**GSTM1, GSTT1, GSTP1, CYP1A1, and NAT1 Polymorphisms, Tobacco Use, and the Risk of Head and Neck Cancer**

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**Abstract**

Squamous cell carcinoma of the head and neck (SCCHN), including the oral cavity, pharynx, and larynx, provides an ideal tumor model to investigate gene-environment interaction. We conducted a hospital-based case-control study including 182 cases with newly diagnosed SCCHN and 202 controls with nonneoplastic conditions of the head and neck that required surgery. Lifetime tobacco use and risk of SCCHN were evaluated in relation to the polymorphisms of GSTM1, GSTT1, GSTP1, CYP1A1, and NAT1. The main effects of genotype were associated with a slightly increased risk of SCCHN for GSTP1 [age-, race-, and sex-adjusted odds ratio (OR), 1.2; confidence interval (CI), 0.8–1.9], GSTT1 (OR, 1.2; CI, 0.7–2.3), and NAT1 (OR, 1.1; CI, 0.7–1.7). The joint effects of genotype combinations showed some excess risk for the combination of the GSTM1 null genotype and the CYP1A1 Ile/Val polymorphism (OR, 2.6; CI, 0.7–10.3). The analysis of the joint effects (interaction) of the “at-risk” genotypes and tobacco use did not reveal any interaction on either the multiplicative or additive scale for GSTM1, GSTP1, or CYP1A1. However, there was a suggestion of an interaction on the additive scale between the pack-years of tobacco use and the GSTT1 null genotype. The combined heterozygote and homozygote NAT1*10 genotypes also had a suggestive interaction with tobacco smoking history. The results of this study suggest a possible gene-environment interaction for certain carcinogen metabolizing enzymes, but larger studies that fully evaluate the interaction are needed.

**Introduction**

The risk of SCCHN, including cancer of the oral cavity, pharynx, and larynx, is strongly associated with tobacco and alcohol consumption. SCCHN thus may provide an ideal tumor system to evaluate gene-environment interaction. Metabolic enzymes that are potentially involved in either the activation (Phase I) or detoxication (Phase II) of chemical carcinogens in tobacco smoke have received a great deal of attention recently as possible genetic susceptibility factors for a variety of cancers (1, 2). Polymorphisms in the genes that code for these enzymes may alter expression or function, thus increasing or decreasing the activation or detoxication of carcinogenic compounds. Tobacco smoke is a complex mixture of carcinogenic compounds, and it contains numerous substrates for these enzymes, such as benzo(a)pyrene, ethylene oxide, and 4-aminobiphenyl. The polymorphisms in combination with environmental exposure have been hypothesized to confer a differential risk of cancer for individuals carrying these genetic variants.

Despite the strong biological plausibility for the role of metabolizing enzymes in the etiology of SCCHN, there have been a relatively small number of epidemiological studies that have evaluated these polymorphisms. The published studies have not provided evidence for a clear pattern of association with several enzyme polymorphisms, although few have directly evaluated the potential interaction between tobacco exposure and the presence of an at-risk genotype. We report the results of a case-control study of SCCHN, including the assessment of GSTM1, GSTT1, GSTP1, CYP1A1, and NAT1 genotypes.

**Materials and Methods**

This was a hospital-based case-control study conducted at the University of North Carolina Hospitals from April 1994 to June 1997. Cases comprised patients newly diagnosed with a first SCCHN. Cases were considered eligible for the study if they were >17 years of age, had pathologically confirmed squamous cell carcinoma of the oral cavity, pharynx, or larynx, or did not have a history of a previous malignant cancer or a diagnosis of a genetic disease or syndrome. Patients with nasopharyngeal cancer were not included. In addition, cases were eligible if they spoke either English or Spanish. A total of 207 eligible cases were identified, 182 (88%) were successfully interviewed, and 25 (12%) refused participation. The primary tumor site was distributed among cases as: 93 oral cavity, 37 pharynx, and 52 larynx.

