Glutathione *S*-Transferases M1, T1, and P1 and Breast Cancer[1]

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Abstract

We examined associations for glutathione *S*-transferases M1 (*GSTM1*), T1 (*GSTT1*), and P1 (*GSTP1*) genotypes and breast cancer in the Carolina Breast Cancer Study, a population-based, case-control study in North Carolina. Odds ratios were close to the null value for each *GST* locus among African-American women (278 cases and 271 controls) and white women (410 cases and 392 controls), as well as pre- and postmenopausal women. For women with a history of breast cancer in one or more first-degree relatives, odds ratios were 2.1 (95% confidence interval, 1.0–4.2) for *GSTM1* null and 1.9 (0.8–4.6) for *GSTT1* null genotypes. Among women with a family history, age at diagnosis was significantly earlier for those with the *GSTM1* null genotype. We did not observe strong evidence for modification of odds ratios for smoking according to *GST* genotypes. There was no evidence for combined effects of *GSTM1*, *GSTT1*, and *GSTP1* genotypes, and there were no combined effects for *GST* genotypes and the catechol *O*-methyltransferase genotype. We conclude that *GSTM1*, *GSTT1*, and *GSTP1* genotypes do not play a strong role in susceptibility to breast cancer. However, the role of *GST* genotypes in age at onset and risk of breast cancer among women with a family history merits further investigation.

Introduction

GSTs[3] are a family of enzymes involved in detoxication of benzo(*a*)pyrene and other carcinogens found in tobacco smoke, cytotoxic drugs, and chemical solvents (1, 2). Deletions in two GST genes, *GSTM1* and *GSTT1*, occur at frequencies of 15% or greater in human populations (3). Individuals who are deletion homozygotes, classified as *GSTM1* null or *GSTT1* null, exhibit absence of enzymatic activity and are hypothesized to be at increased risk for the carcinogenic effects of a wide range of environmental exposures. Associations between *GSTM1* and *GSTT1* null genotypes and cancer of the lung, bladder, and colon have been reported, but results are inconsistent across studies (2–5). An amino acid substitution variant in a third *GST* gene, *GSTP1* codon 105 *Ile→Val*, has been identified recently that encodes an enzyme with reduced catalytic activity (6). The *GSTP1 Val* allele is common in human populations but has not been extensively examined in association with cancer.

Several previous studies investigated *GSTM1* and *GSTT1* genotypes and breast cancer risk (reviewed in Refs. 4, 7, and 8), and one study examined the role of *GSTP1* genotype (9). We examined the relation of *GSTM1*, *GSTT1*, and *GSTP1* genotypes and breast cancer risk in the CBCS, a large, population-based, case-control study of African-American and white women residents of North Carolina. To address issues raised by previous studies, we estimated main effects for each *GST* locus; conducted analyses stratified on smoking, family history, and other factors; and determined age at onset according to *GST* genotype and family history. We investigated joint effects for combinations of *GST* genotypes, as well as joint effects for *GST* genotypes and the *COMT* genotype, a gene involved in detoxication of catechol estrogens (10).

Materials and Methods

Study Population. The CBCS is a population-based, case-control study of breast cancer conducted in 24 counties of North Carolina (11). Incident cases of invasive breast cancer among women of ages 20–74 were identified in cooperation with the North Carolina Central Cancer Registry, and controls were identified using Division of Motor Vehicles and Medicare beneficiary lists. Randomized recruitment (12) was used to oversample younger women and African-American women. Between 1993 and 1996, 889 cases of primary invasive breast cancer and 841 population-based controls were enrolled. Overall response rates (number of completed interviews/number of eligible women) were 74% for cases and 53% for controls (13). In-person interviews were conducted in participants' homes. Over 98% of women who were interviewed agreed to provide a blood sample.

Genotyping for *GSTs* was conducted for the first 688 cases and 663 controls enrolled in the CBCS (278 African-American cases and 271 African-American controls; 410 white cases and 392 white controls). There were no appreciable differences in risk factors for breast cancer or response rates between participants genotyped for *GSTs* and the remaining participants in the CBCS (data not shown).

