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Polymorphisms in the microsomal epoxide hydrolase gene: role in lung cancer susceptibility and prognosis

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ABSTRACT

Aims and background. The aim of this study was to investigate the relationship between *EPHXI* exon 3 Tyr113His and exon 4 His139Arg polymorphisms, predicted microsomal epoxide hydrolase (mEH) activity, and lung cancer development. mEH is a protective enzyme involved in oxidative defences against a number of environmental chemicals and pollutants, but it is also responsible for the xenobiotic activation of carcinogens.

Methods We investigated the two polymorphisms of the mEH gene (*EPHX1*) in 58 lung cancer patients and 41 controls using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results. The exon 3 Tyr113His polymorphism was associated with lung cancer (P <0.001). The frequency of the His113His homozygote genotype in exon 3 was significantly increased in patients compared with controls (P <0.001). In contrast, there was no significant difference in exon 4 polymorphisms between patients and controls. When the exon 3 and 4 polymorphisms were considered together, the combined *EPHX1* His113His113/His139His139 genotype (very low predicted enzyme activity) was found to be associated with an increased risk of lung cancer (P = 0.044, OR = 3.063, CI = 0.932-10.069). We observed that patients with T3 + T4 tumors had an approximately 3-fold higher risk of the Tyr113/His113 genotype than patients with T1 + T2 tumors. Lung cancer patients carrying a heterozygote Tyr113/His113 genotype had a 2-fold increased risk of lymph node metastases (P = 0.051).

Conclusion. These findings suggest that the exon 3 Tyr113His and exon 4 His139Arg polymorphisms of EPHXI may be associated with a increased risk of lung cancer and a worse prognosis. Free full text available at www.tumorionline.it

Key words: microsomal epoxide hydrolase, lung cancer, exons 3 and 4 polymorphism, metastasis.

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Introduction

Microsomal epoxide hydrolase (mEH) is of great importance in a variety of detoxification processes and in the metabolism of endogenous and exogenous compounds[1]. The enzyme hydrolyzes epoxides, yielding trans-dihydrodiols. Such hydrolysis usually has a detoxifying effect, but in some instances trans-dihydrodiols generated from polycyclic aromatic hydrocarbons (PAHs) are highly toxic and mutagenic. Therefore, mEH has a dual role in the detoxification and activation of procarcinogens, depending on the substrate[2,3]. mEH is expressed in all tissues, and the highest concentrations have been found in the gonads, kidney, lung, liver and bronchial epithelic cells[4-6].

Genetic variations in metabolic activation or detoxification enzymes have been thought to contribute to individual differences in lung cancer susceptibility[7]. Poly-

morphic sites within the mEH gene (*EPHX1*), localized on chromosome 1q (1q 42.1) resulting in variation of amino acid residues 113 (Tyr/His) and 139 (Arg/His) have been identified. However, these mutations do not affect the specific activity of the mEH enzyme. It is suggested that the amino acid substitution may result in altered protein stability[8]. In exon 3, a C-to-T transition resulting in a Tyr113His substitution is associated with a 40-50% decrease in the *in vitro* activity of mEH. This allelic conversion has been referred to as the "slow" allele. The second variant is characterized by a C-to-A transition in exon 4 causing a His139Arg substitution and a 25% increase of enzyme activity. This allele has been called the "fast" allele[9]. With regard to *EPHX1*, the literature shows relatively inconsistent findings on the association between susceptibility to lung cancer and *EPHX1* polymorphism[10-12].

The aim of this study was to investigate a possible correlation between exon 3 and exon 4 polymorphism of the *EPHX1* gene and lung cancer development.

Materials and methods

Study population

A total of 58 lung cancer patients and 41 controls were included in this study. The mean ages of the lung cancer patients and controls were 58.0 ± 9.4 years and 59.1 ± 4.0 years, respectively. The percentage of males was 87.9% for patients and 43.9% for controls. None of the subjects in the control group were smokers. The histological diagnosis was squamous cell carcinoma and nonsquamous (i.e., adenocarcinoma, large cell carcinoma and other histologies) in 58.3% and 33.3% of patients, respectively. Nineteen of 21 patients with squamous cell carcinoma were smokers (90.5%) and 9 of 12 patients with adenocarcinoma (75%) were smokers.

