CYPIA1 and GSTM1 Polymorphisms and Oral Cancer Risk

Jong Y. Park, Joshua E. Muscat, Qing Ren, Stimson P. Schantz, Robert D. Harwick, Jordan C. Stern, Virginia Pike, John P. Richie, Jr., and Philip Lazarus

Abstract
The importance of both the CYPIA1 exon 7 (ile:val) and GSTM1 (0/0) polymorphisms in oral cancer susceptibility was assessed by examining polymorphic prevalences in 135 patients with oral cancer and 135 noncancer controls frequency-matched by age at diagnosis (≥5 years), race, sex, and institute of patient recruitment. The prevalence of the GSTM1 (0/0) genotype was approximately 51% in both cases and controls. The prevalence of the CYPIA1 (ile:val) polymorphism (including both the (ile:val) and (val:val) genotypes) was significantly higher in cases as compared to controls (17.6% versus 7.6%, respectively; crude odds ratio, 2.6; confidence interval, 1.2-5.7). No association was observed between polymorphic prevalence and levels of smoking or alcohol consumption in cases. These results suggest that the GSTM1 null genotype is not associated with oral cancer risk. These results also suggest that individuals with the CYPIA1 exon 7 (ile:val) polymorphism are at increased risk for oral cancer, and that this risk may not be influenced by differences in exposure to tobacco smoke.

Introduction
Differences in individual susceptibility to cancer may result from activity-dependent genetic differences in XMEs. Both the GSTM1 and CYPIA1 genes, which code for enzymes involved in the detoxification or activation of the strong tobacco carcinogen B[a]P (8), exhibit similar metabolic activities toward the major tobacco carcinogen, B[a]P (8). Nevertheless, the CYPIA1 and GSTM1 polymorphisms have been associated with an increased risk for tobacco-related diseases such as lung cancer.

Oral cancer is a tobacco-related disease that represents a significant problem based upon its high incidence in many parts of the world, the poor survival rates associated with this type of malignancy, and the severe functional and cosmetic defects accompanying the treatment of this disease. Few studies on reliable genetic markers for individual susceptibility to oral cancer have as yet been reported. Two preliminary studies examining GSTM1 (0/0) genotype as a determinant for oral cancer risk have been described, but these studies have produced conflicting results concerning the importance of this genotype in oral cancer susceptibility (14, 15). A link between susceptibility to oral cancer and altered expression of XMEs is, however, supported by the relatively low incidence of oral and laryngeal cancer patients exhibiting detectable levels of GST μ protein (16).

In the present case-control study of 135 patients with squamous cell carcinoma of the oral cavity or larynx and 135 matched noncancer controls, we explored potential relationships between XME genotype and oral cancer risk. We have examined: (a) the importance of both the GSTM1 (0/0) and the CYPIA1 exon 7 (ile:val) polymorphisms as markers for oral cancer risk; and (b) the association between XME genotype prevalence and exposure to risk factors important in oral cancer induction.

Materials and Methods
Study Populations. All cases were Caucasian patients diagnosed with primary squamous cell carcinoma of the oral cavity and were recruited during 1994 to 1996 from three institutions: Temple University Hospital (Philadelphia, PA); MSKCC (New York, NY); and NYEEI (New York, NY). The number of cases recruited into the study from each of the participating institutes was as follows: Temple University Hospital, 49; MSKCC, 66; NYEEI, 20 (see Table 1). Incident cases, defined as subjects diagnosed within 1 year prior to recruitment into the study, comprised...
Table 1

<table>
<thead>
<tr>
<th>Distribution of oral cancer patients and frequency-matched controls according to demographic characteristics</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td><strong>n</strong></td>
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<td>-------</td>
</tr>
<tr>
<td>Oral cancer patients</td>
</tr>
<tr>
<td>Temple</td>
</tr>
<tr>
<td>Controls</td>
</tr>
<tr>
<td>Noncancer patients</td>
</tr>
</tbody>
</table>

*a* Includes smokers of cigarettes, cigars, and pipes.

