

Lung cancer risk in relation to genetic polymorphisms of microsomal epoxide hydrolase among African-Americans and Caucasians in Los Angeles County

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Received 5 August 1999; received in revised form 19 November 1999; accepted 23 November 1999

Abstract

Microsomal epoxide hydrolase participates in the metabolism of benzo[a]pyrene, an important carcinogen in tobacco smoke. Two relatively common polymorphisms of the microsomal epoxide hydrolase gene that influence enzyme activity have been described. An association between genetic variation in microsomal epoxide hydrolase and lung cancer risk has been reported in one of two studies of Caucasians. We examined the relation between these two polymorphisms and lung cancer risk among 337 incident cases and 700 population controls of African-American and Caucasian ethnicity enrolled in a case-control study in Los Angeles County. African-Americans, homozygous for the exon 3 variant allele conferring reduced activity, were at decreased risk of lung cancer (odds ratio (OR) = 0.08, 95% CI 0.01–0.62). When data from both the exon 3 and exon 4 polymorphisms were combined into indices of predicted microsomal epoxide hydrolase activity, a decreased risk was seen among African-American subjects with very low predicted activity OR = 0.10 (95% CI 0.01–0.83). No comparable association was seen among Caucasians. Although the three published results for Caucasians are somewhat variable, the association among African-Americans in these data provides some support for the hypothesis that genetically reduced microsomal epoxide hydrolase activity may be protective against lung cancer. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Ephx; MEH; Susceptibility; Variant introduction

1. Introduction

Microsomal epoxide hydrolase (mEH) is involved in the biotransformation of ben-

zo[a]pyrene, a major carcinogen in tobacco smoke, as well as other xenobiotics. Microsomal epoxide hydrolase is generally regarded as detoxicating but can produce reactive electrophiles in concert with the action of cytochromes P450 [1]. Humans exhibit marked interindividual variability in mEH activity. Two mEH polymorphisms are relatively common, and are functionally signifi-

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cant *in vitro* [1,2]. One variant is characterized by substitution of histidine for tyrosine at position 113 in exon 3 (Tyr113→His113). A polymorphism in exon 4 results in substitution of arginine for histidine at position 139 (His139→Arg139).

One recent report suggested that mEH genotypes that would be expected to be associated with reduced enzyme activity are associated with decreased risk of lung cancer [3]. In contrast, Smith and Harrison, reported no association with lung cancer [4]. Of interest, Pastorelli et al. [5] recently reported that subjects with very low predicted mEH activity based on mEH genotypes have reduced levels of adducts of benzo[a]pyrene diol epoxide, the ultimate carcinogen metabolite of benzo[a]pyrene. They proposed that subjects with very low mEH activity might be at reduced risk of cancer [5].

We tested the hypothesis that genotypes of mEH that predict low activity decrease risk of lung cancer among cases of incident lung cancer and population controls from a study of genetic susceptibility to lung cancer among African-Americans and Caucasians in Los Angeles County.

2. Materials and Methods

Details of the study methods have been previously published [6,7]. In brief, we enrolled incident cases of lung cancer diagnosed at one of 35 hospitals in Los Angeles County between 1 September 1990 and 6 January 1994. Controls under age 65 years were sampled from driver's license lists, those over 65 years from lists of Medicare beneficiaries. We frequency matched on age, sex, and ethnicity. Participation was sought by repeated mailings, supplemented by phone calls and home visits to nonrespondents. Eligible subjects were Caucasian or African-American residents of Los Angeles County, CA, aged 40–84 years and able to complete a questionnaire in English. Eligible cases had no prior history of cancer, other than nonmelanoma skin cancer.

We identified 859 potentially eligible cases. Of these, 207 had died by the time physicians received our request for permission to contact them.

The physicians declined permission for us to contact 36 of the 652 patients not known to be ineligible. Among the remaining 616, we were unable to locate 92, 158 declined to participate and we ended the study before we could enroll 10 cases who agreed to participate. Thus, we enrolled 356 eligible cases — 167 African-Americans and 189 Caucasians.

We sent letters to 3193 potential controls asking them to write their phone number on an enclosed postcard and return it. We sent up to four letters if we received no response. We looked up phone numbers of all nonrespondents and tried 12 times to call each subject with a listed phone number. We then attempted to visit the addresses of all subjects not reached after 12 tries as well as all unlisted subjects.

