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Genetic polymorphisms of *GSTO2*, *GSTM1*, and *GSTT1* and risk of gastric cancer

Mohammad Masoudi · Iraj Saadat · Shahpour Omidvari · Mostafa Saadat

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Abstract *Objective* The glutathione *S*-transferases (GSTs) are a superfamily of proteins that participates in detoxification. The GSTs were dividing into several classes including omega (*GSTO*), mu (*GSTM*) and theta (*GSTT*) classes. In human *GSTO2*, *GSTM1*, and *GSTT1* are polymorphic. In order to study whether *GSTO2*, *GSTM1*, and *GSTT1* polymorphisms are associated with increased gastric cancer risk in Iranian patients, the present case–control study was done. *Methods* Genomic DNA was extracted from peripheral blood of 67 gastric cancer patients and 134 control subjects. The genotyping was performed using PCR-based method. The possible association of gastric cancer with the *GSTO2 N142D* polymorphism was estimated with assuming additive, dominant, and recessive effect of the variant *142D* allele. To investigate whether profiles of GST genotypes are associated with the risk of gastric cancer, we used unconditional logistic regression analysis. *Results* The *GSTO2 142D* allele in additive, dominant and recessive models was not associated with the risk. Because *GSTM1*, *GSTT1*, and *GSTO2* genes belong to low-penetrance genes which might be involved in the carcinogenesis, patients and controls without family of cancer in first-degree relatives were also analyzes separately. To investigate whether profiles of GST genotypes are associated with the risk of gastric cancer, we used unconditional logistic regression analysis with *GSTM1*, *GSTT1*, and *GSTO2* genotypes as predictor factors. The *GSTO2 DD* genotype was associated with decreased risk as compared to *GSTO2 NN* genotype (OR = 0.21, 95% CI: 0.05–0.92, $P = 0.038$). *Conclusions* Present findings show that *GSTO2 DD* genotype decreases the risk of gastric cancer in individuals without history of cancer in their first-degree relatives.

Keywords: Gastric cancer · Genetic polymorphism · *GSTM1* · *GSTO2* · *GSTT1* · Iran

M. Masoudi · I. Saadat · M. Saadat (✉)
Department of Biology, College of Sciences, Shiraz University, Shiraz 71454, Iran
e-mail: saadat@susc.ac.ir; msaadat41@yahoo.com

I. Saadat · M. Saadat
Institute of Biotechnology, Shiraz University, Shiraz, Iran

S. Omidvari
Department of Chemotherapy, Shiraz University of Medical Sciences, Shiraz, Iran

Introduction

The glutathione *S*-transferases (GSTs) are a superfamily of proteins that participate in phase II detoxification. They have important functions such as removal of reactive oxygen species, regeneration of *S*-thiolated proteins and conjugation of glutathione to endogenous and exogenous electrophile substrates [1, 2]. The GSTs were dividing into several classes including omega (*GSTO*), mu (*GSTM*) and theta (*GSTT*) classes [2].

The GSTO is a new identified subfamily of GSTs that has some different characteristics in structure and function from the other members of GST superfamily. They have a cysteine residue in their active site in contrast to serine or tyrosine that is in active sites of other subfamilies [3]. In addition, they catalyze the reduction of monomethylarsonic acid to monomethylarsonous acid that is the rate-limiting step in detoxification of inorganic arsenic [4].

GSTO class has two members, named *GSTO1* and *GSTO2*. The human GSTO2 is a protein with 243 amino acid residues. In human, the *GSTO2* is polymorphic with an *N142D* substitution in the coding region [5]. It is reported that the GSTO2 Asp142 (D142) variant allozyme

showed 20% reduction in level of expression compared with the level of the GSTO2 wild type (N142) allozyme [6]. There were few studies, which investigated the association of *GSTO2 N142D* polymorphism and risk of several types of cancers, such as breast cancer, hepatocellular carcinoma, colorectal cancer, basal cell skin carcinoma, and ovarian cancer [7–10]. However, there is no data about the association between the genetic polymorphism of *GSTO2 N142D* and gastric cancer risk.