*b* Significantly different than controls (P < 0.0001) by two-sample t test (2-tailed).

approximately 61% (n = 82) of all oral cancer patients. Greater than 90% of incident cases (n = 75) were interviewed as an in-patient in one of the participating hospitals. Greater than 95% of eligible incident cases were enrolled over the course of the study period. The average time (mean ± SE) between diagnosis and recruitment for prevalent cases was 8.94 ± 1.00 years. Oral cancer was defined as squamous cell carcinoma of the oral cavity (n = 112) including gingiva (including hard palate and dorsal tongue), floor of the mouth, inner lip, soft palate, buccal mucosa, ventrolateral tongue, tonsil, oropharynx, and larynx (n = 23). For all cases, confirmation of tumor type was performed by the Department of Pathology at Temple University, the Department of Pathology at MSKCC, or the Department of Pathology at NYEE1.

Controls were defined as individuals without any prior diagnosis of cancer or present respiratory ailment. “Healthy” controls (63%; n = 85) consisted of friends, spouses, and spousal family members of cancer patients and subjects receiving non-disease-related dental treatment. Other controls recruited for this study included out-patients treated for non-cancer-related hearing or vision problems or non-respiratory allergic reactions at the Ear, Nose, and Throat Clinics of the participating institutions (12.6%; n = 17), as well as hospital in-patients (24.4%; n = 33) treated for trauma-related injuries. Greater than 95% of eligible controls consented to participate in the study. Controls were frequency-matched by age at diagnosis (± 5 years), sex, and race of the cases, as well as institution of case recruitment.

We collected demographic information as well as information on tobacco use and alcohol consumption for all cases and controls by administering a short questionnaire by subject interview. Smoking levels were calculated as the sum of cigarettes, pipes, and cigars smoked according to the equivalents adopted previously, i.e., 20 cigarettes = 4 cigars = 5 pipes = 1 pack (17), with our data calculated as pack-years (1 pack/day for 1 year = 1 pack-year). Never-smokers were defined as subjects who smoked <1 pack/year. Individuals using other forms of tobacco (i.e., snuff or chewing tobacco) were not included in the tobacco correlation analysis. For many of the subjects that were interviewed, we could not obtain accurate lifetime alcohol use data but were able to collect determinations on the peak amount of alcohol consumption. All cases who were drinkers drank for a minimum of 10 years. Alcohol consumption was classified as shots per day, where one shot = 12.9 g of 43% alcohol, which is roughly equivalent to 1 ounce of 86-proof hard liquor, one 3.6-ounce glass of wine, or one 12-ounce can of beer. Never-drinkers were subjects who consumed <1 shot/day. Ex-drinkers were subjects who quit drink-

ing >5 years prior to recruitment into the study. Clinical data pertaining to tumor site were collected from patient charts for all oral cancer subjects recruited into the study. Informed consent was obtained from all subjects. Sample Collection and Processing. Buccal cell samples, isolated after surgery from prevalent (n = 53) as well as some incident (n = 15) cases at a follow-up examination, or normal oral tissue adjacent to the excised tumor isolated at the time of surgery from incident cases (n = 67), were used for the analysis of polymorphic genotype frequencies in cases, whereas buccal cells were collected for the analysis in matched controls. Buccal cell samples were collected in sterile 0.9% saline by scraping buccal mucosa with either a toothbrush or tongue suppressor after the subject rinsed one to two times with sterile, autoclaved water. Collected samples were stored at 4°C until centrifugation (1000 × g for 5 min) on-site within 1 h after collection. Cell pellets were stored at −20°C until use. DNA was isolated by incubation overnight with protease K (0.1 mg/ml) in 1% SDS at 50°C, extracted with phenol:chloroform, and precipitated with ethanol as described previously (18). Normal oral tissue obtained from cases during surgery was immediately frozen at −70°C until snap-cooled in liquid nitrogen and homogenized in protease K-containing buffer.

To prevent contamination and cross-contamination during PCR, all PCRs were performed using fresh, sterile autoclaved tips, tubes, and double-distilled water. Careful attention was given throughout to prevent cross-contamination between samples during DNA purification and isolation. The purification of DNA samples was performed in groups of 10–20 in a location distant from the workstation where PCR amplifications were performed and processed with a new set of pipetmen. All equipment used for tissue blending and homogenization was washed in a bath of concentrated chromic:sulfuric acid, rinsed three times in autoclaved double-distilled water and once in 70% ethanol, air-dried, and autoclaved after each tissue sample was processed as described above.

