Glutathione S-Transferase Polymorphisms and Risk of Differentiated Thyroid Carcinomas

A Case-Control Analysis

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Objective: To determine the association between glutathione S-transferase (GST) polymorphisms and the risk of differentiated thyroid carcinoma (DTC) and benign thyroid tumors.

Design: Case-control study.

Setting: Tertiary care cancer center.

Patients: Two hundred one patients with DTC, 103 patients with benign thyroid tumors, and 680 cancer-free control subjects.

Main Outcome Measures: Results of a polymerase chain reaction–based assay for genotyping. A multivariate logistic regression analysis was performed with adjustment for age, sex, ethnicity, tobacco use, and alcohol use.

Results: The patients with DTC were younger, more likely to be female and nonwhite, and less likely to smoke or consume alcohol than the controls. Overall, 55.2% of the DTC cases and 52.6% of the controls were null for the gene for GST-µ1 (GSTM1) (P=.52), and 25.4% of the DTC subjects and 20.6% of the controls were null for the GST-θ1 gene (GSTT1) (P=.15). However, 15.9% of the DTC cases but only 9.4% of the controls were null for both genes (P=.009). In addition, the results of the adjusted multivariate regression analysis suggested that having both null genotypes was associated with an increased risk for DTC (odds ratio [OR], 2.1 [95% confidence interval, 1.3-3.5; P=.003]). This was particularly true for women (OR, 2.5), current smokers (OR, 3.6), and nonwhites (OR, 5.6). A similar analysis demonstrated a nonsignificant association between these genotypes and benign thyroid tumors (OR, 1.5 [95% confidence interval, 0.7-3.0; P=.30]).

Conclusions: Our results suggest that the simultaneous presence of the GSTM1- and GSTT1-null genotypes is a susceptibility factor for DTC. Such knowledge may ultimately help refine cancer prevention efforts; however, larger studies are needed to verify these findings.

has been investigated for several GST isoenzymes, particularly GST-µ1 (GSTM1 gene) and GST-01 (GSTT1 gene). Both genes possess null genotypes with no enzyme activity. Epidemiologic studies have found that individuals with homozygous deletions of these genes (ie, GSTM1 null or GSTT1 null) have an increased risk of cancer at a number of different body sites, including the head and neck, lungs, breasts, and brain.

In this molecular epidemiologic case-control study, we explored the association between GSTM1- and GSTT1-null genotypes and the risk of DTC, with adjustments for age, sex, ethnicity, and tobacco and alcohol use. We hypothesized that the lack of GST-µ1 and GST-01 isoenzymes puts individuals at risk of DTC by limiting their ability to detoxify carcinogens resulting from potential exposures or products of oxidative stress. Subjects with benign thyroid disease (BTD) and those without a history of cancer were included in this study as intermediate comparison and control groups, respectively.

### METHODS

#### STUDY SUBJECTS

This was a tertiary cancer center–based, case-control study. From November 1, 1999, to October 31, 2004, patients who presented to the Head and Neck Surgery Clinic at The University of Texas M. D. Anderson Cancer Center with a diagnosis of DTC or a thyroid mass suggestive of DTC were recruited into a molecular epidemiologic study of nonsquamous cell carcinoma of the head and neck before undergoing definitive surgical therapy. Final histopathologic diagnoses were obtained from a review of the medical records. Patients whose final histopathologic diagnoses were not DTC (eg, anaplastic, medullary, or mucoepidermoid carcinoma or lymphoma of the thyroid) were excluded. Patients with BTD, as determined on pathologic examination, were included as an intermediate comparison group. We also included cancer-free control subjects who had been recruited from among spouses and other visitors who accompanied patients for a molecular epidemiologic study of head and neck squamous cell carcinoma from November 1, 1996, to March 31, 2004.

Each study subject had completed a self-administered questionnaire, providing demographic, socioeconomic, risk exposure, and family medical history data. Smokers were defined as those subjects who had smoked more than 100 cigarettes in their lifetimes. Subjects who had quit smoking more than 1 year before enrollment in the study were classified as former smokers. Those who had smoked alcohol for more than 1 year before enrollment were defined as drinkers; those drinkers who had not drunk alcohol for more than 1 year before enrollment were defined as former drinkers; and all other drinkers were considered current drinkers. Ethnicity was categorized by the subject as non-Hispanic white, Hispanic, African American, or Asian.