GSTM1 and *GSTT1* are members of the GSTμ and GSTθ classes, respectively. In human, the *GSTM1* and *GSTT1* are polymorphic and the null alleles result in a lack of corresponding enzyme activities. Homozygosity for these null alleles (named the null genotypes) associated with increased risk of several multifactorial diseases such as several types of cancers [11–14], asthma [15], and cataract [16, 17]. Oxidative stress is involved in pathogenesis of these diseases. The *GSTO2*, *GSTM1* and *GSTT1*, as low-penetrance susceptibility genes may act additively or multiplicatively, increasing the risk of gastric cancer. There is no published article describing the association between the *GSTO2 N142D* genetic polymorphism and gastric cancer risk. Therefore, the present case–control study was done.

Materials and methods

Subjects

The present study consisted of 67 subjects (22 females, 45 males) with pathologically confirmed primary gastric cancer that were recruited from chemotherapy department of Nemazi hospital in Shiraz (southern Iran), from October 1999 to August 2000 and from July 2004 to July 2005. Sex and age frequency-matched controls were randomly selected from the healthy blood donors. A total of 134 healthy individuals (44 females, 90 males) were included in the study. Exclusion criteria for controls included previous history of cancer and psychiatric disorders. The mean age (SD; Min–Max) of the patients and the controls was 57.7 (13.2; 24–82) years and 57.8 (8.6; 42–82) years, respectively. There was no significant difference between case and control groups ($t = 0.03$, df $= 199$, $P = 0.973$). Because Iranian population is one of the most heterogeneous populations, we select our patients and controls from Persian Muslims living in Fars province (southern Iran). Informed consent was obtained from each subject before the study.

Each subject (patients and controls) was asked to complete a self-administered questionnaire establishing demographic information, cigarette smoking status and family history of cancer in first-degree relatives. Each subject with at least one first-degree relative with cancer was considered as a case with positive family history.

DNA extraction and genotyping analysis

Genomic DNA was extracted from whole blood samples. Genotypic analysis for the *GSTO2 N142D* polymorphism was determined by PCR-RFLP method. Primers for amplifying the *GSTO2* polymorphic site were 5′-AACC CTCCTAAAGCACCC-3′ and 5′-GCCTGTGAAAGCTG GTGTTAG-3′. PCR was performed in 20 µl final volume with 1.5 mM $MgCl_2$, 0.2 mM each dNTPs, 0.5 pmol each primer, 0.2 µl of 10× PCR buffer (Cinagene Co., Iran) and 1 unit of *Taq* DNA polymerase. 94°C for 5 minutes as initial denaturation, 35 cycles of 94°C for 1 min; annealing 62.5°C for 1 min and 72°C for 1 min was thermal condition of PCR. Amplified segments were treated with *Mbo*I restriction enzyme and were incubated at 37°C for one overnight. Using 2.5% agarose gel stained with ethidium bromide; *NN*, *ND* and *DD* genotypes shown 420, 420/287/133 and 287/133 bp bands, respectively.

The PCR conditions for determining the *GSTM1* and *GSTT1* genotypes were the same as that reported previously. The absence of amplified product was consistent with the homozygous null genotype of *GSTM1* and *GSTT1*. Successful amplification by β-globin specific primers confirmed the proper function of the PCR reaction. Evaluating the polymorphism and laboratory quality control were the same as that reported previously [13].

Statistical analysis

Selected characteristics were compared between cases and controls by using independent samples *t*-test and χ^2-test. A Chi-square test was performed for the polymorphism to determine if the control sample demonstrated Hardy–Weinberg equilibrium. The risk of cancer associated with the *GSTO2* polymorphism was estimated. We first estimated the risk of the genotypes *ND* and *DD* compared with the wild-type *NN* homozygote, and then evaluated the risk of *DD* versus (*ND* + *NN*) and (*DD* + *ND*) versus *NN*, which assumed recessive and dominant effects, respectively, of the variant *142D* allele. Unconditional logistic regression was used to calculate odd ratios (ORs) and 95% of confidence intervals (CIs) for the various genotypes. Data analysis was performed using SPSS software version 11.5. A probability of $P < 0.05$ was considered statistically significant. All *P*-values were two-tailed.

