Genetic Polymorphisms in *GSTM1*, *GSTP1*, and *GSTT1* and the Risk for Breast Cancer: Results from the Shanghai Breast Cancer Study and Meta-Analysis

Kathleen M. Egan,[1] Qiuyin Cai,[1] Xiao-Ou Shu,[1] Fan Jin,[2] Tian-Li Zhu,[2] Qi Dai,[1] Yu-Tang Gao,[2] and Wei Zheng[1]

[1]Department of Medicine and Vanderbilt-Ingram Cancer Center, Vanderbilt University, Nashville, Tennessee, and [2]Department of Epidemiology, Shanghai Cancer Institute, Shanghai, China

Abstract

Purpose: **We studied the relation of breast cancer to common deletion mutations in *GSTM1* and *GSTT1* and the functional *Ile^{105}Val* polymorphism in *GSTP1* in a large, population-based case-control study conducted in China and performed a meta-analysis to summarize the literature.**

Experimental Design: **In the case-control study, a total of 1144 breast cancer cases and 1221 community controls were genotyped for *GSTM1*, *GSTP1*, and *GSTT1* using PCR-based methods. Associations of genotypes and breast cancer were evaluated in logistic regression models. Meta-analysis odds ratios (ORs) were estimated using a fixed effects model.**

Results: **In the case-control study, associations were null for *GSTM1* [age-adjusted OR 0.97, 95% confidence interval (CI): 0.82–1.14] and *GSTT1* (OR 0.97, 95% CI: 0.83–1.15). A significant increase in risk was observed among homozygotes for the variant *Ile^{105}Val* polymorphism (OR 1.92, 95% CI: 1.21–3.04). No combined effects of *GSTM1*, *GSTP1*, and *GSTT1* genotypes or interactions with potential effect modifiers were detected. All results were similar in pre- and postmenopausal women and for early versus advanced stage breast cancer. The meta-analysis, based predominately on Caucasian women, supported null results for the homozygous deletion variant in *GSTM1* (summary OR 1.05; combining 19 studies) and *GSTT1* (summary OR 1.11; 15 studies). Meta-analysis results for the homozygous *GSTP1* variant indicated no overall association (summary OR 1.04; 10 studies), although results varied significantly across studies ($P = 0.009$).**

Conclusions: **This large case-control study provides strong support for earlier studies showing no overall association of the *GSTM1* and *GSTT1* deletion polymorphisms with breast cancer risk. The *GSTP1* variant may be relevant to breast cancer risk in Asian populations.**

Introduction

Glutathione *S*-transferase (GST) is a family of genes with a critical function in the protection against electrophiles and the products of oxidative stress (1). The GSTs are involved in the metabolism of many xenobiotics, including an array of environmental carcinogens and chemotherapeutic agents and endogenously derived reactive oxygen species. GSTs are widely distributed in nature and are found in essentially all eukaryotic species. The four major families of GSTs, distinguished on the basis of primary structure, are designated as α, μ, π, and θ and are encoded by the *GSTA*, *GSTM*, *GSTP*, and *GSTT* genes, respectively. Of these, class π and μ predominate in the breast (2–4). Humans possess a single functional class π GST gene, whereas human class α, μ, and θ families contain multiple distinct genes, sharing ~55, 65, and 50% identity, respectively. Mechanisms for biochemical protection by GSTs involve both conjugation of electrophilic compounds with glutathione facilitating their transport from the cell and reduction of organic hydroperoxides that contribute to oxidative stress (1). Certain epoxides formed from polycyclic aromatic hydrocarbons in cigarette smoke are substrates for class μ and π GST. The activated metabolites of the heterocyclic amines, carcinogens formed by cooking meat at high temperatures, are detoxified through conjugation by α and θ transferases. In addition to providing protection against exogenous chemicals, the GSTs are also involved in the protection of cells from oxidative damage, including free radicals generated through the metabolic redox cycle of catechol estrogens (5).

At least 20 isoenzymatic forms of GST have been identified, and many of them show genetically based individual variability of enzyme activity. The *GSTM1* and *GSTT1* genes both exhibit deletion polymorphisms (6, 7). Homozygous deletions of these genes, referred to as *GSTM1–0* and *GSTT1–0*, respectively, result in complete absence of enzyme activity. An A→G polymorphism at nucleotide 313 in the *GSTP1* gene leads to an amino acid change (*Ile^{105}Val*). The polymorphism resides at the substrate binding site and has been associated with reduced activity of the enzyme *in vitro* (8).

