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Genetic Polymorphisms in Catechol-*O*-Methyltransferase, Menopausal Status, and Breast Cancer Risk[1]

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ABSTRACT

Polymorphic catechol-*O*-methyltransferase (COMT) catalyzes the *O*-methylation of estrogen catechols. In a case-control study, we evaluated the association of the low-activity allele ($COMT^{Met}$) with breast cancer risk. Compared to women with $COMT^{Val/Val}$, $COMT^{Met/Met}$ was associated with an increased risk among premenopausal women [odds ratio (OR), 2.1; confidence interval (CI), 1.4–4.3] but was inversely associated with postmenopausal risk (OR, 0.4; CI, 0.2–0.7). The association of risk with at least one low-activity $COMT^{Met}$ allele was strongest among the heaviest premenopausal women (OR, 5.7; CI, 1.1–30.1) and among the leanest postmenopausal women (OR, 0.3; CI, 0.1–0.7), suggesting that COMT, mediated by body mass index, may be playing differential roles in human breast carcinogenesis, dependent upon menopausal status.

INTRODUCTION

It is widely believed that estrogen exposure is an important etiological agent in breast carcinogenesis. However, studies of excreted estrogens or estrogen metabolites have demonstrated only weak associations between high levels of plasma or urinary estrogens and breast cancer risk (1–3). The catechol estrogens (*i.e.*, 2-hydroxy estrogens) are the major metabolites of estrogens in humans and animals (4). The 2- and 4-catechol estrogens have been reported to demonstrate both cancer-promoting and -inhibiting activities through interactions with the estrogen receptor or with macromolecules (*i.e.*, cellular proteins and DNA; Refs. 3–9). Interindividual differences in steroid metabolism have been noted and attributed to both genetic polymorphisms in and differential expression of metabolizing enzymes that hydroxylate and conjugate the steroid hormones (4, 10–12). COMT[3] is one of several phase II enzymes involved in the conjugation and inactivation of the catechol estrogens (13). COMT is found in various mammalian tissues, with high levels in liver and kidney and significant amounts in RBCs, endometrium, and breast (14). An amino acid change (valine to methionine) at position 158/108 in the membrane-bound/cytosolic form of the protein has been linked to decreased methylation activity of the enzyme (15). This amino acid change is believed to be closely associated with the observed trimodal distribution of COMT enzyme activity in the human population associated with high $COMT^{Val/Val}$, intermediate $COMT^{Val/Met}$, and low COMT $COMT^{Met/Met}$ activity toward certain combination therapeutics used in Parkinson's disease (16, 17). Because the genetic polymorphism in COMT correlates with decreased enzyme activity and because COMT is a major conjugation pathway for the catechol estrogens, we sought to determine whether polymorphisms in the *COMT* gene may be associated with increased risk of breast cancer and whether the association between genotypes and risk may be modified by menopausal status and body mass index.

MATERIALS AND METHODS

Study Population. These research data were collected from an earlier case-control study (1986–1991) of 617 premenopausal and 933 postmenopausal Caucasian women in Western New York; the detailed methods have been reported (18, 19). The protocol for the study was reviewed by the Institutional Review Board of the State University of New York at Buffalo and of all of the participating hospitals. Informed consent was received from all participants for interview and medical record review. Women diagnosed with incident, primary, histologically confirmed breast cancer were frequency-matched by age and county of residence with controls randomly selected from the New York State Motor Vehicle lists (<65 years) and the Health Care Finance Administration rolls (>65 years). Interview data included medical, reproductive, and lifestyle histories. Approximately 45% of premenopausal and 63% of postmenopausal women provided blood samples. DNA was extracted from blood clots, as reported previously, and analyzed for *COMT* genotype in case and control specimens having adequate DNA.