Results and discussion

The general characteristics of cases and controls are summarized in Table 1. Family history of cancer in first-degree relatives did not significantly differ between cases and controls ($P = 0.152$). However, smoking status differed

significantly between them ($P = 0.004$). This finding is consistent with published reports from several populations [18–20].

Detailed genotype distributions are summarized in Table 2. Patient and control groups were initially divided into two sex groups. Since no statistically difference was observed between sex groups for the frequency of the *GSTO2 N142D*, *GSTM1*, and *GSTT1* genotypes, the sex groups were pooled (data not shown).

The allelic frequency of *142D* in our control group was estimated equal to 0.369. The genotypes of *GSTO2 N142D* in the control group were in Hardy–Weinberg equilibrium ($\chi^2 = 0.067$, df = 1, $P = 0.95$). The frequency of *GSTO2 142D* allele was reported from several populations [5–10]. The frequency of *142D* allele in our samples is similar to those of Caucasians, but has essential difference with African and American-African populations [5–10].

Table 1 Selected characteristics of study subjects

Characteristics	Categories	Cases (67)	Controls ($n = 134$)	P*
Age (years)	Mean ± SD	57.7 + 13.2	57.8 + 8.6	0.973
Sex	Female	22	44	1.00
	Male	45	90	
Cigarette smoking	Non-smoker	38	90	0.004
	Smoker	24	21	
	Missing	5	23	
FH[a]	Negative	52	53	0.152
	Positive	11	5	
	Missing	4	76	

* P associated with either Student's t-test or χ^2-test

[a] Family history of cancer in first-degree relatives

Table 2 Association between *GSTO2*, *GSTM1*, and *GSTT1* polymorphisms and gastric cancer risk

Polymorphisms	Case	Control	OR	95% CI	P-value
GSTO2 N142D genotypes					
NN	33	54	1.0	–	–
ND	29	61	0.78	0.42–1.44	0.426
DD	5	19	0.43	0.15–1.26	0.125
GSTM1 genotypes					
Intact genotype	30	74	1.0	–	–
Homozygous null genotype	37	60	1.52	0.84–2.74	0.163
GSTT1 genotypes					
Intact genotype	42	96	1.0	–	–
Homozygous null genotype	50	38	1.50	0.81–2.79	0.198

The *ND* (OR = 0.78, 95% CI: 0.42–1.44, $P = 0.426$) and *DD* (OR = 0.43, 95% CI: 0.15–1.26, $P = 0.125$) genotypes were not associated with an increased risk as compared to *NN* genotype. There was no linear trend for presence of 0, 1, and 2 of the *142D* allele and risk of gastric cancer ($\chi^2 = 2.404$, $P = 0.121$). In order to show whether the *142D* allele has a dominant and/or recessive effect, we performed additional two comparisons. Assuming the recessive effect of the *D* allele (comparison between *DD* versus *NN* + *ND*), *DD* genotype did not increase the risk of the cancer (OR = 0.69, 95% CI: 0.38–1.26, $P = 0.228$). Assuming the dominant effect of the *D* allele (comparison between *DD* + *ND* versus *NN*), *DD* + *ND* genotypes were not associated with the risk of gastric cancer (OR = 0.49, 95% CI: 0.17–1.37, $P = 0.173$).

The frequencies of homozygous null genotypes of *GSTM1* and *GSTT1* in the control group were 44.8 and 28.4%, respectively, which were in accordance with recent data from our country [11–13, 15–18, 21]. In patient group, the frequencies of the homozygous null genotypes of *GSTM1* and *GSTT1* were higher in comparison with those of the control group, but these differences were not significant (For *GSTM1*: OR = 1.52, 95% CI: 0.84–2.74, $P = 0.163$; for *GSTT1*: OR = 1.50, 95% CI: 0.81–2.79, $P = 0.198$).

