Genetic Polymorphisms in the Catechol Estrogen Metabolism Pathway and Breast Cancer Risk

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Abstract

Background: This study investigated whether single nucleotide polymorphisms (SNP) in genes within the catechol estrogen metabolism pathway altered the risk of breast cancer alone or in combination, as well as whether menopausal hormone therapy modified the effect of these SNPs on breast cancer risk.
Methods: In a population-based case-control study of breast cancer, 891 cases and 878 controls were genotyped for six functional SNPs in the COMT, CYP1B1, GSTM1, GSTP1, and GSTT1 genes.
Results: Women homozygous with the T allele in CYP1B1*2 (Ser[119]; rs1056827) were at 1.69 (95% confidence interval, 1.17-2.46) times the risk of women homozygous with the G allele; women homozygous with the G allele in GSTP1 (Val[105]; rs1695) were at 0.73 (95% confidence interval, 0.54-0.99) times the risk of breast cancer compared with women homozygous with the A allele. No other SNPs tested were associated with breast cancer to any appreciable degree. Potential gene-gene and gene-hormone therapy interactions were investigated.
Conclusion: With the exception of GSTP1 and possibly CYP1B1*2, our findings do not provide support for the role of genetic variation in the catechol estrogen metabolism pathway and breast cancer risk in postmenopausal women. (Cancer Epidemiol Biomarkers Prev 2009;18(5):1461–7)

Introduction

A vast accumulation of data has shown that estrogen-related exposures play a role in breast cancer etiology (1, 2). Conceivably, this could be due in part to the mutagenic effects of the intermediate metabolites of estrogen within the catechol estrogen (CE) pathway. There is evidence that genotoxicity may operate through the formation of reactive estrogen metabolites, namely CE semiquinones and quinones that damage DNA via the formation of superoxide radicals and depurinating DNA adducts, although it is unclear what proportion of estradiol is converted into CEs (3-6).

It is plausible that the genotoxic potential of estrogen varies across individuals and may be influenced by genetic variation within the CE metabolism pathway. Specifically, CYP1B1 plays a role in the conversion of 17β-estradiol (E_2) into 4-OHE_2 CE (3, 5, 7-9). Several single nucleotide polymorphisms (SNP) within CYP1B1 have been shown to have functional effects on the catalytic properties of the CYP1B1 enzyme (9, 10), and variant alleles of CYP1B1*2 (Ala[119]Ser) and CYP1B1*3 (Leu[432]Val) have been observed to be associated with

breast cancer risk in some (11, 12), but not all, previous studies (13-15). Catechol-O-methyltransferase (COMT) is involved in methylating (and thereby inactivating) CEs (5, 16). The COMT Met[158] allele has been hypothesized to produce an enzyme with reduced functionality, although previous epidemiologic studies have not shown an association with breast cancer (9, 14). If CEs are inactivated, they can be easily oxidized into CE semiquinones and then into CE quinones (5). Members of the glutathione S-transferase (GST) family are thought to play a role in the conjugation of CE quinones, with GSTP1 being the predominant GST enzyme found in the breast (16, 17). The GSTP1 Val[105] allele has been shown to confer different catalytic enzyme activity (16). However, the epidemiologic findings regarding breast cancer risk have been mixed (14, 18-21). Null mutations have been characterized for both GSTM1 and GSTT1. For GSTM1, one pooled analysis reported a marginally significant, modestly increased risk of breast cancer associated with the null variant (14), although no increased risk was observed in a meta-analysis (22). For the GSTT1 null variant, no pooled analyses have observed an association with breast cancer risk (14).

The amount of CE accumulating in the breast tissue from daily exposure to estrogen supplied by estrogen-progestin therapy (EPT) or estrogen-alone therapy (ET) may conceivably vary among postmenopausal women and may be affected by the variation in genes within the CE metabolism pathway. Several studies have investigated interactions between genes in this pathway, as well as gene-EPT interactions, but no specific gene-gene combination has been observed to be associated with breast cancer in more than one study (19-21, 23-25). We investigated whether variation within five genes in

the CE metabolism pathway was associated with an increased risk of breast cancer and whether EPT and/or ET modified the effect of genotype on breast cancer risk.

Materials and Methods

Study Design and Data Collection. The study participants were recruited for a population-based case-control study, the Puget Sound Area Breast Cancer Evaluation (PACE) study of invasive breast cancer among postmenopausal women of 65 to 79 y of age in western Washington State. The methods have been described in detail previously (26) and thus are summarized only briefly here. Eligible case participants were (*a*) women 65 to 79 y of age when diagnosed with primary, invasive breast cancer between April 1, 1997, and May 31, 1999; (*b*) residents of the three county (King, Pierce, and Snohomish counties) Seattle-Tacoma metropolitan area at diagnosis; (*c*) women with no previous history of *in situ* or invasive breast cancer; and (*d*) women with the ability to communicate in English. All cases were ascertained through the Cancer Surveillance System, the population-based tumor registry serving the Seattle-Puget Sound region of western Washington State and a participant in the Surveillance, Epidemiology, and End Results Program of the National Cancer Institute. The PACE study interviewed 975 cases (80.6%) of those identified through the Cancer Surveillance System.

