
Other system disease	44	(36.1)
Lung and other system disease	17	(13.9)
None	30	(24.6)
Smoking status		
Current smoker	77	(63.1)
Former smoker	35	(28.7)
Life-long non-smoker	10	(8.2)
Smoking history (among current smokers)		
<39 pack/years	16	
40-59 pack/years	20	
60-79 pack/years	13	
>80 pack/years	28	

other hand, the *GSTM1* null genotype was over represented in adenocarcinoma (21 out of 30 cases) compared to other histological types (p=0.046, OR: 2.67, 95% CIs: 1.01-7.08) (Table III). Furthermore, the *GSTM1* null genotype was encountered significantly more frequently in lung cancer patients with history of COPD compared to patients with history of other chronic lung disease (Fisher's exact test, p=0.023, OR: 8.00, 95% CIs: 1.33-48.18) (Table III). No association was detected among the genotype frequencies of the XME genes, when patients with history of COPD were compared to patients without prior respiratory history. The *GSTT1* null genotype did not correlate with the histological type of lung cancer. On the contrary, an association was detected between *GSTT1* status and age, among lung cancer patients: the 21 patients with the *GSTT1* null genotype had significantly higher age than the 101 patients bearing the gene (mean age 70.1 vs 63.5, one-way ANOVA p=0.002).

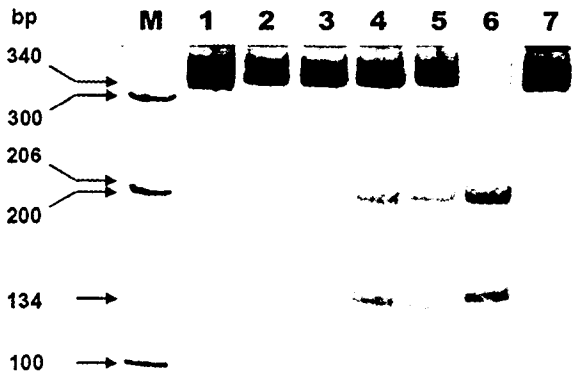

Figure 1. Representative examples of *CYP1A1 Msp*I 3'-UTR polymorphism analysis. M, DNA molecular size standard. Lanes 1 and 7, uncut *CYP1A1* PCR product. Lanes 2 and 3, homozygous wild-type samples; lanes 4 and 5, heterozygous samples (wt/m1). Lane 6, homozygous m1 sample.

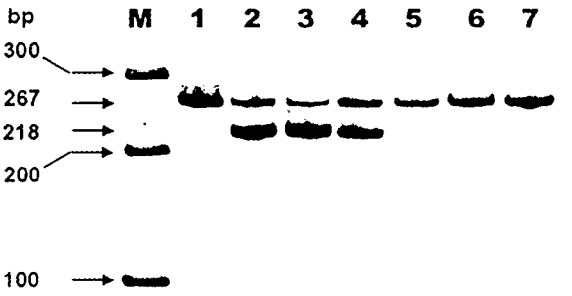

Figure 2. Representative examples of multiplex PCR assays for *GSTM1* polymorphism and ß-globin. M, DNA molecular size standard. Lane 1, ß-globin (singleplex). Lanes 2-4, GSTM1(+); lanes 5-7, GSTM1(-).

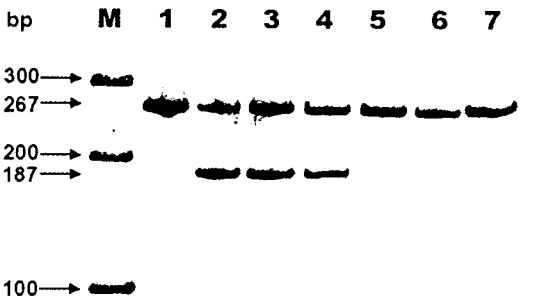

Figure 3. Representative examples of multiplex PCR assays for *GSTT1* polymorphism and ß-globin. M, DNA molecular size standard. Lane 1, ß-globin (singleplex). Lanes 2-4, GSTT1(+); lanes 5-7, GSTT1(-).

The distribution of genotype combinations for the three genes among the study population is presented in Table II. Twelve different genotype variations may arise from the possible combinations of *CYP1A1* (homozygous wt, homozygous m1, heterozygous), *GSTM1* (homozygous deletion or allele present) and *GSTM1* (homozygous deletion or allele present), and 11 out of those were observed in this

Table II. Genotype distribution among study population.
A, Single genotypes.