After institutional review board–approved informed consent had been obtained, each participant had donated 20 mL of blood for cell culture and DNA extraction (before the initiation of treatment, if applicable).

#### GENOTYPING OF GSTM1 AND GSTT1

A leukocyte cell pellet was obtained from the buffy coat by centrifugation of 1 mL of whole blood. The pellet was used for genomic DNA extraction with a DNA blood kit (Qiagen DNA Blood Mini Kit; Qiagen, Inc, Valencia, Calif) according to the manufacturer’s instructions. We used a multiplex polymerase chain reaction (PCR) assay to simultaneously determine the presence or absence of the GSTM1 and GSTT1 genes and the dihydrofolate reductase (DHFR) gene as an internal control for amplification failure secondary to DNA degradation. The primers used for GSTM1 were 5’-GAA TTC CTC GCT AAG CTG ATA CAG GTC GGT G-3’ and 5’-GTT GGC CTC AAA TAT ACG GTG G-3’, generating a 480-base pair (bp) fragment. For GSTT1, the primers used were 5’-TTC CTT ACT CCT GGT CCT CAC ATC TC-3’ and 5’-TCA CGG CAT GCG CAT CGG-3’, generating a 215-bp fragment. The primers used for DHFR were 5’-CAT CCG CAA GAA CGG GCA CTT-3’ and 5’-ACC GAA GCC TCC ACC CAG TTG-3’, generating a 280-bp fragment. The absence of a 480- or a 215-bp band in the PCR assay indicated the presence of a GSTM1-null or a GSTT1-null genotype, respectively. When no band was evident at 280 bp, the PCR assay was considered unsuccessful, most likely owing to degraded DNA because DHFR is typically easily amplified.

The GSTM1, GSTT1, and DHFR genes were coamplified in a 40-µL reaction mixture containing 100 ng of genomic DNA as the template, 3.5 pmol of each GSTM1 primer, 2.9 pmol of each GSTT1 primer, 6.2 pmol of each DHFR primer, 0.1 mM deoxynucleoside triphosphate, 1× PCR buffer (50 mM potassium chloride, 10 mM Tris hydrochloride [pH 9.0 at 25°C]; 0.1% Triton X-100, and 1.5 mM magnesium chloride), and 1.0 U of Taq DNA polymerase (Sigma-Aldrich Corp, St Louis, Mo). The PCR profile consisted of an initial melting step of 95°C for 5 minutes; 35 cycles of 95°C for 30 seconds, 58°C for 35 seconds, and 72°C for 60 seconds; and a final elongation step of 72°C for 10 minutes. The PCR products were separated on a 2% agarose gel and photographed with a digital imaging system (IS-1000; Alpha Innotech Co, San Leandro, Calif).

#### STATISTICAL ANALYSIS

The demographic characteristics of the participants (the DTC, BTD, and control groups) were compared using a χ² analysis. Age distribution was compared using an unpaired t test with a Satterthwaite adjustment for unequal variances. The GST genotypes were first analyzed as a dichotomized variable, with GSTM1 null genotype and 1 being the other genotype. Univariate types were first analyzed as a dichotomized variable, with 0 being the null genotype and 1 being the other genotype. Univariate analyses were performed with commercially available software (SAS, version 9.0 [SAS Institute, Inc, Cary, NC] or STATA, version 7.0 [StataCorp, College Station, Tex]). A P value of .05 was preset as the level of significance.

#### RESULTS

We included 201 patients with DTC, 103 patients with BTD, and 680 cancer-free controls in this study. The demographic characteristics of case subjects and controls are shown in Table 1. The mean±SD age of the controls was 54.1±12.3 (median, 54.0; range, 20-85) years; the DTC subjects, 45.6±14.6 (median, 44.0; range,
18-86) years ($P < .001$); and the BTD subjects, 48.1 $\pm$ 14.4 (median, 48.0; range, 18-83) years ($P < .001$). The DTC cases included 179 with papillary thyroid carcinoma (89.0%), 15 with follicular thyroid carcinoma (7.5%), and 7 with Hürthle cell carcinoma (3.5%). Radiation exposure history was not available for all study subjects, but only 6 patients with DTC and 4 with BTD had a known history of radiation exposure.

As shown in Table 1, there were higher proportions of young patients (age, $\leq$ 45 years), women, Hispanic patients, never smokers, and never drinkers among the DTC subjects compared with the controls. There were also more young patients and women in the BTD group than in the control group. The differential distributions of these demographic and exposure variables were adjusted for with multivariate logistic regression models and subgroup statistical analyses.