Laboratory Analysis. DNA was extracted from peripheral blood lymphocytes using standard methods (14). Genotyping for *GSTM1*, *GSTT1*, and *GSTP1* was conducted using PCR-RFLP methods, as described in Helzlsouer *et al.* (9), with slight

Received 11/2/99; revised 3/8/00; accepted 4/11/00.
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[1] Supported by the Specialized Program of Research Excellence in Breast Cancer, NIH/NCI P50-CA58223; Pesticides and Breast Cancer in North Carolina, NIH/NIEHS R01-ES07128; and Environment and Breast Cancer Program, R21-CA66201.
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[3] The abbreviations used are: GST, glutathione *S*-transferase; CBCS, Carolina Breast Cancer Study; BMI, body mass index; CI, confidence interval; COMT, catechol *O*-methyltransferase; df, degrees of freedom; ETS, environmental tobacco smoke; OR, odds ratio.

Table 1 Genotype frequencies and ORs for breast cancer for *GSTM1*, *GSTP1*, and *GSTT1* among African-American and white participants

Genotype	Cases			Controls			OR[a] (95% CI)
	n	Frequency	(95% CI)	*n*	Frequency	(95% CI)	
A. African-Americans	$N = 278$			$N = 271$			
GSTM1							
Present	197	0.75	(0.70–0.80)	187	0.72	(0.67–0.78)	Referent
Null	66	0.25	(0.20–0.30)	72	0.28	(0.22–0.33)	0.9 (0.6–1.3)
Missing	15			12			
χ^2 test (1 df):[b] $P = 0.48$							
GSTT1							
Present	210	0.80	(0.75–0.85)	216	0.83	(0.79–0.88)	Referent
Null	52	0.20	(0.15–0.25)	43	0.17	(0.12–0.21)	1.3 (0.8–2.0)
Missing	16			12			
χ^2 test (1 df): $P = 0.34$							
GSTP1							
Ile/Ile	61	0.25	(0.19–0.30)	54	0.22	(0.17–0.27)	Referent
Ile/Val	131	0.53	(0.47–0.59)	135	0.55	(0.48–0.61)	0.9 (0.6–1.3)
Val/Val	56	0.22	(0.17–0.28)	58	0.23	(0.18–0.29)	0.9 (0.5–1.4)
Missing	30			24			
χ^2 test (2 df): $P = 0.77$							
B. Whites	$N = 410$			$N = 392$			
GSTM1							
Present	189	0.49	(0.44–0.54)	177	0.48	(0.43–0.53)	Referent
Null	194	0.51	(0.46–0.56)	192	0.52	(0.47–0.57)	0.9 (0.7–1.2)
Missing	27			23			
χ^2 test (1 df):[b] $P = 0.71$							
GSTT1							
Present	331	0.85	(0.82–0.89)	312	0.84	(0.80–0.87)	Referent
Null	58	0.15	(0.11–0.18)	61	0.16	(0.13–0.20)	0.8 (0.6–1.2)
Missing	21			19			
χ^2 test (1 df): $P = 0.58$							
GSTP1							
Ile/Ile	178	0.48	(0.43–0.54)	141	0.40	(0.35–0.46)	Referent
Ile/Val	155	0.42	(0.37–0.47)	169	0.49	(0.43–0.54)	0.7 (0.5–1.0)
Val/Val	35	0.10	(0.07–0.13)	38	0.11	(0.08–0.14)	0.7 (0.4–1.2)
Missing	42			44			
χ^2 test (2 df): $P = 0.11$							

[a] Adjusted for age.
[b] Comparing genotype frequencies in cases *versus* controls.

modifications for *GSTT1*; the forward primer sequence was 5′-gcc ctg gct agt tgc tga ag, the reverse primer was 5′-gca tct gat ttg ggg acc aca, and the annealing temperature was 59°C.

The genotyping assays for *GSTM1* and *GSTT1* classify individuals with one or two copies of the relevant gene as "present" and individuals with homozygous deletions as "null." The assay for *GSTP1* classifies individuals according to the alleles *Ile* and *Val*. Assays that were unreadable for each locus are reported as "missing." Approximately half of the samples listed as missing did not amplify for the relevant locus, and the remaining amplified too poorly to assign genotypes. The proportions of missing values were similar in cases and controls (5% of cases and 4% of controls were missing for *GSTM1;* 6% of cases and 4% of controls for *GSTT1;* 11% of cases and 9% of controls for *GSTP1*). Missing values for genotypes were not related to smoking or other covariates (data not shown). Sensitivity analyses were conducted by replacing missing values with different genotype combinations, and ORs remained within the confidence limits presented.

