The Interaction between Microsomal Epoxide Hydrolase Polymorphisms and Cumulative Cigarette Smoking in Different Histological Subtypes of Lung Cancer[1]

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Abstract

Microsomal epoxide hydrolase (*mEH*) is involved in the metabolism of environmental and tobacco carcinogens. Smaller studies found inconsistent results in the relationship between *mEH* polymorphisms and lung cancer risk. We investigated the two polymorphisms of *mEH* in 974 Caucasian lung cancer patients and 1142 controls using PCR-RFLP techniques. The results were analyzed using generalized additive models and logistic regression, adjusting for relevant covariates. There was no overall relationship between *mEH* genotypes and lung cancer risk. The adjusted odds ratio (OR) of the very low activity genotype *versus* that of other genotypes combined was 1.00 [95% confidence interval (CI), 0.74–1.34]. However, gene-environment interaction analyses revealed that the ORs decreased as cumulative smoking (defined as square root of pack-years) increased. When pack-years = 0, the OR was 1.89 (95% CI, 1.08–3.28). When pack-years = 28.5, the OR was 1.00 (95% CI, 0.76–1.32), and when pack-years = 80, the OR decreased to 0.65 (95% CI, 0.42–1.00). When cases were stratified according to histological subtypes, the interaction between *mEH* genotype and cumulative smoking was statistically significant ($P < 0.01$) for the 222 squamous cell carcinoma cases, whereas it was not significant ($P = 0.18$) for the 432 adenocarcinoma cases. In conclusion, cumulative cigarette smoking plays a pivotal role in the association between *mEH* polymorphisms and lung cancer risk, altering the direction of risk (in the case of the very low activity genotype) from a risk factor in nonsmokers to a relatively protective factor in heavy smokers.

Introduction

Xenobiotic-metabolizing *mEH*[3], one of four distinct epoxide hydrolases, catalyzes the hydrolysis of arene, alkene, and aliphatic epoxides to less reactive and more water soluble dihydrodiols through the *trans* addition of water (1–3). Although this hydrolysis is generally considered to represent a detoxification reaction, some PAHs, including BPDE, are in fact more reactive carcinogenic species (4). Therefore, *mEH* plays a dual role in the detoxification and activation of procarcinogens, and its role in carcinogenesis may depend on exposures to different environmental substrates.

There are two polymorphisms that affect enzyme activity in the human *mEH* gene. One variant is characterized by substitution of histidine for tyrosine at amino acid position 113 (*EH3*), and the other is substitution of arginine for histidine at position 139 (*EH4*), conferring low and high activity, respectively (1). Higher activity *mEH* genotypes were associated with elevated lung cancer risk in several studies (5–8), whereas low activity mEH somewhat increased lung cancer risk in another study (9). One small study reported no relationship between *mEH* genotypes and lung cancer (10). Thus far, the relationship between *mEH* genotypes and lung cancer risk has not been studied in a large numbers of subjects.

Tobacco-associated carcinogens, including PAHs, are known to induce mEH activity (11, 12). Furthermore, we recently evaluated (13) another genetic polymorphism, NADPH: quinone oxidoreductase 1, in lung cancer that was dependent on smoking status and cumulative smoking exposure. Thus, we hypothesized that smoking history may alter the relationship between *mEH* genotypes and lung cancer risk through exposure to different substrates differentially metabolized by mEH. *mEH* was strongly related to SCC of the lung in a Chinese population (7); therefore, we postulated that various histological subtypes of lung cancer may also have different relationships with *mEH* polymorphisms. Drawing from a large sample population, we tested these hypotheses using gene-environment interaction analyses and in analyses where the cases were stratified by histological subgroups.

Materials and Methods

Population. The study was approved by the Human Subjects Committees of Massachusetts General Hospital and the Harvard School of Public Health. Eligible cases included all of the

Received 12/20/00; revised 2/22/01; accepted 2/26/01.
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[1] Supported by NIH Grants CA74386, ES/CA 06409, ES00002, and TW 00828.
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[3] The abbreviations used are: *mEH*, microsomal epoxide hydrolase; PAH, polycyclic aromatic hydrocarbon; BPDE, (±)*r*-7,*t*-8-dihydroxy-*t*-9, *t*-10-epoxy-7,8,9,10-tetrahydrobenzo(*a*)pyrene; *EH3*, *mEH* exon 3; *EH4*, *mEH* exon 4; SCC, squamous cell carcinoma; VL, very low activity genotype group of *mEH*; GAM, generalized additive model; OR, odds ratio; CI, confidence interval; AC, adenocarcinoma; HWE, Hardy-Weinberg equilibrium; SR-PY, square root of pack-years.