The percentages of GSTM1- and GSTT1-null cases were higher in the DTC group compared with the control group (55.2% vs 52.6% and 25.4% vs 20.6%, respectively), but these differences were not statistically significant ($P = .009$). Of significance, however, was that 15.9% of the DTC cases and only 9.4% of the controls were null for both the GSTM1 and GSTT1 genes. Compared with the controls, the BTD group also had higher percentages of GSTM1-null, GSTT1-null, and both-null
cases, although these differences were not statistically significant.

After dichotomization of the data into null and positive genotypes, the calculated crude ORs for the GSTM1-null genotype as a risk factor for DTC and BTD showed a minimal, statistically insignificant risk increase (OR, 1.1 for both groups) (Table 3). The findings for the GSTT1-null genotype were similar. However, the crude OR for the combined GSTM1/GSTT1-null genotype as a risk factor for DTC was 1.8 (95% CI, 1.2-2.9) and was statistically significant (P = .01). The crude OR for the both-null genotype as a risk factor for BTD (1.3) was lower than that for DTC and did not reach statistical significance (P = .48). When the data sets were trichotomized into 3 groups (GSTM1/GSTT1 positive; GSTM1 or GSTT1 null; and GSTM1/GSTT1 null), the combined GSTM1/GSTT1-null genotype was associated with a statistically significant risk for DTC, with a crude OR of 1.7 (95% CI, 1.1-2.9; P = .03). However, there was no dose-response relationship between the number of null genotypes and the risk for DTC. When trichotomized, having either null genotype was associated with a 1.3-fold higher risk of BTD, and having the combined null genotype was associated with a 1.5-fold higher risk of BTD (P = .29 and P = .28, respectively).

A multivariate logistic regression analysis was then performed to adjust for the residual effects of the variables listed in Table 1, including age, sex, ethnicity, smoking status, and alcohol status (Table 3). After adjustment, having the combined GSTM1/GSTT1-null genotype remained a significant risk factor for DTC in the dichotomized and trichotomized data analyses (OR, 2.1 [95% CI, 1.3-3.5; P = .003] and OR, 1.9 [95% CI, 1.1-3.3; P = .02], respectively). The risks for the BTD group were intermediate but were not statistically significant (ORs, 1.5 and 1.6, respectively).

After stratification for the potential confounding variables listed in Table 1, some notable differences in genotype frequency distributions were seen between subgroups of DTC cases compared with the controls (Table 4). For all subgroups (age, sex, ethnicity, tobacco use, and alcohol use), patients with DTC had a higher frequency of GSTM1/GSTT1-null findings than did the controls. This difference was significant for patients with DTC who were young (≤45 years), female, non-white, and current drinkers. The difference was of borderline significance for patients who were older than 45 years, male, and current and never smokers. Increasing tobacco use was associated with an increasing GSTM1/GSTT1-null genotype frequency in DTC cases but not in controls. In a similar subgroup analysis for the BTD group, higher frequencies of the GSTM1/GSTT1-null genotype were observed for nearly all BTD subgroups compared with controls, with the exception of former smokers and current drinkers, and this difference was significant only for current smokers (P = .02, data not shown).

Genotype risk analysis was then performed for patients with DTC stratified by the potential confounding variables and adjusted by multivariate logistic regression analysis (Table 4). The combined GSTM1/GSTT1-null genotype was associated with a significant risk for DTC for those who were 45 years or younger, female, non-white, and current drinkers. For subjects who were older, male, and current and never smokers, there was a borderline association. After multivariate adjustment, the increased risk estimate remained statistically significant for patients who were 45 years or younger, female, non-white, never smokers, and current never drinkers, and a borderline association was found for current smokers. A similar genotype frequency and risk analysis for the BTD group did not demonstrate a significant risk association in any of the subgroups (data not shown).