The address was incorrect for 752 subjects and we were unable to obtain a usable address for these. We determined that 157 persons to whom we sent letters were not eligible because they no longer lived in Los Angeles County. Of the 2284 subjects with valid addresses we obtained a response on 1573. Responses for these 1573 indicated that 94 were ineligible, 71 were deceased, 351 declined to participate and 1057 expressed willingness to participate. Among these 1057 subjects, 46 could not be reached before the end of the study, 831 were eligible on screening and 180 were ineligible. We were unable to enroll 100 of the 831 eligible and willing subjects before we terminated data collection. Because the DMV tape does not contain race identifiers and because Caucasians are more numerous than African-Americans in the county, we identified more than our target number of Caucasians. However, we continued to solicit potential subjects in order to identify additional African-Americans. We enrolled 731 eligible controls: 473 Caucasians and 258 African-Americans.

Subjects completed a questionnaire on risk factors for lung cancer and provided a blood sample. The median time from diagnosis of cases to enrollment was 3.7 months and the maximum was 10 months. The protocol was reviewed and approved by the Human Subjects Committee at the University of Southern California.

Among the 356 eligible cases and 731 eligible controls enrolled, we obtained a DNA sample adequate to assign genotypes for the exon 3 and exon 4 polymorphisms of epoxide hydrolase and the *GSTM1* homozygous deletion polymorphism on 337 cases (182 Caucasian and 155 African-American) and 700 controls (458 Caucasian and 242 African-American).

DNA was prepared from buffy-coat samples using standard methods as previously described [6]. The exon 3 polymorphism was detected by PCR using a mismatched primer that created a restriction site for *A_lw26I* in the mutant allele. PCR was carried out with the primers 5'-AGCAGGTGGAGATTCTCAAGAG-3' and 5'-GTTCTCATGACATACATCCC-3' using the general methods described previously [8]. Gel electrophoresis of the digest gave one band (105 bp) for wild-type individuals, two bands (105 bp and 87 bp) for heterozygotes, and the smaller fragment (87 bp) only for homozygous mutant subjects.

The exon 4 polymorphism of microsomal epoxide hydrolase was determined by PCR-RFLP analysis with *RsaI* as described by Hassett et al. [1]. The homozygous deletion of the *GSTM1* gene was detected by the polymerase-chain-reaction (PCR) method described by Zhong et al. [9].

Odds ratios and 95% confidence intervals were calculated by unconditional logistic regression [10] using Epilog Plus, version 3.05 (Epicenter Software, Pasadena, CA). Within each ethnic group, terms for the frequency matching variables of age and sex were included. Smoking was modeled as the natural logarithm (ln) of pack-years plus a product term for the ln of pack-years by the ln of years since quitting smoking, as previously described [6].

3. Results

The mean age was 64 years (SD = 10) for cases, and 63 (SD = 8) for controls. The percentage of males was 59% for cases, and 65% for controls. As expected, cases had markedly greater smoking history than controls [6] — 5% of cases versus 34% of controls were never smokers. Current

smoking among controls (23%) was similar to that observed among persons of comparable age and ethnicity in a contemporary national survey [11].

Data on the mEH exon 3 and exon 4 polymorphisms are shown in Table 1. The frequency of the exon 3 His113 allele was 0.208 for African-Americans and 0.279 for Caucasians. The exon 4 Arg139 allele frequency was 0.289 for African-Americans and 0.191 for Caucasians. The genotype frequencies were in Hardy-Weinberg equilibrium for both polymorphisms in both ethnic groups. Among controls, the exon 3 and exon 4 genotypes were unrelated to smoking in both ethnic groups (data not shown).

Based on what is known about the functional significance of these variants, one would predict diminished mEH activity for the exon 3 *His113/His113* genotype and increased mEH activity for the exon 4 *Arg139/Arg139* genotype [1]. Among African-Americans, only one case was *His113/His113* at exon 3, in comparison to 12 controls, giving an adjusted odds ratio of 0.08 (95% CI 0.01–0.62). We found no comparable association for Caucasians. Because of this difference in association, we performed all analyses separately by ethnic group. The exon 4 polymorphism was not appreciably related to lung cancer risk in either ethnic group.

Because both polymorphisms affect function and because stratification of the data on one polymorphism by another results in very small numbers, it is reasonable to combine the data on the exon 3 and exon 4 polymorphisms to provide an index of predicted activity. Based on the in vitro data presented for haplotypes by Hassett et al. [1], three different indices of predicted activity have been presented in previous studies [3–5]. Each of these indices is based on the assumption that the exon 3 Tyr113 (wild-type or wt) allele confers normal activity while the His113 allele is 'slow'. For the exon 4 polymorphism, His139 allele is considered the normal activity or wild-type allele and the Arg139 allele is 'fast'. For comparability with these studies, we report on all three indices.