Positive and negative control samples were included with each batch of samples (one batch, 94 samples). Gels were scored by two different readers, and discordant samples were repeated. Reliability was assessed by selecting a random sample of 10% of samples from each batch. Batches with <95% agreement were rerun.

Methods for genotyping of *COMT* and associations with breast cancer in the CBCS have been reported previously (15).

Statistical Analysis. Genotype frequencies and 95% CI for *GSTM1*, *GSTT1*, and *GSTP1* were calculated as the proportion of individuals with a given genotype divided by the total number of participants. For *GSTP1*, allele frequencies and 95% CI were calculated as the number of alleles divided by the number of chromosomes, and tests for Hardy-Weinberg equilibrium were conducted by comparing observed and expected genotype frequencies using a χ^2 test (16).

Adjusted OR for breast cancer and 95% CI were calculated from unconditional logistic regression models using SAS (version 6.11; SAS Institute, Cary NC). PROC GENMOD was used to incorporate offset terms derived from the sampling probabilities used to identify eligible participants (12) and to adjust for race (as two-level categorical variable) and age (as an 11-level ordinal variable reflecting 5-year age categories). Race was defined according to self-report.

Table 2 ORs for *GSTM1*, *GSTT1*, and *GSTP1* and breast cancer, stratified by menopausal status

Genotype	Overall		Premenopausal		Postmenopausal	
	Cases/Controls	OR[a] (95% CI)	Cases/Controls	OR[a] (95% CI)	Cases/Controls	OR[a] (95% CI)
GSTM1						
Present	386/364	Referent	193/161	Referent	193/203	Referent
Null	260/264	0.9 (0.7–1.1)	131/133	0.8 (0.6–1.1)	129/131	1.0 (0.8–1.4)
Missing	42/35		25/13		17/22	
GSTT1						
Present	541/528	Referent	278/249	Referent	263/279	Referent
Null	110/104	1.0 (0.7–1.3)	52/44	1.0 (0.7–1.6)	58/60	1.0 (0.7–1.5)
Missing	37/31		19/14		18/17	
GSTP1						
Ile/Ile	239/195	Referent	120/88	Referent	119/107	Referent
Ile/Val	286/304	0.8 (0.6–1.0)	142/145	0.7 (0.5–1.0)	144/159	0.8 (0.6–1.1)
Val/Val	91/96	0.8 (0.5–1.1)	43/36	0.9 (0.5–1.5)	48/60	0.7 (0.4–1.1)
Missing	72/68		44/38		28/30	

[a] Adjusted for age and race.

Multivariable logistic regression models were used to adjust for potential confounding factors. Covariates included menopausal status, a composite of parity and age at first full-term pregnancy, breastfeeding, family history of breast cancer, history of breast biopsy, smoking, and alcohol consumption, as described previously (17). Family history was defined as having one or more first-degree relatives with breast cancer and was not verified by contacting relatives or reviewing pathology reports from relatives. Women were classified as exposed to ETS if they reported living with a smoker when they were 18 years or older and unexposed if they did not live with a smoker. Our assessment of ETS exposure did not include information on occupational, leisure, or recreational exposure. With the exception of ORs for smoking variables, ORs did not change after adjustment for additional covariates and therefore are adjusted only for sampling fractions, age, and race (where appropriate) in this report.

ORs for *GST* genotypes and breast cancer were calculated after stratifying on menopausal status, family history of breast cancer, use of hormone replacement therapy, alcohol consumption, and BMI. We conducted stratified analyses in this manner to compare our results with Helzlsouer *et al.* (9). For BMI, we stratified on the median among controls in our study (27.7 kg/m^2), as well as the cutpoint used by Helzlsouer *et al.* (Ref. 9; 24.7 kg/m^2). ORs for smoking and breast cancer were stratified on *GST* genotype to compare our results with our previous study of *N*-acetyl transferase genotypes and breast cancer (17). Thus, the method of stratification differed across tables but was necessary to compare results across studies. Interpretation of results did not differ when a uniform method of stratification based on a single common referent group was used (results not shown).

Joint effects of *GST* genotypes were estimated using the *a priori* low-risk genotype combination (*GSTM1* present, *GSTT1* present, and *GSTP1 Ile/Ile*) as a common referent group. Joint effects of *GST* genotype and *COMT* were assessed using the low-risk *GST* genotype in combination with *COMT HH* or *HL* genotypes as a common referent group. The *COMT L* allele encodes a thermolabile form of the enzyme that displays reduced ability to inactivate catechol estrogens through *O*-methylation (10).

