Interaction models of CYP1A1, GSTM1 polymorphisms and tobacco smoking in intestinal gastric cancer

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AIM: To explore the interaction models of the cytochrome P-450 (CYP) 1A1 Val variant and glutathione S-transferase (GST) M1 null polymorphisms with tobacco smoking in the occurrence of intestinal gastric cancer.

METHODS: A community-based case-control study was conducted in Yangzhong. Subjects included 114 intestinal types of gastric cancer with endoscopic and pathological diagnosis during January 1997 and December 1998, and 693 controls selected from their spouse, siblings or siblings-in-law who had no history of digestive system cancer. Logistic regression was used to estimate the interaction models.

RESULTS: The frequency of the CYP1A1 Val variant allele in cases did not differ from that in controls. The OR of GSTM1 null genotype was 2.0 (95% confidence interval [95% CI]: 1.2-3.1, P<0.01). It showed a significant type 2 form of interaction model when both CYP1A1 Val variant allele and former tobacco smoking existed (i.e., among the multiplicative effects, the disease risk is increased by the tobacco exposure alone but not by the CYP1A1 variant alone). The interaction index γ was 2.8, and OR_{adj} (95% CI) was 5.0 (1.9-13.4). GSTM1 null genotype and former tobacco smoking were significant in a type 4 interaction model (i.e., among the multiplicative effects, the disease risk is increased by GSTM1 null genotype or smoking; Intestinal gastric cancer).

CONCLUSION: Different interaction models of CYP1A1 Val variant allele and GSTM1 null genotype with tobacco smoking will contribute to understanding carcinogenic mechanism, but there is a need to further investigate in larger scale studies.

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Key words: Interaction models; CYP1A1; GSTM1; Tobacco smoking; Intestinal gastric cancer

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INTRODUCTION

Worldwide, gastric cancer is the second in rank overall (798 000 new cases per year), and ranks second in males, fourth in females. It remains the most common malignancy in many countries of the world[1,2], though the frequency of incidence and mortality is declining in almost all populations. Thirty-eight percent of gastric cancer cases occurred in China, where it remains to be most common and is the leading cause of cancer death in both sexes[3]. Yangzhong is among the areas with the highest gastric cancer mortality and incidence rate in south-east of China[4]. The crude mortality rate of gastric cancer changed from 96.9 to 110.9/100 000 during 1991 and 1997, and the average adjusted incidence rate in the same period was over 115/100 000 (unadjusted rate is 155.5/100 000). Previous studies have shown that high incidence rate of gastric cancer is associated with exposure to environmental factors (tobacco, alcohol consumption, and H pylori infection), and individual susceptibility[5,6].

Genetic polymorphisms in cytochrome P-450 (CYP) 1A1 and glutathione S-transferase (GST) M1 genes that metabolize known and potentially carcinogenic environmental exposures may affect enzymatic activities and alter an individual's ability to metabolize pro-carcinogenic and related compounds, which may change the biologic effect of exposures[7-9]. A large number of studies have examined the role of polymorphisms in CYP1A1, GSTM1, and cancer risk, including gastric cancer[6,10], but the results are equivocal[11-14]. The interaction between GSTM1 null genotype and tobacco smoking for the risk of gastric cancer has only been explored in three studies, with inconsistent evidence of departure from a
multiplicative model, possibly because of the small size (70, 91, and 136 gastric cancer cases, respectively), and the gene-environmental interaction (GEI) models best describing the risk of gastric cancer is not clear\[6,11,14\]. For this reason, we conducted a community-based case-control study in a Chinese population. Genetic polymorphisms in \textit{CYP1A1} and \textit{GSTM1} were analyzed to test the hypothesis that these genotypes have different interaction models with tobacco smoking in the development of intestinal gastric cancer.

