Glutathione *S*-transferases M1, T1, and P1 and Breast Cancer: A Pooled Analysis

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Abstract

The glutathione *S*-transferase (*GST*) genes are involved in the metabolism of various carcinogens. Deletion polymorphisms in the genes *GSTM1* and *GSTT1* and a base transition polymorphism at codon 105 (Ile→Val) in *GSTP1* were investigated in relation to breast cancer risk. Tobacco smoking and reproductive factors were examined as potential effect modifiers. Individual data from seven case-control studies were pooled within the International Collaborative Study on Genetic Susceptibility to Environmental Carcinogens. To measure the effect of GSTs on breast cancer risk, odds ratios and 95% confidence intervals were computed adjusting for study center and age. The modifying effect was investigated by stratification on variables of smoking habits and reproductive history. A total of 2,048 cases with breast cancer and 1,969 controls were analyzed. The relative odds ratio (95% confidence interval) of breast cancer was 0.98 (0.86–1.12) with the *GSTM1* null, 1.11 (0.87–1.41) with the *GSTT1* null, 1.01 (0.79–1.28) with *GSTP1* heterozygous mutants, and 0.93 (0.62–1.38) with *GSTP1* homozygous mutants. Stratification by smoking or reproductive factors did not reveal a modifying effect of these variables, nor was there any association between *GSTM1* and age at diagnosis of breast cancer. This is the largest study investigating susceptibility to breast cancer due to polymorphisms in the *GST* genes. The results conclusively show that single gene *GST* polymorphisms do not confer a substantial risk of breast cancer to its carriers. Furthermore, GSTs did not interact with smoking or reproductive history to modify cancer risk. (Cancer Epidemiol Biomarkers Prev 2004;13(9):1473–9)

Introduction

Inherited differences in the capacity of xenobiotic metabolizing enzymes might be an important factor of genetic susceptibility to cancer. Glutathione *S*-transferases (GST) are phase II enzymes involved in the detoxification of a broad range of toxic and potentially carcinogenic compounds (1). In humans, five classes of GST enzymes have been identified (GST classes α, μ, π, σ, and θ). Each class is encoded by a separate gene or gene family. Allelic variants for each of these genes may result in less effective or absent enzymatic detoxification and thus increase susceptibility to cancer, although the exact biochemical processes are not yet fully understood.

The *GSTM1* gene, coding for cytosolic GST class μ enzyme, is located on chromosome 1p13.3 (2) and includes a deletion polymorphism that, in the homozygous state (*GSTM1* null), results in the total absence of a functional gene product (3). Several studies have shown high agreement between the *GSTM1* null genotype and a lack of GST class μ function. *GSTM1* is expressed in various tissues, mainly liver, stomach, and brain. The frequency of the *GSTM1* null genotype varies across ethnic groups and was reported to be ~50% in Caucasians (4-6). The *GSTT1* gene (chromosome 22q11.2; ref. 7) also has an inactivating homozygous deletion polymorphism (8). Homozygosity for the deletion is present in ~11% to 18% of Caucasians (9). In humans, the *GSTT1* enzyme is primarily expressed in liver and erythrocytes. In *GSTP1* (chromosome 11q13; ref. 10), an amino acid transition has been reported at codon 105 (A313G→Ile105Val), leading to expression of an active but functionally different protein (11, 12). The *GSTP1* encoded enzyme GST class π is mainly found in spleen, heart, and lung tissue. Both GST classes π and μ enzymes are also expressed in breast cancer tissue (13).

Several environmental risk factors have been previously associated with increased susceptibility to breast cancer. Hormonal factors that play an important role in cell growth and several aspects of reproductive history, characterized by elevated and prolonged estrogen levels, are associated with breast cancer risk. Nulliparity, lack of or reduced breast-feeding, older age at first birth, early age at menarche, and late age at menopause increase

Received 3/16/04; revised 8/6/04; accepted 4/12/04.

Grant support: IARC Special Training Award (F.D. Vogl) and European Commission grant CAN/96/33919.