Tests for interaction on multiplicative and additive scales were performed by comparing ORs for the joint effects of genotype and environmental factors (or joint effects for genotypes) using a common referent group (18). No evidence of departure from additive or multiplicative effects was seen (results not shown).

χ^2 tests were used to compare the prevalence of *GST* genotypes across stages of breast cancer among cases, and *t* tests were used to compare mean age at diagnosis among cases according to *GST* genotypes. Trend tests were conducted by calculating *P*s for the β coefficient in a logistic regression model with the exposure coded as an ordinal variable. All *P*s are two-sided.

Results

Characteristics of participants in the CBCS have been described previously (17). Briefly, onset of menarche prior to age 12, nulliparity, first full-term pregnancy at age 26 or older, breast cancer in a first-degree relative, and smoking cigarettes for >20 years were positively associated with breast cancer, whereas breastfeeding showed an inverse association. Genotype frequencies for *GSTM1*, *GSTT1*, and *GSTP1* in the CBCS are presented in Table 1. Estimates among controls are similar to previous studies, including a higher prevalence of *GSTM1* null genotype among white compared with African-American controls (2, 4). Allele frequencies for the *GSTP1* Val allele were 0.49 (95% CI 0.45–0.53) in African-American cases and 0.51 (0.46–0.55) in African-American controls ($P = 0.6$), and 0.31 (0.27–0.34) in white cases and 0.35 (0.32–0.39) in white controls ($P = 0.1$). We did not observe significant departures from Hardy-Weinberg equilibrium for *GSTP1* genotypes among African-American cases ($P = 0.8$), African-American controls ($P = 0.5$), white cases ($P = 0.9$), or white controls ($P = 0.8$).

We did not observe significant case-control differences in genotype frequencies for *GSTM1*, *GSTT1*, or *GSTP1* among African-American or white women (Table 1). ORs for *GSTM1*, *GSTT1*, and *GSTP1* genotypes and breast cancer among African-American and white women are presented in Table 1. Results were close to the null for each *GST* locus. Similar results were found among pre- and postmenopausal women (Table 2). Results of subsequent analyses were similar among African-American and white women; therefore, results are presented for both races combined to increase precision.

GSTM1 null and *GSTT1* null genotypes were positively associated with breast cancer among women with a family history of breast cancer (Table 3). ORs for *GSTP1* did not differ according to family history. There were no differences in ORs for any of the *GST* loci when we stratified by use of hormone

Table 3 ORs for *GSTM1*, *GSTT1*, and *GSTP1* genotypes and breast cancer, stratified by family history, hormone replacement therapy, alcohol consumption, and body mass index

	GSTM1 Null OR[a] (95% CI)	*GSTM1* Present OR (95% CI)	*GSTT1* Null OR (95% CI)	*GSTT1* Present OR (95% CI)	*GSTP1 Val/Val* OR (95% CI)	*GSTP1 Ile/Val* OR (95% CI)	*GSTP1 Ile/Ile* OR (95% CI)
Family history[b]							
No	0.8 (0.6–1.0)	Referent	0.9 (0.6–1.2)	Referent	0.8 (0.5–1.2)	0.8 (0.6–1.0)	Referent
Yes	2.1 (1.0–4.2)	Referent	1.9 (0.8–4.6)	Referent	0.6 (0.2–1.7)	0.7 (0.4–1.5)	Referent
Hormone replacement therapy							
Never	0.8 (0.6–1.1)	Referent	1.0 (0.7–1.5)	Referent	0.8 (0.5–1.2)	0.8 (0.6–1.1)	Referent
Ever	1.1 (0.7–1.7)	Referent	0.9 (0.5–1.6)	Referent	0.8 (0.4–1.5)	0.8 (0.5–1.3)	Referent
Alcohol consumption							
No	1.0 (0.6–1.5)	Referent	0.9 (0.5–1.5)	Referent	0.6 (0.3–1.2)	0.8 (0.5–1.2)	Referent
Yes	0.8 (0.6–1.1)	Referent	1.0 (0.7–1.5)	Referent	0.9 (0.6–1.3)	0.8 (0.6–1.1)	Referent
Body mass index							
≤27.7 kg/m^2	0.9 (0.7–1.3)	Referent	0.9 (0.6–1.3)	Referent	0.7 (0.4–1.2)	0.9 (0.6–1.2)	Referent
>27.7 kg/m^2	0.8 (0.6–1.2)	Referent	1.0 (0.6–1.6)	Referent	0.8 (0.5–1.4)	0.7 (0.5–1.1)	Referent

[a] Adjusted for age and race.
[b] One or more first-degree relatives with breast cancer.

