# Polymorphisms of the glutathione S-transferase P1 gene and head and neck cancer susceptibility

# POLYMORPHISMS OF THE GLUTATHIONE S-TRANSFERASE P1 GENE AND HEAD AND NECK CANCER SUSCEPTIBILITY

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*Accepted 20 May 2002*
*Published online 4 November 2002 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/hed.10182*

**Abstract:** *Background.* Factors determining the individual susceptibility to head and neck squamous cell carcinoma (HNSCC) are still largely unknown. An imbalance between enzymes involved in the toxification and detoxification of (pre)-carcinogens closely related to HNSCC, which may appear during smoking and alcohol consumption, may play a role. Genetic polymorphisms in glutathione S-transferases (GSTs) often result in altered detoxification, which may contribute to individual susceptibility to HNSCC.

*Methods.* We studied the frequencies of polymorphic variants in the GSTP1 gene in 235 patients with HNSCC and 285 healthy controls. In addition, data on exposure to alcohol and tobacco consumption were recorded. DNA was extracted from whole blood, and polymerase chain reaction–based methods were used to detect genetic polymorphisms.

*Results.* In patients with HNSCC and control groups, the homozygous GSTP1 BB genotype was observed in 12.3% and 13.6%, respectively. No statistical differences were found for the GSTP1 AA and GSTP1 AB/GSTP1BB genotypes.

*Conclusions.* Our study showed that genetic polymorphisms of GSTP1 are not associated with altered susceptibility to HNSCC. © 2002 Wiley Periodicals, Inc. *Head Neck* **25:** 37–43, 2003

**Keywords:** head and neck cancer; glutathione S-transferase P1; phase II enzyme; genetic polymorphism

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An imbalance in phase I drug metabolism enzymes, such as cytochromes P450 (CYPs), or phase II detoxification enzymes, such as glutathione S-transferases (GSTs), may contribute to the individual susceptibility to head and neck squamous cell carcinoma (HNSCC). These enzymes, for instance, are involved in the toxification and detoxification of metabolites of polycyclic aromatic hydrocarbons (PAHs), one of the primary carcinogens of tobacco smoke.[1] Most PAHs first require activation by phase I enzymes to become potential carcinogens that are subsequently detoxified by phase II enzymes.

As a result of conjugation with glutathione, the potential carcinogens are eliminated, and DNA or other important biomolecules are protected against damage or adduct formation.[2–5] In humans, the GST enzymes can be divided into five main classes: Alpha (GSTA), Mu (GSTM), Pi (GSTP), Theta (GSTT), and Zeta (GSTZ). Each class consists of one or more isoenzymes with different, but sometimes overlapping, substrate specificity.[4,5] At present, genetic polymorphisms have been demonstrated for *GSTM1, GSTT1,* and *GSTP1*.[5–9]

Polymorphisms in the *GSTP1* gene consist of the variant genotypes *GSTP1 AB* and *GSTP1 BB* next to the wild-type *GSTP1 AA*. The frequency of *GSTP1 AA* in healthy controls ranges from 42%–69%.[9–14] A transition of adenine (A) to guanine (G) at nucleotide 313 in exon 5 of the *GSTP1* gene results in substitution of isoleucine (Ile) to valine (Val) at position 104 in the amino acid sequence of the protein.[7,15,16] The valine variants exhibit lower specific activity and affinity for the electrophilic substrates of the enzyme.[7,13,15] The valine-containing homozygous variant, *GSTP1 BB,* occurs in approximately 10% of healthy controls, whereas the heterozygous Ile-Val variant, *GSTP1 AB,* occurs in approximately 35% of controls.[9–14]

The *GSTP1* gene encodes for the isoenzyme GSTP1-1. The GSTP1-1 enzyme level has been extensively studied in relation to tobacco-associated malignancies. The GSTP1-1 enzyme is overexpressed in many preneoplastic and neoplastic lesions. Elevated tissue levels of GSTP1-1 enzyme have been found in stomach, colorectal, bladder, oral, pharynx, larynx, lung, skin, and breast tumors compared with normal tissues of matched controls.[5,17–22] The GSTP1-1 enzyme may be involved in the resistance to chemotherapeutic agents and radiotherapy.[5,23–25] Because GSTP1-1 is also involved in the metabolism and subsequent removal of anticancer drugs, high levels of GSTP1-1 in tumors may contribute to drug resistance in several different cancers.[26–28] However, there are also contradictory reports.

