RESEARCH COMMUNICATION

Polymorphisms in GSTM1, GSTTI and GSTP1 and Nasopharyngeal Cancer in the East of China: a Case-control Study

Jiang Yan[1], Li Na[1*],Dong Pin[2], Zhang Niankai[1], SunYan[1], Han Min[1], Wen Junfeng[1], Chen Min[1]

Abstract

Aim: The study was performed to assess the potential role of GSTM1, GSTT1 and GSTP1 polymorphisms in the risk of nasopharyngeal cancer in Chinese population. Method: We collected 182 cases undergoing pathologic examination and 366 controls from the affiliated hospital of Medical College of Qingdao University from April 2006 to July 2010. Genotyping was based upon duplex polymerase-chain-reactions with the PCR-CTPP method. Results: More smokers were found in NPC patients than controls, and a higher IgA/VCA+ . Individuals carrying null GSTM1 and GSTT1 had 1.76 and 2.01 fold risk of NPC when compared with non-null genotypes, respectively. A non-significant increase risk of NPC was found in individuals with 1b/1b genotype when compared with 1a/1a genotype (OR=1.32, 95%CI=0.60-2.94). When compared with non-null GSTM1 and GSTT1 genotypes, the combination of null/null GSTM1 and GSTT1 genotypes showed moderate increased risk of NPC (OR=3.03, 95% CI=1.74-5.08). Conclusion: Our study provides evidence that genetic deletion of GSTM1 and GSTT1 may contribute to increased susceptibility to NPC in Chinese population, while GSTP1 may not. Our findings provide information relevant to the prevention of NPC.

Keywords: GSTM1 - GSTT1 - GSTP1 – polymorphisms - nasopharyngeal cancer - Chinese population

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Introduction

Nasopharyngeal carcinoma (NPC) is relatively rare on a world scale, but it is endemic in a few well-defined populations (Parkin et al., 2002). In 2002, there were 80,000 new cases worldwide, accounting for 0.7% of all cancers and making it the 23rd most common new cancer in the world (Parkin et al., 2005). In contrast, it was the fourth most common new malignancy in Hong Kong (Parkin et al., 2002). Based on geographic distribution, the age-standardized incidence rate of nasopharyngeal carcinoma for both males and females is < 1 per 100,000 person-years in most regions. However, dramatically elevated rates are observed in the Cantonese population of southern China (including Hong Kong), and intermediate rates are observed in several indigenous populations in South East Asia and in natives of the Arctic region, North Africa and the Middle East (Parkin et al., 2002). The wide geographic variation at an international levels of NPC in terms of incidence and mortality suggested the role of genetic and environmental factors in the pathogenesis of this cancer.

Recently evidence indicated that carcinogen-metabolizing genes and DNA-repair genes may play critical roles in determining individual susceptibility to cancers. Polymorphisms in these genes encoding the enzymes, possibly by altering their expression and function, may increase or decrease carcinogen activation or detoxication and modulate DNA repair.

Xenobiotics can be detoxified by phase II enzymes, such as GSTM1 and GSTT1 which have been suggested to be involved in detoxification of polycyclic aromatic hydrocarbons (PAHs) and benzo(a)pyrene (Schneider et al., 2004), which could detoxify carcinogens and reactive oxygen species (Rebbeck, 1997). Individuals who have homozygous deletions for GSTM1 and GSTT1 gene have no GSTM1 and GSTT1 enzyme activity. Lack of these enzymes may potentially increase cancer susceptibility because of a decreased ability to detoxify carcinogens such as benzo[α]pyrene-7,8-diol epoxide, the activated form of benzo[α]pyrene. The misses substitution Ile105Val results from an A3G base substitution at nucleotide 313. The Val105 form of the GSTP1 enzyme may be 2–3 times less stable than the canonical Ile105 form (Johansson et al., 1998) and may be associated with a higher level of DNA adducts (Ryberg et al., 1997). Number of published

*[1]Department of Otolaryngology-Head and Neck Surgery, the Affiliated Hospital of Medical College, Qingdao University, Qingdao, [2]Department of Otolaryngology-Head and Neck Surgery, Shanghai First People's Hospital, Shanghai Jiao Tong University, Shanghai, China *For correspondence: linadr2011@yahoo.com.cn*

studies have focused on GSTM1, GSTTI and GSTP1 genetic variation with respect to NPC, but yielded conflicting results. Whether GSTM1, GSTT1 and GSTP1 polymorphisms are risk factors for NPC remains largely uncertain. We therefore conducted a matched case-control study in east of China to investigate the association of GSTM1, GSTT1 and GSTP1 polymorphisms with NPC susceptibility.