Gene allele	Cases Frequency	Controls Frequency	Odds ratio	95% CI
CYP1A1				
Wt	0.73	0.73	1.02	0.61-1.72
wt/m1	0.23	0.25	0.86	0.50-1.47
m1	0.04	0.02	0.40	0.09-1.71
GSTM1				
Null	0.52	0.54	0.91	0.58-1.45
Present	0.48	0.46	1.05	0.82-1.34
GSTT1				
Null	0.17	0.11	1.64	0.85-3.18
Present	0.83	0.89	0.93	0.85-1.03

B, Genotype combinations.

Genotype			Lung cancer cases		Controls			
CYP1A1	*GSTM1*	*GSTT1*	No. cases	Frequency	No. cases	Frequency	Odds ratio	95% CI
Wt	+	+	33	0.27	52	0.30	0.90	0.52-1.55
wt/m1	+	+	12	0.09	21	0.11	0.82	0.36-1.83
m1	+	+	2	0.02	2	0.01	1.47	0.15-14.78
Wt	-	+	42	0.34	62	0.35	0.98	0.59-1.64
wt/m1	-	+	10	0.08	20	0.11	0.71	0.30-1.66
m1	-	+	2	0.02	1	0.01	2.95	0.21-83.05
Wt	+	-	7	0.06	5	0.03	2.11	0.58-7.86
wt/m1	+	-	5	0.04	2	0.01	3.76	0.64-28.49
m1	+	-	0	0.00	0	0.00		
Wt	-	-	7	0.06	11	0.06	0.92	0.31-2.67
wt/m1	-	-	1	0.01	2	0.01	0.73	0.03-10.35
m1	-	-	1	0.01	0	0.00		
Total			122	1.00	178	1.00		

Table III. Associations of GSTM1 genotype with histological type, and with past respiratory history of lung cancer patients.

Patient group	No. of cases GSTM1 genotype		p-value	Odds ratio	95% CIs
	Null	Allele present			
Adenocarcinoma	21	9	0.046	2.67	1.01-7.08
Other histological types[a]	21	24			
History of COPD	14	7	0.023	8.00	1.32-48.18
History of other lung disease	2	8			

[a]Including only definite types (i.e. small cell, squamous). Poorly differentiated cases, as well as NSCLC cases, otherwise non-specified, were excluded from analysis.

Table IV. Associations of three rarely encountered genotypic combinations of XME genes [*CYP1A1*(wt/m1)/*GSTM1*(+)/*GSTT1*(-), *CYP1A1*(m1)/*GSTM1*(-)/*GSTT1*(+) and *CYP1A1*(wt)/*GSTM1*(+)/*GSTT1*(-)] with smoking status, among current smokers with lung cancer.

Record of exposure	Genotype combinations		p-value	Odds ratio	95% CIs
	Rare	Other			
	No. of cases				
High (>80 pack/years)	6	22	0.053	4.18	0.82-23.68
Low (<40 pack/years)	3	46			

study. Four combinations occur with high, similar frequencies in patients and controls, together exceeding 80% of cases (Table II). The remaining 7 genotypic combinations are represented in ≤5% each, in both study groups. It is of note that 3 out of those 7 rare genotypic combinations, that is *CYP1A1*(wt/m1)/*GSTM1*(+)/*GSTT1*(-), *CYP1A1*(m1)/*GSTM1*(-)/*GSTT1*(+) and *CYP1A1*(wt)/ *GSTM1*(+)/*GSTT1*(-) (each including at least one homozygous deletion) had higher frequencies among patients than among controls, and were associated with increased risk for lung cancer (odds ratios 3.76, 2.95, and 2.11, respectively), although the differences did not reach statistic significance, apparently due to the small size of sample in each category.

Genotype distributions for the XME genes examined in the present study could not be associated with the smoking history of the patients (only 10 patients were non-smokers). However, when smokers were analyzed into quartiles according to the distribution of our samples, a difference was detected between patients in the lowest and the highest smoke consumption quartiles, marginally failing to reach statistical significance: presence of the three rare genotype combinations described above was more frequently detected among current smokers with record of exposure >80 pack/years (6 cases among 28 patients) than among current smokers with exposure <40 pack/years (3 cases among 49 patients) (Fisher's exact test, p=0.053, OR: 4.18, 95% CIs: 0.82-23.68) (Table IV). No significant associations could be established between any genotype combinations and histological type, TNM stage, degree of differentiation, family history, residence setting, occupational risk history, response to treatment, and survival (data not shown).