Blood samples were collected from all 58 patients with a histologically proven diagnosis of non-small cell lung carcinoma. The controls were selected among people with no proven malignant disease or disease history attending outpatient clinics and clinics. The main medical diagnoses among the control subjects were rheumatological, cardiovascular and non-neoplastic diseases such as trauma. We asked each study participant to complete a structured questionnaire to collect demographic information. All study participants gave their written informed consent. Controls and cases were interviewed regarding age, sex, smoking status, history of cancer, chronic diseases, and family history of cancer. Only individuals without a history of cancer and chronic respiratory disease were eligible to participate as controls. Only current smokers and never smokers were recruited in this study. All data, including pathological diagnoses and surgical findings, were recorded. Histological and staging information was confirmed by manual review of the pathology reports and clinical charts. The histologies of the lung tumors were determined according to the WHO classification[13]. This study protocol was approved by our local ethics committee.

Isolation of DNA

DNA was isolated from blood leukocytes in 10 mL EDTA by the method of Miller *et al.* based on sodium dodecyl sulfate lysis, ammonium acetate extraction, and ethanol precipitation[14]. Two separate polymerase chain reactions (PCRs) were used to detect the 2 mutations in the *EPHX1* gene, namely His139Arg in exon 4[10] and Tyr113His in exon 3[15].

Identification of the EPHX1 Tyr/His 113 variant (exon 3 polymorphism)

Template DNA (0.5-1.0 µg) was used in a PCR under sterile conditions. 0.4 mol/L of each primer was used for the reaction. For exon 3, the forward primer was 5'-GAT CGA TAA GTT CCG TTT CAC C-3' and the reverse primer was 5'-ATC CTT AGT CTT GAA GTG AGG AT-3' (MBI Fermentas) in a volume of 25 µL containing 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH:8.4), 0.16 mM each of dNTP (MBI Fermentas), and 1 unit of Taq polymerase (MBI Fermentas). Amplification was performed by initial denaturation at 94 ºC for 5 minutes, followed by 35 cycles with denaturation steps at 94 ºC for 40 seconds, annealing at 52.6 ºC for 50 seconds, and extension at 72 ºC for 30 seconds. The PCR program was completed by a final extension cycle at 72 ºC for 5 minutes. The PCR product exhibited a 160 base-pair fragment. PCR product (10 µL) was digested with 3U EcoRV (MBI Fermentas), and visualized by electrophoresis on 3% agarose containing 0.5 mg/mL ethidium bromide. When an EcoRV restriction site was present, the 160-bp fragment was digested into 2 lengths of 140 and 20 bp. The homozygous wild-type (Tyr113/Tyr113) had no such cutting site, heterozygous individuals (Tyr113/-His113) had 3 bands, and the homozygous variant type (His113/His113) had 2 bands. The *EPHX1* exon 3 polymorphism was typed by visualization under ultraviolet light and photographed with a Polaroid camera.

Identification of the EPHX1 His/Arg139 variant (exon 4 polymorphism)

For exon 4, the forward primer was 5'-GGG GTA CCA GAG CCT GAC CGT-3' and the reverse primer was 5'-AAC ACC GGG CCC ACC CTT GGC-3' (MBI Fermentas). PCR was performed in a 25-µL final volume containing 2 mM $MgCl_2$, 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 0.2 mM each of dNTP (MBI Fermentas), and 1.5 unit of Taq polymerase (MBI Fermentas). DNA was denatured at 94 ºC for 5 minutes. Thirty-five cycles of amplification began with denaturation at 94 ºC for 30 seconds, primer annealing at 62 ºC for 30 seconds, and extension at 72 ºC

for 45 seconds followed by a final extension step of 5 minutes at 72 ºC. Following overnight digestion of the 15-µL PCR product with 10 U RsaI (MBI Fermentas), the product was visualized by electrophoresis on 3% agarose gel. The His139-coding wild-type allele was identified by 2 DNA bands (295 and 62 bp), whereas the Arg139 allele resulted in 3 DNA bands after digestion (174, 121, and 62 bp) and the heterozygous allele produced all 4 DNA bands (295, 174, 121, and 62 bp). In order to verify our polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) results, we repeated the PCR-RFLP stage twice for each of selected subjects. Pursuant to Smith and Harrison[16], we classified the predicted mEH activity as very low, low, intermediate, or high, as indicated in Table 1.

