Human Glutathione S-transferase A1 Polymorphism and Susceptibility to Oral Squamous Cell Carcinoma in Japanese

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Abstract

Objectives: Glutathione S-transferase (GST) A1 catalyses the activated heterocyclic aromatic amine carcinogen *N*-acetoxy-2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (N-OAc-PhIP). This case-control study was carried out to examine whether the genetic polymorphism of GSTA1 is associated with the risk of oral squamous cell carcinoma among Japanese people in relation to their smoking status.

Methods: In this study, 97 Japanese oral squamous cell carcinoma patients and 457 healthy controls were compared for the frequencies of the *GSTA1* genotypes (*A: -567T,-69C,-52G, *B: -567G,-69T,-52A).

Results: The frequencies of *GSTA1* *A/*B+*B/*B genotypes were 32.3% in male cancer patients and 11.4% in female cancer patients, compared with 20.1% in the male control group (Odds ratio (OR)=1.86; 95% confidence interval (CI) 0.99–3.46) and 23.1% in the female control group (OR=0.58; 95% CI 0.18–1.81). The *GSTA1* *A/*B+*B/*B genotypes were associated with an 86% increased risk of oral squamous cell carcinoma among males, albeit without statistical significance. Also, among male smokers, the frequency of *GSTA1* *A/*B+*B/*B genotypes was significantly higher among the oral squamous cell carcinoma patients (33.3%) than among the controls (19.6%). The OR of the male smokers with the *GSTA1* *A/*B+*B/*B genotypes for oral squamous cell carcinoma was 1.97 (95% CI 1.02–3.79).

Conclusions: We present the first evidence of an association between *GSTA1**B and oral squamous cell carcinoma among smokers. This study suggests that the *GSTA1* polymorphism and tobacco smoke-derived PhIP are associated with oral squamous cell carcinoma susceptibility among male smokers.

Key words: *GSTA1*, polymorphism, oral squamous cell carcinoma, smoking, PhIP

Introduction

Oral squamous cell carcinoma, the main type of oral cancer, is among the ten most common cancers in the world (1) and represents a significant problem based on the severe functional and cosmetic defects accompanying treatment. Exposure

to tobacco smoke and alcohol are two of the leading causes of this malignancy (2–4). The oral tissue is particularly susceptible from the standpoint of carcinogen exposure such as tobacco-derived 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and benzo[*a*]pyrene.

Most environmental carcinogens are metabolized via complex enzymatic mechanisms involving activation and inactivation. In some of these metabolisms, there are genetic differences, and these individual variations may modulate cancer risk. The glutathione S-transferase (GST) is a large family consisting of phase II enzymes that facilitate the detoxification of various carcinogens, therapeutic drugs, environmental toxins and products of oxidative stress.

GSTA1, an alpha class enzyme, catalyses the glutathione-dependent detoxification of the activated heterocyclic aromatic amine carcinogen *N*-acetoxy-PhIP (5). Recently, a polymor-

Abbreviations: GST, Glutathione S-transferase; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; OR, Odds ratio; 95% CI, 95% confidence interval.

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phism of the *GSTA1* gene localized on chromosome 6p12 has been discovered, and its activity is due to differences in *GSTA1**A (-567T,-69C,-52G) and *GSTA1**B (-567G,-69T,-52A) polymorphisms (6).

This case-control study was carried out to examine whether the genetic polymorphism of a major phase II enzymes, GSTA1, is associated with the risk of oral squamous cell carcinoma.

Materials and Methods

Subjects

The patient group comprised 97 oral squamous cell carcinoma patients (tongue n=36, floor of mouth n=18, alveolus and gingiva n=17, buccal mucosa n=12, soft palate n=5, other regions of the oral cavity n=9) (62 men, 35 women; mean age 62.8 years) from Kitakyushu City, Japan. Consecutive patients who visited the University of Occupational and Environmental Health Hospital and had been histologically diagnosed during the period from September 1992 to January 1997. None of these patients refused to participate in this study. The differentiation grades of oral squamous cell carcinomas were well differentiated (n=55), moderately differentiated (n=34), poorly differentiated (n=5), and unknown (n=3).

