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Polymorphisms for microsomal epoxide hydrolase and genetic susceptibility to COPD

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Abstract

Although smoking is the major causal factor in the development of chronic obstructive pulmonary disease (COPD), only 10–20% of chronic heavy cigarette smokers develop symptomatic COPD, which suggests the presence of genetic susceptibility. The human microsomal epoxide hydrolase (EH) is a metabolizing enzyme which involves the process of numerous reactive epoxide intermediates and contains polymorphic alleles which are associated with altered EH activity and may be linked to increased risk for COPD. To determine whether the *EH* polymorphisms contributed to increased risk for COPD, prevalence of the *EH* codons 113 and 139 polymorphisms were compared between COPD patients and controls using a PCR-RFLP analysis using genomic DNA isolated from 131 COPD patients and 262 individually matched controls by age (± 5 years) among Caucasians with 1:2 ratio. Significantly increased risk for COPD was observed for subjects with the *EH*^{113His/His} genotypes (OR =2.4, 95% CI=1.1–5.1). These results were consistent with the fact that a significant trend towards increased risk was observed with predicted less protective *EH* codon 113 genotypes ($p = 0.03$, trend test). A similar association was not observed for *EH* codon 139 polymorphism. As expected, a significant correlation between smoking dose and severity of COPD was observed ($p < 0.001$). These results suggest that *EH* codon 113 polymorphism may modify risk for COPD.

Keywords

chronic obstructive pulmonary disease; epoxide hydrolase; genetic polymorphism; genetic susceptibility

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a major health problem with increasing prevalence and mortality. While cigarette smoking is the main risk factor for the development of COPD, only 10–20% of chronic heavy cigarette smokers develop symptomatic COPD which indicates the presence of genetic predisposing factors in its pathogenesis (1). Despite the clinical importance of COPD and its predisposed condition of lung cancer (2), the genetic susceptibility of this lung disease has not been not fully investigated. The genes which have been implicated in the development of COPD are

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involved in protease imbalance, metabolism of tobacco toxic materials and the inflammatory process (3).

Microsomal epoxide hydrolase (EH) is one of enzymes involved that metabolize environmental reactive epoxide intermediates. This process is controversial because it produces not only the dihydrodiol derivatives which substrates for additional metabolism to highly reactive and carcinogenic compounds from tobacco toxic materials, such as benzo[a]pyrene (BaP), but also less toxic water-soluble transhydrodiol derivatives. Hence, although EH metabolizes tobacco carcinogens such as polycyclic aromatic hydrocarbons (PAHs) into highly mutagenic diol epoxides (4, 5), EH is considered a protective enzyme involved in defense from oxidative damage against a number of environmental substances (6–8).

Among 11 single nucleotide polymorphisms in the coding region of the *EH* gene reported (9), functional analysis performed on two non-synonymous polymorphisms at codons 113 (Tyr>His) and 139 (His>Arg), and both have been associated with alterations in EH activity (10). The *EH*^{113His} variant is associated with a 40% decrease in EH activity while the *EH*^{139Arg} variant enhances enzyme activity by 25% via an increase in EH protein stability (10). These polymorphic alleles have previously been linked to risk changes for various smoking related cancers (11, 12), including lung cancer (9, 13, 14).

Results from previous epidemiological studies that investigated an association between *EH* polymorphisms and risk for COPD are inconsistent. Significant associations between low EH activity genotypes and COPD risk were reported in several studies, (6, 15–18) but these findings were not observed in other studies (19–22). Together with the finding that EH is expressed in lung tissues (23), these data suggest that *EH* polymorphisms may play important roles in risk for lung diseases.

In this present study, data are presented investigating the effects of *EH* genotypes on COPD risk examined both alone and in combination with two polymorphisms in *EH* gene.