The relations between various indices of predicted mEH activity and lung cancer are shown in

Table 1
Lung cancer risk in relation to microsomal epoxide hydrolase (mEH) exon 3 and exon 4 polymorphisms

mEH polymorphism	Whites				Blacks			
	Case	Control	Crude OR	Adjusted OR (95% CI ^a)	Case	Control	Crude OR	Adjusted OR (95% CI ^a)
<i>Exon 3</i>								
<i>Tyr113/Tyr113</i> (wt/wt)	85	237	1.00	1.00	106	153	1.00	1.00
<i>Tyr113/His113</i> (wt/slow)	82	184	1.24	1.35 (0.89–2.05)	48	77	0.90	0.90 (0.58–1.40)
<i>His113/His113</i> (slow/slow)	15	37	1.13	0.99 (0.46–2.14)	1	12	0.12	0.08 (0.01–0.62)
<i>Exon 4</i>								
<i>His139/His139</i> (wt/wt)	125	302	1.00	1.00	70	119	1.00	1.00
<i>His139/Arg139</i> (wt/fast)	50	136	0.89	0.87 (0.55–1.37)	70	105	1.13	1.06 (0.66–1.71)
<i>Arg139/Arg139</i> (fast/fast)	7	20	0.85	0.63 (0.23–1.77)	15	18	1.42	1.05 (0.45–2.49)
Total	182	458			155	242		

^a Odds ratio and 95% confidence interval. Adjusted models include terms for age, gender and smoking (the natural logarithm of pack-years and the product of the natural logarithms of pack-years and years since quitting smoking).

Table 2. Because the results are very similar with and without adjustment for the frequency matching factors (age and sex) and smoking, and because the unadjusted odds ratios (OR) can be calculated from the table counts, only the adjusted OR are presented. First we classified subjects as low, medium or high activity, based on the categorization of Benhamou et al [3]. Using this index [3], we did not confirm an elevated risk with increasing predicted activity among Caucasians. Among African-Americans, a modest increased risk for the subjects with high activity was not statistically significant (OR = 1.45, 95% CI 0.77–2.74).

Smith and Harrison [4] categorized individuals with exon 3 slow/slow as ‘very slow’, regardless of exon 4 genotype, and compared this group to all others. This dichotomy amounts to simply collapsing the wt/wt and wt/slow categories for exon 3 into a single reference category. Given the exon 3 findings in Table 1, we again observed no appreciable association among Caucasians (OR = 0.87, 95% CI 0.41–1.83) and the association

among African-Americans remains (OR = 0.08, 95% CI 0.01–0.63) (Table 2). Pastorelli et al. [5] found lower levels of adducts of benzo[a]pyrene diol epoxide (BDPE) among subjects predicted to have reduced activity on the basis of being exon 3 slow/slow and carrying no exon 4 fast allele than in other subjects, and predicted that these subjects might be at reduced risk of cancer. Very low activity was not associated with lung-cancer risk among Caucasians (Table 2). However, among African-Americans, this category was at lower risk (OR = 0.10, 95%, CI 0.01–0.83).

For comparability with Benhamou et al. [3], we present data on predicted phenotype stratified by histology, smoking status, and GSTM1 genotype. Among Caucasians with either squamous or small cell carcinoma, the group studied by Benhamou et al. [3], there was a suggestion of a decreased risk with increasing predicted activity (OR for high activity = 0.38, 95%, CI 0.15–0.98). However, analyses with the indices of predicted very low activity proposed by either Smith and Harrison [4] (OR = 1.00, 95%, CI 0.38–2.61) or Pastorelli

Table 2
Lung cancer risk in relation to several indices of microsomal epoxide hydrolase (mEH) activity predicted from exon 3 and exon 4 genotypes

Activity	Whites			Blacks		
	Cases	Controls	OR (95% CI) ^a	Cases	Controls	OR (95% CI)
<i>Based on Benhamou et al. [3]^b</i>						
Low	67	165	1.00	26	54	1.00
Intermediate	88	194	0.95 (0.61–1.49)	65	101	1.46 (0.78–2.74)
High	27	99	0.64 (0.35–1.15)	64	87	1.45 (0.77–2.74)
<i>Based on Smith and Harrison [4]</i>						
‘Very slow’	15	37	0.87 (0.41–1.83)	1	12	0.08 (0.01–0.63)
All others	167	421	1.00	154	230	1.00
<i>Based on Pastorelli et al. [5]</i>						
Low	12	27	1.01 (0.43–2.34)	1	9	0.10 (0.01–0.83)
All others	170	431	1.00	154	233	1.00
Total	182	458		155	242	

^a Odds ratio and 95% confidence interval. Adjusted for age (continuous), gender and smoking modelled as the natural logarithm of pack-years and the product of the natural logarithms of pack-years and years since quitting smoking.