The deletion mutations in *GSTM1* and *GSTT1* and the *I^{105}V* variant in *GSTP1* have been investigated for associations with breast cancer in a large number of studies. Several studies reported about a 20–50% elevated risk associated with the null *GSTM1* genotype (9–11), and a stronger association was reported in two other studies (12, 13). However, the majority of studies reported no relation or even a possible inverse association of the null *GSTM1* variant with breast cancer (14–26). The *GSTT1* polymorphism has also been investigated in many

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Requests for reprints: Dr. Kathleen M. Egan, Vanderbilt University Medical Center, Suite 6000, Medical Center East, Nashville, TN 37232-8300. Phone: (615) 936-1640; Fax: (615) 936-1269; E-mail: Kathleen.Egan@Vanderbilt.edu.

studies, also with conflicting results (10–13, 15, 17–24, 26). A positive relationship of the *GSTP1 Val/Val* genotype with breast cancer was reported initially (12, 27), although this finding was not replicated in most subsequent studies (10, 18, 19, 21, 22, 28, 29). Only a few studies had statistical power to examine interactions among GST polymorphisms and the potentially modifying influences of environmental and endogenous substrates for GST. To further explore these relationships, we analyzed data from the large Shanghai Breast Cancer Study, a population-based case-control study of breast cancer conducted in China. To place the current findings in context of the large literature on GST polymorphisms in relation to breast cancer risk, we also performed a meta-analysis.

Materials and Methods

Study Subjects. Case and control women in this study were participants of the Shanghai Breast Cancer Study, a population-based case-control study (30). The study protocol was approved by committees of relevant institutions for the use of human subjects in research. All case patients and control subjects were permanent residents of urban Shanghai between the age of 25 and 64 years; controls had no history of breast cancer. Through a rapid case-ascertainment system, supplemented by the population-based Shanghai Tumor Registry, 1602 eligible case patients with incident breast cancer, diagnosed between August 1996 and March 1998, were identified, and in-person interviews were completed for 1459 (91%) of them. The major reasons for nonparticipation were refusal (109 case patients, 6.8%), death before the interview (17 case patients, 1.1%), and the inability to locate (17 case patients, 1.1%). Cancer diagnoses for all patients were confirmed by two senior study pathologists through a review of tumor slides.

Control subjects were randomly selected from the female general population and were frequency matched to case patients by age (5-year intervals). The number of control subjects in each age-specific stratum was determined in advance according to the most recent data on the age distributions of the breast cancer patients available from the Shanghai Tumor Registry. The Shanghai Resident Registry, which keeps registry cards for all adult residents in urban Shanghai, was used to randomly select control subjects. In-person interviews were completed for 1556 (90%) of the 1724 eligible control subjects identified. Excluded from the study were 168 potential control subjects because of refusal ($n = 166$; 9.6%) or death or a prior cancer diagnosis ($n = 2$; 0.1%).

A structured questionnaire was used to elicit detailed information on demographic factors, menstrual and reproductive histories, hormone use, dietary habits, prior disease history, physical activity, tobacco and alcohol use, and family history of cancer. All participants were also measured for their current weight, circumferences of the waist and hip, and heights while sitting and standing. Blood samples were obtained from 1193 (82%) case patients and 1310 (84%) control subjects who completed the in-person interviews (31, 32). Of these, genotyping for one or more of the GST polymorphisms was successfully completed for 1143 (96%) case and 1221 (93%) control subjects.

Genotyping Method. Genomic DNA was extracted from buffy coat fractions using the Puregene DNA isolation Kit (Gentra Systems, Minneapolis, MN) following the manufacturer's protocol. DNA concentration was measured by PicoGreen dsDNA Quantitation kit (Molecular Probes, Eugene, OR). Five to 10 ng of genomic DNA were used for each PCR. The laboratory staff was blind to the identity of the subject. Quality control samples (water, CEPH 1347-02 DNA, as well as blinded and unblinded DNA samples) were included in genotyping assays.