### Table 3. GST Genotype Risk Estimates

<table>
<thead>
<tr>
<th>Genotype Risk Estimates</th>
<th>DTC Group, OR (95% CI); P Value</th>
<th>BTD Group, OR (95% CI); P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dichotomized</strong></td>
<td>Crude</td>
<td>Adjusted*</td>
</tr>
<tr>
<td>GSTM1 positive</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>GSTM1 null</td>
<td>1.1 (0.8-1.5); .52</td>
<td>1.1 (0.8-1.5); .58</td>
</tr>
<tr>
<td>GSTT1 positive</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>GSTT1 null</td>
<td>1.3 (0.9-1.9); .15</td>
<td>1.4 (0.9-2.0); .12</td>
</tr>
<tr>
<td>GSTM1 positive and/or GSTT1 positive</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Both null</td>
<td>1.8 (1.2-2.9); .01</td>
<td>2.1 (1.3-3.5); .003</td>
</tr>
<tr>
<td><strong>Trichotomized</strong></td>
<td>Crude</td>
<td>Adjusted*</td>
</tr>
<tr>
<td>Neither null</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Either null</td>
<td>0.9 (0.7-1.3); .63</td>
<td>0.8 (0.6-1.2); .37</td>
</tr>
<tr>
<td>Both null</td>
<td>1.7 (1.1-2.9); .93</td>
<td>1.9 (1.1-3.3); .92</td>
</tr>
</tbody>
</table>

**Abbreviations:** BTD, benign thyroid tumors; CI, confidence interval; DTC, differentiated thyroid carcinoma; GST, glutathione S-transferase; GSTM1, gene for GST-M1; GSTT1, gene for GST-T1; OR, odds ratio.

*Adjusted for age, sex, ethnicity, tobacco use, and alcohol use.

In this molecular epidemiologic case-control study of patients with DTC, patients with BTD, and cancer-free controls, we demonstrated an association between the combined GSTM1/GSTT1-null genotype and an increased risk of DTC. This finding was confirmed after adjustment for age, sex, ethnicity, smoking, and alcohol use and in our subgroup analyses.

Genetic polymorphisms of drug-metabolizing enzymes are related to cancer risks secondary to their differ-
fering abilities to activate and deactivate environmental carcinogens and mutagens. The GST enzymes have been shown to protect organisms from reactive oxygen compound damage through their abilities to bind with glutathione, and variations in the efficiencies of these enzymes may influence cancer risks in certain populations. Approximately 50% and 20% of the white population in the United States have the GSTM1- and GSTT1-null genotypes, respectively, although considerable allele frequency differences exist among various ethnicities. We found a similar proportion of GSTM1- and GSTT1-null genotypes in the control population of our study (52.6% and 20.6%, respectively). The fact that radiation exposure is a clear risk factor for DTC further implicates an organism’s ability to neutralize reactive oxygen species as a potential risk factor for thyroid carcinoma.

We found that subjects with the combined GSTM1/GSTT1-null genotype had a statistically significantly elevated risk for DTC compared with the controls, although the magnitude of association was modest (adjusted OR, 2.1). The GSTM1/GSTT1-null genotype was also found to be associated with an elevated risk of BTD, with an OR that was less than that of DTC, as expected for an intermediate-risk group (adjusted OR, 1.5), but the difference was not statistically significant. The findings suggest a synergistic effect of the GSTM1- and GSTT1-null genotypes. This should not be a surprise given the number and complexity of isoenzymes in the GST system and their variability in expression. Different GST isoenzymes can have overlapping specificity for substrates and a certain amount of redundancy in function, so a deficiency in the activity of a single GST isoenzyme may be compensated for by another isoenzyme. Consequently, lack of function in the GST system may be associated with increased cancer risk only if multiple isoenzymes are disabled.

Results of the risk association between GST polymorphisms and thyroid carcinoma in the medical literature to date have been somewhat conflicting. Our findings are consistent with those reported by Morari et al, who compared 67 patients with thyroid carcinoma with 300 cancer-free controls and found a 2.6-fold increased risk for thyroid carcinoma for those with the GSTM1/GSTT1-null genotype. A study of 103 patients with thyroid carcinoma and 204 healthy matched controls similarly found that the simultaneous presence of GSTM1-null, GSTT1-null, and GST-π1 (GSTP1) Ile/Ile polymorphisms led to a 2.9-fold increased risk of thyroid carcinoma. However, in that study, the combination of GSTM1- and GSTT1-null genotypes was not associated with a significant increase in risk for thyroid carcinoma. In another study involving 134 patients with thyroid carcinoma and 116 controls, the presence of GSTM1, GSTT1, and GSTP1 polymorphisms, individually and in combination, was not associated with risk for thyroid carcinoma (combined GSTM1/GSTT1-null OR, 1.09 [95% CI, 0.49-2.44; P = .83]).

