

CYP1A1 and *GSTP1* polymorphisms in an oral cancer case-control study

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Abstract

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CYP1A1 and *GSTP1* polymorphisms have been associated with a higher risk to develop several cancers, including oral squamous cell carcinoma (OSCC), which is closely related to tobacco and alcohol consumption. Both genes code for enzymes that have an important role in activating or detoxifying carcinogenic elements found in tobacco and other compounds, and polymorphic variants of these genes may result in alterations of the enzymatic activity. The *CYP1A1* gene codes for the enzyme aryl hydrocarbon hydroxylase, which is responsible for the metabolism of polycyclic aromatic hydrocarbons. The investigated polymorphism, Ile/Val, seems to increase the activity of the enzyme in homozygous individuals, leading to an accumulation of carcinogens. The Ile/Val polymorphism occurs because of an A→G transition at exon 7, resulting in the *CYP1A1*2B* allele. The *GSTP1*B* variant shows an A→G transition at exon 5, changing the amino acid Ile to Val, with a reduced catalytic activity of the enzyme. Due to this reduction, the carriers of mutant alleles lost the capability to metabolize carcinogens, which could be responsible for a higher susceptibility to cancer. We conducted a case-control study in a group of 72 cases with newly diagnosed OSCC and 60 healthy controls matched for age, gender, smoking habits, and ethnicity. We used PCR methods to identify the allelic variants *CYP1A1*2B* and *GSTP1*B*. The data obtained showed no statistically significant association of allelic or genotypic variants of *CYP1A1*2B* (OR = 1.06; 95% CI = 0.49-2.29) and *GSTP1*B* (OR = 1.40; 95% CI = 0.70-2.79) with OSCC.

Key words

- Oral carcinoma
- Biometabolism genes
- *CYP1A1*
- *GSTP1*
- Gene polymorphism

Introduction

Oral squamous cell carcinoma (OSCC) is highly prevalent among head and neck cancers and is a major cause of cancer morbidity and mortality worldwide. Tobacco and alco-

hol are the most important risk factors. Epidemiologic studies have indicated that various polymorphic genes such as cytochrome P450 (CYPs) and glutathione-S-transferase (GSTs) code for enzymes that are crucial in the carcinogen activation and detoxification processes.

The P450 family comprises at least five subfamilies, designated A to E (1). During biotransformation, the coded enzymes mediate phase I reactions in which xenobiotics are activated to reactive intermediate substances. The *CYP1A1* gene, located in chromosome 15q22-24 (2), codes for the enzyme aryl hydrocarbon hydroxylase, which is responsible for the metabolism of polycyclic aromatic hydrocarbons present in cigarette smoke, transforming them into carcinogens. Two described polymorphisms, *MspI* and Ile/Val (or exon 7 polymorphism), seem to increase the activity of the enzyme in homozygous individuals. The Ile/Val polymorphism occurs because of an A→G transition at position 4889, resulting in the *CYP1A1**2B allele.

The GST family codes for enzymes that are crucial in the carcinogen detoxification process (phase II), mediating the conjugation of electrophilic compounds with reduced glutathione and facilitating the excretion of many xenobiotics. The enzymes are coded for at least five distinct loci - α , μ , θ , π , and γ . *GSTP1*, located in chromosome 11q18 (3), may be of particular relevance for cancer susceptibility. Board et al. (4) described three different alleles: *GSTP1**A (wild type), *GSTP1**B and *GSTP1**C. The *GSTP1**B variant shows an A→G transition at position +313, codon 105, exon 5, changing the amino acid Ile to Val. The *GSTP1**C allele has 2 transitions, the same as those observed in the *GSTP1**B allele and a second one, located at position +314, codon 106, exon 6, changing the amino acid Ala to Val. The Ile₁₀₅→Val₁₀₅ substitution in the *B and *C variants reduces the catalytic activity of the enzyme (5). Due to this reduction in enzyme activity, the carriers of mutant alleles lost the capability to metabolize carcinogens, which could be responsible for a higher susceptibility to cancer.

In the present study, we analyzed two polymorphisms, *CYP1A1* Ile/Val and *GSTP1* BsmI, in order to obtain the gene frequen-

cies in a south Brazilian sample, conducting a case-control study to determine if there is an association between these markers and oral cancer.