A multiplex PCR protocol was used to analyze simultaneously for the presence or absence of *GSTM1* and *GSTT1* genes (33). The *Albumin* gene was used as an internal control. The internal control amplified *Albumin* fragment was 350 bp in length, whereas presence of the *GSTM1* and *GSTT1* genes were identified by 215 and 480 bp fragments, respectively. Although these assays did not distinguish between heterozygote and homozygote positive genotypes, they conclusively identify the null genotypes. The *GSTP1* $A^{313}G$ polymorphism was determined by PCR-RFLP method reported previously (28). The PCR products were digested by *Bsm*AI restriction endonuclease. The A to G substitution at nucleotide position 313 creates a *Bsm*AI restriction site. The PCR product with G allele was digested to two fragments (148 and 41 bp), whereas the PCR product with A allele remained undigested (189 bp). Blinded duplicates were included in the genotyping: concordance rates were 94.5% for *GSTM1* (55 replicates); 98.2% for *GSTP1* (55 replicates); and 95.2% for *GSTT1* (57 replicates).

Statistical Analysis. Odds ratios (ORs) and 95% confidence intervals (CIs) were obtained by logistic regression; multivariate models included terms for age, benign breast disease, body mass index, waist-hip ratio, and number of menstrual cycles (estimated by subtracting age at menarche and time spent pregnant from age at menopause or current age, if premenopausal). Few women reported ever drinking alcohol (<4%). The few ever-active smokers (2%) were excluded. Analyses stratifying by indicators of endogenous estrogen exposure or environmental exposure to GST substrates were conducted to evaluate the potential modifying effects of these variables on associations between GST genotypes and breast cancer risk. Multiplicative interactions were examined by introducing cross-product terms for (dichotomized) risk factors and GST genotype (AA *versus* AG/GG for *GSTP1*).

To summarize the literature on GST polymorphisms in relation to breast cancer, we performed a meta-analysis. The meta-analysis was limited to published data. To identify relevant literature, we searched Medline through June 2003 using broad combinations of key words. We cross-referenced literature cited in research articles and checked review articles of breast cancer genetics, GST polymorphisms, and cancer susceptibility genes for studies not otherwise identified. To derive summary ORs and 95% CIs, we used routines available in STATA (34). Results presented are based on the method of Mantel and Haenszel (35), which assumes fixed or invariant effects of the genotype on cancer risk across populations. Meta-analysis ORs were estimated for all women combined and by menopausal status. We tested for publication bias using the method of Begg and Mazumdar (36), which evaluates whether there is correlation between effect estimates (ORs) and study variances in the published literature.

Case-Control Study Results. The distributions of selected demographic characteristics and major risk factors for breast cancer are shown in Table 1. Cases and controls were comparable in age (median: 47 years in both groups) and education (43% completed high school in both groups). Population differences between cases and controls reflected typical risk factors for breast cancer. Approximately one-third of the women were postmenopausal.

As shown in Table 2, allele frequencies for the individual GSTs were similar to those reported previously in other Asian populations. Approximately one-half of the women had the null

Table 1 Comparison of cases and controls by selected demographic factors and major risk factors for breast cancer, Shanghai Breast Cancer Study, 1996–1998

	Cases (n = 1144)	Controls (n = 1221)	P^a
Demographic factors			
Age (yr)[b]	47 (42, 53)	47 (40, 54)	0.26
Education ≥ high school (%)	43	43	0.16
Established risk factors			
First-degree relative with breast cancer (%)	3.5	2.4	0.11
Ever diagnosed with breast fibroadenoma (%)	9.9	5.2	<0.01
No regular leisure physical activity (%)	81	74	<0.01
Waist-to-hip ratio[b]	0.81 (0.77–0.84)	0.80 (0.76–0.84)	<0.01
Body mass index[b]	23.2 (21.2–25.5)	22.8 (20.8–25.1)	0.02
Age at menarche (yr)[b]	14 (13, 16)	15 (13, 16)	<0.01
Postmenopausal (%)	30	34	0.01
Age at menopause (%)[b,c]	49 (47, 51)	49 (46, 50)	<0.01
No live births (%)	6.8	4.1	<0.01
Age at first live birth (yr)[b,d]	27 (24, 29)	26 (24, 28)	<0.01

[a] P derived from t tests (continuous variables) or the χ^2 test (categorical variables).
[b] Median (25th, 75th percentile) values are presented.
[c] Among postmenopausal women.
[d] Among parous women.

genotype for *GSTM1* or *GSTT1* in both cases and controls. Case-control comparisons revealed no differences in genotype prevalence for *GSTM1* or *GSTT1*: age-adjusted ORs were close to one, and CIs excluded large influences of these genotypes on breast cancer risk. For *GSTP1*, allele frequencies for the A to G substitution at nucleotide position 313 were 21% in cases and 18% in controls. Heterozygotes for the variant G allele had a modestly elevated relative risk (OR 1.08, 95% CI: 0.91–1.29), whereas the less common homozygotes had nearly twice the risk for breast cancer (OR 1.92, 95% CI: 1.21–3.04) when compared with those without the substitution. Results were similar regardless of stage at breast cancer diagnosis (data not shown). All results were essentially unchanged after adjustment for breast cancer risk factors (data not shown).