Discussion

In the present study, we examined the genotyping profile for the *CYP1A1, GSTM1*, and *GSTT1* genes, all involved in the metabolism of common carcinogens, in a series of Greek lung cancer patients and healthy controls. Several polymorphisms of the genes encoding for these enzymes have been reported with increased frequency among lung cancer patients, in different ethnicity and environmental backgrounds (4,8,9,18,23). It must be emphasized that racial variation clearly exists in the distribution of polymorphisms of XME genes (15,16,23). Previously, we determined the frequencies of XME genes in a series of Greek breast cancer patients, furthermore identifying frequent transcriptional deregulation of those genes in breast cancer (24). To the best of our knowledge, this is the first report on the genotype frequency of these genes in Greek lung cancer patients. We found that the frequency of m1 allele of *CYP1A1* was 16%, of *GSTM1*(-) was 52% and of *GSTT1*(-) was 17% among lung cancer patients. Previous studies on Caucasian lung cancer patients have estimated that frequencies of *CYP1A1, GSTM1* and *GSTT1* were 14%, 48-54%, and 13-26%, respectively (17,18,25-27).

In the present study, higher prevalence of the *CYP1A1* homozygous *Msp*I genotype was recorded among patients compared to controls (4.1% vs 1.69%, respectively). This difference failed to reach statistical significance, apparently due to the size of the sample. In a recently published pooled analysis of 22 case-control studies, a clear association between the *CYP1A1* homozygous *Msp*I genotype and lung cancer was detected in Caucasians (28), confirming the finding of our study.

In contrast with *CYP1A1*, data on lung cancer risk attributed to *GSTM1* and *GSTT1* genes are less consistent. In the present study, no association between the allelic frequencies of those genes and lung cancer risk was observed, which is consistent with previously reported data (27). In a recent meta-analysis, marginal correlation between the presence of *GSTM1* null genotype and lung cancer risk was reported, without, however, evidence of interaction between *GSTM1* genotype and tobacco consumption (17). The frequency of *GSTM1* null genotype in our study was slightly lower in lung cancer patients than in controls, and interestingly, in a previous study in Caucasians, *GSTM1* homozygous (-) genotype has been correlated with decreased risk for lung cancer (29).

Perhaps the most interesting finding of the present study was the detection of three rare genotypic combinations with higher frequency among lung cancer patients compared to controls. Lung cancer was associated with those combinations of rare genotypes stronger than with any individual genotype in our study, as well as than with any of the genotypic combinations commonly represented in our study groups. Although differences did not reach statistical significance, our results are consistent with previously reported data (14,15,18). In a previous study on European patients, lack of association of separate genotypes with lung cancer was in contrast with increased lung cancer susceptibility in persons showing concurrent lack of the *GSTM1* and *GSTT1* genes (27). A recent meta-analysis on *CYP1A1* and *GSTM1* genes among non-smokers with lung cancer gave also similar

results (30). It seems, therefore, that rather than the individual genotypes, which present small if any contribution to the absolute risk of cancer, the combination of rare XME genotypes - consisting of accumulated genetic alterations, involving at least one homozygous deletion of GST genes - should be considered more appropriate for cancer risk assessment.

Contradictory data on the association between XME genotypes and lung cancer risk according to smoking status exist in the literature. In a recent study on Caucasian lung cancer patients diagnosed before 45 years of age, the association between *CYP1A1* genotype and lung cancer was confined to never smokers (25). Conversely, in a large meta-analysis where Caucasian patients were stratified according to duration of smoking, the increase in the risk of lung cancer was greater in patients carrying the *CYP1A1* m1 genotype, than among the individuals with the homozygous reference allele (28). On the other hand, another recent meta-analysis, including large cohorts of patients, concluded that *GSTM1* genotype is not a modifier of the effect of smoking on lung cancer risk (17). In the present study, we found the three genotypic combinations over-represented in lung cancer patients, furthermore correlated with smoke exposure, the difference between heavy (exposure >80 pack/years) and light smokers (exposure <40 pack/years) marginally failing to reach statistical significance, obviously due to the small number of cases in each group. This result is consistent with reports from two previous studies on GST genes, using exposure cut-off of 30 pack/years and 35 pack/years, respectively (18,31). In all studies, however, the relatively small number of patients with combinations of rare genotypes, as well as of light smokers, limits the validity of the estimates. As more studies on the role of XME genes on lung cancer development become available, future larger meta-analyses are expected to elucidate the relationship between specific genotypes of those genes and tobacco exposure.