histologically confirmed newly diagnosed lung cancer patients presenting to the Thoracic Surgery, Hematology-Oncology, and Pulmonary Units at Massachusetts General Hospital between December 1992 and December 1999. Controls were the friends of lung cancer cases, the spouses of lung cancer cases, or friends or spouses of other cardiothoracic-surgery patients in the hospital with no specific matching characteristics. This selection process provided a natural balance between the cases and controls, with no overmatching for potential confounding factors such as smoking status, ethnicity, race, socioeconomic class, or age (13, 14). Over 95% of both cases and controls in this population were Caucasians. Over 85% of eligible cases and 90% of eligible controls were enrolled in this study.

Trained interviewers, using a modified standardized American Thoracic Society respiratory questionnaire (15), obtained occupational, dietary, and detailed smoking information. Lifetime smoking histories included the number of years of smoking, average cigarettes/day, and the time since cessation of smoking for ex-smokers. If the participant was unable to fill out the questionnaires at the time of investigation, we used mail or telephone correspondence to obtain complete data.

Gene Polymorphism of *EH3* and *EH4*. Blood samples were obtained from each case or control via venipuncture. Within 24 h, DNA was extracted from the blood samples according to standard protocols described previously (14).

Genotyping was performed by investigators who were blinded to the subjects' case or control status. Two separate PCR assays were used to detect the polymorphisms in *EH3* and in *EH4*. The assay for the *EH3* variant used the primer pair (5′-GATCGATAAGTTCCGTTTCACC-3′) and (5′-ATCCTTAGTCTTGAAGTGAGGAT-3′), and a *Eco*RV restriction enzyme site was cleaved in the wild-type allele (7, 10). A *Rsa*I restriction enzyme site was cleaved in the variant alleles for *EH4* using the primer pair (5′-GGGGTACCAGAGCCTGACCGT-3′) and (5′-AACACCGGGCCCACCCTTGGC-3′; Ref. 1).

PCR assays were carried out using a Perkin-Elmer thermocycler in PCR buffer [100 ng of DNA, 3.5 mmol/liter magnesium chloride, 20 mmol/liter Tris-HCl (pH 8.6), 50 mmol/liter KCl, 12.5 μmol/liter of each deoxynucleotide triphosphate, 0.1% BSA, 100 ng of primers, and 1.5 units of *Taq* polymerase] for a final volume of 25 μl. Each PCR product was digested with 10 units of the appropriate restriction enzyme (New England Biolabs, Beverly, MA). Digested PCR products were separated by size on a 2.0% agarose gel (Sigma Chemical Co., St. Louis, MO). RFLP bands were visualized through ethidium bromide staining and UV illumination. For *EH3*, *Tyr113* wild-type alleles produced two bands (140 and 22 bp), *His113* homozygous alleles were identified by a single band (undigested 162 bp), and heterozygotes displayed three bands (162, 140, and 22 bp). For *EH4*, *His139* wild-type alleles were identified by two DNA bands (295 and 62 bp), *Arg139* homozygous alleles displayed three bands (174, 121, and 62 bp), and heterozygotes produced all of the four DNA bands (295, 174, 121, and 62 bp). The main cycling parameters included: 31 cycles of 94°C for 30 s and 56°C (for *EH3*) or 62°C (for *EH4*) for 30 s, followed by 72°C for 60 s.

Variant Forms of *mEH* as Surrogate of Enzyme Activity. The population was divided into four different enzyme activity groups (very low, low, intermediate, and high) according to the gene polymorphisms in *EH3* and *EH4* (1, 5, 10). VL consisted of individuals with *His/His* in both *EH3* and *EH4*. The low activity genotype group consisted of *His/His* in *EH3* and either *His/Arg* or *Arg/Arg* in *EH4* or *Tyr/His* in *EH3* and *His/His* in *EH4*. The intermediate activity genotype group consisted of *Tyr/His* in *EH3* and *His/Arg* in *EH4* or *Tyr/Tyr* in *EH3* and *His/His* in *EH4*, and the high activity genotype group consisted of individuals with *Tyr/Tyr* in *EH3* and either *His/Arg* or *Arg/Arg* in *EH4* or *Tyr/His* in *EH3* and *Arg/Arg* in *EH4*.

