Tumor Biol. (2011) 32:215–222
DOI 10.1007/s13277-010-0115-8

RESEARCH ARTICLE

Genetic polymorphisms of metabolic enzymes—CYP1A1, CYP2D6, GSTM1, and GSTT1, and gastric carcinoma susceptibility

Ya-ping Luo · Han-chun Chen · Md. Asaduzzaman Khan · Fang-zhi Chen · Xin-xing Wan · Bo Tan · Fang-dan Ou-Yang · Dian-zheng Zhang

Received: 27 August 2010 / Accepted: 16 September 2010 / Published online: 29 September 2010

Abstract Genetic polymorphisms in metabolic enzymes are associated with numerous cancers. In this study, the relationships between genetic polymorphisms of phase I metabolic enzymes including cytochrome P450 1A1 (CYP1A1), CYP2D6 and phase II metabolic enzymes such as glutathione *S*-transferase M1 (GSTM1) and GSTT1 and gastric carcinoma susceptibility were investigated. Genomic DNA was isolated from the peripheral blood of 129 healthy controls and 123 gastric carcinoma patients from Han ethnic group of Hunan Province located in Central South China. The genetic polymorphisms of the above mentioned enzymes were analyzed using PCR-RFLP techniques. There was no significant difference among the frequencies of *CYP1A1* and/or *CYP2D6* gene's wild type, heterozygous or homozygous mutations between the gastric carcinoma group and control group. But the differences among the frequencies of *GSTM1* and *GSTT1* null genotype between the gastric carcinoma and control group were significant (both $P<0.05$). Also there were significant differences in the frequencies of *GSTM1* null in high/high–middle differentiated, middle differentiated, middle–low differentiated and low differentiated gastric tumor separately. *GSTM1* null showed an increased risk in middle–low differentiated and low differentiated gastric carcinoma type, but *GSTT1* null was not a risk factor for the four pathological types of gastric carcinoma mentioned above. We report here that the genotypes of *CYP1A1* and *CYP2D6* are not associated with gastric carcinoma risk; *GSTM1* null, but not *GSTT1* null inheritably increases risk of some pathological types of gastric carcinoma in Han ethnic population of Hunan Province.

Keywords Cytochrome P450 · Glutathione *S*-transferase · Genetic polymorphism · Gastric carcinoma

Y.-p. Luo · H.-c. Chen (✉) · M. A. Khan · X.-x. Wan · B. Tan · F.-d. Ou-Yang · D.-z. Zhang
Department of Biochemistry,
School of Biological Science and Technology,
Central South University,
Changsha, Hunan 410013, China
e-mail: chenhanchun@mail.csu.edu.cn

Y.-p. Luo
e-mail: luo_yaping@sohu.com

M. A. Khan
e-mail: asadkhanbmj@yahoo.com

X.-x. Wan
e-mail: daynstars@gmail.com

B. Tan
e-mail: 71058538@qq.com

F.-d. Ou-Yang
e-mail: guguxiaowanzi@yahoo.com.cn

D.-z. Zhang
e-mail: dianzhengzh@pcom.edu

F.-z. Chen
Department of Urology, Second Xiangya Hospital,
Central South University,
Changsha, Hunan 410011, China
e-mail: fzch82@yahoo.com

D.-z. Zhang
Department of Biochemistry/Molecular Biology,
Philadelphia College of Osteopathic Medicine,
Philadelphia, PA 19131, USA

Introduction

Multiple lines of evidence indicate that the development of the cancers is the results of both cumulative effect of

environmental risk factors and genetic susceptibility of the individual [1]. The environment–gene interaction in carcinogenesis is also well reflected by metabolic enzymes involved in the inactivation and/or detoxification of environmental carcinogens. Most of the carcinogens are metabolically inactivated by detoxification enzymes. Therefore, inherited variations in genes encoding the carcinogen-metabolizing enzymes may alter enzymatic activity and subsequently the carcinogens activation and/or deactivation [2]. Individual susceptibility to cancer is likely to be affected by the genotypes of biotransformation enzymes which represent significant ethnic differences in the frequency of alleles [3].