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Table 1. Studies contributing to the pooled analysis and information provided on investigated variables

	Reference	Cases/controls	Country	Source of controls	*GSTM1*	
					Cases (% Null)	Controls (% Null)
1	(26)	283/338	USA	Population	283 (51.2)	338 (50.6)
2	(30)	827/262	Norway	Population	816 (51.6)	196 (45.4)
3	*	130/122	USA	Population	130 (39.2)	122 (52.5)
4	(29)	361/437	France	Population + Hospital	361 (55.7)	437 (51.3)
5	(27)	164/209	USA	Hospital	164 (55.5)	209 (59.8)
6	(28)	79/123	Brazil	Hospital	79 (41.8)	123 (52.9)
7	(31, 32)	204/478	USA	Population	200 (48.5)	474 (52.5)
Total		2,048/1,969			2033 (51.1)	1899 (52.0)

*T. Rebbeck et al., unpublished data.

breast cancer risk (14). High body mass index (BMI) and possibly low physical exercise are also risk factors of postmenopausal breast cancer, acting via interference with hormonal levels (15). Cigarette smoking is an established risk factor for several cancers including the respiratory and upper aerodigestive tract, kidney, bladder, stomach, and pancreas (16).

Enzymes belonging to the GST classes μ, π, and θ are involved in the detoxification of benzo(*a*)pyrene and other polycyclic aromatic hydrocarbons found in tobacco smoke and have received considerable attention in relation to smoking-related cancers. An association between the *GST* null phenotype and cancer susceptibility was described for lung cancer (17, 18), colorectal cancer, and bladder cancer (19, 20). Polycyclic aromatic hydrocarbons induced mammary tumors in animal models, and polycyclic aromatic hydrocarbon-DNA adducts have been identified in human mammary epithelial cells. A recent case-control study suggested an increased risk of breast cancer in relation to polycyclic aromatic hydrocarbon-DNA adducts (21). Although tobacco smoking does not seem to be a risk factor of breast cancer in studies of unselected populations, the possibility of an increased risk in genetically predisposed groups remains (22). GSTs are also involved in detoxifying reactive compounds generated during estrogen metabolism.

Few studies have addressed *GST* polymorphisms and breast cancer, although a meta-analysis of studies published before 1997 has suggested a slight risk increase in carriers of the *GSTM1* null polymorphism, which was only significant in the youngest age group (23).

The Genetic Susceptibility to Environmental Carcinogen (GSEC) Study, an international collaborative project, has been initiated to investigate the relationships of polymorphisms in genes that metabolize environmental carcinogens and cancers at different sites (24). The established database offered the opportunity for investigating the association of polymorphisms in the *GSTM1*, *GSTT1*, and *GSTP1* and breast cancer using individual data of several studies and taking into account the potential modifying effect of reproductive factors and tobacco consumption.

Material and Methods

Data Collection. The present analysis was based on the original data of seven case-control studies on *GST* genotypes and breast cancer, which were provided by the investigators to the GSEC Study (24, 25). Investigators who had published case-control studies on metabolic gene polymorphisms and cancer up to June 1999 were identified through Medline and invited to provide data on all subjects (including published as well as unpublished data). The results of six of the seven studies including breast cancer had been published previously (26-32). Two groups have published their results after they had submitted data to the GSEC. The number of subjects contained in the published reports may differ slightly from the number included in this pooled analysis. The present study was restricted to women of Caucasian origin. For every subject, case-control status and genotype at the *GSTM1*, *GSTT1*, and *GSTP1* loci were submitted for the pooled analysis. All studies used PCR to detect polymorphisms. Although in some studies different PCR protocols were used, the laboratory procedures in general should be comparable. If available, additional information on age, anthropometric measures (body weight and height), family history of cancer, smoking status, and reproductive factors was provided. Prior to analysis, the data were checked and coded in a standard fashion and entered into a common database.