Because the amino acid changes are the result of the polymorphisms close to the area of hydrophobic binding site for electrophiles, the homozygous *GSTP1 BB* and heterozygous *GSTP1 AB* gene products result in a decreased specific activity and affinity for electrophilic compounds.[7] Thus, the altered metabolic activity of these enzymes could influence susceptibility to head and neck cancer. Earlier we investigated the possible involvement in HNSCC and in benign head and neck lesions of genetic polymorphisms in *CYP1A1, GSTM1,* and *GSTT1,* but we found no enhanced rates of *GST* null genotypes in the cancer population.[29] Earlier work from our group showed that the GSTP1-1 enzyme, in a quantitative sense, is by far the most important detoxification enzyme in human head and neck tissues.[30] Therefore, we investigated genetic polymorphisms in *GSTP1* in a large series of patients with HNSCC and in matched healthy controls. We also evaluated exposure to alcohol and tobacco of these subjects and evaluated the potential effect modification between *GSTP1* polymorphisms and these environmental exposures.

## MATERIALS AND METHODS

**Patients and Controls.** This case-control study was conducted in the patient referral region of Maastricht University Hospital. A total of 235 Caucasian patients (185 men, 50 women) with primary HNSCC were recruited during their scheduled admission at the Department of Otolaryngology, Head and Neck Surgery of the Maastricht University Hospital between 1994–1998 (mean age 59.1 years; range, 23–86 years). This group consists of 100 patients with laryngeal carcinoma (mean age, 59.9 years; range, 35–85 years), 114 patients with oral cavity/oropharyngeal carcinoma (mean age, 58.0 years; range, 23–86 years), and a group of 21 patients (mean age, 61.4 years; range, 41–82 years) with hypopharyngeal carcinoma.

From the same base population, a group of 285 healthy Caucasian blood donors (199 men, 86 women) were recruited at the Blood Transfusion Centre Limburg and served as a control group, frequency matched on age in 10-year age groups (mean age, 50 years; range, 19–91). The investigations were approved by the Medical Ethical Review Committee of the Academic Hospital of the University of Maastricht, and informed consent was obtained from all patients and controls according to the Helsinki Declaration II (1975, revised 1983).

**Exposure Assessment, Blood Sampling, and Assessment of Genetic Polymorphisms.** Information on tobacco use and alcohol consumption was collected for all patients and controls by interview. The amount of pack years (PY) was calculated as the number of years smoking × the number of packs a day (assuming 20 cigarettes = 1 pack). Never smokers were defined as subjects who had smoked less than 1 pack year cumulatively.

Blood was collected by venapuncture in sterile siliconized EDTA K3 (15%) 4-mL Vacutainer tubes (Becton Dickinson, San Jose, CA). Immediately after collection, whole blood was stored at –20°C until use. Genomic DNA was isolated from whole blood using the Wizard genomic DNA purification kit, according to the instructions of the manufacturer (Promega, Madison, WI).

Genetic polymorphisms were detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). For studying the genetic polymorphism in *GSTP1,* a primer set (105F and 105R) was designed so that the presence of the A to G substitution at codon 313 resulted in the appearance of an *Alw261* restriction enzyme site.[9] All primers were synthesized by Life Technologies (Breda, The Netherlands). All chemicals needed for PCR were purchased from Promega (Madison, WI).

**Statistical Analysis.** The statistical program SPSS 8.0 for Windows was used. Information on *GSTP1* genotype, smoking, or alcohol was missing in 0, 0, and 11 of the cases and 0, 3, and 1 of controls, respectively. For the purpose of the analysis, the PY of cigarette smoking were categorized as 0, 1–20, 21–40, 41–60, and 60+ PY or in increments of 10 PY. Alcohol consumption was categorized as 0–9, 10–50, and 51+ g/ day. Because there were only three subjects who indicated never to have consumed alcohol, the first category consists of 0–9 g/day. Analyses were conducted for the total group of patients with HNSCC, as well as for oral cavity/oropharyngeal and laryngeal carcinoma separately. Because of small numbers of hypopharyngeal carcinoma, this group was not evaluated separately.