In the present study, patients with the *GSTT1* null genotype were diagnosed with lung cancer at significantly older age than patients bearing the wild-type allele. Although data on association of XME gene polymorphisms and age are limited, it has recently been reported that lung cancer patients younger than 45 years exhibit more frequently the *GSTT1* null genotype, as well as the *CYP1A1* variant allele, compared to age-matched controls (25).

Presence of *CYP1A1* m1 allele in our study was negatively associated with squamous cell carcinoma compared to adenocarcinoma, while the presence of *GSTM1* null genotype correlated with adenocarcinoma. Although these results appear in discordance with previously reported data (32,33), it seems that a definite association between polymorphisms of XME genes and histological type of lung cancer cannot be established: in two recently published meta-analyses involving large number of cases the association between the homozygous *CYP1A1* m1 variant and lung cancer was equally strong for squamous cell carcinomas and adenocarcinomas among Caucasians (28), while no correlation between *GSTM1* null genotype and histological type of lung cancer could be established (17). It seems therefore that susceptibility to the effect of carcinogens, arising from polymorphisms in XME genes, shows no predilection for any histological type of lung cancer.

An interesting finding of the present study was the significant association between the *GSTM1* null genotype and history of COPD in lung cancer patients, compared to patients with history of other lung disease. The association between *GSTM1* gene and COPD has been reported in the past in several populations, including cohorts of Caucasian patients (34,35). The connection between *GSTM1* gene and COPD should be considered on the basis that both lung cancer and COPD have a causative relationship with smoke exposure. This hypothesis was hard to test in the present study, as only 10 lung cancer patients were non-smokers, nevertheless, it is supported by the lack of association between the genotypes examined when lung cancer patients with pre-existing COPD were compared to patients with free respiratory history. It is of note that, in contrast with *GSTM1* gene, no associations between COPD and either *CYP1A1*, or *GSTT1* exist so far in the literature. Therefore, the difference in frequency of deletions of *GSTM1* gene between COPD patients and patients with other previous respiratory disease could imply the existence of different pathogenetic mechanisms for lung cancer development.

In the present study, no association between XME genotypes and other clinicoepidemiological parameters of lung cancer cases could be established. This result is in accordance with the limited data existing so far in the literature. In one Northern European study, no associations between *CYP1A1* gene polymorphisms and family history could be revealed (36). Furthermore, a meta-analysis on asbestos exposure in relation to *GSTM1* and *GSTT1* polymorphisms on Caucasians could not support the hypothesis that the risk of lung cancer after asbestos exposure differs according to those genotypes (37). In another study, performed on an Asian population, NSCLC patients with at least one *CYP1A1* m1 allele (and in particular smokers and those with advanced disease) were associated with a shortened survival compared to the wild-type homozygous patients, and this effect was enhanced when the *CYP1A1* genotype was combined with the *GSTM1* null genotype (38). The failure of the present study to establish any correlations between XME genes and survival or response to therapy could be attributed to the short follow-up period, as well as the multiple treatment modalities used, moreover lung cancer is a malignancy with poor overall prognosis if not diagnosed early.

In conclusion, we studied the genotyping profile of XME genes in a series of Greek lung cancer patients and healthy controls. Homozygous *CYP1A1* m1 genotype and *GSTT1* homozygous deletion were more frequent in patients than in controls, however, no definite associations between the risk for lung cancer and any individual genotype could be established. It is rather the combination of rare genotypes - due to accumulated deviations from the common XME genotypes - that tends to correlate with lung cancer, mainly in association with high tobacco consumption. These findings did not reach statistical power in the present study; though results from recently published meta-analyses are also consistent with this hypothesis. Combined rare XME genotypes in patients with previous history of COPD, as well as differences between lung cancer histological types, may account for involvement of these genes in the pathogenesis of distinct lung malignancies. As data on XME genes become

increasingly available, larger molecular epidemiology studies are expected to provide further insight into the role of XME genes in lung carcinogenesis.

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