Genotypes at the *GSTM1* and *GSTT1* loci were coded positive if at least one functional allele was present and null in the case of a homozygous deletion. The number of homozygote and heterozygote carriers of *GSTM1* and *GSTT1* wild-type alleles was not provided. *GSTP1* genotype was classified in three categories: the homozygous wild-type, the heterozygous, and the homozygous mutant. BMI was calculated according to the standard formula: weight (kg)/height $(m)^2$. Information on cumulative tobacco smoking was expressed as pack-years (daily amount multiplied by duration in years and divided by 20). Available information on reproductive factors included parity (nulliparous versus parous, i.e., at least one birth), menopausal status (premenopausal versus postmenopausal), age at menopause, and age at menarche. Family history of breast cancer was considered positive if a woman had at least one first-degree relative with breast cancer.

Statistical Analysis. Crude odds ratios (OR) and 95% confidence intervals (95% CI) for the risk of breast cancer associated with *GSTM1* null and *GSTT1* null were calculated for each study by means of meta-analysis. Fixed and random effects models were fitted. The *Q* test

Table 1. Continued

GSTT1		*GSTP1*		Availability of information (%)				
Cases (% Null)	Controls (% Null)	Cases (% Mutant)	Controls (% Mutant)	Age	BMI	Smoking	Reproduction	Family history
—	—	—	—	98.6	97.6	98.4	98.6	98.6
794 (17.8)	196 (17.4)	769 (11.1)	258 (10.9)	—	—	—	—	—
—	—	—	—	—	—	—	—	—
—	—	—	—	100.0	—	—	—	—
164 (28.7)	209 (25.8)	—	—	100.0	97.1	96.3	85.0	71.1
79 (19.0)	123 (25.2)	—	—	100.0	—	97.5	94.6	98.0
155 (26.5)	325 (20.0)	152 (5.9)	327 (8.3)	100.0	—	98.5	100.0	—
1192 (20.5)	853 (21.6)	921 (10.2)	585 (9.4)	66.4	24.1	45.8	44.9	26.8

was done to assess heterogeneity between individual studies (33). Meta-analysis was based on the data submitted to the GSEC Study. Publication bias was assessed by a funnel plot and Begg's and Egger's test (34, 35).

Differences in the proportions of exposed and unexposed individuals among cases and controls were assessed using the χ^2 test. Differences in the distribution of continuous variables (age and BMI) were investigated with the Wilcoxon rank sum test due to the non-normal distributions of these variables. Genotype frequencies of *GSTP1* were tested for Hardy-Weinberg equilibrium by comparing observed and expected frequencies using a χ^2 test. All significance tests were two sided at the 0.05 level.

To measure the effect of genotypes and potential risk factors (age, family history, BMI, smoking, and reproductive factors) on the risk of breast cancer, ORs and 95% CIs were estimated using unconditional logistic regression. All ORs were adjusted for study center because of possible population stratification and possible study-specific misclassification of genotype (e.g., due to differences in the laboratory protocols).

To evaluate a possible modifying effect of tobacco consumption and reproductive factors on the association between GSTs and breast cancer, separate analyses were conducted after stratifying by categories of ever versus never smokers, parous versus nulliparous women, and premenopausal versus postmenopausal women. The continuous variables pack-years in ever smokers, age at menarche, and age at menopause were each categorized into two groups based on the division at the median among controls. To assess a modifying effect of GST genotype on the effect of smoking, ORs for the risk induced by smoking were estimated stratifying by genotype. To address an effect on age at onset, the distributions of age at diagnosis among cases were compared by genotype. ORs were calculated stratifying at tertiles of age in controls (<51, 51–61, and ≥62 years).

Interaction between GSTs and variables used for stratification was formally assessed by adding a product term to a model containing the main effects of *GST* genotype and the categories of the stratification variable. Models with and without interaction term were compared by using the likelihood ratio test. Analyses were repeated restricting to postmenopausal women. This set of analyses does not include *GSTP1* because only one study group provided information on menopausal status and *GSTP1* (31).

Finally, genotypes were combined to assess a potential synergism of polymorphisms of different *GST* classes. ORs were computed for the effect of each possible combination of wild-type and null among *GSTM1*, *GSTT1*, and *GSTP1* genotype and the risk of breast cancer. Analyses were carried out using Stata statistical software (Stata, College Station, TX; ref. 36).