Materials and methods

Study Populations and Sample Processing

One hundred thirty one COPD patients, and 262 individually matched with 1:2 ratio by age (± 5 years) among Caucasians were recruited from a cancer-screening clinic affiliated with the H. Lee Moffitt Cancer Center (Tampa, FL). At this center, which screens approximately 23,000 subjects annually, routine screenings are performed for cancers of the breast, prostate, lung, colorectum, cervix and skin. The eligible subjects were healthy non-cancer subjects. Ineligible were those with a history of any cancer other than non-melanoma skin cancer. All participants agreed to undergo spirometry, with measurement of forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC). The initial eligibility for COPD included a degree of pulmonary obstruction based upon ratio of FEV1/FVC. Study population was divided into two groups, one consisting of controls (70%) and the other of COPD patients (<70%).

Protocols involving the analysis of blood specimens were approved by the institutional review boards at the participating institutes, and informed consent was obtained from all subjects.

Detailed questionnaire data have been collected from all subjects through one-on-one patient interviews. A questionnaire was administered to all subjects that contained questions on demographics, and life-long smoking habits. Tobacco use was categorized into pack-years

(py) for smokers of cigarettes (1pack/day for 1year = 1py). Blood samples were collected from all subjects for genotyping assays. DNA was isolated from blood by incubation overnight with proteinase K (0.1 mg/ml) in 1% sodium dodecyl sulfate at 50°C, extracted with phenol:chloroform, and ethanol precipitation as previously described (24).

Genotyping Assays

All subjects were screened for the presence of the *EH* codons at 113 and 139 polymorphisms by a modified PCR-restriction fragment length polymorphism (RFLP) analysis similar to that described previously using primers homologous to exon 3 of the *EH* gene to generate a 172bp fragment and *AspI* restriction enzyme treatment for codon 113 polymorphism and using primers homologous to exon 4 and intron 4 sequences in the *EH* gene utilized to generate a 286bp fragment and *RsaI* restriction enzyme for codon 139 polymorphism (12).

Statistical Analysis

The risks of COPD in relation to *EH* genotypes were estimated using the chi-square test and conditional logistic regression to calculate ORs and 95% CIs. Conditional logistic regression models were fit to examine the relationship between the log odds of COPD and each covariate in the whole population and also in different subgroups after with/without adjusting for the following factors: age, smoking, and sex. Subjects were categorized into three groups based on the predicted activity of their *EH* genotype as described previously: (10) the slow *EH* activity genotype (113His/His139His/His, and 113His/His139His/Arg), normal *EH* activity genotypes (113His/Tyr139His/His, 113Tyr/Tyr139His/His, and 113His/Tyr139His/Arg) and fast *EH* activity genotype (113Tyr/Tyr139His/Arg, 113Tyr/Tyr139Arg/Arg, and 113His/Tyr139Arg/Arg). The χ^2 analysis was used to test for allelic prevalence, gender distribution and for a deviation of genotype distribution from the Hardy-Weinberg equilibrium. The χ^2 test for trend was used to examine potential associations between predicted high-risk *EH* genotypes and COPD risk. The Student's t-test (2-tailed) was used for comparing the smoking (py), age, and FEV1/FVC ratio variables between COPD patients and controls. The Pearson's test was used to examine correlation between smoking level and severity of COPD. The statistical computer software SAS (version 8e; SAS Institute, Cary, NC, USA) was used to perform all statistical analyses. All statistical tests were two-sided.

RESULTS

A total of 393 Caucasians with/without COPD were entered into this study. The mean ages of control and COPD groups were 61 and 64, and approximately 25% of COPD and 35% of controls were females (Table 1). As expected, COPD groups had a significantly higher level of cigarette consumption than control group ($p < 0.001$). Furthermore, level of smoking consumption significantly correlates with severity of COPD ($p < 0.001$) (Figure 1).

The genotyping was determined by the combined data obtained from individual PCR-RFLP analysis of the codons 113 and 139 polymorphisms. In controls, the allelic frequencies of both *EH*^{113His} and *EH*^{139Arg} variant were 0.24. These allelic frequencies are similar to those observed in previous studies of Caucasians (7, 13, 17, 18), but significantly different from the Asian population (15, 16, 19, 21, 22, 25).

A significant difference in *EH*^{113His} allelic frequency was observed between control and COPD groups. A frequency of the *EH*^{113His} allele was 0.32 in COPD group and 0.24 in controls (0.24) ($p=0.02$), while the *EH*^{139Arg} allelic frequency was similar in both groups (COPD: 0.20 vs. controls: 0.24; $p=0.2$). The prevalence of these polymorphisms among

controls followed the Hardy-Weinberg equilibrium ($p=0.89$ for *EH* codon 113 polymorphism, $p=0.95$ for *EH* codon 139 polymorphism).

