PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

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• ORIGINAL RESEARCH •

Glutathione S-transferases M1, T1 genotypes and the risk gastric cancer: A case-control study

Lin Cai[1], Shun-Zhang Yu[2] and Zuo-Feng Zhang[3]

[1]Department of Epidemiology, Fujian Medical University, Fuzhou 350004, Fujian Province, China
[2]Department of Epidemiology, Shanghai Medical University, Shanghai 200032,China
[3]Department of Epidemiology, UCLA School of Public Health, Los Angeles California, USA
Project supported by Natural Science Foundation of Fujian Province, China, No. C001009
Correspondence to Lin Cai, Department of Epidemiology, Fujian Medical University, Fuzhou 350004, Fujian Province, China. zjcailin@pub5.fz.fj.cn
Tel: 0086-591-3569264
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Abstract

AIM **Glutathione S-transferases (GSTs) are involved in the detoxification of many potential carcinogens and appear to play a critical role in the protection from the effects of carcinogens. The contribution of glutathione S-transferases M1 and T1 genotypes to susceptibility to the risk of gastric cancer and their interaction with cigarette smoking are still unclear. The aim of this study was to determine whether there was any relationship between genetic polymorphisms of GSTM1 and GSTT1 and gastric cancer.**

METHODS **A population based case-control study was carried out in a high-risk area, Changle County, Fujian Province, China. The epidemiological data were collected by a standard questionnaire and blood samples were obtained from 95 incidence gastric cancer cases and 94 healthy controls. A polymerase chain reaction method was used to detect the presence or absence of the *GSTM1* and *GSTT1* genes in genomic DNA. Logistic regression model was employed in the data analysis.**

RESULTS **An increase in risk for gastric cancer was found among carriers of *GSTM1* null genotype. The adjusted odds ratio (OR) was 2.63 [95% Confidence Interval (95% CI) 1.17-5.88], after controlling for age, gender, cigarette smoking, alcohol drinking, and fish sauce intake. The frequency of *GSTT1* null genotype in cancer cases (43.16%) was not significantly different from that in controls (50.00%). However, the risk for gastric cancer in those with *GSTM1* null and *GSTT1* non-null genotype was significantly higher than in those with both *GSTM1* and *GSTT1* non-null genotype (OR = 2.77, 95% CI 1.15-6.77). Compared with those subjects who never smoked and had normal GSTM1 genotype, ORs were 1.60 (95% CI: 0.62-4.19) for never smokers with *GSTM1* null type, 2.33 (95% CI 0.88-6.28) for smokers with normal *GSTM1*, and 8.06 (95% CI 2.83-23.67) for smokers with *GSTM1* null type.**

CONCLUSIONS ***GSTM1* gene polymorphisms may be associated with genetic susceptibility of stomach cancer and may modulate tobacco-related carcinogenesis of gastric cancer.**

Subject headings glutathione transferase/genetics; genotype; polymorphism (genetics); stomach neoplasm/genetics; case control studies

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INTRODUCTION

Glutathione S-transferases (*GSTs*), a supergene family of detoxification enzymes, appear to form a protection mechanism against chemical carcinogenesis. In human tissues this family consists of four multigene classes, referred to as alpha, mu, pi, and theta. The *GSTM1* gene is classified into the mu class and the *GSTT1* gene belongs to the theta class. They detoxify reactive chemical species, such as polycyclic aromatic hydrocarbon epoxides by catalyzing their conjugation to glutathione. Genes coding for *GSTM1* and *GSTT1* proteins are polymorphic in humans and these genes are absent in 10% -60% of different ethnic populations[1,2]. Accumulating evidence indicates that susceptibility to cancer is mediated by genetically determined differences in the effectiveness of detoxification of potential carcinogens. Genetic differences are likely to be a major source of interindividual variation in susceptibility to cancer[3].

Gastric cancer is the most common cancer in whole China[4-8], especially in Changle County, Fujian Province, China[9,10]. Previous studies have shown that a number of environmental risk factors may play a role in a multistep and multifactorial process[11-13]. Tobacco smoking has been considered a potential risk factor for gastric cancer[14]. Few data have so far been reported on the risk of gastric cancer associated with genetic and environmental exposures. To evaluate the relationships between *GSTM1/GSTT1* and gastric cancer, a molecular epidemiological study was conducted in Changle County.

MATERIALS AND METHODS

Study subjects

Cases and controls were all residents in Changle County, China, which is one of areas with the highest rates of gastric cancer in the world. All primary gastric cancers (n=95) were histologically confirmed or diagnosed by operation between January 1996 to March 1998. Population controls (n = 94) were randomly selected from the same geographical region, and matched to cases by their gender and age. The field staff conducted face-to-face interviews. Cases and controls were interviewed in the same manner using a standard epidemiological questionnaire. Blood samples (5mL) were collected.