Pairwise analyses indicated no significant increase in risk for any combination of putative at-risk genotypes (Table 3). Furthermore, no dose response was observed according to these variants; women with all three at-risk genotypes had no increase in risk, combining AG and GG genotypes for *GSTP1* (too few women were homozygous to be separated in analysis), when compared with women with three low-risk genotypes (Table 3). All results were similar in pre- and postmenopausal women (data not shown).

Table 4 shows age-adjusted ORs stratified by potential risk modifiers. Overall, ORs were not materially affected by GST genotype for various indices of estrogen exposure, including menopausal status, number of menstrual cycles, and body mass (Table 4); no significant interaction was observed for body mass index in pre- or postmenopausal women, when considered separately (data not shown). ORs for oral contraceptive use and waist-to-hip ratio were similarly unchanged stratifying by these genotypes (data not shown). The one statistically significant interaction for menstrual cycles and *GSTM1* suggested a potentially abrogating influence of the GSTM1 protein on breast cancer associated with ovarian estrogen exposure. Interactions with sources of environmental exposure to polycyclic aromatic hydrocarbon and heterocyclic amines also were considered (Table 4): exposure to passive tobacco smoke in the workplace or from the husband smoking had little influence on breast cancer, regardless of GST genotype. Results for meat cooking preference were similarly null, although there was evidence for a modest potential interaction involving *GSTT1* (P for interaction = 0.03). No significant interactions were noted for waist-to-hip ratio or for oral contraceptive use (data not shown).

Results of Meta-Analysis. Summary ORs for the individual GST genotypes are shown in Table 5. A total of 5950 breast cancer cases and 6601 controls was genotyped for the *GSTM1* null variant in 19 published studies. The summary results from the meta-analysis indicated no relationship of the *GSTM1* null variant with breast cancer risk overall (OR 1.05, 95% CI: 0.98–1.13) or in premenopausal women (OR 0.95). In postmenopausal women, the OR was minimally, although significantly elevated (OR 1.14). ORs for *GSTT1* were also modestly elevated overall (OR 1.11) and in pre- (OR 1.13) and postmenopausal (OR 1.10) women; a significant test for heterogeneity in the unstratified analysis (P = 0.03) indicated substantial variability in the published results for the *GSTT1* deletion. The meta-analysis for the homozygous $I^{105}V$ variant in *GSTP1* indicated no overall association of this variant with breast

Table 2 Polymorphisms of *GSTM1*, *GSTP1*, and *GSTT1* and breast cancer risk, Shanghai Breast Cancer Study, 1996–1998

	No. of case subjects (%)	No. of control subjects (%)	All women (n = 2365)	Premenopausal (n = 1563)	Postmenopausal (n = 757)
				Odds Ratio (95% CI)[a]	
GSTM1					
Present	497 (43.8)	523 (43.4)	1.0 (ref)	1.0 (ref)	1.0 (ref)
null	638 (56.2)	683 (56.6)	0.97 (0.82, 1.14)	0.90 (0.74, 1.11)	1.05 (0.79, 1.40)
missing	58	104			
GSTP1					
Ile/Ile	723 (63.5)	809 (66.8)	1.0 (ref)	1.0 (ref)	1.0 (ref)
Ile/Val	363 (31.9)	371 (30.6)	1.08 (0.91, 1.29)	1.02 (0.82, 1.27)	1.14 (0.83, 1.57)
Val/Val	53 (4.6)	31 (2.6)	1.92 (1.21, 3.04)	2.12 (1.21, 3.71)	1.96 (0.82, 4.71)
missing	54	99			
P, trend			0.02	0.09	0.15
GSTT1					
Present	579 (51.0)	614 (50.7)	1.0 (ref)	1.0 (ref)	1.0 (ref)
null	557 (49.0)	596 (49.3)	0.97 (0.83, 1.15)	1.00 (0.82, 1.23)	0.95 (0.71, 1.28)
missing	57	100			

[a] OR, odds ratio adjusted for age; CI, confidence interval. Menopause information was missing for 45 women.

