Effects of CYP2D6 Activity and Tobacco on Larynx Cancer Risk

Simone Benhamou, Christine Bouchardy, Catherine Paoletti, and Pierre Dayer

Unit of Cancer Epidemiology (INSERM U351), Institut Gustave-Roussy, 39 rue Camille Desmoulins, 94805 Villejuif Cedex, France (S. B., C. P.); and Geneva Cancer Registry, 55 boulevard de la Cluse, 1205 Geneva [C. B.]; and Division of Clinical Pharmacology, University Hospital of Geneva, 21 rue Michel-du-Crest, 1211 Geneva, Switzerland [P. D.]

Abstract

The genetically determined capacity of the cytochrome P450 CYP2D6 is suspected to be involved in the activation of tobacco carcinogens. From a multicentric case-control study carried out to analyze the interaction between host and environmental factors on tobacco-related cancers, we reported recently that the effect of tobacco on lung cancer risk rose with increasing CYP2D6 activity, and the effect of CYP2D6 activity rose with increasing tobacco consumption. The aim of the present report was to investigate whether results on lung cancer could be observed for larynx cancer, from a study on 140 cases and 157 controls. A weak interaction between increasing levels of both CYP2D6 activity and average daily consumption of tobacco was found (P = 0.12). The only significant interaction between these two factors was observed when CYP2D6 activity was considered with the two conventional phenotypes (P < 0.05). A dose-response effect of tobacco on larynx cancer risk was found only among one-third of the smokers with the highest level of CYP2D6 activity, and CYP2D6 was a risk factor only among heavy smokers. The highest risk for larynx cancer was then observed among smokers having both the highest levels of CYP2D6 activity and daily consumption of tobacco.

The interaction between CYP2D6 activity and tobacco was weaker for larynx cancer than that reported previously for lung cancer. However, the similarities in the results found for these two cancers, i.e., a greater effect of tobacco among smokers with the highest CYP2D6 activity, reinforce the hypothesis that this activity could modify the effect of tobacco on cancer risk.

Introduction

Individual susceptibility to tobacco-related cancers might depend partly on genetic capacity in activation of tobacco carcinogens (1). The genetically determined activity of the cytochrome P450 CYP2D6 is suspected to be involved in this activation (2). Then the effect of tobacco on cancer risk should depend on CYP2D6 activity, and vice versa. From a case-control study undertaken to evaluate the interaction between host and environmental factors on tobacco-induced lung cancer, we recently reported a strong interaction on lung cancer risk between the average daily consumption of tobacco and CYP2D6 activity (3). The effect of tobacco on lung cancer risk rose with increasing levels of CYP2D6 activity, and vice versa. The well-known increase in risk associated with increased levels of smoking was observed only among one-third of smokers having the highest level of CYP2D6 capacity. Similarly, CYP2D6 activity was found to be a risk factor for lung cancer only among the heaviest smokers.

Epidemiological studies have also strongly implicated tobacco smoking as a causative factor in larynx carcinogenesis (4). The concomitant effect of CYP2D6 activity and tobacco consumption on larynx cancer risk, however, has never been evaluated. The aim of the present report was to investigate whether results on lung cancer could be observed for larynx cancer, thus reinforcing the hypothesis that CYP2D6 activity could modify the effect of tobacco on cancer risk.

