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

Lin Cai¹, Shun-Zhang Yu² and Zu-Feng Zhang³

¹Department of Epidemiology, Fujian Medical University, Fuzhou 350004, Fujian Province, China
²Department of Epidemiology, Shanghai Medical University, Shanghai 200032, China
³Department of Epidemiology, UCLA School of Public Health, Los Angeles California, USA

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

Cai L, Yu SZ, Zhang ZF. Glutathione S-transferases M1, T1 genotypes and the risk of gastric cancer: A case-control study. World J Gastroenterol, 2001;7(4):506-509

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, especially in Changle County, Fujian Province, China [5,6]. Previous studies have shown that a number of environmental risk factors may play a role in a multistep and multifactorial process [7,8,9]. Tobacco smoking has been considered a potential risk factor for gastric cancer [10]. 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 and 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 MgCl₂, 50 mmol·L⁻¹ KCI, 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’-GCTTCACTGTTATGGAGTTC-3’ and 5’-GAGATGAGTCCTCAGATT-3’, which produced a 157 base pair band. The GSTT1 primers were 5’-TTCTTTACTGGTCTCTCAGC-3’ and 5’-TCACCGATCATGCGCCAGCA-3’, which produced a 480-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°C for 8 min, followed by 35 cycles of 94°C for 30s, 60°C for 40s and 72°C for 1 min with a final extension at 72°C 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 was 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

| Age groups / yr | Cases (n=95) | Controls (n=94) |
|-----------------|--------------|----------------|
|                 | n (%)        | n (%)          |
| <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        |

**Table 2** Association between GSTM1 and gastric cancer risk

| Gender          | Nonnull | n (%) | null | n (%) |
|-----------------|---------|-------|------|-------|
| Male            | 81      | (85.3)| 82   | (87.2)|
| Female          | 14      | (14.7)| 12   | (12.8)|

**Table 3**

| College         | Contr | Case |
|-----------------|-------|------|
|                 | 51    | 35   |
|                 | 54.3  | 36.8 |

**Crude OR (95% CI)** 2.03 (1.13-3.65)

**Adjusted OR (95% CI)** 2.03 (1.13-3.68)

**Adjusted OR (95% CI)** 2.47 (1.21-5.03)

**Adjusted OR (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).
compounds, particularly epoxides. These lipophilic compounds with electrophilic centers, including cytotoxic and genotoxic reactions.[38] Polycyclic aromatic hydrocarbons, N-nitrosamines, found in cigarette smoke and tobacco smoke-derived carcinogens, the risk of gastric cancer 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 GSTM1 null phenotype. The subjects which have been exposed to cigarette smoking and GSTM1 null genotypes had 8.06 fold risk to develop gastric cancer (Table 4).

**GSTM1 null genotype and smoking**

Because GSTM1 may play an important role in the metabolism of tobacco smoke-derived carcinogens, the risk of gastric cancer associated with the polymorphisms 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 GSTM1 null phenotype. The subjects which have been exposed to cigarette smoking and GSTM1 null genotypes had 8.06 fold risk to develop gastric cancer (Table 4).

**Table 3 Association between gastric cancer and combinations of GSTM1 and GSTT1 genotypes**

| Genotype        | Case Contr | OR(95% CI) |
|-----------------|------------|------------|
| Non-null        | Null       | n %        | n %        | 1.00 |
| Null            | Non-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) |

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

| Genotype | Smoke | Contr | Case | OR (95% CI) |
|----------|-------|-------|------|-------------|
| 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     | 1.00 |
| 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 carcinogens 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 S-derkvist E, Sun XF. Glutathione S-transferase T1 and M1 genotypes in normal mucoa, transitional mucosa and colorectal adenocarcinoma. J Cancer Pred Oncol, 1999;8:135-138.

1. Setiawan VW, Zhang ZF, Yu GP, Li YL, Lu ML, Tsai CJ, Cordova D, Wang MR, Guo CH, Yu SZ, Kurtz RC. GSTT1 and GSTM1 null genotypes and the risk of gastric cancer: a Case-control study in a Chinese population. Cancer Epidemiology, Biomarkers & Prevention, 2000;9:73-77.

