Research Article

Cancer Epidemiology, Biomarkers & Prevention

# Genetically Lowered Microsomal Epoxide Hydrolase Activity and Tobacco-Related Cancer in 47,000 Individuals

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## Abstract

**Background:** Two functional polymorphisms of the microsomal epoxide hydrolase (mEH) gene (*EPHX1*), Tyr113His (rs1051740) and His139Arg (rs2234922), have variably been found to influence susceptibility to various cancer forms. We tested whether genetically lowered mEH activity affects risk of developing cancer in the general population.

**Methods:** We genotyped 47,089 individuals from the Danish general population for the Tyr113His and His139Arg polymorphisms in the *EPHX1* gene and divided them into groups with predicted fast, intermediate, and slow mEH activity. Using Cox proportional hazards models, we calculated HRs for 26 individual cancer diagnoses and for groups of any cancer, tobacco-related cancers, estrogen-related female cancers, and other cancers.

**Results:** Of the 47,089 individuals, 7,590 experienced a cancer event, and of these, 1,466 were tobacco-related. After multifactorial adjustment, the HRs (95% CI) for tobacco-related cancer were 1.1 (0.8–1.5) and 1.5 (1.1–2.0) in individuals with intermediate and slow mEH activity versus individuals with the fast phenotype ($P_{trend} = 0.003$). The corresponding HRs among ever-smokers were 1.1 (0.8–1.5) and 1.5 (1.1–2.0; $P_{trend} = 0.003$), whereas HRs among never-smokers did not differ from 1.0.

**Conclusions:** Our results indicate that genetically lowered mEH activity is associated with increased risk of developing tobacco-related cancer among smokers in the general population; however, additional studies are needed to confirm our findings.

**Impact:** To our knowledge, this is the largest study to investigate the association of mEH phenotype and genotype with tobacco-related cancers combined in the general population. *Cancer Epidemiol Biomarkers Prev; 20(8); 1673–82. ©2011 AACR.*

## Introduction

Cancer is one of the leading causes of morbidity and mortality (1). Oxidative stress is involved in the pathogenesis of many different cancers (2). During oxidative stress, there is an overload of reactive oxygen species (ROS) in the organism. It is thought that genetically altered activity of the enzymes responsible for detoxification by neutralization of these ROS could be associated with individual cancer risk (3).

The microsomal epoxide hydrolase (mEH) enzyme encoded by the *EPHX1* gene is one such detoxifying enzyme. It has an established role in oxidative processes through detoxification of smoking-induced oxidative substances (4), and this makes it a candidate gene for risk of developing tobacco-related cancers. Two well-characterized variants of the *EPHX1* gene have been shown to alter enzyme activity considerably (5, 6). Homozygosity for the exon3 Tyr113His (rs1051740) variant allele has been found to decrease enzyme activity by 40% compared with noncarriers, whereas homozygosity for the exon4 His139Arg (rs2234922) variant allele increases enzyme activity by 25% compared with noncarriers (6). Combination of these polymorphisms results in 3 distinct phenotypes with fast, intermediate, or slow enzyme activity (5). Therefore, mEH has been investigated in relation to various cancer forms but with very inconsistent results (2–5, 7–26).

In the present study, we used data from the Copenhagen City Heart Study ($n = 10,038$) and the Copenhagen General Population Study ($n = 37,051$) to test the hypothesis that genetically lowered mEH activity is associated with risk of developing cancer and that this relationship could depend on smoking history.

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## Materials and Methods

### Study population

In this population-based study, we studied randomly selected white individuals of Danish descent ($N = 47{,}089$) consisting of participants from the Copenhagen City Heart Study ($n = 10{,}038$) and the Copenhagen General Population Study ($n = 37{,}051$) combined (27–29). In brief, the Copenhagen City Heart Study is a study of the Danish general population initiated in 1976–1978, with follow-up examinations in 1981–1983, 1991–1994, and 2001–2003; DNA was collected in 1991–1994 and 2001–2003. The Copenhagen General Population Study is another similar study of the Danish general population initiated in 2003 and still recruiting, with DNA being obtained at study entry. Individuals in both studies were randomly selected on the basis of the national Danish Civil Registration System to reflect the Copenhagen general population aged 20 to 80 years and older. Information on morbidity of each participant was obtained by linkage of registry records to the national Danish Cancer Registry and the national Danish Patient Registry, using each participant's unique Central Person Register number (28, 29). We obtained information on all individuals from establishment of the national Danish Cancer Registry in 1943 and establishment of the Danish Patient Registry in 1976 for cancer events until the occurrence of disease, death, or emigration from Denmark up to May 2009 (end of follow-up). During the study period, we had 100% follow-up and the maximum surveillance period was 63 years. All subjects answered similar questionnaires and had objective and clinical parameters measured by the same methods.