Statistical Analysis. We restricted our analysis to Caucasians with complete information on age, gender, smoking status (current, ex-, or non-), pack-years of smoking, and, for ex-smokers, years since smoking cessation. GAM (16) was used to examine the relationship between the odds of lung cancer risk and each continuous covariate. GAM extends the generalized linear models framework, such as logistic regression, by allowing the relationship between the outcome and each covariate to be an unspecified but smooth function. Using GAM, we created plots of the log odds of lung cancer *versus* the smooth function of each covariate in S-plus (17), after adjusting for other covariates. If any plot showed a departure from linearity, we fitted a second GAM model using a parametric transformation of the covariate suggested by the original plot and examined the linear relationship between the transformed covariate and the log odds of lung cancer risk. Then, we used logistic regression models to assess the independent association between *mEH* activity genotypes and the risk of all lung cancer and with histological subtypes (AC and SCC) that had an adequate sample size for subanalyses. We adjusted for possibly transformed confounding factors such as age (years), gender, smoking status, pack-years of smoking, and years since smoking cessation. Years since smoking cessation were defined as 0 for both current and nonsmokers. On the basis of our sample size, we also tested models that included possible interactions among *mEH* genotype, smoking status, and pack-years of smoking for all lung cancers (and for AC and SCC). When appropriate, OR and 95% CI for the risk of lung cancer by the *mEH* polymorphisms were calculated from these models. A lack of fit test was performed to summarize the goodness-of-fit of each logistic regression model (18).

Results

A total of 974 lung cancer patients and 1142 controls were included in this study. Histological data were available for 828 cases: 52.2% were AC, 26.8% were SCC, 7.7% were large-cell carcinoma, 6.9% were small-cell carcinoma, and 6.4% either were mixed histological subtypes or had more than one primary tumor. The frequencies of different histology subgroups reflect recent increases in incidence of AC in North America and the rising proportion of cases that are females and ex-smokers seen in more recent years (19–22). The frequencies of the main ethnic groups were Anglo-Saxon (44%), Italian/Greek (17%), French (10%), German (5%), and mixed (10%), and this ethnic distribution was similar in cases and controls and for different histological subtypes in cases. The mean age (± SD) of cases was 65.1 ± 10.7 years (range, 30–89), and the mean age of controls was 58.7 ± 12.3 years (range, 27–96). Among cases, the mean pack-years for ex-smokers were 55.2 ± 35.9, and for current smokers they were 63.9 ± 36.1. Among controls, pack-years were 29.1 ± 27.8 and 38.5 ± 25.6, respectively. The mean years since smoking cessation for ex-smokers were 13.7 ± 10.8 in cases and 19.5 ± 12.1 in controls. For *EH3*, the frequencies of *Tyr/Tyr*, *Tyr/His*, and *His/His* were 47.7, 34.1, and 18.2% in cases and 50.9, 31.1, and 18.0% in controls, respectively. For *EH4*, the frequencies of *His/His*, *His/Arg*, and *Arg/Arg* were 66.0, 30.8, and 3.2% in cases and 67.6, 28.6, and 3.8% in controls, respectively. χ^2 analysis of observed and expected frequencies in the controls suggests that the alleles of

Table 1 *mEH* genotype frequencies of cases and controls stratified by age, gender, pack-years, smoking status, and histological subtypes