2. Dong CH, Yu SZ, Chen GC, Zhao DM, Hu Y. Association of polymorphisms of glutathione S-transferase M1 and T1 genotypes with elevated aflatoxin and increased risk of primary liver cancer. Huaxue Xiaohua Zazhi, 1998;6:463-466.

3. Deng DJ, Chang YS, Li JY, Pan KF, Zhang JS, Li T, Zhao L, Zhang L, Ma JL, You WC. Comparison of total N-nitrosamides in fasting gastric juice from subjects in high and low risk areas for gastric cancer. Zhonghua Zhongliu Zazhi, 1997;19:96-99.

4. Xia HX. Association between Helicobacter pylori and gastric cancer: current knowledge and future research. World J Gastroenterol, 1998;4:93-96.

5. Wang SJ, Wen DG, Zhang J, Man X, Liu H. Intensify standard-based Helicobacter pylori screening to prevent gastric cancer. Shijie Huaren Xiaohua Zazhi, 1999;8:262-265.

6. Niu WX, Qin XY, Liu H, ‘Park’ WP. Clinicalopathological analysis of patients with gastric cancer in 1200 cases. World J Gastroenterol, 2001;7:281-284.

7. Lu HD, Wang QZ, Pan YR, Zhou TS, Xu ZX, Ke TW. Comparative study of serum Zn, Cu and Se contents between healthy people and patients in high, middle and low incidence areas of gastric cancer of Fujian Province. World J Gastroenterol, 1999;5:84-86.

8. Chen ZC, Zheng TR, Chen JS, Wu JP, Zhang QZ, Chen JB. Evaluation of ten-year results of cancer prevention and treatment in Changle City with high incidence of gastric cancer. Zhonghua Zhongliu Zazhi, 2000;2:111-135.

9. Cao GH, Yan SM, Yuan ZK, Wu L, Liu YF. A study of the relationship between trace element Mo and gastric cancer. World J Gastroenterol, 1998;4:516-518.

10. Xia HX. Association between Helicobacter pylori and gastric cancer: current knowledge and future research. World J Gastroenterol, 1998;4:93-96.

**REFERENCES**

1. Zhang H, Ahmadi A, Arbman G, Zdolsek J, Carstensen J, Nordenskj ld B, S-derkvist E, Sun XF. Glutathione S-transferase T1 and M1 genotypes in normal mucosa, transitional mucosa and colorectal adenocarcinoma. J Cancer Pred Oncol, 1999;8:135-138.

2. Setiawan VW, Zhang ZF, Yu GP, Li YL, Lu ML, Tsai CJ, Cordova D, Wang MR, Guo CH, Yu SZ, Kurtz RC. GSTT1 and GSTM1 null genotypes and the risk of gastric cancer: a Case-control study in a Chinese population. Cancer Epidemiology, Biomarkers & Prevention, 2000;9:73-77.

3. Dong CH, Yu SZ, Chen GC, Zhao DM, Hu Y. Association of polymorphisms of glutathione S-transferase M1 and T1 genotypes with elevated aflatoxin and increased risk of primary liver cancer. Huaxue Xiaohua Zazhi, 1998;6:463-466.

4. Deng DJ, Chang YS, Li JY, Pan KF, Zhang JS, Li T, Zhao L, Zhang L, Ma JL, You WC. Comparison of total N-nitrosamides in fasting gastric juice from subjects in high and low risk areas for gastric cancer. Zhonghua Zhongliu Zazhi, 1997;19:96-99.

5. Xia HX. Association between Helicobacter pylori and gastric cancer: current knowledge and future research. World J Gastroenterol, 1998;4:93-96.

6. Wang SJ, Wen DG, Zhang J, Man X, Liu H. Intensify standard-based therapy for esophageal and stomach cancer in tumor hospitals. World J Gastroenterol, 2001;7:80-82.

7. Wang Q, Jin PH, Lin GW, Xu SR. Cost-effectiveness of population-based Helicobacter pylori screening to prevent gastric cancer. Shijie Huaren Xiaohua Zazhi, 2000;8:262-265.

8. Niu WX, Qin XY, Liu H, ‘Park’ WP. Clinicalopathological analysis of patients with gastric cancer in 1200 cases. World J Gastroenterol, 2001;7:281-284.