**MATERIALS AND METHODS**

**Subjects**
All gastric cancer patients and controls in this study are Han ethnic Chinese selected from Yangzhong County. Gastric cancer was prevalent in cases diagnosed according to International Classification of Diseases for Oncology IX, code = 151, and classified by the criteria of Laurén\[15\]. One hundred and fourteen intestinal gastric cancer patients (76 men and 38 women; mean age and SD 59.4±9.9 years) were identified by endoscopic and pathological diagnosis in Yangzhong City Municipal Hospital from January 1997 to December 1998. To reduce misclassification of the histological types, two pathologists reviewed and confirmed all diagnosed cases. Controls were selected from case’s siblings (150 male and 140 female) and non-blood relatives (403 spouses and 140 female) and without digestive tract cancers. Both kinds of controls differed slightly in demographic features, and their results were combined to increase the sample size and decrease type I error. The study was approved by the regional ethics committees of Yangzhong. All participants were given an explanation of the study and informed consent was obtained. Study subjects completed a questionnaire administered by specially trained interviewers through a face-to-face interview. The questionnaire was designed to elicit detailed information on tobacco smoking habit, alcohol drinking habit, family history of cancer, and occupational exposures. Cigarette smokers were defined as subjects who reported smoking of at least one cigarette per day for 1 year or more, or whose accumulated tobacco cigarette consumption was over 18 packs per year. Former smokers were those who had stopped smoking for one or more years before the interview. Cumulative smoking exposure (pack years) was defined as one pack per day for 1 year equals 1 pack years.

**CYP1A1 and GSTM1 genotypes**
DNA extraction was performed using Puregene DNA isolation kits (Genta Systems, Minneapolis, MN, USA). PCR-RFLP approach was used to detect the 7th \textit{CYP1A1} \textit{Ile} /\textit{Val} variant at position 4 889. PCR was used to amplify the transcription regulatory region of \textit{CYP1A1} that includes the restriction enzyme recognition site for \textit{HincII}. The two allele-specific primers are respectively F5'-TCTT ACCTG- AAGGTTTCTCACCC-3' (Tm = 63.0) and R5'-TTTTT TTTTGTCAAACCTCAGG GGTCATA-3' (Tm = 65.1) modified from those previously reported\[16\]. The homozygous null polymorphism of \textit{GSTM1} was determined using a PCR approach as previously reported\[16\].

**Statistical analysis**
The relative associations between cases and controls were assessed by crude odds ratio (OR), interaction OR\(_\text{int}\) and the corresponding 95%CI. Unconditional logistic regression analysis was performed to assess the association between the \textit{CYP1A1} \textit{Ile}/\textit{Val} and \textit{GSTM1} null polymorphism and intestinal gastric cancer after adjusting for confounding factors. All models included as co-variables: gender, age (entered as a continuous variable), living areas (relative lower or higher incidence areas in Yangzhong), education level (years), former tobacco smoking, former alcohol drinking, BMI (weight [kg]/height [m\(^2\)]) and family history of cancer. Test of trend was calculated through logistic models based on semi-continuous and dummy variables. A common way to describe the interaction between the effect of an environmental agent and a genetic risk factor is to use a term called interaction index (\(\gamma\)), which is determined by coefficient (\(\beta\)) in a multiple logistic regression model. The coefficient of this interactive term was calculated according to the method of Taioli \textit{et al}\[17\]. If the types of interaction belong to multiplicative effects suggested by Khoury and Ottman\[18-20\], it included several types of interactions. Such as type 1 interaction which means that the disease risk is increased only in the presence of the genotype and the environmental exposure. Type 2 interaction: the disease risk is increased by the environmental exposure alone but not by the genotype alone. Type 3 interaction: the disease risk is increased by the genotype in the absence of the environmental exposure but not by the environment alone. Type 4 interaction means that the disease risk is increased by genotype or environmental exposure alone\[18-20\]. False positive report probability (FPRP) was used as an index for judging the noteworthy or not noteworthy results at the 0.5 FPRP level among significant GEI results\[21\]. All data analysis was performed with the SAS package Genmod (SAS Institute, Cary, NC, USA) for the personal computer.

