RESEARCH COMMUNICATION

Glutathione S-Transferase Polymorphisms in Breast Cancers of Thai Patients

Tanett Pongtheerat[1]*, Maneerut Treetrisool[1], Wichai Purisa[2]

Abstract

Breast cancer is the most common cancer among women worldwide and second in Thailand. Glutathione S-transferase (GST) enzymes involved in the detoxification of reactive metabolites of carcinogens may be important in modulating susceptibility to cancers. This study aimed to determine the influence of genetic polymorphisms of glutathione S-transferase T1, M1, P1 and A1 on breast cancer in Thai patients. Links with clinico-pathological characteristics were also analyzed. The results showed no association between GSTs polymorphism and overall susceptibility to breast cancer in Thai patients ($P \geq 0.05$). However, the data pointed to a relation of the GSTP1(Ile105Val) polymorphism with progesterone receptor status ($P = 0.04$) and age at diagnosis ($P = 0.03$) of breast cancer cases. In summary, this is the first study to report association between glutathione S-transferase T1, M1, P1 and A1 genes polymorphism and breast cancer in Thai patients. While GST genotypes may not be associated with susceptibility, a GSTP1 polymorphism(Ile105Val) may be related to progression of breast cancer.

Key Words: Glutathione S-transferase (GSTs) - multiplex PCR - breast cancer

Asian Pacific J Cancer Prev, **10**, 127-132

Introduction

Breast cancer is the most common malignancy and cause of death in the western world. In Thailand, the incidence is 17.2/100,000 population, second only to cervical cancer in women. The incidence rate appears to be highest in Bangkok followed by Chiangmai, Lampang, Songkhla, and Khon Kaen (Sriprung et al., 2006). There are many factors involved in breast cancer development, such as longtime exposure of estrogen in her life resulting in hormonal imbalance, and dietary factors, like high intake of polyunsaturated fatty acids. Approximately 10-15 % of breast cancers are linked to inherited genes (Dunning et al., 1999).

The inherited metabolic capacity of glutathione S-transferases (GSTs) may be important in this context. Glutathione S-transferases are a superfamily of enzymes that are involved in the detoxification of reactive metabolites of carcinogens such as polycyclic aromatic hydrocarbons. Polymorphisms within genes which result in GSTs null phenotypes greatly affect the function of enzymes (Kang 2003, Kim et al., 2004). GSTM1 polymorphism has been identified with three alleles (GSTM1*0, GSTM1*A and GSTM1*B), of which GSTM1*0 is a null allele consisting of complete deletion of the GSTM1 gene. Individuals who are homozygous for this allele are unable to produce the GSTM1 protein(Hivonen et al., 1993). GSTT1 is also polymorphic and presents two alleles, GSTT1*1 active allele and the GSTT1*0 null gene. Like GSTM1, GSTT1*0 is a non-functional allele resulting from the deletion of the GSTT1 gene unable to produce the GSTT1 protein (Chenevix-Trench et al., 2002, Naoe et al., 2002).

A single nucleotide polymorphism in the GSTP1 gene causes substitution of isoleucine to valine at amino acid codon 105 (Ile105Val). The valine allele is associated with a decreased activity of the enzyme compared with isoleucine allele (Allan et al., 2001). The polymorphism in GSTA1 consisting of four mutational sites are apparently linked on the proximal promoter. The variants named as: GSTA1*A (-631G/T, -567T, -69C, -52G) and hGSTA1*B (-631G, -567G, -69T, -52A) (Ping et al., 2006).

In the present study, we used multiplex-PCR to determine gene deletion within glutathione S-transferase T1 (GSTT1), M1 (GSTM1) and PCR-RFLP to determine the polymorphism of the GSTP1 and GSTA1 in Thai breast cancer patients and healthy women. The correlation between genotypes polymorphism of GSTs genes and breast cancer were also analyzed.

Materials and Methods

Sample collection and DNA extraction

DNA samples were obtained from 43 Thai breast cancer patients tissues. Samples were collected from Pramongkutkloa Hospital. A control group was included, composed of 56 healthy females in Rangsit University, and DNA was extracted by salting out method (Miller et al.,1988).

