Polymorphisms in Genes Involved in Sex Hormone Metabolism, Estrogen Plus Progestin Hormone Therapy Use, and Risk of Postmenopausal Breast Cancer

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Abstract

Hormone therapy, estrogen plus progestin (E+P) particularly, is associated with increased risk of breast cancer. Functionally relevant polymorphisms in genes involved in sex hormone metabolism may alter exposure to exogenous sex hormones and affect risk of postmenopausal breast cancer. We evaluated associations of common polymorphisms in genes involved in estrogen and/or progesterone metabolism, E+P use, and their interactions with breast cancer risk in a case-control study of postmenopausal women (324 cases; 651 controls) nested within the VITAL cohort. None of the polymorphisms studied was, by itself, statistically significantly associated with breast cancer risk. E+P use was significantly associated with increased breast cancer risk (≥10 years versus never; odds ratio, 1.9; 95% confidence interval, 1.3-2.8; P_{trend} = 0.0002). Statistically significant interactions between *CYP1A1* Ile^{462}Val ($P_{interaction}$ = 0.04), *CYP1A1 Msp*I ($P_{interaction}$ = 0.003), *CYP1B1* Val^{432}Leu ($P_{interaction}$ = 0.007), *CYP1B1* Asn^{453}Ser ($P_{interaction}$ = 0.04) and *PGR* Val^{660}Leu ($P_{interaction}$ = 0.01), and E+P use were observed. The increased risk of breast cancer associated with E+P use was greater among women with at least one rare allele of the *CYP1A1* Ile^{462}Val, *CYP1A1 Msp*I, *CYP1B1* Asn^{453}Ser, and *PGR* Val^{660}Leu polymorphisms than among women homozygous for the common allele of these polymorphisms. Risk of breast cancer increased little with increasing years of E+P use among women with at least one *CYP1B1* Val^{432} allele; a large increase in risk was seen among women homozygous for *CYP1B1* Leu^{432}. Our results support the hypothesis that specific polymorphisms in genes involved in sex hormone metabolism may modify the effect of E+P use on breast cancer risk. (Cancer Epidemiol Biomarkers Prev 2008;17(7):1751–9)

Introduction

Breast cancer is one of the most common malignancies among women in the Western world. It is well established that sex hormones play a critical role in the etiology of breast cancer. Levels of endogenous sex hormones are strongly associated with increased breast cancer risk in postmenopausal women (1), and known risk factors for breast cancer, such as age at menarche, parity, and age at menopause, can be viewed as markers of lifetime exposure to endogenous hormones (2). Epidemiologic studies have also provided strong evidence that postmenopausal hormone therapy use, particularly the use of estrogen plus progestin (E+P), is associated with increased breast cancer risk (3-6).

Functionally relevant polymorphisms in genes involved in the metabolism of sex hormones may well alter a woman's exposure to estrogens and progestogens, and, thus, affect the risk of developing breast cancer. Candidate genes include *CYP17* and *CYP19*, which are involved in estrogen synthesis; *SHBG*, which encodes a protein, sex hormone-binding globulin, that binds to and restricts the biological activity of estradiol; *CYP1A1* and *CYP1B1*, which encode phase I enzymes that catalyze the synthesis of catechol estrogens and the highly reactive estrogen quinones; *COMT, SULT1A1*, and *GSTP1*, which are involved in the inactivation of catechol estrogens and estrogen quinones; and the hormone receptor genes *ESR1* and *PGR*, which code for receptors that bind to and form complexes with estrogens and progestogens, respectively. Polymorphisms resulting in changes in protein functionality are present in all these genes, and statistically significant associations with circulating sex hormone levels have been reported for some genetic variants (7-9). Nonetheless, results from studies that evaluated associations between the genetic variants and breast cancer risk have been inconsistent (10, 11).

Failure to consider exposure to relevant environmental factors, e.g., postmenopausal hormone therapy, may

Received 2/20/08; revised 4/29/08; accepted 5/5/08.

Grant support: NIH grant RO1 CA74846, Oklahoma Center for the Advancement of Science and Technology AR05.1025, Presbyterian Health Foundation, Oklahoma Life Sciences Fund, and Swisher Family Trust.

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doi:10.1158/1055-9965.EPI-08-0168

explain the heterogeneity in results of genetic association studies. As eloquently shown by Low et al. (12), the effect on breast cancer risk of certain genetic variants may become detectable only in the presence of specific environmental factors. Information on the effect of interactions between polymorphisms in hormone metabolism genes and use of hormone therapy on risk of developing breast cancer is currently limited. Further insight into this is important, as it may eventually result in the ability to identify postmenopausal women who are particularly susceptible to breast cancer if exposed to exogenous hormones. We conducted a nested case-control study to evaluate whether the effect of postmenopausal hormone therapy use on breast cancer risk was modified by common polymorphisms in genes involved in estrogen and/or progesterone metabolism. The following single-nucleotide polymorphisms (SNP) were investigated: *COMT* Val$^{108/158}$Met, *CYP1A1* Ile^{462}Val, *CYP1A1 Msp*I, *CYP17A1* 5′ untranslated region (5′UTR), *CYP19A1* Arg^{264}Cys, *CYP19A1* 3′UTR, *CYP1B1* Arg^{48}Gly, *CYP1B1* Val^{432}Leu, *CYP1B1* Asn^{453}Ser, *ESR1* Ser^{10}Ser, *GSTP1* Ile^{105}Val, *PGR* +331G/A, *PGR* Val^{660}Leu, *SHBG* 5′UTR, *SHBG* Asp^{356}Asn, and *SULT1A1* Arg^{213}His.