^b For this variable, the low activity category includes subjects with the following genotype combinations: exon 3 slow/slow with exon 4 either wt/wt or wt/fast; exon 3 wt/slow and exon 4 wt/wt. The intermediate activity category includes subjects with exon 3 slow/slow with exon 4 fast/fast; exon 3 wt/wt with exon 4 wt/wt; exon 3 wt/slow with exon 4 wt/fast. The high activity category includes subjects with genotypes exon 3 wt/wt with exon 4 wt/fast; exon 3 wt/wt with exon 4 fast/fast and exon 3 wt/slow with exon 4 fast/fast.

(OR = 0.81, 95%, CI 0.25–2.67) [5] did not confirm this association. Among African-Americans there was a slight suggestion of an increase risk for higher mEH activity for adenocarcinoma, but there was no trend and neither the individual estimates nor the difference in association between histology categories was statistically significant.

Limiting the analysis to smokers did not appreciably change the results (Table 3). This is not surprising given the small number of nonsmoking cases and the overall lack of association between smoking and predicted phenotype among the controls. We found no appreciable difference in the association between mEH genotype and lung cancer risk according to lifetime smoking history stratified on the median number of pack-years for all smokers in the study (35 pack-years).

We previously reported on the relation between the genetic polymorphism of *GSTM1* and lung cancer among these subjects [6]. Because *GSTM1* and *mEH* are both involved in the metabolism of benzo[a]pyrene, we also examined polymorphisms of *mEH* and *GSTM1* together. Like Benhamou et al. [3], we found no evidence that the association between mEH polymorphisms and lung cancer risk differed by *GSTM1* genotype (Table 3).

4. Discussion

When we examined the relation between lung cancer risk and individual mEH genotypes, the only suggestion of an association was a decreased risk among African-Americans homozygous for the exon 3 slow allele. When data on the exon 4 and exon 3 polymorphisms are incorporated into an index of predicted activity, African-American subjects with very low predicted activity by virtue of having the slow/slow genotype for exon 3 without carrying any exon 4 fast allele were at significantly decreased risk of lung cancer. No comparable association was seen among Caucasians.

Two previous publications included data on the association between lung cancer and the epoxide hydrolase polymorphism, both in European Caucasians. Smith and Harrison [4] studied 144 re-

sected lung cancer patients, the majority of whom (65%) had pathologic evidence of emphysema consistent with the very heavy smoking history typical of lung cancer cases. Their data suggest an increased risk of lung cancer for subjects with ‘very slow’ predicted activity: OR = 3.9 (95%, CI 1.9–7.8) for all lung cancers, OR = 1.9 (95%, CI 0.6–5.9) for lung cancer without emphysema and 5.0 (95%, CI 2.3–10.9) for lung cancer with emphysema. The authors interpreted their data as showing an increased risk for low activity in relation to emphysema but no association with lung cancer. Benhamou et al. [3], in their study of squamous and small cell lung cancer, reported an association in the opposite direction — an increased risk with higher predicted activity.

In our group of Caucasians the frequencies of the exon 3 and exon 4 variant alleles are similar to the two previous studies. However, we found no overall association for indices of predicted activity used by either Benhamou [3] or Smith and Harrison [4]. Thus, the three studies appear to give somewhat different impression of the association between epoxide hydrolase genotype and lung cancer risk in Caucasians. The three studies have rather different designs. Issues related to differences in study design between this study and that of Benhamou et al. [3] have been previously discussed [12] but do not obviously explain why the results should differ from each other or from the study of Smith and Harrison [4]. Given the modest number of Caucasian cases in each study (about 150), variability will result from chance alone. Thus, the publication of results from multiple studies, including studies with a null result, is necessary to properly evaluate the putative association. It should be noted that in all three studies, predictions of activity are based on measurements of protein levels and enzyme activity for the four possible haplotypes expressed *in vitro* [1]. Though Laurenzana et al. [13] recently published evidence in human liver samples confirming the original observation of Hassett et al [1], another study on activity in human livers indicated that *in vivo* levels may not be exactly as predicted from the *in vitro* data [14]. Genetic polymorphisms in the 5′ flanking sequence that may contribute to the variability of mEH expression have recently been described [15].