*[1]Unit of Biochemistry, Department of Medical Sciences, Faculty of Science, Rangsit University, Patumthani, [2]Research Division, National Cancer Institute of Thailand, Bangkok, Thailand *For Correspondence: dtanett@yahoo.com*

Multiplex-PCR for GSTM1 and GSTT1

Genetic polymorphisms assays for Glutathione S-transferase GSTT1 and GSTM1 were detected by Multiplex polymerase chain reaction (mutiplex-PCR). This technique was used to detect the presence or absence of the GSTT1, GSTM1 genes in DNA sample (Deng et al., 2005, Abdel-Rahman et al., 1996).

Genotypes for the GSTM1 and GSTT1 deletions were determined by polymerase chain reaction (PCR) on the genomic DNA. Primers for the 5'region of GSTM1 (5'-GTT GGG CTC AAA TAT ACG GTG G-3') and the 3'region (5'-GAA CTC CCT GAA AAG CTA AAG C-3') were used to amplify a 215 bp fragment. Primers for the 5' region of GSTT1 (5'-TTC CTT ACT GGT CCT CAC ATC TC-3') and the 3' region (5'-TCA CCG GAT GGC CAG CA-3') were used to amplify a 480 bp fragment.

As an internal control exon 7 of the CYP1A1 gene was co-amplifiled using the primers 5'-GAA CTG CCA CTT CAG CTG TCT-3' and 5'-CAG CTG CAT TTG GAA GTG CTC-3'(Abdel-Rahman et al., 1996). The 25 µl multiplex-PCR of GSTM1 and CYP1A1 reaction mixture containing 20 pmol of each primers, 5 µl of 10x buffer, 8 µl of 1.25 mM dNTPs , 3.5 mM of MgCl2 , 2.5 U of Taq polymerase and 100 ng of template DNA. The amplified conditions for GSTM1 and CYP1A1, initial denaturation at 94˚C for 5 minutes, 35 PCR cycles were done. Amplification conditions were strand separation at 94 ˚C for 2 min, primer annealing at 59˚C for 1 minute, extension at 72˚C for 1 minute, and a final elongation step of 72˚C for 10 minutes.

The 25 ml multiplex-PCR of GSTT1 and CYP1A1 reaction mixture containing 20 pmol of each primers, 5 ml of 10x buffer, 8 µl of 1.25 mM dNTPs, 3.5 mM of MgCl2 , 2.5 U of Taq polymerase and 100 ng of template DNA. The amplified conditions for GSTT1 and CYP1A1 genes were initial denaturation at 94˚C for 5 minutes and 35 PCR cycles were done. Amplification conditions were strand separation at 94 ˚C for 2 min, primer annealing at 66˚C for 1 minute, extension at 72˚C for 1 minute, and a final elongation step of 72˚C for 10 minutes.

Detection of GSTM1 and GSTT1 Polymorphisms

The amplified products were detected by 2.0% agarose gel and visualized by staining with ethidium bromide. The presence or absence of GSTM1 and GSTT1 genes were detected by presence or absence of a band at 215 bp (corresponding to GSTM1) and a band at 480 bp (corresponding to GSTT1). A band at 312 bp (corresponding to CYP1A1) always present and was used as an internal control.

PCR for GSTP1and GSTA1

The polymorphism of the GSTP1 and GSTA1 genes were analyzed using the PCR-RFLP Technique. Primers for the 5'region of GSTP1 (5'-TCCCCAGTGACTGTGTGTTG-3') and the 3' region (5'-GAAGCCCCTTTCTTTGTTCA-3') were used to amplify a 224 bp fragment.by PCR in a mixture 25 µl containing 20 pmol primer, 5 µl of 10x buffer, 8 µl 1.25 mM dNTPs, 1.5 mM of $MgCl_2$, 2.5 U of Taq polymerase and 100 ng of template DNA. Following initial denaturation at 94˚C for 5 minutes, amplification conditions include strand separation at 94 ˚C for 30 seconds, primer annealing at 60˚C for 30 seconds, polymerization at 70˚C for 30 seconds, 30 PCR cycles were done and a final elongation step of 72˚C for 7 minutes. Amplification leads to a 224 bp band and visualized by staining with ethidium bromide (Gatedee et al., 2007).