	Case (%)					Control (%)				
	n	Very low	Low	Intermediate	High	*n*	Very low	Low	Intermediate	High
Total	974	14.4	27.1	38.0	20.5	1142	13.8	25.7	41.2	19.4
Age										
<55	177	16.4	20.9	40.7	22.0	425	13.7	25.2	40.9	20.2
55–64	260	14.6	28.1	36.5	20.8	299	12.4	23.8	42.8	21.1
≥65	537	13.6	28.7	37.8	19.9	418	14.8	27.5	40.2	17.5
Gender										
Female	443	14.2	26.0	37.0	22.8	617	14.4	25.9	39.9	19.8
Male	531	14.5	28.1	38.8	18.6	525	13.0	25.3	42.7	19.1
Pack-yr										
0	58	19.0	22.4	43.1	15.5	402	12.4	27.6	39.3	20.7
1–19	96	19.8	15.6	38.5	26.0	299	11.4	23.8	42.5	22.4
20–39	199	14.6	29.7	38.7	17.1	218	17.9	24.8	44.0	13.3
40–59	243	12.8	25.5	38.3	23.5	121	11.6	28.1	41.3	19.0
60–79	150	12.7	28.0	36.7	22.7	48	18.8	18.8	45.8	16.7
≥80	228	13.6	32.0	36.4	18.0	54	20.4	25.9	31.5	22.2
Smoking Status[a]										
Ex-smokers	519	15.0	28.7	34.5	21.8	529	13.8	24.6	42.0	19.7
Current-smokers	397	12.9	25.7	41.8	19.7	211	16.1	24.6	42.7	16.6
Histology[b]										
AC	432	15.3	26.4	37.0	21.3					
SCC	222	11.3	26.1	41.0	21.6					
Others	174	14.9	28.2	35.1	21.8					

[a] Nonsmokers were individuals with pack-years = 0.
[b] Histological data were available for 828 cases.

Table 2 Lung cancer risk of VL *versus* other genotype groups

	Cases (n = 974)	Controls (n = 1142)	Overall crude OR (95% CI)	Overall adjusted OR (95% CI)[a]
All of the cases *vs.* controls				
VL *vs.* other genotypes combined	140/834	157/985	1.05 (0.82–1.35)	1.00 (0.74–1.34)
VL *vs.* low	140/264	157/293	0.99 (0.75–1.31)	1.02 (0.72–1.42)
VL *vs.* intermediate	140/370	157/470	1.13 (0.87–1.48)	1.06 (0.77–1.46)
VL *vs.* high	140/200	157/222	0.99 (0.74–1.33)	0.86 (0.60–1.22)
Histological subtype-specific cases *vs.* controls (VL/other genotypes combined)[b]				
AC	66/366	157/985	1.13 (0.83–1.55)	0.96 (0.67–1.35)
SCC	25/197	157/985	0.80 (0.51–1.25)	0.72 (0.42–1.24)

[a] Logistic regression model including the following variables: age, gender, SR-PY, smoking status (current smokers, ex-smokers, and nonsmokers), and years since smoking cessation.
[b] Other histological subtypes were not analyzed because of small sample size.

EH4 were in HWE ($P > 0.05$), whereas the alleles of *EH3* were not ($P < 0.01$). There was a trend for the frequencies of VL to decrease among cases and increase among controls as pack-years increased (Table 1).

GAM plots showed a nonlinear association between lung cancer risk and pack-years of smoking, and a linear association was found when the pack-years were square root transformed. SR-PY (as a continuous variable) was then used in our logistic regression models. Because similar results were found when the low, intermediate, and high activity genotype groups were compared with VL, we combined the three groups into one group.

No overall relationship between *mEH* genotypes (as a surrogate marker of mEH activity) and lung cancer risk was found (Table 2). The crude OR of VL *versus* other genotypes combined was 1.05 (95% CI, 0.82–1.35). After adjustment for age, gender, SR-PY, smoking status, and years since quitting smoking, the OR was 1.00 (95% CI, 0.74–1.34). When cases were stratified according to histological types, the adjusted OR was 0.96 (95% CI, 0.67–1.35) for AC and 0.72 (95% CI, 0.42–1.24) for SCC.

For the gene-environment interaction analyses, we first included all of the possible interactions among the three variables of *mEH* genotype, smoking status, and SR-PY in the logistic regression model. The 3-way interaction and the two 2-way interactions between smoking status and genotype were not significant, and these were removed from the model. The final model included the interactions between *mEH* genotype and SR-PY ($P < 0.01$) and between smoking status and SR-PY ($P = 0.065$) and was adjusted for age, gender, SR-PY, smoking status, and years since smoking cessation. In this model, the ORs of VL *versus* all of the other genotypes decreased as SR-PY increased (Table 3). At 0 pack-years (nonsmokers), the OR was 1.89 (95% CI, 1.08–3.28), indicating that VL had a higher risk of lung cancer than other the genotypes combined. The OR equaled 1 (95% CI, 0.76–1.32) at pack-years equal to 28.5. At pack-years of 80, the OR decreased to 0.65 (95% CI, 0.42–1.00). In addition, the lung cancer risk of all of the *mEH* genotype groups increased both in ex-smokers and current smokers as pack-years increased, but the rate of increase was greater in current smokers (Fig. 1).