Table 3
Lung cancer risk in relation to predicted microsomal epoxide hydrolase (mEH) activity by histology, GSTM1 genotype and smoking history

Category	mEH predicted activity	Whites				Blacks			
		Cases	Controls	OR ^a	(95% CI)	Cases	Controls	OR	(95% CI)
<i>Histology</i>									
Squamous + Small	Low	33	165	1.00		12	54	1.00	
	Med	36	194	0.85	(0.47–1.54)	19	101	0.81	(0.34–1.93)
	High	7	99	0.38	(0.15–0.98)	20	87	0.94	(0.39–2.25)
Adenocarcinoma and other histologies	Low	34	165	1.00		14	54	1.00	
	Med	52	194	1.04	(0.61–1.78)	46	101	2.05	(0.97–4.34)
	High	20	99	0.89	(0.46–1.74)	44	87	1.86	(0.87–3.99)
<i>GSTM1 genotype</i>									
GSTM1 positive	Low	32	74	1.00		19	38	1.00	
	Med	43	97	0.82	(0.42–1.61)	44	76	1.47	(0.70–3.10)
	High	15	47	0.67	(0.28–1.60)	49	61	1.75	(0.82–3.71)
GSTM1 null	Low	35	91	1.00		7	16	1.00	
	Med	45	97	1.01	(0.55–1.86)	21	25	1.36	(0.42–4.47)
	High	12	52	0.59	(0.26–1.33)	15	26	0.84	(0.25–2.90)
<i>Smoking</i>									
All smokers	Low	63	110	1.00		25	42	1.00	
	Med	85	122	1.00	(0.63–1.59)	61	68	1.47	(0.77–2.83)
	High	27	63	0.70	(0.38–1.29)	61	56	1.53	(0.79–2.97)
Smokers <35 pack-yrs	Low	18	62	1.00		13	27	1.00	
	Med	23	62	0.88	(0.39–2.00)	32	46	1.31	(0.55–3.15)
	High	11	40	0.71	(0.27–1.86)	28	25	1.48	(0.60–3.62)
Smokers 35+ pack-yrs	Low	45	48	1.00		12	15	1.00	
	Med	62	60	1.05	(0.59–1.85)	29	22	1.69	(0.63–4.54)
	High	16	23	0.66	(0.30–1.46)	33	21	1.67	(0.61–4.52)

^a Odds ratio and 95% confidence interval. Adjusted for age (continuous), gender and smoking modelled as the natural logarithm of pack-years and the product of the natural logarithms of pack-years and years since quitting smoking.

Ours are the only published data on African-American subjects. Among African-Americans, we find an association in the same direction to what Benhamou et al. [3] observed in their Caucasians — decreased risk for low activity. The finding of an association among African-Americans, but not Caucasians, in our study raises concern in interpreting the association as causal. However, our finding is in the direction predicted by Pastorelli et al. [5] in their study of BDPE adducts in relation to predicted mEH phenotype. 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 in different frequencies in the different groups, or perhaps linkage disequilibrium with another gene that is causally related to lung cancer. With appropriate caution in interpretation of the result in both studies, our data in African-Americans could be viewed as consistent with the finding of Benhamou et al. [3] in supporting a role for genetic variation of epoxide hydrolase in lung cancer risk.

Acknowledgements

The authors acknowledge the efforts of Jan Lowery and Lena Masri in project management, Corinne Singer, Regina Olivas-Ho, Kisha Barnes and Steven Grossman for enrollment of subjects, and Catherine Carpenter and Lisa Aubry for computer programming. We thank the physicians and staff at participating hospitals for their cooperation. Data collection and *GSTM1* genotyping was funded by grants 1RT-140 and 3RT-0403 from the State of California Tobacco-Related Disease Research Program to S.J. London. Epoxide hydrolase genotyping was funded by a UK Cancer Research Campaign grant to A.K. Daly. Data analysis was supported by the Division of Intramural Research at the National Institute of Environmental Health Sciences. Case ascertainment was supported in part by the California Public Health Foundation, subcontract 050-F-8709, which is supported by the California Department of Health Services as part of its

state-wide cancer-reporting program mandated by Health and Safety Code Sections 210 and 211.3. The ideas and opinions expressed herein are those of the authors and no endorsement by the State of California, Department of Health Services, or the California Public Health Foundation is intended, or should be inferred. Case ascertainment was also supported in part by the Division of Cancer Prevention and Control, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, under the assigned contract number N01-CN-25403.

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