Results

The baseline characteristics of the seven case-control studies on *GST* and breast cancer available in the GSEC database are shown in Table 1. In total, 2,048 cases with invasive breast cancer and 1,969 controls were included in the pooled analysis. All studies investigated the association between *GSTM1* genotype and breast cancer; four studies provided genotypes of *GSTT1*, comprising 50.9% of study subjects; and two studies provided genotypes of *GSTP1*, comprising 37.5% of study subjects (Table 1). Overall, the *GSTM1* null genotype was found in 52.0% of controls, with similar proportion in each individual study (P for difference in proportions = 0.25). The *GSTT1* null genotype was found in 21.6% of controls, ranging from 17.4 in the Norwegian study (30) to 25.8% in a study in the United States (ref. 27; P = 0.12). A homozygous mutant genotype of *GSTP1* was present in 9.4% of controls (Table 1). No departure from Hardy-Weinberg equilibrium was found for *GSTP1* genotypes in each study and both among cases and controls.

The meta-analytic OR (95% CI) of the effect of *GSTM1* on breast cancer risk in the random effects model was 0.94 (0.77–1.15). The individual ORs in the studies ranged from 0.59 to 1.28 with considerably overlapping 95% CIs (Q test: P = 0.056). 95% CIs were not significant, except for one small study (unpublished) with OR (95% CI) of 0.59 (0.36–0.96). There was an indication of publication bias in the funnel plot (Fig. 1; Egger's test, P = 0.011; Begg's test, P = 0.035). Meta-analysis of the four studies providing *GSTT1* genotypes yielded an OR (95% CI) of 1.11 (0.87–1.42). None of the single-study ORs were significant.

The availability of information on variables investigated in the pooled analysis is shown in Table 1. Cases were, on average, 2 years older than controls (median age 59 versus 57 years; P <0.001). Eighty-five (17%) cases and 40 (7%) controls had a positive family history of breast cancer (P <0.001). No significant difference between

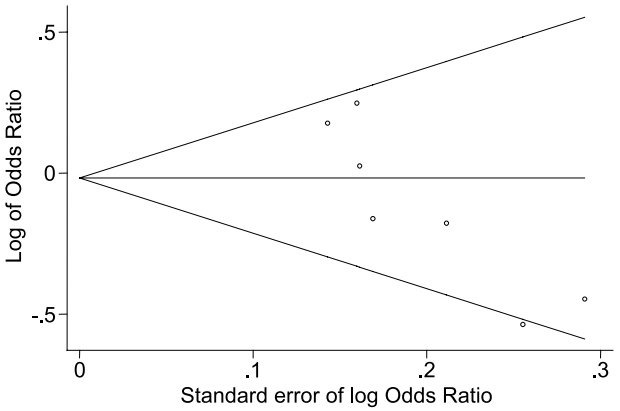

Figure 1. Begg's funnel plot for seven studies contributing data to GSEC.

cases and controls was found for BMI (median 25 kg/m^2 in each cases and controls; P = 0.24), proportion of smokers (43% versus 39%; P = 0.10), number of pack-years (median 22.5 versus 27; P = 0.50), parity (82% versus 81%; P = 0.73), age at menarche (13 years in each group; P = 0.95), and menopausal status (74% versus 77% postmenopausal women; P = 0.28). The age at menopause seemed to be slightly higher in cases than in controls but the difference was not significant (median 49 versus 47 years; P = 0.07).

A positive family history of breast cancer conferred a >2-fold increased risk of breast cancer (OR, 2.45; 95% CI, 1.64–3.66). Neither BMI (OR, 0.88; 95% CI, 0.68–1.13), ever smoking (OR, 1.07; 95% CI, 0.88–1.30), and postmenopausal status (OR, 0.78; 95% CI, 0.56–1.06) nor parity (OR, 1.09; 95% CI, 0.68–1.76) was associated with breast cancer risk. ORs were recalculated controlling for age (categorized at tertiles in controls) but differed little from the previous estimates (results not shown).