Cytochrome P450s (CYP450 or CYPs) are phase I biotransformation enzymes and these enzymes play a key role in the metabolism of drugs and environmental chemicals including food contaminants. Several CYP enzymes metabolically activate precarcinogens to genotoxic intermediates [4]. It has been hypothesized that polymorphism in the *CYP* genes could affect the individual's capacity in converting precarcinogens into carcinogens and this be one of the important contributing factor to individual's susceptibility to cancer development [5]. CYP1A1 participates in the metabolism of a vast number of xenobiotics, among which hydroxylation at a vacant position of an aromatic ring is considered to be the hallmark of the initiation of carcinogenesis; it has been reported that the formation of highly reactive intermediates can cause oncogenic mutations in experimental animals and humans [6]. It was revealed experimentally that the polymorphism of *Msp* I restriction site owing to a T–C variation in the 3′ non-coding region of *CYP1A1* allele is associated with increased catalytic activity and increased DNA adducts in cord blood and placenta of newborns [7]. CYP2D6 is involved in the metabolism of various therapeutic drugs and is a possible susceptibility factor for certain environmental reagent-induced diseases [8]. Ethnicity is an important contributing factor in the occurrence of CYP2D6 variability. In African-Americans, the frequency of CYP2D6 with poor metabolize activity is greater than that in the white populations. The occurrence of CYP2D6 with ultra rapid metabolizers appears to be greater amongst Middle Eastern and North African populations [9, 10]. Among the variants, the detrimental mutation of the *CYP2D6Ch* gene is C188-T, causing a Pro34-Ser amino acid substitution in the highly conserved region. This substitution results in lower CYP2D6 stability, and thus, decreased enzymatic activity [11]. Johansson [12] demonstrated that the lower capacity to metabolize the carcinogens in Chinese was well correlated with a higher frequency of the C188-T mutation in *CYP2D6* gene.

Glutathione *S*-transferases (GSTs) are phase II biotransformation enzymes, which catalyze the conjugation of reduced glutathione via a sulfhydryl group to electrophilic centers on a wide variety of substrates [13]. This activity is responsible for detoxifying both xenobiotics and endogenously generated compounds such as peroxidized lipids. Therefore, GSTs play a pivotal role in the elimination of toxic chemicals [14]. The GSTM1 and GSTT1 enzymes have been shown to alleviate cellular damage from cytotoxic substances in vitro [15]. Individuals with total or partial deletion of *GSTM1* and/or *GSTT1* showed reduced ability in detoxifying electrophilic carcinogens [16]. The *GSTM1* or *GSTT1* null, which results from the entire *GSTM1* or *GSTT1* gene deletion mutation, results in decreased capability in detoxifying some of the environmental carcinogens. It has been observed that *GSTM1* null may affect individual susceptibility to cancer [17]. *GSTT1* null has also been proved to be a risk factor for individual susceptibility to some cancers [7, 18].

Alcohol consumption, tobacco smoking, as well as some dietary factors believed to be related to the development of gastric carcinoma. It is conceivable that polymorphisms of the metabolic enzymes responsible in metabolizing dietary or smoking carcinogens are associated with gastric carcinoma [19]. The reported mechanisms of carcinogenic role of alcohol are production of acetaldehyde, which is a weak mutagen and carcinogen; induction of CYP2E1 and associated oxidative stress and conversion of procarcinogens to carcinogens; depletion of *S*-adenosylmethionine and consequently, induction of global DNA hypomethylation; induction of increased production of inhibitory guanine nucleotide regulatory proteins and components of extracellular signal-regulated kinase-mitogen-activated protein kinase signaling etc. [20]. Nicotine (major component of tobacco smoking) may promote carcinogenesis through activation of extracellular signal-regulated kinase/cyclooxygenase-2/vascular endothelial growth factor signaling pathway [20].

Therefore, numerous researches about the relationship between the polymorphisms of metabolic enzymes and cancer susceptibility have been conducted. However, the findings are controversial due to different reasons including the populations selected and their ethnicities. We were particularly interested in the risk factors of gastric carcinoma in the Han ethnic population in Changsha City of Hunan Province of China. A molecular epidemiological investigation aimed to figure out the possible relationship between the genetic polymorphisms of the genes, *CYP1A1*, *CYP2D6*, *GSTM1*, and *GSTT1*, and the susceptibility of gastric cancer was conducted. The genotype analyses of the two-phase metabolic enzymes would provide a rational strategy for the risk estimation and prevention of gastric carcinoma in the Han ethnic population of Central South China.

Materials and methods

Grouping of participants

This case-controlled study included 123 patients with gastric carcinoma and 129 healthy controls. The healthy controls in this study were defined as people who are cancer- and hematological disease-free. Both the patients and controls were selected from the Han ethnic group born and lived in Changsha City of Hunan Province, P. R. China. The 123 patients were all pathologically confirmed at Xiangya Hospital, of them, 51 were women and 72 were men with an average age of 55.2 years old. Among 123 patients, 35 were with high/high–middle differentiation (low grade tumor), 22 with middle differentiation (intermediate grade), 17 with middle–low differentiation (high grade), and 49 were with low differentiation (highest grade) tumor type. The National Cancer Institute Fact sheet was followed for differentiation typing [http://www.cancer.gov/images/documents/21b8921a-b275-46b4-8dde-957b7218f2ab/Fs5_9.pdf]. The 129 controls were recruited from citizens taking part in a health screening survey carried out at Xiangya School of Medicine, of them, 49 were women and 80 were men with an average age of 53.7 years old.