Eligible control participants for the PACE Study were identified through the Center for Medicare and Medicaid Services' list of Social Security recipients from the general population of the same three county Metropolitan Seattle area from which the cases were drawn. The Center for Medicare and Medicaid Services Social Security list includes all individuals 65 y and older residing in the United States who are eligible for Medicare benefits, including women whose income precludes eligibility to receive funds and women who elect not to receive cash benefits. The controls for this study were randomly selected from the Center for Medicare and Medicaid Services rolls and were frequency-matched to the expected distribution of cases by 5-y age group. Of the 1,365 controls selected, 1,007 women (73.8%) were interviewed.

Data collection for cases and controls was done in an identical manner. For each participant, upon the attainment of informed consent, a structured, in-person interview was conducted by a trained study interviewer on established and suspected breast cancer risk factors, including demographic characteristics, reproductive history, menstrual history, hormonal contraception history, medical history, certain medications, weight and height history, lifestyle factors, family history of cancer, and hormonal therapy use. The questions on hormonal therapy were extensive and solicited information on lifetime use, including drug name, start and stop dates of use, separate and combined uses of estrogen and progestin, strength, and monthly pattern of pill use. All interview questions were limited to events occurring before each participant's diagnosis date (reference date for controls). Within the 5-y age groups on which controls were frequency-matched to cases, reference dates for controls were assigned in a distribution matching the cases' expected diagnosis dates.

Blood samples were provided by 891 (91.4%) of the interviewed cases and 878 (87.1%) of the interviewed controls. This study was approved by the institutional review board of the Fred Hutchinson Cancer Research Center.

Laboratory Methods. DNA was extracted from buffy coats using manual phenol chloroform method. Genotyping was done at the Functional Genomics Laboratory of the Center for Ecogenetics and Environmental Health at the University of Washington.

The 5'-nuclease TaqMan Detection System–based assays were developed to discriminate the following alleles: *CYP1B1*2* (Ala[119]Ser), *CYP1B1*3* (Leu[432]Val), *COMT* Val[158]Met, and *GSTP1* Ile[105]Val (Supplementary Table S1A). Primers and dual-labeled allele-specific probes were designed through Assays-by-DesignSM Service-SNP Genotyping by Applied Biosystems, Inc. Each TaqMan minor groove binder probe consisted of an oligonucleotide labeled both with a particular 5' reporter dye and a 3' nonfluorescent quencher. Amplification was done by initial denaturation at 95°C for 10 min, followed by 40 cycles of amplification at 92°C for 15 s and annealing at 60°C for 1 min. End-point analysis was done on ABI 7900 Sequence Detection System (Applied Biosystems, Inc.) to determine the genotypes.

The presence or absence of the GST mu (*GSTM1*) and theta (*GSPT1*) genes was determined using a multiplex PCR (27). Briefly, two sets of primers were used to amplify a 215-bp segment of the *GSTM1* gene and a 480-bp segment of the *GSTT1* gene. Primers were also included to amplify a 268-bp segment of the *β-globin* gene, which was used as a positive control for the PCR. The products were separated by electrophoresis with ethidium bromide–stained 2.5% agarose gel (ISC BioExpress) and genotyped by visual inspection using UV illumination.

Positive controls consisting of DNA aliquots representing wild-type/wild-type, wild-type/mutant, mutant/mutant genotypes (characterized by DNA-sequencing), and a negative control (no DNA) were included in each assay. In addition, randomly selected samples (10%) were identified for replicate testing and were integrated randomly into the genotyping plates. Laboratory staff members were blinded to case-control status and to replicate status.

Among the 10% of the samples for which genotypes were replicated for quality control assessment, the level of agreement between the samples was 100% for the *COMT* Val[158]Met alleles, 100% for *CYP1B1*2* Ala[119]Ser, 94.7% for *CYP1B1*3* Leu[432]Val, 93.2% for *GSTM1*0*, 96.2% for *GSTP1* Ile[105]Val, and 96.4% for *GSTT1*0*. When discordance occurred, the genotype was coded as a missing variable. Additionally, the amount of missing data (including those removed for quality control purposes) for each genotype was 0.0% (*COMT*), 2.0% (*CYP1B1*2*), 0.6% (*CYP1B1*3*), 0.5% (*GSTM1*), 0.2% (*GSTP1*), and 0.2% (*GSTT1*).

Statistical Methods. The EPT variable was categorized as never use, short-term use (<60 mo of use), and long-term use (=60 mo of use). The ET variable was similarly categorized as never use, short-term use, and long-term use. In addition, analysis examining ET use excluded any women who had ever used EPT due to the association observed between EPT use and breast cancer risk in this data set.

Concordance between replicate samples included for quality control purposes was determined for each SNP. Testing for deviation from Hardy-Weinberg equilibrium was done within population controls using a χ^2 goodness of fit test. Mantel-Haenszel χ^2 test was used for tests of associations in bivariate analyses.