Laboratory Analysis. To determine the polymorphic *COMT* genotype, DNA was subjected to PCR as described (20). Briefly, the reaction conditions included buffer [10 mM Tris-HCl (pH 8.3), 50 mM KCl], 2 mM MgCl2, 0.2 mM 2′-deoxynucleoside-3′-triphosphate (Boehringer Mannheim, Indianapolis Indiana)], 2.5 units of Taq DNA polymerase (Promega Corp., Madison, WI), and primers specific for *COMT* (10 pmol each; 5′-ACTGTGGCTACTCAGCTGTG-3′ and 5′-CCTTTTTCCAGGTCTGACAA-3′) in a total reaction volume of 100 μl using 100 ng of sample DNA. PCR products (169 bp) were digested with Hsp92II (Promega) and analyzed by gel electrophoresis (2.5% Metaphor agarose; FMC BioProducts, Rockland, ME). Digestion of the *COMT* product with Hsp92II gives rise to fragment sizes of 114, 23, and 32 bp for the high-activity allele and 96, 23, 32, and 18 bp for the low-activity allele. This assay was validated by confirming inheritance patterns in eight family lines encompassing three generations (National Institute General Medical Scientist Human Genetic Mutant Cell Repository; Coriell Institute, Camden, NJ). All assays were conducted and interpreted by two reviewers (P. T. and A. S.) blinded to case-control status.

Statistical Analysis. Student's *t* tests were performed to assess mean differences in reproductive and lifestyle factors by *COMT* genotypes within case and control groups. ORs and 95% CIs were calculated using unconditional logistic regression to evaluate associations between *COMT* genotypes and breast cancer risk separately for premenopausal and postmenopausal women. ORs were adjusted for age, education, age at menarche, age at first pregnancy, reported family history of breast cancer, body mass index, and age at menopause for postmenopausal women. Possible modification of risk by body mass was evaluated by calculating ORs for genotype and breast cancer risk within tertiles of body mass index, determined by the distribution among controls.

RESULTS

Genotype data for *COMT* were available for 281 women with breast cancer and 289 community controls. For the most part, asso-

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[3] The abbreviations used are: COMT, catechol *O*-methyltransferase; BMI, body mass index; OR, odds ratio; CI, confidence interval; HRT, hormone replacement therapy.

Table 1 *Case and control differences in putative risk factors for breast cancer within the entire study set and the subset for which COMT data were available*

	All data		With COMT results	
	Case	Control	Case	Control
	Premenopausal			
Age	45.8 (4)[a]	46.1 (4)	46.2 (4)	46.8 (4)
Education	14 (3)	14 (3)	14 (3)	14 (3)
Age at menarche	12.5 (1.6)	12.8 (1.7)	12.5 (1.6)[b]	13.0 (1.8)
Age at first pregnancy	23.6 (5)[b]	22.4 (5)	24.0 (5)[b]	22.2 (4)
BMI	25.1 (5.7)	25.8 (5.2)	24.7 (5.4)	25.8 (5)
Family history of breast cancer	13%[b]	7%	16%[b]	5%
	Postmenopausal			
Age	62.8 (8)	63.5 (8)	62.9 (8)	63.3 (7)
Education	12 (3)	12 (3)	13 (3)	12 (3)
Age at menarche	12.8 (1.5)	12.9 (1.6)	12.8 (1.6)	12.8 (1.6)
Age at first pregnancy	24.2 (5)[b]	24.2 (5)	24.4 (5)	24.1 (5)
Age at menopause	47.3 (6.1)	46.5 (6.4)	47.8 (5.8)	47.0 (6)
BMI	26.5 (5.4)[b]	25.7 (5.2)	25.7 (5.2)	25.6 (4.8)
Family history of breast cancer	16%[b]	8%	15%[b]	9%

[a] Mean (SD).
[b] $P < 0.05$.

ciations between putative risk factors for breast cancer (*i.e.*, those for which logistic models were adjusted) were similar within the larger data set and the subset for which *COMT* data were available. Values for cases and controls within each group, by menopausal status, are shown in Table 1. Results of the study of the association between *COMT* genotype and breast cancer risk, evaluated by menopausal status, are shown in Table 2. Marked differences in the association of *COMT* genotypes with risk were noted between premenopausal and postmenopausal women. Premenopausal cases were less likely than controls to be homozygous for the high-activity $COMT^{Val}$ allele, 20% *versus* 36%, respectively. Heterozygosity was more frequent in the cases than in the controls, with an adjusted OR of 2.7 (95% CI, 1.5–5.1). However, there was no gene-dose effect; women who were $COMT^{Met/Met}$ had no additional increase in risk (OR, 2.1; CI 1.4–4.3). When $COMT^{Met/Val}$ individuals were combined with the $COMT^{Met/Met}$ genotype, those with at least one low-activity allele showed significantly increased risk (OR, 2.4; CI, 1.4–4.3).

