The Null Genotype of Glutathione S-Transferase M1 and T1 Locus Increases the Risk for Thyroid Cancer

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Abstract
Susceptibility to chemical carcinogens plays an important role in the development of most cancers. Several polymorphisms of human drug-metabolizing enzymes influence this individual susceptibility. The genes that encode the isoenzymes of the glutathione s-transferase (GST) system present a polymorphic inheritance. The GST mu 1 (GSTM1) and GST theta 1 (GSTT1) genes have a null allele variant in which the entire gene is absent. The null genotype for both enzymes has been associated with many different types of tumors. To look for the influence of the inheritance pattern of these enzymes on thyroid cancer risk, we used a triplex PCR that included beta-globin gene as a DNA quality control to compare 300 normal individuals of our population to 116 goiter patients. There were 49 cases of benign and 67 cases of malignant nodules: 50 papillary and 17 follicular carcinomas. Comparison between thyroid tumor specimens and normal corresponding samples of 35 cancer patients demonstrated identical patterns, suggesting that the GST system is not involved in the process of follicular dedifferentiation. There was no statistical difference between the prevalence of the deleted alleles in the normal individuals and in the goiter patients. However, papillary carcinoma patients (10%) and follicular carcinoma patients (17%) presented a higher prevalence of the null genotype than the normal population individuals (5%; P < 0.05). We found a 2.6 increased risk of thyroid cancer in individuals with the GSTT1 and GSTM1 combined null inheritance, suggesting that this genotype may be associated with an increased susceptibility to thyroid cancer.

Introduction
The majority of human tumors is considered to be a result of the interaction between environmental factors and personal genetic susceptibility (1, 2). However, people vary greatly in their likelihood of developing cancer in response to natural hazards. Individual differences in susceptibility to carcinogens play an essential role in the development of sporadic cancer. The biochemical basis for this susceptibility is related to genetic polymorphisms that normally occur in the general population regarding genes involved in predisposition to a specific cancer, in the metabolic activation or detoxification of environmental genotoxins, and in controlling DNA repair or cellular damage (3–5).

The etiology of thyroid cancer is markedly uncertain. Exposure to ionizing radiation, especially in childhood, remains the only factor clearly associated with benign and malignant thyroid tumors in humans (6). However, there is strong epidemiological evidence pointing toward the involvement of geographic, ethnic, and dietary factors in the risk of sporadic thyroid cancer (7). A variety of drugs, pesticides, goitrogenic xenobiotics, and chemicals have been shown to increase the incidence of thyroid tumors in rodents (8–10). However, chemicals have seldom been associated with human thyroid cancer, in contrast to lung, bladder, and many other cancers. No increase in the risk of human thyroid cancer has ever been consistently observed with any drug (6, 7, 11). On the contrary, a number of studies have reported a reduced risk of thyroid cancer in women who smoke cigarettes (12, 13).

The GST system consists of a large multigenic group of detoxifying enzymes, the activity of which, catalyzing the conjugation of toxic and mutagenic compounds with glutathione, is essential for cell protection (14). Conversely, this important service may be disadvantageous during chemotherapy where GSTs have been associated with multidrug resistance of tumor cells (15, 16). At present, five classes of isoenzymes have been identified: alpha, mu, pi, sigma, and theta. The genes that encode the GST enzyme system are polymorphic in the general population (14–17). Both the GSTM1 and GSTT1 genes have a null variant allele in which the entire gene is absent. Persons with homozygous deletions of either the GSTM1 or the GSTT1 locus have no functional activity of the respective enzyme. Epidemiological studies suggest that individuals who are homozygous null have an increased risk of developing cancer at a number of sites like lung, bladder, colon, and breast (18).

The primary objective of this study was to test the hypothesis that individuals with an inherited homozygous deletion of the GSTT1 and/or the GSTM1 genes are at an increased risk of thyroid cancer. We conducted a prospective case control study in which we compared the proportion of GSTT1 and GSTM1 null genotypes between a group of patients with benign and malignant thyroid tumors and a control group. Heterogeneity of risk according to clinical and morphological subtypes of

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The abbreviations used are: GST, glutathione S-transferase; GSTT1, GST T1 locus; GSTM1, GST M1 locus; HC-FCM/UNICAMP, University Hospital–School of Medicine of the State University of Campinas; FC, follicular carcinoma; PC, papillary carcinoma.
null genotype increases thyroid cancer risk

Materials and Methods

Subjects. The study was approved by the Ethics Committee of the HC-FCM/UNICAMP, and informed written consent was obtained from a total of 416 individuals from our region, considered to have a normal iodine intake. Because of the highly heterogeneous ethnic composition of the Brazilian population, we included a large control group of 300 individuals (99 males and 201 females, 16–78 years old, 35 ± 23 years old) selected from the general population. To obtain a comparable control group with respect to gender proportion and the range of ages, we selected two to three women for every man who presented himself to donate blood, because thyroid cancer occurs more frequently in women than in men. Data on lifetime occupational history, smoking history, general health conditions, previous diseases, and other anamnestic data were obtained through interviews. Individuals with history of previous thyroid disease, exposure to radiation, and antecedents of malignancy were excluded. There were 252 healthy blood donors (78 males and 174 females, 18–60 years old, 31 ± 21 years old), who provided a representative group of the general population that seeks medical assistance in this region, and 48 volunteers (21 males and 27 females, 16–78 years old, 36 ± 25 years old) recruited among students and co-workers from the State University of Campinas. One hundred sixteen patients consecutively referred to the outpatient clinic of the University Hospital (HC-FCM/UNICAMP) for thyroid disease evaluation, who agreed to participate, were enrolled in the study. The study population included 49 cases of benign thyroid lesions [multi-who agreed to participate, were enrolled in the study. The study population included 49 cases of benign thyroid lesions [multi-

Statistical Analysis. The analysis was conducted using statistical software (Statistical Analysis System, version 8.1. 1999–2000; SAS Institute, Inc., Cary, NC). χ² or Fisher’s exact tests were used to examine homogeneity between cases and controls.