Genomic DNA isolation from peripheral blood cells

Heparinized peripheral blood samples were collected and the genomic DNAs were purified using standard sodium dodecyl sulfate-proteinase K treatment and phenol–chloroform extraction [21]. The purified DNAs were dissolved in tris–ethylenediaminetetraacetic acid buffer (TE buffer, pH 7.4) and the concentration of DNA was estimated by absorbance at 260 nm and adjusted to 100 ng/ml. The quality of DNA was assessed by agarose gel electrophoresis.

Amplification of *CYP1A1, CYP2D6, GSTM1*, and *GSTT1* genes

The allele sequences of *CYP1A1*, *CYP2D6*, *GSTM1*, and *GSTT1* polymorphic variants were obtained from Genbank. PCR primers (Table 1) for various alleles of each variant mentioned above were designed using software Primer Premier 5. PCR was carried out in a total volume of 50 ml containing 100 ng of DNA template, 10 pmol/L of each forward (F) and reverse (R) primer, 200 mmol/L of each deoxyribonucleotide triphosphate and 2 units of Taq polymerase (Roche Basel, Switzerland). The reaction conditions for *CYP1A1* and *GSTT1* variant allele amplification were as follows. The mixture was incubated for 5 min at 94°C to denature the template DNA; and then the PCR reactions were carried out for 30 cycles by denaturing at 94°C for 30 s, annealing for 30 s at 58°C, and extension for 30 s at 72°C, and a final extension period of 5 min at 72°C was performed after the PCR cycles finished. For analysis of *CYP2D6* and *GSTM1* genes, annealing temperature was set at 62°C and cycle number was 32. Five microliters of PCR products were loaded on a 1.5% agarose gel with 0.5 mg/ml of ethidium bromide. The DNA bands were visualized and photographed under UV light after electrophoresis.

Mutation analysis of *CYP1A1* and *CYP2D6* genes

To detect the mutation in *CYP1A1* and *CYP2D6* alleles, the related PCR product was digested with *Msp* I or *Kpn* I and the restriction fragment length polymorphism (RFLP) was conducted. Ten microliters of PCR product and 10 units of restriction enzyme were used for each digestion reaction. All the reactions were carried out overnight at 37°C. All digested products were separated on 2% or 3% agarose gel

Table 1 PCR primers for amplification of CYP1A1, CYP2D6, GSTM1, and GSTT1 genes

Primer name	Primer sequence	Product size (bp)
CYP1A1-F	5′-CAGTGAAGAGGTGTAGCCGCT-3′	340
CYP1A1-R	5′-TAGGAGTCTTGTCTCATGCCT-3′	
CYP2D6-F	5′-CACTGGCTCCAACGCTGGGCTGCACGGTA-3′	210
CYP2D6-R	5′-TAGCTTCTGGTCCAGCCTGTGGTTTCA-3′	
GSTM1-F1	5′-GCAGGAAACAAGGTAAAGG-3′	994
GSTM1-R1	5′-AGGCAGTAGAATCGCTTG-3′	
GSTM1-F2	5′-GAACTCCCTGAAAAGCTAAAGC-3′	220
GSTM1-R2	5′-GTTGGGCTCAAATATACGGTGG-3′	
GSTT1-F1	5′-GAGTAGAGGAAAGGGAATGG-3′	491
GSTT1-R1	5′-TGCACGATAGGTCACCTGAG-3′	
GSTT1-F2	5′-TTCCTTACTGGTCCTCACATCTC-3′	459
GSTT1-R2	5′-TCACCGGATCATGGCCAGCA-3′	
β-actin-F	5′-TGACGGTCAGGTCATCACTATCGGCAATGA-3′	260
β-actin-R	5′-TTGATCTTCATCGTGATAGGAGCGAGGGCA-3′	

F forward primer; *R* reverse primer

with 0.5 mg/ml of ethidium bromide. For PCR-RFLP analysis, the corresponding sequenced PCR products were used as controls.

Mutation analysis of *GSTM1* and *GSTT1* genes

The absence of *GSTM1*- or *GSTT1*-specific fragments indicated the corresponding null genotype, which was confirmed by using β-actin-specific primers to amplify the specific fragment as internal control. To further verify the gene deletion, a second PCR was performed using another pair of PCR primers in extension for the same gene (Table 1).

