

Role of glutathione S-transferase omega gene polymorphisms in breast-cancer risk

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ABSTRACT

Background/aims. Genetically influenced variations in the levels of activity and/or expression of some members of the glutathione S-transferase (GST) family have been identified as risk factors for cancer. One, GST omega (GSTO), has been found in a very limited number of studies. The aim of the present study was to investigate the influence of *GSTO1* and *GSTO2* polymorphisms on breast cancer risk.

Methods. DNA isolated from the blood of 101 patients with breast cancer and 151 healthy controls was investigated for *GSTO1* and *GSTO2* polymorphisms by polymerase chain reaction-restriction-fragment length polymorphism.

Results. Univariate and multivariate analyses showed no association between *GSTO1* and *GSTO2* genotypes and the risk of breast cancer. A higher prevalence of wild-type *GSTO1* (A140/A140) was significantly correlated with advanced-stage breast cancer (OR = 0.1, 95% CI, 0.01-0.77), but the presence of the genotype did not correlate with patient age at diagnosis, menopausal status, tumor size, lymph node metastasis, or estrogen-receptor status. No association was found between *GSTO2* genotype and clinicopathological features.

Conclusions. The results of the study suggest that *GSTO1* and *GSTO2* variants are not associated with breast cancer risk, but that wild-type *GSTO1* (A140/A140) is likely among cases at an advanced stage.

Introduction

Breast cancer is the second most common cancer of women in Thailand, accounting for about 15% of all female cancers. Its incidence has been increasing¹. The etiology of breast cancer is still poorly understood, although several risk factors are well established, including high estrogen exposure, life-style risk factors (e.g., alcohol and diet), and family history². Since human breast cancer results from genetic-environmental interactions, genetic factors need to be identified for a more accurate evaluation of overall breast cancer risk. Polymorphisms in breast cancer susceptibility genes with low penetrance have a greater contribution to breast tumorigenesis when combined with environmental exposure³.

Glutathione S-transferases (GST), a family of phase-II detoxification enzymes, play important roles in protecting cells against environmental carcinogens. Eight classes of cytosolic, soluble GST have been identified in humans: alpha, mu, pi, theta, zeta, sigma, and omega^{4,5}, whereas GST kappa is located in the mitochondria⁶ and peroxisomes⁷. In contrast, unlike other human GST, GST omega (GSTO) mediates glutathione-dependent thioltransferase and dehydroascorbate reductase activities, which are similar to those catalyzed by the glutaredoxins. In addition, GSTO catalyzes the reduction of monomethylarsonic acid, the rate-limiting reaction in the biotransformation of inorganic arsenic⁸, a notorious environmental carcinogen.

In humans, two expressed genes (*GSTO1* and *GSTO2*) and a pseudogene *GSTO3p* have been identified in the omega class GST. The *GSTO1* and *GSTO2* genes contain six exons, spanning 12.5 and 24.5 kb, respectively, and lie 7.5 kb apart on chromosome

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10q24.3. Two *GSTO* gene polymorphisms, *GSTO1**A140D and *GSTO2**N142D, have been identified in population studies⁹. Both polymorphisms were investigated in a single, small-scale (30 cases, 33 controls) study, which found the *GSTO1* polymorphism alone related to breast cancer¹⁰.

These findings are of epidemiologic importance, as it has been suggested that carriers of the *GSTO1* and *GSTO2* variant alleles account for 8-34% and 23-86%, respectively, of the general population⁹⁻¹³. In addition, only a few *GSTO* polymorphisms have been reported in the literature. The current study was conducted to evaluate any association between genetic *GSTO1* and *GSTO2* polymorphisms with breast cancer risk among Thai women. Since a number of key factors are involved in the etiology of breast cancer, multivariate analysis was used to elicit and adjust for the confounding effects of such factors. An association between gene polymorphisms and patient clinicopathological characteristics was also observed.