The association between *GSTP1* polymorphisms, smoking, alcohol, and HNSCC was modeled through multivariate logistic regression analysis, controlling for age and gender. First, analyses were conducted to examine the associations between *GSTP1* genotype and HNSCC, controlling for age, gender, smoking, and alcohol. Specifically, we evaluated whether subjects with *GSTP1 AA* are less susceptible to HNSCC than those with *GSTP1 AB/BB*. Likewise associations between smoking and HNSCC or alcohol consumption and HNSCC were examined, controlling for age, gender, and alcohol or smoking, respectively. The strength of the associations was estimated as odds ratios combined with 95% confidence intervals. Subsequently, logistic regression analyses were performed in which the association between smoking or alcohol and HNSCC (and its subgroups) was evaluated within subgroups of the *GSTP1* genotype, the wild-type *GSTP1 AA* group, and the combined heterozygote/homozygote *GSTP1 AB/BB* group. Likelihood ratio tests were conducted to test statistical significance of regression model terms, as well as to test for trends and interactions. Two-sided *p* values are reported throughout. Finally, the interaction between smoking and alcohol was evaluated, specifically within subgroups of the *GSTP1* genotype.

**Table 1.** Frequency distribution of *GSTP1* genotypes in HNSCC cases and controls.

| *GSTP1* genotype | Controls *N* (%) | All HNSCC cases *N* (%) | Oral/oro-pharynx *N* (%) | Larynx *N* (%) |
|---|---|---|---|---|
| *AA* | 125 (43.9) | 116 (49.4) | 53 (46.5) | 51 (51.0) |
| *AB* | 121 (42.5) | 90 (38.3) | 48 (42.1) | 36 (36.0) |
| *BB* | 39 (13.6) | 29 (12.3) | 13 (11.4) | 13 (13.0) |
| Total | 285 | 235 | 114 | 100 |

## RESULTS

Table 1 shows the frequency analyses for the A to G substitution at codon 313 in the *GSTP1* gene, which was examined in 235 HNSCC patients and 285 controls. The *GSTP1 BB* frequency is low in the control group, as well as in the total HNSCC group or its subgroups. To maintain values for appropriate statistical analysis, we combined the frequencies for *GSTP1 AB* and *GSTP1 BB* (Tables 3–5).In Table 2, the frequency distribution is given on the smoking habits and the alcohol con-

**Table 2.** Frequency distribution of smoking and alcohol consumption in HNSCC cases and controls.

| Covariable | Controls *N* (%) | All HNSCC cases *N* (%) | Oral/oro-pharynx *N* (%) | Larynx *N* (%) |
|---|---|---|---|---|
| Smoking pack years | | | | |
| 0 | 86 (30.2) | 13 (5.5) | 7 (6.1) | 3 (3.0) |
| 1–20 | 130 (45.6) | 36 (15.3) | 20 (17.5) | 13 (13.0) |
| 21–40 | 60 (21.1) | 113 (48.1) | 52 (45.6) | 53 (53.0) |
| 41–60+ | 9 (3.2) | 73 (31.1) | 35 (30.7) | 31 (31.0) |
| Total | 285 | 235 | 114 | 100 |
| Alcohol g/day | | | | |
| 0–9 | 139 (48.8) | 18 (8.0) | 7 (6.3) | 10 (10.9) |
| 10–50 | 142 (49.8) | 106 (47.4) | 44 (39.6) | 54 (58.7) |
| 51+ | 3 (1.1) | 100 (44.6) | 60 (54.1) | 28 (30.4) |
| Total | 284 | 224 | 111 | 92 |

**Table 3.** Association between alcohol, smoking, and *GSTP1* genotype and HNSCC (total group of cases and subgroups of oral/oropharyngeal and laryngeal cancer).