Control subjects included patients seen in the same clinic with conditions requiring surgery. These major conditions included chronic sinusitis, nasal obstruction, and obstructive sleep apnea. Eligibility criteria for controls were the same as for cases. In addition, patients with the aspirin triad (nasal polypsis, asthma, and aspirin sensitivity) were also excluded. Controls were frequency-matched with cases on age (20–44, 45–49, 50–54, 55–59, 60–64, 65–69, 70 + years) and gender. A total of 236 eligible controls were identified, and 202 participated (86%) in the study.

An in-person interview was conducted with each subject in...
the hospital clinic by a trained interviewer. The interview consisted of questions related to lifetime tobacco and alcohol consumption (not including the year before diagnosis), occupation, medical history, family history of cancer, demographics, and diet. In addition, a blood sample and buccal swab sample of exfoliated oral cells were obtained with informed consent at that time. Smokers were defined as those subjects that reported that they had smoked a total of ≥100 cigarettes over their lifetime. In addition, subjects were asked about the use of cigars, pipes, chewing tobacco, and snuff. Cigarette smokers were asked the number of cigarettes smoked per day, on average, the age they began smoking and the number of years of smoking, and whether the cigarettes were filter or nonfilter. In the analysis, tobacco use was represented in several forms, including average number of cigarettes smoked per day, years of smoking, and pack-years. These variables were also separately constructed for filter and nonfilter cigarettes. Subjects using only other tobacco products were included in the analysis of tobacco use (ever/never) but not in the analyses of intensity or duration of cigarette use. A lifetime history of alcohol consumption (beer, wine, and liquor) was obtained, and variables corresponding to average weekly use, years of use, and drink-years were derived. Variable cutpoints were selected using the distribution among controls and the published literature, and they were confirmed with nonparametric regression (3).

Blood samples or buccal swabs were not obtained from a total of 13 subjects (6, 3.3% of cases and 7, 3.5% of controls). Blood was collected in one yellow-top 8.5-mL vacutainer tube. Plasma, buffy coat, and red cells were separated and stored at −70°C within 24 h of collection. The buffy coat was thawed, and DNA was extracted using the ABI Nucleic Acid Purification System (Applied Biosystems, Foster City, CA). DNA samples were evaluated for quantity by spectrophotometry and quality by a 1% agarose gel run. Samples were stored at 4°C until genotyping. Genotyping of samples was carried out using 50 ng of genomic DNA per assay and published PCR-based methods. Genotyping was performed primarily using DNA from blood samples, and when these were unavailable, buccal cell samples were used. DNA was extracted from buccal swab samples using the Qiagen method (Qiagen, Inc., Chatsworth, CA). When there was difficulty in determining the genotype for a particular sample, both blood and buccal cell samples were used. GSTM1 and GSTT1 genotypes were determined using the multiplex PCR method of Chen et al. (4). This technique does not distinguish between heterozygote and homozygote GSTM1- or GSTT1-positive genotypes, but it conclusively identifies null genotypes. The GSTP1 (Ile105Val) genotype was determined using the PCR-RFLP method of Watson et al. (5). NAT1 genotypes (NAT1*4, NAT1*10, NAT1*11) were determined using the PCR-based methods of Bell et al. (6). CYP1A1 (Ile/Val; CYP1A1*2R) polymorphism was detected using PCR primers (1A1F, GGCTGCTGCCTTCTCATC; 1A1R, AAA-GACCTCAGCCGGGTAA), standard PCR conditions, and annealing at 53°C. The reverse primer contained a mismatched base (italic) that formed a partial MaelIII (Bering Mannheim, Indianapolis IN) restriction site in the presence of the G nucleotide at position 4889 that characterizes the CYP1A1 valine allele. Digestion of the PCR product with MaelIII produced genotype-specific band patterns on agarose gels. GST and NAT genotypes could not be determined for two cases (one for GSTs and one for NAT1) and zero controls. The CYP1A1 genotype could not be determined for 7 cases and 11 controls.