Table 3 Lung cancer risk (OR and 95% CI) of VL *versus* other genotype groups at different pack-years[a]

	Pack-yr of smoking							P^b
	0	20	40	60	80	100	120	
VL *vs.* other genotypes	1.89 (1.08–3.28)	1.11 (0.83–1.48)	0.89 (0.66–1.19)	0.75 (0.53–1.07)	0.65 (0.42–1.00)	0.57 (0.35–0.95)	0.51 (0.29–0.91)	<0.01
VL *vs.* low	2.49 (1.26–4.92)	1.19 (0.84–1.69)	0.88 (0.62–1.24)	0.69 (0.46–1.05)	0.57 (0.34–0.94)	0.48 (0.26–0.87)	0.41 (0.21–0.81)	<0.01
VL *vs.* intermediate	1.74 (0.95–3.20)	1.15 (0.84–1.58)	0.97 (0.70–1.33)	0.85 (0.58–1.25)	0.76 (0.48–1.21)	0.69 (0.40–1.19)	0.63 (0.34–1.18)	0.06
VL *vs.* high	1.59 (0.80–3.14)	0.95 (0.66–1.36)	0.77 (0.53–1.11)	0.65 (0.42–1.02)	0.57 (0.33–0.97)	0.50 (0.27–0.95)	0.45 (0.22–0.93)	0.04

[a] Logistic regression model including the following variables: age, gender, SR-PY, smoking status (current smokers, ex-smokers, and nonsmokers), years since smoking cessation, genotype, interaction between genotype and SR-PY, and interaction between smoking status and SR-PY.
[b] *P* for the interaction term between genotype groups and SR-PY in the logistic regression model.

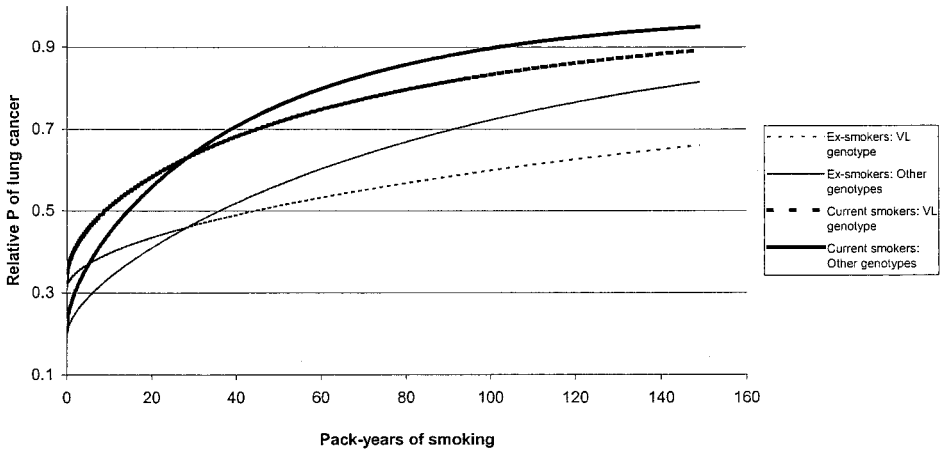

Fig. 1. Lung cancer risk of *mEH* VL and of other genotypes combined at different pack-years. From a model that includes the following variables: age, gender, SR-PY, smoking status, years since smoking cessation, the interaction between genotype and SR-PY, and the interaction between smoking status and SR-PY. The risks were calculated for a 65-year-old male or female and for an ex-smoker who quit smoking 17 years ago. The probabilities are relative to the case-control sample, in which approximately half are cases.