In contrast to premenopausal women among whom the $COMT^{Met}$ low-activity allele was associated with increased risk, postmenopausal women with breast cancer were more likely than controls to be $COMT^{Val/Val}$ (29% *versus* 19%), and an inverse association was most pronounced among those who were $COMT^{Met/Met}$ (OR, 0.4; 95% CI, 0.2–0.7). When $COMT^{Met/Val}$ individuals were combined with individuals who were $COMT^{Met/Met}$, having one or two low-activity alleles significantly decreased risk (OR, 0.5; CI, 0.3–0.9). Taken together, these data suggest that the role of the high- and low-activity *COMT* alleles in breast carcinogenesis may vary by menopausal status.

Because there appears to be a consistent effect documented in the literature (21, 22) of BMI on breast cancer risk by menopausal status (with higher BMI associated with increased risk for postmenopausal women but a slight decreased or no risk for premenopausal women) and because hormonal levels may be linked to BMI, particularly in postmenopausal women, we sought to more closely evaluate associations between BMI, *COMT*, and breast cancer risk. As shown in Table 3, the low-activity $COMT^{Met}$ allele was most strongly associated with risk among the heaviest premenopausal women (OR, 5.7; CI, 1.1–30.1), whereas in postmenopausal women, an inverse association with *COMT* and risk was strongest in the leanest women with at least one low-activity allele (OR, 0.3; CI, 0.1–0.7). It is also possible that *COMT* activity could modify the association between HRT and breast cancer risk. HRT was not a risk factor in these data and was not added to the multivariate model. Neither was there any modification of that association by *COMT* genotypes (data not shown).

Finally, we determined the distribution of *COMT* genotypes and their relationship to breast cancer risk among all women independent of menopausal status. Genotype data for *COMT* were available for 281 women with breast cancer and 289 community controls. As shown in Table 4, there was no association between *COMT* genotypes and breast cancer risk for women who were heterozygous ($COMT^{Val/Met}$) or homozygous for the low-activity allele ($COMT^{Met/Met}$) when women were grouped independent of menopausal status.

DISCUSSION

In this study, we found that the genetic polymorphism in *COMT* associated with enzyme activity was differentially associated with breast cancer risk among premenopausal and postmenopausal women. Statistically significant increased risk was observed among premenopausal women with the low-activity allele, whereas there was decreased risk among postmenopausal women with this genotype. When stratified by BMI, the low-activity *COMT* allele was associated with significantly increased risk among the heaviest premenopausal women, which is the group thought to be at lowest risk, although the confidence interval was wide. Similarly, although there was an inverse relationship between *COMT* and postmenopausal breast cancer risk, this effect was attenuated in the heaviest postmenopausal women. We observed no association between *COMT* genotypes and breast cancer risk when premenopausal and postmenopausal women were combined (Table 4). This further supports arguments from a number of studies suggesting that breast cancer etiology may differ between premenopausal and postmenopausal women, warranting the careful classification and separation of women by menopausal status in studies of breast cancer risk factors. Lastly, it should be noted that no gene-dose effect was observed in these data. The lack of a gene-dose effect is common to these types of genotype-based studies that serve as indicators of "lifetime" phenotype. Several mechanisms may account for this lack of gene-dose effect, including the pharmacokinetic considerations that determine the rate-limiting steps in the met-

Table 2 *COMT[a] genetic polymorphisms and risk of breast cancer by menopausal status: Western New York Breast Cancer Study: 1986–1991*