Statistical analysis

All statistical analyses were carried out using SPSS version 7.5 for Windows. Numeric values were analyzed by Student's *t*-test. Differences in characteristics between lung cancer patients and controls were assessed with Fisher's exact test, as well as disparities in genotype and allele frequencies. The frequencies of *EPHX1* (both exon 3 and exon 4) alleles were estimated by gene counting methods. The relative associations between lung cancer patients and controls were assessed by calculating crude Gart's odds ratios (ODs) and 95% confidence intervals (95%CI). The threshold for significance was $P<0.05$. Multivariate analysis was performed to examine the effect of smoking on the development of lung cancer.

Results

Data on the *EPHX1* exon 3 and exon 4 polymorphisms are shown in Table 2. In the study group, the frequencies obtained for the exon 3 *EPHX1* Tyr113 and His113 alleles were 0.33 and 0.67, respectively. The allele frequencies for the exon 4 *EPHX1* polymorphism were 0.75 and 0.25 for His139 and Arg139, respectively. In the control group, the frequencies for the exon 3 *EPHX1* polymorphism were 0.68 and 0.32 for Tyr113 and His113, respectively. In this group, the frequencies for the exon 4 polymorphism were 0.73 and 0.27 for His139 and Arg139, respectively. The distribution of the exon 3 genotypes in controls and lung cancer patients was found to be highly significantly different ($P<0.001$). The prevalence of *EPHX1* exon 3 His/His homozygosity was 44.8% (26/58) in lung cancer patients and 17.1% (7/41) in controls. This difference was statistically significant ($P = 0.004$; OR = 2.626, 95%CI = 1.262-5.462) (Table 2).

Table 1 - Predicted mEH enzyme activity based on classification of Smith and Harrison[16]

Exon 4 (His139Arg polymorphism)	Exon 3 (Tyr113His polymorphism)		
Genotypes	Tyr/Tyr	Tyr/His	His/His
His/His	Intermediate	Low	Very low
His/Arg	High	Intermediate	Very low
Arg/Arg	High	Low	Very low

mEH, microsomal epoxide hydrolase; His, histidine; Arg, arginine; Tyr, tyrosine.

The frequency of the *EPHX1* exon 3 His allele in the lung cancer patient and control groups was 89.7% (52/58) and 51.2% (19/41), respectively. The distribution of the *EPHX1* exon 3 His allele was significantly different between patients and controls ($P<0.001$; OR = 1.935,95% CI = 1.376-2.720). The Arg139Arg variant of *EPHX1* was rare in both patients 5.2% (3/58) and controls 7.3% (3/41). No relationship was found between exon 4 *EPHX1* polymorphism and lung cancer ($P = 0.690$).

We have investigated whether a combination of the Tyr113His and His139Arg genotypes was associated with lung cancer. Our results showed that the Tyr113/Tyr113 and His139/His139 genotype combination was the most common genotype in controls (13/41; 31.7%). We found that the Tyr113/Tyr113 and His139/His139 genotype combination (with intermediate predicted mEH activity) was associated with a decreased risk in the lung cancer group compared to all other combinations ($P = 0.003$; OR = 0.272, 95%CI = 0.105-0.704). The His113/His113 and His139/His139 genotype (with very low predicted mEH activity) was more common in the lung cancer group ($P = 0.044$; OR = 3.063, 95%CI = 0.932-10.069) compared to all other combinations. His113/His113 and either His139/His139 or His139/Arg139 and His113/His113 and Arg139/Arg 139 genotype combinations (very low predicted mEH activity) were found more frequently in the lung cancer group compared to all other combinations ($P = 0.004$; OR = 2.626; 95%CI = 1.262-5.462). A significant negative correlation between genotype combinations that predicted high mEH activity (Tyr113/Tyr113 and His139/ Arg139; Tyr113/Tyr113 and Arg139/Arg139) and lung cancer risk was found compared to all other combinations ($P = 0.001$; OR = 0.079; 95%CI = 0.010-0.596) (Table 2).