Odds ratios (OR) and the corresponding 95% confidence intervals (95% CI) were calculated using unconditional logistic regression. ORs were adjusted for age and year of diagnosis because these were frequency-matched variables. The Wald's test statistic was used to test trends in models containing variables with multiple levels. We also used the Wald's test statistic to determine if the trend in ORs associated with various genotypes differed by hormone therapy use.

In the multigene analysis, we considered only SNPs statistically significantly associated with breast cancer in the single gene analyses. Similarly, only when a single-gene or multigene model was statistically significantly associated with breast cancer risk did we investigate the potential for a gene-hormone therapy interaction. The exception to this was the investigation of interactions that have been previously reported in the literature.

Interaction terms were investigated using the likelihood ratio test. We estimated CYP1B1 haplotype frequencies and ORs and 95% CIs associated with breast cancer. Haplotype estimation was done using Phase v.2.1. The CYP1B1 haplotype with the highest frequency (G at A119S and G at L432V) served as the reference category.

We accounted for multiple testing in our analyses by using the false-positive reporting probability (FPRP) and preset the FPRP-level criterion at 20% (based on the number of studies previously investigating these genes and our adequate sample size). We evaluated the FPRP using prior probabilities ranging from 0.1 to 0.01 (28).

Results

Demographic and hormone-related characteristics of breast cancer cases and controls are presented in Table 1. Similar to findings previously reported in the overall PACE study (26), cases had a higher body mass index and were more likely to be long-term users of EPT than controls.

In the single-gene analyses, women homozygous for the CYP1B1*2 Ser allele (T/T) had a 1.69-fold increased risk of breast cancer (95% CI, 1.17-2.46) and the heterozygotes (G/T) were at no increased risk (OR, 0.99; 95% CI, 0.81-1.20) compared with homozygous wild-types (Table 2). Women homozygous for the GSTP1 Val allele (G/G) had a 0.73-fold increased risk of breast cancer (95% CI, 0.54-0.99) and the heterozygotes (A/G) had no altered risk (OR, 1.04; 95% CI, 0.85-1.27) compared with the homozygous wild-type. The COMT Met[158] allele (rs4680), CYP1B1 Val[432] allele (rs1056836), GSTM1 null allele, and GSTT1 null allele were not observed to be associated with risk of breast cancer.

We investigated the possibility of reporting a false-positive association between breast cancer and the SNPs in our study by calculating a FPRP for prior probabilities ranging from 0.1, 0.05, to 0.01. We observed FPRPs equal to 0.11, 0.21, and 0.58, at these prior probability levels, respectively, for CYP1B1*2, and FPRPs equal to 0.06,

0.11, and 0.39, respectively, for GSTP1, indicating that the FPRP was within the 20% criterion set a priori for both of these SNPs at a prior probability of 0.1 but not at 0.01.

There was no indication that the associations seen for the homozygous variant allele of CYP1B1*2 differed according to the use of EPT or ET (Table 3). For GSTP1, the reduced risk of breast cancer among women homozygous for the Val allele was limited to never users of ET (P for interaction = 0.004). Although we had data on duration and recency of use, our sample size was too limited to investigate gene-disease associations stratified by EPT duration and recency.

The two genes found to have associations with breast cancer, CYP1B1*2 and GSTP1, were assessed for a possible gene-gene interaction. However, we observed no evidence of this (P = 0.36; Table 4). We also observed

Table 1. Characteristics of breast cancer cases and controls

Characteristic	Case n (%)*,†	Control n (%)*,†
	$n = 891$	$n = 878$
Age at reference date (y)		
65-69	278 (31.2)	278 (31.7)
70-74	613 (68.8)	600 (68.3)
Age at menopause (y) ‡		
<39	74 (9.6)	98 (11.2)
40-44	115 (14.9)	130 (14.8)
45-49	233 (30.1)	229 (26.1)
50-54	257 (33.2)	239 (27.2)
≥55	95 (12.3)	103 (11.7)
Missing data	117	79
Age at first full-term pregnancy (y)		
<30	720 (81.1)	726 (82.8)
≥30	168 (18.9)	151 (17.2)
Missing data	3	1
Cause of menopause		
Natural menopause	508 (57.9)	519 (59.9)
Induced menopause	122 (13.9)	124 (14.3)
Simple hysterectomy	207 (23.6)	198 (22.8)
Other	41 (4.7)	26 (3.0)
Missing data	13	11
BMI quartiles		
16.00-22.96	169 (19.5)	211 (24.8)
22.97-26.01	221 (25.6)	213 (25.1)
26.02-30.11	244 (28.2)	211 (24.8)
30.12-48.70	231 (26.7)	214 (25.2)
Missing data	26	29
Mean BMI	27.5 (5.7)	26.9 (5.4)
EPT		
Never use	648 (73.0)	697 (79.8)
<60 mo of use	80 (9.0)	80 (9.2)
≥60 mo of use	160 (18.0)	96 (11.0)
Missing data	3	5
ET		
Never use	407 (46.1)	412 (47.2)
<60 mo of use	157 (17.8)	176 (20.2)
≥60 mo of use	319 (36.1)	284 (32.6)
Missing data	8	6
Race		
Caucasian	854 (95.9)	814 (92.7)
African American	11 (1.2)	27 (3.1)
Asian	17 (1.9)	21 (2.4)
Other/unknown	9 (1.0)	16 (1.8)

Abbreviation: BMI, body mass index.
* With the exception of mean body mass index: mean (SD).
† Some percentages do not sum to 100% due to rounding.
‡ Using data imputed from hormone therapy use and bilateral oophorectomy status.

