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Advances in Brief

High-Activity Microsomal Epoxide Hydrolase Genotypes and the Risk of Oral, Pharynx, and Larynx Cancers[1]

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Abstract

Human microsomal epoxide hydrolase (mEH), encoded by the *EPHX1* gene, is involved in the metabolism of tobacco carcinogens. We investigated the effect of exon 3 and 4 polymorphisms of the *EPHX1* gene in 121 patients with cancers of the oral cavity/pharynx, 129 patients with cancer of the larynx, and 172 non-cancer controls, all Caucasian regular smokers. The potential modifying role of previously analyzed *GSTM1*, *GSTM3*, and *GSTP1* genotypes was also examined. Compared with the putative low-activity genotypes, odds ratios (ORs) associated with predicted intermediate and high mEH activity genotypes were significantly increased for oropharyngeal cancers [OR = 1.8; 95% confidence interval (CI) = 1.0–3.3; and OR = 2.1; 95% CI = 1.0–4.5, respectively; P_{trend} = 0.03] and laryngeal cancers (OR = 1.7; 95% CI = 1.0–3.1; and OR = 2.4; 95% CI = 1.1–5.1, respectively; P_{trend} = 0.02). Moreover, a positive interaction was found between mEH activity and *GSTM3* genotype for laryngeal cancer. The combined *EPHX1* high activity-associated genotype and *GSTM3* (*AB* or *BB*) genotype conferred a 13.1-fold risk (95% CI = 3.5–48.4) compared with the concurrent presence of the *EPHX1* low activity-associated genotype and the *GSTM3 AA* genotype. Thus, *EPHX1* polymorphisms may be one of the factors of importance in susceptibility to smoking-related cancers of the upper aerodigestive tract.

Introduction

France is among the countries with the highest incidences of cancers of the upper aerodigestive tract in men (1). Tobacco smoking and alcohol consumption are the main risk factors for these malignancies (2). However, the risk of developing smoking-related cancers varies between individuals, and there is increasing evidence that polymorphisms in the genes that encode enzymes involved in the metabolism of tobacco carcinogens could modify this risk (3). mEH,[3] encoded by the *EPHX1* gene, catalyzes the hydrolysis of reactive epoxide intermediates (3). This reaction usually is regarded as a detoxifying pathway because the metabolites produced are less reactive and can be more easily excreted. mEH also intervenes in the metabolic activation of the polycyclic aromatic hydrocarbons abundant in tobacco smoke, thereby triggering the formation of highly reactive metabolites (4). Two variant *EPHX1* alleles have been associated with altered mEH activity in Caucasians; substitution of histidine for tyrosine at residue 113 (exon 3 polymorphism) decreases mEH activity, whereas substitution of arginine for histidine at residue 139 (exon 4 polymorphism) enhances enzyme activity, probably by affecting the stability of the mEH protein. When both mutations are present, mEH activity approximately equals normal (5). The mEH enzyme is expressed in the upper aerodigestive tract (6), which makes it an interesting candidate as a modifier of smoking-associated disorders at this site. However, few studies have investigated the role of mEH in the onset of several smoking-associated cancers, and the results of these studies were inconclusive (7, 8) We recently reported an almost 3-fold risk of lung cancer associated with predicted high mEH activity based on data obtained in a multicentric hospital-based case-control study (9). We investigated in this study the relationships between *EPHX1* polymorphisms and cancers of the upper aerodigestive tract and whether the effects of these polymorphisms were modified by *GSTM1*, *GSTM3*, and *GSTP1* genotypes.