Materials and Methods

Cases and controls were recruited from the affiliated hospital of medical college of Qingdao university during the period of April 2006 to July 2010. Nasopharyngeal carcinoma cases were hospitalized patients at the the affiliated hospital of medical college of Qingdao university and outpatients in the same hospital in Qingdao, and NPC cases were defined with nasopharyngeal carcinoma by pathologic examination. Among a total of 201 eligible cases, 182 were interviewed with a participation rate of 90.05%. Controls were the people who requested the health examinations in our hospital at the same period. Controls were required to be without any history of any type of cancer and frequency matched by five year age groups, with a control to case ratio of two, whenever possible. Among a total of 395 eligible controls, 366 were successfully interviewed with a participation rate of 92.65%. 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. Trained interviewers conducted face-to-face interviews using a structured questionnaire to collect information on sociodemographic characteristics and potential confounding factors. Collected potential confounders mainly included education level, tobacco smoking (Yes and No), alcohol use (Yes and No) and family history. Cancer patients were asked to refer about habits a year before the disease diagnosed.

Genomic DNA Extraction

Blood samples were from patients and controls and stored at -20°C. DNA was extracted from whole blood or lymphoblastoid cell lines using a Wizard Genomic DNA purification Kit. More than 80% of the genotypes were determined from DNA directly extracted from whole blood.

Genotyping of GSTM1, GSTTI and GSTP1

Genotyping was based upon duplex polymerase-chain-reaction with the confronting-two-pairprimer (PCR-CTPP) method (Harries et al., 1997; Hung et al., 1997). Briefly, the sequences of primers used for polymorphism of GSTM1, GSTTI and GSTP1 were amplified by using the following primers. The primers of GSTM1 were 5'-CTGCCCTACTTGATTGATGGG-3' and 5'-CTGGATT- GTAGCGATCATGC-3'. The primers of GSTT1 were 5'-TCACCGGATCATGGCCAGCA-3' and 5'-TTCCTTACTGGTCCTCACATCTC-3'. The primers were 5'-ACCCCAGGGCTCTATGGGAA-3' and 5'-TGAGGGCACAAGAAGCCCCT-3'. Each 25 μL reaction mixture contained 1.3 U Tag biocatalysts, 1.8 mmol/L $MgCl_2$, 0.24 mmol/L dNTPs, 15 pmol of each primer and 5-8 μL template. The PCR conditions were as follows: initial denaturation at 94°C for 4 min, followed by 35 cycles at 94°C for 40 s, at 55°C for 30 s, at 72°C for 40 s, and a final extension at 72°C for 10 min. After transient centrifugation, agarose electrophoresis was conducted.

Statistical analysis

Differences in the distributions of demographic characteristics, selected variables, and frequencies of genotypes of the GSTM1, GSTTI and GSTP1 polymorphism between the cases and controls were evaluated by using the Student's t-test (for continuous variables) or chi-square test (for categorical variables). The associations between the GSTM1, GSTTI and GSTP1 genotypes and risk of RCC were estimated by computing odds ratios (ORs) and their 95% confidence intervals (CIs) from unconditional logistic regression analysis with the adjustment for possible confounders, including sex, age, smoking, drinking, family history and EBV/IgA/VCA antibody status. Hardy–Weinberg equilibrium was tested using a goodness-of fit chi-square test. $p<0.05$ was considered statistically significant, and all statistical tests were two sided. All the statistical analyses were performed with the software Stata version 9 (Stata, College Station, TX).

Results

The characteristics of the 182 NPC cases and 366 controls enrolled in this study are shown in Table 1. There was no statistically significant difference between the cases and controls in terms of age, sex and drinking. NPC cases had more smokers than that in controls, and NPC cases also showed higher positive rate of IgA/VCA and more cancer history of first relatives than controls.

Frequency distributions of GSTM1, GSTTI and GSTP1 genotypes and their association with NPC risk are shown in Table 2. The null GSTM1 and GSTT1 genotypes were detected in 53.3% and 65.93% of the NPC cases,

Table 1. Characteristics of Study Subjects by the Case-control Status

Characteristics	Cases N=182(%)	Controls N=366(%)	Test statistics	P
Sex				
Male	132	250	1.02	0.31
Female	50	116		
Age (mean±SD)	48.3±4.6	47.9±4.8	0.09	0.17
Smoking				
Yes	84	47	74.2	<0.001
No	98	319		
Drinking				
Yes	63	56	26.7	<0.001
No	119	310		
Cancer history of first relatives				
Yes	14	2	21.9	<0.001
No	168	364		
IgA/VCA+	167	105	193.4	<0.001
IgA/VCA-	15	261		

Table 2. Relationship Between Polymorphism of GSTM1, GSTTI and GSTP1 and NPC Risk

Genetic polymorphisms	Cases N=182(%)	Controls N=366(%)	OR (95% CI)[1]
GSTM1			
Non-null	85 (46.7)	215 (58.7)	1.0 (Reference)
Null	97 (53.3)	157 (42.9)	1.76 (1.13-2.57)
GSTT1			
Non-null	62 (34.1)	186 (50.8)	1.0 (Reference)
Null	120 (65.9)	180 (49.2)	2.01 (1.39-2.99)
GSTP1			
1a/1a	113 (62.1)	236 (64.5)	1.0 (Reference)
1a/1b	55 (30.2)	109 (29.8)	1.02 (0.70-1.59)
1b/1b	14 (7.69)	21 (5.74)	1.32 (0.60-2.94)