Table 4 ORs for smoking and breast cancer, stratified by *GSTM1*, *GSTT1*, and *GSTP1* genotypes

	GSTM1 Null OR[a] (95% CI)	*GSTM1* Present OR (95% CI)	*GSTT1* Null OR (95% CI)	*GSTT1* Present OR (95% CI)	*GSTP1 Ile/Val* and *Val/Val* OR (95% CI)	*GSTP1 Ile/Ile* OR (95% CI)
Active smoking status						
Never	Referent	Referent	Referent	Referent	Referent	Referent
Current	1.2 (0.7–1.9)	0.7 (0.4–1.0)	0.3 (0.1–0.9)	0.9 (0.6–1.3)	0.8 (0.6–1.3)	0.7 (0.4–1.2)
Former	1.2 (0.7–1.8)	1.4 (0.9–2.0)	1.5 (0.7–3.0)	1.2 (0.9–1.7)	1.1 (0.8–1.5)	1.7 (1.0–2.7)
Usual amount smoked (packs/day)						
Never	Referent	Referent	Referent	Referent	Referent	Referent
<1/2	1.2 (0.7–2.1)	1.0 (0.6–1.5)	0.7 (0.3–1.7)	1.1 (0.8–1.6)	0.9 (0.6–1.3)	1.1 (0.6–2.0)
1/2–1	1.4 (0.8–2.4)	1.1 (0.7–1.7)	0.8 (0.3–1.9)	1.2 (0.9–1.8)	1.0 (0.7–1.6)	1.7 (0.9–3.2)
>1	0.9 (0.5–1.6)	1.1 (0.6–1.8)	1.9 (0.7–5.5)	0.9 (0.6–1.3)	1.0 (0.6–1.7)	0.7 (0.4–1.3)
Trend test	$P = 0.8$	$P = 0.8$	$P = 0.5$	$P = 0.8$	$P = 0.9$	$P = 0.9$
Duration of smoking (years)						
≤10	1.0 (0.6–1.8)	1.0 (0.6–1.5)	0.8 (0.3–2.3)	1.0 (0.7–1.5)	0.7 (0.4–1.1)	1.3 (0.6–2.6)
11–20	0.7 (0.4–1.3)	1.0 (0.6–1.6)	1.0 (0.4–2.6)	0.8 (0.5–1.2)	0.9 (0.5–1.5)	0.8 (0.4–1.4)
>20	1.7 (1.0–2.8)	1.1 (0.7–1.7)	0.9 (0.4–2.2)	1.3 (0.9–1.8)	1.3 (0.9–2.0)	1.4 (0.8–2.3)
Trend test	$P = 0.1$	$P = 0.7$	$P = 0.9$	$P = 0.3$	$P = 0.3$	$P = 0.5$
Former smokers: time since cessation (yr)						
≤3	1.7 (0.8–3.6)	2.3 (1.2–4.5)	2.2 (0.7–7.1)	2.0 (1.1–3.5)	1.6 (0.9–3.1)	3.3 (1.2–8.8)
4–19	0.9 (0.5–1.6)	1.3 (0.8–2.1)	1.4 (0.5–3.9)	1.1 (0.7–1.6)	1.0 (0.6–1.5)	1.6 (0.8–3.1)
>20	1.4 (0.7–3.0)	0.8 (0.5–1.6)	1.3 (0.4–3.8)	1.1 (0.6–1.8)	0.9 (0.5–1.6)	1.2 (0.6–2.7)
Trend test	$P = 0.6$	$P = 0.7$	$P = 0.5$	$P = 0.6$	$P = 0.8$	$P = 0.2$
Never active smokers						
Unexposed to active or ETS	Referent	Referent	Referent	Referent	Referent	Referent
Exposed to ETS after age 18	0.8 (0.4–1.4)	1.3 (0.8–2.0)	1.9 (0.7–5.4)	1.0 (0.7–1.5)	1.0 (0.6–1.5)	1.1 (0.6–2.1)

[a] Adjusted for age, race, menopausal status, age at first full-term pregnancy/parity composite, breastfeeding, family history of breast cancer, biopsy, and alcohol consumption.

replacement therapy, alcohol consumption, or BMI (Table 3). Results for BMI were unchanged when we used strata for BMI as defined by Helzlsouer *et al.* (Ref. 9; data not shown). We also did not observe differences in ORs for *GST* genotypes when we stratified by consumption of fruits and vegetables (data not shown).