Statistics analysis

Genotype frequencies were reported as percentages. The difference in frequencies between groups was compared by using χ^2 test. A P value of 0.05 was considered statistically significant. The correlation between genetic polymorphisms of the studied metabolic enzymes and risk of gastric carcinoma was assessed by means of the odds ratio (OR) with 95% confidence intervals (95% CI) calculated by unconditional multivariate logistic regression. All computations were calculated by using the statistical package of SPSS (SPSS Inc, Chicago, Illinois, USA) version 13.

Results

The demographic characteristics of the gastric carcinoma cases and healthy controls are shown in Table 2.

The PCR products and the *Msp* I (C▼CGG) restriction map showed three genotypes of *CYP1A1* alleles which were wild type (W) without *Msp* I restriction site, heterozygous mutant (W/M) with *Msp* I restriction site in one allele and homozygous mutant (M/M) with *Msp* I restriction sites in both alleles, respectively. Furthermore, PCR-RFLP analysis by digesting the PCR products of *CYP2D6* alleles with *Kpn* I (GGTAC▼C) revealed that there were also three genotypes in this gene, wild type (W) with *Kpn* I restriction sites in both alleles, heterozygous mutant (W/M) with *Kpn* I restriction site in one of the alleles and homozygous mutant (M/M) without *Kpn* I restriction site (Fig. 1). *GSTM1* and *GSTT1* genotypes were analyzed by PCR amplification as described in materials and methods. The absence of *GSTM1* or *GSTT1*-specific fragments indicated the deletion mutation (Fig. 2). The β-actin gene was used as an internal control to confirm the presence of amplifiable DNA template in the reaction mixture.

There was no significant difference among the frequencies of *CYP1A1* and/or *CYP2D6* gene's wild type (W), heterozygous mutant (W/M) and homozygous mutant

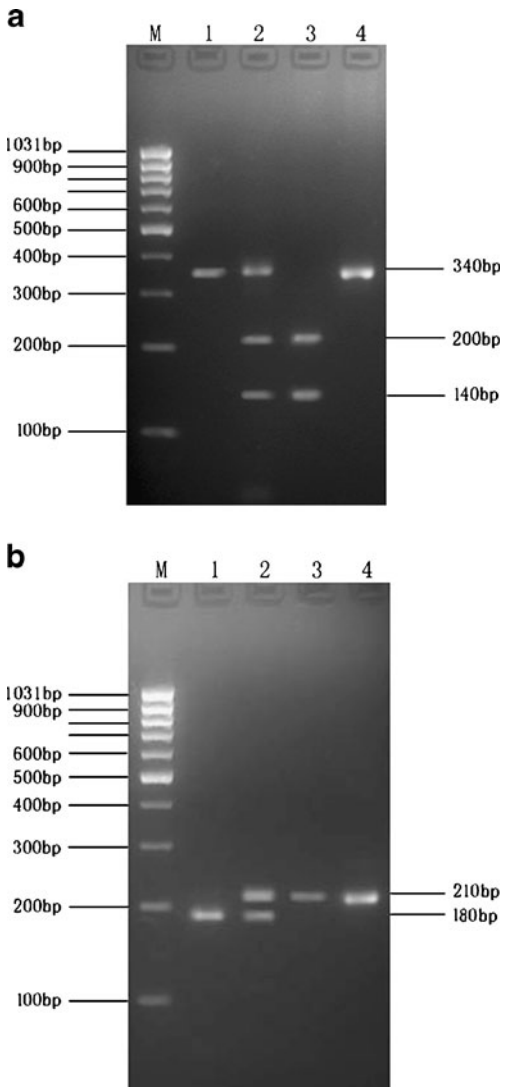

Fig. 1 Agarose gel electrophoresis of PCR products from **a** CYP1A1 alleles and the Msp I restriction fragments [*1* wild type, *2* heterozygous mutant, *3* homozygous mutant, *4* PCR products without Msp I digestion]. **b** CYP2D6 alleles and the Kpn I restriction fragments [*1* wild type, *2* heterozygous mutant, *3* homozygous mutant, *4* PCR products without Kpn I digestion]

Table 2 Sex and age distribution in gastric carcinoma cases and controls

	Cases (n=123)	Control (n=129)	P value
Sex			
Male	72(58.5%)	80(62.0%)	0.540[a]
Female	51(41.5%)	49(38.0%)	
Age (years)	27~76	23~70	0.063[b]
$\bar{x} \pm s$	55.2±10.6	53.7±12.3	

