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Catechol-*O*-methyltransferase and breast cancer risk

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Recent studies suggest that a polymorphism in catechol-*O*-methyltransferase (*COMT*) is associated with increased risk of breast cancer. Methylation by COMT is the principal pathway for inactivation of catechol estrogens, which are hypothesized to participate in estrogen-induced carcinogenesis. We examined the association of *COMT* genotype and breast cancer risk in a population-based, case–control study of invasive breast cancer in North Carolina. The study population consisted of 654 cases and 642 controls, with approximately equal numbers of African-American and white women and women under the age of 50 and aged 50 or over. Contrary to previous reports, we did not observe an association between one or more copies of the low activity *COMT* allele (*COMT-L*) and breast cancer risk. Multivariate relative risks (RRs) were 0.8 (95% confidence interval: 0.6–1.1) for *COMT-HL* and 0.8 (0.6–1.1) for *COMT-LL*, compared with the *COMT-HH* genotype. RRs for *COMT* did not differ among African-American and white women and we did not observe strong modification of RR estimates by menopausal status, body mass index, physical activity or other covariates. Our results suggest that *COMT* genotype is not related to breast cancer risk.

Introduction

Reproductive hormones appear to play an important role in the development of breast cancer (1). Many established breast cancer risk factors (including early age at menarche and late age at menopause) are indicators of cumulative exposure to estrogen and progesterone (2). Additional risk factors, such as obesity and physical activity, are also hypothesized to contribute to breast cancer risk by modifying lifetime exposure to endogenous estrogen (3–6).

To uncover new risk factors for breast cancer, recent epidemiological studies have focused on environmental (7) and genetic factors (8,9) that modify metabolism of estrogen. The natural estrogen, 17β-estradiol (E2), in conjunction with other hormones, stimulates epithelial proliferation in the breast (10,11). Women with higher levels of circulating estradiol are reported to be at higher risk for subsequent breast cancer, but the biological mechanisms are unknown (12). One potential mechanism for the carcinogenicity of estradiol is C-2 hydroxylation by cytochrome P450 enzymes to form catechol estrogen (13). Accumulation of catechol estrogens leads to oxidative DNA damage (14), which may increase risk of breast cancer (15). The principal pathway for inactivation of catechol estrogens is *O*-methylation by catechol-*O*-methyltransferase (COMT) (16,17). Thus, several authors have predicted that reduced COMT activity would increase the risk of breast cancer (10,14).

Lachman *et al*. (18) recently identified a polymorphism in the *COMT* gene, a G→A transition at codon 158, which leads to substitution of methionine for valine. The Val→Met *COMT* allele, designated *COMT-L*, encodes a thermolabile form of the enzyme with reduced activity. Approximately 25% of Caucasians are homozygous for the low activity allele (*COMT-LL*) and exhibit a 3- to 4-fold reduction in methylation of catechol substrates. Individuals who are homozygous for the wild-type allele (*COMT-HH*) demonstrate high COMT activity and heterozygotes (*COMT-HL*) show intermediate activity (18). Two published epidemiological studies have investigated the relationship between *COMT* genotype and breast cancer risk (19,20). Lavigne *et al*. (19) reported a positive association with *COMT-LL* genotype among post-menopausal women and an inverse association among pre-menopausal women. For post-menopausal women, multivariate relative risks (RRs) for women with one (*COMT-HL*) or two (*COMT-LL*) copies of the low activity *COMT* allele were 1.70 [95% confidence interval (CI): 0.77–3.75] and 2.18 (95% CI: 0.93–5.11), respectively, compared with women with two copies of the wild-type allele (*COMT-HH*). The RR for *COMT-LL* was stronger among post-menopausal women with a high body mass index (BMI) (RR = 3.58, 95% CI: 1.07–11.98). Among pre-menopausal women, multivariate RR = 0.57 (95% CI: 0.14–2.40) for *COMT-HL* and 0.24 (95% CI: 0.04–1.51) for *COMT-LL*. Thompson *et al*. (20) reported positive associations for the *COMT-HL* (RR = 2.7, 95% CI: 1.5–5.1) and *COMT-LL* (RR = 2.1, 95% CI: 1.0–4.4) genotypes among pre-menopausal women. For post-menopausal women, the corresponding RRs were 0.6 (95% CI: 0.4–1.2) and 0.4 (95% CI: 0.2–0.7). The authors also observed modification of RRs by BMI: risk was highest among pre-menopausal women with a high BMI (RR = 5.7, 95% CI: 1.1–30.1) and lowest among post-menopausal women with a low BMI (RR = 0.3, 95% CI: 0.1–0.7).