Materials and Methods

Details of the study have been described previously (10). In brief, Caucasian individuals were recruited between 1988 and 1992 in 10 French hospitals. Peripheral blood samples were available from 121 patients with cancers of the oral cavity/pharynx (67 oral cancers, 50 oro- or hypopharyngeal cancers, and 4 unspecified or unclassifiable cancers of the oral cavity and pharynx), 129 patients with cancers of the larynx (55 supraglottic, 47 glottic/subglottic, and 27 unspecified or unclassifiable larynx cancers), and 172 control individuals. Only incident cases with histologically confirmed primary squamous cell carcinoma were included. The control group was frequency matched on age, sex, and hospital. The main diagnoses among control individuals were rheumatological (33%), infectious and parasitic diseases (10%), respiratory (9%), cardiovascular (8%), digestive diseases (6%), and traumatological diseases (6%). The main reasons for admission were related to general symptoms (7%) for the other categories. Severe liver diseases (total bilirubin >60 μmol/l, or serum glutamic-oxaloacetic transaminase or glutamic-pyruvic transaminase >150 units/l, or serum phosphatase alkaline >600 units/ml) were exclusion criteria for both cases and control subjects. All study subjects were regular smokers, defined as people having smoked at least five cigarettes (or cigars or pipes) per day for at least 5 years. Detailed information on recent and past tobacco use and alcohol consumption was recorded during a personal standardized interview. The daily consumption of each type of tobacco was expressed in g/day (1 g for cigarette, 2 g for cigar, and 3 g for pipe). The average daily consumption of tobacco smoking was calculated by dividing the cumulative lifetime tobacco consumption by the overall duration of smoking. The consumption of alcoholic beverages was expressed in grams of pure ethanol (4.0, 9.4, 14.5, and 31.7 g for 0.1 liter of beer, wine, cider, aperitif, and hard liquor, respectively). The average daily consumption of alcohol was calculated by dividing the cumulative daily consumption of alcohol (the sum of the number of grams of ethanol per day multiplied by the number of years that the quantity was drunk) by the overall duration of drinking (11). The main characteristics of the study population are presented in Table 1.

Blood samples were collected into EDTA tubes and stored at −20°C until total WBC DNA was extracted, using standard protocols. The *EPHX1* genotyping assay was performed blinded to the subjects' case-control status. After

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[3] The abbreviations used are: mEH, microsomal epoxide hydrolase; GST, glutathione *S*-transferase; OR, odds ratio; CI, confidence interval.

Table 1 *Main characteristics of the study population*

	Oral/pharyngeal cancer patients (n = 121)	Laryngeal cancer patients (n = 129)	Control individuals (n = 172)
Male (%)	113 (93%)	127 (98%)	163 (95%)
Age, years			
Mean (SD)	54.4 (10.2)	55.0 (9.4)	54.9 (11.1)
Range	25–89	22–85	25–88
Mean daily alcohol consumption, g/day (SD)	111.8 (71.5)[a]	98.1 (69.9)[b]	77.1 (64.0)
Mean duration of alcohol drinking, years (SD)	30.3 (12.6)	30.1 (14.1)	29.0 (16.5)
Mean daily tobacco consumption, g/day (SD)	28.2 (13.6)[c]	30.4 (15.8)[b]	25.1 (12.5)
Mean duration of smoking, years (SD)	33.9 (10.2)	34.6 (8.8)[c]	32.2 (11.6)

[a] P = 0.0001, Wilcoxon's rank-sum test (comparison with control individuals).
[b] $P < 0.01$, Wilcoxon's rank-sum test (comparison with control individuals).
[c] $P < 0.05$, t test (comparison with control individuals).

two separate PCR reactions, the variant allele correlating with increased mEH activity (*Arg139*) was determined by the presence of a *Rsa*I restriction site (6), and the allele correlating with decreased activity (*His113*) was determined by the presence of an *Asp*I site (12). Three levels of predicted mEH activity were assigned according to *in vitro* expression of the variant alleles (8, 9). *GSTM1*, *GSTM3*, and *GSTP1* genotypes had been determined previously as described (11). ORs and their 95% CIs were calculated by unconditional logistic regression, with sex, age (<50, 50–54, 55–59, 60–64, and ≥65 years), daily consumption of tobacco in g/day (20, 21–30, and ≥31), duration of smoking in years (25, 26–34, and ≥35), exclusive cigarette smoking (no/yes), and daily consumption of alcohol in g/day (40, 41–80, 81–120, and ≥121) as confounding factors. All of the cutoff points were defined according to the distributions in the control population so that sufficient numbers of individuals were included in each subgroup. We evaluated the gene-dosage effect (*i.e.*, increasing risks according to predicted intermediate and high mEH activity compared with predicted low activity) by linear trend tests (13). All P values reported are two-sided.