Genotyping Assays. The presence of the GSTM1 null polymorphism [homozygous (0/0)] was screened in all subjects (n = 135 each for both cases and controls) using a three primer-based PCR assay (19). This assay enabled us to screen for both the GSTM1 and GSTM4 genes simultaneously in one PCR reaction. The GSTM4 gene is a nonpolymorphic μ-class gene exhibiting high homology (>90%) with GSTM1 (20) in the 5' sense primer site but little homology with the 3' antisense primer site. In this PCR assay, we screened for exon 4–5 sequences in both the GSTM1 and GSTM4 genes. Because GSTM4 should be present in all cells, the amplification of this gene hence provides a positive control for each reaction. Samples where only the GSTM4 gene was amplified were considered homozygous null (0/0) for GSTM1. The standard PCR was performed similar to that described previously (19) in a 100-μl reaction volume containing 25–50 ng of purified genomic DNA, 2.5 mM Tris-HCl (pH 8.0), 5 mM KCl, 0.1 mM 2-mercaptoethanol, 10 mM MgCl2, 0.2 μg of each of the deoxynucleotide triphosphates, 1.5 units of Taq DNA polymerase (Amersham Corp.), 400 ng of sense primer (22, 5'-CGCCCCATCTGGATT-GATGG-3') and 200 ng of each antisense primer (23) (GSTM4-specific), 5'-ATCTTCTCTTTCTGCTTC-3', and 24y (GSTM1-specific), 5'-TTCTGGATTTAGCAGATCA-3'. The reaction mixtures underwent the following incubations in a GeneAmp 9600 thermocycler (Perkin-Elmer): 1 cycle of 95°C for 2 min, 32 cycles of 94°C for 20 s, 59°C for 30 s, and 72°C for 30 s, followed by a final cycle of 10 min at 72°C.
Samples were electrophoresed on 8% native polyacrylamide gels, stained with ethidium bromide, and examined over UV light. This analysis was performed independently on two separate occasions for 25% of all samples to confirm GSTM1 status. The results of the second round of GSTM1 analysis were identical to those obtained for the first round of analysis. GSTM1 PCR amplifications were not performed for one control due to insufficient template DNA, and analysis of GSTM1 data were confined to 134 matched pairs of cases and controls (except for analysis of risk factor associations, where 135 cases were included).

We screened all subjects for the presence of the (ile:val) polymorphism in exon 7 of the CYPIA1 gene using PCR/SSCP, a technique used routinely for the screening of single base pair changes in small DNA fragments (21). Briefly, PCR of CYPIA1 exon 7 sequences were performed in similar conditions to those described above for GSTM1, using 0.5 μl of [α-32P]dCTP (New England Nuclear) and 10 mM of both sense and antisense (5′-AAGAATTCCTACCGGGCA-3′) primers in a similar 35-cycle PCR program with an annealing temperature of 56°C (13). The resulting PCR product was 210 bp, sufficiently small in size for base pair change detection by SSCP analysis. PCR products were purified by band isolation after electrophoresis on 8% native polyacrylamide gels, ethidium bromide staining, incubation overnight in 0.5 M NH4HAc/1 mM EDTA (pH 8.0) at 37°C, and ethanol precipitation. Purified PCR products were dried and resuspended in 4 μl of gel loading buffer [95% formamide, 20 mM EDTA (pH 8.0), 0.05% bromphenol blue, and 0.05% xylene cyanol] and heated at 100°C for 10 min. Samples were immediately loaded onto a 0.4-mm thick 6% native polyacrylamide gel with 10% glycerol and electrophoresed at 30 W for 16–20 h using a sequencing-type apparatus. After electrophoresis, gels were dried and autoradiographed for 16–40 h. A second round of SSCP analysis was performed on 27 samples [i.e., 10%: 3 (ile/val) and 24 (ile/ile)] randomly selected from 270 screened samples (cases and controls) for the purpose of confirming previous results. The results of the second round of SSCP analysis of those 27 samples were identical to those obtained for the first round of SSCP analysis.