The studies were approved by Herlev Hospital and Danish ethical committees and were conducted according to the Declaration of Helsinki. Written informed consent was obtained from all participants.

### Genotyping

TaqMan assays analyzed on the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems Inc.) were used to genotype 2 polymorphisms in the *EPHX1* gene (Tyr113His, *113T > C*, g.19537412*T > C*, rs1051740, and His139Arg, *139A > G*, g.19544185*A > G*, rs2234922; ref. 30). These 2 single-nucleotide polymorphisms (SNP) were chosen because they are functional and have previously been investigated in relation to the endpoints under investigation in the current study. Furthermore, these 2 SNPs tag the entire coding region of the *EPHX1* gene (31). The binding of primers and probes was not influenced by the codon *119* polymorphism (sequences reported in Supplementary Table S1; ref. 32). Because we carried out reruns twice, call rates were 99.98% for both polymorphisms. Genotyping was verified by DNA sequencing (MegaBase; Pharmacia), which showed 100% concordance with the TaqMan genotyping results.

### Other covariates

Participants filled out a questionnaire with information on smoking history and alcohol consumption (divided into categories of <4 drinks/wk and ≥4 drinks/wk). Women also stated their menopausal status, nulliparity, and whether they underwent hormone replacement therapy (HRT). Participants were divided on the basis of their smoking history into ever-smokers (further divided into current and former smokers) and never-smokers. Pack-years were (number of cigarettes smoked per day × number of years smoked)/20 and adjusted for as a continuous variable. Smoking intensity was based on the number of cigarettes smoked per day (cpd); strong intensity was more than 25 cpd, whereas mild intensity was less than 25 cpd. Body mass index (BMI) was measured weight divided by measured height squared ($kg/m^2$).

### Endpoints

The national Danish Cancer Registry identifies 98% of all cancers in Denmark (33–35), whereas the Danish Patient Registry identifies all diseases in relation to hospitalization, including cancer. Dates of deaths were obtained from the Danish Civil Registration System, which is also 100% complete (28, 29). Cancer diagnoses were defined according to the World Health Organization International Classification of Diseases (ICD) 7th edition, and were categorized as tobacco-related cancer (previously associated with tobacco smoking), female cancer (previously associated with estrogen imbalance), and a remainder group of other cancer types. Tobacco-related cancers were lung cancer (ICD-7 162–164, 462.2–462.4), bladder cancer (ICD-7 180.1–180.2, 181), oral cancer (ICD-7 141–148, 442), larynx cancer (ICD-7 160, 161, 262.0), esophagus cancer (ICD-7 150), stomach cancer (ICD-7 151), pancreas cancer (ICD-7 157, 457), kidney cancer (ICD-7 180.0, 180.3), and leukemia (ICD-7 204, 214, 404.0, 503.0, 504.4), because there is suggestive evidence that each of these cancers might be associated with tobacco smoking (36). We had information only on leukemia subtypes in 64 cases [acute lymphoblastic leukemia (ALL), $n = 1$; chronic lymphocytic leukemia (CLL), $n = 36$; acute myeloid leukemia (AML), $n = 12$; chronic myeloid leukemia (CML), $n = 6$; other specified or unspecified leukemias, $n = 9$]. Of these, only AML has previously been associated with smoking; however, results in the present study were similar if leukemia was excluded from the tobacco-related cancer category. Female cancers were breast cancer (ICD-7 170, 470), ovarian cancer (ICD-7 175, 176.9, 375.0, 475.0), cervix cancer (ICD-7 171), and corpus uteri cancer (ICD-7 172–174, 472.0). Other cancers included colorectal cancer (ICD-7 153, 154, 253, 453, 454), liver cancer (ICD-7 155, 455), brain cancer (ICD-7 093, 193, 293, 393.1, 493, 195.4), thyroid cancer (ICD-7 194, 195), skin cancer (ICD-7 140, 191), prostate cancer (ICD-7 177, 477), testis cancer (ICD-7 278, 378, 478), melanoma (ICD-7 190, 299.6, 460, 498), Hodgkin's disease (ICD-7 201), non–Hodgkin's lymphoma (ICD-7 198.0, 200, 202.0, 750.1, 751, 752.0, 745.0,

770.1, 771.0, 778.1, 791.5, 792.0, 793.0, 762.1, 796.6), multiple myeloma (ICD-7 203, 205), sarcoma (ICD-7 196, 197.0, 851.0–899.9), and others (ICD-7 158, 159, 176, 179).

