RESEARCH COMMUNICATION

Glutathione S-transferase Gene Polymorphisms and Risk of Gastric Cancer in a Chinese Population

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Abstract

Aim: The potential role of GSTM1, GSTT1 and GSTP1 polymorphisms in risk of gastric cancer in Chinese was studied. Methods: We collected 194 gastric cancers by pathologic examination and 412 controls from southern China during January 2007 to January 2011. Genotyping was based upon duplex polymerase-chain-reaction withthe PCR-CTPP method. Results: Individuals carrying null GSTM1 and GSTT1 had 1.49 and 1.96 fold risk sof gastric cancer when compared with respective non-null genotypes. We also found a non-significant 37% excess risk of gastric cancer among carriers of GSTP1 1b/1b genotype when compared with 1a/1a genotype (OR=1.37, 95% CI=0.81-2.25). The combination of null/null GSTM1 and GSTT1 genotypes showed higher increased risk of gastric cancer (OR=3.17, 95% CI=1.68-4.21). Moreover, cancers in ever smokers and ever drinkers were observed to be strongly associated with null GSTM1 and GSTT1, and a significant cancer risk was observed in positive *H.pylori* infection individuals with null GSTT1. Conclusion: Our study provided evidence that genetic deletion of GSTM1 and GSTT1 may contribute to increased susceptibility to gastric cancer in our Chinese population, while the GSTP1a/b polymorphism may not.

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

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Introduction

About one million new cases of stomach cancer were estimated to have occured in 2008 (988 000 cases, 7.8% of the total), making it currently the fourth most common malignancy in the world, behind cancers of the lung, breast and colo-rectum. More than 70% of cases (713 000 cases) occur in developing countries (467 000 in men, 246 000 in women), and half the world total occurs in Eastern Asia (mainly in China). Age-standardised incidence rates are about twice as high in men as in women, ranging from 3.9 in Northern Africa to 42.4 in Eastern Asia for men, and from 2.2 in Southern Africa to 18.3 in Eastern Asia for women (IARC, 2011). The wide geographic variation at an international levels of gastric cancer in terms of incidence and mortality suggested the role of genetic and environmental factors in the pathogenesis of this cancer.

Gastric carcinogenesis is a multistep and multifactorial process (Correa, 1992). Howver, its exact mechanism is still unclear. It is known to be associated with risk factors such as atrophic gastritis, intestinal metaplasia, Helicobacter pylori (H. pylori) infection, exposure to toxins and carcinogens, dietary habits, and genotype of the individual (Nomura, 1996). *H.pylori* infection is associated with an gastric cancer and is classified as a group I carcinogen by the World Health Organization (IARC, 1994). About 50-80% of the world's population is infected with *H.pylori*, but only about 1% of infected individuals develop malignancy (Graham et al., 1991; Parsonnet et al., 1997). Moreover, in some Asian countries, despite a high prevalence of H.pylori infection, the annual incidence of gastric cancer is low, such as India and Pakistan (Singh and Ghoshal, 2006). This indicates a role of host factors in gastric carcinogenesis. Dietary and environmental carcinogens play an important part in gastric carcinogensis. Carcinogens and toxins entering in our body are metabolized via the xenobiotic pathway which is an important defense mechanism against carcinogensis.

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 –T1 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

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of benzo[α]pyrene. The missense 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). Numbers of published studies have focused on GSTM1, GSTTI and GSTP1 genetic variation with respect to gastric cancer, however, there are conflicting reports on their association. Whether GSTM1, GSTT1 and GSTP1 polymorphisms are risk factors for gastric cancer remains largely uncertain, particularly in the Chinese population. Therefore, the aim of the present study was to determine the association of GSTM1, GSTT1 and GSTP1 genes with patients with gastric cancer

Materials and Methods

Subjects

Cases and controls were recruited from the Daping hospital of the Third Military Medical University and the affiliated hospital of Southern Medical University during the period of January 2007 to January 2011. A total of 194 histological confirmed gastric cancer cases, who aged 40-75 years. 436 controls were recruited from health individuals visiting hospital for routine physical examination. Finally, the overall sample comprised 412 controls aged 35-77 years, corresponding to a participation proportion of 94.5%. Controls were eligible for this analysis if there was no malignant tumors or digestive tract disorders free.