Age has been implicated as a modifier of thyroid carcinoma’s clinical behavior, but a younger age at diagnosis may also be a marker of genetic susceptibility. Canbay et al, in a study of the GSTM1 genotype, found that the GSTM1-null genotype alone may be a susceptibility factor for thyroid carcinoma in patients younger than 40 years. However, no correlation between age at onset and risk for thyroid carcinoma was seen for the GSTM1 and GSTT1 genotypes in other studies. The combined GSTM1- and GSTT1-null genotype was associated with an increased risk for DTC in patients who were 45 years or younger in our subgroup analysis. This finding appears to be consistent with the hypothesis that the absence of the GST enzyme leads to increased susceptibility to cancer, resulting in an earlier onset of the disease.

Table 4. GSTM1/GSTT1-Null Genotype Frequency Distribution and Risk Estimates for DTC and Control Groups Stratified by Selected Factors

<table>
<thead>
<tr>
<th>Variable</th>
<th>GSTM1/GSTT1 Null, % of Subjects</th>
<th>Crude OR (95% CI); P Value</th>
<th>Adjusted OR (95% CI); P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTC Group</td>
<td>Control Group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>≤45</td>
<td>15.7</td>
<td>2.3 (1.1-4.9); .04</td>
</tr>
<tr>
<td></td>
<td>&gt;45</td>
<td>16.1</td>
<td>1.7 (0.9-3.2); .09</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>16.9</td>
<td>1.8 (0.9-3.7); .09</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>15.4</td>
<td>2.0 (1.0-3.9); .04</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>Non-Hispanic white</td>
<td>14.7</td>
<td>1.5 (0.9-2.7); .13</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>18.5</td>
<td>3.2 (1.3-8.0); .01</td>
</tr>
<tr>
<td>Tobacco use</td>
<td>Current</td>
<td>22.2</td>
<td>3.3 (0.9-12.3); .07</td>
</tr>
<tr>
<td></td>
<td>Former</td>
<td>16.3</td>
<td>1.6 (0.7-3.9); .27</td>
</tr>
<tr>
<td></td>
<td>Never</td>
<td>15.0</td>
<td>1.7 (1.0-3.2); .07</td>
</tr>
<tr>
<td>Alcohol use</td>
<td>Current</td>
<td>22.2</td>
<td>3.0 (1.4-6.5); .006</td>
</tr>
<tr>
<td></td>
<td>Former</td>
<td>13.0</td>
<td>1.0 (0.3-3.8); .99</td>
</tr>
<tr>
<td></td>
<td>Never</td>
<td>13.8</td>
<td>1.7 (0.9-3.2); .12</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; DTC, differentiated thyroid carcinoma; GST, glutathione S-transferase; GSTM1, gene for GST-π1; GSTT1, gene for GST-π1; OR, odds ratio.

*Calculated by χ2 analysis comparing DTC with control subjects.

†Adjusted for age, sex, ethnicity, tobacco use, and alcohol use.

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The generally higher incidence of thyroid diseases among women is not yet completely understood; reproductive and hormonal factors have been suggested as potential explanations but are supported by limited evidence. In our subgroup analyses, women with the GSTM1/GSTT1-null genotype had a significantly elevated risk for DTC compared with the controls, although this difference may be a reflection of the small sample size of the subgroups.

The findings of this study should be viewed with caution for several reasons. First, it is possible that these findings may be a result of chance, secondary to the relatively small sample size, so confirmation of these findings with a larger population is needed. Next, there may be additional genetic and environmental confounders whose effects are not yet clearly understood and that were not adjusted for. These include a variety of drugs and chemicals that have been shown to increase the risk of thyroid carcinoma in rodents but have not yet been found to be associated with thyroid carcinoma in humans. Furthermore, possible selection biases could have resulted in a study population that did not necessarily reflect the genetic characteristics of DTC or BTD found in the general population. This is certainly a possibility, given that there was a statistically significant difference in the distribution of age, sex, ethnicity, and tobacco and alcohol use between the cases and controls. However, our age and sex distributions were similar to those reported for DTC in general.

The findings of this molecular epidemiologic study suggest that the simultaneous presence of the GSTM1- and GSTT1-null genotypes is associated with an increased risk for DTC. This finding further implicates a possible relationship between alteration in the detoxification ability of the GST enzyme family and the development of DTC. Such knowledge may aid in screening among individuals at risk for DTC and ultimately refine cancer prevention efforts. However, additional, preferably prospective studies with larger samples are needed to verify these findings and further elucidate the complex interactions among demographic, exposure, genotype, and phenotype characteristics.

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REFERENCES