Table 2. The risk of breast cancer associated with genetic variation as investigated in single-gene models

Gene	Genotype	Case *n* (%)	Control *n* (%)	OR (95% CI)
COMT	G/G (Val/Val)	224 (25.1)	211 (24.0)	1.0 (reference)
	G/A (Val/Met)	427 (48.0)	431 (49.1)	0.94 (0.74-1.18)
	A/A (Met/Met)	240 (26.9)	236 (26.9)	0.97 (0.75-1.26)
CYP1B1*2	G/G (Ala/Ala)	452 (51.5)	454 (53.0)	1.0 (reference)
	G/T (Ala/Ser)	341 (38.8)	353 (41.2)	0.99 (0.81-1.20)
	T/T (Ser/Ser)	84 (9.6)	50 (5.8)	1.69 (1.17-2.46)
CYP1B1*3	C/C (Leu/Leu)	289 (32.6)	271 (31.0)	1.0 (reference)
	C/G (Leu/Val)	409 (46.2)	427 (48.9)	0.90 (0.73-1.11)
	G/G (Val/Val)	188 (21.2)	176 (20.1)	1.00 (0.77-1.30)
GSTP1	A/A (Ile/Ile)	382 (42.9)	366 (41.8)	1.0 (reference)
	A/G (Ile/Val)	417 (46.8)	390 (44.6)	1.04 (0.85-1.27)
	G/G (Val/Val)	92 (10.3)	119 (13.6)	0.73 (0.54-0.99)
GSTM1	Present	421 (47.4)	415 (47.4)	1.0 (reference)
	Null	467 (52.5)	460 (52.6)	1.00 (0.83-1.21)
GSTT1	Present	744 (83.5)	738 (84.2)	1.0 (reference)
	Null	147 (16.5)	139 (15.8)	1.04 (0.81-1.34)

NOTE: OR adjusted for age and year of diagnosis (reference date for controls).

no elevation in risk associated with the CYP1B1*2-CYP1B1*3 haplotypes (compared with GG: OR, 1.0; 95% CI, 0.8-1.1, for GC; OR, 1.0; 95% CI, 0.8-1.3, for TG; OR, 1.1; 95% CI, 0.9-1.3, for TC; data not shown).

Additionally, we investigated possible interactions between genes previously reported in the literature (19-21, 23-25) but did not detect any among the following combined gene variables: GSTT1-GSTM1, COMT-GSTP1, COMT-GSTT1, COMT-GSTM1, COMT-GSTM1-GSTT1, and GSTP1-GSTM1-GSTT1 (Supplementary Table S1).

We also investigated a possible EPT interaction with combined genes as reported by Mitrunen et al. and did not observe evidence that the risk of breast cancer associated with combined genes varied according to EPT use within the COMT-GSTP1 combined variable (P = 0.19; Supplementary Table S2). With the COMT-GSTT1 and COMT-GSTM1 combined variables, although we observed a greater risk in short-term users of EPT than was predicted by their separate associations (P = 0.004 and 0.001, respectively), the pattern of risk when stratified by EPT was not what we would have expected *a priori* (comparing 2 with 0 high-risk genotypes in COMT-GSTT1: OR, 0.8; 95% CI, 0.5-1.4, for never users; OR, 24.4; 95% CI, 3.0-200.2, for EPT use <30 months; OR, 0.8; 95% CI, 0.3-2.4, for EPT use of 30+ months; in COMT-GSTM1: OR, 0.7; 95% CI, 0.4-1.1, for never users; OR, 8.5; 95% CI, 1.4-52.4, for EPT use <30 months; and OR, 1.3; 95% CI, 0.7-2.3, for EPT use of 30+ months), admittedly with a constrained sample size for this analysis.

Our results were unchanged when limited to White women for all of the analyses presented above.

Discussion

Our study observed that variants in the CYP1B1 and GSTP1 genes were associated with breast cancer risk. In interpreting these results, we must first consider the potential limitations of our study. Laboratory errors resulting in nondifferential misclassification of genotypes could bias ORs toward the null. However, quality control analyses indicated high correlations between replicate samples. Also, allele distributions were consistent with Hardy-Weinberg equilibrium and there was a minimal amount of missing genotype data.

Another potential limitation was the omission of a portion of the interviewed cases (8.6%) and controls (12.9%) who were otherwise eligible for the parent study but did not donate a blood specimen. Comparison of the women who donated blood with all participants interviewed is reassuring in that there were no discernible differences in risk-factor distributions and risk estimates, nor did the cases who donated blood vary from the entire case series in terms of stage and other disease features. Another possible source of selection bias, however, could arise from the 19.4% of cases and 26.2% of controls who were identified by the Cancer Surveillance System but did not participate in the parent study. For analyses that consider interview data, the ability of women to recall past exposures is a potential concern. Specific tools designed to assist recall were used in this study, including a lifetime calendar and colored photographs of medications. As a check on the quality of recall in PACE participants, we previously compared self-reported data on several categories of medications with pharmacy records and found overall agreement to be quite good (29).