Recently, it has been shown that exercise increases the proportion of catechol estrogens that undergo *O*-methylation by COMT (21). Cigarette smoking may alter circulating levels of endogenous estrogens (22). Therefore, we hypothesized that level of smoking and physical activity might modify the effects of *COMT* genotype on breast cancer risk. We investigated the role of *COMT* genotype in a population-based, case–control study of breast cancer among African-American and white

Abbreviations: BMI, body mass index; CBCS, Carolina Breast Cancer Study; COMT, catechol-*O*-methyltransferase; CI, confidence interval; E2, 17β-estradiol; HRT, hormone replacement therapy; LRT, likelihood ratio test; OC, oral contraceptive; RR, relative risk.

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women. We examined modification of RRs for *COMT* and breast cancer by menopausal status, BMI, use of exogenous hormones, level of physical activity and smoking history.

Materials and methods

Study population

The Carolina Breast Cancer Study (CBCS) is a population-based, case–control study of breast cancer in North Carolina (23). Women with a first diagnosis of histologically confirmed, invasive breast cancer were identified through a rapid ascertainment system with the help of the North Carolina Central Cancer Registry (24). Controls were selected from lists provided by the NC Division of Motor Vehicles (women aged 20–64 years) and the US Health Care Financing Administration (women aged 65–74 years). Randomized recruitment (25) was used to select approximately equal numbers of African-American and white women, as well as equal numbers of women younger than age 50 and aged 50 or older, among cases and controls. Controls were frequency-matched to cases by race and 5 year age group. During phase 1 of the CBCS (May 1993–December 1996), 889 cases and 841 controls were enrolled. Contact rates (number of women contacted/number of women identified as eligible to participate) were 97% for cases and 81% for controls. Cooperation rates (number of completed interviews/number of women contacted and eligible to participate) were 77% for cases and 68% for controls. Overall response rates [number of completed interviews/(number of women enrolled in study – ineligible and deceased women)] were 74% for cases and 53% for controls. In-person interviews were conducted in participants' homes by trained nurse interviewers and information collected on reproductive history, diet and lifestyle factors, a detailed family history of cancer and occupational history. Approximately 98% of participants who were interviewed agreed to give a 30 ml blood sample at the time of interview. Informed consent to obtain DNA was sought using a form approved by the Institutional Review Board of the UNC School of Medicine. For the present analysis of *COMT* genotype, we included the first 682 cases and 663 controls enrolled in the CBCS who agreed to provide blood samples.

Laboratory methods

DNA was extracted from peripheral blood leukocytes according to standard methods (26). A PCR-based restriction fragment length polymorphism assay was used to detect the presence of the G→A transition at position 1947 in *COMT* (accession no. Z26491) (18). PCR was used to amplify a 185 bp fragment of genomic DNA containing the polymorphism. The primer sequences were 5′-GGA GCT GGG GGC CTA CTG TG-3′ (CMT.1814.U) and 5′-GGC CCT TTT TCC AGG TCT GAC A-3′ (CMT.1978.L). Samples of 50 ng genomic DNA were added to a 15 ml PCR reaction mixture containing deionized water, 1× PCR buffer, 2 mM $MgCl_2$, 0.2 mM each dNTP, 0.8 µmol CMT.1814.U primer, 0.16 µmol CMT.1978.L primer and 0.28 U Taq DNA polymerase, combined with an equal volume of TaqStart antibody (Clontech Laboratories Inc., Palo Alto, CA). Amplification was performed on a GeneAmp 9600 thermocycler (Perkin-Elmer, Foster City, CA) under the following conditions: initial denaturation, 4 min at 94°C; amplification, 30 s at 94°C, 30 s at 61°C, 30 s at 72°C (30 cycles); final extension, 4 min at 72°C. PCR amplification was conducted using a 96-well plate which included 90 samples, two non-template controls and two positive amplification/genotype controls within each batch.

The resulting PCR products were subjected to restriction digestion for 3 h at 37°C using 5 U *Nla*III. After restriction digestion, 5 µl loading dye (50 µl 1% xylene cyanol in 1 ml 30% Ficoll/H_2O) were added to each sample. The digested products were resolved at 90 V for 30–45 min on a 3% Metaphor agarose gel (FMC BioProducts, Rockland, ME) containing 0.5 µg/ml ethidium bromide. A 100 bp marker was used as a size standard for each gel lane. The gel was visualized under UV light using an Eagle Eye II still video system (Stratagene, La Jolla, CA) and the resulting photographs were digitally enhanced to provide better fragment resolution.