Statistical Analysis. The risk of oral cancer in relation to GSTM1 null and CYPIA1 (ile/val) polymorphic prevalences was calculated using ORs and 95% CIs based upon χ2 and Fisher’s exact test. For comparisons of levels of smoking in cases versus controls, Student’s t test was used.

Results
A total of 135 cases and 135 controls were entered into the study, of which 66% were male (Table 1). The mean age of the control group (60.9 years; range, 28–91) was identical to that of the case group (mean age, 60.9; range, 28–91; Table 1). Approximately 78% of cases and 41% of controls ever smoked cigarettes, with 17% of cases and 53% of controls having never smoked any form of tobacco (including cigars and pipe; data not shown). As expected, cases had a significantly higher level of overall tobacco consumption (including cigarettes, cigars and pipes; P < 0.00001; see Table 1) and smoked a significantly higher number of total cigarettes (mean ± SD pack year, 42.0 ± 31.3; P < 0.00001) as compared to controls (mean ± SD cigarette-only pack-years, 13.6 ± 27.3). One case was a snuff dipper.

The distribution of GSTM1 (0/0) and CYPIA1 (ile/val) polymorphic genotypes among oral cancer patients and controls is shown in Table 2. Informative PCR amplifications were observed for GSTM1 in 135 cases and 133 matched controls. For matched cases and controls (n = 133), the homozygous null (0/0) GSTM1 genotype, as evidenced by the sole presence of a 202-bp fragment corresponding to GSTM4 (see Fig. 1A), was observed in 51% of subjects in both groups. In controls, there was no significant difference in the GSTM1 (0/0) prevalence in males and females (50.6% versus 52.2%, respectively). Similarly, no significant difference in GSTM1 genotype frequency was observed when comparing cases with controls when stratified by sex (P < 0.70 for males; P < 0.60 for females; Table 1). Among cases, there was a similar prevalence of the GSTM1 (0/0) genotype in prevalent (28 of 82) versus incident (40 of 82) cases. When differentiating by major tumor sites, there appeared to be an increased prevalence of the GSTM1 (0/0) genotype in prevalent (28 of 82) versus incident (40 of 82) cases. When differentiating by major tumor sites, there appeared to be an increased prevalence of the GSTM1 (0/0) genotype in prevalent (28 of 82) versus incident (40 of 82) cases. When differentiating by major tumor sites, there appeared to be an increased prevalence of the GSTM1 (0/0) genotype in prevalent (28 of 82) versus incident (40 of 82) cases. When differentiating by major tumor sites, there appeared to be an increased prevalence of the GSTM1 (0/0) genotype in prevalent (28 of 82) versus incident (40 of 82) cases. When differentiating by major tumor sites, there appeared to be an increased prevalence of the GSTM1 (0/0) genotype in prevalent (28 of 82) versus incident (40 of 82) cases. When differentiating by major tumor sites, there appeared to be an increased prevalence of the GSTM1 (0/0) genotype in prevalent (28 of 82) versus incident (40 of 82) cases. When differentiating by major tumor sites, there appeared to be an increased prevalence of the GSTM1 (0/0) genotype in prevalent (28 of 82) versus incident (40 of 82) cases. When differentiating by major tumor sites, there appeared to be an increased prevalence of the GSTM1 (0/0) genotype in prevalent (28 of 82) versus incident (40 of 82) cases. When differentiating by major tumor sites, there appeared to be an increased prevalence of the GSTM1 (0/0) genotype in prevalent (28 of 82) versus incident (40 of 82) cases. When differentiating by major tumor sites, there appeared to be an increased prevalence of the GSTM1 (0/0) genotype in prevalent (28 of 82) versus incident (40 of 82) cases.

