**GSTM1 and GSTT1 Gene Deletions and the Risk for Nasopharyngeal Carcinoma in Han Chinese**

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**Abstract**

Southern China is a major nasopharyngeal carcinoma–endemic region. Environmental factors and genetic susceptibility contribute to nasopharyngeal carcinoma development in this area. Polymorphic deletions of GSTM1 and GSTT1 genes involved in the detoxification of potentially carcinogenic agents may be a risk factor for nasopharyngeal carcinoma. To investigate the roles of genetic variations of GSTM1 and GSTT1 in nasopharyngeal carcinoma susceptibility in the Chinese population, we conducted a case-control study of 350 nasopharyngeal carcinoma cases and 622 controls. GSTM1 and GSTT1 deletion variants were genotyped by multiplex PCR assays. Logistic regression analysis was used to estimate odds ratios and 95% confidence intervals (95% CI). No significant association was observed for either GSTM1- or GSTT1-null genotype independently in the contribution to nasopharyngeal carcinoma risk. To explore possible joint effects of the GSTM1- and GSTT1-null polymorphisms with each other and with other risk factors for nasopharyngeal carcinoma, we examined the association between each combined genotype and the risk for nasopharyngeal carcinoma stratified by gender and EBV replication status. We found that individuals who carried GSTM1/GSTT1–double null genotype had a higher risk for nasopharyngeal carcinoma in the male population (odds ratio, 1.76; 95% confidence interval, 1.04-2.97; P = 0.03); however, this was not significant after correction for multiple comparisons. No statistical difference was found between cases and controls in females and the subpopulation positive for immunoglobulin A antibodies to EBV capsid antigen for combined genotypes. Our results suggest that the GSTM1/GSTT1–double null genotype may be a risk factor for nasopharyngeal carcinoma among males in southern China, but this result warrants confirmation in other studies. (Cancer Epidemiol Biomarkers Prev 2008;17(7):1760–3)

**Introduction**

Nasopharyngeal carcinoma is a leading cause of cancer deaths in the Cantonese population of southern China and is the 8th cause of cancer mortality overall in China (1, 2). Nasopharyngeal carcinoma is a fast-growing tumor characterized by a high frequency of nodal and distant metastasis at diagnosis. Nasopharyngeal carcinoma is thought to be caused by the combined effects of EBV, environmental carcinogens, and genetic susceptibility. Case-control studies have indicated a strong role for environmental factors, including traditional southern Chinese foods such as salted fish and other preserved foods containing volatile nitrosamine, which are commonly consumed in high–nasopharyngeal carcinoma incidence areas (3). An individual’s effectiveness in the detoxification of these chemicals is in part ascribed to genetic differences of metabolic activity that may influence susceptibility to malignant disease.

Glutathione S-transferases constitute a superfamily of ubiquitous, multifunctional enzymes, which play a key role in cellular detoxification. Glutathione S-transferases are widely distributed in nature and are found in essentially all eukaryotic species. GSTM1 and GSTT1 are known to be highly polymorphic. This genetic variation may change an individual’s susceptibility to carcinogens and toxins, as well as affect the toxicity and efficacy of certain drugs (4). GSTM1 is located on chromosome 1p13.3 and is a homologous recombination involving left and right 4.2-kb repeats, resulting in a 16-kb deletion containing the entire GSTM1 gene. GSTT1 is located at 22q11.2 and, like GSTM1, is a deletion produced by a homologous recombination event involving left and right 403-bp repeats, resulting in a ~54-kb deletion containing the entire GSTT1 gene (5). Homozygous deletions of these genes, referred to as GSTM1 null and GSTT1 null, respectively, result in lack of enzyme activity. Null mutations of these genes have been associated with increased risk for a number of cancers in some studies (6-11), but not in others (12-16). Two studies have reported modest associations between nasopharyngeal carcinoma and GSTM1 and/or GSTT1 deletions; however, these studies were quite small, with less than 100 nasopharyngeal carcinoma cases (17, 18).

Here, we conducted a case-control study with 350 nasopharyngeal carcinoma cases and 622 controls to determine if deletions of the GSTM1 or GSTT1 genes are...
associated with nasopharyngeal carcinoma risk in southern Chinese. We also tested for the joint effects of these genes with the known nasopharyngeal carcinoma risk factors of chronic EBV replication and sex.

Materials and Methods

Patients and Controls. Cases and controls were recruited from an area along the Xijiang River in Guangxi province of southern China from April 2000 to June 2001. Nasopharyngeal carcinoma cases were defined with nasopharyngeal carcinoma by pathologic examination. Controls were the case’s spouse or geographically matched residents who were nasopharyngeal carcinoma–free at the time of study enrollment. Nasopharyngeal carcinoma cases were hospitalized patients at the Wuzhou Red Cross Hospital in Wuzhou City and outpatients at the Cangwu Institute for Nasopharyngeal Carcinoma Control and Prevention in Cangwu County. All participants self-identified as Han Chinese and self-reported 6 or more months of residency in Guangdong or Guangxi Province of China. Immunoglobulin A antibodies to EBV capsid antigen (EBV/IgA/VCA) and immunoglobulin A antibodies to EBV early antigen were confirmed by serologic testing at the time of study enrollment. Blood samples were obtained from 350 nasopharyngeal carcinoma cases (234 males and 116 females) and 622 controls (267 males and 355 females). The mean age was 45 ± 11 and 46 ± 10 years for nasopharyngeal carcinoma cases and controls. Internal review board approval was obtained from all participating institutions, and informed consent was obtained from each study participant.

Genomic DNA Extraction. DNA was extracted from whole blood or lymphoblastoid cell lines using a QIAamp DNA blood maxi kit (Qiagen; catalog 51194). More than 80% of the genotypes were determined from DNA directly extracted from whole blood.