Table 3 Combinations of at-risk genotypes for *GSTM1*, *GSTP1*, and *GSTT1* in relation to breast cancer: Shanghai Breast Cancer Study

GSTM1	*GSTT1*	*GSTP1*	No. of case subjects (n = 1132)	No. of control subjects (n = 1193)	OR (95% CI)[a]
All putative low-risk genotypes					
Present	Present	Ile/Ile genotype	154	163	1.0 (referent)
One putative high-risk genotype					
Null	Present	Ile/Ile	207	238	0.90 (0.67–1.20)
Present	Null	Ile/Ile	160	173	0.95 (0.70–1.30)
Present	Present	Ile/Val or Val/Val	91	100	0.91 (0.63–1.31)
Two putative high-risk genotypes					
Null	Null	Ile/Ile	198	221	0.91 (0.68–1.23)
Null	Present	Ile/Val or Val/Val	125	102	1.30 (0.92–1.83)
Present	Null	Ile/Val or Val/Val	92	80	1.21 (0.83–1.76)
All three putative high-risk genotypes					
Null	Null	Ile/Val or Val/Val	105	116	0.89 (0.63–1.26)

[a] OR, age-adjusted odds ratio; CI, confidence interval; GST, glutathione *S*-transferase.

cancer (OR 1.04), although there was highly significant heterogeneity in the contributing studies (P = 0.009). Excluding the current data, the overall OR for *GSTP1* was slightly inverse (OR 0.92, 95% CI: 0.75–1.13), and heterogeneity was attenuated (P = 0.08).

In addition to the current report, seven studies (10, 12, 13, 17–19, 21) considered combinations of *GSTM1* and *GSTT1*, and four of these (12, 18, 19, 21) presented results for all three genotypes. The combined OR for deletion mutations in both *GSTM1* and *GSTT1*, compared with none (based on 522 exposed cases; 523 exposed controls), was 1.08 (95% CI: 0.92–1.27). The combined OR for having three variant alleles (combining AG or GG for *GSTP1*) compared with none (based on 177 exposed cases; 185 exposed controls) was 0.99 (95% CI: 0.77–1.28; individual studies shown in Fig. 1).

The test for publication bias was marginally significant for *GSTT1* overall (P = 0.048) and in studies of stratified results for postmenopausal women (P = 0.061). There was no similar evidence of publication bias for *GSTM1* (P = 0.243) or *GSTP1* (P = 0.938). Fig. 2 displays a funnel plot for the 15 studies that examined the *GSTT1* polymorphism and breast cancer risk included in the meta-analysis. Asymmetry around the horizontal line for the meta-analysis OR, toward the right of the graph, indicates a pattern of more elevated ORs among the smaller, less precise published studies.

Discussion

The current case-control study of women in urban Shanghai is the largest and most comprehensive examination of GST polymorphisms in relation to breast cancer risk. Results indicate no overall relationship of the *GSTM1* or *GSTT1* deletion variants with breast cancer risk in premenopausal or in postmenopausal women. In contrast, women with homozygous *GSTP1* ^{105}*Val* substitution were at significantly increased risk for breast cancer when compared with those with two wild-type alleles;

Table 4 Polymorphisms of *GSTM1*, *GSTP1*, and *GSTT1* and breast cancer risk according to potential risk modifiers, Shanghai Breast Cancer Study, 1996–1998