### Statistical analyses

We used STATA/SE 10.1 for all statistical analyses. For trend tests, individual genotypes were coded 0 → 2, with homozygosity for the common allele as the reference. On the basis of the predicted mEH activity as described by Benhamou and colleagues (5), participants were divided into 3 groups encoded 0 → 2 (fast → slow; fast: *113TT/139AG*, *113TT/139GG*, *113TC/139GG*; intermediate: *113TT/139AA*, *113TC/139AG*, *113CC/139GG*; slow: *113TC/139AA*, *113CC/139AG*, *113CC/139AA*). We chose *a priori* to analyze these phenotype groupings to maximize statistical power and to simplify data presentation and interpretation; however, if we examined each of the 9 genotype combinations separately, results were similar to those presented for the 3 phenotype groupings.

We analyzed the relationship of *EPHX1* phenotype and genotype with risk of developing cancer by using Kaplan–Meyer curves, log-rank statistics, and Cox regression. HRs for any cancer, tobacco-related cancer, female cancers, and other cancers combined and individually, were calculated using Cox proportional hazards models adjusted for age, sex, tobacco smoking (number of pack-years), and alcohol consumption. Analyses of female cancers were furthermore adjusted for nulliparity, menopausal status, and HRT. Besides smoking, all covariates were treated as dichotomous exposures. The proportional hazards assumption was examined visually by plotting log(cumulative hazard) as a function of age; no violations were observed. We used age as time scale, thus analyzing age at event by using left truncation (i.e., delayed entry). Age differences are thereby automatically adjusted for. Individuals were censored at the relevant cancer endpoint, death, emigration, or at May 2009, whichever came first. An interaction test was conducted by likelihood-ratio test of models including or not including the interaction term between smoking (ever vs. never) and mEH phenotype on risk of developing tobacco-related cancer.

## Results

Of the 47,089 individuals, 7,590 experienced a cancer event, and of these, 1,466 were tobacco-related. Characteristics of participants are presented according to *EPHX1* phenotype and genotype in Table 1. Age of onset for tobacco-related cancers did not differ according to either *EPHX1* phenotype or genotype (Table 1). The age distribution within our study population makes for a relatively high proportion of postmenopausal women and also a low nulliparity rate. After Bonferroni correction, there were no significant *P* values for the distribution of characteristics according to mEH phenotype or genotype. Genotype frequencies were in accordance with the Hardy–Weinberg equilibrium and corresponded well with those previously reported for Caucasians (37, 38). Of the 47,089 participants, 9,670 had the fast phenotype, 20,922 had the intermediate phenotype, and 16,497 had the slow phenotype.

### Tobacco-related cancer

Figure 1 shows the cumulative incidence of tobacco-related cancer among fast, intermediate, and slow mEH activity individuals (log-rank: $P_{trend} = 0.06$). Our data indicate an increased cumulative incidence of tobacco-related cancer for the slow mEH phenotype with increasing age; this result, however, was not statistically significant.

Figure 2 shows risk of developing tobacco-related cancer according to *EPHX1* phenotype and genotype with adjustment for age and sex only (left panel), multifactorial adjustment (middle panel), and multifactorial and smoking adjustment (right panel). After adjustment for age, sex, BMI, alcohol consumption, and smoking, the HRs (95% CI) for tobacco-related cancer were 1.1 (0.8–1.5) in individuals with intermediate mEH activity and 1.5 (1.1–2.0) for slow mEH activity versus individuals with the fast mEH phenotype ($P_{trend} = 0.003$). For *EPHX1* genotypes, HRs (95% CI) were 1.4 (1.1–1.8) in *113TC* heterozygotes and 1.2 (0.8–1.7) in *113CC* homozygotes versus noncarriers ($P_{trend} = 0.02$). The corresponding HRs were 0.9 (0.7–1.1) in *139AG* heterozygotes and 0.8 (0.5–1.4) in *139GG* homozygotes ($P_{trend} = 0.25$).

We also calculated HRs for ever-smokers and various subgroups of smokers and for never-smokers separately (Fig. 3). As expected, the association seen in the entire study population could be assigned to the ever-smokers. In ever-smokers, and after multifactorial adjustment, HRs were 1.1 (0.8–1.5) and 1.5 (1.1–2.0) in individuals with intermediate and slow mEH activity versus individuals with the fast mEH phenotype ($P_{trend} = 0.003$). For never-smokers, HRs did not differ from 1.0. Accordingly, smoking and mEH phenotype interacted on risk of developing tobacco-related cancer (likelihood-ratio test: $P < 0.001$).