Multivariate logistic regression was used to obtain the OR estimate and 95% CI for the main effects of tobacco and each enzyme genotype (7). An adjustment was made for the potential confounding effects of age, sex, race (black, white), and alcohol consumption (average number of drinks per week). The joint effects (interaction) of tobacco and each of the genes were evaluated on the additive and multiplicative scales. Dummy variables were created, each representing the combination of a tobacco consumption category and genotype, with nonsmokers having the wild-type genotype as the referent category. Adjusted ORs generated in this manner were evaluated for deviation from the expected null value on the additive or multiplicative scale. To further quantify departures from additivity, the ICR (also previously called the relative excess risk for interaction) and 95% CI were estimated (8, 9). In addition, interaction terms including genotype and smoking duration or amount, ungrouped, were evaluated for statistical significance with logistic regression.

Results

Cases were more likely than controls to be male (76% versus 56%) and black (38% versus 14%). The mean age of cases was 59.5 years and 56.8 years for controls (Table 1). More cases were less than age 45 years at diagnosis (13% versus 56%) and black (38% versus 31%). Cases were more likely than controls to be male (76% versus 56%), although the distribution of subjects ≥59 years was similar (46% of cases; 47% of controls). Consumption of ≥40 drinks/week produced a nearly 6-fold increase in risk (OR, 5.9; CI, 2.0–17.7). Table 2 presents the main effects of tobacco use, adjusted for age, race, sex, and alcohol use. A total of 19 cases and 20 controls failed to report their history of smoking with respect to amount or duration. Also, 25 subjects (13 cases and 15 controls) used tobacco products other than cigarettes and were counted as missing for the analysis of the amount and duration of cigarette use. As expected, persons with a history of tobacco use were at an increased risk of SCCHN (adjusted OR, 4.1; 95% CI, 2.0–8.7). All of the cigarette use variables showed an indication of a dose-response gradient, with a 7-fold increased risk for ≥40 total years of cigarette use.

| Table 1 Distribution of demographic factors and alcohol use among cases and controls |
|-----------------|-----------------|-----------------|
| Factor          | No. of cases    | %               |
| Gender          |                 |                 |
| Male            | 139             | 76              |
| Female          | 43              | 24              |
| Race            |                 |                 |
| White           | 112             | 62              |
| Nonwhite        | 70              | 38              |
| Age (yrs)       |                 |                 |
| 20–44           | 23              | 13              |
| 45–49           | 18              | 10              |
| 50–54           | 21              | 12              |
| 55–59           | 28              | 15              |
| 60–64           | 21              | 12              |
| 65–69           | 26              | 14              |
| 70+             | 76              | 20              |
| Total years of alcohol use | 32 | 18 | 94 | 47 |
| 0–9             | 58              | 33              |
| 10–29           | 86              | 49              |
| Average drinks per wk | 32 | 19 | 94 | 47 |
| 1–19            | 51              | 31              |
| 20–39           | 46              | 28              |
| 40+             | 38              | 23              |

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in risk according to the site of the tumor, although the effect of the GSTM1 null genotype was stronger among cases with laryngeal cancer (OR, 1.9; CI, 0.9–3.8).

Table 4 presents the results of the analysis of the joint effects or interaction between tobacco use and GSTT1. The ORs are for each combination of tobacco use and genotype, they are relative to those nonusers without the null deleted genotype, and they are adjusted for age, sex, race, and tobacco use. When analyzed simply as tobacco users compared with nonusers, there was no indication of any increased risk among users with the null genotype (OR, 5.1 versus 4.9 among users with the GSTT1 gene present). However, analysis of pack-years of use showed some suggestion of an interaction. Among those individuals with ≥40 years of smoking and the null genotype, the OR was 13.4 (CI, 3.6–50.4) versus 5.4 (CI, 2.0–14.2) among those with the gene present. The OR of 13 can be compared with that expected multiplicative OR of 14 and the additive OR of 7.1. The ICR that measures the extent of the departure from the additive null is 9.7 (a value of 0 indicates no excess; CI, −11.4 to 30.9). However, caution should be used in interpreting these results given the imprecision of the estimates. The interaction term including GSTT1 and pack-years (not categorized) was not statistically significant (P = 0.89).