ORs for smoking and breast cancer, stratified by *GST* genotype, are presented in Table 4. In a previous paper that estimated main effects for smoking variables (17), we reported weak positive associations with breast cancer among former smokers, women who smoked for >20 years, and former smokers who had quit smoking within the last 3 years. There was little evidence of modification of these associations by *GST* genotype. The ORs for smoking longer than 20 years was slightly higher among women with *GSTM1* null genotype compared with those with *GSTM1* present. ORs for smoking more than one pack per day and exposure to ETS were higher among women with *GSTT1* null genotype compared with those with *GSTT1* present. There was an inverse association for current smoking among women with *GSTT1* null genotype. ORs for former smokers and former smokers who had quit within the past 3 years were higher for women with *GSTP1 Ile/Ile* genotype compared with women with one or more copy of the *GSTP1 Val* allele. However, for each of these results, associations remained weak, and confidence intervals were wide, leading to considerable overlap for the groups being compared. Results were similar for pre- and postmenopausal women (data not shown).

ORs for combinations *GST* genotypes and breast cancer are presented in Table 5. Compared with the putative lowest

Table 5 ORs for combinations of *GSTM1*, *GSTT1*, and *GSTP1* genotypes and breast cancer

GST genotype			Cases/controls	OR[a] (95% CI)
GSTM1	*GSTT1*	*GSTP1*		
Present	Present	*Ile/Ile*	104/86	Referent
Null	Present	*Ile/Ile*	83/76	0.9 (0.6–1.4)
Present	Null	*Ile/Ile*	18/12	1.2 (0.6–2.7)
Present	Present	*Ile/Val* or *Val/Val*	174/179	0.8 (0.6–1.2)
Null	Null	*Ile/Ile*	18/10	1.2 (0.5–2.9)
Null	Present	*Ile/Val* or *Val/Val*	111/120	0.7 (0.5–1.1)
Present	Null	*Ile/Val* or *Val/Val*	42/41	0.9 (0.5–1.5)
Null	Null	*Ile/Val* or *Val/Val*	20/31	0.5 (0.3–1.0)
Missing			118/108	

[a] Adjusted for age and race.

Table 6 ORs for combinations of *COMT* and *GST* genotypes and breast cancer

Genotype		Cases/controls	OR[a] (95% CI)
COMT	*GSTM1*		
HH or *HL*	Present	299/273	Referent
LL	Present	70/84	0.8 (0.5–1.1)
HH or *HL*	Null	198/204	0.8 (0.6–1.1)
LL	Null	54/54	0.9 (0.6–1.3)
Missing		67/48	

Genotype		Cases/controls	OR[a] (95% CI)
COMT	*GSTT1*		
HH or *HL*	Present	418/400	Referent
LL	Present	103/117	0.8 (0.6–1.1)
HH or *HL*	Null	87/83	0.9 (0.7–1.3)
LL	Null	17/19	0.9 (0.4–1.7)
Missing		63/44	

Genotype		Cases/controls	OR[a] (95% CI)
COMT	*GSTP1*		
HH or *HL*	Ile/Ile	181/143	Referent
LL	Ile/Ile	48/47	0.8 (0.5–1.3)
HH or *HL*	Ile/Val or Val/Val	287/304	0.8 (0.6–1.0)
LL	Ile/Val or Val/Val	72/82	0.7 (0.5–1.0)
Missing		100/87	

[a] Adjusted for age and race.

risk group (*GSTM1* present, *GSTT1* present, and *GSTP1 Ile/Ile)*, ORs were close to 1.0 for all combinations of *GST* genotypes except the putative highest risk group (*GSTM1* null, *GSTT1* null, and *GSTP1 Ile/Val* or *Val/Val*), for which we observed an inverse association with breast cancer risk. ORs were close to the null for all combinations of *GST* and *COMT* genotypes (Table 6).