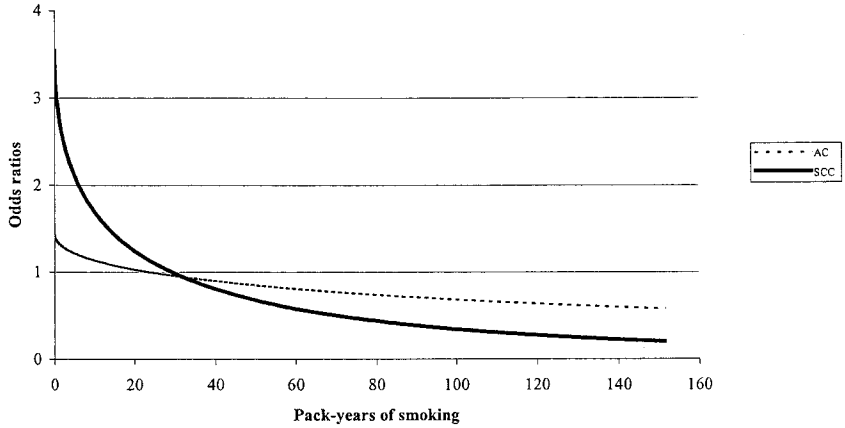

Fig. 2. ORs of risk of AC and SCC for *mEH* VL *versus* other genotypes combined at different pack-years. From a model that includes the following variables: age, gender, SR-PY, smoking status, years since smoking cessation, the interaction between genotype and SR-PY, and the interactions between smoking status and SR-PY.

The interaction between *mEH* genotype and SR-PY was in the same direction when the different histological subtypes, AC and SCC, were separately compared with all of the controls. However, the interaction between *mEH* genotype and SR-PY was statistically significant ($P < 0.01$) in SCC, but not in AC ($P = 0.18$). Fig. 2 illustrates that as pack-years increased, the ORs of VL *versus* the other genotypes decreased slightly for the AC group and dramatically for the SCC group. When pack-years = 0, the ORs were 1.43 (95% CI, 0.73–2.83) for AC cases and 3.56 (95% CI, 1.03–12.36) for SCC cases. The ORs decreased to unity (OR = 1) when pack-years increased to 24 for AC cases (95% CI, 0.71–1.41) and 29 for SCC cases (95% CI, 0.58–1.74).

Discussion

Lung cancer is the leading cause of cancer death among both men and women in the United States, accounting for an estimated 164,100 new cases and 156,900 deaths in the year 2000. Although smoking, radon, asbestos, diet, non-neoplastic lung disease, and other environmental/occupational carcinogenic exposures have been reported to be associated with the development of lung cancer (23), the precise genes that affect individual susceptibility to the carcinogenic effects of these agents remain undetermined. In this study, we explored the relationship between *mEH* genetic polymorphisms and the risk of lung cancer in a hospital-based case-control study. A major strength in our study is the large sample size, which allowed us to

perform previously unreported analyses of gene-environment interactions with smoking variables that were collected systematically. Our results indicate that the *mEH* genotype, which confers very low enzyme activity, increased the risk of lung cancer in nonsmokers and light smokers and decreased the risk in heavy smokers.

The mEH enzyme is an important Phase II biotransformation enzyme, and it is highly expressed in several human tissues including the lung, where it catalyzes the hydrolysis of various epoxides and reactive epoxide intermediates into less reactive and more water soluble dihydrodiols, which are then excreted from the body (1–3, 10). Hence, mEH is a protective enzyme involved in general oxidative defenses against a number of environmental chemicals and pollutants (10, 24). However, mEH is also involved in the xenobiotic activation of tobacco carcinogens. Combined with cytochrome P-450, mEH can metabolize PAHs into highly mutagenic and carcinogenic diol-epoxides (4, 25, 26). Thus, the activation or inactivation effects of mEH depend on the specific compounds being metabolized.

In nonsmokers, environmental pollutants may play an important role in the development of lung cancer. Examples of these chemicals include alkene, arene, or reactive epoxide intermediates, which are detoxified by mEH, and explain why VL increases the risk of lung cancer compared with all of the other genotypes. For smokers, PAHs [*e.g.*, benzo(*a*)pyrene] are activated by mEH into reactive intermediates (27), and compared with VL, higher mEH activity leads to higher concentrations of BPDE, BPDE-serum albumin adducts, and DNA adducts in the body (25). In addition, cigarette smoking can significantly induce the activity of mEH (11, 12), further magnifying these processes. This theory may explain why the ORs of VL *versus* all of the other genotypes decreased as cumulative smoking dose levels increased and why VL was protective against the risk of lung cancer in heavy smokers. As pack-years increased, the increased lung cancer risk was more pronounced in current smokers than in ex-smokers; thus, continuing to smoke is an additional risk factor for lung cancer.