We know that *GSTM1*, *GSTT1*, and *GSTO2* genes belong to low-penetrance genes, which might be involved in the carcinogenesis [1, 11]. Therefore, patients and controls that had positive family history for cancer were excluded. Since one or more high-risk genes might have inherited in these patients and controls. To investigate whether profiles of GST genotypes are associated with the risk of gastric cancer, we used unconditional logistic regression analysis. The *GSTM1*, *GSTT1*, and *GSTO2* genotypes were used as predictor factors. Table 3 shows ORs and 95% CI for various genotypes based on fitted model. The *GSTO2 DD* genotype was associated with decreased risk as compared to *GSTO2 NN* genotype (OR = 0.21, 95% CI: 0.05–0.92, $P = 0.038$). The homozygous null genotype of *GSTT1* (OR = 2.34, 95% CI:

Table 3 Polymorphisms at *GST* loci and risk of gastric cancer in unrelated patients and controls using multivariate logistic regression analysis

Polymorphisms	OR	95% CI	P-value
GSTO2 ND	0.79	0.35–1.84	0.598
GSTO2 DD	0.21	0.05–0.92	0.038
GSTT1 homozygous null genotype	2.34	0.94–5.85	0.068
GSTM1 homozygous null genotype	1.06	0.48–2.35	0.885

Note: *GSTO2* genotypes were compared with *GSTO2 NN* genotype. The *GSTT1* and *GSTM1* homozygous null genotypes were compared with their intact genotypes

0.94–5.85, $P = 0.068$) and also the homozygous null genotype of *GSTM1* (OR = 1.06, 95% CI: 0.48–2.35, $P = 0.885$) were not associated with risk of gastric cancer. If smoking habits (which differed significantly between patients and controls) were included in the unconditional logistic regression analysis as another predictor of gastric cancer risk, then same results were obtained. At that time the *GSTO2 DD* genotype was associated with decreased risk as compared to *GSTO2 NN* genotype (OR = 0.21, 95% CI: 0.05−0.96, $P = 0.043$).

In conclusion, our present findings show that *GSTO2 DD* genotype decrease the risk of gastric cancer in individuals without history of cancer in their first degree relatives. It should be noted that there were few studies that investigated association between *GSTO2 N142D* polymorphism and risk of several types of cancers. There was no significant association between the polymorphism and risk of several types of cancers such as breast cancer, hepatocellular carcinoma, colorectal cancer, basal cell skin carcinoma, and ovarian cancer [7–10]. Our present finding is consistent with these reports, if study only *GSTO2 N142D* polymorphism; and it is not consistent with these reports, if study *GSTO2 N142D* polymorphism and other polymorphisms of GSTs, such as *GSTM1* and *GSTT1*. It may be suggested that profiles of *GST* genotypes are important in gastric carcinogenesis.

The limitations of our study could not be ignored. The small sample size (and missing data for some subjects) may be criticized. Our study might not have sufficient power to detect an association between genetic polymorphisms of GSTs and gastric cancer due to small sample size. Also, there are several other single nucleotide polymorphisms for *GSTO2* (in addition to *N142D*) in humans, which were not studied in the present work. Finally, gastric cancers are divided into proximal (cardia) and distal (noncardia) cancers that have different epidemiology [22]. This classification was not taken in consideration in the present study. Conducting future research using larger sample size and considering other potential related variables is highly recommended.