GSTM1 and GSTT1 Assay

DNA was isolated from peripheral white blood cells by proteinase K (Huamei Biotechnology, Inc.) digestion and phenol / chloroform extractions. The PCR reactions were performed in 50μL of a solution containing PCR buffer (1.5 mmol·L^{-1} $MgCl_2$, 50 mmol·L^{-1} KCl, 10 mmol·L^{-1} Tris-HCl, pH 8.3), 200 μmol·L^{-1} of each dNTP, 1 μmol·L^{-1} of each primer, 200ng of template DNA, and 2.5 unit of TAQ DNA polymerase (Promega). Primer sequences for *GSTM1* were 5'-GCTTCACGTGTTATGGAGGTTC-3' and 5'-GAGATGAAGTCCTCCAGATTT-3', which produced a 157 base pair band. The *GSTT1* primers were 5'-TTCCTTACTGGTCCTCACATCTC-3' and 5'-TCACCGGATCATGGCCAGCA-3'-3, which produced a 480-base pair band. β-globin was used as an internal positive control, which was amplified with the following primers: 5'-CAACTTCATCCACGTTCACC-3' and 5'-GAAGAGCCAAGGACAGGTAC-3' and produced a 268-base pair band. The primers were synthesized by Sangon and PCR amplifications were carried out in a Thermal Cycler (Perkin Elmer 4800). Main cycling parameters were 94℃ for 8 min, followed by 35 cycles of 94℃ for 30s, 60℃ for 40s and 72℃ for 1 min with a final extension at 72℃ for 10 min. PCR products were detected by electrophoresis in agarose gels (2g·L^{-1} for *GSTM1* and 12g·L^{-1} for *GSTT1*).

Statistical analysis

The Chi-square method was used to test the frequencies of *GSTM1* and *GSTT1* genotypes. ORs and 95% CIs were calculated by logistic regression analysis controlling for possible confounding factors.

RESULTS

GSTM1 and *GSTT1* null genotypes are indicated by the absence of a 157bp band and 480 bp band, respectively. β-globin (268bp) indicating the presence of DNA is co-amplified in all the samples (Figures 1, 2).

Main characteristics of subjects

The main characteristics of cases and controls are presented in Table 1, the distribution of sex and age among cases and controls were not statistically significant (P>0.05).

Table 1 Main characteristics of cases and controls

	Cases (n=95)		Controls (n=94)	
	n	(%)	n	(%)
Age groups / yr				
<50	21	(22.1)	22	(23.4)
50 - 59	23	(24.2)	22	(23.4)
60 - 69	33	(34.7)	34	(36.2)
⩾70	18	(19.0)	16	(17.0)
Mean age	59 ± 11		58 ± 11	
Age range	32 - 78		34 - 79	
Gender				
Male	81	(85.3)	82	(87.2)
Female	14	(14.7)	12	(12.8)
Education				
College	1	(1.1)	1	(1.1)
High school	15	(15.8)	63	(67.0)
Elementary school	61	(64.2)	22	(23.4)
Illiterate	18	(19.0)	8	(8.5)

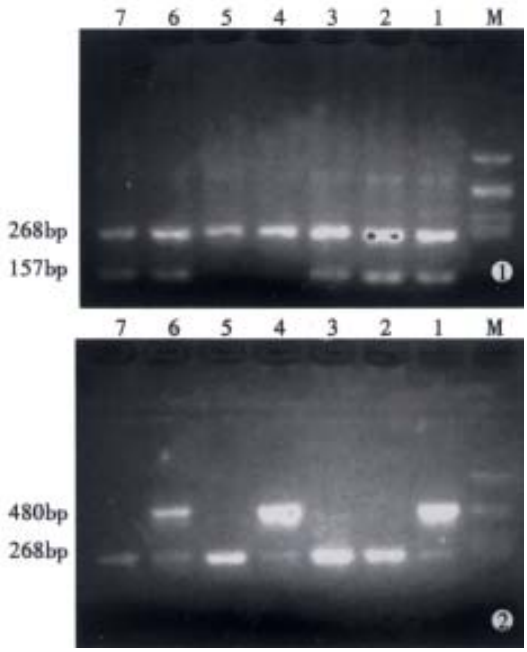

Figure 1 Agarose gel electrophoresis of PCR products. 157bp fragment: *GSTM1*; 268bp fragment: β-globin. Lane M: marker; Lanes 4 and 5:*GSTM1* null; Lanes 1, 2, 3, 6 and 7: *GSTM1* non null.

Figure 2 Agarose gel electrophoresis of PCR products. 480bp fragment: *GSTT1*; 268bp fragment: β-globin. Lane M: marker; Lanes 1, 4, and 6: nun-null; Lanes 2, 3, 5 and 7: *GSTT1* null.

GSTM1 and GSTT1 genotype frequencies in cases and controls

The results showed that GSTM1 null genotype distributed unevenly between gastric cancer cases and controls. The frequency of *GSTM1* null was significantly increased in gastric cancer cases compared with the general controls (χ^2 = 5.75, P=0.0165, Table 2).