[a] χ^2 test

[b] t test between two samples

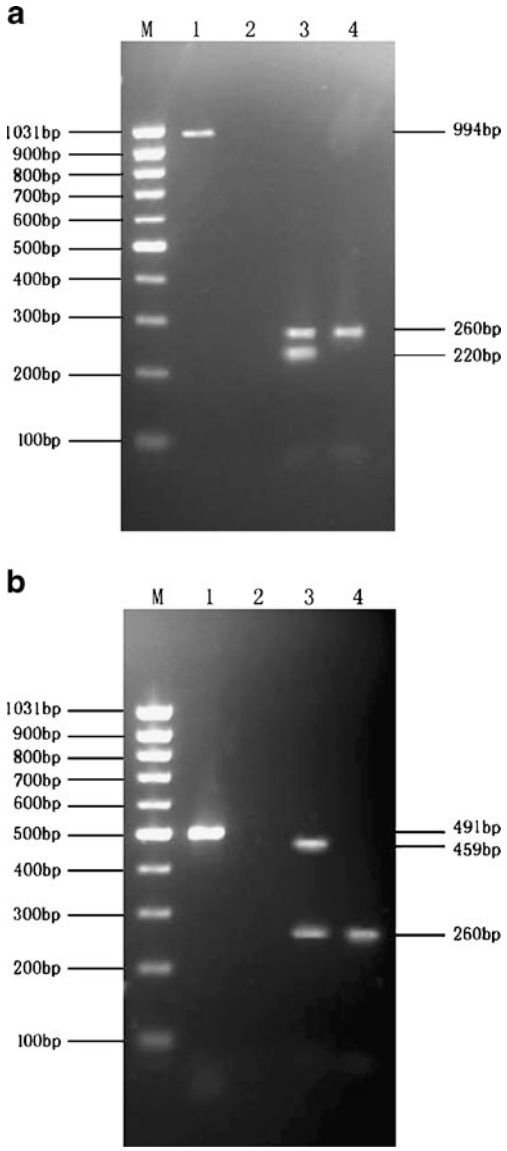

Fig. 2 Agarose gel electrophoresis of PCR products from **a** GSTM1 alleles and β-actin gene [*M* 100 bp DNA ladder marker, *1* GSTM1-F1/R1, *2* GSTM1-F1/R1 (deletion), *3* GSTM1-F2/R2/β-actin, *4* GSTM1-F2/R2 (deletion)/β-actin]. **b** GSTT1 alleles and β-actin gene [*M* 100 bp DNA ladder marker, *1* GSTT1-F1/R1, *2* GSTT1-F1/R1 (deletion), *3* GSTT1-F2/R2/β-actin, *4* GSTT1-F2/R2 (deletion)/β-actin]

(M/M) between the gastric carcinoma group and healthy control group. The difference in the frequencies of *GSTM1* null (*GSTM1*−/−) was significant (P=0.001), and there were significant differences in the frequencies of *GSTM1* null (*GSTM1*−/−) in four kinds of clinical adenocarcinoma pathology types: high/high–middle differentiated tumor (77.1%, P=0.030), middle differentiated tumor (81.8%, P=0.034), middle–low differentiated tumor (70.6%, P=0.019) and low differentiated tumor (73.5%, P=0.039), separately. The difference in *GSTT1* null (*GSTT1*−/−) was also significant (P=0.039), but there were no significant differences (P>0.05) in the frequencies of *GSTT1* null in the mentioned four kinds of clinical adenocarcinoma pathology types: high/high–middle differentiated tumor (60.0%, P=0.328), middle differentiated tumor (68.2%, P=0.149), middle–low differentiated tumor (64.7%, P=0.333), and low differentiated tumor (61.2%, P=0.191), respectively.

Excluding the influence from other factors, *GSTM1* null showed an increased risk of middle–low differentiated gastric carcinoma (OR=2.532, 95% C.I.: 1.738−6.692) and low differentiated gastric tumor (OR=1.960, 95% C.I.: 1.911−4.220). However, *GSTT1* null showed no influence to development of the four pathological types of gastric carcinoma mentioned above and the influence of homozygous or heterozygous mutation of *CYP1A1 Msp* I site and/or *CYP2D6 Kpn* I site was not significant on any type of differentiated gastric tumors (Table 3).