A large number of individual studies have reported on associations between genes in this pathway and breast cancer risk (11-13, 15, 18, 20-25, 30-36). However, no genome-wide association studies have reported findings for regions encompassing these genes (37, 38). In addition to several large individual studies, a meta-analysis of CYP1B1*2 found no association between breast cancer risk and the Ala/Ser genotype [OR, 1.1 (95% CI, 0.9-1.2)], or the Ser/Ser genotype (OR, 1.0; 95% CI, 0.8-1.2; refs. 9, 11, 13, 15, 31, 33). Findings from haplotype analyses have not been any more compelling. Among three large studies investigating the risk of breast cancer associated with haplotypes in the CYP1B1 gene, one observed a 1.5-fold increased risk comparing women homozygous for G,T,C at positions 48, 119, and 432, respectively, with those with the most common haplotype (95% CI, 1.0-2.1; P = 0.03). The two other studies reported no alteration in breast cancer risk in any CYP1B1 haplotype (15, 31, 33). The generally negative findings from previous studies argue that the 1.7-fold increased risk of breast cancer that we observed among women with the CYP1B1*2 Ser allele should be interpreted with caution.

Table 3. The risk of breast cancer associated with CYP1B1*2 and GSTP1 Ile[105]Val stratified by EPT and ET use

Genotype	Case*	Control*	OR[†] (95% CI)	P[‡]
CYP1B1				
Never EPT users				
G/G	317 (49.5)	346 (51.0)	1.0 (reference)	
G/T	259 (40.5)	291 (42.9)	0.98 (0.78-1.23)	
T/T	64 (6.9)	42 (6.2)	1.63 (1.07-2.48)	0.14
Ever EPT users				
G/G	133 (56.8)	105 (60.7)	1.0 (reference)	
G/T	81 (34.6)	60 (34.7)	1.07 (0.70-1.62)	
T/T	20 (8.6)	8 (4.6)	1.90 (0.80-4.51)	0.001
			$P_{int} = 0.96$	
Never ET users				
G/G	134 (49.3)	155 (50.2)	1.0 (reference)	
G/T	113 (41.5)	137 (44.3)	0.98 (0.70-1.38)	
T/T	25 (9.2)	17 (5.5)	1.69 (0.87-3.27)	0.91
Ever ET users[§]				
G/G	179 (49.3)	191 (51.6)	1.0 (reference)	
G/T	145 (39.9)	154 (41.6)	1.01 (0.74-1.38)	
T/T	39 (10.7)	25 (6.8)	1.57 (0.91-2.72)	0.23
			$P_{int} = 0.64$	
Missing	14 (1.6)	21 (2.4)		
GSTP1				
Never EPT users				
A/A	284 (43.8)	284 (40.8)	1.0 (reference)	
A/G	293 (45.2)	315 (45.2)	0.94 (0.75-1.18)	
G/G	71 (11.0)	95 (13.6)	0.73 (0.51-1.03)	0.11
Ever EPT users				
A/A	97 (40.4)	80 (45.4)	1.0 (reference)	
A/G	122 (50.8)	72 (40.9)	1.40 (0.92-2.11)	
G/G	21 (8.8)	24 (13.6)	0.73 (0.38-1.41)	0.97
			$P_{int} = 0.41$	
Never ET users				
A/A	125 (45.3)	103 (32.8)	1.0 (reference)	
A/G	125 (45.3)	166 (52.9)	0.63 (0.44-0.90)	
G/G	26 (9.4)	45 (14.3)	0.45 (0.26-0.78)	0.001
Ever ET users[§]				
A/A	55 (59.8)	37 (40.2)	1.0 (reference)	
A/G	77 (75.5)	25 (24.5)	1.30 (0.96-1.78)	
G/G	12 (46.2)	14 (53.8)	0.97 (0.61-1.54)	0.53
			$P_{int} = 0.004$	
Missing	0 (0.0)	3 (0.3)		

* Some percentages do not sum to 100% due to rounding.
† Adjusted for age and year of diagnosis (reference date for controls).
‡ P value for test of trend.
§ Limited to exclusive users of ET.

For *GSTP1*, a pooled analysis of 301 cases and 397 controls reported that the G/G genotype (Val homozygotes) was associated with an increased risk of breast cancer (OR, 1.86; 95% CI, 1.05-3.3; ref. 14), but two larger studies have observed either a decreased risk (OR, 0.6; 95% CI, 0.3-1.0) or no association among the Val homozygotes (OR, 0.9; 95% CI, 0.5-1.4; refs. 19, 20). However, among postmenopausal women, these studies have observed borderline decreased risks associated with Val homozygotes compared with wild-type homozygotes (OR, 0.5; 95% CI, 0.2-1.1 and OR, 0.7; 95% CI, 0.4-1.1; refs. 19, 20). In the two studies that are stratified by use of hormone therapy, one reported a greater reduction in breast cancer risk observed among ever users of ET (OR, 0.2; 95% CI, 0.04-0.8) than never users (OR, 0.6; 95% CI, 0.2-2.0; ref. 20). However, another study observed no differential association with Val homozygosity according to use of EPT (OR, 0.8; 95% CI, 0.5-1.2, for never users; OR, 0.8; 95% CI, 0.4-1.5, for ever users; ref. 20).