The analysis of the individual patient data from seven case-control studies yielded no evidence for an increased risk of breast cancer associated with the null genotype at the *GSTM1* locus (OR, 0.98; 95% CI, 0.86–1.12, adjusted for study center). This estimate is similar to the summary OR obtained in the meta-analysis (OR, 0.94; 95% CI, 0.77–1.15). No association was found between the *GSTT1* null genotype and breast cancer (OR, 1.11; 95% CI, 0.87–1.41) and there was no risk increase for either the heterozygous (OR, 1.01; 95% CI, 0.79–1.28) or the homozygous (OR, 0.93; 95% CI, 0.62–1.38) mutant genotype at the *GSTP1* locus (Table 2).

In stratified analyses, no risk modification was found with regard to family history of breast cancer. For *GSTM1* null, the OR (95% CI) was 0.68 (0.30–1.53) among those with a positive history and 0.89 (0.69–1.16) among those with a negative family history. The respective ORs (95% CIs) for *GSTT1* null were 1.70 (0.38–13.47) and 0.90 (0.57–1.42).

Smoking status, parity, age at menarche, and menopause also did not show any effect modification. There was no evidence of interaction between *GST* genotypes and these stratifying variables. Detailed results are given in Tables 3 and 4. In the group of individuals who had smoked <27 pack-years seemed to be a significant 2.7-fold increased cancer risk associated with *GSTT1* null (95% CI, 1.13–6.37).

Higher age at menopause (stratified at 47 years) had no modifying effect on risk. In a separate analysis addressing the risk conferred by smoking and stratifying by *GST* genotypes, there was no evidence of a modifying effect (results not shown). All analyses were repeated restricting the sample to postmenopausal women but did not reveal any significant risk increase or modifying effect (details not shown).

The median age at diagnosis of breast cancer was 58 years for carriers of *GSTM1* null compared with 60 years in cases with wild-type *GSTM1* (P = 0.09). There was no significant difference in the distribution of age at diagnosis according to genotype after stratification by family history. ORs (95% CIs) for the risk of breast cancer stratified by age group were 1.01 (0.77–1.32) for the youngest group, 0.92 (0.65–1.30) for the middle group, and 1.04 (0.81–1.35) for the highest age group.

The effect of combined *GST* genotypes on the risk of breast cancer was assessed in a separate set of analyses permuting genotypes in *GSTM1*, *GSTT1*, and *GSTP1*. Results are shown in Table 5. The combination of *GSTT1* null and *GSTP1* mutant yielded a nonsignificant 2-fold increased risk. Compared with wild-type for both, the addition of *GSTM1* null suggested no risk modification.

Discussion

This analysis of pooled individual data revealed no increased risk of breast cancer associated with *GST* null genotypes at the *GSTM1* and *GSTT1* loci and the codon 105 polymorphism at the *GSTP1* locus. This is consistent with the results of the published studies that contributed data to the GSEC database and in which no risk increase was found (26, 27, 30). Other studies, published recently, have also failed to detect an association between GSTs and breast cancer (37, 38). An earlier meta-analysis of three studies revealed a significant risk increase for *GSTM1* null only in the youngest age group (23). We did not observe any risk difference by age group in the pooled analysis. Among cases, the age distributions of age at onset were not significantly different by *GSTM1* genotype. Our results are based on the analysis of case-control data from Caucasian women. A large case-control study in Asian women from Shanghai found no

Table 2. Analysis of *GST* genotypes and the risk of breast cancer

	Cases/controls	OR* (95% CI)
GSTM1+	994/912	1.0†
GSTM1−	1,039/987	0.98 (0.86–1.12)
GSTT1+	948/669	1.0†
GSTT1−	244/184	1.11 (0.87–1.41)
*GSTP1*wt	427/289	1.0†
*GSTP1*het	400/241	1.01 (0.79–1.28)
*GSTP1*mut	94/55	0.93 (0.62–1.38)

NOTE: Abbreviations: wt, wild-type; het, heterozygous mutant; hom, homozygous mutant.
*Adjusted for study center.
†Reference category.