Discussion

Biotransformation of toxic xenobiotics to their nontoxic forms is considered as the most important step in the processing of the potentially toxic chemical substances toward their elimination [7]. Xenobiotics are metabolized typically in a two-phase reaction: activation and deactivation. In phase I, carcinogens are activated mainly by the CYP450 enzyme family and in phase II, usually under the catalysis of conjugation enzymes, like GSTs, the activated carcinogen is processed into more hydrophilic metabolites which are much easier for excretion [22]. The conjugation enzymes such as GSTs usually play important roles in the latter phase. Genetic polymorphisms of metabolic enzymes resulted from either partial or complete deficiencies significantly affect their catalytic activity and subsequently the levels of activated carcinogens. On the other hand, these activated carcinogens could affect the expression and function of the genes in DNA-repairing, oncogenes and/or tumor suppressor genes. Altogether, these effects could lead to cell damage and tumor genesis by affecting cellular growth, differentiation and apoptosis [23]. Thus, inter-individual variability in xenobiotic metabolism has been associated with different susceptibility to toxicity in response to a given environmental factor. Individuals with certain genotypes that evoke increased metabolic activation of carcinogens and decreased detoxification are more susceptible to environmental carcinogens and have higher risk of cancer development.

Genetic predisposition of CYP enzymes is known to be associated with gastro-esophageal cancer [24], however the association is controversial. There were even reports showed that there was no significant association between the polymorphic expression of CYP1A1, CYP2D6, and other CYP enzymes and gastric carcinoma risk [4, 25]. But significant association between *CYP1A1* polymorphism and

Table 3 Frequency of CYP1A1, CYP2D6, GSTM1, and GSTT1 genotypes and influence of the genotypes to the risk of gastric carcinomas

Group	Control	Gastric cancer	High/high–middle differentiation	Middle differentiation	Middle–low differentiation	Low differentiation
Cases (*n*)	129	123	35	22	17	49
CYP1A1						
W (%)	47(36.4)	38(30.9)	9(25.7)	6(27.3)	6(35.3)	16(32.6)
WM (%)	54(41.9)	61(49.6)	18(51.4)	12(54.5)	8(47.1)	24(49.0)
MM (%)	28(21.7)	24(19.5)	8(22.9)	4(18.2)	3(17.6)	9(18.4)
P:case/control in WM plus MM		0.427	0.326	0.556	0.860	0.768
OR_1:(WM/W)			1.711	1.736	1.170	1.315
95% $C.I_1$: (WM/W)			0.698–4.194	0.599–5.030	0.375–3.652	0.620–2.787
OR_2:(MM/W)			1.509	1.100	0.753	0.913
95% $C.I_2$: (MM/W)			0.517–4.403	0.284–4.265	0.170–3.333	0.352–2.369
CYP2D6						
W (%)	19(14.7)	22(17.9)	8(22.9)	4(18.2)	3(17.7)	8(16.3)
WM (%)	77(59.7)	71(57.7)	18(51.4)	13(59.1)	10(58.8)	30(61.2)
MM(%)	33(25.6)	30(24.4)	9(25.7)	5(22.7)	4(23.5)	11(22.5)
P:case/control in WM plus MM		0.612	0.373	0.924	0.965	0.975
OR_1:(WM/W)			0.543	0.830	0.789	0.860
95% $C.I_1$: (WM/W)			0.200–1.471	0.236–2.920	0.191–3.255	0.333–2.221
OR_2:(MM/W)			0.639	0.724	0.769	0.763
95% $C.I_2$: (MM/W)			0.209–1.953	0.171–3.065	0.152–3.895	0.258–2.261
GSTM1						
(+/+) plus (+/−) (%)	58(45.0)	30(24.4)	8(22.9)	4(18.2)	5(29.4)	13(26.5)
(−/−) (%)	71(55.0)	93(75.6)	27(77.1)	18(81.8)	12(70.6)	36(73.5)
P:case/control in null genotype		0.001	0.030	0.034	0.019	0.039
OR:(−/−)/(+/+) plus (+/−)			1.212	1.412	2.532	1.960
95% C.I:(−/−)/(+/+) plus (+/−)			0.525–2.797	0.504–3.954	1.738–6.692	1.911–4.220
GSTT1						
(+/+) plus (+/−) (%)	66(51.2)	46(37.4)	14(40.0)	7(31.8)	6(35.3)	19(38.8)
(−/−) (%)	63(48.8)	77(62.6)	21(60.0)	15(68.2)	11(64.7)	30(61.2)
P:Case/Control in null genotype		0.039	0.328	0.149	0.333	0.191
OR:(−/−)/(+/+) plus (+/−)			1.082	1.086	1.630	0.907
95% C.I:(−/−)/(+/+) plus (+/−)			0.459–2.554	0.399–2.953	0.670–5.505	0.438–1.880

W wild type, *WM* heterozygous mutation, *MM* homozygous mutation, + presence, − deletion, *(+/−)* heterozygous deletion, *(−/−)* null genotype, *OR* odd ratio, *C.I* confidence interval

gastric cancer risk was found in European population [26]. Polymorphism in *CYP1A1* was also not found to be associated with gastric carcinoma patients in USA [27] and Japan [28]. Possible explanations to the controversial results include sample size and detecting method, ethnicity, and random scatter around the null hypothesis. In our study, we did not find any significant relationship among the genetic polymorphisms in *CYP1A1* and/or *CYP2D6* between gastric carcinoma patients and healthy controls from the central southern region of China. It may be that the polymorphisms of these two genes are not susceptible to gastric carcinoma risk because of some specific genetic background or environmental influences. Further investigations are required to clarify the exact cause.