The CYPIA1 (ile/val) heterozygote and (val/val) homozygote could be easily distinguished from the wild-type CYPIA1 (ile/ile) homozygote by PCR/SSCP analysis as shown in Fig. 1B. Informative PCR amplifications for CYPIA1 were observed for 134 cases and 131 matched controls. The prevalence of this polymorphism in the control group was 7.6% (Table 2). For matched cases and controls (n = 131 each), the prevalence of the CYPIA1 exon 7 (ile:val) polymorphism was significantly higher in cases (17.6%; crude OR, 2.6; CI, 1.2–5.7), and this was most prevalent in females (26.1%; OR, 3.7; CI, 1.1–12.5). The frequency rate of the CYPIA1 (ile:val) polymorphism was similar in prevalent (8 of 50) versus incident (15 of 81) cases in patients with both oral cavity and laryngeal cancers (Table 2). Similar to that observed in previous studies (15, 16), the frequency of the (val/val) homozygote was extremely low in the Caucasian population, with no controls and only one case exhibiting this genotype (only the polymorphic shifted bands were observed on an SSCP autoradiograph; results not shown). For the 131 matched cases and controls that gave informative results, a synergistic increase in risk was not observed in subjects with the combined CYPIA1 (ile/val)/GSTM1 (0/0) genotype (Table 2).

The influence of two major risk factors for the induction of cancer was calculated using ORs and 95% CIs based upon χ2 and Fisher’s exact test. For comparisons of levels of smoking in cases versus controls, Student’s t test was used.

Table 2: Prevalence of GSTM1 null (0/0) and CYPIA1 exon 7 (ile:val + val/val) genotypes in oral cancer patients and frequency-matched controls.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases n (total %)</th>
<th>Controls n (total %)</th>
<th>OR (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTM1 (0/0)</td>
<td>68/133 (51.1)</td>
<td>68/133 (51.1)</td>
<td>1.0 (0.6–1.7)</td>
</tr>
<tr>
<td>Male</td>
<td>47/87 (54.0)</td>
<td>44/87 (50.6)</td>
<td>1.1 (0.6–2.1)</td>
</tr>
<tr>
<td>Female</td>
<td>21/46 (45.7)</td>
<td>24/46 (52.2)</td>
<td>0.8 (0.4–1.8)</td>
</tr>
<tr>
<td>CYPIA1 (val)</td>
<td>23/131 (17.6)</td>
<td>10/131 (7.6)</td>
<td>2.6 (1.2–5.7)</td>
</tr>
<tr>
<td>Male</td>
<td>11/85 (12.9)</td>
<td>6/85 (7.1)</td>
<td>2.0 (1.0–5.7)</td>
</tr>
<tr>
<td>Female</td>
<td>12/46 (26.1)</td>
<td>4/46 (8.7)</td>
<td>3.7 (1.1–12.5)</td>
</tr>
<tr>
<td>CYPIA1 (ile/GSTM1)</td>
<td>52/131 (39.7)</td>
<td>60/131 (45.8)</td>
<td>0.8 (0.5–1.3)</td>
</tr>
<tr>
<td>CYPIA1 (ile/GSTM1)</td>
<td>56/131 (42.7)</td>
<td>61/131 (46.6)</td>
<td>0.9 (0.5–1.4)</td>
</tr>
<tr>
<td>CYPIA1 (val/GSTM1)</td>
<td>12/131 (9.2)</td>
<td>3/131 (2.3)</td>
<td>4.3 (1.2–15.6)</td>
</tr>
<tr>
<td>OCCE GSTM1 (0/0)</td>
<td>55/109 (50.5)</td>
<td>58/109 (53.2)</td>
<td>0.9 (0.5–1.5)</td>
</tr>
<tr>
<td>OCCE CYPIA1 (val)</td>
<td>10/108 (9.3)</td>
<td>9/108 (7.4)</td>
<td>2.5 (1.0–6.0)</td>
</tr>
<tr>
<td>LaryngCa GSTM1 (0/0)</td>
<td>13/23 (56.5)</td>
<td>10/24 (41.7)</td>
<td>1.7 (0.5–5.1)</td>
</tr>
<tr>
<td>LaryngCa CYPIA1 (val)</td>
<td>5/23 (21.7)</td>
<td>2/23 (8.7)</td>
<td>2.9 (0.5–16.9)</td>
</tr>
</tbody>
</table>

