Yonsei Medical Journal
Vol. 48, No. 2, pp. 233 - 239, 2007

# Polymorphisms of *CYP1A1* and *GSTM1* Genes and Susceptibility to Oral Cancer

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**Purpose:** Oral cancer is the fifth most common form of cancer in the world and comprises 6.5% of all cancer deaths. Since one of the major risk factors for oral cancer is tobacco use, we hypothesized that polymorphic genes coding for tobacco carcinogen-metabolizing enzymes may play a role in oral cancer susceptibility. **Materials and Methods:** To investigate the association between polymorphisms of the *CYP1A1* and *GSTM1* genes and risks for oral squamous cell carcinoma (OSCC) in the Korean population, the prevalence of the *CYP1A1* MspI and *GSTM1* null polymorphisms were examined in 72 patients with histologically confirmed primary OSCC, as well as in 221 healthy control subjects. **Results:** A significant risk increase for oral cancer was observed among subjects with the homozygous *CYP1A1* (*m2/m2*) genotype (OR = 3.8, 95% CI = 1.9-7.7), but not the *GSTM1* null genotype (OR = 0.7, 95% CI = 0.4-1.3). Risk for oral cancer was significantly increased in subjects with the homozygous *CYP1A1* (*m2/m2*) genotype, regardless of smoking history (smokers; OR = 4.4; 95% CI = 1.2-16.3; non-smokers OR = 4.9; 95% CI = 1.9-12.5). Using the potentially most protective genotype *GSTM1* (+)/*CYP1A1* [(*m1/m1*)+(*m1/m2*)] as the reference group, an increased risk for oral cancer was observed among subjects with the *GSTM1* (+)/*CYP1A1* (*m2/m2*) (OR= 2.0, 95% CI=0.8-5.2), and *GSTM1* (-)/ *CYP1A1* (*m2/m2*) (OR= 4.9, 95% CI = 1.5-15.5) genotypes ($p < 0.009$, ($\chi^2$ trend test). **Conclusion:** Our results suggest that individuals with a genotype of *CYP1A1* (*m2/m2*) and *GSTM1* (-) are highly susceptible for OSCC and that the *CYP1A1* (*m2/m2*) genotype is closely associated with increased risk of OSCC in Koreans.

Received June 29, 2006
Accepted September 5, 2006

*This study was supported by a grant from the Research Fund of Yonsei University College of Dentistry to Kwang-Kyun Park for 2005.*

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**Key Words:** Tobacco carcinogen-metabolizing enzyme, *CYP1A1*, *GSTM1*, genetic polymorphism, oral squamous cell carcinoma

## INTRODUCTION

Oral cancer is a tobacco-related disease whose high incidence represents a significant problem in many parts of the world, with its poor survival rates, and severe functional and cosmetic defects accompanying its treatment. Most tobacco carcinogens require metabolic activation by cytochrome P450s (CYPs) for conversion into their reactive electrophilic intermediates[1] and detoxification by glutathione S-transferases (GSTs) to produce water-soluble, excretable compounds.[2] Variations in metabolism of these compounds are often associated with genetic polymorphisms in genes coding for enzymes involved in the metabolic activation or detoxification of tobacco carcinogens. Large differences in the prevalence of certain genetic polymorphisms have been described between ethnic and racial groups for several metabolizing enzyme genes, and it has been suggested that some of these polymorphisms may affect enzyme activity, which in turn may influence individual cancer risk.[2-7]

Tobacco carcinogens such as polycyclic aromatic hydrocarbons and tobacco-specific nitrosamines are primarily metabolized to their activated intermediates by the cytochrome P450-dependent mono-oxygenases. Several polymorphic cytochrome P450 enzymes involved in the activation

of tobacco carcinogens have been examined for their potential association with increased risk for oral cancer.[8-11] The *CYP1A1* gene codes for the enzyme aryl hydrocarbon hydroxylase, which is involved in the biotransformation of various aromatic procarcinogens in cigarette smoke, including benzo[a]pyrene (BaP), to highly electrophilic and carcinogenic phenolic products and epoxides.[1,12] Certain variant genotypes of *CYP1A1* gene which cause enhanced enzymatic activity appear to play a role in susceptibility to adduct formation and, presumably, cancer risk. The *CYP1A1* alleles containing the MspI polymorphic variants have been linked to increased formation of BaP-7,8-diol-epoxide adducts in white blood cells from coke oven workers.[13] The *CYP1A1* MspI polymorphism, which results from a single base pair change at nucleotide position 264 from the poly (A) signal in the 3′ untranslated region of the *CYP1A1* gene, is found in 5-30% of the population[6,7,14-17] and has been linked to susceptibility for smoking-related cancers, such as oral and lung cancers.[14,18,19]