Table 2 presents risk estimates for mEH phenotypes and smoking status for individual tobacco-related cancers and the composite endpoint of all 9 tobacco-related cancers combined. Of the 1,466 tobacco-related cancer events, 39% were lung cancer events. Histology data were available only for a limited number of lung cancer cases. In subgroup analyses of adenocarcinoma versus other histologies, we observed a trend that the slow mEH phenotype may be protective of this specific type of lung cancer, whereas results for other histologic types of lung cancer were similar to the results for lung cancer overall (Table 2). Results for individual tobacco-related cancers generally showed a trend toward increased risk for the slow versus fast mEH phenotype (Table 2, left panels) proportional to the relative risk conferred by current versus never tobacco smoking (Table 2, right panel); however, the effect size of mEH phenotype was more modest than for smoking status. All results for tobacco-related

**Table 1.** Characteristics of study participants from the general population at time of DNA collection

| Characteristic | mEH phenotype | | | | *EPHX1 113* genotype | | | | *EPHX1 139* genotype | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fast | Normal | Slow | *P* | *TT* | *TC* | *CC* | *P* | *AA* | *AG* | *GG* | *P* |
| N | 9,670 | 20,922 | 16,497 | | 23,060 | 19,762 | 4,267 | | 29,473 | 15,463 | 2,153 | |
| Sex, female | 54 | 54 | 54 | 0.55 | 54 | 54 | 54 | 0.43 | 54 | 54 | 55 | 0.48 |
| Age, y | 62 (51–70) | 62 (51–71) | 62 (51–70) | 0.73 | 62 (51–71) | 62 (51–70) | 62 (51–70) | 0.36 | 62 (51–71) | 62 (51–70) | 62 (51–70) | 0.45 |
| Age of onset for tobacco-related cancers, y | 64 (53–73) | 64 (53–74) | 64 (53–74) | 0.12 | 64 (53–74) | 64 (53–74) | 63 (53–73) | 0.81 | 64 (53–74) | 64 (53–73) | 64 (53–73) | 0.80 |
| BMI | 26 (23–28) | 26 (23–28) | 25 (23–28) | 0.36 | 26 (23–28) | 26 (23–28) | 25 (23–28) | 0.44 | 25 (23–28) | 25 (23–28) | 26 (23–28) | 0.66 |
| Ever-smokers | 64 | 64 | 64 | 0.93 | 64 | 64 | 64 | 0.74 | 64 | 64 | 63 | 0.73 |
| Pack-years | 22 (10–35) | 22 (10–35) | 22 (10–34) | 0.43 | 22 (10–35) | 22 (10–34) | 22 (10–35) | 0.7 | 22 (10–35) | 22 (10–35) | 22 (10–35) | 1 |
| Alcohol drinkers[a] | 69 | 69 | 68 | 0.03[b] | 69 | 68 | 67 | 0.01[b] | 68 | 69 | 69 | 0.62 |
| Nulliparity[c] | 17 | 17 | 17 | 0.51 | 17 | 17 | 16 | 0.68 | 17 | 17 | 16 | 0.55 |
| Menopause[c] | 69 | 68 | 69 | 0.27 | 68 | 69 | 68 | 0.03 | 68 | 69 | 70 | 0.4 |
| HRT[c] | 16 | 15 | 16 | 0.16 | 15 | 16 | 15 | 0.96 | 15 | 16 | 15 | 0.17 |

NOTE: Values are median (interquartile range) or percentage. *P* values were calculated using Pearson's $\chi^2$ tests for categorical variables and Kruskal–Wallis 1-way ANOVA tests for continuous variables.

[a]Alcohol drinkers are individuals who consume more than 48 g of alcohol per week.

[b]After Bonferroni correction for multiple comparison, the required value for significance was $P < 0.05/27 = 0.002$.

[c]Women only.

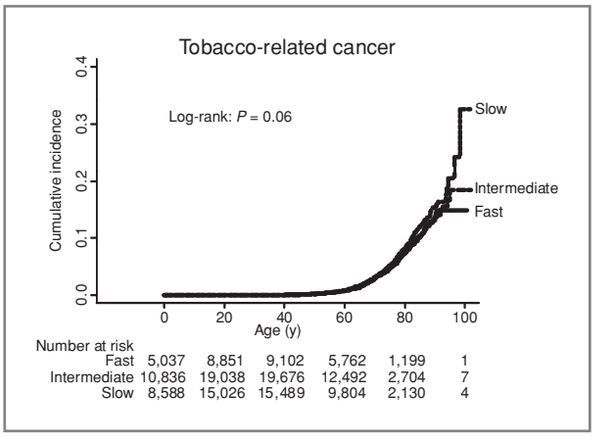


**Figure 1.** Cumulative incidence based on Kaplan–Meyer estimates of tobacco-related cancer as a function of age according to mEH phenotype. Based on 47,089 individuals from the Danish general population with up to 63 years of surveillance for cancer development.

cancer reported above were similar if leukemia was excluded from the tobacco-related cancers category.

### Female and other cancers

The risk of developing female cancers and other cancers was investigated in a similar fashion as tobacco-related cancers. With or without adjustment, HRs in these 2 groups did not differ from 1.0 for mEH phenotype or *EPHX1* genotypes ($P_{trend}$ = 0.37–0.90; Supplementary Fig. S1). The risk estimates for individual cancers according to mEH phenotype are listed in Supplementary Table S2. None of the HRs differed significantly from 1.0.