Primers for the 5'region of GSTA1 (5'-GCATCAGCT TGC CCT TCA-3') and the 3' region (5'-AAACGC TGT CACCGT CCTG-3') were used to amplify a 400 bp fragment. DNA was amplified by PCR in a mixture 25 µl containing 20 pmol primer, 5 µl of 10x buffer, 8 ml 1.25 mM dNTPs, 1.5mM of MgCl2 , 2.5 U of Taq polymerase and 100 ng of template DNA. Following initial denaturation at 94˚C for 5 minutes, amplification conditions include strand separation at 94 ˚C for 30 seconds, primer annealing at 60˚C for 30 seconds, polymerization at 70˚C for 30 seconds, 30 PCR cycles were done and a final elongation step of 72˚C for 7 minutes. Amplification leads to a 224 bp band and visualized by staining with ethidium bromide (Ping et al., 2006).

RFLP Detection of GSTP1 and GSTA1 Polymorphisms

The A to G polymorphism of GSTP1 introduces a restriction site recognized by the BsmA1 restriction enzyme. Digestion of the PCR product with 10 U BsmA1 in a 10 µl volume for 18 h at 37˚C. Results either in retention of the 224 bp product or complete digestion to 146 bp and 78 bp fragments corresponding to individuals homozygous for the Ile or Val alleles, respectively. The presence of all three fragments corresponded to individuals heterozygous at codon 105. Products were separated on 3.0% agarose and visualized with ethidium bromide.

For GSTA1 genes polymorphisms analysis, digestions (20 µl) were performed for 18 h at 37˚C using 5 units restriction enzyme EarI and 4 µl unpurified PCR product as supplied with the restriction enzyme. Aseptic distilled water substitutes the restriction enzyme acted as the negative control. The digested products were separated on 3% agarose gel stained with ethidium bromide for 1 h. The wild type allele (C) had no EarI site and was still 400 bp. Ninety-two bp nucleotide was removed from the variant allele (T), which yielded a 308 bp fragment.

Statistical Analysis

Genotype frequencies in control group and patients were compared using Chi-square test. Binary logistic regression was used to examine the relationship between genotype of control and disease. The correlation between clinico-pathological parameters of the patients and genetic polymorphisms of GSTs genes were analyzed by Chi-square test. P-value lower than 0.05 was considered significant.

Results

GSTM1 and GSTT1 gene polymorphisms

The genetic polymorphisms of GSTM1 and GSTT1

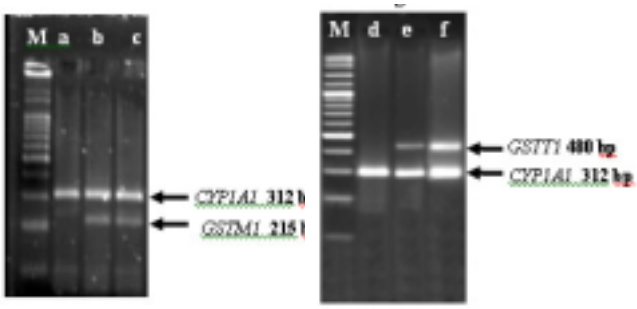

Figure 1. Multiplex-PCR Amplified Products for GSTM1 and GSTT1. (Left) Lane a is null type (GSTM1 0/0), lane b and c are wild type of GSTM1 (GSTM1 +/+); (Right) picture of multiplex-PCR amplified product of GSTT1 and CYP1A1; lane d is null type (GSTT1 0/0), lane e and f are wild type of GSTT1 (GSTT1 +/+), lane M is 100 bp marker

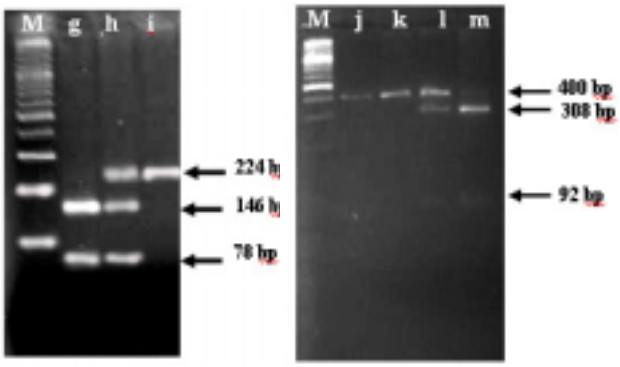

Figure 2. PCR-RFLP Products for GSTP1 and GSTA1. (Left) PCR-RFLP pattern of GSTP1; lane g is homozygous mutant genotype(G/G), lane h is heterozygous genotype (A/G), lane i is wild homozygous genotype (A/A). (Right) PCR-RFLP pattern of GSTA1; lane j-k is wild homozygous genotype (C/C), lane l is heterozygous genotype(C/T), lane m is homozygous mutant genotype(T/T), lane M is 100 bp marker