Materials and Methods

Study Population. This study is a nested case-control study within the VITamins And Lifestyle (VITAL) cohort. Details of the VITAL study design and methods have been described elsewhere (13). Briefly, men and women were eligible to join the VITAL cohort if they were between 50 and 76 years old and living in the Seattle-Puget Sound area of Washington State. Recruitment was conducted by mail from October 2000 to December 2002, using names purchased from a commercial mailing list.

Among eligible women, the response proportion was 25.6% with a total of 40,339 women included in the cohort. For this nested case-control study, cases were postmenopausal women who had been diagnosed with a first primary breast cancer (invasive or *in situ*) in the period from baseline to December 31, 2003, and had provided a buccal cell sample. By linkage to the western Washington Surveillance, Epidemiology, and End Results (SEER) cancer registry, we identified 514 women in the VITAL cohort who had been diagnosed with breast cancer in the period from baseline to December 31, 2003.

Of these women, 127 were excluded because they did not provide a buccal cell sample; 48 were excluded because they had reported a prior breast cancer on their baseline questionnaire; 4 were excluded because of rare histologies (i.e., sarcoma, phyllodes, or lymphoma); and 1 was excluded because the breast cancer risk factor page of the baseline questionnaire was not completed, leaving 334 cases. There were 36,096 women in the VITAL cohort who had not been diagnosed with any type of cancer since baseline and had never been diagnosed with breast cancer.

Of these possible controls, 11,798 were excluded because they did not provide a buccal cell sample, and 185 were excluded because the breast cancer risk factor page of the baseline questionnaire was not completed. Two controls for each case were randomly selected from the remaining 24,113 possible controls by frequency matching on age at baseline (in 5-year intervals) and race resulting in 668 controls. We also ensured that the follow-up times of the controls (time from baseline to death, a move out of area, or December 31, 2003) were greater than or equal to the follow-up times of the cases (time from baseline to breast cancer diagnosis). For the current analyses, we subsequently excluded all women who were not postmenopausal, leaving a final total of 324 cases and 651 controls. The study protocol was reviewed and approved by the Fred Hutchinson Cancer Research Center Institutional Review Board.

Data Collection. Information on medical history, reproductive history, lifestyle factors, diet, personal characteristics, and family history of breast cancer was collected at baseline using a 24-page self-administered, sex-specific questionnaire. Body mass index was computed as weight (in kg) divided by height squared (in meters). Women were asked if their natural mother and/or their sister(s) (not adopted, step, or half) had been diagnosed with breast cancer, and family history of breast cancer was computed as follows: no affected first-degree relatives; one affected first-degree relative; or two or more affected first-degree relatives.

Women were assumed to be postmenopausal if they had no periods in the year before baseline, had ever used hormone therapy, had had a bilateral oophorectomy, or were 60 years or older at baseline. Age at menopause was set to age at which menstrual periods ended or age of first use of hormone therapy, whichever came first. Women who reported a hysterectomy without bilateral oophorectomy were considered to be postmenopausal if they had ever used hormone therapy or were 55 years or older at baseline. For those women, age at menopause was set to the age at which they first used hormone therapy (if before age 55 years); otherwise, it was set to missing. Hormone therapy use was ascertained by asking women about use of prescription estrogen and progestin as pills or patches, excluding oral contraceptives. Information was obtained on hormone therapy use status (never, former, current), age at first use (in 5 categories: 39 or younger, 40-44, 45-49, 50-54, 55 or older), and years of use (in 5 categories: less than one, 1-4, 5-9, 10-14, 15 or more). Past hormone therapy use for less than 1 year was considered no use (never). Current use of hormone therapy for less than 1 year was classified as 1 to 4 years of use. Years of E+P use and years of estrogen-only use were computed separately. Years of E+P use include only those periods in which a woman used estrogen and progestin.

These women could also have had periods of exposure to unopposed estrogen; however, these exposures were not counted in years of E+P use. Years of E+P use was set to missing for women who reported periods of unopposed progestin. Women who reported using estrogen but never used it in combination with progestin were classified as never E+P users. Information on cancers, deaths, and moves out of the area were obtained by linking the VITAL cohort to public databases (western Washington SEER cancer registry, Washington State death file, and the National Change of Address system). Information on cancer stage, grade, histology, and estrogen receptor and progesterone receptor (PR) status was obtained from the SEER registry.

