RESEARCH ARTICLE

Glulathione-S-transferases Gene Polymorphism in Prediction of Gastric Cancer Risk by Smoking and Helicobacter Pylori Infection Status

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Abstract

Aim: To evaluate the association of glutathione S-transferases gene polymorphisms with the risk of gastric cancer, with reference to smoking and *Helicobacter pylori* infection. Methods: We conducted a 1:1 matched case-control study with 410 gastric cancer cases and 410 cancer-free controls. Polymorphisms of GSTM1, GSTT1 and GSTP1 were determined using PCR-CTPP. Results: The GSTM1 and GSTT1 null genotypes were significantly associated with the risk of gastric cancer after adjusting for potential confounding factors (OR=1.68, 95% CI=1.32-2.23 for null GSTM1, OR=1.73; 95% CI=1.24-2.13 for null GSTT1). The combination of null GSTM1 and null GSTT1 conferred an elevated risk (OR=2.54, 95% CI=1.55-3.39). However, no association was found for GSTP1 polymorphism The smoking modified the association of GSTM1 and GSTT1 null genotypes with the risk of gastric cancer. Conclusion: GSTM1 and GSTT1 null genotypes are associated with increased risk of gastric cancer, and smoking modifies the association.

Keywords: GST M1 - GST T1 - GST P1 - polymorphism - gastric cancer - smoking - *H. pylori*

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Introduction

Gastric cancer is the second leading cause of death from cancer throughout the world. It is reported that about one million new cases of gastric cancer occurred in 2008, behind cancers of the lung, breast and colo-rectum. Moreover, more than 70% of cases occur in Eastern Asian countries, mainly in China. However, the incidence rates of adenocarcinomas of the proximal gastric and distal esophageal cancer are increasing in Western world. The wide geographic variation at an international levels of gastric cancer in terms of incidence and mortality indicated that the genetic and environmental factors may play a role in pathogenesis of this cancer.

Helicobacter pylori, which has been classified as group I carcinogen by World Health Organization, is recognized as one of the most important risk factors for gastric carcinogenesis (Correa, 1992; IARC, 1994). Almost 50% to 80% of the world's population infected with *H.pylori*, but only 1% of them developed gastric cancer (Graham et al., 1991; Parsonnet et al., 1997). This suggested the host genetic and environmental factors may modulate the risk of gastric cancer in association with *H.pylori* infection (Singh et al., 2006; Ghoshal et al., 2007; Ghoshal et al., 2008).

Glutathione S-transferases (GSTs), a supergene family of phase II detoxification enzymes, appear to form a protection mechanism against chemical carcinogenesis (Hayes et al., 1995). Human cytosolic GSTs are involved in metabolism of many xenobiotics, including an array of environmental carcinogens, chemotherapeutic agents, and endogenously derived reactive oxygen species (Ali-Osman et al., 1997; Garte et al., 2001; Aydemir et al., 2007; Zhou et al., 2009). Single nucleotide polymorphisms (SNPs) exist in GST genes, resulting in differential expression of the gene product (Pemble et al., 1994; Hayes et al.,1995; Ali-Osman et al., 1997; Hayes et al., 2000). GSTM1, GSTT1 and GSTP1 genes of the GST super gene family exhibit polymorphisms, which are associated with reduced enzyme activity (Pemble et al, 1994; Hayes et al., 1995; Ali-Osman et al., 1997). The results on the association of GST polymorphisms with gastric cancer are contradictory by previous studies (Colombo et al., 2004; Tamer et al., 2005 You et al., 2005; Martinez et a;., 2006). The difference might be due to ethnic variations, or differences in expression of GST genes.

The alone GST polymorphisms may not be sufficient in carcinogenesis of gastric cancer, one of the factors known to be associated with reduced GST activity is *H.pylori* infection, and the *H.pylori* along with variant GST genotypes may enhance the risk of cancer. Another factors is smoking. The polycyclic aromatic hydrocarbons

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(PAH) are the main carcinogens in tobacco smoke. Ultimate carcinogene, PAH-DE, can be detoxified through conjugation with glutathione by GSTs, which are phase II enzymes (Bartsch et al., 2000).

Therefore, we aimed to study the GST polymorphisms, GSTM1, GSTT1 and GSTP1 in gastric cancer, and explore the role of GST polymorphism in combination with H.pylori infection and smoking in the risk of gastric cancer.

Materials and Methods

Subjects

The study included 410 newly diagnosed cancer cases and 410 controls. The cases were histological confirmed in the General Hospital of Chengdu Military Area, between April 2007 and April 2011. Case with secondary or recurrent tumors was excluded.

The control group consisted of participants in the health examination center from April 2007 and April 2008, and they were matched with the cases by age and sex. All the cases and controls signed the formed consent and then provided their blood in our study.

The ethics committee of the General Hospital of Chengdu Military Area reviewed and approved the study.

Blood samples and DNA collection

All participants provided 5ml blood, and the blood were stored at -20 °C. Genomic DNA was extracted using a Qiagen Blood Kit (Qiagen, Chastworth, CA) according to the manufacturer's protocol.