	GSTM1[a]		*GSTT1*[a]		*GSTP1*[a]	
	Present	Null	Present	Null	AA	AG/GG
Menopausal status						
Pre	1.0 (ref)	0.89 (0.73–1.08)	1.0 (ref)	1.01 (0.82–1.23)	1.0 (ref)	1.09 (0.88–1.34)
Post	0.64 (0.48–0.87)	0.69 (0.51–0.92)	0.66 (0.45–0.98)	0.65 (0.43–0.92)	0.70 (0.54–0.91)	0.84 (0.61–1.15)
P, interaction	0.35		0.72		0.67	
Menstrual cycles[b]						
<370	1.0 (ref)	0.76 (0.60–0.97)	1.0 (ref)	1.01 (0.79–1.28)	1.0 (ref)	1.12 (0.87–1.44)
≥370	1.07 (0.79–1.45)	1.26 (0.94–1.68)	1.40 (1.05–1.87)	1.35 (1.01–1.80)	1.36 (1.04–1.77)	1.56 (1.16–2.10)
P, interaction	0.01		0.79		0.89	
Body mass index (kg/m^2)						
<22.8	1.0 (ref)	0.96 (0.75–1.22)	1.0 (ref)	1.01 (0.80–1.28)	1.0 (ref)	1.01 (0.79–1.30)
≥22.8	1.18 (0.91–1.51)	1.14 (0.90–1.44)	1.21 (0.96–1.54)	1.22 (0.91–1.46)	1.09 (0.88–1.34)	1.37 (1.07–1.76)
P, interaction	0.94		0.69		0.22	
Passive smoke exposure[c]						
None	1.0 (ref)	1.04 (0.70–1.54)	1.0 (ref)	0.90 (0.61–1.32)	1.0 (ref)	1.27 (0.84–1.92)
Any	1.01 (0.71–1.42)	0.94 (0.67–1.32)	0.92 (0.67–1.26)	0.88 (0.64–1.22)	1.06 (0.80–1.41)	0.95 (0.70–1.29)
P, interaction	0.65		0.61		0.15	
Deep fried meat						
Never	1.0 (ref)	1.06 (0.80–1.41)	1.0 (ref)	1.21 (0.91–1.61)	1.0 (ref)	1.08 (0.81–1.46)
Usually-Always	1.16 (0.86–1.56)	0.96 (0.73–1.26)	1.24 (0.94–1.63)	0.96 (0.73–1.27)	1.07 (0.84–1.36)	0.95 (0.71–1.27)
P, interaction	0.22		0.03		0.35	

[a] Odds ratios adjusted for age. Odds ratio (95% confidence interval).
[b] Number of menstrual cycles = [(current age or age at menopause in menopausal women-age at menarche) × 13] − live births × 9; women with unknown menopausal status were excluded.
[c] Exposure in workplace or to husband smoking.

Table 5 Results of meta-analysis of studies examining *GST* polymorphisms and breast cancer risk

Gene (variant)	Group	No. of Studies[a]	No. of case subjects	No. of control subjects	Summary odds ratio (95% confidence interval)	Test for heterogeneity	References
GSTM1 (deletion)	All women	19	5,950	6,601	1.05 (0.98, 1.13)	0.56	9–26
	Premenopausal	10	2,033	2,485	0.95 (0.84, 1.07)	0.28	10–13,16–18,21,23
	Postmenopausal	11	2,521	2,963	1.14 (1.02, 1.27)	0.07	10,12–14,16–18,21,23,24
GSTT1 (deletion)	All women	15	4,873	5,245	1.11 (1.01, 1.22)	0.03	10–13,15,17–24,26
	Premenopausal	9	1,810	2,151	1.13 (0.98, 1.31)	0.05	10–13,17,18,21,23
	Postmenopausal	9	2,473	2,160	1.10 (0.96, 1.27)	0.20	10,12,13,17,18,21,23,24
GSTP1[b] (^{105}Val homozygote)	All women	10	2,136	2,282	1.04 (0.87, 1.25)	0.009	10,12,18,19,21,22,27–29
	Premenopausal	5	849	1,016	1.21 (0.89, 1.65)	0.11	10,12,18,21
	Postmenopausal	6	900	1,110	1.01 (0.78, 1.30)	0.02	10,12,18,21,28

[a] Current case-control study is included in the analysis.
[b] Odds ratio for *GSTP1* for homozygous variant are compared to homozygous wild-type as referent.

heterozygotes for this variant had no excess risk. Results suggest that these genotypes have no additive or multiplicative relationship with breast cancer risk: no association was demonstrated for any combination of putative at-risk genotypes (although too few women were homozygous for the *GSTP1* substitution for a full evaluation of relationships with this genotype). Furthermore, we observed no consistent interactions of these genotypes with known or potential GST substrates, including exposures to carcinogens in sidestream smoke or heterocyclic amines in cooked meat (although a potential interaction of the *GSTT1* deletion with meat cooking was suggested in the data). A statistically significant interaction with cyclic estrogen exposure was observed only for *GSTM1*. Taken together, results for the GST deletion variants are in line with the meta-analysis and suggest no overall relationship of these genotypes with breast cancer risk; in contrast, this is the first study to show a statistically significant positive association of breast cancer with the functional *GSTP1* substitution variant.