	Case *n* (%)	Control *n* (%)	OR (CI)[b]	OR (CI)[c]
		Premenopausal		
$COMT^{Val/Val}$	28 (20)	48 (36)	1.0	1.0
$COMT^{Val/Met}$	84 (60)	57 (42)	2.5 (1.4–4.6)	2.7 (1.5–5.1)
$COMT^{Met/Met}$	29 (20)	29 (22)	1.7 (0.8–3.4)	2.1 (1.0–4.4)
$COMT^{Val/Val}$	28 (20)	48 (36)	1.0	1.0
$COMT^{Val/Met}$ and $COMT^{Met/Met}$	113 (80)	86 (64)	2.2 (1.3–3.7)	2.4 (1.4–4.3)
		Postmenopausal		
$COMT^{Val/Val}$	41 (29)	30 (19)	1.0	1.0
$COMT^{Val/Met}$	75 (54)	82 (53)	0.7 (0.4–1.2)	0.6 (0.4–1.2)
$COMT^{Met/Met}$	24 (17)	43 (28)	0.4 (0.2–0.8)	0.4 (0.2–0.7)
$COMT^{Val/Val}$	41 (29)	30 (19)	1.0	1.0
$COMT^{Val/Met}$ and $COMT^{Met/Met}$	99 (71)	125 (81)	0.6 (0.3–1.0)	0.5 (0.3–0.9)

[a] $COMT^{Val/Val}$ is associated with the high-activity phenotype, $COMT^{Val/Met}$ with the intermediate-activity phenotype, and $COMT^{Met/Met}$ with the low-activity phenotype.
[b] ORs and 95% CIs calculated by unconditional logistic regression, adjusted for age and education.
[c] ORs and 95% CIs calculated by unconditional logistic regression, adjusted for age, education, age at menarche, age at pregnancy, age at menopause, BMI, and family history of breast cancer.

Table 3 *Effect of COMT genotype on risk, within tertiles of BMI: Western New York Breast Cancer Study, 1986–1991*

BMI *COMT* genotype	≤23		23–27		>27	
	Ca/Co[a]	OR (CI)[b]	Ca/Co	OR (CI)	Ca/Co	OR (CI) 2
			Premenopausal			
$COMT^{Val/Val}$	19/18	1.0	8/16	1.0	2/28	1.0
$COMT^{Val/Met}$ and $COMT^{Met/Met}$	55/34	1.8 (0.8–4.1)	29/22	3.3 (1.1–9.8)	14/30	5.7 (1.1–30.1)
			Postmenopausal			
$COMT^{Val/Val}$	19/11	1.0	8/7	1.0	14/12	1.0
$COMT^{Val/Met}$ and $COMT^{Met/Met}$	31/50	0.3 (0.1–0.7)	42/43	0.5 (0.1–1.6)	26/32	0.8 (0.2–2.2)

[a] Number of cases/number of controls.
[b] ORs and 95% CIs calculated by unconditional logistic regression, adjusted for age, education, age at menarche, age at first pregnancy, family history of breast cancer, and age at menopause (postmenopausal women only).

abolic pathway. For COMT, this may be cofactor availability (*i.e.*, *S*-adenosyl-methionine) and/or differential regulation of gene expression accounting for overlapping enzyme activity among the three genotypes (14).

These data compared with that recently reported by Lavigne *et al.* (23) are in direct contrast. In that study of *COMT* and breast cancer risk, they found increased risk with the low-activity allele among postmenopausal women and an inverse association with premenopausal breast cancer risk. It is the nature of epidemiological studies that there will be inconsistencies in results from one study to another, and conclusions should not be drawn until similar findings are observed in a number of studies. Conflicting studies may be due to a number of factors, including the population evaluated, the choice of a control group, various biases resulting in random or systematic error, and small sample sizes. Although the Lavigne analyses were from a cohort study, our data were derived from a case-control study, which may be subject to biases common to such studies. However, the original study was extremely well designed, and controls were from the community, frequency-matched to cases on age and county of residence. The group with *COMT* data did not vary substantially from this larger group, as shown in Table 1. There is little reason to believe that case-control or cohort design would impact on results of studies of genetics and risk, because genotype is fixed and thus not affected by recall bias. Furthermore, our data are derived from 281 cases and 289 controls, almost three times that of the study of Lavigne, containing 111 cases and 111 controls. Larger sample size may more clearly elucidate relationships, and results may be less subject to type I or type II errors. It is also possible that the composition of the study population could affect results. Prevalence of *COMT* genotypes varies markedly with ethnicity, and it is possible that participants from western New York were from different ethnic backgrounds than those in Maryland (24). In our data, larger proportions of postmenopausal women were first- or second-generation Italians or Germans, which could, in combination with small numbers, influence the distribution of the *COMT* polymorphism in the Caucasian population, skewing genotype distribution in these and other similar data sets (15, 20, 25–27).