Patients and methods

Patients

In this case-control study, 101 cases of histologically diagnosed breast cancer were recruited from the National Cancer Institute, Bangkok, Thailand. A control group of 151 healthy individuals was selected from women who came to the same hospital for an annual health checkup. Informed consent was obtained from all individuals: demographic and anthropometric data, reproductive and medical history, physical activity, occupation, and dietary data were obtained by an interview. The study was reviewed and approved by the Ethics Committee of the National Cancer Institute, Bangkok. Clinical stage was classified according to the American Joint Committee on Cancer TNM staging system.

DNA isolation

A blood sample of approximately 7 ml was collected from each patient and control. Genomic DNA was isolated from buffy coats using a QIAmp DNA extract kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The DNA was quantitated by a spectrophotometer and stored at -80 °C.

Genotype analysis

Genotyping was performed by polymerase chain reaction-restriction-fragment length polymorphism. *GSTO1* and *GSTO2* genotypes were determined as previously described¹⁰. All analyses were blinded to case or control status. Histopathological features (i.e., tumor size, lymph node metastasis, staging and estrogen receptor status assessed using immunohistochem-

istry) were analyzed at the Pathological Division of the National Cancer Institute in Bangkok.

Statistical analysis

The observed genotype frequencies were compared with those calculated from Hardy-Weinberg equilibrium ($p^2+2pq+q^2$; where p is the frequency of the variant allele and $q = 1-p$) using the chi-square of goodness-of-fit with one degree of freedom, with respect to the distribution of the allele groups under study. Chi-squared and Fisher's exact tests were used to evaluate the differences between cases and controls, for age, menopausal status, pregnancy, breast feeding, oral contraceptive use, body mass index (BMI), tobacco smoking, alcohol consumption and education. Odds ratios (OR) and 95% confidence intervals (CI) were calculated by unconditional logistic regression to evaluate associations between *GSTO1* and *GSTO2* genotypes and breast cancer risk. The OR were adjusted for the potential confounders comprising age, menopausal status, pregnancy, BMI, and education. Associations between the genotypes and clinicopathological parameters were analyzed using OR and 95% CI estimated by the logistic regression model.

Results

The characteristics of the case and control cohorts are shown in Table 1. There were no significant differences between cases and controls regarding breast feeding, oral contraceptive use, smoking, or drinking alcohol. However, age ≥ 40 years, postmenopause, pregnancy, BMI ≥ 25 kg/m², and low educational level were more prevalent among cases than controls. The allelic frequencies for *GSTO1* and *GSTO2* polymorphisms were determined for both study populations. For *GSTO1*, the frequencies of the wild-type A140 alleles were 0.89 and 0.88 for cases and controls, respectively. For *GSTO2*, the frequencies of the N142 alleles were 0.77 for both cases and controls. The genotypic frequencies of the different *GSTO1* and *GSTO2* polymorphisms in cancer and control populations agreed with the Hardy-Weinberg equilibrium ($P > 0.1$).

Univariate and multivariate logistic regression analysis of the overall effect of the different *GSTO1* and *GSTO2* polymorphisms did not support an association between the presence of one polymorphism genotype and individual susceptibility to breast cancer, since the OR of *GSTO1* and *GSTO2* genotypes did not reach the level of statistical significance (Table 2).

The relationships of clinicopathological parameters with *GSTO1* and *GSTO2* genotypes are shown in Tables 3 and 4, respectively. A significant association was found between higher prevalence of wild-type *GSTO1* (A140/A140) and advanced stage breast cancer (OR =

Table 1 - General characteristics for the breast cancer cases (n = 101) and control population (n = 151)

Characteristics	Cases No. (%)	Controls No. (%)	P
Age			0.000
<40	12 (11.9)	79 (52.3)	
40-60	77 (76.2)	65 (43.0)	
>60	12 (11.9)	7 (4.6)	
Menopause			0.002
Pre	52 (51.5)	107 (70.9)	
Post	49 (48.5)	44 (29.1)	
Pregnancy			0.001
No	27 (26.7)	73 (48.3)	
Yes	74 (73.3)	78 (51.7)	
Breast feeding			0.076
No	12 (17.9)	6 (8.0)	
Yes	55 (82.1)	69 (92.0)	
Oral contraceptive use			0.685
No	63 (63.0)	100 (66.2)	
Yes	37 (37.0)	51 (33.8)	
Body mass index (kg/m ²)			0.014
<25	64 (63.4)	118 (78.1)	
≥25	37 (36.6)	33 (21.9)	
Tobacco smoking			0.359
No	98 (97.0)	149 (98.7)	
Yes	3 (3.0)	2 (1.3)	
Alcohol consumption			0.661
No	92 (91.1)	135 (89.4)	
Yes	9 (8.9)	16 (10.6)	
Education			0.000
≤9 years	60 (59.4)	43 (28.5)	
>9 years	41 (40.6)	108 (71.5)	