Materials and Methods

Subjects. The design of the multicentric case-control study on host factors associated with lung cancer was reported previously (3). In the course of this study, all eligible Caucasian patients with histologically confirmed squamous primary larynx cancers were also recruited. A unique control group consisting of all eligible Caucasian patients without previous or actual malignant disease was included. The main medical diagnoses in the control population were rheumatological, cardiovascular, respiratory, infectious, and parasitic diseases. Control individuals were recruited according to age, sex and hospital distributions observed in cases. Patients were recruited by one of the seven trained study interviewers who determined eligibility through a short questionnaire. Each interviewer had to include both cases and controls. All larynx cancer patients and controls were regular smokers, defined as people having smoked five cigarettes or more (or cigars or pipes) per day for at least 5 years. For both cases and controls, the main exclusion criteria were: refusal to give informed consent, inability to take oral medications or to be interviewed, presence of severe renal or liver disease or chronic heart failure, and use of medications known or suspected to interfere with the phenotyping test (these include neuroleptics; antidepressants; antiarrhythmics; β-blockers; and drugs containing cimetidine, debrisoquine, dextromethorphan, dextropropoxyphene, diiltiazem, guanoxan, phenacine, phenformine, or phenotiazine). For both eligible cases and controls, all other medications administered during the last week before phenotyping test were abstracted from medical records. A questionnaire was filled out for each subject from personal interview where information on lifetime tobacco...
and alcohol consumption, personal medical history, current use of medications, and occupations was recorded.

**Phenotyping Assays.** Individual CYP2D6 activity was measured through the use of the probe drug dextromethorphan, a nonprescription cough suppressant, according to the method already described (3, 5). All phenotyping analyses were performed blindly in the Department of Clinical Pharmacology, University Hospital of Geneva, Switzerland. The CYP2D6 activity was expressed as the MR of dextromethorphan to its metabolite dextromethorphan.

**Statistical Analysis.** The primary site of larynx tumor was coded according to the ICD-O (6). Because of possible differences in carcinogenic effects of tobacco and alcohol on larynx subsites, the analyses were performed for larynx cancer considered as a whole and separately for two subgroups; the first subgroup was composed of supraglottic cancers (ICD-O 161.1), and the second of glottic and subglottic cancers (ICD-O 161.0 and 161.2).

Statistical analysis was performed through unconditional logistic regression (7) to estimate the RRs and 95% CIs. All models were log-linear, fitted using the generalized linear interaction modeling statistical package (8).

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; Ref. 9). 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 g of pure ethanol (4.0, 9.4, 14.5, and 31.7 g, respectively, for 0.1 liter of beer, wine, cider, aperitif, and hard liquor; Ref. 10). The average daily consumption of alcohol was calculated by dividing the cumulative daily consumption of alcohol (the sum of the number of g of ethanol per day multiplied by the number of years that the quantity was drunk) by the overall duration of drinking.

Ex-smokers (or ex-drinkers) were defined as people having stopped smoking (or drinking) at least 1 year prior to the diagnosis. For them, the duration of smoking (or drinking) and the average daily consumption of tobacco (or alcohol) were calculated from the age at the beginning until the age at cessation. For the others, they were calculated from the age at beginning until the age at diagnosis.

To analyze the interaction between CYP2D6 and tobacco levels, CYP2D6 activity was studied both as a continuous variable (the logarithm of the MR) and as a categorical one in three levels according to the tertile of the control distribution (log MR ≥ −1.8; −2.5 ≤ log MR < −1.8; and log MR < −2.5). Because most of the previous studies analyzed CYP2D6 activity into two levels, reflecting the bimodal distribution of the MR in the general population (11), this conventional classification of phenotype "extensive" (MR ≤ 0.3) versus "poor" (MR > 0.3) metabolizers was also studied. The average daily consumption of tobacco was expressed as a continuous variable and as a categorical one (≤20, 21–30, >30).

The interaction between CYP2D6 activity and average daily consumption of tobacco was studied according to the method previously described (7). The potential linear variation of the effect of daily consumption of tobacco with increasing level of CYP2D6 activity was evaluated by a one-sided trend test. Similar analyses were performed using CYP2D6 phenotypes "extensive" versus "poor" metabolizers. However, the constraint of this classification implies very few poor metabolizers, and it was not possible to study the effect of CYP2D6 phenotype with more than two classes of tobacco consumption.

All models were adjusted for age, the main effects of the studied risk factors, and variables related to tobacco, alcohol, and concurrent medications administered during the last week before phenotyping test (sedatives; antihypertensive and diuretic drugs; cardiotonics; anti-infectious drugs; analgesics; vitamins and trace elements; other drugs).