A large number of studies have considered GST polymorphism in relation to breast cancer risk, with conflicting results. Rebbeck (37) has reviewed the early literature on the GST deletion variants in relation to cancer risk and noted a number of design issues that limit the interpretation of many of these studies. The earliest literature often relied on undefined convenience samples for case and control selection, with the possibility for selection bias on age, ethnicity, and other factors. Selection bias is also possible in hospital-based studies because the GSTs are likely to be related to the risk for other cancers and chronic diseases linked to smoking. The importance of matching for age was also noted: Rebbeck (37) cited studies showing increased frequency of the *GSTM1-0* in hospital controls *versus* postmortem or geriatric series, suggesting negative selection of this genotype with advancing age (38, 39). An additional potential source of bias in studies of *GST* and cancer risk relates to their possible role in therapeutic response. The GSTs are involved in the metabolism of front-line chemotherapeutic agents, including nitrogen mustards and alkylating agents among others (1). Response to therapy may be governed at least in part by the genes driving the metabolism of and therefore exposure to sustained therapeutic levels of these drugs. A number of studies have suggested an improved prognosis in breast cancer associated with reduced expression of the GSTs (30–44). Inclusion of prevalent breast cancers in etiologic studies could lead to biased results by selecting for case women with prognostically favorable genotypes. Finally, the majority of studies were small and many lacked statistical power to detect moderate associations of GST with breast cancer risk or interactions with GST substrates (45). These design issues would have contributed to heterogeneous results in the literature regarding the contribution of these genes to breast cancer risk.

The current case-control study of women in urban Shang-

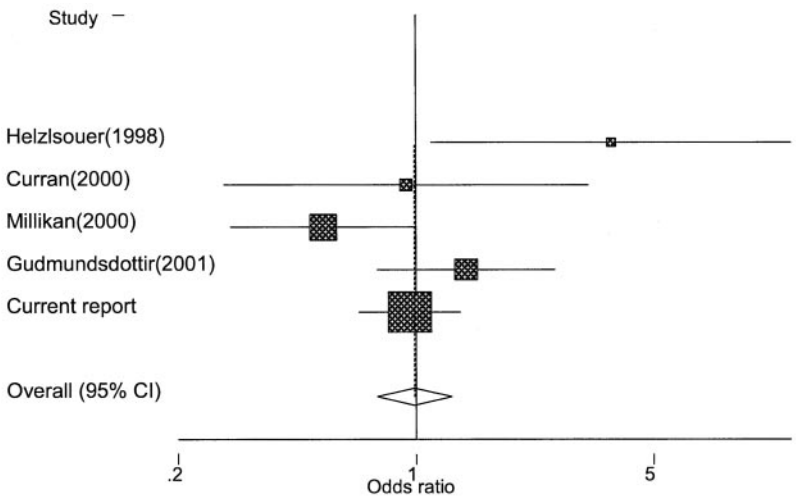

Fig. 1. Meta-analysis of combined *GSTM1*, *GSTP1*, and *GSTT1* polymorphisms in breast cancer risk based on a total of five studies. Overall odds ratio and confidence interval (CI) estimated using the method of Mantel and Haenszel. Odds ratios less than unity indicate protective associations of the three variants with breast cancer risk. Larger squares indicate more influential observations. (Raw data and results of individual studies are available from the authors upon request.)

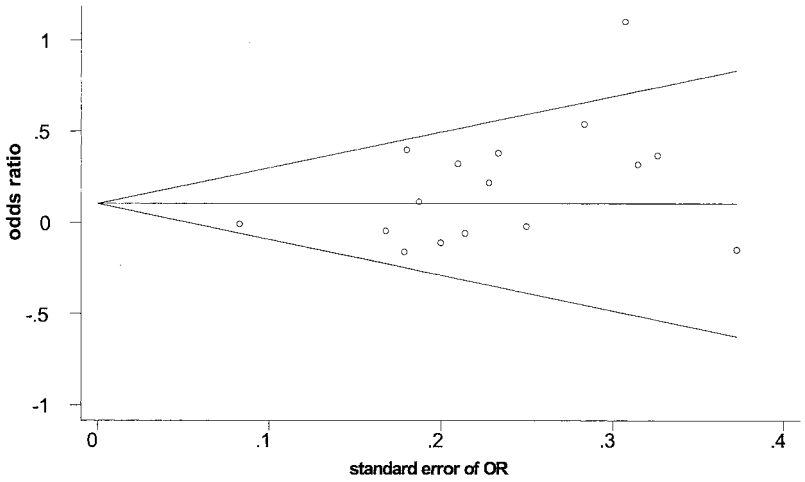

Fig. 2. Funnel plot for *GSTT1* based on 15 studies. The graph plots odds ratios (ORs) *versus* SEs for the studies evaluated. The *horizontal line* is drawn at the meta-analysis OR. Asymmetry on the right of the graph (where studies with a high SE are plotted) may indicate publication bias.