Material and Methods

Human subjects

The patients (N = 72; 61 males and 11 females, mean \pm SD age 57.26 ± 11.43 years) were recruited from Erasto Gaertner Hospital (Curitiba, south of Brazil), Instituto do Câncer de Londrina and Centro Odontológico Universitário Norte do Paraná (Londrina, south of Brazil), centers specializing in the treatment of cancer, between July 2002 and November 2004. The study was approved by the local Ethics Committee. The patients and controls signed informed consent documents and answered a questionnaire about their life style, socioeconomic level, ethnicity, age, occupation, and family history of cancer, among other questions. Also, they had to fulfill the following inclusion criteria: absence of previous radiotherapy or chemotherapy and confirmation of OSCC by anatomopathologic study. Among the controls (N = 60; 49 males and 11 females, median age 53.68 ± 12.07 years), individuals with a history of any malignant disease were excluded from the study.

Laboratory methods

DNA was extracted by a salting-out method (6). PCR primers were designed according to the Genome Data Bank (GDB; Table 1).

CYP1A1 Ile/Val polymorphism. Allele-specific PCR was performed as follows: 100 ng/ μ L DNA was amplified in a total reaction volume of 50 μ L containing 20 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl₂, 2 mM of each deoxynucleotide triphosphate, 100 ng/ μ L of each primer (W + A or W + G), and 1.25 U of AmpliTaq DNA polymerase. PCR

was carried out in an Eppendorf Gradient Thermocycler after 5 min of pretreatment at 94°C, with 30 cycles of 1 min at 94°C and 2 min at 70°C, followed by 5 min at 72°C. PCR products (20 µL) were subjected to electrophoresis on 2% agarose gel at 100 V for 50 min and visualized using ethidium bromide. The primers, named A and G, only differ in one base (A or G - reflecting the polymorphism) were used in two different reactions with the reverse wild primer (W), yielding fragments with the same molecular weight (340 bp). In Figure 1 we can see two lines for each individual, the first one showing amplification of the wild allele (Ile) and the second the variant allele (Val).

GSTP1 BsmI polymorphism. The PCR-RFLP method was applied as follows: 100 ng/µL DNA was amplified in a total reaction volume of 25 µL containing 20 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl₂, 2 mM of each deoxynucleotide triphosphate, 100 ng/µL of each primer (*P105F* and *P105R*), and 1.25 U *AmpliTaq* DNA polymerase. PCR was carried out in an Eppendorf Gradient Thermocycler, with 5 min of pretreatment at 95°C, 30 cycles of 30 s at 57°C, 30 s at 72°C, and 30 s at 94°C, followed by 5 min at 72°C. After amplification, PCR products (20 µL) were cleaved by 5 U *BsmI* enzyme at 55°C for 2 h and then subjected to electrophoresis on 3.5% agarose gel at 40 V for 2 h and visualized using ethidium bromide. The primers *P105F* and *P105R* generated an amplified fragment of 176 bp corresponding to the wild genotype (Ile/Ile). After cleavage with the restriction enzyme *BsmI*, individuals with the variant allele (Ile/Val) will show two bands of 85 and 91 bp, respectively. Figure 2 shows that individual number 1 has both alleles cleaved (mutant homozygote) showing two bands (81 and 91 bp), 2 and 4 have only one allele cleaved (heterozygotes) showing three bands (176, 91, and 81 bp), and number 3 has no alleles cleaved (wild homozygote) showing a 176-bp fragment.

Table 1. Oligonucleotide primers used in this study.

Gene	Primer	Sequence
<i>CYP1A1</i>	W	5'-GAA AGG CTG GGT CCA CCC TCT-3'
	A	5'-AAG ACC TCC CAG CGG GCA AT-3'
	G	5'-AAG ACC TCC CAG CGG GCA AC-3'
<i>GSTP1</i>	<i>P105F</i>	5'-ACC CCA GGG CTC TAT GGG AA-3'
	<i>P105R</i>	5'-TGA GGG CAC AAG AAG CCC CT-3'

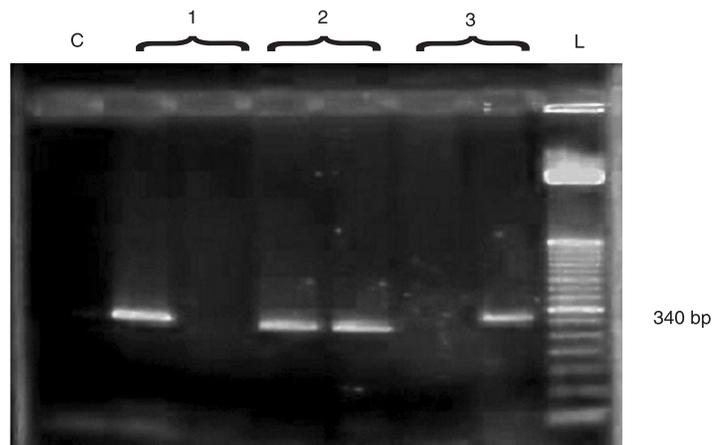


Figure 1. Banding pattern of Ile/Val polymorphism (*CYP1A1* gene). L = ladder, 50 bp; C = negative control; 1 = wild homozygote; 2 = Ile/Val heterozygote; 3 = Val/Val mutant homozygote.