0.1, 95% CI, 0.01-0.77). No other clinicopathological parameter was significantly associated with *GSTO1* genotype. No significant association was found between *GSTO2* polymorphism and age of patient at diagnosis, menopausal status, tumor size, lymph node metastasis, stage, or estrogen receptor status.

Discussion

Differences between populations in the genotypic distribution of both *GSTO1* and *GSTO2* polymorphisms have been shown. For *GSTO1*, the frequency of the D140 allele was high in European Australians (f = 0.34) and low in Chinese (f = 0.17), Brazilians (f = 0.16), Mexicans (f = 0.12), Japanese (f = 0.12), and Africans (f = 0.08). In *GSTO2*, the D142 allele occurred most commonly in Africans (f = 0.86), with Australians (f = 0.31) and Chinese (f = 0.27) being similar⁹⁻¹³. In the present study, the frequencies for these alleles (D140, f = 0.12; D142, f = 0.23) among the controls were consistent with those for Asian populations.

Common low-penetrance cancer-susceptibility genes, acting together with endogenous and life-style risk factors, are likely to account for most sporadic breast cancers, which comprise the majority of all breast cancers¹⁴. Such genes can be identified by studying the biochemical or physiological pathways that are postulated to be involved in breast tumorigenesis. Polymorphisms in breast cancer susceptibility genes with low penetrance have a greater contribution to breast carcinogenesis in combination with exogenous and endogenous exposures³. Candidate polymorphic genes including *GSTO*, which reduce enzyme activity and lower capacity to biotransform inorganic arsenic, could play a role in breast carcinogenesis.

Arsenic is an obvious environmental carcinogen, and contamination of drinking water with inorganic arsenic is a worldwide health problem. Methylation is the main detoxification pathway for inorganic arsenic. Marked variations in methylation capacity have been found, and arsenic susceptibility has been noted among some individuals. *GSTO* genetic polymorphisms may contribute to such a variation between individuals¹⁵.

Only two previously published studies have demonstrated an association between *GSTO* polymorphism and

Table 2 - OR and 95% CI for *GSTO1* and *GSTO2* genotypes and breast cancer association

Genotype	Cases	Controls	OR crude	P	OR adjusted	P
<i>GSTO1</i>						
A140/A140	80	117	1.00 (Reference)		1.00 (Reference)	
A140/D140	20	33	0.85 (0.46-1.59)	0.62	0.93 (0.46-1.88)	0.83
D140/D140	1	1	1.45 (0.09-23.52)	0.79	0.97 (0.06-17.29)	0.99
A140/D140 + D140/D140			0.87 (0.47-1.60)	0.66	0.93 (0.46-1.86)	0.83
A140 allele frequency	0.89	0.88				
D140 allele frequency	0.11	0.12				
<i>GSTO2</i>						
N142/N142	59	86	1.00 (Reference)		1.00 (Reference)	
N142/D142	38	60	0.95 (0.56-1.60)	0.84	0.85 (0.47-1.55)	0.60
D142/D142	4	5	0.88 (0.20-3.80)	0.86	1.32 (0.24-7.14)	0.75
N142/D142 + D142/D142			0.94 (0.57-1.57)	0.82	0.88 (0.49-1.58)	0.67
N142 allele frequency	0.77	0.77				
D142 allele frequency	0.23	0.23				

In parenthesis, 95% CI. OR, Odds ratio.