Specimen Collection, DNA Isolation, and Genotyping Assays. Buccal cells were collected as a source of DNA using cytobrushes. Three months after the

completed baseline questionnaire was received, a DNA kit, containing three sterile cytobrushes each packaged in a separate bar-coded tube, detailed instructions with pictures, and a consent form, was mailed to the VITAL participants. Nonrespondents were mailed a second kit about 1 year after the first. DNA was extracted from the cytobrushes (one brush per participant) using QIAamp mini kits (Qiagen, Inc.) according to the manufacturer's protocol for buccal swabs with some modifications, as previously described (14). Cases and controls were genotyped for 16 different SNPs: *COMT* Val$^{108/158}$Met (rs4680), *CYP1A1* Ile^{462}Val (rs1048943), *CYP1A1 Msp*I (rs4646903), *CYP17A1* 5′UTR (rs743572), *CYP19A1* Arg^{264}Cys (rs700519), *CYP19A1* 3′UTR (rs10046), *CYP1B1* Arg^{48}Gly (rs10012), *CYP1B1* Val^{432}Leu (rs1056836), *CYP1B1* Asn^{453}Ser (rs1800440), *ESR1* Ser^{10}Ser (rs2077647), *GSTP1* Ile^{105}Val (rs1695), *PGR* +331G/A (rs10895068), *PGR* Val^{660}Leu (rs1042838), *SHBG* 5′UTR (rs1799941), *SHBG* Asp^{356}Asn (rs6259), and *SULT1A1* Arg^{213}His (rs9282861).

All genotypes were determined by microsphere-based, allele-specific primer extension (ASPE) assays followed by analysis on the Luminex 100 flow cytometer (Luminex) as previously described (15). The DNA samples were preamplified by multiplex PCR done in an Eppendorf Mastercycler using HotStar *Taq* DNA polymerase (Qiagen). Annealing and extension temperatures were optimized for each multiplex primer set. All ASPE assays had reproducibility rates >99.4%. Primer sequences, ASPE capture probe sequences, and specific genotyping conditions are available from the authors upon request. Apart from *COMT* Val$^{108/158}$Met, all SNPs had <2.5% samples that failed to be genotyped. For *COMT* Val$^{108/158}$Met, 9.8% of the samples failed. Samples that failed to be genotyped were scored as missing. Minor allele frequencies among controls were >0.05 for all SNPs except for *CYP1A1* Ile^{462}Val (minor allele frequency = 0.04) and *CYP19A1* Arg^{264}Cys (minor allele frequency = 0.04).

Statistical Analysis. Differences in baseline characteristics between cases and controls were assessed using χ^2 tests. Observed genotype frequencies in the control population were tested for deviation from Hardy-Weinberg equilibrium with the χ^2 goodness-of-fit test. With the exception of *SULT1A1* Arg^{213}His (P = 0.02), all SNPs were in Hardy-Weinberg equilibrium. Odds ratios for breast cancer risk and the corresponding 95% confidence intervals were calculated using multiple logistic regression models. Because the number of rare-allele homozygotes was relatively small, we combined heterozygotes and rare-allele homozygotes in the logistic regression analyses; common-allele homozygotes were used as reference group. Linear trend was assessed using numerical scores assigned to the ordered categories (i.e., 1 to the first category, 2 to the second, and so on) as continuous variable in the model. Effect modification by the different genetic variants was investigated for the association of E+P use (never, 1-<10, ≥10) with breast cancer risk. Log-likelihood ratio tests were calculated to test for statistical significance of multiplicative interactions, comparing models that differed only by the cross-product term of the hormone therapy use trend variable and genotype (as two groups, as described above).

Analyses conducted to evaluate associations between the different genetic variants and breast cancer risk were adjusted for age at baseline (<55, 55-<65, ≥65 years) and race (White, other), i.e., the matching variables. All other analyses were adjusted for age at baseline (<55, 55-<65, ≥65 years), race (White, other), body mass index (<25.0 kg/m^2, 25.0-<30.0 kg/m^2, ≥30.0 kg/m^2, unknown), cigarette smoking (never, former, current, unknown), number of first-degree relatives with history of breast cancer (none, 1, 2+, unknown), prior breast biopsy (no, yes), and age at menopause (≤44, 45-49, ≥50 years, unknown). Additional adjustment for other known breast cancer risk factors (e.g., parity) did not change the estimates significantly (i.e., not more than 10%). All significance tests were two sided; P values <0.05 were considered statistically significant. All analyses were done with use of the SAS statistical software package (SAS version 9.1.3, SAS Institute, Inc.).

Results

The study population consisted of 324 first primary breast cancer cases and 651 controls; all participants were postmenopausal. Baseline characteristics of the study population are presented in Table 1. Cases reported having had a prior breast biopsy statistically significantly more often than controls. They also had significantly lower parity. Mean age at diagnosis among cases was 64.9 (± 6.9) years (not in table). The majority of tumors were invasive (79.3%), were well differentiated to moderately differentiated (grade I/II; 55.9%), had ductal histology (60.8%), and were estrogen receptor and PR positive (59.9%; not in table).

Table 2 shows results of case-control comparisons conducted to assess main effects of the different genotypes on breast cancer risk. None of the 16 genetic variants examined was, by itself, statistically significantly associated with breast cancer risk in our study population. Similarly, no statistically significant associations were observed with specific tumor subgroups (data not shown). We also assessed the main effect of postmenopausal hormone therapy use. Years of E+P use was statistically significantly associated with increased breast cancer risk (Table 3).