| Risk factor | Total HNSCC *N* = 235 OR (95% CI) | Oral/oropharynx *N* = 114 OR (95% CI) | Larynx *N* = 100 OR (95% CI) |
|---|---|---|---|
| Alcohol g/day | | | |
| 0–9 | 1.0* (ref) | 1.0* (ref) | 1.0* (ref) |
| 10–50 | 5.36 (2.68–10.72) | 8.23 (3.05–22.20) | 5.00 (1.97–12.68) |
| 51+ | 194.16 (49.21–766.31) | 420.03 (87.47–2016.92) | 113.36 (22.91–560.84) |
| Test for trend, *p* value | <.001 | <.001 | <.001 |
| Smoking Pack years | | | |
| Increment 10 PY | 1.80† (1.51–2.14) | 1.63† (1.34–1.97) | 1.85† (1.51–2.26) |
| *GSTP1 AB/BB* | 1.0‡ (ref) | 1.0‡ (ref) | 1.0‡ (ref) |
| *GSTP1 AA* | 0.99 (0.58–1.71) | 0.78 (0.38–1.60) | 0.90 (0.46–1.79) |

**Odds ratio, adjusted for age, gender, smoking, and* GSTP1 *genotype.*
*†Odds ratio, adjusted for age, gender, alcohol consumption, and* GSTP1 *genotype.*
*‡Odds ratio, adjusted for age, gender, alcohol consumption, and smoking.*
*Abbreviations: CI, confidence interval; OR, odds ratio; ref, reference value.*

sumption in HNSCC cases, subgroups of HNSCC, and the control group. The HNSCC group in total, as well as the two subgroups, show a significantly higher tobacco exposure compared with the control group. The control group shows significantly lower alcohol consumption than the HNSCC cases (total or subgroups). In the oral/oropharyngeal subgroup, the rate of daily alcohol consumption more than 51 g/day is much higher compared with the laryngeal subgroup (54.1% vs 30.4%, respectively). Alcohol consumption more than 51 g/day hardly exists in the control group.

Table 3 shows that the odds ratios (OR) for alcohol or smoking are similar in the HNSCC group as a total and the two subgroups, except for alcohol consumption more than 51 g/day, when there is a higher OR in the oral/oropharyngeal group. To provide appropriate statistical values, smoking is displayed with an increment of 10 PY. When no adjustment is made for smoking and alcohol (but only for age and gender), the OR for *GSTP1 AA* vs *GSTP1 AB/BB* will be 1.31 in the total HNSCC group.

The association between alcohol and smoking

**Table 4.** Association between alcohol consumption, smoking, and HNSCC in subgroups of *GSTP1* genotype.

| Risk factor | Total HNSCC OR (95% CI) | Oral/oropharynx OR (95% CI) | Larynx OR (95% CI) |
|---|---|---|---|
| Subgroup *GSTP1 AA* (108 cases, 122 controls) | | | |
| Alcohol g/day | | | |
| 0–9 | 1.0* (ref) | 1.0* (ref) | 1.0* (ref) |
| 10–50 | 7.89 (2.75–22.68) | 15.88 (2.66–94.66) | 6.95 (1.76–27.50) |
| 51+ | 232.24 (38.22–1407.08) | 696.88 (70.08–6930.15) | 140.16 (16.22–1211.12) |
| Test for trend, *p* value | <.001 | <.001 | <.001 |
| Smoking pack years | | | |
| Increment 10 PY | 1.51† (1.21–1.80) | 1.34† (1.10–1.80) | 1.63† (1.21–1.97) |
| Subgroup *GSTP1 AB/BB* (116 cases, 159 controls) | | | |
| Alcohol g/day | | | |
| 0–9 | 1.0* (ref) | 1.0* (ref) | 1.0* (ref) |
| 10–50 | 4.21 (1.65–10.70) | 5.57 (1.64–18.89) | 3.70 (1.07–12.84) |
| 51+ | 191.67 (20.88–1759.33) | 307.88 (26.42–3588.09) | 74.82 (6.76–828.31) |
| Test for trend, *p* value | <.001 | <.001 | <.001 |
| Smoking pack years | | | |
| Increment 10 PY | 2.28† (1.73–3.01) | 2.41† (1.67–3.47) | 2.46† (1.74–3.49) |