were analyzed by multiplex-PCR technique and the result showed that there were null genotypes in both control and the patients with breast cancer groups (Figure 1). The overall frequencies of null genotype GSTM1 in controls and in patients with breast cancer were 42.9% and 35.0%, respectively (Table 1). The difference between control and case of the GSTM1 was not statistically significant (P≥ 0.05). GSTT1 genotype frequency was similar in both studied groups (47.2% and 41.9%).

The correlation between clinico-pathological parameters of the patients and genetic polymorphisms of GSTM1 and GSTT1 were analyzed and shown in Table 2 and Table 3. The association between genetic polymorphisms (GSTM1 and GSTT1) of patients and clinico-pathological parameters comprising lymph node, estrogen receptor (ER), progesterone receptor (PR), HER-2, tumer size and age at diagnosis of patients showed no significant difference.

RFLP Detection of GSTP1 and GSTA1 genes polymorphisms

The genetic polymorphisms of GSTP1 and GSTA1 were analyzed by PCR-RFLP technique and the result showed that there were three types of polymorphism in both control and the patients with breast cancer (Figure 2). The overall frequencies of mutant GSTP1 and GSTA1

Table 1. Gene Polymorphisms of GSTM1 and GSTT1 in Breast Cancer Patients and Controls

	GSTM1		GSTT1	
	Control	Cancer	Control	Cancer
Wild type (+/+)	32 (57.1)	26 (65.0)	28 (52.8)	25 (58.1)
Mutant (0/0)	24 (42.9)	14 (35.0)	25 (47.2)	18 (41.9)
P value	0.43		0.60	
Total	56	40	53	43

Table 2. Gene Polymorphism of GSTM1 and Clinico-Pathological Parameters of Breast Cancer Cases

	LN		ER		PR		HER-2		Size		Age	
	+	-	+	-	+	-	+	-	≤3	>3	≤45	>45
Wild	6	7	4	9	4	8	11	1	7	6	5	9
Mutant	12	10	6	15	4	17	12	8	17	4	6	4
P value	0.63		0.89		0.35		0.05		0.09		0.64	

LN, lymph node; ER, estrogen receptor; PR, progesterone receptor; HER-2, HER-2 protein

Table 3. Gene Polymorphism of GSTT1 and Clinico-Pathological Parameters of Breast Cancer Cases

	LN		ER		PR		HER-2		Size		Age	
	+	-	+	-	+	-	+	-	≤3	>3	≤45	>45
Wild	9	6	5	10	5	9	11	3	10	5	6	10
Mutant	12	13	7	17	4	16	13	10	16	6	5	13
P value	0.46		0.78		0.30		0.17		0.69		0.55	

LN, lymph node; ER, estrogen receptor; PR, progesterone receptor; HER-2, HER-2 protein

genotypes of controls were 39.7% and 10.7% respectively as well as those of cases were 30.3 and 12.5% respectively (Table 4). The differences between control and case were not statistically significant (P≥ 0.05).

The correlation between genetic polymorphisms of GSTP1 and GSTA1 and clinico-pathological parameters of the patients as analyzed as shown in Table 5 and Table 6. The results showed that the association between genetic polymorphisms of GSTP1 were significantly associated with progesterone receptor (PR+, P = 0.04) and age of the patients at diagnosis(>45, P = 0.03). However, the

Table 4. Gene Polymorphisms of GSTP1 and GSTA 1 in Breast Cancer Patients and Controls

	GSTP1		GSTA1	
	Control	Cancer	Control	Cancer
Wild type (+/+)	32 (60.3)	30 (69.7)	42 (89.3)	35 (87.5)
Mutant (0/0)	21 (39.7)	13 (30.3)	3 (10.7)	5 (12.5)
P value	0.33		0.36	
Total	53	43	45	40

Table 5. Gene Polymorphism of GSTP1 and Clinico-Pathological Parameters of Breast Cancer Cases