Genotyping. GSTM1- and GSTT1-deletion genotypes were determined by a multiplex PCR protocol described by Arand et al. (19), and results were recorded as each of the 15-nanograms of target DNA was amplified in total volume of 15-μL PCR mixtures consisting of 10 mmol/L Tris-HCL buffer, 50 mol/L KCl, 2.0 mol/L MgCl2, 0.2 mol/L deoxynucleotide triphosphate, 3 μg/mL of each GSTM1, 1 μg/mL of each GSTT1 primer, 0.6 μg/mL of each albumin primer, and 5 units of Taq polymerase in 96-well plates. Thermal cycling conditions were 94°C for 2 minutes, followed by 30 cycles at 94°C for 1 minute, 64°C for 1 minute, 72°C for 1 minute, and then a final extension of 72°C for 5 minutes. The 215-bp GSTM1 and the 480-bp GSTT1 fragments were coamplified with the 350-bp albumin fragments in the same reaction. The albumin fragments served as a positive control for the success of the amplification reaction. The absence of either GSTM1 or GSTT1 fragments indicated the corresponding null genotype. PCR products were electrophoresed on 4% agarose gel.

Statistics. All statistical analyses were carried out using SAS 9.1 software. Present or null gene frequencies were computed and compared between case and controls with the Pearson’s χ² test or Fisher’s exact test. Odd ratios, 95% confidence intervals (95% CI), and P values were computed by logistic regression, and all results were adjusted for age. To investigate the influence of sex and EBV/IgA/VCA antibody status, we have also analyzed the associations between GSTM1, GSTT1-null genotype, and the occurrence of nasopharyngeal carcinoma in male, female, and EBV/IgA/VCA-positive subpopulations. Because only 16 nasopharyngeal carcinoma cases were EBV/IgA/VCA seronegative, these were not included in the analysis. Joint effects between GSTM1-null and GSTT1-null genotypes and sex or EBV/IgA/VCA antibody status (as a biomarker of EBV replication) were tested. The P values presented were shown without adjustment for multiple tests. After adjustment using a Bonferroni correction for 20 independent tests, P ≤ 0.0025 was considered significant.

Results

The deletion polymorphisms for GSTM1 and GSTT1 were genotyped in 350 nasopharyngeal carcinoma cases and 622 controls. Genotypes were obtained for more than 95% of the participants. Table 1 lists the genotype distribution of the GSTM1, GSTT1, and GSTM1/GSTT1 in the total cohort, stratified by sex. The GSTM1- and GSTT1-null genotypes were detected in 57.1% and 46.9% of the participants, respectively. GSTM1/GSTT1 double nulls were detected in 26.7% of the study population. There was no significant difference (P, 0.39-0.88) in the GSTM1-null, GSTT1-null, and the GSTM1/GSTT1–double null genotypes between males and females.

Table 1 presents the distribution of the GSTM1- and GSTT1-null genotypes in cases and controls, and the odds ratios for the association of GSTM1, GSTT1, and nasopharyngeal carcinoma. No significant difference in the frequencies of GSTM1- and GSTT1-null genotypes was observed between cases and controls. We stratified the analysis by sex and by EBV/IgA/VCA status to test for joint effects. No significant difference in frequencies of the GSTM1-null or GSTT1-null genotypes was found between cases and controls in these different subgroups (Table 2).

To investigate the joint effects of GSTM1- and GSTT1-null genotypes, the association between each combined
genotype and the risk for nasopharyngeal carcinoma was tested again, stratifying for sex and EBV/IgA/VCA status (Table 3). Using the GSTM1/GSTT1 double positive as a reference, a relationship between risk for nasopharyngeal carcinoma (odds ratio, 1.76) but only in males. The effects of the GSTM1- and GSTT1-null genotypes were examined for association with nasopharyngeal carcinoma risk. The frequency of the GSTM1-null genotype was 56% in our control population, similar to Europeans (53%) but much higher than in African-Americans (27%; ref. 5). The frequency of the GSTT1-null genotype was 46% in our control population, higher than in Europeans (22%) and in African-Americans (21%; refs. 11, 20). We observed no significant association for GSTM1- or GSTT1-null genotypes either independently or jointly with nasopharyngeal carcinoma. GSTM1- and GSTT1-null genotypes, separately or in combination, do not contribute to overall nasopharyngeal carcinoma risk in this population of females or in persons with EBV reactivation. Only the GSTM1/GSTT1-double null genotype combination showed a tendency to increase risk for nasopharyngeal carcinoma (odds ratio, 1.76) but only in males.

Several studies have provided evidence that glutathione S-transferase isoforms exhibiting overlapping substr-
genotypes will clarify the role of smoking-gene interactions in nasopharyngeal carcinoma.

A limitation of our study is that we did not consider gene copy number in the analysis because homozygotes cannot be detected by the genotyping assay used. We were assessing the role of homozygosity for the null mutations and comparing the null group to individuals carrying either one or two copies of the gene. It is possible that if the effects were additive or dominant, we may have missed associations. A second limitation is that no smoking exposure data are available for this group of nasopharyngeal carcinoma cases and controls to directly assess the interactions between these genetic factors and smoking.

No previous study has systematically assessed the effects of GSTM1/GSTT1 – double null genotypes with sex and EBV replication status in nasopharyngeal carcinoma. Studies with detailed data on environmental risk factors for nasopharyngeal carcinoma, such as salted fish and other preserved meat consumption, smoking, and occupational exposures to carcinogens, are needed to fully understand the role of GSTM1 and GSTT1 gene copy number in nasopharyngeal carcinoma disease.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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