The mu class of GST enzymes plays an important role in the detoxification of BaP and other polycyclic aromatic hydrocarbons. The absence of *GSTM1* enzyme activity for the detoxification (phase II) reaction is caused by homozygous deletion (null genotypes) of the respective genes[20] and results in the accumulation of activated carcinogens that can bind covalently to DNA. The polymorphic *GSTM1* null genotype has been found in 20-50% of populations of various ethnic origins, and this genotype has been correlated with risk for various tobacco-related cancers among Caucasians,[4,21-23] Japanese[10,11,24] and Indians.[25] Significant associations were also found between the *GSTM1* null genotype and the risk for oral squamous cell carcinoma (OSCC) in several studies.[10,26] However, a lack of association between the *GSTM1* polymorphism and oral cancer in Caucasians has been reported.[8,27] These conflicting results may be due to ethnic differences in the allelic frequency of the *GSTM1* polymorphism.

The present case-control study was done to investigate the potential role of *CYP1A1* and *GSTM1* gene polymorphisms in the risk for OSCC in Koreans.

## MATERIALS AND METHODS

### Study populations and sample processing

All cases (n = 72) comprised patients who had been histopathologically diagnosed for OSCC in the Department of Oral and Maxillofacial Surgery, Yonsei University College of Dentistry (Seoul, Korea) between 1998 and 2000. Controls (n = 221) without any precancerous or cancerous lesions were recruited at a public school or College of Dentistry during routine dental screening. For all subjects, oral rinse samples were used for controls (n = 171) and oral biopsy samples collected during routine preventive dental screening or post treatment at dental clinics from the cases (n = 72) and a portion of the controls (n = 50) were used for the analysis of polymorphic genotypes. Buccal cells were collected from the 171 healthy controls by the mouthwash method as follows:[28] 1 hr after brushing their teeth, the control subjects rinsed their mouths vigorously for 1 min with 10 mL of undiluted mouthwash (Listerine, Warner-Lambert Consumer Healthcare, NJ, USA) and expelled it into a strerile 50-mL tube. The collected mouthwash was centrifuged at 2,700 rpm for 15 min, the supernatant discarded, and the pellet washed with 25 mL of TE buffer (10 mM Tris, pH 8.0, 10 mM EDTA, pH 8.0). The suspension was centrifuged again and the pellet used for DNA extraction.

A short questionnaire was administered to all subjects with questions on demographic information and environmental risk factors such as life-long smoking habits and alcohol consumption. The demographic data of both groups are presented in Table 1. Tobacco smoke exposure was measured in pack-years [1 pack-year = 1 pack (20 cigarettes)/day for 1 years]. This study was approved by the institutional review board at our institute and informed consent was obtained from all subjects.

### Genotyping analysis

Genomic DNA was isolated from oral tissue samples or buccal cells by proteinase K digestion and phenol-chloroform extraction as previously described.[29] The *CYP1A1* MspI polymorphism

**Table 1.** Age, Gender and Smoking History of Controls and Oral Cancer Patients

| | Healthy controls | Oral cancer patients |
|---|---|---|
| Subjects No. | 221 | 72 (100%) |
| Age (yrs) | 35.4 ± 17.5 (20 - 84) | 62.5 ± 12.3 (20 - 88) |
| Mean ± SD* (range) | | |
| Gender | | |
| Male | 113 (51%) | 46 (64%) |
| Female | 108 (49%) | 26 (36%) |
| Smoking Status† | | |
| Non smoker | 181 (82%) | 33 (52%) |
| Light smoker (≤ 20 py) | 30 (14%) | 15 (24%) |
| Heavy smoker (> 20 py) | 10 (4%) | 15 (24%) |