**RESULTS**

**\textit{CYP1A1, GSTM1} polymorphisms and risk of intestinal gastric cancer**
Compared with controls, patients were significantly older (median age 59 years for cases and 53 years for controls) and with a BMI less than 20. Former tobacco smoking is significantly associated with the risk of intestinal gastric cancer (OR = 2.4, 95%CI: 1.0-5.8). A significant dose-response was observed in relation to increasing gastric cancer risk, especially in 20 pack-years or more smokers (Table 1). The frequencies of \textit{CYP1A1} \textit{Ile}/\textit{Val} and \textit{Val}/\textit{Val} genotypes in controls were 33.4% and 5.6%, respectively, which showed no significant difference from that in cases (32.1% and 5.4%, respectively). The homozygote null \textit{GSTM1} genotype was observed in 63.4% of cases, which was significantly higher than that in controls (53.5%). The adjusted OR was 2.0 (95%CI: 1.2-3.1).

**Interaction models of \textit{CYP1A1} and \textit{GSTM1} polymorphisms with former tobacco smoking**
Among former tobacco smokers with \textit{CYP1A1} \textit{Ile} or \textit{GSTM1} present genotypes, the OR\(_\text{int}\) of intestinal gastric...
With both exposure to tobacco smoking and the CYP1A1 Val allele or GSTM1 null genotype, the OReg of suffering intestinal gastric cancer increased sharply, 5.0 (95%CI: 1.9-13.4) and 8.4 (95%CI: 3.4-20.9), respectively. The \( \gamma \) were 2.8 and 3.4, respectively, which showed multiplicative effects of type 2 GEI model for CYP1A1 and type 4 model for GSTM1 (Table 2).

## DISCUSSION

A large number of molecular epidemiological studies completed in the past decade have identified the relative etiologic roles of the CYP1A1 Val variant allele and GSTM1 null genotype for cancer risk (including gastric cancer), although some results indicate no overall associations, only specific relationships were found in subgroups, such as in smokers, \( H pylori \)-infected patients or low consumption of

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### Table 1 Characteristics of the interviewed subjects

| Characteristics                  | Gastric cancer cases (114) | Control subjects (693) | OR (95%CI) |
|----------------------------------|-----------------------------|------------------------|------------|
| Median age yr±SD                 | 59±10                       | 53±10                  | 1.1 (1.0-1.1) |
| Min (yr)                         | 35                          | 30                     |            |
| Max (yr)                         | 82                          | 78                     |            |
| Gender                           |                             |                        |            |
| Female (%)                       | 38 (33)                     | 383 (55)               | 1.0 (Ref.) |
| Male (%)                         | 76 (67)                     | 310 (45)               | 1.2 (0.7-2.1) |
| F/M ratio                        | 1.2                         | 1.08                   |            |
| Educational level                |                             |                        |            |
| >6 yr                            | 44 (38.6)                   | 246 (35.5)             | 1.0 (Ref.) |
| ≥8 yr                            | 70 (61.4)                   | 447 (64.5)             | 1.0 (0.6-1.7) |
| Occupation                       |                             |                        |            |
| Manual                           | 51 (44.7)                   | 375 (54.1)             | 1.0 (Ref.) |
| Office                           | 9 (7.9)                     | 87 (12.6)              | 0.6 (0.2-1.3) |
| Retired                          | 54 (47.4)                   | 231 (33.3)             | 1.2 (0.7-1.9) |
| Marriage status                  |                             |                        |            |
| Married                          | 100 (87.7)                  | 649 (94.7)             | 1.0 (Ref.) |
| Divorce or bereft spouse         | 14 (12.3)                   | 36 (5.3)               | 1.4 (0.6-3.0) |
| Family history of cancer         |                             |                        |            |
| No                               | 84 (76.4)                   | 613 (90.3)             | 1.0 (Ref.) |
| Yes                              | 26 (23.6)                   | 66 (9.7)               | 3.9 (2.1-7.3) |
| BMI (kg/m²)                      |                             |                        |            |
| <20                              | 56 (49.1)                   | 95 (13.7)              | 1.0 (Ref.) |
| 20-23                            | 44 (38.6)                   | 254 (36.7)             | 0.5 (0.2-0.9) |
| Tobacco smoking habits           |                             |                        |            |
| Never                            | 51 (44.7)                   | 437 (63.0)             | 1.0 (Ref.) |
| Current                          | 36 (31.6)                   | 225 (32.5)             | 0.6 (0.3-1.3) |
| Former                           | 27 (23.7)                   | 31 (4.5)               | 2.4 (1.0-5.8) |
| Alcohol drinking habits          |                             |                        |            |
| Never                            | 78 (68.5)                   | 505 (72.9)             | 1.0 (Ref.) |
| Current                          | 11 (9.6)                    | 156 (22.5)             | 0.2 (0.1-0.5) |
| Former                           | 25 (21.9)                   | 32 (4.6)               | 2.3 (1.2-4.7) |
| Plasma \( H pylori \) CagA antibody |                         |                        |            |
| Negative                         | 136 (82.4)                  | 107 (49.3)             | 1.0 (Ref.) |
| Positive                         | 29 (17.6)                   | 110 (50.7)             | 0.2 (0.1-0.3) |
| CYP1A1 genotype                  |                             |                        |            |
| Ile/Ile                          | 70 (62.5)                   | 412 (61.0)             | 1.0 (Ref.) |
| Ile/Val                          | 36 (32.1)                   | 226 (33.4)             | 0.9 (0.5-1.4) |
| Val/Val                          | 6 (5.4)                     | 38 (5.6)               | 0.7 (0.2-1.8) |
| GSTM1 genotype                   |                             |                        |            |
| Present                          | 41 (36.6)                   | 314 (46.5)             | 1.0 (Ref.) |
| Null                             | 71 (63.4)                   | 361 (53.5)             | 2.0 (1.2-3.1) |