<table>
<thead>
<tr>
<th>Histology</th>
<th>Age (mean ± SD)</th>
<th>Sex</th>
<th>Color</th>
<th>Previous thyroid disease</th>
<th>Smokers</th>
<th>Use of medicines</th>
<th>Diagnosis (presence of metastasis)</th>
<th>Follow-up (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCs</td>
<td>42 ± 13</td>
<td>12</td>
<td>38</td>
<td>34/16</td>
<td>4</td>
<td>13/11</td>
<td>23/8</td>
<td>15</td>
</tr>
<tr>
<td>FCs</td>
<td>57 ± 17</td>
<td>7</td>
<td>10</td>
<td>16/4</td>
<td>1</td>
<td>3/4</td>
<td>3/9</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 1. Distribution of thyroid carcinoma patients according to their histology, clinical features including age (X ± SD in years), gender (F, female; M, male), color (W, white; NW, non-white), the history of previous thyroid benign diseases, smoke habits, use of medicines, the presence of lymph node involvement and distant metastasis by the time of the diagnosis, and the diagnosis of recurrence and/or distant metastasis during the follow-up.

The null genotype increases thyroid cancer risk.
Discussion

The recognition of factors designed to identify people at risk of developing cancer is essential for good medical practice. Five to 10% of the population presents detectable nodules during their life span, mainly in iodine-deficient communities (6, 7, 20). However, most of these nodules will prove to be benign because thyroid cancer is responsible for only 0.6% to 1.6%, respectively, of all kinds of cancers that occur in men and women in the United States (6, 7). The environment has the principal role in causing sporadic cancer (2). Acting in concert with individual susceptibility, environmental factors such as smoking, diet, and pollutants play a role in most human cancers (1).

Variations of thyroid cancer incidence in different geographic and ethnic groups suggest that environmental factors may influence the thyroid tumorigenesis process, but available data regarding carcinogenic products are conflicting. Several chemicals produce thyroid neoplasia in rodents, generally acting through two basic mechanisms. The first involves a direct carcinogenic effect activating oncogenes, inactivating tumor suppressor genes, and producing specific alterations in the expression and function of genes involved in cell growth, differentiation, and life span. The second involves chemicals
that, through a variety of mechanisms, disrupt thyroid function and produce neoplasia secondary to hormone imbalance (21). However, there are important species-specific differences in thyroid gland physiology between humans and rodents. Broad screening of more than 200 drugs for carcinogenicity revealed that just two, griseofulvin and senna, were associated with increased risk of thyroid carcinoma in humans (22). Spirolactone and vitamin D, but not calcium supplements, were found to be significantly associated with thyroid cancer, mostly medullary carcinoma (23). Nutritional goitrogens intake, like vegetables containing cyanogenic glucosides (most forms of cabbage, cauliflower, broccoli, and other members of the cruciferous family), was not associated with increased risk of thyroid carcinoma in humans and may even exert a protective effect (23). There is no epidemiological evidence of increased risk of thyroid cancer in smokers. On the contrary, recent data suggest a protective effect of smoking, perhaps involving an effect on thyroid-stimulating hormone and estrogen metabolism (12, 13).

Individuals with a homozygous deletion of the GSTT1 and GSTM1 genes lack enzymatic conjugation of foreign compounds with glutathione. This results in diminished ability to detoxify many environmental carcinogens, including 1,3-buta diene, ethylene oxide, epoxides, and monohalomethanes. Absence of GSTT1 and of GSTM1 activity in blood, corresponding to the absence of GSTT1 and GSTM1 null genotypes, have been associated with carcinogen-induced and background chromosomal changes in some case-control studies in lung, bladder, and colon cancers, particularly mediating the risk of smoke-related cancers (24–26). We were not able to find any literature data regarding the role of detoxifying enzymes in thyroid tumors. Therefore, we carried out a study of benign and malignant thyroid lesions involving the GSTT1 and GSTM1 genes. We studied a large control group that presented a genotype profile similar to that previously described in our population, confirming its high heterogeneity (27, 28). Our data on thyroid nodules indicate a high prevalence of the combined null genotype in malignant lesions in FC (17%) and PC (10%), significantly higher than in the control population ($P < 0.05$). We were not able to find any association between clinical features, histology, parameters of aggressiveness at diagnosis or during follow-up, and the genotype. Moreover, genotypes of tumor and normal autologous cells were always identical, indicating that GST gene inheritance does not play any role in the follicular transformation process. However, an estimated 2.6-fold greater risk of malignant thyroid nodules was observed in individuals with combined null genotypes. These data suggest that individuals with GSTT1 and GSTM1 combined null inheritance may be genetically predisposed for an increased risk of developing thyroid cancer.

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References

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