Genotypeing

The GSTM1, GSTT1 and GSTM1 genotyping was performed by duplex polymerase-chain-reaction with the confronting-two-pairprimer (PCR-CTPP) method (Harries et al., 1997; Hung et al., 1997). The sequences of primers used for polymorphism of GSTM1, GSTTI and GSTP1 were amplified by primers described previous (Zhang et al., 2011). 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'. The 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.

H.pylori infection Diagnosis

The H. pylori infection was diagnosed by enzyme linked immunoabsorbent assay (ELISA) for IgG antibodies (HpIgG ELISA) using commercially available kit (Genesis Diagnostics, Cambridgeshire, UK) as per manufacturer's instructions on sera obtained from 5 mL blood. The sensitivity and specificity of the kit was 91% and 100%, respectively.

Statistical analysis

Statistical analysis was performed by using SPSS 16.0. The descriptive data for the major characteristics of study groups are expressed as mean and percent. We used t-tests to determine statistical differences in the continuous variables and chi-square test for the categorical variables. We compared differences in genotype distribution of GSTM1, GSTT1 and GSTP1 among cases and controls, as well as tests for Hardy-Weinberg equilibrium in controls. Adjusted odds ratios (OR) and their 95% confidence intervals (95% CI) of the association between genotype and gastric cancer were calculated by using conditional logistic regression models with adjustments for potential confounding factors, including sex, age, smoking, drinking and H.pylori. The interaction of genotype with smoking and H.pylori and smoking was estimated by using the logistic regression model. P were considered statistically significant which was less than or equal to 0.05.

Results

We included 410 cases of gastric cancer and 410 controls in the present study. The demographic characteristics of subjects are shown in Table 1. The proportion of smoking and drinking in the cancer cases was significantly higher than that in the controls (P<0.05), and the proportion of positive H.pylori infection was significant higher than that in controls.

The frequency distributions of the null GSTM1 genotype in the cases and controls were 58.6% and 50.6%, respectively. The frequency distributions of the null GSTT1 genotype in cases and controls were 42.5% and 57.5%, respectively. The GSTP1 Ile/ Ile, Ile/Val and Val/Val were 53.8%, 26.4% and 19.8% in cases, respectively, and were 55.9%, 28.2% and 15.9% in controls, respectively. Significant difference was observed in the frequencies of the GSTM1 genotypes, while no significant difference was found in GSTT1 and GSTP1. The GSTM1 and GSTT1 null genotypes were significantly associated with the risk of gastric cancer after adjusted for potential confounding factors (OR=1.68, 95% CI=1.32-2.23 for null GSTM1, OR=1.73; 95% CI=1.24-2.13 for null GSTT1, Table 2). The GSTP1 Ile/Val and Val/Val

Table 1. Distributions of Demographic Characteristics

Characteristics	Cases N=410(%)	Controls N=410(%)	P
Age (years)	49.5±4.9	48.7±5.0	
≤65	256(62.4)	251(61.2)	0.72
>65	154(37.6)	159(38.8)	
Sex			
Female	133(32.5)	133(32.5)	-
Male	277(67.5)	277(67.5)	
Smoking			
Ever	169(41.3)	93(22.6)	<0.05
No	241(58.7)	317(77.4)	
Drinking			
Ever	145(35.3)	117(28.6)	<0.05
No	265(64.7)	293(71.4)	
H.pylori infection			
Positive	282(68.7)	211(51.5)	<0.05
Negative	128(31.3)	199(48.5)	

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

Genetic polymorphisms	Cases N=410(%)	Controls N=410(%)	OR (95% CI)	Adjusted OR (95% CI)[1]
GSTM1				
Present	170(41.4)	203(49.4)	1.0 (Ref.)	1.0 (Ref.)
Null	240(58.6)	207(50.6)	1.38(1.04-1.84)	1.68(1.32-2.23)
GSTT1				
Present	174(42.5)	208(50.7)	1.0 (Ref.)	1.0 (Ref.)
Null	236(57.5)	202(49.3)	1.40(1.05-1.86)	1.73(1.24-2.13)
GSTP1				
Ile/ Ile	221(53.8)	229(55.9)	1.0 (Ref.)	1.0 (Ref.)
Ile/Val	108(26.4)	116(28.2)	0.96(0.69-1.35)	1.03(0.72-1.65)
Val/Val	81(19.8)	65(15.9)	1.29(0.87-1.91)	1.37(0.91-2.13)

[1]Adjusted for sex, age, drinking, smoking and H.pylori infection

Table 3. Combination of GSTM1 and GSTT1 Genotype Frequencies and Their Association with Risk of Gastric Cancer

Genetic polymorphisms	Cases N=410(%)	Controls N=410(%)	OR (95% CI)	Adjusted OR (95% CI)[1]
GSTM1/GSTT1				
Present/Present	65(15.9)	94(22.9)	1.0 (Ref.)	1.0 (Ref.)
Present/Null	105(25.6)	109(26.6)	1.39(0.90-2.16)	1.54(0.95-2.54)
Null/ Present	109(26.6)	114(27.8)	1.38(0.89-2.13)	1.51(0.92-2.43)
Null/Null	131(32.0)	98(23.9)	1.94(1.26-2.98)	2.54(1.55-3.39)