Table 3. ORs of breast cancer associated with *GST* genotypes (*GSTM1*, *GSTT1*, and *GSTP1*) stratified by smoking exposure (A: ever/never smoking; B: pack-years smoked)

	Cases/controls	OR* (95% CI)	Cases/controls	OR* (95% CI)	Cases/controls	OR* (95% CI)	Interaction *P*
A	Total†		Never Smokers		Ever Smokers		
GSTM1+	347/528	1.0‡	199/316	1.0‡	148/212	1.0‡	
GSTM1−	361/595	0.91 (0.75–1.10)	202/365	0.85 (0.66–1.09)	159/230	1.00 (0.74–1.34)	0.44
GSTT1+	286/495	1.0‡	186/321	1.0‡	100/174	1.0‡	
GSTT1−	101/146	1.17 (0.87–1.57)	65/101	1.06 (0.74–1.53)	36/45	1.39 (0.84–2.30)	0.41
*GSTP1*wt	85/165	1.0‡	57/117	1.0‡	28/48	1.0‡	
*GSTP1*het	55/131	0.81 (0.54–1.23)	36/92	0.80 (0.49–1.32)	19/39	0.84 (0.41–1.72)	
*GSTP1*mut	9/26	0.67 (0.30–1.50)	6/19	0.65 (0.25–1.71)	3/7	0.73 (0.18–3.07)	0.99
B	Total†		<27 Pack-Years		≥27 Pack-Years		
GSTM1+	120/182	1.0‡	71/101	1.0‡	49/81	1.0‡	
GSTM1−	140/198	1.09 (0.79–1.50)	81/88	1.33 (0.86–2.05)	59/110	0.84 (0.52–1.37)	0.16
GSTT1+	73/132	1.0‡	42/60	1.0‡	31/72	1.0‡	
GSTT1−	27/34	1.43 (0.80–2.56)	19/10	2.69 (1.13–6.37)	8/24	0.79 (0.32–1.96)	0.051
*GSTP1*wt	28/48	1.0‡	17/17	1.0‡	11/31	1.0‡	
*GSTP1*het	19/39	0.84 (0.41–1.72)	14/20	0.70 (0.27–1.83)	5/19	0.74 (0.22–2.47)	
*GSTP1*mut	3/7	0.73 (0.18–3.07)	2/5	0.40 (0.07–2.35)	1/2	1.41 (0.12–17.12)	0.73

NOTE: Abbreviations: wt, wild-type; het, heterozygous mutant; hom, homozygous mutant.
*ORs were adjusted for study center, except for the analysis of *GSTP1*, because both smoking and *GSTP1* genotype were assessed only in one study.
† The total number of cases and controls in these columns refers to subjects with available information on smoking habits.
‡ Reference category.

association between *GSTM1* or *GSTT1* genotypes and breast cancer but observed a significant risk increase among homozygotes for the *GSTP1* polymorphism (39), the latter not being confirmed in our analysis. These authors also did a meta-analysis of 19 published studies (predominantly Caucasian women, according to the authors) and found no association with *GSTM1*, *GSTT1*, or *GSTP1* (39).

We found no evidence of interaction between *GST* polymorphisms and smoking. There was a suggestion of an increased risk for *GSTT1* null carriers who smoked <27 pack-years. This association was not observed when controlling for age. Published studies have yielded no (40) or only weak evidence of a modifying effect of smoking in postmenopausal women (22). Our positive result might also be due to chance in a small subgroup.