The *COMT-LL* genotype was represented by 114, 35 and 36 bp fragments; *COMT-HH* by 96, 35, 36 and 18 bp fragments; *COMT-HL* by 114, 96, 35, 36 and 18 bp fragments. The presence of the constant 35 and 36 bp fragments served as an internal control for restriction digestion. The 18 bp fragment was difficult to visualize because of both its small size and co-migration with the similarly sized primer residue; however, detection of this fragment was not critical in determining genotypes. Genotypes were determined based upon independent scoring of the results by two reviewers who were unaware of case/control status. Samples that failed to produce decisive results after the first assay run were repeated. In addition to the internal controls included within each batch, genotyping was repeated on 5% of samples in a random fashion. Repeat assays were 100% concordant with initial results. The *COMT* assay was unreadable for 28 cases and 21 controls due to poor PCR amplification, thus results are presented for 654 cases and 642 controls.

Statistical analysis

Adjusted odds ratios and 95% CIs were calculated from unconditional logistic regression models and used to estimate RRs (27). PROC GENMOD of the software package SAS (v.6.11; SAS Institute, Cary, NC) allowed for incorporation of offset terms derived from the sampling probabilities used to identify eligible participants (25) and for adjustment for race (as a 2 level categorical variable) and age (as an 11 level ordinal variable that reflected 5 year age categories).

A reference date of the date of diagnosis (for cases) or selection (for controls) was used to define exposure status for all study participants. Race was classified according to self-report. For the present analysis, we classified women as African-American and white. Among women classified as white, we included nine American Indians, eight Asian Americans, four Hispanic Americans and two women who listed their race as 'multi-racial'. Menopausal status was defined as follows: for women under the age of 50 years, post-menopausal status was assigned to women who had undergone natural menopause, bilateral oophorectomy or irradiation of the ovaries; for women aged 50 years or older, menopausal status was assigned on the basis of cessation of menstruation, except for women taking hormone replacement therapy (HRT), who were classified as post-menopausal regardless of whether their menstrual periods had stopped.

Current BMI, a measure of obesity, was calculated as weight divided by the square of height (kg/m^2), using measurements taken at the time of interview. To investigate interaction between *COMT* genotype and BMI, the study population was dichotomized based upon the median value of BMI among controls (27.8 kg/m^2). BMI was included as a continuous variable for adjustment in multivariate models. Recent physical activity was based upon a dichotomous (yes/no) response to the question, 'In the last three months (prior to reference date), did you do anything on a weekly basis to help keep you physically fit?' We inquired about frequency and type of recent physical activity, but too few women engaged in vigorous physical activity to permit analyses using multiple levels of this variable. Participants were also asked about their level of physical activity at age 12: women who reported they were less physically active than their peers were compared with women who were as active or more active at this age. Women were also asked whether they participated in vigorous sports or serious athletic training at age 12 (dichotomous). Lifetime use of HRT or oral contraceptives (OCs) were categorized as 'ever' (3 months or more of use) versus 'never' for stratified analyses and as continuous variables (months) for adjustment in multivariate models.

We compared observed *COMT* genotype frequencies to expected genotype frequencies calculated on the basis of observed allelic frequencies, assuming a Hardy–Weinberg equilibrium. Departure from a Hardy–Weinberg equilibrium was tested among African-American and white case and control groups using a goodness-of-fit χ^2 test (28). In order to compare our results with those of Lavigne *et al*. (19) and Thompson *et al*. (20), we calculated RRs for *COMT* and breast cancer by comparing women with *COMT-HL* and *COMT-LL* genotypes with *COMT-HH* as the reference group. We calculated overall RRs, as well as separate estimates for pre-menopausal and post-menopausal women and African-American and white women. Multivariate logistic models were used to adjust for potential confounding factors (27). Covariates included age at menarche (continuous), parity (three categories), breast feeding (ever/never), family history of breast cancer (yes/no for first-degree relative), benign breast biopsy (yes/no), duration of HRT use (continuous), duration of OC use (continuous), BMI (continuous), recent physical activity (yes/no), duration of smoking (4 levels) and alcohol consumption. In this population, relatively few women consumed large amounts of alcohol, thus analyses of this variable are based upon a dichotomous (ever/never) categorization only. Participants with missing values for any of the variables in a regression model were not included in such analyses. We also adjusted for smoking as a 3 level variable (never/current/past), but results were unchanged.