The ethics committee of Third Military Medical University and Southern Medical University reviewed and approved the study, and informed consent was obtained from all participants.

Date collection

Face to face interview was performed for all subjects. Two interviewers were trained and were not aware of the study hypothesis. Cancer patients were asked to refer about dietary habit a year before diagnosis. The information on sociodemographic characteristics and potential confounding factors were collected, including education level, tobacco smoking (Ever and No), alcohol use (Ever and No) and family history.

H.pylori detection

A blood sample was drawn and serum was kept frozen at -20°C. Anti-H. pylori serum IgG titres were quantified by ELISA. Participants were classified as negative if they had <16RU ml-1, as borderline if their antibody concentration was between 16 and 22 RU ml-1 and as positive if this was ≥22 RU ml-1, according to the manufacturer's instructions. In our analysis, subjects with borderline IgG titres were classified as infected.

Genomic DNA Exaction

For DNA extraction, 5 ml blood were provided by each collected subjects. Blood samples were stored at -20°C. DNA was extracted from whole blood or lymphoblastoid cell lines usingthe Qiagen Blood Kit (Qiagen, Chastworth, CA) according to the manufacturer's instructions. More than 80% of the genotypes were determined from DNA directly extracted from whole blood.

Table 1. Distributions of Demographic Characteristics, Selected Variables

Characteristics	Cases(%) N=194	Controls(%) N=412	p
Age	46.4±5.2	45.8±6.4	0.127
Sex			
Male	122(62.9%)	243(59.0%)	0.375
Female	72(37.1%)	169(41.0%)	
Smoking			
Ever	62(32.0%)	78(18.9%)	<0.001
No	132(68.0%)	334(81.1%)	
Drinking			
Ever	91(46.9%)	140(34.0%)	<0.001
No	103(53.1%)	272(66.0%)	
Family cancer history of first relative			
Yes	29(14.9%)	16(3.9%)	<0.001
No	165(85.1%)	396(96.1%)	
H.pylori infection			
Positive	138(71.1%)	214(51.9%)	<0.001
Negative	56(28.9%)	198(48.1%)	

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'-GAACTCCCTGAAAAGCTAAGC-3' and 5'-GTTGGGGCTCAAATATACGGTGG-3'. The primers of GSTT1 were 5'-TTCCTTACTGGTCCTCACATCTC-3' and 5'-TCACCGGATCAGGCCAGCA-3'. The primers of GSTP1 were 5'-ACCCCAGGGCTCTATGGGAA-3' and 5'-TGAGGGCACAAGAAGCCCCT-3'. PCR conditions included initial denaturation at 958C for 5 min followed by 35 cycles of 94°C for 30 s, 58.5°C for 25 s, and 72°C for 30 s. Final extension was done at 72°C for 5 min.

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 gastric cancer 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 and family history.

Results

The characteristics of the 194 gastric cancer cases and 412 controls were enrolled in this study (Table 1). There was no significant difference between the cases and controls in terms of age and sex. Gastric cancer cases

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

Genetic polymorphisms	Cases N=194(%)	Controls N=412(%)	OR (95% CI)	Adjusted OR (95% CI)*
GSTM1				
Non-null	89(45.9%)	218(52.9%)	1.0(Reference)	1.0(Reference)
Null	105(54.1%)	194(47.1%)	1.32(0.93-1.90)	1.49(1.04-2.28)
GSTT1				
Non-null	80(41.2%)	214(51.9%)	1.0(Reference)	1.0(Reference)
Null	114(58.8%)	198(48.1%)	1.54(1.08-2.21)	1.96(1.32-2.50)
GSTP1				
1a/1a	107(55.2%)	235(57.0%)	1.0(Reference)	1.0(Reference)
1a/1b	52(26.8%)	115(27.9%)	0.99(0.65-1.51)	1.03(0.73-1.74)
1b/1b	35(18.0%)	62(15.1%)	1.24(0.75-2.04)	1.37(0.81-2.25)

*Adjusted for sex, age, drinking, smoking, family cancer history and H.pylori infection.