\( ^1 \)showed significant difference after adjusting co-variables.
Among gastric cancer cases, smoking-related DNA-adduct levels were higher in smokers than in non-smokers[24], and a positive finding in the present study even with a prior probability less than 0.01 (data not shown). These results suggest that the risk of intestinal gastric cancer is not well understood, tobacco-specific nitrosamines and other nitroso-compounds, plus other carcinogens contained in tobacco and tobacco smoke, are swallowed and may thus be involved in the process of gastric carcinogenesis[22,23]. Among gastric cancer cases, smoking-related DNA-adduct levels were higher in smokers than in non-smokers[25], and a study in China found smoking to be a risk factor for intestinal metaplasia and gastric dysplasia arising from chronic atrophic gastritis[26]. The biological mechanisms responsible for different interaction models observed in our study were not known, but it was possible to speculate that these interaction effects might be a reflection for individuals with and without environmental susceptible genotypes. This analytical approach may be used to determine higher risk individuals and take further preventive measures to decrease cancer risks in these susceptible individuals.

One limit of the study is the small number of subjects in some cells when GEI models were analyzed. The models resulting from the present study might be affected by chance, and need to be identified by more large-scale molecular epidemiological studies. Another potential limit was the

Table 2  Interaction models of CYP1A1 and GSTM1 polymorphism with former smoker in intestinal gastric cancer

| Genotype | Former smoker | Cases | Controls | ORd | 95%CI |
|----------|---------------|-------|----------|-----|-------|
| Ille     | No            | 57    | 391      | 1.0 | Ref.  |
| Val      | Yes           | 13    | 21       | 1.8 | 0.7-4.3 |
| Ille     | Yes           | 10    | 5.0e6 | 5.0-1.9-13.4 |
| Present  | No            | 31    | 302      | 1.0 | Ref.  |
| Null     | Yes           | 17    | 16       | 8.4e6 | 3.4-20.9 |

1Bold with gray fill color indicate “noteworthy at the 0.5 FPRP level”; 2with no fill color indicate “not noteworthy at the 0.5 FPRP level”.