**Results**

Because only five larynx cancer patients were enrolled among females, the analysis was restricted to males, i.e., 140 larynx cancer cases and 157 controls. Of the cases, 44% were classified as supraglottic cancers, 36% as glottic/subglottic cancers, and 21% as unspecified or unclassifiable cancers (i.e., tumor that overlaps the boundaries of two or more subcategories and whose point of origin cannot be determined or is not otherwise specified).

The mean age was similar among larynx cases (55.8 years, SD = 9.2) and controls (54.3 years, SD = 10.3). More than 80% of the study population were exclusive cigarette smokers, the rest being almost entirely cigarette smokers associated with another tobacco product. Cases had a higher average daily consumption of tobacco than controls (30.2 g/day, SD = 14.9 versus 26.4 g/day, SD = 13.2; P < 0.03) and a longer duration of smoking (35.5 years, SD = 9.0 versus 31.7 years, SD = 10.6; P < 0.001). The mean daily consumption of alcohol was slightly higher among larynx cases than among controls (102.3 g/day, SD = 78.5 versus 93.0 g/day, SD = 79.3), and more cases than controls reported a high daily consumption of ethanol defined as 80 g of ethanol/day or more (64% versus 44%; P < 0.01).

After adjusting on age and on all variables related to tobacco and alcohol as confounding factors, the estimates of the RRs of larynx cancer were not increased significantly for smokers with medium or high CYP2D6 activity compared to those having low CYP2D6 activity. Results were quite similar when considering separately supraglottic or glottic/subglottic cancers (Table 1).

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Table 1. Number of cases and controls and estimates of adjusted RRs* (95% CI) of larynx cancer according to level of CYP2D6 activity

<table>
<thead>
<tr>
<th>Level of CYP2D6 activity</th>
<th>Low (log MR ≥ −1.8)</th>
<th>Medium (−2.5 ≤ log MR &lt; −1.8)</th>
<th>High (log MR &lt; −2.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All larynx cancersa</td>
<td>45/51</td>
<td>37/50</td>
<td>58/53</td>
</tr>
<tr>
<td>Supraglottic cancersb</td>
<td>20/51</td>
<td>15/50</td>
<td>26/53</td>
</tr>
<tr>
<td>Glottic/subglottic cancersd</td>
<td>14/51</td>
<td>16/50</td>
<td>20/53</td>
</tr>
</tbody>
</table>

* Adjusted for age (<50, 50-54, 55-59, 60-64, and ≥65), smoking status (ex-smokers and current smokers); inhalation (no/yes); age at smoking initiation (>18, 17-18, and ≤16), duration of smoking (<25, 25-29, 30-34, 35-39, and ≥40 years), daily consumption of tobacco in g (≥20, 21–30, and >30), daily consumption of alcohol in grams; duration of alcohol drinking in years; drinking status (never drinkers, ex-drinkers, and current drinkers).

1 Adjusted for log MR; ICD-O. International Classification of Diseases for Oncology: RR, relative risk; 95% CI, 95% confidence interval; NNK, 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanone.
A nonsignificant interaction on the risk of larynx cancer was observed between CYP2D6 activity and average daily consumption of tobacco (P = 0.12). Similar results were found for supraglottic (P = 0.13) and glottic/subglottic cancers (P = 0.13). These findings were not modified when the continuous variables were used rather than the categorical variables in three levels in logistic regression. RR of larynx cancer were estimated according to the average daily consumption of tobacco within each level of CYP2D6 activity (Table 2). Larynx cancer risks were not increased significantly with daily consumption of tobacco among smokers with low or medium CYP2D6 activity. In contrast, among smokers with high CYP2D6 activity, there was a tendency for increased risks with increasing levels of smoking (trend test, P < 0.08). RR of larynx cancer were 1.4 for smokers of 21-30 g of tobacco/day and 2.1 for smokers of more than 30 g of tobacco/day compared with smokers of 20 g or less. Similarly, in the two subgroups of larynx cancer, the dose-response effect of daily consumption of tobacco was found only among smokers with high CYP2D6 activity: for smokers of more than 30 g of tobacco/day compared with smokers of 20 g or less, the RR of supraglottic cancers and of glottic and subglottic cancers were 3.3 (95% CI, 0.9-12.2) and 2.1 (95% CI, 0.5-9.1), respectively.