Mean age at diagnosis of breast cancer according to family history and *GST* genotypes is presented in Table 7. For women with a family history of breast cancer, mean age at breast cancer diagnosis was significantly earlier (49.1 years) among women with *GSTM1* null genotype compared with women with *GSTM1* present (56.3 years). Less extreme but similar results were obtained for women with a family history of breast cancer and one or two *GSTP1 Val* alleles (52.7 years *versus* 56.8 years for *GSTP1 Ile/Ile* genotype). There was virtually no difference in mean ages at diagnosis by *GST* genotype among women who did not report a family history of breast cancer.

Genotype frequencies among breast cancer cases did not differ according to stage at diagnosis ($P = 0.9$ for *GSTM1*, $P = 0.9$ for *GSTT1* and $P = 0.8$ for *GSTP1*). Additionally, ORs for *GSTM1*, *GSTT1* and *GSTP1* and breast cancer did not differ when we stratified cases by stage at diagnosis (data not shown).

Table 7 Age at diagnosis of breast cancer among cases, according to *GST* genotype and family history

GST genotype	*N*	Age at diagnosis		*P*[a]
		Mean	(SD)	
A. Positive family history[b]				
GSTM1				
Null	22	49.1	(13.0)	
Present	49	56.3	(11.6)	0.02
GSTT1				
Null	10	57.4	(8.8)	
Present	62	53.5	(13.0)	0.4
GSTP1				
Ile/Val or *Val/Val*	43	52.7	(13.4)	
Ile/Ile	25	56.8	(11.0)	0.2
B. No family history				
GSTM1				
Null	231	50.4	(12.1)	
Present	301	50.7	(11.0)	0.8
GSTT1				
Null	92	50.3	(11.5)	
Present	443	50.7	(11.5)	0.7
GSTP1				
Ile/Val or *Val/Val*	344	51.2	(11.7)	
Ile/Ile	162	50.8	(11.6)	0.7

[a] *t* test.
[b] One or more first-degree relatives with breast cancer.

Discussion

We examined the relation of *GSTM1*, *GSTT1*, and *GSTP1* genotypes and breast cancer risk in a population-based, case-control study of African-American and white women in North Carolina. *GSTM1*, *GSTT1*, and *GSTP1* genotypes were not associated with breast cancer risk in African-American or white women, or among pre- or postmenopausal women. Two previous studies reported no overall association between *GSTM1* genotype and breast cancer risk (19, 20). Helzlsouer *et al.* (9) and Charrier *et al.* (21) reported positive associations for the *GSTM1* null genotype among postmenopausal but not premenopausal women, whereas Ambrosone *et al.* (22) reported a positive association for *GSTM1* null genotype among younger postmenopausal women. In contrast, Garcia-Closas *et al.* (23) reported no association for *GSTM1* null genotype in pre- or postmenopausal women. Helzlsouer *et al.* (9) reported no association for *GSTT1* null genotype and breast cancer in pre- or postmenopausal women, whereas Garcia-Closas *et al.* (23) observed an inverse association for *GSTT1* null genotype among premenopausal women. All of the aforementioned studies were conducted primarily among white women. In the single previous study to include substantial numbers of African-American women, Bailey *et al.* (24) observed no association between *GSTM1* null or *GSTT1* null genotypes and breast cancer among African-American or white women. Only one previous study investigated *GSTP1* and breast cancer (9). The authors reported a positive association for *GSTP1* Val/Val genotype in postmenopausal women. It is likely that the differences in results across studies are attributable to chance, because they are based upon small subgroups of women.

ORs for *GSTM1* null and *GSTT1* null genotypes were elevated slightly among women with family history of breast cancer, and age at diagnosis was lower among women with a family history and *GSTM1* null genotype. Helzlsouer *et al.* (9) reported 2-fold elevated ORs for all three *GST* genes among women with a family history of breast cancer, whereas Kelsey *et al.* (20) reported no modification of ORs for *GSTM1* by family history. The positive associations for *GSTM1* and *GSTT1* genotypes among women with a family history could be attributable to unmeasured genetic or environmental factors that interact with *GST* genes to increase risk of breast cancer and/or age at onset. Family-based studies that incorporate genotyping and environmental exposure assessment are the ideal study design to test such a hypothesis (25). We did not estimate joint effects for *GST* genotypes and *BRCA1* or *BRCA2* status because of the small number of *BRCA* carriers in the CBCS. In fact, the majority of CBCS cases with a family history did not carry mutations in *BRCA1* (26). Our results for family history could be biased, because we did not verify family history information using medical records.