The presence of at least one exon 4 Arg139 variant allele was associated with a 2.1-fold increased risk in our smoker lung cancer patients ($P = 0.016$; OR = 2.1; 95%CI = 1.091-4.041). Heterozygosity of the exon 4 genotype (His139Arg) was found to be a risk factor for lung cancer compared to homozygous genotypes ($P = 0.006$, OR = 2.644; 95%CI = 1.218-5.741). However, no significant relationship between exon 3 genotypes and lung cancer was found. When we analyzed lung cancer patients who were smokers, the Tyr113/His113 and His139/Arg139 (with intermediate predicted mEH activity) genotype combination indicated a higher risk compared to nonsmoker patients ($P = 0.026$; OR = 4.2, 95%CI = 0.992-17.781) (Table 3).

The Tyr113/His113 and either His139/His139 or Arg139/Arg139 (low predicted mEH activity) genotype

Table 2 - Risk of lung cancer associated with *EPHX1* genotypes and predicted mEH activity

Genotypes	Patients n (%)	Controls n (%)	OR[#] (95%CI)	*P* value
EPHX1 exon 3				
Tyr113/Tyr113	6 (10.3)	22 (53.7)	0.193 (0.086-0.433)	0.001***
Tyr113/His113	26 (44.8)	12 (29.3)	1.532 (0.879-2.668)	0.117
His113/His113	26 (44.8)	7 (17.1)	2.626 (1.262-5.462)	0.004**
EPHX1 exon 4				
His139/His139	32 (55.2)	22 (53.7)	1.028 (0.712-1.484)	0.882
His139/Arg139	23 (39.7)	16 (39)	1.016 (0.618-1.671)	0.950
Arg139/Arg139	3 (5.2)	3 (7.3)	0.707 (0.150-3.329)	0.690
Combined *EPHX1* genotypes				
Tyr113/Tyr113 and His139/His139	5 (8.6)	13 (31.7)	0.272 (0.105-0.704)	0.003**
Tyr113/Tyr113 and His139/Arg139	1 (1.7)	8 (19.5)	0.088 (0.011-0.680)	0.003**
Tyr113/Tyr113 and Arg139/Arg139	0 (0)	1 (2.4)	-	0.414
Tyr113/His113 and His139/His139	14 (24.1)	6 (14.6)	1.649 (0.692-3.932)	0.246
Tyr113/His113 and His139/Arg139	11 (19)	4 (9.8)	1.944 (0.665-5.680)	0.208
Tyr113/His113 and Arg139/Arg139	1 (1.7)	2 (4.9)	0.353 (0.033-3.769)	0.568
His113/His113 and His139/His139	13 (22.4)	3 (7.3)	3.063 (0.932-10.069)	0.044*
His113/His113 and His139/Arg139	11 (19)	4 (9.8)	1.944 (0.665-5.680)	0.208
His113/His113 and Arg139/Arg139	2 (3.4)	0 (0)	-	0.519
Predicted mEH activity[##]				
Very low	26 (44.8)	7 (17.1)	2.626 (1.262-5.462)	0.004*
Low	15 (25.9)	8 (19.5)	1.325 (0.620-2.832)	0.461
Intermediate	16 (27.6)	17 (41.5)	0.665 (0.383-1.157)	0.149
High	1 (1.7)	9 (22)	0.079 (0.010-0.596)	0.001***

n, number of subjects.
**P* value <0.05; ***P* value <0.01; *P* value <0.001.
[#]Odds ratio computed between selected genotype/predicted mEH activity *versus* all other genotypes/predicted mEH activities in corresponding group.
[##]Classification of predicted mEH activity is as follows: very low: His113/His113 and either His 139/His139 or His139/Arg or Arg139/Arg139; low: Tyr113/His113 and either His139/His139 or Arg139/Arg139; intermediate: Tyr113/Tyr113 and His139/His139, Tyr113/His113 and His139/Arg139; high: Tyr113/Tyr113 and either His139/Arg139 or Arg139/Arg139.
EPHX1, epoxide hydrolase; Tyr, tyrosine; His, histidine; Arg, arginine.