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

Genetic polymorphisms	Cases N=194(%)	Controls N=412(%)	OR (95% CI)	Adjusted OR (95% CI)*
GSTM1/GSTT1				
Non-null/ Non-null	42 (21.5)	110(31.42)	1.0 (Reference)	1.0 (Reference)
Non-null/Null	47 (24.4)	108(27.32)	1.14(0.68-1.93)	1.32(0.82-2.06)
Null/ Non-null	38 (19.7)	104(19.40)	0.96(0.55-1.65)	1.03(0.72-1.94)
Null/Null	67 (37.91)	90(21.86)	1.94(1.18-3.23)	3.17(1.68-4.21)

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

Table 4. Stratified Analyses Between GSTM1 and GSTT1 Gene Polymorphism and the Risk of Gastric Cancer

Variables	Cases/Controls 110/226	Adjusted OR1 (95% CI) GSTM1 Non-null	Null	Adjusted OR1 (95% CI) GSTT1 Non-null	Null
Smoking					
Ever	62/78	1.0(Reference)	1.61(1.10-2.34)	1.0(Reference)	2.36(1.31-3.09)
No	132/334	1.0(Reference)	1.26(0.87-1.81)	1.0(Reference)	1.67(1.09-2.27)
Drinking					
Ever	91/140	1.0(Reference)	1.70(1.05-2.14)	1.0(Reference)	2.43(1.29-3.30)
No	103/272	1.0(Reference)	1.32(0.88-1.63)	1.0(Reference)	1.52(0.88-1.87)
Family history of cancer, N (%)					
Yes	29/16	1.0(Reference)	1.33(0.96-1.91)	1.0(Reference)	2.28(0.79-3.61)
No	165/396	1.0(Reference)	1.48(0.95-2.04)	1.0(Reference)	1.69(0.83-1.85)
H.pylori infection, N (%)					
Yes	138/214	1.0(Reference)	1.42(0.94-1.85)	1.0(Reference)	2.15(1.07-3.21)
No	56/198	1.0(Reference)	1.25(0.81-1.74)	1.0(Reference)	1.59(0.94-1.87)

had more smokers and drinkers than those in controls, and cancer cases showed higher positive rate of H.pylori infection and more cancer history of first relatives than controls($p<0.001$).

Frequency distributions of GSTM1, GSTTI and GSTP1 genotypes and their association with gastric cancer are shown in Table 2. Among controls, the frequency of GSTM1, GSTTI and GSTP1 variant allele was 47.1%, 48.1% and 43% and the genotype distributions did not deviate from Hardy–Weinberg equilibrium ($p = 0.20, 0.32$ and 0.06, respectively). The null GSTM1 and GSTT1 genotypes were detected in 54.1% and 58.8% of the gastric cancer cases, respectively, which were significantly higher than those in controls ($p<0.05$). The frequencies of GSTP1 1a/1a, 1a/1b and 1b/1b in the gastric cancer patients were 55.2%, 26.8% and 18.0%, respectively, which show significant difference compared with those in controls ($p<0.05$). Individuals carrying null GSTM1 and null GSTT1 had 1.49 and 1.96 fold risk of gastric cancer when compared with Non-null genotypes, respectively. We found a non-significant 37% excess risk of gastric cancer among carriers of GSTP1 1b/1b genotype when compared with 1a/1a genotype (OR=1.37, 95%CI=0.81-2.25).