*Standard deviation.
†Smoking information from nine cases is not available.
py, the number of pack/day x years of smoking.

was identified by PCR-restriction fragment length polymorphism (PCR-RFLP),[30] testing for substitution of CCGG for CTGG in the MspI site at base 264 from the additional polyadenylation signal in the 3′-flanking region. Using two primers (5′-TAG GAGTCTTGTCTCATGCCT-3′ and 5′-CAGTGAA GAGGTGTAGCCGCT-3′), PCR-amplification was performed using 30 cycles of 1 min at 95℃ for denaturation, 1 min at 65℃ for primer annealing and 1min at 72℃ for primer extension. The PCR products were digested with *MspI* and subjected to electrophoresis on a 2.0% agarose gel. The *CYP1A1* (*m1/m1*) genotype (wild type) was characterized by a 340 bp fragment, polymorphic homozygous *CYP1A1* (*m2/m2*) genotype by 140 and 200 bp fragments, and heterozygous *CYP1A1* (*m1/m2*) genotype by 140, 200 and 340 bp fragments, respectively.

The *GSTM1* genotypes were also determined by PCR analysis.[30] Two primers (5′-GAACTCCCTGA AAAGCTAAAGC-3′ and 5′-GTTGGGCTCAAAT ATACGGTGG-3′) were used for 30 cycles of the amplification with 1 min at 94℃ for denaturation, 1min at 59℃ for primer annealing, 1min at 72℃ for extension. The PCR products were electrophoresed on a 2.0% agarose gel and the 215 bp fragment in the *GSTM1*-positive genome was identified by ethidium bromide staining. A 268 bp fragment of the β-goblin gene was co-amplified as an internal control. The primers for β-goblin were 5′-CAACTTCATCCACGTTCACC-3′ and 5′-GAA GAGCCAAGGACAGGTAC-3′. The prevalence of the homozygous or heterozygous genotype of the complete *GSTM1* gene [*GSTM1*(+)], or the homozygous deficient gene [*GSTM1* (-)] was compared between OSCC patients and healthy controls.

### Statistical analysis

The risk of oral cancer in relation to polymorphic prevalence was estimated using conditional logistic regression to calculate the odds ratios (ORs) and 95% confidence intervals (CIs). The chi-squared test for trends was used in combined genotype analysis, and was implemented as appropriate for the analysis of categorical variables, genotypes and case status. The statistical computer software SPSS (ver. 11.5) was used to perform all statistical analyses (SPSS, 2003).[31]

## RESULTS

A total of 72 cases and 221 controls were entered into this study (Table 1). The distribution of genotypes of *CYP1A1* and *GSTM1* among the

**Table 2.** Distribution of *CYP1A1* and *GSTM1* Genotypes and Risk for Oral Cancer Stratified by Smoking Behavior

| Genotypes | Total | | | Smokers | | | Non-smokers | | |
|---|---|---|---|---|---|---|---|---|---|
| | Cases | Controls | OR (95% CI) | Cases | Controls | OR (95% CI) | Cases | Controls | OR (95% CI) |
| *CYP1A1* (*m1/m1*) | 20 (28)* | 49 (30) | 1.0 (reference) | 8 (30) | 9 (26) | 1.0 (reference) | 9 (26) | 40 (31) | 1.0 (reference) |
| *CYP1A1* (*m1/m2*) | 30 (42) | 97 (60) | 0.8 (0.4-1.4) | 9 (33) | 21 (62) | 0.5 (0.1-1.7) | 12 (39) | 76 (59) | 0.7 (0.3-1.8) |
| *CYP1A1* (*m2/m2*) | 22 (30) | 17 (10) | 3.3 (1.4-10) | 10 (37) | 4 (12) | 2.8 (0.6-12.6) | 11 (35) | 13 (10) | 3.8 (1.3-11.1) |
| *CYP1A1* [(*m1/m1*)+(*m1/m2*)] | 50 (70) | 146 (90) | 1.0 (reference) | 17 (63) | 30 (88) | 1.0 (reference) | 20 (65) | 116 (90) | 1.0 (reference) |
| *CYP1A1* (*m2/m2*) | 22 (30) | 17 (10) | 3.8 (1.9-7.7) | 10 (37) | 4 (12) | 4.4 (1.2-16.3) | 11 (35) | 13 (10) | 4.9 (1.9-12.5) |
| *GSTM1* (+)† | 35 (49) | 86 (41) | 1.0 (reference) | 20 (65) | 12 (35) | 1.0 (reference) | 11 (34) | 53 (45) | 1.0 (reference) |
| *GSTM1* (-)‡ | 37 (51) | 123 (59) | 0.7 (0.4-1.3) | 11 (35) | 22 (65) | 0.3 (0.1-0.8) | 21 (66) | 64 (55) | 1.6 (0.7-3.6) |