Fifty percent (47/94) of individual in the controls exhibited the *GSTT*1 null genotype, and 43.2% (41/95) in gastric cancer cases. The frequencies of *GSTT*1 genotypes in cases and population controls were not significantly different (OR = 0.76, 95% CI 0.1 ~ 1.4). The odds ratio of gastric cancer associated with the combined genotypes of the polymorphisms of *GSTM*1 and *GSTT*1 are shown in Table 3. Persons who carried the *GSTM*1 null genotype and *GSTT*1 non-null had a higher risk of gastric cancer. The odds ratio was 2.77.

Table 2 Association between *GSTM1* and gastric cancer risk

	*GSTM*1 genotype				
	Nonnull	n	(%)	null	n	(%)
Contr		51	54.3		43	45.7
Case		35	36.8		60	63.2
Crude OR (95% CI)		2.03 (1.13-3.65)				
Adjusted OR[a] (95% CI)		2.03 (1.13-3.68)				
Adjusted OR[b] (95% CI)		2.47 (1.21-5.03)				
Adjusted OR[c] (95% CI)		2.63 (1.17-5.88)				

a: Logistic regression adjusted for age and sex; b: Adjusted for age, sex, cigarette smoking and alcohol drinking (yes / no); c: Adjusted for age, sex, cigarette smoking, alcohol drinking (yes / no), and fish sauce intake (continuous).

Table 3 Association between gastric cancer and combinations of *GSTM*1 and *GSTT*1 genotypes

*GSTM*1	*GSTT*1	Case		Contr		OR(95% CI)
		n	%	*n*	%	
Non-null	Non-null	21	22.1	30	31.9	1.00
Non-null	Null	14	14.7	21	22.3	0.95 (0.36~2.50)
Null	Null	27	28.4	26	27.7	1.48 (0.64~3.47)
Null	Non-null	33	34.7	17	18.1	2.77(1.15~6.77)

GSTM1 null genotype and smoking

Because *GSTM*1 may play an important role in the metabolism of tobacco smoke-derived carcinogens, the risk of gastric cancer associated with the polymophisms of metabolic enzymes may depend on the individuals' smoking status. We compared smokers with and without gastric cancer and found that the increased susceptibility to gastric cancer in smokers with *GSTM*1 null phenotype. The subjects which have been exposed to cigarette smoking and *GSTM*1 null genotypes had 8.06 fold risk to develop gastric cancer (Table 4).

Table 4 Risk of gastric cancer in relation to *GSTM1* genotypes by e smoking

Genotype	Smoke	Contr		Case		OR (95% CI)
		n	%	*n*	%	
Nonnull	No	28	29.8	12	12.6	1.00
Null	No	32	34.0	22	23.2	1.60 (0.62-4.19)
Nonnull	Yes	23	24.5	23	24.2	2.33 (0.88-6.28)
Null	Yes	11	11.7	38	40.0	8.06 (2.83-23.9)

DISCUSSION

Changle County is a hyperendemic area of gastric cancer. Familial aggregation of gastric cancer in this area has been reported in previous studies[15,16]. This familial tendency toward gastric cancer may result from a common environment shared by familial members of inherited genetic susceptibility[17]. Gastric cancer is a multistage process[18]. each caused by numbers of factors[19-31]. Environmental and host factors may all contribute to the etiology of gastric cancer[32]. The relationship between polymorphisms of genes involved in carcinogen metabolism and individual susceptibility to the mutagenic and carcinogenic actions of specific chemical exposure is a new field of research[33-35].

Recent studies reported genes that on code enzymes involved in the metabolism of carcioogens or environmental toxins may be related to an increased risk of cancer in some individuals[36,37]. GSTs are multifunctional proteins that catalyze many reactions between glutathione (GSH) and lipophilic compounds with electrophilic centers, including cytotoxic and genotoxic reactions[38]. Polycyclic aromatic hydrocarbons, N-nitrosomines, found in cigarette smoke and food, are potential human carcinogens[39,40]. Deficiency of detoxifying enzymes may affect the metabolic fates of these chemicals and raise cancer risks in exposed individuals[41]. The *GSTM*1 enzyme is involved in detoxifying a number of carcinogenic electrophiles, such as the epoxides of polycyclic aromatic hydrocarbons. Individuals with the homozygous *GSTM*1 null genotypes express no protein and are expected to have reduced abilities of detoxification of hazardous compounds, particularly epoxides.

In this study, *GSTT*1 gene deletion was not associated with gastric cancer. We observed evidence of a relationship between null genotype of *GSTM*1 and risk of gastric cancer. The *GSTM*1 genotype exhibited a higher frequency of gene deletions in cases than in controls. The finding suggests that *GSTM*1 may play a role in gastric cancer susceptibility. Gastric cancer, which is associated with exposure to smoking, may be more striking in individuals who carrying the null genotype *GSTM*1. This result suggests that intervention against smoking may be important for the prevention of gastric cancer in high incidence area because the *GSTM*1 is present in a majority of persons and the potential population impact may be important. However, these results should be considered preliminary. Larger studies will be needed to confirm potential gene-environment interactions.

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