To determine whether the *EH* polymorphisms contributed to increased risk for COPD, we examined the prevalence of *EH* polymorphic alleles in COPD patients versus control subjects. Significantly increased risk for COPD was observed for subjects with the *EH*^{I13His/His} genotype (OR=2.6, 95% CI=1.2–5.2) (Table 2). This risk was not affected by adjusting for other potential confounding factors (age, sex, and smoking level) via regression analysis (OR_{adjusted}=2.4, 95% CI=1.1–5.1). These results were consistent with the fact that a significant trend towards increased risk was observed with predicted less protective *EH* codon 113 genotypes ($p = 0.03$, trend test, Table 2). A similar association was not observed for *EH* codon 139 polymorphism. Using the presumed fast *EH* activity genotypes as the reference group, a near significant increased risk was observed with slow, presumed less protective, *EH* genotypes (OR= 2.2, 95%CI=0.9–5.2, $p=0.07$, Table 2).

To examine the association between *EH* genotypes and COPD risk by exposure to the environmental risk factor, smoking, we stratified study subjects by *EH* genotypes and different smoking levels. The differences in risk associated with the *EH* genotypes were modified by smoking history (Table 2). The association between *EH* genotypes and COPD was examined based upon smoking level. Smokers were stratified into two groups based upon lifetime smoking history divided at the median number of pack-years for subjects (47 py; Table 2). The *EH*^{I13His/His} genotype was associated with an increased risk for COPD in smokers with a history of <47 py (OR = 4.6, 95% CI = 1.3 – 15.5; Table 2). A significant association was also observed in the low-dose group when stratifying tobacco use at the median number of pack-years for controls in our data set (47 py). No association between *EH* codon 113 genotypes and COPD risk was observed in subjects with a greater dose of lifetime smoking (i.e., ≥ 47 py, OR = 1.2, 95% CI = 0.5 – 3.2; Table 2). No association between *EH* 139 polymorphism genotypes and COPD risk was observed after stratifying by smoking.

DISCUSSION

Epoxide hydrolase is an enzyme that functions to catalyze the addition of water to an epoxide to form the vicinal dihydrodiol. EH is known to play a central role in the formation of precursors of even more deleterious products in the metabolisms for polycyclic aromatic hydrocarbon to diol bay region epoxides (8). However, enzymatic hydration often results in metabolites of lower reactivity or metabolites which can be conjugated and excreted. Therefore, in most cases, the action of EH is considered as detoxifying process.

Similar to that observed in previous reports of association between smoking and COPD risk (26, 27), we observed a significant correlation between smoking dose and severity of COPD. This correlation is dose-dependent, with significantly increased severity of COPD with smoking levels (Table 1).

Our data suggested an association between *EH* codon 113 polymorphism and risk for smoking related COPD. These results are biologically plausible and consistent with a critical role for EH in the metabolism of tobacco oxidative damage and in tobacco-related lung diseases. We observed a stronger association between *EH*^{I13His/His} genotype and COPD among light smokers (<47pys). This pattern is similar to that observed in previous studies examining polymorphic genotypes and lung cancer risk. The *glutathione S transferase M1* (*GSTM1*) (0/0) (28, 29), the *CYP1A1* exon 7 and *MspI* polymorphisms (29, 30), and *CYP2D6* polymorphisms (31) have all been shown to be associated with increased risk for lung cancer in light but not heavy smokers. Therefore, genetic variations in the ability to

metabolize tobacco smoke toxins are most important in determining risk for lung disease at low levels of exposure, and may be less relevant at higher smoking doses where high levels of tobacco toxin exposure overwhelm polymorphism-induced differences in enzyme activity and/or expression.