Subsequently, we investigated whether the different genetic variants modified the relationship between years of E+P use and breast cancer risk. To see the joint effect of genotype and postmenopausal hormone therapy use on breast cancer risk more clearly, a single reference group [lowest level of E+P use (never) and homozygous for common allele] was used. Results are shown in Table 4. Statistically significant interactions between *CYP1A1* Ile^{462}Val, *CYP1A1 Msp*I, *CYP1B1* Val^{432}Leu, *CYP1B1* Asn^{453}Ser and *PGR* Val^{660}Leu, and years of E+P use were observed. Among women with at least one rare allele of the *CYP1A1* Ile^{462}Val, *CYP1A1 Msp*I, *CYP1B1* Asn^{453}Ser, and *PGR* Val^{660}Leu polymorphisms, the increased risk of breast cancer associated with years of E+P use was greater than among women homozygous for the common allele of these polymorphisms. Women with at least one *CYP1B1* Val^{432} allele had a statistically significantly increased risk of breast cancer compared with the reference group, but their risk increased very little with increasing duration of E+P use. A large increase in risk with increasing years of E+P use was seen among women homozygous for the common allele of the

Table 1. Baseline characteristics of postmenopausal breast cancer cases and controls

	Cases (n_{total} = 324)*, *n* (%)	Controls (n_{total} = 651)*, *n* (%)	P †
Age (y)			0.96
≤55	33 (10.2)	70 (10.8)	
55-<65	135 (41.7)	269 (41.3)	
≥65	156 (48.2)	312 (47.9)	
Race: Caucasian	308 (95.1)	619 (95.1)	0.99
Education: some college or more	239 (74.9)	476 (74.1)	0.79
Married/living with partner	221 (69.1)	455 (71.2)	0.49
Body mass index (kg/m^2)			0.27
<25.0	140 (45.3)	245 (39.9)	
25.0-<30.0	102 (33.0)	216 (35.2)	
30.0 or ≥30	67 (21.7)	153 (24.9)	
Smoking			0.54
Never	162 (50.3)	348 (53.9)	
Former	141 (43.8)	259 (40.1)	
Current	19 (5.9)	39 (6.0)	
Family history of breast cancer			0.36
None	256 (81.0)	539 (84.2)	
1 first-degree relative with breast cancer	52 (16.5)	91 (14.2)	
2+ first-degree relatives with breast cancer	8 (2.5)	10 (1.6)	
Mammogram within last 2 y	300 (93.2)	609 (93.8)	0.69
Prior breast biopsy	90 (28.0)	141 (21.7)	0.03
Surgical menopause	53 (16.4)	113 (17.4)	0.70
Ever used oral contraceptives	215 (67.2)	419 (64.7)	0.44
Age at menarche (y)			0.67
≤11	65 (20.1)	119 (18.4)	
12	91 (28.2)	176 (27.2)	
13	101 (31.3)	197 (30.5)	
≥14	66 (20.4)	154 (23.8)	
Age at first birth (y)			0.05
≤19	46 (14.2)	103 (9.9)	
20-24	129 (39.8)	295 (45.8)	
25-29	73 (22.5)	142 (22.1)	
≥30	35 (10.8)	40 (6.2)	
No births	41 (12.7)	64 (9.9)	
Parity			0.01
Nulliparous	41 (13.4)	64 (10.8)	
1	35 (11.4)	60 (10.2)	
2	108 (35.3)	177 (30.0)	
3	73 (23.9)	131 (22.2)	
4+	49 (16.0)	159 (26.9)	
Age at menopause (y)			0.34
≤44	66 (21.8)	154 (26.1)	
45-49	94 (31.0)	167 (28.3)	
≥50	143 (47.2)	269 (45.6)	

*The numbers do not always add up to the total number of cases and controls due to missing information.
†χ^2 test.

CYP1B1 Val^{432}Leu polymorphism. When we limited the analyses for the five statistically significant variants to women with invasive breast cancer, the results were similar and the interaction with years of E+P use remained statistically significant for all variants except *CYP1B1* Asn^{453}Ser (data not shown). We assessed linkage disequilibrium between *CYP1A1* Ile^{462}Val and *CYP1A1 Msp*I, and between *CYP1B1* Val^{432}Leu and *CYP1B1* Asn^{453}Ser: r^2 = 0.3 for both pairs.

Discussion

In this nested case-control study, we evaluated associations of 16 common SNPs in genes important in sex hormone metabolism, years of E+P use, and their interactions with risk of postmenopausal breast cancer. None of the polymorphisms examined was, by itself, statistically significantly associated with breast cancer risk. Consistent with results from other studies (3-6), years of E+P use was statistically significantly associated with increased breast cancer risk in our study population. We observed statistically significant gene-environment interactions between each of *CYP1A1* Ile^{462}Val, *CYP1A1 Msp*I, *CYP1B1* Val^{432}Leu, *CYP1B1* Asn^{453}Ser, and *PGR* Val^{660}Leu on the one hand, and years of E+P use on the other hand, suggesting that the effect of E+P use on breast cancer risk is modified by these polymorphisms.