Our study, the largest-to-date study examining the association between *GSTP1* and breast cancer risk, observed women with the *GSTP1* Val/Val genotype generally to be at a reduced risk of breast cancer, and is in broad agreement with the findings of other epidemiologic studies reported for postmenopausal women. We evaluated the probability that this finding represented a false-positive association and calculated the likelihood of that to be 11% at a prior probability of 0.05 (at lower prior probabilities, the false-reporting probability is higher). Thus, if the prior probability of an association between the *GSTP1* Val/Val allele is at least 5% (given the *a priori* biological rationale for investigating SNPs within this gene, it is reasonable to assume the prior probability is 5% or greater), the probability of this finding being a false-positive association is relatively small.

Additionally, although it is plausible that the reduction in breast cancer risk associated with the *GSTP1* Val/Val genotype is most pronounced in never users of ET, these findings would need to be replicated before any inferences can be made. Adding complexity to the issue are the conflicting findings demonstrating varying levels of catalytic activity between the variant protein and the wild-type protein. Some showed a higher V_{max} for the Val[105] isoform, whereas others showed a lower catalytic efficiency for the Val[105] isoform compared with wild-type (39-42).

Our null findings with *COMT*, *GSTM1*, and *GSTT1* and breast cancer are in broad agreement with previous reports from pooled and meta-analyses (9, 14, 22). Also, if account is taken of the effect modification by age in the HuGE review of *CYP1B1*3* (for Val/Leu compared with Leu/Leu genotypes in Caucasians women >60 years of age: OR, 1.1; 95% CI, 0.8-1.4; 51-54 years of age: OR, 2.5; 95% CI, 1.1-5.7; 55-59 years of age: OR, 1.9; 95% CI, 1.1-3.6; ref. 43), then our findings in this study of women ages 65 years and older are consistent with previous works as well.

Previous studies have also investigated possible gene-gene combinations within this set of genes as there is strong biological rationale for considering the joint effects of genes within this shared pathway, with several studies (18, 20, 23-25, 44) but not all (21), reporting associations between high-risk genotypes from combined genes in this pathway and breast cancer risk. However, with the exception of two studies reporting marginally significant

Table 4. The risk of breast cancer associated with genetic variation modeled as CYP1B1*2-GSTP1 gene-gene interactions

Case n (%)*	Control n (%)*	No. high-risk genotypes[†]		OR[‡] (95% CI)
		GSTP1	CYP1B1*2	
79 (9.0)	108 (12.6)	0	0	1.0 (reference)
10 (1.1)	8 (0.9)	0	1	1.81 (0.64-5.07)
714 (81.4)	696 (81.5)	1	0	1.53 (0.99-2.35)
74 (8.4)	42 (4.9)	1	1	2.78 (1.46-5.32)
				$P_{int} = 0.36$

* Some percentages do not sum to 100% due to rounding.
† High-risk genotypes as determined in the single-gene analyses: A/A and A/G for *GSTP1* and T/T for *CYP1B1*.
‡ Adjusted for age and year of diagnosis (reference date for controls).

increased risks of breast cancer associated with combined *GSTP1*, *GSTM1*, and *GSTT1*, no specific gene-gene combination has been observed in more than one study (18, 20, 23-25, 44). We investigated but did not detect any gene-gene interactions within the CE pathway.

A previous study reported on a potential interaction between combined gene variables and EPT in breast cancer (24). Specifically, among EPT users of >30 months, Mitrunen et al. observed the risk of breast cancer to be heightened in relation to *COMT-GSTM1* (OR, 9.1; 95% CI, 1.8-45.0), *COMT-GSTT1* (OR, 8.4; 95% CI, 1.4-49.0), and *COMT-GSTP1* (OR, 7.0; 95% CI, 1.2-40.6) comparing 2 with 0 high-risk genotypes. An increased risk was not similarly observed among EPT users of <30 months (*COMT-GSTM1*: OR, 0.7; 95% CI, 0.2-3.5, comparing 2 with 0 high-risk genotypes; *COMT-GSTT1*: OR, 1.7; 95% CI, 0.3-10.5; and *COMT-GSTP1*: OR, 2.3; 95% CI, 0.4-14.1). Although we observed a greater risk associated with two high-risk genotypes for short-term users of EPT for both the *COMT-GSTT1* combined variable and the *COMT-GSTM1* combined variable, these patterns of risk were not what we had hypothesized *a priori* (nor were the patterns in agreement with those reported by Mitrunen et al.), and thus, we cannot draw any conclusions based on these findings.