Table 3 - Association of *GSTO1* polymorphism with clinicopathological parameters

Parameter	No.	A140/A140	A140/D140 + D140/D140	OR (95% CI)	P
Age					
<40	12	8	4		
40-60	77	62	15	0.48 (0.13-1.82)	0.28
>60	12	10	2	0.40 (0.06-2.77)	0.35
Menopausal status					
Pre	52	41	11		
Post	49	39	10	0.96 (0.37-2.50)	0.10
Tumor size (cm)					
≤2	34	25	9		
>2	67	55	12	0.61 (0.23-1.62)	0.32
Lymph node metastasis					
Negative	40	34	6		
Positive	61	46	15	1.85 (0.65-5.25)	0.25
Stage					
I + II	73	53	20		
III + IV	28	27	1	0.10 (0.01-0.77)	0.03
ER					
Positive	45	36	9		
Negative	38	30	8	1.01 (0.37-3.11)	0.91

OR, Odds ratios.

Table 4 - Association of *GSTO2* polymorphism with clinicopathological parameters

Parameter	N	N142/N142	N142/D142 + D142/D142	OR(95% CI)	P
Age					
<40	12	7	5		
40-60	77	43	34	1.11 (0.32-3.80)	0.87
>60	12	9	3	0.47 (0.08-2.66)	0.39
Menopausal status					
Pre	52	27	25		
Post	49	32	17	0.57 (0.26-1.28)	0.17
Tumor size (cm)					
≤2	34	20	14		
>2	67	39	28	1.03 (0.44-2.37)	0.95
LN metastasis					
Negative	40	25	15		
Positive	61	34	27	1.32 (0.59-2.99)	0.50
Stage					
I + II	73	40	33		
III + IV	28	19	9	0.57 (0.23-1.44)	0.24
ER					
Positive	45	28	17		
Negative	38	20	18	1.48 (0.62-3.56)	0.38

OR, Odds ratios.

breast cancer risk. One found a significantly higher risk of breast cancer among heterozygous or homozygous carriers of *GSTO1***D140*, but not carriers of *GSTO2***D142*. However, the study was conducted in small groups of Thai women¹⁰. The second study, of Danish women, found that homozygous carriage of *GSTO1***D140* was significantly associated with higher risk, especially for estrogen-receptor-positive breast cancer¹⁶.

In contrast, our findings did not show a significant role for the *GSTO* gene, or its polymorphisms, in breast cancer risk. The main differences between the two pre-

vious studies and the present one were the sample size and the menopausal status of the study populations. In the first study¹⁰, 30 cases and 33 controls were recruited. In the second¹⁶, all participants were postmenopausal women. In contrast, in the present study, 101 patients and 151 healthy controls, either premenopausal or postmenopausal women, were enrolled. Thus, although no association of *GSTO1* genotype with breast cancer risk was found in our study, such an association cannot currently be excluded for postmenopausal women, especially for estrogen-receptor-positive breast cancer.

To our knowledge, no association between *GSTO1* polymorphism and the clinicopathological features of patients with breast cancer has been reported. Therefore, in the present study, we investigated the association of *GSTO1* and *GSTO2* genotypes with a number of clinical parameters in the total case group. It was found that wild-type *GSTO1* (A140/A140) correlated with advanced stage breast cancer. Recently, a study demonstrated that mouse *GSTO1* overexpressed in a lymphoma cell line with resistance to radiation and chemotherapeutics¹⁷. Since *GSTO1* appears to be involved in drug and xenobiotic metabolism, it would be of great interest to investigate further whether resistance to radiation or chemotherapy occurs in carriers of wild-type *GSTO1*, particularly those with advanced stage breast cancer. Elucidating this relationship should lead to the improved clinical management of these patients.

In conclusion, no clear association between *GSTO1* or *GSTO2* polymorphism and breast cancer risk was found in the present study. However, we found wild-type *GSTO1* (A140/A140) more frequently among patients with advanced stage breast cancer.