The control group comprised 457 individuals who had visited local medical clinics in Kitakyushu City and Miyazaki City between September 1993 and April 2002 for regular medical health check-up (including blood and urine analyses). There were 284 males and 173 females (mean age 67.2±12.2 years). While no specific age matching was carried out, the mean age of the patients was similar to that of the control subjects. The control subjects had no current or previous diagnosis of cancer. All participants completed a questionnaire administered by a trained interviewer, covering medical, residential, occupational and smoking states. We divided the subjects into smokers (those who had ever smoked) and non-smokers (those who had never smoked) until the time of the interview. Alcohol consumption information, an important risk factor for oral squamous cell carcinoma, was collected from oral squamous cell carcinoma patients but, unfortunately, not from control subjects. Data on other oral squamous cell carcinoma risk factors, such as dietary habits, oral hygiene, dental health and infection with human papilloma virus were not available. All of the patients and the control subjects were given an explanation of the nature of the study and informed

consent was obtained. This study was approved by the ethics committees of the University of Occupational and Environmental Health and the University of Miyazaki.

Genotyping

Genomic DNA was isolated from peripheral leukocytes by proteinase K digestion and phenol/chloroform extraction (7). The *GSTA1* genotype was determined by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) according to Coles et al. (6). Briefly, the primers used in the PCR amplification were the sense primer (5'-TGT TGA TTG TTT GCC TGA AAT T-3') and the antisense primer (5'-GTT AAA CGC TGT CAC CCG TCC T-3'). Thirty nanograms of DNA was amplified in a total volume of 30 µl containing 16.7 pmol of each primer, 0.8 U of Taq polymerase, 2 mM MgCl₂, and PCR buffer. The amplification was performed by denaturing at 94°C for 5 min, followed by 35 cycles at 94°C for 60 sec, annealing at 64°C for 60 sec and 72°C for 60 sec in a Perkin-Elmer 9700 (Norwalk USA). The amplification products (20 µl) were digested with 10 U of restriction endonuclease EarI at 37°C for 12 hrs. Digest patterns were determined by resolution on a 2% agarose gel.

Statistical analysis

We used a chi-square test to compare the *GSTA1* gene polymorphism in the oral squamous cell carcinoma patients with the expected gene distribution from the healthy control individuals. Crude odds ratio (OR) and 95% confidence interval (CI) for the *GSTA1* genotype were calculated separately by sex. OR was adjusted for age and smoking status using multiple logistic regression analysis. The chi-square test was used for the probability of the Hardy-Weinberg equilibrium. We also analyzed an interaction between *GSTA1* polymorphism and smoking status. All statistical analyses were based on two-tailed probabilities. Values of p<0.05 were considered statistically significant. SPSS II for Windows software (version 11.0J, SPSS Japan, Tokyo, Japan) was used for statistical analysis.

Results

The frequency of the *GSTA1* genotype in relation to oral squamous cell carcinoma is shown in Table 1. Genotype distribution in controls was in the Hardy-Weinberg equilibrium (p=0.615). The frequencies of *GSTA1* *A/*B+*B/*B genotypes were 32.3% in male cancer cases and 11.4% in female cancer

Table 1 *GSTA1* genotype frequencies among Japanese oral squamous cell carcinoma patients and control individuals

	<i>GSTA1</i> genotype	Oral squamous cell carcinoma	Control	Crude odds ratio (95% CI)	Odds ratio ^a (95% CI)
Male	<i>GSTA1</i> *A/*A	67.7% (42)	79.9% (227)	1 (reference)	1 (reference)
	<i>GSTA1</i> *A/*B	30.6% (19)	18.0% (51)	2.02 (1.09–3.75) ^b	1.97 (1.04–3.72) ^b
	<i>GSTA1</i> *B/*B	1.6% (1)	2.1% (6)	0.76 (0.09–6.42)	0.77 (0.09–6.78)
	<i>GSTA1</i> *A/*B+*B/*B	32.3% (20)	20.1% (57)	1.90 (1.03–3.48) ^b	1.86 (0.99–3.46) ^c
Female	<i>GSTA1</i> *A/*A	88.6% (31)	76.9% (133)	1 (reference)	1 (reference)
	<i>GSTA1</i> *A/*B	11.4% (4)	22.5% (39)	0.44 (0.15–1.33)	0.61 (0.19–1.93)
	<i>GSTA1</i> *B/*B	0% (0)	0.6% (1)	—	—
	<i>GSTA1</i> *A/*B+*B/*B	11.4% (4)	23.1% (40)	0.43 (0.14–1.29)	0.58 (0.18–1.81)

^a Odds ratio compared with *GSTA1* *A/*A genotype (adjusted for age and smoking status). ^b p<0.05. ^c p=0.05.