Previous studies that explored the relationship between *mEH* genotypes and lung cancer risk found inconsistent results. Among three Caucasian studies, only the French study of 150 smoking cases found that higher activity of *mEH* was a risk factor for lung cancer (5). A study based in the Los Angeles area (96% of the 182 cases were smokers) and an English study (50 smoker cases) found no significant relationship between *mEH* genotype and lung cancer risk (8, 10). A Taiwanese study, in which 57% of the 132 cases were smokers, found that higher activity of *mEH* was associated with higher risk of SCC (7), whereas a Beijing study, in which 26% of the 76 cases were smokers, suggested that *EH4* heterozygotes were associated with an increased risk of lung cancer (6). An African-American study, in which 95% of the 155 cases were smokers, found that the VL genotype decreased the risk of lung cancer (8). Additional data from a phenotype study suggested that low enzyme activity increased lung cancer risk, although the data were not conclusive ($P > 0.05$; Ref. 9). The significant interaction between *mEH* genotype and SR-PY observed in our study may partly explain some of these inconsistent results.

The interaction between *mEH* genotype and cumulative smoking exposure in lung cancer risk was seen primarily in SCC and not in AC. There are two potential explanations for this finding: AC cases had smaller pack-years on average than SCC cases, thus any potential relationship between SR-PY and *mEH* genotype may be more difficult to identify. Alternatively, SCC development may be associated with tobacco smoke PAH exposures that are metabolized by mEH, whereas AC may be more associated with exposure to tobacco-specific nitrosamines or other substrates that are not metabolized by mEH (28).

We classified the activity of mEH into four different groups based on *in vitro* data. Because the structural differences encoded by the *mEH* genetic variants may have only modest impact on the specific activity of the enzyme *in vivo* (29) and the activity of mEH varies more than 50-fold in Caucasians (30), genotype alone is insufficient to account for the variation of mEH activity seen in population studies (31). In addition, other enzymes such as CYP1A1, CYP1B1, CYP2E1, and glutathione *S*-transferase may interact with mEH function (25, 26, 32). These reasons may explain why no "dose-response" relationship was found among different genotype activity groups in our study. Our analysis that compared VL with other genotypes was based on empirical patterns in our data. However, the differential association between VL and other genotypes was also found in another study (8). Furthermore, subjects with the VL genotype were reported to have reduced BPDE levels when compared with other genotype groups (25).

In our Caucasian control population, the alleles of amino acid 113 were not in HWE. Similar results were reported previously in both Caucasian (1) and Chinese female populations (33), although other studies had control populations that were consistent with HWE (5–10). A random 5% of our samples were retested using an alternative method that used different primers, PCR conditions, and digestion enzymes (34, 35). No discordant results were found to suggest a misclassification bias. In our control population, *EH4* and other genes (NADPH: quinone oxidoreductase 1, *N*-acetyltrasferase-2, and *p53*) were all in HWE, arguing against a selection bias. We further performed an *EH4*-only analysis using the same logistic regression models, and this subanalysis found a similar trend for the interaction between *EH4* genotype (*His/His* compared with *Arg/Arg*) and SR-PY ($P = 0.11$; data not shown). A case-only analysis, which negated the need for any controls, also found a similar trend between *mEH* genotype and SR-PY ($P = 0.15$; data not shown). The reason for this unusual finding of *EH3* in some control populations requires further study.

In conclusion, this is the first study to explore gene-environment interactions between *mEH* genotype and cigarette smoking, using pack-years as a continuous variable. Our results suggest that cumulative cigarette smoking exposures play pivotal roles in the association between *mEH* polymorphisms and lung cancer risk, altering the direction of risk (in the case of VL) from a risk factor in nonsmokers to a protective factor in heavy smokers. Additional studies should explore other gene-gene interactions or joint effects between *mEH* and polymorphisms in other metabolizing genes, in relationship to histology and different smoking exposures.

Acknowledgments

We thank the following staff members of the Lung Cancer Susceptibility Group: Barbara Bean, Andrea Solomon, Linda Lineback, Lucy Ann Principe, Nick Weidemann, Dr. Rong Fan, Dr. Yang Sai, Stephanie Shih, and Maria Fragoso.

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Cancer Epidemiol Biomarkers Prev 2001;10:461-466.