Table 3  FPRP values for interactions between GSTM1 and former tobacco smoking in intestinal gastric cancer

| GSTM1 | Former | Observed | Assuming | Prior probability |
|-------|--------|----------|----------|------------------|
|       | Smoking | OR       | OR       | 0.25 | 0.1  | 0.01 | 0.001 | 0.0001 | 0.00001 |
| GSTM1 null | No     | 1.9       | 1.2       | 0.4811 | 0.7561 | 0.968 | 0.997 | 1.000 | 1.000 |
|        |        | 1.5       | 0.151     | 0.348 | 0.854 | 0.983 | 0.998 | 1.000 | 1.000 |
|        |        | 2.0       | 0.050     | 0.136 | 0.634 | 0.946 | 0.994 | 0.999 | 0.999 |
| GSTM1 present | Yes     | 3.5       | 2.4       | 0.202 | 0.432 | 0.893 | 0.988 | 0.999 | 1.000 |
|        |        | 3.0       | 0.157     | 0.322 | 0.840 | 0.981 | 0.998 | 1.000 | 1.000 |
|        |        | 4.0       | 0.093     | 0.236 | 0.773 | 0.972 | 0.997 | 1.000 | 1.000 |
| GSTM1 null | Yes     | 8.4       | 4.8       | 0.000 | 0.000 | 0.004 | 0.040 | 0.293 | 0.805 |
|        |        | 6.0       | 0.000     | 0.000 | 0.002 | 0.020 | 0.168 | 0.669 | 0.87|
|        |        | 8.4       | 0.000     | 0.000 | 0.001 | 0.009 | 0.087 | 0.487 | 0.487 |

fruit[6-10]. Other studies have reported contrary findings[11-14]. These inconsistent results might be contributed to ethnically diverse populations involved in studies, different histological subtypes of cases used, and different co-variables categorized the sub-population. Until now, only a few studies discussed interactions between tobacco smoking and the polymorphisms in the occurrence of gastric cancer[6-10]. Other studies have reported contrary findings[11-14].
selection of controls, which include case’s siblings. This kind of selection might create over-matching because siblings were more likely to have the same genotypes as cases than non-related controls, therefore leading to some loss of statistical efficiency, i.e. larger sample sizes required to attain the same statistical precision\cite{24}. But others consider that the use of sibling controls generally improves efficiency for GEI evaluations\cite{25,26}.

In conclusion, our studies identified two kinds of GEI models between the CYP1A1 Val variant allele, GSTM1 null genotype and tobacco smoking. Although the proposed carcinogen metabolism pathway seems a plausible explanation of our findings, further large studies are required to replicate these observations.