The frequency distribution of the log MR showed a typical bimodal distribution both among cases and controls, with values ranging from −0.4 to 0.63. Analyses under the classification of phenotype “extensive” (MR ≤ 0.3) versus “poor” (MR > 0.3) metabolizers provided a significant interaction on larynx cancer risk between average daily consumption of tobacco and CYP2D6 phenotype (P < 0.05). The adjusted risk of larynx cancer associated with “extensive” compared to “poor” phenotype was 0.4 (95% CI, 0.1–1.5) among smokers of 20 g of tobacco/day or less and increased to 2.7 (95% CI, 0.7–11.2) among smokers of more than 20 g.

No significant interaction was found between CYP2D6 activity and duration of smoking or other measures of tobacco exposure. Because the results were not affected by adjustment on medications administered before the phenotyping test, the RRs were presented without such adjustment.

### Table 2: Number of cases/controls and estimates of adjusted RR* (95% CI) of larynx cancer according to average daily consumption of tobacco by level of CYP2D6 activity

<table>
<thead>
<tr>
<th>Daily consumption of tobacco (g/day)</th>
<th>Level of CYP2D6 activity</th>
<th>Low log MR</th>
<th>Medium log MR</th>
<th>High log MR</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤20</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>20/17</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>21–30</td>
<td>0.3 (0.1–1.0)</td>
<td>0.6 (0.2–2.1)</td>
<td>1.4 (0.5–3.9)</td>
<td></td>
</tr>
<tr>
<td>7/16</td>
<td>7/10</td>
<td>15/13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;30</td>
<td>0.9 (0.3–2.3)</td>
<td>0.6 (0.2–1.7)</td>
<td>2.1 (0.8–6.0)</td>
<td></td>
</tr>
<tr>
<td>18/18</td>
<td>12/20</td>
<td>19/10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Trend test (P value)**
- NS*
- <0.08

*A Adjusted for age (<50, 50–54, 55–59, 60–64, and ≥65), smoking status (ex-smokers and current smokers): inhalation (no/yes), age at smoking initiation (>18, 17–18, and ≤16), duration of smoking (<25, 25–29, 30–34, 35–39, and ≥40 years), daily consumption of alcohol in g: duration of alcohol drinking in years: drinking status (never drinkers, ex-drinkers, and current drinkers).

**Discussion**

Tobacco represents the main causative factor for lung cancer and also considerably increases the risk for larynx cancer. These similarities in exogenous risk factors for lung and larynx cancers may then also extend to certain host factors. Crespi et al. (2) demonstrated that an important carcinogen in tobacco, NNK, is activated by CYP2D6 enzyme. In comparison to other P450 enzymes, the activity of P450 2D6 in the activation of NNK is rather low (12, 13). Thus, its importance in this activation is not known. If this cytochrome really affects smoking induced cancers by activating tobacco procarcinogens, then it should modify the effect of tobacco on the risk of lung as well as larynx cancer. The effect of smoking on those tobacco-related cancers should increase with CYP2D6 activity; the highest risk should be observed among individuals having the highest levels of both smoking and CYP2D6 activity. On the contrary, because low CYP2D6 oxidators have little or no capacity to activate this tobacco carcinogen in this metabolic way, the effect of tobacco should be minimized among these individuals. Regarding lung cancer, we reported previously a significant interaction between tobacco and CYP2D6 activity showing that increasing levels of daily tobacco consumption strongly modified the lung cancer risk only among smokers with high CYP2D6 activity and had no significant effect among smokers with low or medium activity. Similar patterns were found for larynx cancer, although the interaction between average daily consumption of tobacco and CYP2D6 activity was only of borderline significance. As found for lung cancer, a dose-response effect of tobacco on larynx cancer risk was found only among one-third of smokers with the highest level of CYP2D6 activity. Within both the low and the medium classes of CYP2D6 activity, no such dose-response was observed. These findings are therefore consistent with the assumption that CYP2D6 is involved in tobacco-related cancers through the activation of a carcinogen compound in tobacco smoke.