Up to date, there are nine independent epidemiological studies investigated association between *EH* polymorphisms and COPD risk using Asians and Caucasians (Table 3). The results of studies are not consistent, and allele frequencies were significantly different among studies. These discrepancies may be differences of race, population selection, and grouping of genotypes. Among six Asian studies, one Chinese study (16) and one Taiwanese study (15) found that slow *EH* activity genotypes were a risk factor for COPD. However, two Japanese studies (19, 22), a Chinese study (32), and a Korean study (25) did not find a significant association between *EH* genotypes and COPD risk, whereas the *EH* polymorphism may be related with COPD severity (15, 17, 22). In two Caucasian studies, performed in the United Kingdom (6), and Spain (18), reported a significant higher risk among COPD patients with slow *EH* genotypes. Finally, Sanford et al. have reported the significant association of the homozygous *EH*^{113His-139His} (very slow) *EH* genotype with rapid decline in lung function (17) (Table 3).

The COPD is a lung disease that likely involves multiple genes in development and progression. We previously reported a gene:gene interaction between the polymorphisms in the *GSTs* and *EH* in smoking related-oral cancer (33). Polymorphisms of non-penetrance genes often contribute a low or moderate relative risk change. Therefore it will be necessary to study other genes involved in tobacco toxin metabolic pathways, such as *GSTs*, and *P450CYPs*. Strength of our study is the use of controls individually matched by age (± 5 years) and race to remove potential confounding effects. A limitation of this study is the potential selection bias of gender because controls were not matched by sex. However, there was no significant difference of genotype distribution between genders, and *EH* gene is located on chromosome 1q42.1 not X or Y. Therefore, it is not likely affect the results of this study. In summary, codon 113 polymorphism in the *EH* gene appear to play a role in susceptibility to COPD.

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Figure 1.
Correlation between smoking level and severity of COPD

Table 1

Distribution of subjects according to demographic characteristics.

	Control	COPD	P value
N	262	131	
Mean age (yr)	61	64	0.001
<50yrs	14 (5) ^I	4 (3)	
50–59	102 (39)	28 (21)	
60–69	106 (40)	64 (49)	
70yrs	40 (16)	35 (27)	
Gender (M/F)	169/93	98/33	0.04
Mean FEV1/FVC Ratio $\pm$ SD	0.77 $\pm$ 0.04	0.56 $\pm$ 0.11	<0.001
Mean smoking (py) $\pm$ SD	49.7 $\pm$ 28.2	64.1 $\pm$ 26.3	<0.001

^I Numbers in parenthesis denote percentages.

Table 2

EH polymorphic variants and COPD risk stratified by smoking level.

EH genotypes		Control	COPD	OR (95%CI)	OR (95%CI) ¹
Total	113Tyr/Tyr	153 (58) ²	67 (51)	1.0 (referent) ³	1.0 (referent)
	113Tyr/His	92 (35)	45 (34)	1.1 (0.7–1.8)	1.2 (0.7–1.9)
	113His/His	17 (7)	19 (15)	2.6 (1.2–5.2)	2.4 (1.1–5.1)
	139His/His	153 (58)	82 (63)	1.0 (referent)	1.0 (referent)
Total	139Arg/His	93 (36)	44 (34)	1.3 (0.8–2.1)	1.3 (0.8–2.1)
	139Arg/Arg	16 (6)	4 (3)	0.8 (0.2–2.5)	0.8 (0.2–2.8)
Smoker <47py	113Tyr/Tyr	85 (56)	20 (51)	1.0 (referent)	1.0 (referent)
	113Tyr/His	60 (39)	11 (28)	0.8 (0.3–1.7)	0.8 (0.4–1.9)
	113His/His	7 (5)	8 (21)	4.9 (1.6–15.0)	4.6 (1.3–15.5)
	113Tyr/Tyr	68 (56)	47 (51)	1.0 (referent)	1.0 (referent)
Smoker 47py	113Tyr/His	32 (39)	34 (28)	1.5 (0.8–2.8)	1.5 (0.8–2.9)
	113His/His	10 (5)	11 (21)	1.6 (0.6–4.0)	1.2 (0.5–3.2)
	139His/His	104 (58)	21 (63)	1.0 (referent)	1.0 (referent)
	139Arg/His	41 (36)	15 (34)	1.8 (0.9–3.9)	1.9 (0.9–4.4)
Smoker <47py	139Arg/Arg	6 (6)	2 (3)	1.7 (0.3–8.7)	1.8 (0.3–10.3)
	139His/His	73 (58)	61 (63)	1.0 (referent)	1.0 (referent)
	139Arg/His	31 (36)	29 (34)	1.1 (0.6–2.1)	1.1 (0.6–2.1)
	139Arg/Arg	5 (6)	2 (3)	0.5 (0.1–2.6)	0.6 (0.1–3.5)
Predicted EH activity ⁴		Control	COPD	OR (95%CI)	OR (95%CI)
Total	Fast	51 (20)	24 (18)	1.0 (referent)	1.0 (referent)
	Normal	191 (74)	87 (67)	1.0 (0.6–1.7)	1.0 (0.5–1.7)
	Slow	17 (6)	19 (15)	2.4 (1.1–5.4)	2.2 (0.9–5.2)