Some excess risk for most of the highest categories of smoking among those with the GSTM1 null genotype can be seen in Table 5, although the differences are very small. No interaction can be seen for GSTP1 at-risk genotypes (Table 6). Neither GSTP1 nor GSTM1 showed an interaction with smoking on the additive scale. The interaction (multiplicative) between GSTM1 or GSTP1 and either duration or amount of smoking (not categorized) was not statistically significant. The combined NAT1*10 heterozygote and homozygote genotypes (NAT1*10/NAT1*4 and NAT1*10/NAT1*10) had a suggestive interaction with some measures of tobacco use (Table 7). The NAT1 at-risk genotype was associated with a nearly 5-fold increased risk among smokers with a history of ≥40 pack-years (ICR, 3.3; CI, −1.44 to 8.1). The strongest joint effect was with total years of cigarette use (data not shown). The observed OR of 11.8 (CI = 2.8–49.4) for ≥40 years of smoking among those with the NAT1 at-risk genotype (OR, 3.3; CI, 1.0–10.2 for those with *4 or *11 alleles) is greater than the expected multiplicative OR of 2.6 and expected additive OR of 1.1. The ICR is 9.2 (CI, −5.9 to 24.4). Additionally, a statistically significant interaction was found between NAT1 and the continuous smoking variables, amount (P = 0.03), and duration (P = 0.002).

There were no subjects with homozygous genotypes for the CYP1A1*2A polymorphism (Val/Val genotype). A total of 13 cases and 12 controls were heterozygotes (Ile/Val). There was no pattern of increased risk of SCCHN among smokers with CYP1A1 heterozygote genotypes relative to non-smokers and those with homozygous normal genotypes (Table 8). OR 4.3 (CI, 1.0–17.9) for the *2A allele was observed, this is less than the expected OR of 4.6 and suggests a modest protective effect. Logistic regression analysis controlling for age, sex, race, and total pack-years of smoking found no significant interaction with any smoking measures (Table 8).

We also examined the risk of SCCHN associated with multiple at-risk genotypes. In general, no elevated ORs were observed for individuals with both at-risk genotypes for two-way combinations of GSTM1, GSTT1, GSTP1, and CYP1A1. An increased, but imprecise point estimate was observed for individuals with both the GSTM1 null genotype and the CYP1A1 Ile/Val polymorphism (OR, 1.9; CI, 0.5–6.9). Analysis of the total number of at-risk genotypes an individual had did not show any meaningful case-control difference.

Discussion
This study confirmed numerous other findings indicating that tobacco use is a strong risk factor in SCCHN. However, we did not find evidence for a strong interaction between tobacco use,

Table 2: Distribution of tobacco use among cases and controls.

<table>
<thead>
<tr>
<th>Tobacco use</th>
<th>No. of cases</th>
<th>%</th>
<th>No. of controls</th>
<th>%</th>
<th>OR*</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never</td>
<td>11</td>
<td>6</td>
<td>74</td>
<td>37</td>
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<td></td>
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<tr>
<td>Ever</td>
<td>171</td>
<td>94</td>
<td>128</td>
<td>63</td>
<td>4.1</td>
<td>2.0–8.7</td>
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<tr>
<td>Average cigarettes per day</td>
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<tr>
<td>0</td>
<td>11</td>
<td>6</td>
<td>74</td>
<td>37</td>
<td></td>
<td></td>
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<td>1–19</td>
<td>46</td>
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<td>44</td>
<td>24</td>
<td>3.3</td>
<td>1.5–7.7</td>
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<td>20+</td>
<td>112</td>
<td>66</td>
<td>69</td>
<td>37</td>
<td>4.6</td>
<td>2.1–10.3</td>
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<td></td>
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<td></td>
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<tr>
<td>0</td>
<td>11</td>
<td>6</td>
<td>74</td>
<td>39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–19</td>
<td>19</td>
<td>11</td>
<td>38</td>
<td>20</td>
<td>2.3</td>
<td>0.9–5.9</td>
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<tr>
<td>20–39</td>
<td>75</td>
<td>44</td>
<td>52</td>
<td>28</td>
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<td>38</td>
<td>23</td>
<td>12</td>
<td>7.2</td>
<td>2.8–18.6</td>
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<td>Pack-years of tobacco use</td>
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</tr>
<tr>
<td>0</td>
<td>11</td>
<td>6</td>
<td>74</td>
<td>39</td>
<td></td>
<td></td>
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<tr>
<td>1–19</td>
<td>36</td>
<td>21</td>
<td>50</td>
<td>26</td>
<td>2.7</td>
<td>1.2–6.2</td>
</tr>
<tr>
<td>20–39</td>
<td>40</td>
<td>24</td>
<td>28</td>
<td>15</td>
<td>4.4</td>
<td>1.8–10.7</td>
</tr>
<tr>
<td>40+</td>
<td>83</td>
<td>49</td>
<td>37</td>
<td>20</td>
<td>5.6</td>
<td>2.4–13.0</td>
</tr>
</tbody>
</table>

