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

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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 gene being present or absent because heterozygotes could not be determined. The *GSTM1*- or *GSTT1*-specific primer set and a primer set for an albumin gene fragment were used for the same amplification reaction. Forty 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 $MgCl_2$, 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 χ^2 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, $P \leq 0.0025$ 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 2 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

Table 1. Distribution of *GSTM1*-, *GSTT1*-, and *GSTM1/GSTT1*-null genotypes

Genotype	Male (%)	Female (%)	Cohort (%)
GSTM1			
Positive	200 (41.6)	199 (44.2)	399 (42.9)
Null	281 (58.4)	251 (55.8)	532 (57.1)
GSTT1			
Positive	260 (54.5)	230 (51.6)	490 (53.1)
Null	217 (45.5)	216 (48.4)	433 (46.9)
GSTM1/GSTT1			
Positive/positive	108 (22.6)	101 (22.7)	201 (22.7)
Positive/null	91 (19.1)	96 (21.6)	187 (20.3)
Null/positive	152 (31.9)	128 (28.8)	280 (30.4)
Null/null	126 (26.4)	120 (27.0)	246 (26.7)

Table 2. Odds ratios for the association of *GSTM1*- and *GSTT1*-null genotypes with nasopharyngeal carcinoma risk

Genotype	Cases (%)	Controls (%)	OR* (95% CI)*	*P**
All subjects				
GSTM1				
Positive	137 (40.2)	262 (44.4)	1.0	
Null	204 (59.8)	328 (55.6)	1.18 (0.90-1.55)	0.23
GSTT1				
Positive	174 (51.5)	316 (54.0)	1.0	
Null	164 (48.5)	269 (46.0)	1.11 (0.85-1.46)	0.44
Male				
GSTM1				
Positive	87 (38.2)	113 (44.7)	1.0	
Null	141 (61.8)	140 (55.3)	1.28 (0.89-1.85)	0.18
GSTT1				
Positive	114 (50.2)	146 (58.4)	1.0	
Null	113 (49.8)	104 (41.6)	1.37 (0.96-1.98)	0.09
Female				
GSTM1				
Positive	50 (44.2)	149 (44.2)	1.0	
Null	63 (55.8)	188 (55.8)	0.99 (0.64-1.52)	0.96
GSTT1				
Positive	60 (54.1)	170 (50.7)	1.0	
Null	51 (45.9)	165 (49.3)	0.9 (0.58-1.38)	0.62
IgA/VCA+				
GSTM1				
Positive	130 (39.9)	120 (44.9)	1.0	
Null	196 (60.1)	147 (55.1)	1.23 (0.89-1.71)	0.22
GSTT1				
Positive	65 (51.1)	145 (54.5)	1.0	
Null	158 (48.9)	121 (45.5)	1.15 (0.83-1.59)	0.41

Abbreviation: OR, odds ratio.
*Adjusted for age but not for multiple comparisons.

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 and the double-null genotype was suggested for males (odds ratio, 1.76; 95% CI, 1.04-2.97; $P = 0.03$); however, this result was not significant after correction for multiple tests.

Discussion

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 substrate specificity with different combinations of various unfavorable deletion genotypes may increase the risk for head and neck cancers (21). *GSTM1/GSTT1* double deletions have been reported to confer a higher risk for head and neck squamous cell carcinoma (22). Similar increases in risk for other cancers have been reported for the combined genotypes of *GSTM1* null and *GSTT1* null (9, 23). Although more than 500 studies have examined the association of the null genotypes for *GSTM1* and *GSTT1* genes with various tumors, very few have investigated the associations between *GSTM1*- and *GSTT1*-null genotypes and the risk for nasopharyngeal carcinoma in Chinese populations. One study showed that *GSTM1*-null genotype was associated with increased risk for nasopharyngeal carcinoma in European-Americans with 83 cases (17); however, the risk factors in China are likely quite different, making comparisons difficult. A Chinese study enrolling 91 cases showed associations between nasopharyngeal carcinoma and *GSTM1*- and *GSTT1*-null genotypes separately and jointly (18); however, we found no support for this finding in our study that included 350 nasopharyngeal carcinoma cases from southern China. The discrepancies between the two studies may reflect differences in overall study design.

Interestingly, we did observe an increase in risk for males who carried the combination of null genotypes for *GSTM1* and *GSTT1*. A 1996 population-based survey in mainland China reported a prevalence of 63% male and 4% female smokers (24). We speculate that smoking may be responsible for the increased risk in males because glutathione *S*-transferase genes have been reported to detoxify nicotine and smoke (13). Previous studies on the interaction of *GSTM1* and *GSTT1* with smoking in tobacco-associated cancer have shown that the deletion of the *GSTM1* and *GSTT1* genes may increase cancer risk in smokers (9, 25), whereas another study reported an interaction with tobacco chewing but not with smoking for head and neck squamous cell carcinoma (22). Further studies on the effects of smoking and *GSTM1* and *GSTT1*

Table 3. Joint effects of *GSTM1/GSTT1* and nasopharyngeal carcinoma

GSTM1/GSTT1	Cases (%)	Controls (%)	OR* (95% CI)*	*P**
All subjects				
Positive/positive	71 (21.0)	138 (23.6)	1.0	
Positive/null	65 (19.2)	122 (20.9)	1.03 (0.68-1.57)	0.90
Null/positive	103 (30.5)	177 (30.3)	1.11 (0.76-1.62)	0.58
Null/null	99 (29.3)	147 (25.2)	1.31 (0.89-1.92)	0.17
Male				
Positive/positive	44 (19.4)	64 (25.6)	1.0	
Positive/null	43 (18.9)	48 (19.2)	1.27 (0.72-2.23)	0.41
Null/positive	70 (30.8)	82 (32.8)	1.20 (0.73-1.99)	0.47
Null/null	70 (30.8)	56 (22.4)	1.76 (1.04-2.97)	0.03
Female				
Positive/positive	27 (24.3)	74 (22.2)	1.0	
Positive/null	22 (19.8)	74 (22.2)	0.82 (0.43-1.56)	0.54
Null/positive	33 (29.7)	95 (28.4)	0.92 (0.51-1.68)	0.80
Null/null	29 (26.1)	91 (27.3)	0.88 (0.48-1.62)	0.69
IgA/VCA+				
Positive/positive	67 (20.7)	62 (23.4)	1.0	
Positive/null	62 (19.2)	57 (21.5)	1.01 (0.61-1.66)	0.98
Null/positive	98 (30.3)	82 (30.9)	1.11 (0.70-1.74)	0.66
Null/null	96 (29.7)	64 (24.2)	1.39 (0.87-2.22)	0.17

*Adjusted for age but not for multiple comparisons.

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 heterozygotes 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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