Our study observed a 27% reduction in breast cancer risk associated with the *GSTP1* Val/Val genotype. However, we are the first study to report a statistically significant decreased risk among postmenopausal women; clearly, replication of our results is needed before any firm conclusion can be drawn. Based on this study and the existing epidemiologic literature, there is little evidence that any SNPs in the CE metabolism pathway, with the exception of *GSTP1*, have a main effect on breast cancer risk and there is little evidence supporting interactions between any of these genes and hormone therapy use.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

References

1. Feigelson HS. Breast cancer: epidemiology and molecular endocrinology. In: Henderson BE, Ponder B, Ross RK. Hormones, genes, and cancer. New York: Oxford University Press; 2003. p. 120 – 38.
2. Thomas HV, Reeves GK, Key TJ. Endogenous estrogen and postmenopausal breast cancer: a quantitative review. Cancer Causes Control 1997;8:922 – 8.
3. Cavalieri EL, Stack DE, Devanesan PD, et al. Molecular origin of cancer: catechol estrogen-3,4-quinones as endogenous tumor initiators. Proc Natl Acad Sci U S A 1997;94:10937 – 42.
4. Yager JD. Endogenous estrogens as carcinogens through metabolic activation. J Natl Cancer Inst Monogr 2000;27:67 – 73.
5. Cavalieri E, Frenkel K, Liehr JG, Rogan E, Roy D. Estrogens as endogenous genotoxic agents-DNA adducts and mutations. J Natl Cancer Inst Monogr 2000;75 – 93.
6. Yager JD, Liehr JG. Molecular mechanisms of estrogen carcinogenesis. Annu Rev Pharmacol Toxicol 1996;36:203 – 32.
7. Goth-Goldstein R, Erdmann CA, Russell M. Cytochrome P4501B1 expression in normal breast tissue. Polycyclic Aromatic Compounds 2003;23:259 – 275.
8. Murray GI, Melvin WT, Greenlee WF, Burke MD. Regulation, function, and tissue-specific expression of cytochrome P450 CYP1B1. Annu Rev Pharmacol Toxicol 2001;41:297 – 316.
9. Wen W, Cai Q, Shu XO, et al. Cytochrome P450 1B1 and catechol-*O*-methyltransferase genetic polymorphisms and breast cancer risk in Chinese women: results from the Shanghai breast cancer study and a meta-analysis. Cancer Epidemiol Biomarkers Prev 2005;14:329 – 35.
10. Mitrunen K, Hirvonen A. Molecular epidemiology of sporadic breast cancer. The role of polymorphic genes involved in oestrogen biosynthesis and metabolism. Mutat Res 2003;544:9 – 41.
11. Watanabe J, Shimada T, Gillam EM, et al. Association of CYP1B1 genetic polymorphism with incidence to breast and lung cancer. Pharmacogenetics 2000;10:25 – 33.
12. Zheng W, Xie DW, Jin F, et al. Genetic polymorphism of cytochrome P450-1B1 and risk of breast cancer. Cancer Epidemiol Biomarkers Prev 2000;9:147 – 50.
13. Rylander-Rudqvist T, Wedren S, Granath F, et al. Cytochrome P450 1B1 gene polymorphisms and postmenopausal breast cancer risk. Carcinogenesis 2003;24:1533 – 9.
14. de Jong MM, Nolte IM, te Meerman GJ, et al. Genes other than BRCA1 and BRCA2 involved in breast cancer susceptibility. J Med Genet 2002;39:225 – 42.
15. Dunning AM, Dowsett M, Healey CS, et al. Polymorphisms associated with circulating sex hormone levels in postmenopausal women. J Natl Cancer Inst 2004;96:936 – 45.
16. Raftogianis R, Creveling C, Weinshilboum R, Weisz J. Estrogen metabolism by conjugation. J Natl Cancer Inst Monogr 2000;27:113 – 24.
17. Kelley MK, Engqvist-Goldstein A, Montali JA, Wheatley JB, Schmidt DE, Kauvar LM. Variability of glutathione S-transferase isoenzyme patterns in matched normal and cancer human breast tissue. Biochem J 1994;304:843 – 8.
18. Helzlsouer KJ, Selmin O, Huang HY, et al. Association between glutathione *S*-transferase M1, P1, and T1 genetic polymorphisms and development of breast cancer. J Natl Cancer Inst 1998;90:512 – 8.
19. Millikan R, Pittman G, Tse CK, Savitz DA, Newman B, Bell D. Glutathione *S*-transferases M1, T1, and P1 and breast cancer. Cancer Epidemiol Biomarkers Prev 2000;9:567 – 73.
20. Mitrunen K, Jourenkova N, Kataja V, et al. Glutathione *S*-transferase M1, M3, P1, and T1 genetic polymorphisms and susceptibility to breast cancer. Cancer Epidemiol Biomarkers Prev 2001;10:229 – 36.
21. Curran JE, Weinstein SR, Griffiths LR. Polymorphisms of glutathione *S*-transferase genes (GSTM1, GSTP1 and GSTT1) and breast cancer susceptibility. Cancer Lett 2000;153:113 – 20.
22. Sull JW, Ohrr H, Kang DR, Nam CM. Glutathione *S*-transferase M1 status and breast cancer risk: a meta-analysis. Yonsei Med J 2004;45:683 – 9.
23. Park SK, Yim DS, Yoon KS, et al. Combined effect of GSTM1, GSTT1, and COMT genotypes in individual breast cancer risk. Breast Cancer Res Treat 2004;88:55 – 62.
24. Mitrunen K, Kataja V, Eskelinen M, et al. Combined COMT and GST genotypes and hormone replacement therapy associated breast cancer risk. Pharmacogenetics 2002;12:67 – 72.
25. Cheng TC, Chen ST, Huang CS, et al. Breast cancer risk associated with genotype polymorphism of the catechol estrogen-metabolizing genes: a multigenic study on cancer susceptibility. Int J Cancer 2005;113:345 – 53.
26. Li CI, Malone KE, Porter PL, et al. Relationship between long durations and different regimens of hormone therapy and risk of breast cancer. JAMA 2003;289:3254 – 63.
27. Sweeney C, Farrow DC, Schwartz SM, Eaton DL, Checkoway H, Vaughan TL. Glutathione *S*-transferase M1, T1, and P1 polymorphisms as risk factors for renal cell carcinoma: a case-control study. Cancer Epidemiol Biomarkers Prev 2000;9:449 – 54.
28. Wacholder S, Chanock S, Garcia-Closas M, El ghormli L, Rothman N. Assessing the probability that a positive report is false: an approach for molecular epidemiology studies. J Natl Cancer Inst 2004;96:434 – 42.
29. Boudreau DM, Daling JR, Malone KE, Gardner JS, Blough DK, Heckbert SR. A validation study of patient interview data and pharmacy records for antihypertensive, statin, and antidepressant medication use among older women. Am J Epidemiol 2004;159:308 – 17.
30. De Vivo I, Hankinson SE, Li L, Colditz GA, Hunter DJ. Association of CYP1B1 polymorphisms and breast cancer risk. Cancer Epidemiol Biomarkers Prev 2002;11:489 – 92.
31. Gaudet MM, Chanock S, Lissowska J, et al. Genetic variation of Cytochrome P450 1B1 (CYP1B1) and risk of breast cancer among Polish women. Pharmacogenet Genomics 2006;16:547 – 53.
32. Kocabas NA, Sardas S, Cholerton S, Daly AK, Karakaya AE. Cytochrome P450 CYP1B1 and catechol O-methyltransferase