Results and Discussion

In the control population, exon 3 and 4 genotype frequencies were in Hardy-Weinberg equilibrium (P = 0.65 and 0.48, respectively; Table 2), and were similar across disease groups. Significant differences in the distribution of exon 3 genotypes were found between controls and both oral/pharynx cancers (P = 0.004) and larynx cancers (P = 0.006), with a significant gene-dose effect for larynx cancer (P_{trend} = 0.006) and to a less extent for oral/pharynx cancer (P_{trend} = 0.17). In contrast, exon 4 genotype distributions were similar in cancer cases and controls.

The distribution of the predicted mEH activity was significantly different between control subjects and oral/pharynx cancer patients (P = 0.03) or larynx cancer patients (P = 0.01; Table 2). A significant increase in risk with increasing mEH activity was found for oral/pharynx cancer (P_{trend} = 0.03) and larynx cancer (P_{trend} = 0.02); in particular, individuals with predicted high activity were at a >2-fold risk of developing these cancers (OR = 2.1; 95% CI = 1.0–4.5 for oral/pharyngeal cancer; and OR = 2.4; 95% CI = 1.1–5.1 for laryngeal cancer). Overall, the present findings are consistent with our previous observations on lung cancer (9). The ORs associated with predicted mEH activity were not modified by the duration of smoking (dichotomized at the approximate median in the control population), combined smoking and alcohol exposures (>20 g tobacco/day, and

Table 2 *Distribution of individuals and ORs of cancer according to EPHX1 genotypes and predicted mEH activity*

	Oral/pharyngeal cancer patients (n = 121)		Laryngeal cancer patients (n = 129)		Control individuals (n = 172)
Genotype	n (%)	OR[a] (95% CI)	n (%)	OR[a] (95% CI)	n (%)
Exon 3 genotypes					
wt_3/wt_3[b]	66 (54.5)	1 (Reference)	72 (55.8)	1 (Reference)	64 (37.2)
$wt_3/slow$	32 (26.5)	0.4 (0.2–0.7)	40 (31.0)	0.4 (0.2–0.7)	77 (44.8)
$slow/slow$	23 (19.0)	0.8 (0.4–1.8)	17 (13.2)	0.5 (0.2–1.1)	31 (18.0)
P for trend		0.17		0.006	
Exon 4 genotypes					
wt_4/wt_4	80 (66.1)	1 (Reference)	84 (65.1)	1 (Reference)	121 (70.3)
$wt_4/rapid$	38 (31.4)	1.1 (0.6–2.0)[c]	41 (31.8)	1.0 (0.6–1.8)[c]	49 (28.5)
$rapid/rapid$	3 (2.5)		4 (3.1)		2 (1.2)
Predicted mEH activity					
Low[d]	42 (34.7)	1 (Reference)	43 (33.3)	1 (Reference)	85 (49.4)
Intermediate[e]	55 (45.5)	1.8 (1.0–3.3)	59 (45.7)	1.7 (1.0–3.1)	65 (37.8)
High[f]	24 (19.8)	2.1 (1.0–4.5)	27 (20.9)	2.4 (1.1–5.1)	22 (12.8)
P for trend		0.03		0.02	

[a] ORs adjusted for sex-, age-, smoking-, and alcohol-related variables. Data on smoking and/or alcohol drinking were missing for six oral/pharynx cancer cases, six larynx cancer cases, and eight control individuals.
[b] *wt*, wild type.
[c] Due to small number of *rapid/rapid* genotype, ORs were calculated for combined $wt_4/rapid$ and *rapid/rapid* genotypes.
[d] *slow/slow* and wt_4/wt_4 genotypes, *slow/slow* and $wt_4/rapid$ genotypes, $wt_3/slow$ and wt_4/wt_4 genotypes.
[e] wt_3/wt_3 and wt_4/wt_4 genotypes, $wt_3/slow$ and $wt_4/rapid$ genotypes.
[f] wt_3/wt_3 and $wt_4/rapid$ genotypes, wt_3/wt_3 and *rapid/rapid* genotypes, $wt_3/slow$ and *rapid/rapid* genotypes.