**Adjusted for age, gender, smoking.*
*†Adjusted for age, gender, and alcohol.*
*Abbreviations: CI, confidence interval; OR, odds ratio; ref, reference value.*

and the *GSTP1* polymorphisms is shown in Table 4. For oral/oropharyngeal cases with the *GSTP1 AA* genotype, alcohol consumption more than doubles the OR compared with the *GSTP1 AB/BB* genotype. This is also shown for laryngeal carcinoma. In the oral/oropharyngeal cases, as well as the laryngeal cases with the *GSTP1 AB/BB* genotype, odds ratios are increased by smoking compared with the *GSTP1 AA* genotype. We tested for interaction of alcohol consumption between subgroups of GSTP1 in the total HNSCC group; LR-X = 0.96, $df = 2$, $p > .05$. Also the interaction of smoking between subgroups of *GSTP1* in the total HNSCC group is calculated; LR-X = 6.04, $df = 1$, $p < .025$.

In Table 5 we show a significant interaction between smoking and alcohol consumption in HNSCC susceptibility when not adjusted for *GSTP1* genotype. The effect of smoking is stronger when more alcohol is consumed (OR increases from 1.27 to 3.53). However, because of small numbers (especially in high alcohol consumption category), interaction between alcohol and smoking and *GSTP1* genotype cannot be fully calculated.

**Table 5.** Interaction between alcohol and smoking regarding risk of HNSCC in total group and in subgroups defined by *GSTP1* genotype.

| Alcohol category | Smoking OR (95% CI) per 10 pack years |
|---|---|
| Total group (224 cases, 281 controls) | |
| Alcohol g/day | |
| 0–9 | 1.27 (1.03–1.57) |
| 10–50 | 2.46 (1.90–3.20) |
| 51+ | 3.53 (1.27–9.81) |
| Test for interaction smoking/alcohol, X2 ($p$) | 18.2 ($p < .01$) |
| Subgroup *GSTP1 AA* (108 cases, 122 controls | |
| Alcohol g/day | |
| 0–9 | 1.24 (0.96–1.60) |
| 10–50 | 2.08 (1.44–3.01) |
| 51+ | np |
| Test for interaction smoking/alcohol, X2 ($p$) | – |
| Subgroup *GSTP1 AB/BB* (116 cases, 159 controls) | |
| Alcohol g/day | |
| 0–9 | 1.43 (0.91–2.28) |
| 10–50 | 2.85 (1.96–4.16) |
| 51+ | 0.57 (0.06–5.48) |
| Test for interaction smoking/alcohol, X2 ($p$) | 3.5 ($p > .05$) |

*Abbreviations: CI, confidence interval; OR, odds ratio; np, no estimation possible.*

## DISCUSSION

Glutathione S-transferases are involved in the detoxification of a wide variety of chemical carcinogens, including those derived from cigarette smoke.[5]

The enzyme GSTP1-1 is known to catalyze the detoxification of PAH in vitro and may be a major enzyme involved in the detoxification of tobacco-related metabolic products in vivo.[5] The expression in tissues may vary, depending on site or localization, and immunohistochemical studies have shown a constant and widespread GSTP1-1 expression throughout pharyngeal and laryngeal squamous cell epithelium in both normal and tumor tissue.[10] Earlier we showed that GSTP1-1 is the most important GST enzyme in human head and neck tissues.[30] Therefore, we studied the occurrence of polymorphisms of this gene in patients with different head and neck tumors and controls.

We previously reported that the *GSTM1* and *GSTT1* null genotypes are not associated with an increased susceptibility for HNSCC.[29] In one of the largest studies performed so far, we found no significant association between risk of HNSCC and occurrence of genetic polymorphisms in the *GSTP1* gene. This was also found for oral/oropharyngeal cancers and laryngeal cancers separately.