## Discussion

In an analysis involving much larger size and scope than earlier work generally focusing on individual cancer types, we observed that genetically lowered mEH activity is associated with increased risk of developing tobacco-related cancer among smokers in the general population.

### Tobacco-related cancer

Because of its role in the metabolism of tobacco smoke constituents, mEH has been investigated in relation to many tobacco-related cancers. To our knowledge, this is the largest study to investigate the association of mEH phenotype and genotype with all tobacco-related cancers combined in the general population. Previous studies have generally focused on individual cancers, typically in case–control studies and with very inconsistent results (2–5, 7–26). Furthermore, many previous studies only investigated individual genotypes and did not report findings on predicted mEH phenotype for cancer groups overall. Our study is, thus, the first of its kind, and no data are available in the current literature for direct comparison with our findings.

It is widely accepted that mEH plays a dual role in the human body, as it both detoxifies and activates a number of carcinogenic compounds (12, 20). Therefore, increased mEH activity might theoretically confer both cancer protection due to increased detoxification or greater risk due to increased carcinogen activation. Our data imply that the detoxifying aspect is the most important, because the slow phenotype is associated with increased risk of developing tobacco-related cancer in our study. For individual genotypes, this tendency was reflected in that the codon *113C* "slow allele" was associated with higher risk of developing tobacco-related cancer, whereas the codon *139G* "fast allele" showed a statistically insignificant trend toward lowered risk. A similar phenomenon has been shown for the genotypes and chronic obstructive pulmonary disease (COPD) in meta-analyses where the *113C* "slow allele" is associated with higher risk for developing COPD whereas the *139G* "fast allele" shows a statistically insignificant trend toward lowered risk (30).

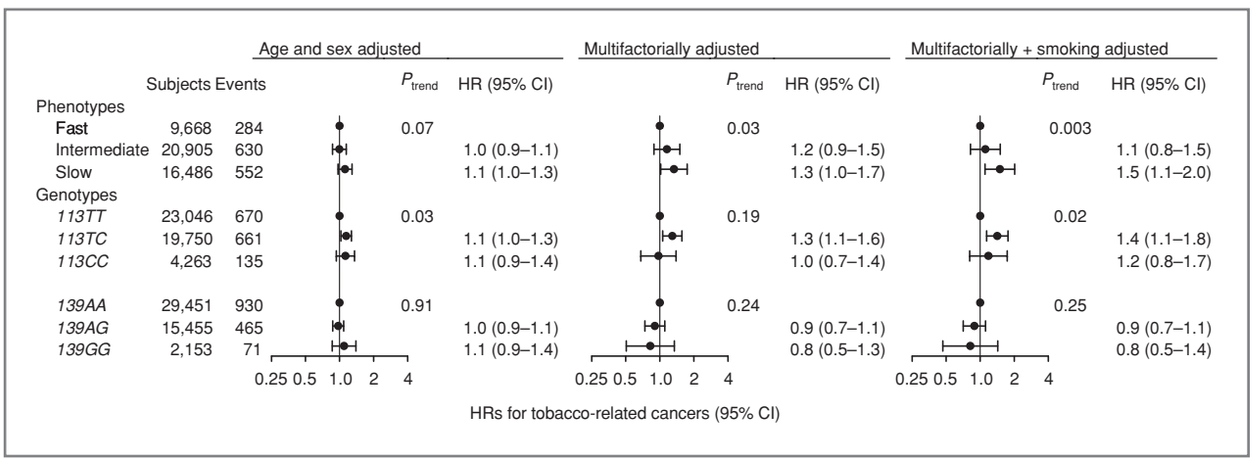


**Figure 2.** Risk of developing tobacco-related cancer in the Danish general population adjusted for age and sex, multifactorially adjusted, or multifactorially + smoking adjusted according to mEH phenotype and genotype. Based on 47,089 individuals from the Danish general population with up to 63 years of surveillance for cancer development.