* (0/0), null genotype for GSTM1.
+ (val), heterozygous (ile/val) + homozygous (val/val) genotypes.
+ (ile), homozygous (ile/ile) genotype.
OCCE, oral cavity cancer.
LaryngCa, laryngeal cancer.
oral cancer, tobacco use, and alcohol consumption on oral cancer risk and polymorphic genotype prevalences are shown in Table 3. Unlike that observed for lung cancer in previous studies (12), the prevalence of the CYP1A1 (ile/val) genotype was significantly higher in never-smoking cases than in "light" (i.e., 1–30 pack-years; OR, 0.30; CI, 0.1–1.0) or "heavy" (i.e., >30 pack-years; OR, 0.33; CI, 0.1–1.0) smoking cases. A similar prevalence in the CYP1A1 (ile/val) genotype was observed in the light and heavy smoking groups (Table 3). A significant association was not observed between case smoking level and the GSTM1 (0/0) genotype alone (Table 3) or when combined with the CYP1A1 (ile/val) genotype (results not shown). Similarly, a significant association was not observed in cases between alcohol consumption and the CYP1A1 (ile/val) or GSTM1 (0/0) genotypes alone (Table 3) or in combination (results not shown). No significant effect was observed for alcohol consumption when stratified by ever-drinkers versus never-drinkers (data not shown) or when stratified by different
levels of alcohol consumption (Table 3). Alcohol consumption did not appear to be a significant confounder of our stratified smoking analysis of polymorphic genotype prevalences in oral cancer cases (Table 4). Attempts were made to elucidate possible correlations between alcohol consumption and genotype frequencies in oral cancer cases using several possible alcohol consumption level cutoffs (one, four, and seven shots/day cut-offs), but no significant associations were found (results not shown).

Table 4  Influence of alcohol consumption on oral cancer risk when stratified by smoking levels and GSTM1 or CYP1A1 exon 7 genotypes

<table>
<thead>
<tr>
<th>Alcohol consumption (shots/day)</th>
<th>Smoking (pack-years)*</th>
<th>CYP1A1</th>
<th>GSTM1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NS (p = 0.01) OR (CI)^a</td>
<td>&gt;4 (p = 0.01) OR (CI)</td>
<td>ND</td>
</tr>
<tr>
<td>CYP1A1 ile</td>
<td>(n = 133)</td>
<td>11 (73)</td>
<td>2 (67)</td>
</tr>
<tr>
<td>GSTM1 +/+</td>
<td>(n = 134)</td>
<td>7 (47)</td>
<td>2 (67)</td>
</tr>
<tr>
<td>n</td>
<td>15</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

* Analysis of cases did not include one tobacco chewer.
+ NS, never-smokers of less than 1 pack-year; ND, never-drinkers of less than one shot/day.
^ p = 0.01. OR and CI were calculated based upon polymorphic prevalence in corresponding ND group.
@ GSTM1 ile/ile genotype.
# GSTM1 ile/val + homozgyous (val/val) genotypes.
^ GSTM1 (0/0) genotype may not play a major role in Bl[a]P detoxification in the oral cavity.

Discussion

Previous studies have suggested that certain genetic polymorphisms present in genes coding for enzymes involved in the metabolism of polycyclic aromatic hydrocarbons such as Bl[a]P may be linked to individual susceptibility to tobacco-related cancers. The GSTM1 null (0/0) polymorphism has been implicated in increased susceptibility to both lung (11) and bladder (22, 23) cancer. The association between the GSTM1 (0/0) genotype and increased risk for head and neck cancer as reported in preliminary studies by Trizna et al. (14) were not confirmed in the present study. The discrepancy between the two studies may be due to several factors, including possible differences in the study populations (i.e., race information was not provided in the Trizna study) and, perhaps most importantly, the study by Trizna et al. (14) was preliminary in nature and involved the analysis of only 42 matched controls (14). Another discrepancy between the two studies may be the different criteria used for case definition (i.e., oral squamous cell carcinoma versus head and neck cancer). We observed no significant increase in the GSTM1 (0/0) genotype in oral cavity cancer cases. However, a noticeable, although not statistically significant, increased risk was observed in cases with laryngeal cancer when compared with matched controls. Therefore, the GSTM1 (0/0) genotype may play an integral role in cancer susceptibility for different organ sites within the head and neck. A larger study will be required to determine whether the GSTM1 (0/0) genotype plays an important role in laryngeal cancer susceptibility.