Table 3 *Number of cases/controls and ORs[a] of cancer according to predicted mEH activity and GSTM3 genotype*

		Oral/pharyngeal cancer patients[b]			Laryngeal cancer patients[c]		
		Predicted mEH activity			Predicted mEH activity		
		Low	Intermediate	High	Low	Intermediate	High
GSTM3	*AA*	1 (Reference)	2.4 (1.2–4.8)	1.6 (0.7–4.1)	1 (Reference)	2.0 (1.0–4.1)	1.1 (0.4–3.1)
	Cases/controls	30/62	40/41	15/17	28/62	35/41	12/17
GSTM3	*AB* or *BB*	1.1 (0.4–2.8)	0.9 (0.4–2.3)	4.2 (1.0–16.9)	2.0 (0.8–5.5)	2.2 (1.0–5.2)	13.1 (3.5–48.4)
	Cases/controls	18/10	12/21	8/5	13/18	22/21	13/5

[a] Data on smoking and/or alcohol drinking were missing for eight control individuals, six oral/pharyngeal cancer cases, and six laryngeal cancer cases; ORs adjusted for sex-, age-, smoking-, and alcohol-related variables.
[b] Interaction test between predicted mEH activity and *GSTM3* genotype (likelihood ratio test, 2 *df*): P = 0.08.
[c] Interaction test between predicted mEH activity and *GSTM3* genotype (likelihood ratio test, 2 *df*): P = 0.03.

>80 g ethanol/day *versus* others), *GSTM1* genotype, or *GSTP1* genotype (data not shown). In contrast, we found a significant interactive effect between predicted mEH activity and *GSTM3* genotype on larynx cancer risk (Table 3). Carriers of both the combined *EPHX1* high activity-associated genotype and the *GSTM3* (*AB* or *BB*) genotype had a 13.1-fold risk (5% CI = 3.5–48.4) compared with individuals with the concurrent presence of the *EPHX1* low activity-associated genotype and the *GSTM3 AA* genotype. When we used low mEH activity as the reference category, a significant increase in risk associated with high mEH activity was observed in carriers of the *GSTM3* (*AB* or *BB*) genotype (OR = 6.4; 95% CI = 1.5–27.3), but not in carriers of the *GSTM3 AA* genotype (OR = 1.1; 95% CI = 0.4–3.1). A similar tendency was shown for oral/pharyngeal cancers, but the interaction test did not reach statistical significance (Table 3). These findings, however, were based on very small numbers and should be confirmed in larger studies.

A potential limitation of our study would be the use of hospital controls, especially if there are any associations between *EPHX1* genotypes and diseases diagnosed in the control group. Nevertheless, no statistically significant association was found within this control group between genotypes and the main diseases diagnosed, although the likelihood of finding a difference in genotype distribution is low. Moreover, if increased mEH activity was associated with smoking-related diseases among controls, this would result in underestimation of the real relative risks. Exclusion of the 28 controls with pulmonary or cardiovascular diseases did not modify the cancer risks associated with mEH activity. In addition, the frequencies of *EPHX1* genotypes reported in our study are comparable to those observed in other Caucasian populations (12). Taken together, this study suggests that *EPHX1* genotypes associated with high mEH activity are associated with increased risk of smoking-related cancers of the oral cavity, pharynx, and larynx, confirming our prior results on lung cancer (9). The observed 2-fold risk of upper aerodigestive tract cancers associated with predicted mEH activity is what we should expect for a low-penetrance susceptibility gene. However, the implications for public health could be very important given the widespread prevalence of alleles associated with high mEH activity in Caucasian populations. Thus, *EPHX1* polymorphism may be a significant genetic determinant of smoking-induced cancers.

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