combination was significantly more frequent in lung cancer patients who were smokers than in nonsmoking patients (*P* = 0.024; OR = 0.339, 95%CI = 0.122-0.943) (Table 3).

The distribution of exon 3 Tyr113His and exon 4 His139Arg genotypes according to familial lung cancer history, tumor stage and lymph node metastasis status in our lung cancer patients is shown in Table 4.

A family history of cancer together with Arg139 alleles (compared to His139/His139 homozygote genotype) were statistically significantly associated with lung cancer (OR = 2.06, 95% CI = 1.268-3.358) (*P* = 0.08). When patients whose first, second or third-degree relatives had any history of cancer were analyzed according to combinations of exon 3 and exon 4 genotypes, we found an increased frequency of the His113His113/His139 Arg139 combined genotype (with a very low predicted mEH activity) (*P* = 0.009; OR = 4.952; 95%CI = 2.030-12.08).

We analyzed exon 3 Tyr113His and exon 4 His139Arg genotypes in the lung cancer group according to tumor stage and lymph node metastases (Table 4). Patients with locally advanced disease (T3 + T4) had approximately 3 times more frequently heterozygous Tyr113/His113 (*P* = 0.001) and homozygous His113/His113 (*P* = 0.016) genotypes than patients with T1 + T2 tumors.

Very low predicted mEH activity was found rarely in patients with T3 + T4 tumors compared to patients with T1 + T2 tumors (*P* = 0.016; OR = 0.275; 95%CI = 0.073-1.030). Lung cancer patients carrying a heterozygous Tyr113/His113 genotype marginally more frequently had an increased risk of having lymph node metastases (*P* = 0.051). Similarly, although there was a relationship between the Tyr113His113/His139Arg139 combined genotype and lymph node metastasis, this relationship was not statistically significant (*P* = 0.141; OR = 2.333; 95%CI = 0.707-7.698).

Discussion

Cancer is caused by specific changes in oncogenes, tumor-suppressor genes, and microRNA genes. Such changes usually occur as somatic events, although germline mutations can predispose a person to heritable or familial cancer[17]. A small increase in the risk of a frequent cancer such as lung cancer can cause a large number of excess lung cancer cases. Cigarette smoking drastically increases the lung cancer risk, but it is known that not all individuals who smoke cigarettes develop lung cancer[18,19]. Genetic differences or polymorphisms

Table 3 - Analysis of *EPHX1* exon 3 and exon 4 genotypes and mEH activity in lung cancer patients stratified by smoking status