Estrogens can influence the development of breast cancer via at least two different pathways. They can stimulate gene expression and cell proliferation via interaction with the estrogen receptor as well as cause DNA damage via their oxidation products (16). The phase I enzymes CYP1A1 and CYP1B1 catalyze the oxidation of estrogens to catechol estrogens and estrogen quinones (17-19). Estrogen quinones can react directly with DNA and form both stable adducts and depurinating adducts that can generate mutations (19-21). These estrogen-derived DNA adducts have been identified in human breast tissue (22, 23). Moreover, catechol

Table 2. Genetic variation and risk of postmenopausal breast cancer: genotype main effects

Gene	Polymorphism (SNP ID)*	Genotype	Cases, n † (%)	Controls, n † (%)	OR (95% CI) ‡
COMT	Val$^{108/158}$Met (rs4680)	Met/Met	81 (28.4)	148 (24.9)	1.0 (Reference)
		Met/Val or Val/Val	204 (71.6)	446 (75.1)	0.8 (0.6-1.1)
CYP1A1	Ile^{462}Val (rs1048943)	Ile/Ile	291 (91.8)	584 (92.3)	1.0 (Reference)
		Ile/Val or Val/Val	26 (8.2)	49 (7.7)	1.1 (0.7-1.8)
CYP1A1	*Msp*I (rs4646903)	T/T	258 (80.9)	495 (77.7)	1.0 (Reference)
		T/C or C/C	61 (19.1)	142 (22.3)	0.8 (0.6-1.1)
CYP17A1	5′UTR (rs743572)	A/A	103 (32.1)	231 (35.6)	1.0 (Reference)
		A/G or G/G	218 (67.9)	417 (64.4)	1.2 (0.9-1.6)
CYP19A1	Arg^{264}Cys (rs700519)	Arg/Arg	292 (91.3)	598 (93.1)	1.0 (Reference)
		Arg/Cys or Cys/Cys	28 (8.8)	44 (6.9)	1.3 (0.8-2.1)
CYP19A1	3′UTR (rs10046)	C/C	73 (23.0)	163 (25.4)	1.0 (Reference)
		C/T or T/T	245 (77.0)	479 (74.6)	1.1 (0.8-1.6)
CYP1B1	Arg^{48}Gly (rs10012)	Arg/Arg	165 (52.4)	334 (52.4)	1.0 (Reference)
		Arg/Gly or Gly/Gly	150 (47.6)	304 (47.6)	1.0 (0.8-1.3)
CYP1B1	Val^{432}Leu (rs1056836)	Leu/Leu	104 (32.5)	222 (34.4)	1.0 (Reference)
		Leu/Val or Val/Val	216 (67.5)	423 (65.6)	1.1 (0.8-1.5)
CYP1B1	Asn^{453}Ser (rs1800440)	Asn/Asn	207 (64.9)	427 (66.4)	1.0 (Reference)
		Asn/Ser or Ser/Ser	112 (35.1)	216 (33.6)	1.1 (0.8-1.4)
ESR1	Ser^{10}Ser (rs2077647)	T/T	86 (26.9)	176 (27.4)	1.0 (Reference)
		C/T + T/T	234 (73.1)	467 (72.6)	1.0 (0.8-1.4)
GSTP1	Ile^{105}Val (rs1695)	Ile/Ile	141 (43.9)	270 (41.7)	1.0 (Reference)
		Ile/Val or Val/Val	180 (56.1)	377 (58.3)	0.9 (0.7-1.2)
PGR	+331G/A (rs10895068)	G/G	294 (91.0)	580 (89.2)	1.0 (Reference)
		G/A or A/A	29 (9.0)	70 (10.8)	0.8 (0.5-1.3)
PGR	Val^{660}Leu (rs1042838)	Val/Val	218 (67.9)	467 (72.5)	1.0 (Reference)
		Leu/Val or Leu/Leu	103 (32.1)	177 (27.5)	1.3 (0.9-1.7)
SHBG	5′UTR (rs1799941)	G/G	175 (54.7)	374 (58.0)	1.0 (Reference)
		G/A or A/A	145 (45.3)	271 (42.0)	1.1 (0.9-1.5)
SHBG	Asp^{356}Asn (rs6259)	Asp/Asp	256 (79.5)	509 (78.5)	1.0 (Reference)
		Asp/Asn or Asn/Asn	66 (20.5)	139 (21.5)	0.9 (0.7-1.3)
SULT1A1	Arg^{213}His (rs9282861)	Arg/Arg	158 (49.4)	300 (46.9)	1.0 (Reference)
		Arg/His or His/His	162 (50.6)	340 (53.1)	0.9 (0.7-1.2)

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval.
*dbSNP build 127, http://www.ncbi.nlm.nih.gov/projects/SNP/.
†The numbers do not add up to the total number of cases and controls due to missing data on genotypes for some participants.
‡Adjusted for age at baseline (<55, 55-<65, ≥65 y) and race (White, other).

estrogens and estrogen quinones can undergo redox cycling, which produces reactive oxygen species that can cause oxidative damage (20). CYP1A1 and CYP1B1 are both expressed at high levels in breast tissue (24, 25).