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References

1. Sheehan D, Meade G, Foley VM et al (2001) Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily. Biochem J 360:1–16
2. Nebert DW, Vasiliou V (2004) Analysis of the glutathione *S*-transferase (GST) gene family. Hum Genomics 1:460–464
3. Board PG, Coggan M, Chelvanayagam G et al (2000) Identification, characterization, and crystal structure of the Omega class glutathione transferases. J Biol Chem 275:24798–24806
4. Wang L, Xu J, Ji C et al (2005) Cloning, expression and characterization of human glutathione *S*-transferase Omega 2. Int J Mol Med 16:19–27
5. Whitbread AK, Tetlow N, Eyre HJ et al (2003) Characterization of the human Omega class glutathione transferase genes and associated polymorphisms. Pharmacogenetics 13:131–144
6. Mukherjee B, Salavaggione OE, Pelleymounter LL et al (2006) Glutathione *S*-transferase omega 1 and omega 2 pharmacogenomics. Drug Metab Dispos 34:1237–1246
7. Marahatta SB, Punyarit P, Bhudisawasdi V et al (2006) Polymorphism of glutathione *S*-transferase omega gene and risk of cancer. Cancer Lett 236:276–281
8. Leite JL, Morari EC, Granja F et al (2007) Influence of the glutathione *S*-transferase gene polymorphisms on the susceptibility to basal cell skin carcinoma. Rev Med Chil 135:301–306
9. Morari EC, Lima AB, Bufalo NE et al (2006) Role of glutathione *S*-transferase and codon 72 of P53 genotypes in epithelial ovarian cancer patients. J Cancer Res Clin Oncol 132:521–528
10. Pongstaporn W, Rochanawutanon M, Wilailak S et al (2006) Genetic alterations in chromosome 10q24.3 and glutathione *S*-transferase omega 2 gene polymorphism in ovarian cancer. J Exp Clin Cancer Res 25:107–114
11. Saadat M (2006) Genetic polymorphisms of glutathione *S*-transferase T1 (GSTT1) and susceptibility to gastric cancer: a meta-analysis. Cancer Sci 97:505–509
12. Saadat I, Saadat M (2000) The glutathione *S*-transferase mu polymorphism and susceptibility to acute lymphocytic leukemia. Cancer Lett 158:43–45
13. Saadat I, Saadat M (20001) Glutathione *S*-transferase M1 and T1 null genotypes and the risk of gastric and colorectal cancers. Cancer Lett 169:21–26
14. Lee SJ, Cho SH, Park SK et al (2002) Combined effect of glutathione *S*-transferase M1 and T1 genotypes on bladder cancer risk. Cancer Lett 177:173–179
15. Saadat M, Saadat I, Saboori Z, Emad A (2004) Combination of *CC16*, *GSTM1*, and *GSTT1* genetic polymorphisms is associated with asthma. J Allergy Clin Immunol 113:996–998
16. Saadat M, Farvardin-Jahromi M (2006) Occupational sunlight exposure, polymorphism of glutathione *S*-transferase M1, and senile cataract risk. Occup Environ Med 63:503–504
17. Saadat M, Farvardin-Jahromi M, Saadat H (2004) Null genotype of glutathione *S*-transferase M1 is associated with senile cataract susceptibility in non-smoker females. Biochem Biophys Res Commun 319:1287–1291
18. Koizumi Y, Tsubono Y, Nakaya N et al (2004) Cigarette smoking and the risk of gastric cancer: a pooled analysis of two prospective studies in Japan. Int J Cancer 112:1049–1055
19. Sjödahl K, Lu Y, Nilsen TI et al (2007) Smoking and alcohol drinking in relation to risk of gastric cancer: a population-based, prospective cohort study. Int J Cancer 120:128–132
20. Crane SJ, Locke GR 3rd, Harmsen WS et al (2007) Subsite-specific risk factors for esophageal and gastric adenocarcinoma. Am J Gastroenterol 102:1596–1602
21. Saadat M, Dadbine-Pour A (2005) Influence of polymorphism of glutathione *S*-transferase M1 on systolic blood pressure of normotensive individuals. Biochem Biophys Res Commun 326:449–454
22. Crew KD, Neugut AI (2006) Epidemiology of gastric cancer. World J Gastroenterol 12:354–362