9. Lu HD, Wang QZ, Pan YR, Zhou TS, Xu ZX, Ke TW. Comparative study of serum Zn, Cu and Se contents between healthy people and patients in high, middle and low incidence areas of gastric cancer of Fujian Province. World J Gastroenterol, 1999;5:84-86.

10. Chen ZC, Zheng TR, Chen JS, Wu JP, Zhang QZ, Chen JB. Evaluation of ten-year results of cancer prevention and treatment in Changle City with high incidence of gastric cancer. Zhonghua Zhongliu Zazhi, 2000;2:111-135.

11. Cao GH, Yan SM, Yuan ZK, Wu L, Liu YF. A study of the relationship between trace element Mo and gastric cancer. World J Gastroenterol, 1998;4:516-518.

12. Cai L, Yu SZ, Ye WM, Yi YN. Fish sauce and gastric cancer: an ecological study in Fujian Province, China. World J Gastroenterol, 2000;6:671-675.

13. Cai L, Yu SZ, Zhang ZF. Helicobacter pylori infection and risk of gastric cancer in Changle County, Fujian Province, China. World J Gastroenterol, 2000;6:374-376.

14. Cai L, Yu SZ. A molecular epidemiologic study on gastric cancer in Changle, Fujian Province. Shijie Huaren Xiaohua Zazhi, 1999;7:652-655.

15. Ye WM, Yi YN, Luo RX, Zhou TS, Lin RT, Chen GD. Diet and gastric cancer: a case-control study in Fujian Province, China. World J Gastroenterol, 1998;4:516-518.

16. Wang QZ, He J, Chen W, Chen Y, Zhou TS, Lin YC. Relationship between different sources of drinking water, water quality improvement and gastric cancer mortality in Changle County-A retrospective-cohort study in high incidence area. World J Gastroenterol, 1998;4:45-47.

17. Ottini L, Palli D, Faichetti M, D’Amico C, Amorosi A, Saieva.
C. Calzolari A, Cimoli F, Tatarelli C, Marchis LD, Masala G, Mariani Costantini R, Cama A. Microsatellite instability in gastric cancer associated with tumor location and family history in a high-risk population from Tuscany. Cancer Res, 1997;57:4523-4529

18 Wang GT. Progress in studies of mechanism of gastric precancerous lesions, carcinogenesis and their reversion. Shijie Huaren Xiaohua Zazhi, 2000;8:1-4

19 Harrison LE, Zhang ZF, Karpeh MS, Sun M, Kurtz RC. The role of dietary factors in the intestinal and diffuse histologic subtypes of gastric adenocarcinoma. Cancer, 1997;80:1021-1028

20 Vecchia CL, Mu-oz SE, Braga C, Fernandez E, Decarli A. Diet diversity and gastric cancer. Int J Cancer, 1997;72:255-257

21 Ward MH, Lopez-Carrillo L. Dietary factors and the risk of gastric cancer in Mexico City. Am J Epidemiol, 1999;149:925-932

22 Ward MH, Sinha R, Heineman EF, Rothman N, Markin R, Zhang ZF, Kurtz RC, Marshall JR. Cigarette smoking and esophageal and gastric cardia adenocarcinoma. Int J Cancer, 1997;71:14-19

23 Zhang ZF, Kurtz RC, Marshall JR. Cigarette smoking and esophageal and gastric cardia adenocarcinoma. J National Cancer Institute, 1997;89:1247-1249

24 Ji BT, Chow WH, Yang G, McLaughlin JK, Zheng W, Shu XO, Jin F, Gao RN, Gao YT, Fraumeni JF Jr. Dietary habits and stomach cancer in Shanghai, China. Int J Cancer, 1998;76:659-664

25 Hill MJ. Nutritional and metabolic aspects of gastrointestinal cancer. Curr Opin Clin Nutr Metab Care, 1998;1:405-407

26 Zhang ZF, Kurtz RC, Yu GP, Sun M, Gargan N, Karpeh M, Jr, Fein JS, Harlap S. Adenocarcinomas of the esophagus and gastric cardia: the role of diet. Nutrition Cancer, 1997;27:298-309