The GSTP1-1 enzyme is overexpressed in many neoplastic lesions, such as malignancies of stomach, colorectum, bladder, oral tissue, pharynx, larynx, lung, skin, and breast compared with corresponding normal tissues.[5,17–22,30] However, when estimating GSTP1-1 serum levels in patients with head and neck cancer, we found significantly higher levels compared with controls, but no association with *GSTP1* genotype was observed.[31] This may strongly indicate that the overexpression of GSTP1-1 in head and neck cancer is not dependent on genotype but most probably transcriptionally regulated.

We found a strong association between smoking and alcohol consumption and HNSCC susceptibility. Assessment of smoking or alcohol consumption is difficult, especially with respect to patient history for the total accumulative exposure. Exposure usually takes place over many years and is not always consistent. In addition, different cigarette brands may yield completely different nicotine and tar exposures. High consumption of alcohol often is not revealed by patients because of its social unacceptance. It there-

fore would help to determine biomarkers such as serum gamma-glutamyltransferase, which could be indicative of recent consumption of alcohol. Development of biomarkers that could indicate the exposure and the period of consumption would be more helpful.

In our control population, *GSTP1 BB* occurs in 13.6% and *GSTP1 AB* in 42.5%. Our data for the healthy controls are in agreement with the genotype frequencies in the control populations reported by other researchers.9–14 Similar observations were found for the HNSCC group (12.3% and 38.3%, respectively).

Our data for HNSCC patients are also in agreement with several other studies performed. Lin et al[14] found that polymorphisms in *GSTP1* were not associated with increased risk for esophageal cancer. They even found a slightly lower occurrence of *GSTP1 AB* or *BB* genotypes in cancer and hyperplasia cases than in controls.[14] Jourenkova-Mironova et al[32] and Matthias et al[10] found no association between the *GSTP1 AB* or *BB* genotypes and risk of larynx cancer. Harries et al[9] reported an association between the occurrence of the *GSTP1* polymorphism and susceptibility to bladder or testicular cancer but found no association with breast or colon cancer. In other studies, no association was found between the *GSTP1* polymorphism and colorectal, lung, or breast cancer.[33,34]

Several other studies, however, revealed a significant correlation between occurrence of *GSTP1* polymorphisms and preneoplastic and neoplastic lesions. Lung cancer patients had a significant higher frequency of *GSTP1 BB* genotype (15.9%) and a lower frequency of *GSTP1 AA* genotype (38.4%) than controls (9.1% and 51.5%, respectively).[12] Homozygous *GSTP1 BB* was found significantly more often in patients with Barrett's esophagus and esophageal adenocarcinoma.[35] Matthias et al[10] reported that *GSTP1* polymorphism influences susceptibility to pharyngeal but not to laryngeal squamous cell carcinoma. However, when comparing our patient groups in a similar way as Matthias et al[10] (oral cavity/oropharyngeal SCC and hypopharyngeal SCC versus laryngeal SCC), we did not find a significant difference in the genotype distributions. Ryberg et al[12] reported that patients with the *GSTP1 BB* genotype had higher DNA adduct levels in lung tissue than the *GSTP1 AA* genotypes. They also found a linear trend between DNA adduct levels and occurrence rates of *GSTP1 B* alleles.

The variant genotypes *GSTP1 AB* and *GSTP1 BB* showed a different specific activity and affinity for several electrophilic substrates but were identical in their affinity for glutathione.[7,15] The properties of the variant genotypes were conducted in "in vitro experiments" with recombinant proteins by Ali Osman et al,[7] which, however, does not necessarily provide adequate information on the enzymes and their reactions "in vivo." In agreement with the in vitro results, Watson et al[13] and van Lieshout et al[35] reported a significantly lower conjugating activity with 1-chloro-2,4-dinitrobenzene (CDNB) in lung and esophageal tissue samples, respectively, in patients with *GSTP1 AB* and *GSTP1 BB* compared with *GSTP1 AA* genotypes.

In summary, we found no evidence for a possible genetic predisposition to HNSCC because of genetic polymorphisms in the *GSTP1* gene. This suggests that the carcinogens involved in the etiology of HNSCC may not be critically dependent on detoxification by GSTP1-1. Further studies on other combinations of polymorphic genotypes in detoxification enzymes and their relation to local and systemic enzyme concentration seem to be justified.

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