GST enzymes are known to metabolize tobacco-related carcinogens. In this study, we report a clear and significant association between *GSTM1* null (deletion) genotype and all types of differentiated gastric carcinoma. Genetic polymorphisms of *GSTM1* and/or *GSTT1*, especially their null genotypes have been reported consistently to be involved in different cancers. It may be that the polymorphisms of these two genes are susceptible to gastric carcinoma risk because of some specific genetic background or environmental influences. Further investigations are required to clarify the exact cause. The involvement of *GSTM1* and/or *GSTT1* null genotypes in esophageal squamous cell carcinoma and gastric cardiac carcinoma among Caucasian population has been reported previously [29]. But there was no significant

difference in the *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms between the gastric carcinoma cases and healthy controls from Korean population [30]. Also, Wideroff [27] found no evidence that the risk of adenocarcinomas of the esophagus or gastric cardia or noncardia stomach varies with *GSTP1*, *GSTM1*, or *GSTT1* genotype among USA population. Like CYP polymorphisms, possible explanations for controversial findings include sample size and detecting method, ethnicity, random scatter around the null hypothesis and different lifestyles.

In this case control study, we have reported a clear pattern of involvement of phase I and phase II metabolic enzymes in gastric carcinoma in Han ethnic population in Hunan, China. However, there are some limitations in our study. As a pilot project, the sample size was limited. Although most of the patients and controls are mainly selected from Changsha city in the province, the socioeconomic levels of this city is relatively high in Hunan Province. Therefore, the socioeconomic effects may not be very well reflected in our study. Investigation in the genetic polymorphism of metabolic enzymes and gastric carcinoma should be further extended with a bigger and more reprehensive population in future studies.

Acknowledgments The work was supported by the grant No. 30371660 from the National Natural Science Foundation of China and the grant CMB No. 99-698 from China Medical Board of New York, Inc.

Conflict of interests There is no conflict of interests regarding this article.