	LN		ER		PR		HER-2		Size		Age	
	+	-	+	-	+	-	+	-	≤3	>3	≤45	>45
Wild	15	15	9	19	4	20	19	9	16	8	13	15
Mutant	6	5	3	8	5	5	5	4	8	2	1	10
P value	0.79		0.76		0.04*		0.50		0.43		0.03*	

LN, lymph node; ER, estrogen receptor; PR, progesterone receptor; HER-2, HER-2 protein; *significant

Table 6. Gene Polymorphism of GSTA1 and Clinico-Pathological Parameters of Breast Cancer Cases

	LN		ER		PR		HER-2		Size		Age	
	+	-	+	-	+	-	+	-	≤3	>3	≤45	>45
Wild	13	14	7	22	6	23	17	12	20	10	12	18
Mutant	3	1	1	3	1	3	3	1	4	0	2	2
P value	0.31		0.97		0.84		0.53		0.17		0.70	

LN, lymph node; ER, estrogen receptor; PR, progesterone receptor; HER-2, HER-2 protein

genetic polymorphisms of GSTA1 were not significantly associated with the studied clinico-pathological parameters.

Discussion

Glutathione S-transferase enzymes involved in the detoxification of reactive metabolites of carcinogens may therefore be important in modulating susceptibility to cancers, especially T1(GSTT1), M1(GSTM1), P1(GSTP1), A1(GSTA1). Many researches indicate that these gene alterations are associated with certain cancers, for example GSTM1 null genotype as associated with ovarian cancer (Spurdle et al., 2001) and the GSTM1 and GSTT1 null genotypes were reported to involve in increasing risk of hepatocellular carcinoma (Deng et al., 2005). GSTP1 polymorphism was found to link to the development of colorectal cancer (Stoehlmacher et al., 2002).

For the breast cancer several studies showed that null GSTT1 genotypes has been associated with an elevated risk of breast cancer and the association might be modified by charred meat intake and cigarette smoking (Zheng et al., 2002). GSTA1 genotypes were associated with increased breast cancer primarily among women with lower consumption of cruciferous vegetables and among current smokers(Ahn et al., 2006). A meta-analysis study of genetic polymorphisms in GST risk for breast cancer in Shanghai breast cancer population showed association of the GSTP1 (Ile105Val) polymorphisms with breast cancer risk (Egan et al., 2004).

However, some reports revealed that GST gene family was not associated with an increased susceptibility to breast cancer. For example, the studies of Unlü et al., 2008 and Samson et al., 2007 demonstrated that the GSTT1, GSTM1null allele and GSTP1 (Ile105Val) were not associated with susceptibility to breast cancer in both Europe and Asian woman.

To our knowledge this is the first time to report genetic polymorphisms of GSTT1, GSTM1, GSTP1 and GSTA1 in Thai patients with breast cancer. Our findings showed no association between GSTM1, GSTT1 deletion and GSTP1, GSTA1 polymorphisms with susceptibility to breast cancer in Thai women ($P \geq 0.05$). In addition, GSTM1, GSTT1and GSTA1 polymorphisms were neither related to clinico-pathological parameters of the patients. However , we found the relationship of GSTP1(Ile105Val) genotype and progesterone receptor ($P = 0.04$) and age of the patients at diagnosis ($P = 0.03$). Some studies (Gilbert et al., 1993 and Colovai et al., 1992) also found that an increased level of GSTP activity was inversely related to hormone progesterone receptor status.

In conclusion, we found no association between GSTM1, GSTT1and GSTA1 genotypes and breast cancer risk. However high frequency of GSTP1 genotype was demonstrated in patients with progesterone receptor negative tumors and older age. The longer sample size showed the further conducted to confirm the findings.

Acknowledgments

This work was supported by the Rangsit University research fund and Faculty of Science, Rangsit University, Thailand.

References

Abdel-Rahman SZ, el-Zein RA, Anwar WA, et al (1996). A multiplex PCR procedure for polymorphic analysis of GSTM1 and GSTT1 genes in population studies. *Cancer Lett*, **107**, 229-33.

Ahn J, Gammon MD, Santella RM, et al (2006). Effects of glutathione S-transferase A1 (GSTA1) genotype and potential modifiers on breast cancer risk. *Carcinogenesis*, **27**, 1876-82.

Allan JM, Wild CP, Rollinson S, et al (2001). Polymorphism in Glutathione S-transferase P1 is associated with susceptibility to chemotherapy - induced leukemia. *Proc Natl Acad Sci USA*, **98**, 11592-7.