hai addresses many of the limitations in previous studies of GST and breast cancer risk. High enrollment rates, the population-based study design, and careful frequency matching on age would have substantially reduced the possibility for selection bias. Moreover, Chinese women living in Shanghai are relatively homogeneous in ethnic background (>98% are classified into a single ethnic group, the Han Chinese). Therefore, any potential confounding by ethnicity (46) would not be a major concern in these data. Finally, large numbers of women were enrolled in the study, and the extensive information available on reproductive and lifestyle risk factors allowed a relatively powerful stratified analysis on several potential effect modifiers. These latter analyses provide little support for interactions expected *a priori*; isolated finding of interactions between *GSTM1* and menstrual cycles and *GSTT1* and meat cooking could be chance observations given the many associations evaluated.

The meta-analysis should provide a reasonable global view of published research on these genotypes in relation to breast cancer. Modestly elevated summary ORs for *GSTT1* (all women) and *GSTM1* (postmenopausal only) were statistically significant because of the large numbers of women included in the analysis. The analysis suggests that at least some of the excess risk for *GSTT1* may be an artifact of publication bias. The meta-analysis also revealed significant heterogeneity in the published results for *GSTT1* and *GSTP1*. This heterogeneity could reflect methodological differences across studies, as noted above. However, it might also indicate population-specific differences in the contribution of these genotypes to breast cancer risk, and if so, summary ORs for these genotypes could be misleading. The outlying results from the current Shanghai study made a substantial contribution to the heterogeneity for *GSTP1*.

The meta-analysis excludes a moderate association of the *GSTP1 Val/Val* genotype with breast cancer risk (upper confidence limit: 1.4); the 2-fold excess in risk for this genotype from the Shanghai study could be due to chance or differences in study design or the ethnic make-up of the study populations. GSTP1 is the predominant GST isoform in the breast (2–4), and overexpression of the protein in breast tumors has been linked to a poorer outcome (42–44). The *Val/Val* genotype is uncommon (~5% in Caucasians (47)), and fewer studies have examined its relationship to breast cancer risk when compared with the other GST variants; it is possible that design issues noted above could have obscured associations in some previous studies. Most research on the relationship of GST polymorphisms and breast cancer risk has been based on Caucasian women. The finding of an excess risk associated with this genotype in Asian women, if not due to chance, could indicate an influence of *GSTP1* on cancer risk that depends on the genetic background or possibly environmental cofactors. In this regard, estrogen levels are substantially lower in Asian women;[3] it may be speculated that environmental carcinogens, including those metabolized specifically by GSTP1, make a larger contribution to breast cancer in populations where estrogen exposure is low. Additional studies in Asian populations may help to clarify these observations.

Because of the importance of the GST enzyme system in defense against potential mammary carcinogens, additional studies should be undertaken to fully elucidate its contribution to breast cancer. Functional polymorphisms in other GSTs should also be studied as these come to light: a recently identified polymorphism in the proximal promoter of *GSTA1*, associated with reduced enzyme expression (48), has been linked to risk for colorectal cancer, particularly among consumers of well-done meat (49). A number of other potentially functional variants in *GSTP1* have been curated,[4] and some may be relevant to breast cancer risk (22, 29); a haplotype-based analysis that encompasses all of the candidate *GSTP1* variants could provide additional insights. Finally, large studies will be needed to gain a clear picture of the contribution of these genes to breast cancer: these studies should consider interactions with genes encoding Phase I enzymes that produce reactive GST substrates (cytochrome monooxygenases) and, potentially, other detoxifying enzyme systems (*i.e.*, glucuronosyl transferases and sulfotransferases) that may work cooperatively with the GSTs to guard the genome from chemical damage.

[3] S. Boyapati, X. O. Shu, Y. T. Gao, Q. Dai, H. Yu, and J. R. Cheng. Correlation of blood sex steroid hormones with body size, body fat distribution, and other known risk factors for breast cancer in Chinese women, submitted for publication.
[4] Internet address: http://cgap.nci.nih.gov.

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Kathleen M. Egan, Qiuyin Cai, Xiao-Ou Shu, et al.

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