* Adjusted for age, race, sex, and average number of drinks of alcohol per week.
metabolizing enzyme polymorphisms, and the risk of SCCHN. There was no clear pattern of increasing risk in the presence of smoking for putative at-risk genotypes of *GSTM1*, *GSTP1*, and *CYP1A1*. Nevertheless, there was a suggestion of some excess risk due to the interaction between smoking and the *GSTT1* null genotype. In addition, a possible interaction with the *NAT1* *10* genotypes was noted. The interaction appeared to be most prominent on the additive scale. The analysis of these interactions was limited by the relatively small number of subjects. The analysis of the joint effects of *GSTM1* and *CYP1A1* genotypes together showed an indication, albeit imprecise, of an increased risk. An increased risk of lung cancer for the combination of *GSTM1* and *CYP1A1* (the MspI allele) has been previously reported in a Japanese study (12). However, a recent oral cancer study did not report an interaction between *GSTM1* and *CYP1A1* (Val/Val; Ref. 13).

There have been 14 published studies that have examined carcinogen metabolizing enzyme genotypes and the risk of SCCHN (13–26). The results have been inconsistent.

Five of 13 studies have found an increased risk (ORs, 1.5–3.9) for the *GSTM1* null genotype (14, 18, 19, 20, 26); five of nine studies reported associations with the *GSTT1* null genotype (ORs, 1.2–2.3; Refs. 14, 17, 20, 24, and 26); and two of three studies evaluated the *GSTP1* genotype and reported an increased risk (ORs, 1.6 –2.0) for the *GSTP1* Ile/Val polymorphism (21, 24). Two studies investigated a polymorphism of the *GSTM3* gene (*GSTM3*B); a weak association was reported for oropharyngeal cancer (OR, 1.3) and a 2-fold elevated OR for cancer of the larynx (24-25). Two studies examined the *CYP1A1* *Ile*/*Ile* polymorphism. Park et al. (13) found an association (OR, 2.6; CI, 1.2–5.7), and the data of Ophuis et al. (22) showed only a weakly increased risk (OR, 1.1; CI, 0.7–1.9). Some studies also evaluated the interaction among multiple genotypes. Hung et al. (17) noted a higher risk of oral cancer among those subjects with the *GSTM1* and/or *GSTT1* null genotypes. Other studies did not report a significant genotype interaction (15, 21, 22).