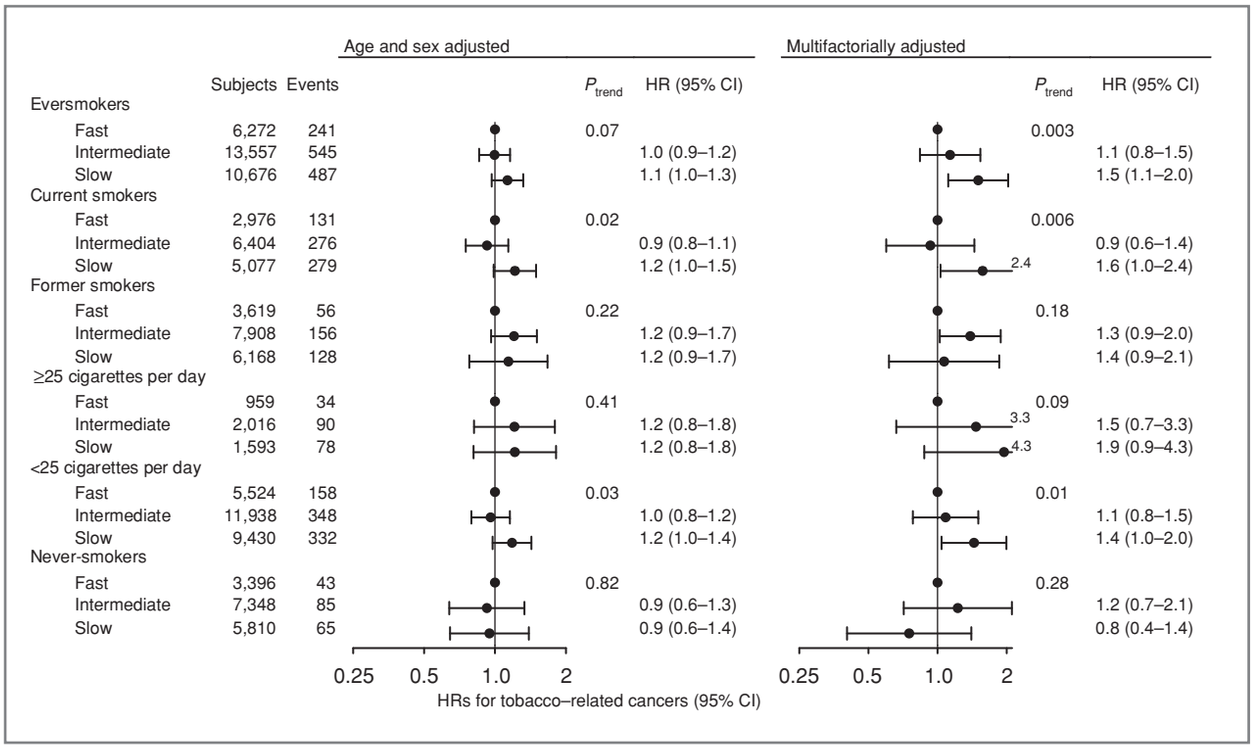


Figure 3. Risk of developing tobacco-related cancer in ever-smokers, various subgroups of smokers, and never-smokers in the Danish general population adjusted for age and sex or multifactorially adjusted according to mEH phenotype. Based on 47,089 individuals from the Danish general population with up to 63 years of surveillance for cancer development.

Among tobacco-related cancers, lung cancer is the most common. Many studies found an association of mEH phenotype/genotype with lung cancer risk (5, 10, 19, 20, 25, 26), particularly early-onset lung cancer (10, 19, 25). In our study, mEH slow versus fast phenotype showed a nonsignificant trend toward increased risk of developing lung cancer whereas the heterozygous codon *113TC* genotype was associated with increased risk of developing overall lung cancer [HR = 1.5 (1.1–2.0); data not shown], but there was no specific association with early-onset lung cancer (data not shown). For lung cancer and other tobacco-related cancers, previous findings are mixed ranging from negative reports to different groups finding opposite associations of the same SNP or mEH phenotype (3, 4, 7, 8, 10–12, 17–20, 23, 25).

## Female and other cancers

As with tobacco-related cancers, female cancers and other cancers as collective groups have not previously been investigated in relation to mEH phenotype and genotype. For female cancers, the interest in mEH lies in its involvement in the estrogen synthetic and metabolic pathways. Studies on individual female cancers and *EPHX1* are largely negative (13, 16, 21), but there have been scattered reports of positive associations of either genotype with risk of developing breast cancer (14, 24). Our findings do not support an association of mEH phenotype or genotype with female cancers as a group or with breast cancer or other female cancers separately.

Our category of other cancers is, of course, very heterogeneous, and findings for individual cancers cannot be generalized across the group. In addition, small study size and heterogeneity likely contribute to the contradictory findings and possibly some of the null findings of previous studies (2–5, 7–15, 17–26). Our data do not support any association with other cancers as a group or individually.