*Numbers in parenthesis denote percentages.
†(+) = homozygous (+/+) and heterozygous (+/0) genotypes.
‡(-) = null genotype for *GSTM1*.

patients with OSCC and healthy controls is shown in Table 2. The genotype distribution for *CYP1A1* MspI polymorphisms among the controls ($p$ = 0.10) followed the expected Hardy-Weinberg distribution. Due to the design of our genotyping analysis, we could not assess the allelic frequency of the *GSTM1* allele.

Patients with OSCC were more likely to have homozygous *CYP1A1* (*m2/m2*) genotypes when compared to controls (OR = 3.8, 95% CI = 1.9-7.7; Table 2). This data corresponded with a significantly higher prevalence of the *CYP1A1 m2* allele (0.51) when compared to controls (0.40, $p$ = 0.023). Conversely, a significant association was not observed between the *GSTM1* (-) genotype and OSCC (OR = 0.7; 95% CI = 0.4-1.3; Table 2).

To evaluate gene-smoking interactions, the prevalence of *CYP1A1* and *GSTM1* genotypes were stratified by smoking history. Oral cancer was significantly increased in subjects with the homozygous *CYP1A1* (*m2/m2*) genotype regardless of smoking history (smokers; OR = 4.4; 95% CI = 1.2-16.3; non-smokers OR = 4.9; 95% CI = 1.9-12.5), whereas it was significantly decreased in smokers with the *GSTM1* null genotype (OR = 0.3; 95% CI = 0.1-0.8) and a similar association was not observed among non-smokers (OR = 1.6; 95% CI = 0.7-3.6; Table 2).

To analyze the association between oral cancer and combined genotypes, the genotype presumed most protective, *GSTM1* (+)/*CYP1A1* [(*m1/m1*)+(*m1/m2*)], was used as the reference group. Increased risk for oral cancer was observed in subjects with the *GSTM1* (+)/*CYP1A1* (*m2/m2*) genotype (OR = 2.0, 95% CI = 0.8 - 5.2), as well as the genotype presumed most dangerous, *GSTM1* (-)/ *CYP1A1* (*m2/m2*) (OR = 4.9, 95% CI = 1.5-15.5) (Table 3). A significant trend towards increased risk was observed in the potentially less protective genotype *GSTM1*/*CYP1A1* ($p < 0.009$, ($\chi^2$ trend test).

## DISCUSSION

Variations in the importance of the *CYP1A1* and *GSTM1* polymorphisms on the increased risk for smoking-related cancers have been demonstrated for different ethnic groups.[8,21,27,32,33] Recently, the association between genetic polymorphisms in *CYP1A1* and *GSTM1* genes and oral cancer risk were studied in several populations.[9-11,34-38] In a Japanese population, individuals with the *CYP1A1* (*m2/m2*) and *GSTM1* null genotype exhibited a remarkably high risk for oral cancer at a low dose level of cigarette smoking, even though the *GSTM1* null genotype is only weakly correlated with oral cancer.[9-11] In contrast, only *GSTM1* and GSTT null genotypes resulting in the deficiency of these gene products was associated with oral cancer in a German population. Interestingly, the