The combination genotype analysis was used to evaluate the possible effect of GSTM1 and GSTT1 genotypes on the risk of gastric cancer in Table 3. When compared with non-null GSTM1 and GSTT1 genotypes, the combination of null/null GSTM1 and GSTT1 genotypes showed higher increased risk of gastric cancer (OR=3.17, 95% CI=1.68-4.21).

Further stratification were conducted regarding smoking, drinking and family history of cancer with polymorphism of GSTM1 and GSTT1 in table 4. Ever smokers and ever drinkers was observed strongly associated with null GSTM1 and GSTT1 (Smokers: Null GSTM1 and GSTT1 genotype, adjusted OR=1.61, 95% CI: 1.10-2.34 and OR=2.36, 95% CI: 1.31-3.09; Drinkers: Null GSTM1 and GSTT1 genotype, adjusted OR=1.70, 95% CI: 1.05-2.14 and OR= 2.43, 95% CI: 1.29-3.30). An increased risk associated with GSTT1 polymorphism varied significantly across different family history. Additionally, despite the suggestion of a strong association between *H.pylori* infection and gastric cancer (Table 3),

only significant higher cancer risk was observed in positive H.pylori infection individuals with null GSTT1 (Positive H.pylori infection, null GSTT1 genotype, adjusted OR= 2.15, 95% CI: 1.07-3.21).

Discussion

To our knowledge, the present study was the first one which examined associations between polymorphisms in GSTM1, GSTTI and GSTP1 and the risk of osteosarcoma in Chinese population. The results suggested the genetic deletion of GSTM1 and GSTT1 may contribute to increase susceptibility to gastric cancer in Chinese population, while GSTP1 polymorphism may not. Moreover, ever smokers and ever drinkers was observed strongly associated with null GSTM1 and GSTT1, and a significant cancer risk was observed in positive H.pylori infection individuals with null GSTT1.

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; Saadat, 2006; Hosgood et al., 2007; Shi et al., 2008). Our present study supported the GSTM1 and GSTT1 deficiency may increase susceptibility to gastric cancer.

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 (Hashibe et al., 2003). GSTM1/ GSTT1 double deletions have been reported to confer a higher risk for head and neck squamous cell carcinoma. Similar increases in risk for gastric and colorectal cancers have been reported for the combined genotypes of GSTM1 null and GSTT1 null (Saadat and Saadat, 2001; Ates et al., 2005). In our study, we did observed an increase in risk for individuals who carried the combination of null genotypes for GSTM1 and GSTT1. Although more than 600 studies have examined the association of null genotypes for GSTM1 and GSTT1 genes with various tumors, very few have investigated 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 gastric cancer in Chinese populations. One study showed that GSTM1 null genotype was associated with increased risk for gastric cancer in Iranians, but no significant increased cancer risk in null GSTT1 genotype (Saadat and Saadat, 2001). However, the risk factors in Chinese are likely quite different, making comparisons difficult. In our study, we found null GSTT1 genotype had high increased risk of gastric cancer in Chinese population. The discrepancies between the two studies may reflect differences in overall study design.

Interestingly, we observed an increase risk for smokers, drinkers and positive H.pylori infection individuals. The glutathione S-transferase genes have been reported to detoxify nicotine and smoke(Schneider et al., 2004). Previous studies on the interaction of GSTM1 and GSTT1 with smoking in tobacco-associated cancer have shown that the deletion of GSTM1 and GSTT1 genes may increase cancer risk in smokers (Kihara et al., 1997; Ates et al., 2005). therefore, the null mutations of GSTs may increase cancer risk in smokers. Also, the positive H.pylori infection and alcohol may have interaction with null GSTs, and thus higher gastric cancer risk was observed in individuals with either H.pylori infection or alcohol and null GSTs.

In conclusion, carriage of null mutations of GSTM1 and GSTT1 might be linked to the risk of gastric cancer, with a tendency for interaction with tobacco smoking, alcohol drinking and H.pylori infection. Our findings provide more information to the prevention of gastric cancer

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