## Limitations

Among the 47,089 individuals included in our study, 16,554 were never-smokers, and of these, just 193 experienced a tobacco-related cancer event. We cannot exclude that the negative finding for this group is a result of low statistical power; however, the interaction between smoking and mEH phenotype on risk of developing tobacco-related cancer indicates that a negative result among never-smokers is plausible. All participants in the present study are Danish whites, and although this eliminates any blurring due to ethnic heterogeneity of the study population, our results may apply to whites only. Another potential limitation of our study is the fact that participants in the Copenhagen City Heart Study were genotyped only if they attended the 1991–1994 or 2001–2003 examinations. If death or morbidity prevented

**Table 2.** Risk of developing tobacco-related cancer

| | | *EPHX1* phenotype | | | | | | Smoking status | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Phenotype** | **Events** | **Age and sex adjusted** | | **Multifactorially adjusted** | | **Multifactorially + smoking adjusted** | | **Category** | **Events** | **Multifactorially + mEH phenotype adjusted** | |
| | | **HR (95% CI)** | **$P_{trend}$** | **HR (95% CI)** | **$P_{trend}$** | **HR (95% CI)** | **$P_{trend}$** | | | **OR (95% CI)** | **$P_{trend}$** |
| Tobacco-related cancers | | | | | | | | | | | |
| Fast | 284 | | 0.07 | | 0.03 | | 0.003 | Never | 193 | | <0.001 |
| Intermediate | 630 | 1.00 (0.87–1.15) | | 1.16 (0.89–1.50) | | 1.11 (0.82–1.50) | | Former | 498 | 1.99 (1.68–2.37) | |
| Slow | 552 | 1.12 (0.97–1.29) | | 1.33 (1.02–1.74) | | 1.49 (1.11–2.02) | | Current | 763 | 5.02 (4.26–5.91) | |
| Lung cancer | | | | | | | | | | | |
| Fast | 109 | | 0.10 | | 0.14 | | 0.14 | Never | 30 | | <0.001 |
| Intermediate | 234 | 0.96 (0.77–1.21) | | 0.92 (0.62–1.36) | | 0.99 (0.84–1.18) | | Former | 142 | 3.95 (2.64–5.91) | |
| Slow | 222 | 1.17 (0.93–1.47) | | 1.25 (0.85–1.85) | | 1.12 (0.94–1.33) | | Current | 389 | 16.4 (11.2–23.9) | |
| Adenocarcinoma[a] | | | | | | | | | | | |
| Fast | 20 | | 0.08 | | 0.03 | | 0.06 | Never | 5 | | <0.001 |
| Intermediate | 31 | 0.69 (0.39–1.21) | | 0.59 (0.33–1.08) | | 0.64 (0.35–1.19) | | Former | 12 | 2.74 (0.88–8.54) | |
| Slow | 20 | 0.57 (0.31–1.06) | | 0.48 (0.25–0.94) | | 0.52 (0.26–1.02) | | Current | 54 | 16.0 (5.78–44.4) | |
| Other histologies[a] | | | | | | | | | | | |
| Fast | 28 | | 0.66 | | 0.71 | | 0.55 | Never | 2 | | <0.001 |
| Intermediate | 60 | 0.96 (0.62–1.51) | | 0.94 (0.59–1.50) | | 0.93 (0.58–1.50) | | Former | 28 | 2.32 (0.34–16.06) | |
| Slow | 44 | 0.90 (0.56–1.45) | | 0.91 (0.56–1.49) | | 0.86 (0.52–1.42) | | Current | 105 | 2.43 (0.39–15.22) | |
| Oral cancer | | | | | | | | | | | |
| Fast | 29 | | 0.92 | | 0.29 | | 0.34 | Never | 33 | | <0.001 |
| Intermediate | 59 | 0.92 (0.59–1.44) | | 0.85 (0.32–2.31) | | 0.38 (0.10–1.41) | | Former | 44 | 1.05 (0.66–1.67) | |
| Slow | 50 | 1.00 (0.63–1.58) | | 1.48 (0.57–3.81) | | 1.31 (0.45–3.76) | | Current | 61 | 2.09 (1.35–3.23) | |
| Larynx cancer | | | | | | | | | | | |
| Fast | 17 | | 0.75 | | 0.27 | | 0.24 | Never | 7 | | <0.001 |
| Intermediate | 39 | 1.04 (0.59–1.83) | | 0.57 (0.18–1.89) | | 0.47 (0.14–1.63) | | Former | 40 | 3.37 (1.49–7.61) | |
| Slow | 32 | 1.09 (0.61–1.97) | | 0.47 (0.13–1.76) | | 0.45 (0.12–1.67) | | Current | 41 | 5.83 (2.59–13.1) | |
| Esophagus cancer | | | | | | | | | | | |
| Fast | 11 | | 0.15 | | 0.11 | | 0.18 | Never | 8 | | <0.001 |
| Intermediate | 24 | 0.98 (0.48–1.99) | | 1.85 (0.39–8.69) | | 1.14 (0.22–5.87) | | Former | 23 | 1.99 (0.88–4.51) | |
| Slow | 29 | 1.51 (0.76–3.03) | | 3.02 (0.66–13.79) | | 2.36 (0.50–11.13) | | Current | 32 | 4.35 (2.00–9.47) | |
| Stomach cancer | | | | | | | | | | | |
| Fast | 20 | | 0.72 | | 0.78 | | 0.34 | Never | 14 | | 0.005 |
| Intermediate | 29 | 0.65 (0.37–1.15) | | 1.21 (0.43–3.41) | | 2.55 (0.57–11.52) | | Former | 40 | 2.34 (1.24–4.44) | |
| Slow | 35 | 1.00 (0.58–1.73) | | 1.20 (0.41–3.51) | | 2.44 (0.52–11.47) | | Current | 30 | 2.61 (1.35–5.02) | |