27 Zhang ZF, Kurtz RC, Sun M, Karpeh M, Yu GP, Gargan N, Fein JS, Georghioupolous SK, Harlap S. Adenocarcinomas of the esophagus and gastric cardia: medical conditions, tobacco, alcohol, and socioeconomic factors. Cancer Epidemiol, Biomarkers & Prevention, 1996;5:761-768

28 Morgner A, Miehlke S, Stolte M, Neubauer A, Alpen B, Thiede C, Klann H, Hiermeier FX, Ell C, Ehninger G, Bayerd-rffer E. Development of early gastric cancer 4 and 5 years after complete remission of Helicobacter pylori-associated gastric low-grade marginal zone B-cell lymphoma of MALT type. World J Gastroenterol, 2001;7:246-253

29 Zhang ZW, Farthing MG. Molecular mechanisms of H. pylori associated gastric carcinogenesis. World J Gastroenterol, 1999;5:369-374

30 Yun J, Guo F, Ebert MPA, Malfertheiner P. Expression of inducible nitric oxide synthase in human gastric cancer. World J Gastroenterol, 1999;5:430-431

31 Miehlke S, Kirsch C, Nagsatos G, Schwanterl M, Oberhuber G, Antos D, Dite P, L-uter J, Labenz J, Leedolter A, Malfertheiner P, Neubauer A, Ehninger G, Stolte M, Bayerd-rffer E. Helicobacter pylori and gastric cancer: current status of the Austrian-Czech-German gastric cancer prevention trial (PRISMA-Study). World J Gastroenterol, 2001;7:243-247

32 Bartsch H, Nair U, Risch A, Rojas M, Wikman H, Alexandrov K. Genetic polymorphism of CYP genes, alone or in combination, as a risk modifier of tobacco-related cancers. Cancer Epidemiology, Biomarkers & Prevention, 2000;9:3-28

33 Harrison DJ, Hubbard AL, MacMillan J, Wylie AH, Smith CAM. Microsomal epoxide hydrolase gene polymorphism and susceptibility to colon cancer. Br J Cancer, 1999;79:168-171

34 Stucker I, de Waziers I, Cenee S, Bignon J, Depierre A, Milleron B, Beaune P, Hemon D. GSTM1, smoking and lung cancer: a case-control study. Int J Epidemiol, 1999;28:829-835

35 Slattery ML, Edwards SL, Samowitz W, Potter J. Associations between family history of cancer and genes coding for metabolizing enzymes (United States). Cancer Causes Control, 2000;11:799-803

36 Slattery ML, Kampman E, Samowitz W, Caan BJ, Potter JD. Interplay between dietary inducers of GST and the GSTM1 genotype in colon cancer. Int J Cancer, 2000;87:728-733

37 Ömer RE, Verhoef L, Van’t Veer P, Idris MO, Kadaru AMY, Kampman E, Bunschoten A, Kok FJ. Peanut butter intake, GSTM1 genotype and hepatocellular carcinoma: a casecontrol study in Sudan. Cancer Causes Control, 2001;12:23-32

38 London SJ, Yuan JM, Chung FL, Gao YT, Coetzee GA, Ross RK, Yu MC. Isothiocyanates, glutathione S-transferase M1 and T1 polymorphisms, and lung-cancer risk: a prospective study of men in Shanghai, China. Lancet, 2000;356:724-729

39 Guo XK, Wang TJ, Gu JF. Effect of esophagus and stomach cancer-preventing vinegar on N-nitrosoprolarine formation in human body. China Natl J New Gastroenterol, 1997;3:269-270

40 Deng DJ, É Z. Overview on recent studies of gastric carcinogenesis: human exposure of N-nitrosamides. Shijie Huaren Xiaohua Zazhi, 2000;8:250-252

41 Jourenkova Mironova N, Voho A, Bouchardy C, Wikman H, Dayer P, Benhamou S, Hirvonen A. Glutathione S-transferase GSTM1, GSTM3, GSTP1 and GSTT1 genotypes and the risk of smoking-related oral and pharyngeal cancers. Int J Cancer, 1999;81:44-48

Edited by Lu HM