Genotypes	Smokers n (%)	Nonsmokers n (%)	OR# (95%CI)	*P* value
EPHX1 exon 3				
Tyr113/Tyr113	2 (6.7)	4 (14.3)	0.467 (0.093-2.352)	0.415
Tyr113/His113	13 (43.3)	13 (46.4)	0.933 (0.527-1.652)	0.813
His113/His113	15 (50)	11 (39.3)	1.273 (0.710-2.280)	0.412
EPHX1 exon 4				
His139/His139	12 (40)	20 (71.4)	0.560 (0.341-0.920)	0.016*
His139/Arg139	17 (56.7)	6 (21.4)	2.644 (1.218-5.741)	0.006**
Arg139/Arg139	1 (3.3)	2 (7.1)	0.467 (0.045-4.867)	0.605
Combined *EPHX1* genotypes				
Tyr113/Tyr113 and His139/His139	2 (6.7)	3 (10.7)	0.622 (0.112-3.452)	0.665
Tyr113/Tyr113 and His139/Arg139	0 (0)	1 (3.6)	-	0.483
Tyr113/Tyr113 and Arg139/Arg139	0 (0)	0 (0)	-	-
Tyr113/His113 and His139/His139	4 (13.3)	10 (35.7)	0.373 (0.132-1.055)	0.047*
Tyr113/His113 and His139/Arg139	9 (30)	2 (7.1)	4.20 (0.992-17.781)	0.026*
Tyr113/His113 and Arg139/Arg139	0 (0)	1 (3.6)	-	0.483
His113/His113 and His139/His139	6 (20)	7 (25)	0.800 (0.306-2.092)	0.648
His113/His113 and His139/Arg139	8 (26.7)	3 (10.7)	2.489 (0.733-8.455)	0.121
His113/His113 and Arg139/Arg139	1 (3.3)	1 (3.6)	-	-
Predicted mEH activity##				
Very low	15 (50)	11 (39.3)	1.273 (0.710-2.280)	0.412
Low	4 (13.3)	11 (39.3)	0.339 (0.122-0.943)	0.024*
Intermediate	11 (36.7)	5 (17.9)	2.053 (0.816-5.169)	0.109
High	0 (0)	1 (3.6)	-	0.483

N, number of subjects. **P* value <0.05; ***P* value <0.01.
#Odds ratio computed between selected genotype/predicted mEH activity *versus* all other genotypes/predicted mEH activities in corresponding group.
##Classification of predicted mEH activity is as follows: very low: His113/His113 and either His 139/His139 or His139/Arg or Arg139/Arg; low: Tyr113/His113 and either His139/His139 or Arg139/Arg139; intermediate: Tyr 113/Tyr113 and His139/His139, Tyr113/His113 and His139/Arg139; high: Tyr113/Tyr113 and either His139/Arg139 or Arg139/Arg139.

EPHX1, epoxide hydrolase; Tyr, tyrosine; His, histidine; Arg, arginine.

Table 4 - Distribution of *EPHX1* exon 3 Tyr113His and exon 4 His139Arg genotypes with clinicopathological features of lung cancer patients

Family history of any kind of cancer	Exon 3 genotypes			Exon 4 genotypes		
	Tyr113/Tyr113 n (%)	Tyr113/His113 n (%)	His113/His113 n (%)	His139/His139 n (%)	His139/Arg139 n (%)	Arg139/Arg139 n (%)
Yes*	1 (16.7)	1 (16.7)	4 (66.7)	1 (16.7)	5 (83.3)	0 (0)
No	5 (9.6)	25 (48.1)	22 (42.3)	31 (59.6)	18 (34.6)	3 (5.8)
T stage						
T3 + T4	0 (0)	11 (84.6)	2 (15.4)	5 (38.5)	8 (61.5)	0 (0)
T1 + T2	4 (16)	7 (28)	14 (56)	13 (52)	11 (44)	1 (4)
Lymph node status						
N (+)	1 (5.3)	12 (63.2)	6 (31.6)	8 (42.1)	11 (57.9)	0 (0)
N (-)	3 (15.8)	6 (31.6)	10 (52.6)	10 (52.6)	8 (42.1)	1 (5.3)

EPHX1, epoxide hydrolase; Tyr, tyrosine; His, histidine; Arg, arginine.
n, number of subjects. *First, second or third-degree relatives with any kind of cancer.

in the genes encoding xenobiotic-metabolizing enzymes may increase an individual's susceptibility to a potential carcinogen[10,20]. Polymorphisms should therefore be thought of as a potentially important public health issue. The mEH enzyme is an important biotransformation system that catalyzes the hydrolysis of a wide variety of xenobiotic epoxides, resulting in the formation of corresponding trans-dihydro derivatives[9]. Substrates for detoxification enzymes include epoxides of environmental toxins, such as carcinogenic PAHs, aromatic amines and benzene[21,22]. Lower activity of *EPHX1* exon 3 genotypes have been linked to a decreased lung cancer risk in several studies[11,23]. Yin *et al.*[7] reported that smokers carrying the exon 3 His113 allele had a higher relative risk of lung cancer in the Taiwanese population. Likewise, we found a significantly increased