References

1. Sriplung H, Sontipong S, Martin N, Wiangnon S, Vootiprux V, Cheirsilpa A, Kanchanabat C, Khuhaprema T: Cancer Incidence in Thailand, 1995-1997. *Asian Pacific J Cancer Prev*, 6: 276-281, 2005.
2. Dumitrescu RG, Cotarla I: Understanding breast cancer risk – where do we stand in 2005? *J Cell Mol Med*, 9: 208-221, 2005.
3. Rothman N, Wacholder S, Caporaso NE, Garcia-Closas M, Buetow K, Fraumeni Jr JF: The use of common genetic polymorphisms to enhance the epidemiologic study of environmental carcinogens. *Biochim Biophys Acta*, 1471: C1-10, 2001.
4. Hayes JD, McLellan LI: Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defence against oxidative stress. *Free Radical Res*, 31: 273-300, 1999.
5. Board PG, Goggan M, Chelvanayagam G, Easteal S, Jermini LS, Schulte GK, Danley DE, Hoth LR, Griffer MC, Kamath AV, Rosner MH, Chrnyk BA, Perregaux DE, Gabel GA, Geoghegan KF, Pandit J: Identification, characterization and crystal structure of omega class glutathione transferase. *J Biol Chem*, 275: 24798-24806, 2000.
6. Ladner JE, Parsons JF, Rife CL, Gilliland GL, Armstrong RN: Parallel evolutionary pathways for glutathione transferases: structure and mechanism of the mitochondrial class kappa enzyme rGSTK1-1. *Biochemistry*, 43: 352-361, 2004.
7. Morel F, Rauch C, Petit E, Piton A, Theret N, Coles B, Guillozo A: Gene and protein characterization of the human glutathione S-transferase kappa and evidence for a peroxisomal localization. *J Biol Chem*, 279: 16246-16253, 2004.
8. Zakharyan RA, Sampayo-Reyes A, Healy SM, Tsapralis G, Board PG, Liebler DC, Aposhian HV: Human monomethylarsonic acid (MMA(V)) reductase is a member of the glutathione S-transferase superfamily. *Chem Res Toxicol*, 14: 1051-1057, 2001.
9. Whitbread AK, Tetlow N, Eyre HJ, Sutherland GR, Board PG: Characterization of the human omega class glutathione transferase genes and associated polymorphisms. *Pharmacogenetics*, 13: 131-144, 2003.
10. Marahatta SB, Punyarit P, Bhudisawasdi V, Paupairoj A, Wongkham S, Petmitr S: Polymorphism of glutathione S-transferase omega gene and risk of cancer. *Cancer Lett*, 236: 276-281, 2006.
11. Hirakawa M, Tanaka T, Hashimoto Y, Kuroda M, Takagi T, Nakamura Y: JSNP: a database of common gene variations in the Japanese population. *Nucleic Acids Res*, 30: 158-162, 2002.
12. Granja F, Morari J, Morari EC, Correa LA, Assumpção LV, Ward LS: GST profiling may be useful in the screening for thyroid nodule malignancy. *Cancer Lett*, 209: 129-137, 2004.
13. Cerda-Flores RM, Budowle B, Jin L, Barton SA, Deka R, Chakraborty R: Maximum likelihood estimates of admixture in northeastern Mexico using 13 short tandem repeat loci. *Am J Human Biol*, 14: 429-439, 2002.
14. Johnson-Thompson MC, Guthrie J: Ongoing research to identify environmental risk factors in breast carcinoma. *Cancer*, 88: 1224-1229, 2000.
15. Yu L, Kalla K, Guthrie E, Vidrine A, Klimecki WT: Genetic variation in genes associated with arsenic metabolism: glutathione S-transferase omega 1-1 and purine nucleoside phosphorylase polymorphisms in European and indigenous Americans. *Environ Health Perspect*, 111: 1421-1427, 2003.
16. Oslen A, Astrup H, Sorensen M, Overvad K, Tjonneland A: Polymorphisms of glutathione S-transferase A1 and O1 and breast cancer among postmenopausal Danish women. *Eur J Cancer Prev*, 17: 225-229, 2008.
17. Giri U, Terry NHA, Kala SV, Lieberman MW, Story MD: Elimination of the differential chemoresistance between the murine B-cell lymphoma LY-ar and LY-as cell lines after arsenic (As₂O₃) exposure via the overexpression of *gstO1* (p28). *Cancer Chemother Pharmacol*, 55: 511-521, 2005.