The observed lack of an association between the GSTM1 (0/0) genotype and susceptibility to oral cancer in our study is similar to that observed in previous studies (15, 24). These results are also consistent with other studies demonstrating low GST μ-GST π protein ratios (−0.09) in normal human oral tissue (16). GST π contains the highest affinity for the toxic metabolite of Bl[a]P, trans-benzo(a)pyrene-anti-7,8 dihydrodiol-9,10 epoxide, as compared with other GST isoforms (25). Therefore, our results are consistent with the hypothesis that GSTM1 may not play a major role in Bl[a]P detoxification in the oral cavity.

Fewer studies have reported on the association between the CYP1A1 exon 7 (ile/val) polymorphism and cancer susceptibility. A link has been suggested between this polymorphism and increased lung cancer risk in both Japanese (12) and Brazilian (13) populations. A linkage between the CYP1A1 (ile/val) polymorphism and susceptibility to lung cancer in Cau- cians was shown in a Finnish study (26) but not in a Swedish population (27). The proportion of controls with the CYP1A1 (ile/val) polymorphism in our study was similar to these populations and the prevalence observed in an American study (28). To the best of our knowledge, ours is the first study examining the CYP1A1 exon 7 (ile/val) polymorphism as a determinant for oral cancer risk. The association between this polymorphism and oral cancer susceptibility observed in the present study is consistent with the high levels of CYP1A1 enzyme present in oral tissue (29). In addition, the higher prevalence of women with the CYP1A1 (ile/val) polymorphism in the oral cancer case group in our study may be related to the higher risk of oral cancer observed in women (30).

The association between polymorphic genotypes in tobacco carcinogen-metabolizing enzymes with smoking and cancer risk was demonstrated previously with both the GSTM1 (0/0) and CYP1A1 (ile/val) polymorphisms and lung cancer susceptibility (11–13). The results of our study suggest that the association between either of these polymorphisms and oral cancer susceptibility may not be directly associated with levels of exposure to tobacco smoke or alcohol consumption. This was not surprising for GSTM1 (0/0), because no association was observed between this polymorphism and oral cancer risk in our study. The fact that the prevalence of the CYP1A1 (ile/val) polymorphism in oral cancer cases did not increase with increased exposure to tobacco smoke is consistent with recent studies demonstrating that, although CYP1A1 ile/val exhibits higher metabolic activities than CYP1A1 ile against certain substrates (6–8), the purified, overexpressed val and ile forms of CYP1A1 enzyme exhibit similar metabolic activities toward Bl[a]P (8). The significance of the higher risk for never-smokers...
with the CYPIA1 (ile/val) genotype as compared to smokers with the same genotype is presently unclear, and we must be cautious when interpreting this result. The number of never-smoking case subjects recruited into our study was relatively low. Therefore, there may have been insufficient subject numbers for an adequate analysis of small but significant associations between smoking and CYPIA1 (ile/val) polymorphic incidence in oral cancer cases in our study. This is particularly relevant because our never-smoking case group included a "never-smoker" who smoked 0.5 pack-years (which, by definition, assigned this subject to the never-smoking classification). Therefore, a larger never-smoking case group will be required to more fully assess this relationship. In addition, a potential confounder for the analysis of smoking versus XME genotype in head and neck cancer may be infection by HPV. HPV has been implicated as a risk factor for several types of head and neck cancers and may be involved in the pathogenesis of between 10 and 20% of all oral cancers (reviewed in Ref. 31). In vitro studies have demonstrated that the E6 protein of oncogenic HPV strains induces the degradation of p53 (32, 33). In addition, some studies on cervical (34), esophageal (35), and paranasal sinus (36) cancer have demonstrated an inverse correlation between the incidence of p53 mutation (34, 36) or protein overexpression (35) and HPV 16 infection. These results are consistent with an HPV 16-induced disruption of p53 expression in certain tumors. Because approximately 37% of head and neck tumors exhibit p53 mutations (37) and p53 mutation incidence in head and neck tumors is directly correlated with increased tobacco use (38, 39), this suggests that infection by HPV may be a strong confounder when examining the relationship between genotypes of tobacco carcinogen-metabolizing enzymes and smoking. Studies examining the association between XME genotype and HPV infection in tumors from our series of head and neck cancer patients are presently underway. This analysis should enable us to better examine the relationship between risk factors such as tobacco use and alcohol consumption and increased susceptibility to XME genotype-dependent oral cancer.

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