References

1. Oliveira C, Seruca R, Carneiro F. Genetics, pathology, and clinics of familial gastric cancer. Int J Surg Pathol. 2006;14:21–33.
2. Boccia S, Sayed-Tabatabaei FA, Persiani R, Gianfagna F, Rausei S, Arzani D, et al. Polymorphisms in metabolic genes, their combination and interaction with tobacco smoke and alcohol consumption and risk of gastric cancer: a case-control study in an Italian population. BMC Cancer. 2007;7:206–13.
3. Medeiros R, Vasconcelos A, Costa S, Pinto D, Ferreira P, Lobo F, et al. Metabolic susceptibility genes and prostate cancer risk in a southern European population: the role of glutathione *S*-transferases GSTM1, GSTM3, and GSTT1 genetic polymorphisms. Prostate. 2004;58:414–20.
4. Agundez JA. Cytochrome P450 gene polymorphism and cancer. Curr Drug Metab. 2004;5:211–24.
5. Roberts-Thomson IC, Butler WJ, Ryan P. Meat, metabolic genotypes and risk for colorectal cancer. Eur J Cancer Prev. 1999;8:207–11.
6. Androutsopoulos VP, Tsatsakis AM, Spandidos DA. Cytochrome P450 CYP1A1: wider roles in cancer progression and prevention. BMC Cancer. 2009;9:187–202.
7. Chen HC, Hu WX, Liu QX, Li WK, Chen FZ, Rao ZZ, et al. Genetic polymorphisms of metabolic enzymes CYP1A1, CYP2D6, GSTM1 and GSTT1 and leukemia susceptibility. Eur J Cancer Prev. 2008;17:251–8.
8. Thuerauf N, Lunkenheimer J. The impact of the CYP2D6-polymorphism on dose recommendations for current antidepressants. Eur Arch Psychiatry Clin Neurosci. 2006;256:287–93.
9. McLellan RA, Oscarson M, Seidegård J, Evans DA Ingelman-Sundberg M. Frequent occurrence of CYP2D6 gene duplication in Saudi Arabians. Pharmacogenetics. 1997;7:187–91.
10. Gaedigk A, Bradford LD, Marcucci KA, Leeder JS. Unique CYP2D6 activity distribution and genotype–phenotype discordance in black Americans. Clin Pharmacol Ther. 2002;72:76–89.
11. Yokoi T, Kamataki T. Genetic polymorphism of drug metabolizing enzymes: new mutations in CYP2D6 and CYP2A6 genes in Japanese. Pharm Res. 1998;15:517–24.
12. Johansson I, Oscarson M, Yue QY, Bertilsson L, Sjöqvist F, Ingelman-Sundberg M. Genetic analysis of the Chinese cytochrome P4502D locus: characterization of variant CYP2D6 genes present in subjects with diminished capacity for debrisoquine hydroxylation. Mol Pharmacol. 1994;46:452–9.
13. Douglas KT. Mechanism of action of glutathione-dependent enzymes. Adv Enzymol Relat Areas Mol Biol. 1987;59:103–67.
14. Hosgood HD, Berndt SI, Lan Q. GST genotypes and lung cancer susceptibility in Asian populations with indoor air pollution exposures: a meta-analysis. Mutat Res. 2007;636:134–43.
15. Norppa H, Hirvonen A, Jarventaus H, Uuskula M, Tasa G, Ojajarvi A, et al. Role of GSTT1 and GSTM1 genotypes in determining individual sensitivity to sister chromatid exchange induction by diepoxybutane in cultured human lymphocytes. Carcinogenesis. 1995;16:1261–4.
16. Bolt HM, Their R. Relevance of the deletion polymorphisms of the glutathione *S*-transferases GSTT1 and GSTM1 in pharmacology and toxicology. Curr Drug Metab. 2006;7:613–28.
17. Clavel J, Bellec S, Rebouissou S, Ménégaux F, Feunteun J, Bonaïti-Pellié C, et al. Childhood leukaemia, polymorphisms of metabolism enzyme genes, and interactions with maternal tobacco, coffee and alcohol consumption during pregnancy. Eur J Cancer Prev. 2005;14:531–40.
18. Saadat M. Genetic polymorphisms of glutathione *S*-transferase T1 (GSTT1) and susceptibility to gastric cancer: a meta-analysis. Cancer Sci. 2006;97:505–9.
19. Sung NY, Choi KS, Park EC, Park K, Lee SY, Lee AK, et al. Smoking, alcohol and gastric cancer risk in Korean men: the National Health Insurance Corporation Study. Br J Cancer. 2007;97:700–4.
20. Purohit V, Khalsa J, Serrano J. Mechanisms of alcohol-associated cancers: introduction and summary of the symposium. Alcohol. 2005;35:155–60.
21. Davis LG, Dibner MD, Battey JF (1986). Preparation of DNA from eukaryotic cells. In: Basic methods in molecular biology. Elsevier, New York, pp 42–50.
22. Hatagima A. Genetic polymorphisms and metabolism of endocrine disruptors in cancer susceptibility. Cad Saúde Pública. 2002;18:357–77.
23. Sinnett D, N'Diaye N, Labuda D, Krajinovic M. Genetic determinants of childhood leukemia. Bull Cancer. 2006;93:857–65.
24. Lao-Sirieix P, Caldas C, Fitzgerald RC. Genetic predisposition to gastro-oesophageal cancer. Curr Opin Genet Dev. 2010; 20:210–17.
25. Canturk P, Caner V, Oruc N, Akarca US, Tepeli E, Cetin OG, et al. The mRNA expression of cytochrome P450 isoforms in human gastric tissue. Hepatogastroenterology. 2010;57:372–6.
26. Agudo A, Sala N, Pera G, Capellá G, Berenguer A, García N, et al. Polymorphisms in metabolic genes related to tobacco smoke and the risk of gastric cancer in the European prospective investigation into cancer and nutrition. Cancer Epidemiol Biomark Prev. 2006;15:2427–34.
27. Wideroff L, Vaughan TL, Farin FM, Gammon MD, Risch H, Stanford JL, et al. GST, NAT1, CYP1A1 polymorphisms and risk

of esophageal and gastric adenocarcinomas. Cancer Detect Prev. 2007;31:233–6.
28. Suzuki S, Muroishi Y, Nakanishi 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; 39:220–30.
29. Zendehdel K, Bahmanyar S, McCarthy S, Nyren O, Andersson B, Ye W. Genetic polymorphisms of glutathione *S*-transferase genes GSTP1, GSTM1, and GSTT1 and risk of esophageal and gastric cardia cancers. Cancer Causes Control. 2009;20:2031–8.
30. Hong SH, Kim JW, Kim HG, Park IK, Ryoo JW, Lee CH, et al. Glutathione *S*-transferases (GSTM1, GSTT1 and GSTP1) and *N*-acetyltransferase 2 polymorphisms and the risk of gastric cancer. J Prev Med Public Health. 2006;39:135–40.