(COMT) genetic polymorphisms and breast cancer susceptibility in a Turkish population. Arch Toxicol 2002;76:643–9.

33. Matyjasik J, Cybulski C, Masojc B, et al. CYP1B1 and predisposition to breast cancer in Poland. Breast Cancer Res Treat 2007;106:383–8.

34. Millikan RC, Pittman GS, Tse CK, et al. Catechol-*O*-methyltransfer-ase and breast cancer risk. Carcinogenesis 1998;19:1943–7.

35. Mitrunen K, Jourenkova N, Kataja V, et al. Polymorphic catechol-*O*-methyltransferase gene and breast cancer risk. Cancer Epidemiol Biomarkers Prev 2001;10:635–40.

36. Thompson PA, Shields PG, Freudenheim JL, et al. Genetic poly-morphisms in catechol-*O*-methyltransferase, menopausal status, and breast cancer risk. Cancer Res 1998;58:2107–10.

37. Hunter DJ, Kraft P, Jacobs KB, et al. A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. Nat Genet 2007;39:870–74.

38. Easton DF, Pooley KA, Dunning AM, et al. Genome-wide association study identifies novel breast cancer susceptibility loci. Nature 2007; 447:1087–93.

39. Ali-Osman F, Akande O, Antoun G, Mao JX, Buolamwini J. Molecular cloning, characterization, and expression in *Escherichia coli* of full-length cDNAs of three human glutathione *S*-transferase Pi gene variants. Evidence for differential catalytic activity of the encoded proteins. J Biol Chem 1997;272:10004–12.

40. Sundberg K, Johansson AS, Stenberg G, et al. Differences in the catalytic efficiencies of allelic variants of glutathione transferase P1-1 towards carcinogenic diol epoxides of polycyclic aromatic hydro-carbons. Carcinogenesis 1998;19:433–6.

41. Zimniak P, Nanduri B, Pikula S, et al. Naturally occurring human glutathione *S*-transferase GSTP1-1 isoforms with isoleucine and valine in position 104 differ in enzymic properties. Eur J Biochem 1994;224:893–9.

42. Eaton DL, Bammler TK. Concise review of the glutathione *S*-transferases and their significance to toxicology. Toxicol Sci 1999;49: 156–64.

43. Paracchini V, Raimondi S, Gram IT, et al. Meta- and Pooled Analyses of the Cytochrome P-450 1B1 Val432Leu Polymorphism and Breast Cancer: A HuGE-GSEC Review. Am J Epidemiol 2006;165:115–25.

44. Spurdle AB, Chang JH, Byrnes GB, et al. A systematic approach to analysing gene-gene interactions: polymorphisms at the microsomal epoxide hydrolase EPHX and glutathione *S*-transferase GSTM1, GSTT1, and GSTP1 loci and breast cancer risk. Cancer Epidemiol Biomarkers Prev 2007;16:769–74.

Genetic Polymorphisms in the Catechol Estrogen Metabolism Pathway and Breast Cancer Risk

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