[1]Adjusted for sex, age, drinking, smoking and H.pylori infection

Table 4. GSTM1 and GSTT1 Gene Polymorphism and the Risk of Gastric Cancer by Smoking and H.pylori Infection

Variables	Cases/ Controls 410/410	Adjusted OR[1] (95% CI) GSTM1 Non-null	GSTM1 Null	Adjusted OR[1] (95% CI) GSTT1 Non-null	GSTT1 Null
Smoking					
Ever	169/93	1.0(Ref.)	1.94(1.54-2.58)	1.0(Ref.)	2.14(1.60-2.74)
No	241/317	1.0(Ref.)	1.30(0.98-1.74)	1.0(Ref.)	1.29(0.97-1.61)
P for interaction			<0.05		<0.05
H.pylori infection, N (%)					
Yes	282/211	1.0(Ref.)	1.63(1.14-1.96)	1.0(Ref.)	1.52(1.13-1.95)
No	128/199	1.0(Ref.)	1.44(1.08-1.93)	1.0(Ref.)	1.38(1.03-1.81)
P for interaction			0.14		0.34

[1]Adjusted for sex, age and drinking

was not significantly associated with the risk of gastric cancer. The combination of null GSTM1 and null GSTT1 was observed a higher risk for gastric cancer (OR=2.54, 95% CI=1.55-3.39, Table 3).

Further stratification was conducted regarding smoking and *H.pylori* in Table 4. The smoking modified the association between GSTM1 and GSTT1 null genotypes and the risk of gastric cancer, That is, smoking individuals with null GSTM1 and GSTT1 genotypes have higher increased risk of gastric cancer. There was significant interaction between smoking and GSTM1 and GSTT1 (P<0.05). We did not find modification of *H.pylori* in the association between these genotypes and cancer risk.

Discussion

The present study investigates the association between GST polymorphisms, GSTM1, GSTT1 and GSTP1 genotypes and susceptibility of gastric cancer, and the modification of smoking and *H.pylori* for GST polymorphisms. Our results showed a significant association between null GSTM1 and GSTT1 genotype and gastric cancer, however, we did not find the association between GSTP1 polymorphism and gastric cancer. An increased risk of gastric cancer was found in individuals with both null GSTM1 and GSTT1 genotypes. Moreover, we found smoking modified the association of polymorphisms of GSTM1 and GSTT1 with the risk of gastric cancer, and the *H.pylori* did not show modification on the association of these polymorphism and cancer risk.

The reports examining the GSTM1 and GSTT1 null genotypes and their association with gastric cancer are quite inconsistent. Several studies suggested that the GSTM1 null genotype increased gastric cancer risk (Katoh et al., 1996; Saadat et al., 2001; Luo et al., 2011), and three previous studies reported that the GSTT1 null genotype increased gastric cancer risk (Setiawan et al., 2000; Palli et al., 2005; Boccia et al., 2007). A meta-analysis study showed GSTM1 gene polymorphism might be risk factors for gastric cancer among Asians. Our study suggested a association exist, and that is in line with the previous results. However, there was previous study showed no significant association with GSTM1 and GSTT1 null genotypes and gastric cancer risk. A previous study conducted in Korea showed GSTM1 and GSTT1 null genotypes had no association with the risk of gastric cancer (Piao et al., 2009). A meta-analysis showed GSTT1 gene polymorphism was association with increased gastric cancer risk, while subjects with both GSTM1 and GSTT1 null genotypes had increased gastric cancer risk compared with those who had non-null genotypes (Chen et al., 2010). Yadav et al reported GSTM1 and GSTT1 status may not influence the risk of gastric cancer (Yadav et al., 2011).

The conflicting results regarding the associations between GSTM1 and GSTT1 null genotypes and risk for gastric cancer may be due to the limited sample size, differences in characteristics of subjects or ethnicities or by chance, and the differences may also be attributable to differences in exposure to environmental factors.

The interaction between tobacco smoking and GST polymorphism was estimated in our study. The PAHs in the tobacco smoking is reported to be detoxified by glutathione by GSTs (Bartsch et al., 2000). Therefore, null genotypes of GSTs would have less activity of detoxification of PAHs, and potentially increase the risk of chemical carcinogenesis. Our results showed smoking did not modify the association between GSTM1 and GSTT1 null genotypes and the risk of gastric cancer.

We did not find that *H.pylori* infection was associated with reduced GST enzyme activity. Previous study showed the *H.pylori* infection was associated with reduced GST enzyme activity, and that eradication of *H.pylori* restored normal enzyme activity (Verhulst et al., 2000). However, our study did not find the modification of *H.pylori* infection on the association of the GST polymorphisms and gastric cancer risk. The reason might be association between GST polymorphism and *H.pylori* infection affected by various factors such as extent of inflammation and dietary habits (Hoensch et al., 2002).

In conclusion, present study suggests the GSTM1 and GSTT1 null genotypes influences the susceptibility of gastric cancer, and smoking modifies the association

between the two genotypes and the risk of gastric cancer.

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