Table 4 *COMT genetic polymorphisms and risk of breast cancer among premenopausal and postmenopausal women combined: Western New York Breast Cancer Study: 1986–1991*

	Case *n* (%)	Control *n* (%)	OR (CI)[a]
$COMT^{Val/Val}$	69 (25)	78 (27)	1.0
$COMT^{Val/Met}$	159 (56)	139 (48)	1.3 (0.9–1.9)
$COMT^{Met/Met}$	53 (19)	72 (25)	0.8 (0.5–1.4)
$COMT^{Val/Val}$	70 (25)	78 (27)	1.0
$COMT^{Val/Met}$ and $COMT^{Met/Met}$	211 (75)	211 (73)	1.1 (0.8–1.6)

[a] ORs and 95% CIs calculated by unconditional logistic regression, adjusted for age, education, age at menarche, age at first pregnancy, BMI, and family history of breast cancer.

Because our results were so similar to those of Lavigne *et al.* (23), except that associations were flipped by menopausal status, we also considered the possibility that there were errors in classification or coding. A thorough review of the original gels, the coding of genotypes within the database, and other variables that could affect results was performed, and this possibility was ruled out. Clearly, there is a need for this hypothesis to be evaluated in other study populations, so that a preponderance of data can further direct research as well as identify subgroups who may be at higher risk and thus, need to be targeted for preventive strategies.

Although the mechanisms are not elucidated, these data suggest that the *COMT* genotypes associated with high, intermediate, and low enzyme activity may contribute to breast cancer etiology. Furthermore, these data indicate that there may be an interaction between BMI, *COMT*, and menopausal status in breast cancer risk. The mechanism of this interaction may be an opposing role of catechol estrogen metabolism in breast cancer etiology, depending on the hormonal environment. We suggest that the differing biological effects of the catechol estrogens reported in the literature (*i.e.*, DNA damaging *versus* growth inhibiting) may be dependent on the levels of circulating estrogens. Therefore, in a high estrogen environment such as in the premenopausal and to some extent in the heaviest postmenopausal women, the presence of higher circulating levels of the catechol compounds (2-OH and 4-OH) of estradiol generated in a low COMT environment may result in higher circulating levels of potentially mutagenic compounds (5, 7, 9). Conversely, low COMT activity may be associated with lower circulating levels of the putative anti-carcinogen, 2-methoxyestradiol (28, 29). In a low-estrogen environment, as in leaner postmenopausal women, higher circulating levels of the unmethylated catechols in a low COMT background may elevate the levels of the putative anticarcinogenic 2-hydroxy estrone (3). It is of interest to note that in leaner postmenopausal women, colorectal cancer risk is reduced by HRT (30) and that HRT appears to maintain the age-related decline in DNA repair capacity (31). The fact that the leanest women appear to benefit more from higher circulating levels of estrogen and estrogen catechols might suggest that some exposure to estrogen postmenopausally is beneficial, but that too little or too much estrogen exposure, as in the premenopausal women, places an individual at increased risk for cancer of the breast and perhaps the colon, the mechanisms of which remain unclear.

In addition to its role in conjugation of estrogenic compounds, COMT acts on a number of other compounds thought to modify cancer risk, including ascorbic acid and certain flavonoids (14, 15, 32). The impact of COMT on breast cancer risk in premenopausal and

postmenopausal women may be reflective of differing etiological events that encompass both endogenous and exogenous exposures.

As discussed above, results from these analyses may be affected by sources of bias that are common to case-control studies. Low participation rates may produce results that are not generalizable to all women. Although it is possible that body mass could differ between those who participated and those who did not, it is unlikely that selection bias would affect overall associations between genetic polymorphisms and breast cancer risk. Of more concern, however, are the relatively small sample numbers in this study, particularly when women were stratified by BMI. Resulting estimates of risk may be unstable, as evidenced by wide CI, due to chance alone. Nonetheless, these data are consistent with biologically plausible interactions and merit further investigation of the associations between hormonal/menopausal status, variability in metabolism of estrogens, and breast cancer risk.

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