Interactions between the two *CYP1A1* polymorphisms investigated in this study, Ile^{462}Val and *Msp*I, and years of E+P use were statistically significant. The Ile^{462}Val polymorphism is located in exon 7 of the *CYP1A1* gene, near the active site of the enzyme (26); the *Msp*I polymorphism, a T→C transition, is located in the 3′ noncoding region (27, 28). Results from functional studies suggest that both the Val variant of *CYP1A1* Ile^{462}Val and the C variant of *CYP1A1 Msp*I may be associated with increased catalytic activity toward estrogens (29, 30); that is, the presence of these variants may well result in increased production of catechol estrogens and estrogen quinones in breast tissue, and, thus, DNA damage. Consistent with this, we observed that the increased risk of breast cancer associated with years of E+P use was greater among women with at least one rare allele of the *CYP1A1* Ile^{462}Val and *CYP1A1 Msp*I polymorphisms. To date, only one other study has evaluated interactions between SNPs in genes involved in hormone metabolism and years of E+P use in the development of breast cancer (31). Rebbeck et al. (31) observed no statistically significant modification of the effect of E+P use on breast cancer risk by any of the polymorphisms that they studied; they evaluated *CYP1A1* Ile^{462}Val but not *CYP1A1 Msp*I. The discrepancy in results might be due to differences in duration of E+P exposure between

Table 3. Postmenopausal hormone therapy use and risk of breast cancer: E+P use main effect

	Cases, n* (%)	Controls, n* (%)	OR (95% CI) †
Years of E+P use			
Never	150 (49.0)	382 (62.6)	1.0 (Reference)
1-<10	78 (25.5)	127 (20.8)	1.5 (1.0-2.2)
10 or more	78 (25.5)	101 (16.6)	1.9 (1.3-2.8)
P_{trend}			0.0002

*The numbers do not add up to the total number of cases and controls due to missing information.
†Adjusted for age at baseline (<55, 55-<65, ≥65 y), race (White, other), body mass index (<25.0 kg/m^2, 25.0-<30.0 kg/m^2, ≥30.0 kg/m^2, unknown), cigarette smoking (never, former, current, unknown), number of first-degree relatives with history of breast cancer (none, 1, 2+, unknown), prior breast biopsy (no, yes), and age at menopause (≤44, 45-49, ≥50 y, unknown).