Adjusted for age, sex, smoking, drinking, cancer history of first relatives and IgA/VCA status

Table 3. Combined Genotype Analysis of GSTM1 and GSTT1 Genotypes on NPC Risk

Genetic polymorphisms	Cases N=182(%)	Controls N=366(%)	OR (95% CI)
GSTM1/GSTT1			
Non-null/ Non-null	34 (18.7)	115 (31.4)	1.0 (Reference)
Non-null/Null	51 (28.0)	100 (27.3)	1.57(0.94-2.82)
Null/ Non-null	28 (15.4)	71 (19.4)	1.35(0.75-2.55)
Null/Null	69 (37.9)	80 (21.9)	3.03(1.74-5.08)

Adjusted for age, sex, smoking, drinking, cancer history of first relatives and IgA/VCA status.

respectively, which were significantly higher than those in controls ($p<0.05$). Individuals carrying null GSTM1 and GSTT1 had 1.76 and 2.01 fold risk of NPC when compared with Non-null genotypes, respectively. The frequencies of GSTP1 1a/1a, 1a/1b and 1b/1b in the NPC patients were 62.09%, 30.22% and 7.69%, respectively, which did not show significant difference compared with those in controls ($p>0.05$). A non-significant increased risk of NPC was found in individuals with 1b/1b genotype when compared with 1a/1a genotype (OR=1.32, 95%CI=0.60-2.94). The frequencies of the GSTM1, GSTTI and GSTP1 polymorphism in controls were according to the Hardy-Weinberg equilibrium ($p=0.23$).

The combination genotype analysis was used to evaluate the possible effect of GSTM1 and GSTT1 genotypes on the risk of NPC table 3. When compared with non-null GSTM1 and GSTT1 genotypes, the combination of null/null GSTM1 and GSTT1 genotypes showed moderate increased risk of NPC (OR=3.03, 95% CI=1.74-5.08), whereas no significantly increased risk in either null GSTM1 or GSTT1.

Discussion

Previous evidence showed the GSTM1, GSTT1 and GSTP1 polymorphisms may have a close association with increased susceptibility to various carcinomas. In the present study, the results suggested the genetic deletion of GSTM1 and GSTT1 may contribute to increase susceptibility to NPC in Chinese population, while GSTP1 polymorphism may not.

GSTs belong to a super-family of detoxification enzymes, which play a role in resisting a large variety of chemical carcinogens and environmental toxicants. Null mutations of GSTM1 and GSTT1, one of the phase II enzymes, are known to abolish enzyme activities and therefore have been linked with increasing incidence of certain cancers, most likely due to increased susceptibilities to environmental toxins and carcinogens. Previous meta-analysis studies indicated that null genotypes of GSTM1 and GSTT1 might have a significant association with increased risks of breast cancer, lung cancer and gastric cancer in Chinese population (Sull et al., 2005; Shi et al., 2008; Hosgood et al., 2007; Saadat, 2006). Our present study supported the GSTM1 deficiency may increase susceptibility to NPC.

Our study showed GSTM1/GSTT1 double deletions have been reported to confer a higher risk for NPC in Chinese population. Similar increased in risk for other cancers have been reported for the combined genotypes of null GSTM1 and GSTT1 (Ates NA et al., 2005; Singh et al., 2008; Saadat et al., 2001). Although many studies have examined the association of null GSTM1 and GSTT1 with various cancers, but few of them investigated the associations between the two genotypes and NPC in Chinese. One study conducted in Guangxi of China indicated a significant association was found between the two genotypes and NPC risk (Deng et al., 2004). Another meta-analysis showed the null GSTM1 polymorphism had 1.42 fold risk for NPC, but no significant increase risk was found in GSTT1 polymorphism. Our results are in line with these findings. However, another two studies conducted in Taiwan and Guangxi of China did not find significant association between the two genotypes and NPC. The discrepancies of these finding sbetween studies may be due to the study design and population selected.

A limitation of our study is that we used the hospital based case-control study, this may introduce selection bias into study. However, controls were selected from health individuals visiting hospital for routine physical examination. These health individuals were workers or staffs who organized to routine physical examination by factors and companies, and these people could represent the residents in Qingdao. A second limitation is that more risk found should be included in this studies, such as dietary and lifestyle factors which play a role in NPC, studies with more detailed data on environmental risk factors for NPC are need to fully understand the role of the two genotypes in NPC risk.

To summarize, this study found genetic deletion of GSTM1 and GSTT1 may contribute to increase susceptibility to NPC in Chinese population, and a higher increased risk was found in both null GSTM1 and GSTT1 genotypes. Our findings provide more information relevant to the prevention of NPC.

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