Table 2 Relationship between *GSTA1* genotype and smoking status among study group

		<i>GSTA1</i> genotype	Oral squamous cell carcinoma	Control	Odds ratio 95% CI ^a
Male	Nonsmokers (85)	<i>GSTA1</i> *A/*A	80.0% (4)	78.8% (63)	1 (reference)
		<i>GSTA1</i> *A/*B+*B/*B	20.0% (1)	21.2% (17)	0.88 (0.09–8.47)
	Smokers (261)	<i>GSTA1</i> *A/*A	66.7% (38)	80.4% (164)	1 (reference)
		<i>GSTA1</i> *A/*B+*B/*B	33.3% (19)	19.6% (40)	1.97 (1.02–3.79) ^b
Female	Nonsmokers (164)	<i>GSTA1</i> *A/*A	85.7% (24)	80.9% (110)	1 (reference)
		<i>GSTA1</i> *A/*B+*B/*B	14.3% (4)	19.1% (26)	0.95 (0.29–3.13)
	Smokers (44)	<i>GSTA1</i> *A/*A	100% (7)	62.2% (23)	1 (reference)
		<i>GSTA1</i> *A/*B+*B/*B	0% (0)	37.8% (14)	—

There was statistical evidence of an interaction between *GSTA1* polymorphism and smoking status among males ($p=0.041$).

^a Odds ratio (95% confidence interval). Odds ratios were calculated by comparison between control individuals and cancer groups for *GSTA1* *A/*B+*B/*B versus *GSTA1* *A/*A, adjusted for age. ^b $p=0.043$.

cases, compared with 20.1% in the male control group (OR=1.86; 95% CI 0.99–3.46) and 23.1% in the female control group (OR=0.58; 95% CI 0.18–1.81). The *GSTA1* *A/*B+*B/*B genotypes were associated with an 86% increased risk of oral squamous cell carcinoma among males, albeit without statistical significance. On the basis of a hypothesized role of GSTs in modulating the effects of carcinogens present in tobacco smoke, we investigated the combined effect of smoking and *GSTA1* polymorphism. In our study, the age-adjusted ORs for smoking were 4.37 (95% CI 1.69–11.33) among males, and 0.64 (95% CI 0.24–1.68) among females (data not shown). Table 2 outlines the relationship between the *GSTA1* genotypes and oral squamous cell carcinoma by stratifying in terms of smoking status. Among male smokers, the frequency of *GSTA1* *A/*B+*B/*B genotypes was significantly higher among oral squamous cell carcinoma cases (33.3%) than among the controls (19.6%). The OR of the male smokers with the *GSTA1* *A/*B+*B/*B genotypes for oral squamous cell carcinoma was 1.97 (95% CI 1.02–3.79). On the other hand, there was no association of the *GSTA1* genotypes with the risk of oral squamous cell carcinoma among male or female nonsmokers (male: OR=0.88; 95% CI 0.09–8.47) (female: OR=0.95; 95% CI 0.29–3.13).

Discussion

Clinically, a link between a chronic consumption of tobacco and oral cancer has been observed for many years. The observation has been supported by numerous epidemiological studies indicating that heavy smoking increases the risk of oral squamous cell carcinoma (8, 9). Our data support previous

studies: the ORs for smokers were 4.37 (95% CI 1.69–11.33) among males and 0.64 (95% CI 0.24–1.68) among females.

GSTA1 is the most abundant GST found in the human liver. A significant decrease in luciferase activity was observed in *GSTA1**B, and the base change at position –52 might explain the different activities of *GSTA1**A and *GSTA1**B promoters (10). In this study, we present the first evidence of an association between *GSTA1**B and oral squamous cell carcinoma among male smokers.

Our results showed that among male smokers the OR of the individuals with the *GSTA1**A/*B+*B/*B genotypes for oral squamous cell carcinoma was 1.97 (95% CI 1.02–3.79). *GSTA1* has been reported to be most efficient in detoxifying *N*-acetoxy-PhIP, and its presence in tobacco smoke is 22.9 ng/cigarette (11). Therefore, we considered that *GSTA1* might play an important role in protecting DNA against tobacco-derived PhIP.

In conclusion, our data show a significant relationship between oral squamous cell carcinoma and genetic polymorphism of *GSTA1* among male smokers. These findings may help in researching the risk and identifying individuals susceptible to oral squamous cell carcinoma.

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