Table 4. Years of E+P use, genetic variation, and risk of breast cancer

Polymorphism	Years of E+P use					
	Never		1-<10		≥10	
	Cases/controls	OR (95% CI)*	Cases/controls	OR (95% CI)*	Cases/controls	OR (95% CI)*
COMT Val$^{108/158}$Met						
Met/Met	37/84	1.0 (Reference)	25/38	1.4 (0.7-2.8)	15/17	2.0 (0.9-4.4)
Met/Val or Val/Val	95/263	0.8 (0.5-1.3)	45/81	1.2 (0.7-2.0)	52/75	1.5 (0.9-2.5)
$P_{interaction}$	0.88					
CYP1A1 Ile^{462}Val						
Ile/Ile	141/341	1.0 (Reference)	66/114	1.3 (0.9-2.0)	68/95	1.7 (1.2-2.5)
Ile/Val or Val/Val	8/31	0.6 (0.3-1.5)	8/10	2.1 (0.8-5.4)	9/5	4.7 (1.5-14.4)
$P_{interaction}$	0.04					
*CYP1A1 Msp*I						
T/T	127/280	1.0 (Reference)	62/98	1.4 (0.9-2.0)	55/85	1.4 (0.9-2.1)
T/C or C/C	23/94	0.6 (0.3-0.9)	12/25	1.1 (0.5-2.3)	22/15	3.2 (1.6-6.5)
$P_{interaction}$	0.003					
CYP17A1 5′ UTR						
A/A	43/131	1.0 (Reference)	26/51	1.5 (0.8-2.8)	30/39	2.3 (1.2-4.1)
A/G or G/G	107/250	1.3 (0.9-2.0)	50/75	2.0 (1.2-3.3)	47/62	2.3 (1.4-3.9)
$P_{interaction}$	0.54					
CYP19A1 Arg^{264}Cys						
Arg/Arg	136/353	1.0 (Reference)	74/116	1.6 (1.1-2.3)	67/91	1.9 (1.3-2.7)
Arg/Cys or Cys/Cys	14/25	1.3 (0.7-2.7)	2/7	0.8 (0.2-4.1)	9/10	2.3 (0.9-5.8)
$P_{interaction}$	0.77					
CYP19A1 3′ UTR						
C/C	33/98	1.0 (Reference)	15/31	1.3 (0.6-2.8)	19/28	2.0 (1.0-4.2)
C/T + T/T	117/278	1.3 (0.8-2.0)	60/94	1.9 (1.1-3.2)	58/73	2.3 (1.4-4.0)
$P_{interaction}$	0.88					
CYP1B1 Arg^{48}Gly						
Arg/Arg	82/197	1.0 (Reference)	38/63	1.4 (0.9-2.3)	38/54	1.6 (1.0-2.7)
Arg/Gly or Gly/Gly	68/179	1.0 (0.7-1.4)	36/60	1.4 (0.8-2.3)	36/45	2.0 (1.2-3.4)
$P_{interaction}$	0.51					
CYP1B1 Val^{432}Leu						
Leu/Leu	40/138	1.0 (Reference)	26/41	2.3 (1.2-4.2)	32/30	3.7 (2.0-6.9)
Leu/Val or Val/Val	109/239	1.7 (1.1-2.6)	50/85	2.0 (1.2-3.4)	45/71	2.2 (1.3-3.8)
$P_{interaction}$	0.007					
CYP1B1 Asn^{453}Ser						
Asn/Asn	105/240	1.0 (Reference)	43/89	1.1 (0.7-1.7)	47/70	1.5 (1.0-2.4)
Asn/Ser or Ser/Ser	45/137	0.8 (0.5-1.1)	32/36	2.0 (1.1-3.5)	30/31	2.2 (1.2-3.8)
$P_{interaction}$	0.04					
ESR1 Ser^{10}Ser						
T/T	39/95	1.0 (Reference)	18/48	0.9 (0.4-1.7)	22/29	1.8 (0.9-3.7)
C/T + T/T	111/284	1.0 (0.6-1.5)	57/76	1.8 (1.1-3.1)	55/72	1.8 (1.1-3.1)
$P_{interaction}$	0.61					
GSTP1 Ile^{105}Val						
Ile/Ile	66/156	1.0 (Reference)	31/55	1.3 (0.7-2.2)	32/43	1.8 (1.0-3.1)
Ile/Val or Val/Val	84/223	0.9 (0.6-1.3)	45/71	1.5 (0.9-2.4)	45/58	1.8 (1.1-2.9)
$P_{interaction}$	0.66					
PGR +331G/A						
G/G	139/344	1.0 (Reference)	73/114	1.5 (1.0-2.2)	67/86	1.9 (1.3 -2.8)
G/A or A/A	11/37	0.7 (0.3-1.4)	4/13	0.7 (0.2-2.2)	11/15	1.7 (0.7-3.8)
$P_{interaction}$	0.74					
PGR Val^{660}Leu						
Val/Val	114/272	1.0 (Reference)	45/88	1.2 (0.8-1.9)	45/75	1.4 (0.9-2.1)
Leu/Val or Leu/Leu	36/105	0.9 (0.6-1.3)	31/38	1.9 (1.1-3.2)	32/26	3.1 (1.8-5.6)
$P_{interaction}$	0.01					
SHBG 5′ UTR						
G/G	87/220	1.0 (Reference)	42/74	1.4 (0.9-2.2)	35/52	1.6 (1.0-2.7)
G/A or A/A	61/157	0.9 (0.6-1.4)	35/53	1.6 (0.9-2.6)	42/48	2.2 (1.3-3.6)
$P_{interaction}$	0.30					
SHBG Asp^{356}Asn						
Asp/Asp	119/309	1.0 (Reference)	58/96	1.5 (1.0-2.3)	66/73	2.3 (1.6-3.5)
Asp/Asn or Asn/Asn	31/71	1.2 (0.7-1.9)	18/30	1.5 (0.8-2.9)	12/28	1.1 (0.5-2.2)
$P_{interaction}$	0.05					
SULT1A1 Arg^{213}His						
Arg/Arg	76/168	1.0 (Reference)	41/65	1.4 (0.8-2.2)	32/50	1.4 (0.8-2.4)
Arg/His or His/His	73/204	0.8 (0.6-1.2)	34/62	1.1 (0.7-1.9)	46/51	1.9 (1.2-3.2)
$P_{interaction}$	0.19					

*Adjusted for age at baseline (<55, 55-<65, ≥65 y), race (White, other), body mass index (<25.0 kg/m^2, 25.0-<30.0 kg/m^2, ≥30.0 kg/m^2, unknown), cigarette smoking (never, former, current, unknown), number of first-degree relatives with history of breast cancer (none, 1, 2+, unknown), prior breast biopsy (no, yes), and age at menopause (≤44, 45-49, ≥50 y, unknown).

the two study populations. Rebbeck et al. defined long-term E+P use as ≥3 years, whereas in our study it was defined as ≥10 years.

Study participants were genotyped for three different polymorphisms in the *CYP1B1* gene: Arg^{48}Gly, Val^{432}Leu, and Asn^{453}Ser. Interactions between the Val^{432}Leu and Asn^{453}Ser polymorphisms and years of E+P use were statistically significant; no significant interaction was observed with Arg^{48}Gly. In the study mentioned above (31), Rebbeck et al. also investigated interactions between the Val^{432}Leu and Asn^{453}Ser polymorphisms and E+P use, with no statistically significant interaction found; they did not evaluate interactions with the Arg^{48}Gly polymorphism. The Arg^{48}Gly polymorphism is located in exon 2 of *CYP1B1* (32). Although it results in an amino acid change, McLellan et al. (33) have shown that, by itself, this substitution does not seem to significantly alter CYP1B1 function, which may explain the lack of interaction seen with years of E+P use. The Val^{432}Leu and Asn^{453}Ser polymorphisms are located in exon 3 of *CYP1B1*, which encodes the heme-binding domain (34).