**Evaluation of only the main effect of the enzyme genotype**

### Table 3  Genotype distribution by case status and race

| Genotype | Whites | | Blacks | | Total  |  
|-----------|--------|-----------------|--------|--------|--------|--------|        |
|           | Genotype | n cases (%) | n controls (%) | OR (CI)  | n cases (%) | n controls (%) | OR (CI)  |
| **GSTM1** | Null | 55 (50) | 76 (45) | 1.2 (0.8–2.0) | 20 (32) | 10 (40) | 0.7 (0.3–1.8) | 1.1 (0.7–1.7) |
|           | Present | 54 (50) | 92 (55) | 1.4 (0.7–2.7) | 14 (22) | 5 (20) | 1.1 (0.4–3.6) | 1.2 (0.7–2.3) |
| **GSTT1** | Null | 18 (17) | 21 (13) | 0.6 (0.2–1.5) | 7 (11) | 5 (20) | 0.9 (0.2–4.7) | 0.6 (0.3–1.4) |
|           | Present | 91 (83) | 147 (88) | 1.3 (0.8–2.2) | 38 (60) | 13 (52) | 1.1 (0.7–1.9) | 1.4 (0.9–2.2) |
| **GSTP1** | Val/Val | 7 (6) | 20 (12) | 1.1 (0.7–1.9) | 7 (11) | 5 (20) | 0.9 (0.2–4.7) | 0.6 (0.3–1.4) |
|           | Ile/Val | 62 (57) | 80 (48) | 1.6 (0.6–3.8) | 38 (60) | 13 (52) | 1.1 (0.7–1.9) | 1.4 (0.9–2.2) |
| **NAT1** | 10/10 | 4 (4) | 13 (8) | 0.6 (0.2–1.9) | 6 (10) | 3 (12) | 0.9 (0.2–5.7) | 0.6 (0.2–1.5) |
|           | 10/4 or 11 | 41 (38) | 56 (33) | 1.1 (0.7–1.9) | 37 (60) | 13 (52) | 2.1 (0.6–7.2) | 1.2 (0.7–1.9) |
|           | 4 or 11  | 64 (59) | 99 (59) | 1.6 (0.6–3.8) | 19 (31) | 9 (36) | 2.1 (0.6–7.2) | 1.2 (0.7–1.9) |
| **CYP1A1** | Ile/Val | 11 (10) | 11 (7) | 1.5 (0.7–2.9) | 2 (3) | 0 (0) | 1.5 (0.6–3.6) | 1.5 (0.6–3.6) |
|           | Ile/Ile  | 97 (90) | 154 (93) | 2.1 (1.1–4.1) | 61 (98) | 24 (100) | 1.5 (0.6–3.6) | 1.5 (0.6–3.6) |

**OR adjusted for age and sex.**

† Referent category.

* Entire sample. OR adjusted for age, sex, and race.

* Could not be estimated.
may mask an underlying interaction with smoking. Given the primary hypothesis of the polymorphisms modifying the risk of the predominant risk factor, smoking, an analysis of interactions is necessary to fully evaluate the role of the genes. Further, examination of the interaction on both the multiplicative and additive scales is important. Unfortunately, only five studies have reported any information on the relationship between genotype, smoking, and the risk of SCCHN. This makes a direct comparison with our study results difficult. Kihara et al. (18) noted an increased risk of the GSTM1 null genotype among

### Table 5  Joint effects of tobacco and GSTM1 polymorphism

<table>
<thead>
<tr>
<th>GSTM1 (present)</th>
<th>GSTM1 (null)</th>
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</thead>
<tbody>
<tr>
<td>No. of cases</td>
<td>No. of cases</td>
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<tr>
<td>Tobacco use</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>6</td>
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<td>Ever</td>
<td>94</td>
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<td>Average cigarettes per day</td>
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<td>1–19</td>
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<tr>
<td>1–19</td>
<td>21</td>
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<tr>
<td>20–39</td>
<td>25</td>
</tr>
<tr>
<td>40+</td>
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<sup>a</sup> OR adjusted for age, race, sex, and average number of drinks of alcohol per week.

### Table 6 Joint effects of tobacco and GSTP1 polymorphism

<table>
<thead>
<tr>
<th>GSTP1 (Ile/Ile)</th>
<th>GSTP1 (Ile/Val or Val/Val)</th>
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<tbody>
<tr>
<td>No. of cases</td>
<td>No. of controls</td>
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<td>Ever</td>
<td>59</td>
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<td>Average cigarettes per day</td>
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<td>1–19</td>
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<td>20–39</td>
<td>17</td>
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<td>40+</td>
<td>24</td>
</tr>
</tbody>
</table>

<sup>a</sup> OR adjusted for age, race, sex, and average number of drinks of alcohol per week.