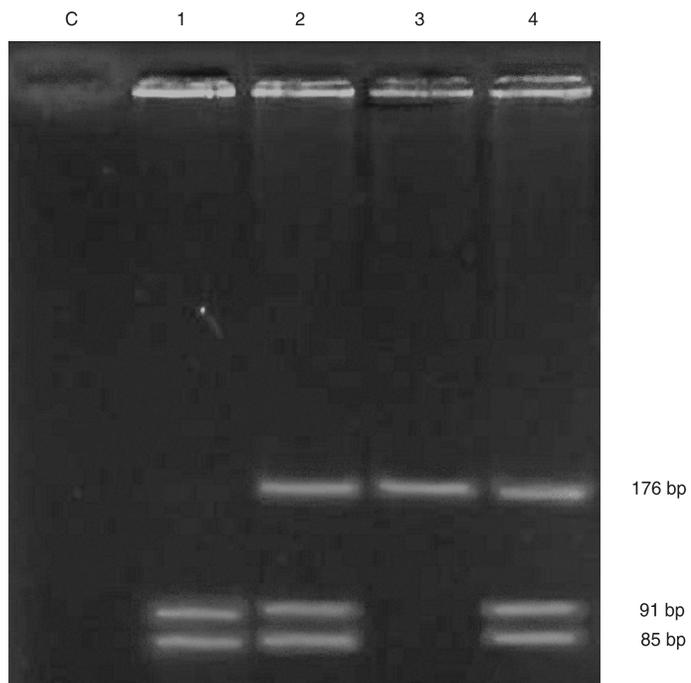


Figure 2. Banding pattern of *BsmI* polymorphism (*GSTP1* gene). C = negative control; 1 = mutant homozygote; 2 and 4 = heterozygotes; 3 = wild homozygote.

Statistical analysis

Statistical analysis was carried out by comparing the allelic frequencies of the *CYP1A1* and *GSTP1* genes between patients with OSCC and controls through a 2 x 2 contingency table in order to obtain the P value. This value was obtained by the Fisher exact test with the RxC program (7). A P value of 0.05 was adopted as significant. The genotypic frequencies observed were also compared with the expected ones based on the Hardy-Weinberg theorem. The chi-square test for homogeneity was applied in order to verify whether males and females were equally distributed among patients and controls, whether the observed genotypes were equally distributed among patients and controls, and in comparison with other studies.

Results

Males (61 patients and 49 controls) and females (11 in each group) were homogeneously distributed among the patient and control groups ($\chi^2_1 = 0.22$; $P > 0.50$). Among the 72 patients analyzed for the *CYP1A1* gene, 52 had the Ile/Ile genotype, 18 the Ile/Val genotype and 2 the Val/Val genotype. Of the 60 controls analyzed for the *CYP1A1* gene, 44 men had the Ile/Ile genotype and 16 had the Ile/Val genotype. The Val/Val genotype was not detected ($\chi^2_2 = 1.72$; $P > 0.30$). For the *GSTP1* gene, 30 patients were +/+, 34 +/-, and 8 -/-. Of the controls, 30 were +/+, 25 +/-, and 5 -/- ($\chi^2_2 = 0.98$; $P > 0.50$).

The allelic frequencies of the mutant variants *CYP1A1*2B* and *GSTP1*B* were 0.15 and 0.35, respectively, in the patient group and 0.13 and 0.29 among the controls, resulting in a total frequency of 0.14 for the *CYP1A1*2B* allele and of 0.32 for the *GSTP1*B* allele considering the patient and control groups as a whole. The genotypic frequencies observed in both groups were also in Hardy-Weinberg equilibrium, showing that the studied samples were obtained

from a population in genetic equilibrium.

The frequencies observed in patients and controls considering both genes together did not demonstrate any significant difference ($\chi^2_8 = 10.25$; $P > 0.20$).