Chenevix-Trench G, Young J, Coggan M, et al (1995). Glutathione S-transferase M1 and T1 polymorphism: susceptibility to colon cancer and age of onset. *Carcinogenesis*, **16**, 1655-7.

Colovai AI, Stoica A, Cinca SA, et al (1992). Glutathione S-transferase activity in human breast tumors. *Neoplasma*, **39**, 249-54.

Deng ZL, Wei YP, Ma Y (2005). Polymorphism of glutathione S-transferase mu 1 and theta 1 genes and hepatocellular carcinoma in Southern Guanxi China. *World J Gastroenterol*, **11**, 272-4.

Dunning AM, Healey CS, Pharoah PDP, et al (1999). A systematic review of genetic polymorphisms and breast cancer risk. *Cancer Epidemiol Biomarkers Prev*, **8**, 843-54.

Egan KM, Cai Q, Shu XO, et al (2004). Genetic polymorphisms in GSTM1, GSTP1, and GSTT1 and the risk for breast cancer: results from the Shanghai Breast Cancer Study and meta-analysis. *Cancer Epidemiol Biomarkers Prev*, **13**, 197-204.

Gatedee J, Pakakassama S, Muangman S, et al (2007). Glutathione S-transferase P1 genotypes, genetic susceptibility and outcome of therapy in thai childhood acute lymphoblastic leukemia. *Asian Pac J Cancer Prev*, **8**, 294-6.

Gilbert L, Elwood LJ, Merino M, et al (1993). A pilot study of pi-class glutathione S-transferase expression in breast cancer: correlation with estrogen receptor expression and prognosis in node-negative breast cancer. *J Clin Oncol*, **11**, 49-58.

Hivonen A, Husgafvel-Pursiainen K, Anttila S, et al (1993). The GSTM1 null genotype as a potential risk modifier for squamous cell carcinoma of lung. *Carcinogenesis*, **14**, 1479-81.

Kang D (2003). Genetic polymorphisms and cancer susceptibility of breast cancer in Korean women. *J Biochem Mol Biol*, **36**, 28-34.

Kim SU, Lee KM, Park SK, et al (2004). Genetic polymorphism of glutathione S-transferase P1 and breast cancer risk. *J Biochem Mol Biol*, **37**, 582-5.

Miller SA, Dykes DD, Polesky HF (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*, **16**, 1215.

Naoe T, Tagawa Y, Kiyoi H, et al (2002). Prognostic significance if the null genotype of glutathione S-transferase T1 in patients with acute myeloid leukemia. *Cancer Epidemiol Biomarkers Prev*, **16**, 203-8.

Ping J, Wang H, Huang M, Liu ZS (2006). Genetic analysis of glutathione S-transferase A1 polymorphism in the Chinese population and the influence of genotype on enzymatic properties. *Toxicol Sci*, **89**, 438-43.

Samson M, Swaminathan R, Rama R, et al (2007). Role of GSTM1 (Null/Present), GSTP1 (Ile105Val) and P53 (Arg72Pro) genetic polymorphisms and the risk of breast cancer: a case control study from South India. *Asian Pac J Cancer*, **8**, 253-7.

Spurdle AB, Webb PM, Purdie DM, et al (2001). Polymorphisms at the glutathione S-transferase GSTM1, GSTT1 and GSTP1 loci: risk of ovarian cancer by histological subtype. *Carcinogenesis*, **22**, 67-72.

Sriprung H, Sontipong S, Martin N, et al (eds)(2003). Cancer in Thailand 1995-1997. Vol III . Bangkok Medical publisher,

Stoehlmacher J, Park DJ, Zhang W, et al (2002). Association between gluthatione S-transferase P1, T1 and M1 genetic polymorphism and survival of patients with metastatic colorectal cancer. *J Natl Cancer Inst*, **94**, 936-42.

Unlü A, Ates NA, Tamer L, et al (2008). Relation of glutathione S-transferase T1, M1 and P1 genotypes and breast cancer risk. *Cell Biochem Funct*, **26**, 643-7.

Zheng T, Holford TR, Zahm SH, et al (2002). Cigarette smoking, glutathione-s-transferase M1 and T1 genetic polymorphisms, and breast cancer risk (United States). *Cancer Causes Control*, **13**, 637-45.