We did not observe modification of ORs for *GST* genotype by use of hormone replacement therapy, alcohol consumption, or body mass index. Our results contrast with Helzlsouer *et al.* (9), who reported a strong positive association for *GSTM1* null genotype among postmenopausal women with BMI >24.47 kg/m^2 and a positive association for *GSTT1* null genotype among women who consumed alcoholic beverages. We did not observe modification of ORs for *GST* genotype by consumption of fruits and vegetables, in agreement with Ambrosone *et al.* (27). Smoking effects were modified only slightly by *GST* genotypes, as in previous studies (9, 20, 22, 23). We only partially addressed exposure to ETS, because the CBCS questionnaire did not include exposure during work or leisure time. Our results suggest that although GST enzymes are expressed in breast tissue (28), polymorphisms in *GST* genes do not play a strong role in modifying the association of smoking and breast cancer.

Helzlsouer *et al.* (9), in a study of 100 cases and 115 controls, reported an OR for breast cancer of 3.77 (95% CI, 1.10–12.88) for the combination of *GSTM1* null + *GSTT1* null + *GSTP1 Ile/Val* or *Val/Val* genotypes, compared with *GSTM1* present + *GSTT1* present + *GSTP1 Ile/Ile.* In contrast, we observed an OR of 0.5 (95% CI, 0.3–1.0) for the same comparison of *GST* genotypes. Garcia-Closas *et al.* (23), in a study of 466 cases and 466 controls, reported ORs close to the null for all combinations of *GSTM1* and *GSTT1* genotypes. Only our study and that of Garcia-Closas *et al.* (23) had 80% power to detect joint effects for *GST* genotypes, and the positive finding in the aforementioned study (9) may represent a chance finding because of the small number of participants (29). Lavigne *et al.* (10), using data from the same study as Helzlsouer *et al.* (9), reported an OR of 4.10 (95% CI 1.17–14.27) for the combination of *COMT LL* + *GSTM1* null genotypes, and an OR of 3.40 (95% CI, 1.17–12.33) for *COMT LL* + *GSTP1 Ile/Val* or *Val/Val* genotypes, whereas we did not observe positive associations for any combination of *COMT* or *GST* genotypes.

We did not observe an association between *GSTM1*, *GSTT1*, or *GSTP1* genotype and stage at diagnosis of breast cancer. Our results are in agreement with Shea *et al.* (30) but contradict Kristensen *et al.* (31) who reported an association between *GSTM1* null and *GSTT1* null genotype status and more advanced stage at diagnosis in breast cancer patients. Kristensen *et al.* (31) reported that patients with *GSTM1* null genotype had shorter overall survival, whereas Kelsey *et al.* (20) reported increased survival for patients with *GSTM1* null genotype. We were unable to examine the relation of *GST* genotype and survival because we do not have information on long-term survival for women in our study.

Our results suggest that *GSTM1*, *GSTT1*, and *GSTP1* genotypes do not play a strong role in susceptibility to breast cancer, in agreement with most previous studies. However, inability to detect effects for *GSTs* could result from failure to include relevant environmental exposures or genes that interact with *GSTs.* The presence of positive associations for *GSTs* in women with a family history suggests that unknown genetic or environmental exposures may modify the effects of *GST* genes, a hypothesis that could be investigated further in family-based association studies. Unmeasured genetic or environmental factors that interact with *GSTs* could also contribute to differences in results across epidemiological studies. A potential role for *GST* genotypes in breast cancer prognosis and response to treatment, as well as the possibility that *GSTM1* status might modify age at onset for breast cancer, also merit further investigation.

Acknowledgments

We gratefully acknowledge the contributions of the nurse interviewers for the Carolina Breast Cancer Study: Martha Beach, Carolyn Dunmore, Dianne Mattingly, Theresa Nalevaiko, Patricia Plummer, Georgette Regan, and Cheryl Robinson. We also acknowledge the invaluable assistance of Susan Jackson and Patricia Moorman as Project Managers of the CBCS and Daynise Skeen for processing of blood samples. We thank three anonymous reviewers for helpful comments on the manuscript.

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Cancer Epidemiol Biomarkers Prev 2000;9:567-573.