*(Continued on the following page)*

**Table 2.** Risk of developing tobacco-related cancer (Cont'd)

| | *EPHX1* phenotype | | | | | | Smoking status | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Phenotype** | **Events** | **Age and sex adjusted** | | **Multifactorially adjusted** | | **Multifactorially + smoking adjusted** | | **Category** | **Events** | **Multifactorially + mEH phenotype adjusted** | |
| | | **HR (95% CI)** | **$P_{trend}$** | **HR (95% CI)** | **$P_{trend}$** | **HR (95% CI)** | **$P_{trend}$** | | | **OR (95% CI)** | **$P_{trend}$** |
| Pancreas cancer | | | | | | | | | | | |
| Fast | 21 | | 0.12 | | 0.33 | | 0.21 | Never | 24 | | <0.001 |
| Intermediate | 45 | 0.95 (0.57–1.60) | | 0.92 (0.39–2.15) | | 0.82 (0.28–2.46) | | Former | 37 | 1.24 (0.73–2.12) | |
| Slow | 51 | 1.39 (0.83–2.30) | | 1.38 (0.61–3.16) | | 1.62 (0.58–4.50) | | Current | 56 | 3.16 (1.93–5.17) | |
| Kidney cancer | | | | | | | | | | | |
| Fast | 17 | | 0.70 | | 0.51 | | - | Never | 14 | | <0.001 |
| Intermediate | 48 | 1.27 (0.73–2.21) | | 2.56 (0.57–11.56) | | N/A | | Former | 32 | 1.82 (0.95–3.51) | |
| Slow | 28 | 0.95 (0.52–1.74) | | 2.08 (0.43–10.03) | | N/A | | Current | 45 | 4.16 (2.23–7.77) | |
| Bladder cancer | | | | | | | | | | | |
| Fast | 47 | | 0.74 | | 0.13 | | 0.13 | Never | 27 | | <0.001 |
| Intermediate | 105 | 1.00 (0.71–1.42) | | 1.83 (0.80–4.18) | | 1.40 (0.60–3.28) | | Former | 106 | 2.41 (1.57–3.71) | |
| Slow | 86 | 1.06 (0.74–1.51) | | 2.00 (0.86–-4.65) | | 1.86 (0.80–4.34) | | Current | 102 | 4.01 (2.61–6.17) | |
| Leukemia[b] | | | | | | | | | | | |
| Fast | 29 | | 0.69 | | 0.68 | | 0.76 | Never | 42 | | 0.18 |
| Intermediate | 71 | 1.11 (0.72–1.70) | | 1.53 (0.75–3.10) | | 2.78 (0.96–8.00) | | Former | 57 | 1.03 (0.69–1.55) | |
| Slow | 47 | 0.94 (0.59–1.49) | | 0.95 (0.43–2.09) | | 1.62 (0.52–5.08) | | Current | 46 | 1.35 (0.88–2.07) | |

NOTE: Multifactorial adjustment included age, sex, BMI, and alcohol consumption (<4 drinks/wk or ≥4 drinks/wk); smoking was adjusted for using pack-years as a continuous variable.

Abbreviation: N/A, not available due to too few subjects.

[a]Histology data were available only on a subset of lung cancer patients. This probably accounts for the observed dissimilarity in risk estimates for subgroups and overall lung cancer. A minority of subjects were excluded from the analyses of both subgroups due to missing information (fast, $n = 1$; intermediate, $n = 8$; slow, $n = 3$).

[b]The proportion of the leukemia patient group of known subtype was composed of the following: AML, $n = 12$; ALL, $n = 1$; CLL, $n = 36$; CML, $n = 6$; others, $n = 9$.

certain individuals from attending these examinations and therefore from being genotyped, biases such as survival and/or selection biases may have occurred. However, the observed *EPHX1* genotype distributions were in Hardy–Weinberg equilibrium and were similar to those observed in the Copenhagen General Population Study, which is not subject to survival or selection bias to the same extent. Furthermore, limiting the analysis to a follow-up period after DNA collection yielded results similar to those presented.

## Conclusion

Our results indicate that genetically lowered mEH activity is associated with increased risk of developing tobacco-related cancer among smokers in the Danish general population; however, additional studies are needed to confirm our findings.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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