¹ ORs were calculated by adjusting for sex, age, and smoking (py).
² Numbers in parenthesis denote percentages.
³ Significant increase in predicted high-risk genotypes as determined by χ^2 -trend test ($p=0.03$).

⁴The slow EH activity genotype (113His/His139His/His, and 113His/His139His/Arg), normal EH activity genotypes (113His/Tyr139His/His, 113Tyr/Tyr139His/His, and 113His/Tyr139His/Arg) and fast EH activity genotype (113Tyr/Tyr139His/Arg, 113Tyr/Tyr139Arg/Arg, and 113His/Tyr139Arg/Arg).

Epidemiological studies for the association between epoxide hydrolase polymorphism and COPD risk

Table 3

First author, year	Ethnicity	Allelic frequencies		Number of		Level of association	reference
		113His	139Arg	COPD	Control		
Cheng et al. 2004	Taiwanese	0.48	0.19	184	212	2.3 (1.1–4.3) ¹	(15)
Xiao et al. 2003	Chinese	0.55	0.09	100	100	3.0 (1.2–7.1) ² 0.9 (0.4–2.0) ³	(16)
Budhi et al. 2003	Japanese	0.42	0.17	63	172	1.4 (0.7–2.7) ⁴ 0.7 (0.4–1.3) ⁵	(19)
Rodriguez et al. 2003	Caucasian (Spain)	0.21	0.19	79	146	7.0 (1.4–34.6) ⁶	(18)
Zhang et al. 2002	Chinese	0.58	0.19	55	52	1.3 (0.6–2.9) ⁷ 1.2 (0.3–4.7) ⁸	(32)
Yim et al., 2000	Korean	0.53	0.14	83	76	0.7 (0.3–1.5) ⁹	(25)
Sandford et al 2001	Caucasian (US)	0.28	0.21	283	308	2.4 (1.1–5.4) ¹⁰	(17)
Yokikawa et al 2000	Japanese	0.44	0.14	40	140	0.9 (0.4–1.9) ⁷ 1.3 (0.5–3.0) ⁵	(22)
Smith et al., 1997	Caucasian (UK)	0.31	0.15	68	203	3.5 (1.5–8.0) ¹¹ 2.0 (0.3–2.4) ⁸	(6)

¹Comparison between the slow/very slow vs normal/fast.

²Comparison between the *EH*¹¹³Tyr/Tyr vs *EH*¹¹³Tyr/His

³Comparison between the *EH*¹³⁹His/Arg vs *EH*¹³⁹His/His.

⁴Comparison between the *EH*¹¹³Tyr/Tyr + *EH*¹¹³Tyr/His vs *EH*¹¹³His/His

⁵Comparison between the *EH*¹³⁹His/His vs *EH*¹³⁹His/Arg + *EH*¹³⁹Arg/Arg

⁶Comparison between the very slow vs slow/normal/fast.

⁷Comparison between the *EH*¹¹³Tyr/Tyr vs *EH*¹¹³Tyr/His + *EH*¹¹³His/His

⁸Comparison between the *EH*¹³⁹His/His + *EH*¹³⁹His/Arg vs *EH*¹³⁹Arg/Arg

⁹Comparison between the slow vs others.

¹⁰Individuals with homozygous *EH*¹¹³His-139His₁ haplotype among fast decliners.