The 432Val and 453Ser variants have been found associated with higher CYP1B1 enzyme activity toward estrogens, resulting in increased formation of 4-hydroxy catechol estrogens than the 432Leu and 453Asn variants, respectively (35, 36). In line with this, in our study, the increased risk of breast cancer associated with years of E+P use was greater among women with the 453Ser variant. However, inconsistent with the reported higher enzyme activity of the 432Val variant, we found that the risk increased very little with increasing years of E+P use among women with at least one *CYP1B1* Val^{432} allele, whereas a large increase in risk was seen among women homozygous for *CYP1B1* Leu^{432}.

Progesterone is, like estrogen, critical to normal breast development. It regulates the expression of genes involved in, among other things, mammary cell growth and differentiation by binding to and forming a complex with the PR (37). The PR exists as two isoforms, PR-A and PR-B, which are both produced from the *PGR* gene via translation initiation at two distinct start codons under the control of separate promoters (38). PR-A and PR-B regulate different subsets of genes involved in particular functional pathways, with PR-B being the more influential transcriptional activator (37). Medroxyprogesterone acetate is by far the most commonly used progestin in hormone therapy in the United States, and most of the women in our study population who used E+P hormone therapy will have taken this progestin. Medroxyprogesterone acetate is structurally related to progesterone and binds with high affinity to the PR (39).

We evaluated two *PGR* polymorphisms in this study: +331G/A and Val^{660}Leu. The +331G/A polymorphism is located in the promoter region of the *PGR* gene. The +331A variant creates a unique transcription start site and has been shown to increase *PGR* transcription and to favor production of PR-B (40). Similar to our results, Feigelson et al. (41) found no statistically significant interaction between E+P use and the +331G/A polymorphism in breast cancer development. Additionally, no statistically significant interaction was observed in relation to changes in breast density (42). Rebbeck et al. (43), in contrast, reported a statistically significant interaction between E+P use and +331G/A but only for the ductal tumor subgroup. When lobular and ductal tumors were grouped together, the interaction was not statistically significant (43). Associations between *PGR* +331G/A and risk of breast cancer have been inconsistent, with most larger studies reporting no association (41, 44, 45).

The interaction between years of E+P use and *PGR* Val^{660}Leu was statistically significant in our study population. The Val^{660}Leu polymorphism is located in exon 4 of the *PGR* gene, and has been reported to be in perfect linkage equilibrium with a silent Hist^{770}Hist polymorphism (rs1042839) in exon 5 and an Alu insertion in intron G. The 660Leu variant of both PR-A and PR-B has been found to be associated with increased transcriptional activity due to increased stability (46). Consistent with this, we observed that the increased risk of breast cancer associated with years of E+P use was greater among women with the 660Leu variant. To our knowledge, this is the first study to evaluate the interaction between postmenopausal hormone therapy use and the Val^{660}Leu polymorphism in development of breast cancer. Van Duijnhoven et al. (42) recently reported no statistically significant interaction in relation to changes in breast density in a European study population. In this study, however, no distinction was made between E+P use and E only use. Additionally, the progestins used in hormone therapy vary among countries and may affect risk of breast cancer differently (47). Regarding associations between *PGR* Val^{660}Leu and risk of breast cancer, several recent reports suggest that by itself the 660Leu variant may be associated with a moderately increased risk of breast cancer overall (44, 45, 48) or in certain age groups (49).

The strengths of our study include the use of prospectively collected exposure data, the matching of cases and controls on age and race, the focus on polymorphisms with putative functional effects, and the availability of information on potential confounders such as age at menopause. Like most observational studies, we relied on self-reports of hormone therapy use and misclassification of E+P use may have occurred. However, given the prospective study design, it is unlikely that this has resulted in systematic bias. Our study population was of medium size and it is possible that some associations and/or interactions were not detected due to insufficient power. Additionally, it is possible that important associations and/or interactions were not identified due to the limited number of SNPs investigated. It should also be noted that multiple comparisons may have led to chance findings: With 16 different statistical tests in Table 3 as well as Table 4, we would have expected one spuriously significant result at $P < 0.05$ in each table. However, instead, we observed no statistically significant main effects of the 16 SNPs in Table 3, whereas there were five statistically significant interactions with years of E+P use in Table 4 (with two significant at <0.01), more than expected by chance. Nonetheless, we cannot rule out the possibility that the reported associations are due to chance and, therefore, they will need to be confirmed in additional studies.

In summary, our data suggest that *CYP1A1* Ile^{462}Val, *CYP1A1 Msp*I, *CYP1B1* Val^{432}Leu, *CYP1B1* Asn^{453}Ser, and *PGR* Val^{660}Leu may modify the risk of breast cancer associated with E+P use. The observed statistically

significant interactions, if confirmed by other studies, support the hypothesis that the effect of E+P use on breast cancer risk may be modified by functional polymorphisms in genes involved in sex hormone metabolism. Additional research is needed to evaluate clinical relevance and the effect of gene-gene interactions. Eventually, enhanced knowledge of interactions between genetic variants and hormone therapy use may result in the ability to effectively identify postmenopausal women who are particularly susceptible to breast cancer when exposed to exogenous hormones.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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Cancer Epidemiol Biomarkers Prev 2008;17:1751-1759.