The association study did not demonstrate a significant association between the genotypes studied and OSCC (*CYP1A1* polymorphism: OR = 1.06, 95% CI = 0.49-2.29; *GSTP1* polymorphism: OR = 1.40, 95% CI = 0.70-2.79).

Discussion

Among malignant diseases, those of epithelial cells contribute to most of the deaths due to cancer worldwide. The association between squamous cell carcinoma of the head and neck (SCCHN) and tobacco has been recognized for a long time and available molecular data suggest that these carcinogens play an important role in malignant transformation. Although these carcinogens may cause a variety of distinct genetic alterations important for the development of SCCHN (8), there is evidence that biometabolism genes may play a significant role in the transformation of benign oral lesions to malignant ones (9).

The *CYP1A1*2B* allele is related to increased susceptibility to the development of lung cancer in Japanese (10) and North-American (11) populations. *GSTP1*B* is associated with an increased risk to develop several types of cancer, including those of the bladder, testicles and prostate (12), larynx and pharynx (13), and lung (14).

We did not find a significant association between oral cancer and the *CYP1A1* (OR = 1.06, 95% CI: 0.49-2.29) and *GSTP1* (OR = 1.40, 95% CI: 0.70-2.79) genotypes. This result is in agreement with Olshan et al. (15), Ophuis et al. (16), and Xie et al. (17), but not with Park et al. (18), who concluded that the Ile/Val polymorphism is associated with an increased risk to develop OSCC. Although the great majority of studies do not demon-

strate a significant association between the Ile/Val polymorphism and OSCC, it is important to emphasize that the biometabolism genes do not act alone in an isolated manner. The evaluation of multiple genes interacting amongst themselves and after exposure to different carcinogens may also be necessary for a better understanding of this phenomenon. We performed a homogeneity test in order to evaluate the significance of the differences in haplotype frequencies (*CYP1A1/GSTP1*) between patients and controls. No significant difference was found ($\chi^2_8 = 10.25$; $P > 0.20$).

Olshan et al. (15) also demonstrated the absence of any additive or multiplicative interaction between the same genotypes and OSCC, in relation to the *GSTP1* genotype alone and SCCHN. We also did not verify any significant evidence of association between the *GSTP1* variants and OSCC (OR = 1.40; 95% CI: 0.70-2.79). Similar data can be found in a meta-analysis performed by Hashibe et al. (19). Previous studies, like those conducted by Katoh et al. (20) and Park et al. (21) have shown an elevated risk to develop OSCC in individuals with the *GSTP1*B* allele, especially in homozygous individuals.

In our study only seven individuals in the patient group were nonsmokers and the data were insufficient to provide any consistent information about the relationship between tobacco and the development of OSCC among the distinct genotypes.

Besides the divergences found among the studies that search for the association of the *GSTP1*B* allele and OSCC, new divergences are arising considering the same allele and other anatomic regions (Ryberg et al. (14); Schneider et al. (22); Abbas et al. (23); Chen et al. (24)). It should be kept in mind that one major factor contributing to the discrepancies found in the analysis of case-control studies could be the interindi-

vidual variability in the levels of enzymatic expression. The enzymatic activity of the GST enzymes varies dramatically among individuals in both normal and cancerous tissues and among tissues in the head and neck. There is also evidence that the methylation level in the promoter region of *GSTP1* is increased in prostate, liver and breast tumors (25). Thus, epigenetic alterations and other factors could be altering the expression of carcinogen-metabolizing enzymes in different individuals and in different ways. The risks associated with the specific genotypes analyzed in these studies may be obscured by these factors. The same could be valid for *CYP1A1* expression and at this time the *CYP1A1* genotype/phenotype relationship is poorly understood.

Another important cause of the divergences between different studies may be the presence of false-positive results due to bias (error type I) or to the lack of statistical power (error type II) in studies with reduced sample sizes or with inadequate controls (26,27). Unfortunately, most of the times obtaining an ideal sample size is not possible for a single research group, but even so, according to the same investigators, the results obtained in many studies can contribute enormously to research if analyzed together with the results obtained in other studies. Heterogeneity of risk between different populations could be another reason for divergences, since positive correlations are found in some populations but not in any other worldwide. Finally, the specific pathways for the activation and detoxification of carcinogens in head and neck tissues have not been characterized. Genotype analysis may provide an insight into the role of specific carcinogen pathways. Thus, future studies, including functional analysis, should be carried out, involving sufficiently large samples to allow a more definitive assessment of gene-environment interactions.

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