### Table 7 Joint effects of tobacco and NAT1 polymorphism

<table>
<thead>
<tr>
<th>NAT1 (4 OR 11/4 OR 11)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>NAT1 (10/4 or 10/10)&lt;sup&gt;b&lt;/sup&gt;</th>
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<td>No. of controls</td>
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<td>Ever</td>
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</tr>
<tr>
<td>20+</td>
<td>54</td>
</tr>
<tr>
<td>Pack-years of tobacco use</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>1–19</td>
<td>18</td>
</tr>
<tr>
<td>20–39</td>
<td>18</td>
</tr>
<tr>
<td>40+</td>
<td>37</td>
</tr>
</tbody>
</table>

<sup>a</sup> OR adjusted for age, race, sex, and average number of drinks of alcohol per week.
<sup>b</sup> Could not be estimated.
smokers compared with nonsmokers. Park et al. (13) did not find a relationship between pack-years of smoking and risk of SCCHN among cases with the CYP1A1 polymorphism or the GSTM1 null genotype. Jourenkova et al. (20) reported finding an increased risk of laryngeal cancer with the GSTM1 null genotype and with a lower amount of smoking and a higher risk with the GSTT1 null genotype and a longer duration of smoking. The same research group reported significant interactions (multiplicative) between smoking (≥31 years of smoking) and the GSTT1 and GSTP1 polymorphisms (14) and no interaction between smoking (amount per week) and GSTM3, GSTP1, and risk of laryngeal cancer (25).

There are several possible explanations for the variation among study results and our failure to find a strong interaction between the enzyme genotypes and smoking. First, as previously noted, despite being among the largest, our study did not have a sufficient number of subjects to allow for the precise estimation of the genotype-smoking interaction. This is especially important because it is likely that these genes do not act isolation, and evaluation of multiple genes interacting with exposure may be required to understand the phenomenon. Second, few studies directly examined the interaction with tobacco; none evaluated departures on the additive scale. Third, the composition of the control groups used in previous studies were variable; some included out-patients from the source hospital for the cases and others used friends or spouses. Only one study appeared to have used population-based controls. Our study identified controls from the same clinic as the cases and included patients with conditions requiring surgery. In the present study, it is possible that some of the diagnoses prevalent among the control population (such as chronic sinusitis) are related to smoking and that these conditions are mediated by the metabolizing enzymes resulting in an underestimation of risk. The main effect of tobacco use in our study, although imprecise, is greater than that found in a large population-based case-control study of oral and pharyngeal cancer conducted in four areas of the United States (27). Additionally, the frequencies of the various enzyme genotypes among control subjects are very similar to those reported for other North Carolina studies (4, 5, 10, 11), and this suggests that it is unlikely that genotype influences the conditions found among clinic controls.

It is possible that interindividual variability in the expression level of metabolizing enzymes in head and neck tissues (that is independent of genotype) could confound this type of case-control analyses. Activity levels of GSTπ and GSTα (not analyzed) vary dramatically between individuals in both normal and cancerous tissues and among tissues in the head and neck (28). Both CYP1A1 and NAT1 activity have been reported to vary in human larynx tissues, and the expression of both enzymes vary widely among people in other tissues (29–33). Variation in CYP1A1 activity has been associated with cigarette smoking exposure (29–30). It has been hypothesized that CYP1A1 and NAT1 polymorphisms detected in this study may be related to differences in expression, but at this time, CYP1A1 and NAT1 genotype/phenotype relationships are still poorly understood. Thus, risks associated with specific genotypes analyzed in this study may be obscured by other factors influencing the expression of carcinogen metabolizing enzymes.

The distribution and disposition of cigarette smoke carcinogens in the oral cavity have not been well studied, and the specific pathways for the activation and detoxication of carcinogens in head and neck tissues have not been characterized.

Genotype analysis may provide an insight into the role of specific carcinogen pathways. The findings of this study related to smoking and GSTT1 and NAT1 and the interaction of CYP1A1 and GSTM1 null genotypes provide some suggestive leads. However, owing to the imprecision of the results from this and previous studies, future efforts should be sufficiently large to allow a more definitive assessment of gene-environment interactions.

Acknowledgments
We thank Cathy Van Doren, Rosemary McAuig, Stacy Geisler, Christopher Lynn, and the staff at Battelle/Centers for Public Health Research and Evaluation for assistance with data collection and Joanna Smith for programming help. In addition, the support of the nurses and surgeons of the Division of Otolaryngology and Head and Neck Surgery is greatly appreciated.

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