**Table 3.** Distribution of Combined *GSTM1* and *CYP1A1* Genotypes Among Study Subjects

| *GSTM1* | *CYP1A1* | Cases | Controls | OR (95% CI) |
|---|---|---|---|---|
| (+)* | [(*m1/m1*) + (*m1/m2*)] | 24 (33)† | 53 (35) | 1.0 (reference)‡ |
| (-)§ | [(*m1/m1*) + (*m1/m2*)] | 26 (36) | 81 (54) | 0.7 (0.4 - 1.4) |
| (+) | (*m2/m2*) | 11 (15) | 12 (8) | 2.0 (0.8 - 5.2) |
| (-) | (*m2/m2*) | 11 (15) | 5 (3) | 4.9 (1.5 - 15.5) |
| (-) | [(*m1/m1*) + (*m1/m2*)] | 26 (70) | 81 (94) | 1.0 (reference) |
| (-) | (*m2/m2*) | 11 (30) | 5 (6) | 6.9 (2.2 - 21.6) |
| (+) | (*m2/m2*) | 11 (50) | 12 (71) | 1.0 (reference) |
| (-) | (*m2/m2*) | 11 (50) | 5 (29) | 2.4 (0.6 - 9.1) |

*(+) = homozygous (+/+) and heterozygous (+/0) genotypes.
†Numbers in parentheses denote percentages.
‡Chi square trend test, $p < 0.009$.
§(-) = null genotype for *GSTM1*.

*CYP1A1* (*m2/m2*) genotype is not seen in patients with oral cancer.[39] In an Indian study, increases in the risk of oral leukoplakia and cancer were observed in individuals with the *GSTM1* null genotype,[35,38] but the combined homozygous and heterozygous mutated genotypes of *CYP1A1* did not show any significant differences between patients with oral leukoplakia and controls among tobacco smokers.[40] It has also been reported that the *GSTM1* null polymorphism plays a significant role in individual risk for oral cancer in the African Americans[25] and Brazilians,[37] but not in Caucasians.[8,26,33] The discrepancy between these results may be due to several factors, including differences between the study populations in tumor site, ethnicity and sample size.

In this study, we investigated the role of singular and combined genotypes of *CYP1A1* and *GSTM1* in the risk for oral cancer in a Korean population. The results from the present study demonstrate that harboring a homozygous *CYP1A1* (*m2/m2*) genotype adds a significant risk increase for oral cancer in both smokers and non-smokers. The lack of an association between the *GSTM1* null genotype and susceptibility to oral cancer in our study is similar to the results reported in previous studies.[8,9,23] As the significance of the protective effect of the *GSTM1* null genotype for smokers is presently unclear, these results need to be confirmed with further studies.

The magnitude of the risk increase from the *CYP1A1* (*m2/m2*) genotype for oral cancer was more evident in subjects who were *GSTM1* null (OR = 4.9, 95% CI = 1.5-15.5) than subjects with the *GSTM1* (+) genotype (OR = 2.0, 95% CI = 0.8-5.2). This data suggests that *CYP1A1* and *GSTM1* gene: gene interactions play a critical role in susceptibility to oral cancer. This interaction can be explained by the risk association for genotypes exhibiting small increases in *CYP1A1* activity only being discernable under circumstances where exposure to BaP-7,8-epoxide is greatest. That the *CYP1A1* genotype plays an important role in oral cancer risk exclusively in *GSTM1* null subjects is consistent with this hypothesis since increased levels of BaP-7,8-epoxide would be present due to decreased rates of detoxification by the *GSTM1* enzyme. As discussed above, no association between the *GSTM1* null polymorphism and oral cancer was observed in the present study. Taken together, these data suggest that the *GSTM1* null genotype is not associated with oral cancer risk regardless of *CYP1A1* genotype {*CYP1A1* (*m2/m2*): OR = 2.4, 95% CI = 0.6-9.1, *CYP1A1* [(*m1/m1*) + (*m1/m2*)]: OR = 0.7, 95% CI = 0.4-1.4}. Therefore, the risk associated with the *GSTM1* null polymorphism may only be discernable when the combined net effect of multiple genotypes results in significant increases in BaP-7,8-epoxide levels.

Several variables could contribute to these con-

flicting results, with the greatest concern being a common problem with molecular epidemiological studies, an inadequate sample size to allow sufficient measurement of attributable risk associated with any given genotype. In summary, this work demonstrated that individuals with the *CYP1A1* (*m2/m2*) *GSTM1* (-) genotype are susceptible for OSCC and the presence of the *CYP1A1* (*m2/m2*) genotype is closely associated with increased risk of OSCC regardless of smoking behavior in Korean populations.

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