Menopausal status and age at menopause did not modify the relation between GSTs and breast cancer risk. Previous studies yielded inconsistent results concerning the potential modifying effect of menopausal status. Two studies have reported a positive association between the homozygous deletion of *GSTM1* and breast cancer risk among postmenopausal women (41) or women ages >50 years (42), whereas another study detected no association neither in postmenopausal nor in premenopausal women (40). Similarly, one of those studies reported a protective effect of *GSTT1* null genotype in premenopausal women (40), but another study failed to show this (41). Such

Table 4. ORs of breast cancer associated with *GST* genotypes (*GSTM1* and *GSTT1*) stratified by reproductive factors (A: parity; B: age at menarche; C: menopausal status)

	Cases/controls	OR* (95% CI)	Cases/controls	OR* (95% CI)	Cases/controls	OR* (95% CI)	Interaction *P*
A	Total†		Nulliparous Women		Parous Women		
GSTM1+	107/106	1.0‡	21/22	1.0‡	86/84	1.0‡	
GSTM1−	109/133	0.80 (0.55–1.16)	17/23	0.78 (0.33–1.88)	92/110	0.81 (0.54–1.22)	0.95
GSTT1+	165/189	1.0‡	27/34	1.0‡	138/155	1.0‡	
GSTT1−	51/50	1.17 (0.75–1.82)	11/11	1.14 (0.42–3.09)	40/39	1.16 (0.70–1.91)	0.93
B	Total†		Menarche ≤13 y		Menarche >13 y		
GSTM1+	189/212	1.0‡	130/152	1.0‡	59/60	1.0‡	
GSTM1−	189/233	0.89 (0.68–1.18)	142/167	0.97 (0.70 –1.35)	47/66	0.73 (0.43–1.23)	0.34
GSTT1+	79/84	1.0‡	56/63	1.0‡	23/21	1.0‡	
GSTT1−	23/25	0.96 (0.50–1.84)	15/19	0.94 (0.43–2.03)	8/6	1.00 (0.28–3.59)	0.93
C	Total†		Premenopausal Women		Postmenopausal Women		
GSTM1+	320/482	1.0‡	61/57	1.0‡	259/425	1.0‡	
GSTM1−	318/521	0.92 (0.75–1.12)	55/67	0.77 (0.46–1.28)	263/454	0.95 (0.76–1.18)	0.45
GSTT1+	235/410	1.0‡	23/32	1.0‡	212/378	1.0‡	
GSTT1−	82/108	1.31 (0.94–1.83)	6/9	0.93 (0.29–2.97)	76/99	1.35 (0.96–1.91)	0.54

*ORs were adjusted for study center.
†The total number of cases and controls in these columns refers to subjects with available information on the stratifying variable.
‡Reference category.

Table 5. ORs for the risk of breast cancer associated with combinations of *GST* polymorphisms (*GSTM1*, *GSTT1*, and *GSTP1*)

GST	Cases/controls*	OR† (95% CI)
M1+ and T1+	460/327	1.0‡
M1 or T1 deficient	607/412	1.13 (0.92–1.40)
M1 and T1 deficient	119/110	0.94 (0.68–1.31)
M1+ and P1+	400/235	1.0‡
M1 or P1 deficient§	459/256	1.10 (0.85–1.42)
M1 and P1 deficient	47/24	0.93 (0.52–1.66)
T1+ and P1+	647/375	1.0‡
T1 or P1 deficient	225/135	0.97 (0.73–1.28)
T1 and P1 deficient	16/5	2.16 (0.70–6.64)
M1+ and T1+ and P1+	313/188	1.0‡
Any one GST deficient	446/250	1.15 (0.88–1.51)
Any two GST deficient	114/69	1.05 (0.71–1.56)
M1, T1, and P1 deficient	9/4	1.41 (0.37–5.41)

*The number of cases and controls in these columns refers to subjects with genotype information on the relevant GSTs.
†Adjusted for study center.
‡Reference category.
§Deficiency for *GSTP1* was here defined as at least one mutant allele.

inconsistent results might be due to limited power in small studies and a tendency to overemphasize positive subgroup findings. Parity and age at menarche did not influence or modify breast cancer risk. The number of children, which probably is a stronger factor for breast cancer than the binary variable parity, could not be examined because this information was available of too few subjects. Family history was a strong risk factor for breast cancer in univariate analysis. Stratification by family history of breast cancer did not modify the OR estimates associated with *GST* genotypes.