risk in subjects with exon 3 His/His homozygosity compared to controls. However, when we stratified our patients by smoking status, there was no significant difference among *EPHX1* exon 3 genotypes. We found no correlation between exon 4 *EPHX1* polymorphism and lung cancer risk. However, previous studies showed that the Arg/Arg genotype at exon 4 was weakly associated with an increased risk of lung cancer among Chinese, Mexican-Americans, blacks and whites[10-12] and with a decreased risk of lung cancer among Chinese and whites[12,24]. We have arranged the combined genotypes into 4 levels of predicted mEH activity (very low, low, intermediate, high) according to Smith and Harrison[16] and calculated the risk associated with a selected genotype/predicted mEH activity compared with all other genotypes/predicted mEH activities. We found an inverse relationship between a genotype combination that predicted high mEH activity and lung cancer risk (P = 0.001). A combined genotype for very low predictied activity was found to be associated with an increased risk of lung cancer. As expected, our lung cancer patients had a markedly greater smoking history than controls. These results are in contrast with the hypothesis that high mEH activity is not protective in smokers with a high cumulative dose of carcinogens derived from smoking[25].

Smith and Harrison[16] reported that most of their patients had pathological evidence of emphysema consistent with the very heavy smoking history typical of lung cancer cases. Their data suggested an increased risk of lung cancer for subjects with very low predicted mEH activity. The authors interpreted their data as showing an increased risk for low activity in relation to emphysema but no association with lung cancer. In our study, we did not classify patients as having emphysema or not. By contrast, Benhamou *et al.*[26] reported an increased risk with higher predicted activity. The discrepancy with our findings could be due to the fact that in our series a minority of patients were nonsmokers and PAHs such as benzo(a)pyrene are activated by mEH into reactive intermediates[25] and higher mEH activity leads to higher concentrations of BPDE, BPDE-serum albumin adducts, and DNA adducts in the body compared with very low activity of mEH[27]. In addition, it has been shown that cigarette smoking can significantly induce the activity of mEH[28]. Previous studies on the relationship between mEH genotypes and lung cancer risk yielded inconsistent results. One study of 150 smoking, Caucasian lung cancer patients found that higher activity of mEH was associated with lung cancer[26]. On the other hand, in a study in which 95% of 155 African-American lung cancer patients were smokers, a very low activity genotype was found to be associated with a decreased risk of lung cancer[23]. Zhou and colleagues[29] analyzed 974 Caucasian lung cancer patients and 1142 controls. They found that there was no overall relationship between *EPHX1* genotypes and lung cancer risk. When we analyzed lung cancer patients after stratifying by smoking status, the highest ratios were found among subjects with Tyr113/His113 and His139/Arg139 (intermediate predicted mEH activity) genotypes compared to all other combinations, and among patients who were heterozygous for the exon 4 genotype compared with individuals with other homozygous genotypes. It must be emphasized that *EPHX1* has been implicated in both protection against and potentation of the effects of carcinogens. Either slow or high *EPHX1* metabolizers should be seriously considered for their ability to simultaneously decrease and increase the bioactivation of specific compounds[30] In nonsmokers, environmental pollutant hydrocarbons or occupational exposure may play a role in lung carcinogenesis. Examples of these chemicals include alkene, arene, and reactive epoxide intermediates, which are detoxified by *EPHX1*. This could explain why the genotype combination His113/His139 (very low predicted activity) increases the risk of lung cancer[26,31]. It is possible that additional factors such as ethnic differences in the distribution of alleles, dietary protein[8,32] or posttranscriptional processing mechanisms come into play[27]. However, it should be noted that we did not assess the enzyme activity. Differences in associations between ethnic subgroups or between study populations can result from linkage disequilibrium with additional allelic variants that modulate overall enzyme activity and may be present at different frequencies in the different groups, or perhaps linkage disequilibrium with another gene that is causally related to lung cancer as suggested by London and colleagues[23]. In our study, this could be a possible factor affecting enzyme activity in addition to the *EPHX1* alleles that were investigated; however, our population was relatively homogeneous and comprised only Caucasians. In our control group, the frequencies for the exon 3 *EPHX1* polymorphism were 0.68 and 0.32 for Tyr113 and His113, respectively, whereas the exon 4 polymorphism frequencies were 0.73 and 0.27 for His139 and Arg139, respectively. In order to clarify the controversial results reported by different research groups, Kiyohara and colleagues[33] conducted a systematic review and meta-analysis and found that the mEH enzyme may act as a phase I enzyme in lung carcinogenesis. They concluded that the low-activity genotype of the *EPHX1* gene is associated with a decreased risk of lung cancer among whites. In our study we have also investigated whether a combination of the Tyr113His and His139Arg genotypes is associated with lung cancer risk. We observed that the Tyr113/Tyr113 and His139/His139 genotype combination was the most common genotype in our controls (13/41; 31.7%). The frequency of the Tyr113/Tyr113 and His139/His139 genotype (with intermediate predicted mEH activity) was found to be decreased. Neither the exon 3 Tyr113His nor the exon 4 His139Arg genotypes were found be associated with a family history of respiratory disease or any kind of can-