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REFERENCES
1. Parkin DM. The global burden of cancer. Seminars Cancer Biol 1998; 8: 219-235
2. Stadlander CT, Waterbor JW. Molecular epidemiology, pathology and prevention of gastric cancer. Carcinogenesis 1999; 20: 2195-2207
3. Setiawan VW, Zhang ZF, Yu GP, Lu QY, Li YL, Lu ML, Wang MR, Guo CH, Yu SZ, Kurtz RC, Hsieh CC. GSTP1 polymorphisms and gastric cancer in a high-risk Chinese population. Cancer Causes Control 2001; 12: 673-681
4. Zhang ZF, Kurtz RC, Klimstra DS, Yu GP, Sun M, Harlap S, Marshall JR. Helicobacter pylori infection on the risk of stomach cancer and chronic atrophic gastritis. Cancer Detect Prev 1999; 23: 357-367
5. Nebert DW, Ingelman-Sundberg M, Daly AK. Genetic epidemiology of environmental toxicity and cancer susceptibility: human allelic polymorphisms in drug-metabolizing enzyme genes, their functional importance, and nomenclature issues. Drug Metab Rev 1999; 31: 467-487
6. Tamer L, Ates NA, Ates C, Ercan B, Elipek T, Yildirim H, Camdeviren H, Atik U, Aydin S. Glutathione S-transferase M1, T1 and PI genetic polymorphisms, cigarette smoking and gastric cancer risk. Cell Biochem Funct 2005; 23: 267-272
7. Gonzalez CA, Sala N, Capella G. Genetic susceptibility and gastric cancer risk. Int J Cancer 2002; 100: 249-260
8. Parl FF. Glutathione S-transferase genotypes and cancer risk. Cancer Lett 2005; 221: 123-129
9. Agundez JA. Cytochrome P450 gene polymorphism and cancer. Curr Drug Metab 2005; 5: 211-224
10. Ng EK, Sung JJ, Ling TK, Ip SM, Lau JY, Chan AC, Liew CT, Chung SC. Helicobacter pylori and the null genotype of glutathione-S-transferase mu1 in patients with gastric adenocarcinoma. Cancer 1998; 82: 268-273
11. Deakin M, Elder J, Hendrickse C. Glutathione S-transferase genotypes and susceptibility to cancer: studies of interactions with GSTM1 in lung, oral, gastric and colorectal cancers. Carcinogenesis 1996; 17: 881-884
12. Suzuki S, Muroishi Y, Nakaniishi I, Oda Y. Relationship between genetic polymorphisms of drug-metabolizing enzymes (CYP1A1, CYP2E1, GSTM1, and NAT2), drinking habits, histological subtypes, and p53 gene point mutations in Japanese patients with gastric cancer. J Gastroenterol 2004; 39: 220-230
13. Wu MS, Chen CJ, Lin MT, Wang HP, Shun CT, Sheu JC, Lin JT. Genetic polymorphisms of cytochrome p450 2E1, glutathione S-transferase M1 and T1, and susceptibility to gastric carcinoma in Taiwan. Int J Colorectal Dis 2002; 17: 338-343
14. 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 Epidemiol Biomarkers Prev 2000; 9: 73-80
15. Lauren P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histoclinical classification. Acta Pathol Microbiol Scand 1965; 64: 31-49
16. Morita S, Yano M, Shiozaki H, Tsujinaka T, Ebisu C, Morimoto T, Kishibuti M, Fujita J, Ogawa A, Taniguchi M, Inoue M, Tamura S, Yamazaki S, Kikkawa N, Mizuno Y, Monden M. CYP1A1, CYP2E1 and GSTM1 polymorphisms are not associated with susceptibility to squamous-cell carcinoma of the esophagus. Int J Cancer 1997; 71: 192-195
17. Taioli E, Zocchetti C, Garte S. Models of interaction between metabolic genes and environmental exposure in cancer susceptibility. Environ Health Perspect 1998; 106: 67-70
18. Khoury MJ, James LM. Population and familial relative risks of disease associated with environmental factors in the presence of gene-environment interaction. Am J Epidemiol 1993; 137: 1241-1250
19. Khoury MJ, Wagener DK. Epidemiological evaluation of the use of genetics to improve the predictive value of disease risk factors. Am J Hum Genet 1995; 56: 835-844
20. Ottman R. An epidemiologic approach to gene-environment interaction. Genet Epidemiol 1990; 7: 177-185
21. Wacholder S, Chanock S, Garcia-Closas M, El Ghormli L, Rothman N. Assessing the probability that a positive report is false: an approach for molecular epidemiology studies. J Natl Cancer Inst 2004; 96: 434-442
22. Hecht SS, Hoffmann D. The relevance of tobacco-specific nitroamines to human cancer. Cancer Surv 1999; 8: 273-294
23. Hecht SS. Tobacco smoke carcinogens and lung cancer. J Natl Cancer Inst 1999; 91: 1194-1210
24. Dyke GW, Craven JL, Hall R, Garner RC. Smoking-related DNA adducts in human gastric cancers. Int J Cancer 1992; 52: 847-850
25. Kneller RW, You WC, Chang YS, Liu WD, Zhang L, Xu GW, Fraumeni JF, Blot WJ. Cigarette smoking and other risk factors for progression of precancerous stomach lesion. J Natl Cancer Inst 1992; 84: 1261-1266
26. Thomas DC, Witte JS. Point: population stratification: a problem for case-control studies of candidate-gene associations? Cancer Epidemiol Biomarkers Prev 2002; 11: 505-512
27. Gauderman W, Witte J, Thomas D. Family-based association studies. Monogr Natl Cancer Inst 1999; 26: 31-37
28. Witte JS, Gauderman WJ, Thomas DC. Asymptotic bias and efficiency in case-control studies of candidate genes and gene-environment interactions: basic family designs. Am J Epidemiol 1999; 148: 693-705