Hypothetically, a combination of deficient GSTs could affect the outcome. Helzlsouer et al. (41), in a study on 110 cases and 113 control women, reported a gene dosage effect of *GSTP1*, wherein increasing number of polymorphic alleles increased breast cancer risk. The Shanghai study cited above described a risk increase for the *GSTP1* polymorphism in the homozygous state, but this was not significant in postmenopausal women (39). Mitrunen et al. (38) investigated 481 cases and 483 controls and described an inverse relation between number of polymorphic alleles and risk. The present analysis, combining data of two studies, did not show any dosage effect of polymorphic *GSTP1* alleles. There was a suggestion that the combination of the *GSTT1* null genotype with a *GSTP1* mutant might increase breast cancer risk, although the 95% CI was wide. Mitrunen et al. also observed a tendency of increased risk with *GSTT1* null plus homozygous mutant *GSTP1* given a null genotype at the *GSTM1* locus (38).

Sources of Confounding, Bias, and Random Error. ORs were adjusted to account for potential confounding by age and study site, but estimates differed only little from the unadjusted values. Slight differences in frequencies of the null genotype of *GSTT1* were observed between studies. In the literature, the reported frequency of the *GSTT1* null genotype ranged from 11% to 18%. The average frequency among controls across the pooled studies was 21.6% with maximum of 25.8% in one study. The outlying frequencies could be the result of misclassification of genotypes or inherent in the different populations investigated (population stratification).

In theory, publication bias could affect the results of the pooled analysis. Publication bias can occur if studies with significant associations are more likely to get published than studies with null results, which are subsequently not included in the pooled analysis. This would lead to biased results (43). We did two formal tests for assessing publication bias. However, the results must be interpreted with caution due to the fairly low power when applied to a few meta-analytically investigated studies. It is unlikely that publication bias does jeopardize the results of the present analysis, because none of the published studies contributing data to the GSEC database had detected a significant positive association of GSTs with breast cancer. Failure to locate and include further null studies cannot bias the presented null results (44). Additionally, data from an unpublished study were included in the pooled analysis. The crude OR for this study (Rebbeck et al.) yielded a significant negative association of *GSTM1* null and breast cancer risk and seemed as outlier on the funnel plot.

Strength and Weaknesses. Pooling and analyzing individual data from original studies has several advantages. Combining the individual efforts of different authors, a large sample size can be obtained. Allowing a type 1 error of 5%, the present study has power greater than 80% to detect an effect size of 1.2 for *GSTM1*, 1.4 for *GSTT1*, and 1.6 for *GSTP1* (in the latter case, considering only homozygous mutants as exposed). This also provides the opportunity to study gene-environment interactions in stratified analysis, whereas smaller studies have not enough power to investigate this. Additionally, one has the possibility to adjust for potential confounders using the individual data. This would not be possible in meta-analysis wherein the study-specific estimates of effect are used to obtain an overall summary estimate.

Uniform coding of all data following a standard protocol is a benefit for the analysis and increases reliability of results. However, the problem of misclassification due to, for example, application of different PCR protocols, as is the case in the present pooled analysis, remains. Some information might get lost if exposure has to be categorized coarsely, as was the case with smoking exposure in this study. A drawback of the present study is that information on alcohol consumption was not available. Alcohol is involved in the etiology of breast cancers (45) and a recent report suggested an interaction of *GST* and alcohol consumption in breast cancer patients (46).

In conclusion, this pooled analysis provides strong evidence that GSTs do not play a major role in susceptibility to breast cancer. Furthermore, there was no evidence for a modifying effect of reproductive factors and exposure to tobacco smoke, in agreement with most previous studies. Taken together, the results of this pooled analysis and the results of the original reports and more recent studies consistently showed no significant association of GSTs with breast cancer risk. Given this strong evidence in favor of the null hypothesis, a clear compelling rationale should be provided to justify further studies into GST polymorphisms and breast cancer risk.

Acknowledgments

We thank Heide A. Stirnadel (Worldwide Epidemiology, GlaxoSmithKline) for helpful comments on an earlier version of the article.

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Cancer Epidemiol Biomarkers Prev 2004;13:1473-1479.