cer, tumor stage, or lymph node status in our lung cancer patients. We found a higher frequency of the combined His113His113/His139Arg139 genotype (with a very low predicted mEH activity) in lung cancer patients whose first, second or third-degree relatives had any history of cancer.

Sandford and colleagues[34] reported that His113/His139 (lower activity haplotype) was associated with a 6-fold increased risk of a rapid decline in lung function. We did not analyze the lung function our patients or investigated the possible relationship between lung function and lung cancer.

What we did investigate was the association between the *EPHX1* exon 3 Tyr113His and exon 4 His139Arg genotypes and exon 3 Tyr113His/exon 4 His139Arg combined genotypes and tumor size and lymph node metastasis in lung cancer patients. We found that lung cancer patients with T3 + T4 tumors had ORs of 3 and 0.3 for Tyr113/His113 heterozygous and His113/His113 homozygous genotypes compared with patients with T1 + T2 tumors, respectively. We also found a decreased risk in patients with very low predicted mEH activity. Patients with locally advanced tumors (T3 + T4) had a significantly lower frequency of very low predicted mEH activity compared to patients with T1 + T2 tumors. Lung cancer patients who were carrying a Tyr113 His113/His139Arg139 combined genotype had a 2.33-fold increased risk of lymph node metastasis, but it was not found to be statistically significant. These findings have not been reported before and it could be speculated that these genotypes play a role in tumor progression and lymph node metastasis, although there has not been any evidence to support this hypothesis. However, this finding is of great importance because nodal involvement is the most important prognosticator in resectable non-small lung cancer[35]. Although we did not analyze the effect of *EPHX1* genotypes on survival, some authors investigated the role of *EPHX1* polymorphism along with glutathione S-transferase (GST) polymorphism as another enzyme taking part in carcinogen detoxification[36]. The distribution of *EPHX1* exon 3 and exon 4 genotypes in controls documented in our study is similar to that reported by Ada *et al.*, who studied a Turkish population[37]. They also documented GST-π polymorphism as a detoxification enzyme. However, the number of cases in our control group was suboptimal for a representation of our national population. Predicted mEH activity distributions were also published from our country[38]. The frequencies of genes for predicted low and intermediate activities were similar to those of our patients, whereas the percentage of genotypes for predicted high activity were reportedly lower comparing to our findings. It could be due to the sample size and non-male control group. However, we did not examine the impact of gender on genotypes.

In conclusion, our study indicated that polymorphism of the *EPHX1* gene might be a modifying factor in lung carcinogenesis and might play a role in the prognosis of patients who underwent resectional surgery. Larger epidemiological studies will be needed to confirm our results and to establish the role of genes that encode additional enzymes participating in the same metabolic pathways in relationship to histology, diet and different smoking history. Larger studies are also needed to clarify the role of *EPHX1* polymorphism in the survival of patients after resectional surgery.

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