Recently, CYP2D6 was also suggested to be involved in nicotine metabolism (14). Extensive metabolizers could require a greater consumption of tobacco than poor metabolizers to maintain nicotine plasma concentrations that satisfy individual craving. These findings could possibly explain a crude increased risk of cancer among CYP2D6 extensive metabolizers. However, these findings cannot explain the interactive effect between smoking and CYP2D6 activity on cancer risk, i.e., that increasing levels of smoking increased the risk of cancer only among individuals with the highest CYP2D6 activity or that CYP2D6 activity is a risk factor only among the heaviest smokers.

The interaction between average daily consumption of tobacco and CYP2D6 activity was weaker for larynx cancer than for lung cancer. CYP2D6 is likely not the only cytochrome involved in the activation of tobacco carcinogens, and its effect could be less important for larynx than for lung cancer. Tobacco compounds are known to form DNA adducts after undergoing metabolic activation, mediated by enzymes P450 1A1, 2C, and 3A4 for polycyclic aromatic hydrocarbons, P450 1A2 for aromatic amines, and several forms of P450 enzymes for nitrosamines (12, 13, 15). Recently, the total adducts in human larynx tissues from smokers were found to correlate strongly with levels of P450 2C, 1A1, and 3A4 in larynx microsomes, but not with P450 2E1 or 2A6, suggesting that polycyclic aromatic hydrocarbons could be the main tobacco carcinogen class for larynx tissue (16).

Alcohol was also implicated strongly as a causative factor in larynx carcinogenesis (4). Other genetically determined en-
zyme activities could play an important role on the occurrence of larynx cancer, e.g., the CYP2E1 polymorphism, involved in the oxidation of ethanol (17).

The similarities in the results for lung and larynx cancers are probably not explained by the use of the same control group, because no association was found within the control group between CYP2D6 activity and either the main medical diagnoses, hospitals, or interviewers. It is therefore unlikely that our results reflect a selection bias in this group.

The phenotyping procedure has some limitation, mainly because of the confounding effects of coadministration of drugs, which then could affect the MR observed for the probe drug. However, all patients who received, or had received during the preceding week, any medication known to interfere with the metabolism of dextromethorphan were carefully excluded from the study. Data on the use of other medications were also recorded and adjusted for. None of these adjustments modified the results presented. Our results should nevertheless be confirmed in additional studies or through direct genotype assessment. In most of the previous studies, the sympathicolytic antihypertensive drug debrisoquine has been administered as a prototypical substrate for determining the CYP2D6 polymorphic activity. We chose to use dextromethorphan, a widely used nonprescription cough suppressant, which has now proved to be the most appropriate probe drug because of its innocuousness and its high specificity for the CYP2D6, thus reducing misclassification in the oxidative level assignment (18, 19).

Only one previous study has investigated the relation between CYP2D6 activity and larynx cancer and reported a 2-fold increased risk associated with the phenotype “extensive” compared to “poor” metabolizers (20). These results are difficult to interpret, because they did not take into account the level of tobacco. In our study, the adjusted RR of larynx cancer was not increased significantly among smokers with the CYP2D6 “extensive” phenotype compared to those with the “poor” phenotype (RR = 1.1; 95% CI, 0.4–2.8). However, the effect of the CYP2D6 “extensive” phenotype differed significantly (P < 0.05) according to the average daily consumption of tobacco. The CYP2D6 “extensive” phenotype was a risk factor for larynx cancer only at a high level of tobacco exposure: among smokers of more than 20 g of tobacco/day, a 2.7-fold increased risk was observed for “extensive” versus “poor” metabolizers.

Our results, in addition to those reported previously on lung cancer, are consistent with the hypothesis that the effect of tobacco smoking could be modified by CYP2D6 activity, and vice versa. Smokers having the highest levels of both tobacco consumption and CYP2D6 activity seem at high risk for both lung and larynx cancers.

Acknowledgments

References
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