We investigated modification of RRs for *COMT* and breast cancer by BMI, use of HRT, OC use, physical activity and smoking. To assess interaction on a multiplicative scale, we compared RRs for *COMT* across strata of each potential modifying variable (using the coding described above) and conducted statistical tests for heterogeneity (27). Next, we fitted separate logistic models with interaction terms between *COMT* and each potential modifying variable. For each variable, we conducted a likelihood ratio test (LRT) comparing the model with interaction terms to a reduced model containing only the main effects (27). To assess interaction on an additive scale, indicator variables were created for each category of joint exposure to *COMT* genotype (*HH*, *HL* and *LL*) and the potential modifying factor, using the hypothesized low risk category as a common reference group. The low risk category was omitted when the indicator variables were incorporated into multivariate models adjusting for the remaining covariates (29,30). Use of a common referent group and evaluation of departure from additive effects has been described

Table I. *COMT* allele and genotype frequencies and RRs and 95% CIs for breast cancer among African-American and white participants in the CBCS

	African-Americans			Whites		
	Cases (n = 265)	Controls (n = 263)		Cases (n = 389)	Controls (n = 379)	
Allele frequencies (= no. of alleles/no. of chromosomes)						
COMT-H	0.69	0.65		0.50	0.47	
COMT-L	0.31	0.35		0.50	0.53	
Genotype frequencies (= no. of participants with genotype/total no. of participants)						
COMT-HH	0.49	0.42		0.27	0.22	
COMT-HL	0.40	0.45		0.47	0.50	
COMT-LL	0.11	0.13		0.26	0.28	
RRs and 95% CI[a]						
COMT-HH	129	110	1.0 (ref)	103	86	1.0 (ref)
COMT-HL	106	119	0.8 (0.5–1.2)	184	188	0.8 (0.6–1.2)
COMT-LL	30	34	0.8 (0.4–1.5)	102	105	0.7 (0.5–1.1)

[a]Adjusted for age, menopausal status, age at menarche, parity, breast feeding, breast cancer in first-degree relative, breast biopsy, alcohol consumption, HRT use, OC use, BMI, smoking and recent physical activity. Models included offset terms to control for sampling probabilities, as described in Materials and methods.

as the most appropriate approach to addressing synergy or interaction of potential component causes (31), the underlying hypothesis for gene–environment interaction (30).

Results

Characteristics of the CBCS population have been described previously (32). African-American controls showed a somewhat lower frequency of the *COMT-L* allele (0.35) than white controls (0.53) (Table I). Among African-American and white cases and controls, genotype frequencies did not differ significantly from expected values under the assumption of a Hardy–Weinberg equilibrium ($P > 0.9$ for each group).

COMT genotype was unrelated to breast cancer risk. The overall adjusted RRs were 0.8 (95% CI: 0.6–1.1) for *COMT-HL* and 0.8 (95% CI: 0.6–1.1) for *COMT-LL*, compared with *COMT-HH*. Adjusted RRs did not differ substantially among African-Americans and whites (Table I) or among pre- and post-menopausal women (Table II). RRs for *COMT* were unchanged when we adjusted for age and race only, for the covariates utilized by Lavigne *et al*. (19) or Thompson *et al*. (20) or when we stratified cases based upon stage at diagnosis (data not shown).

RRs for the joint effects of *COMT* genotype and current BMI are presented for pre- and post-menopausal women in Table II. Using participants with *COMT-HH* genotype and BMI less than or equal to the median as a common reference group, we did not observe positive associations for any combination of *COMT* genotype and BMI. Furthermore, *COMT* genotype was unrelated to breast cancer risk among post-menopausal women with a BMI greater than the median. Results were unchanged when we employed a variety of cut-off points for BMI, including those specified by Lavigne *et al*. (19) and Thompson *et al*. (20) (data not shown). Similar results were obtained for the joint effects of *COMT* genotype and recent physical activity (Table II). We also did not observe evidence of modification of RRs on a multiplicative scale (for LRT $P = 0.76$ for interaction between *COMT* and BMI and $P = 0.59$ for interaction between *COMT* and recent physical activity). We did not observe modification of RRs for *COMT* genotype on additive or multiplicative scales when we considered physical activity at age 12, current use of HRT, OC use or smoking (data not shown).

Discussion

We did not observe a positive relationship between *COMT* genotype and breast cancer risk, overall or among subgroups of women defined by race or menopausal status. Our results differ from two recent epidemiological studies, which reported increased breast cancer risk among women with one or two copies of the low activity *COMT* allele (*COMT-L*). Lavigne *et al*. (19) reported increased breast cancer risk for the *COMT-LL* genotype among post-menopausal women with BMI greater than the median. Thompson *et al*. (20) observed a positive association for the *COMT-HL* and *COMT-LL* genotypes among pre-menopausal women with high BMI. Our results provided no evidence for a positive association between *COMT-HL* or *COMT-LL* genotype and breast cancer risk among pre- or post-menopausal women nor among pre- or post-menopausal women with BMI greater than the median. Thompson *et al*. (20) also reported an inverse association for the *COMT-HL* and *COMT-LL* genotypes among post-menopausal women with low BMI, whereas we did not observe such an association. We also did not observe a positive association for *COMT* genotype among women who were physically inactive (recently or at age 12), who reported use of HRT or OC or who smoked cigarettes.

The distribution of *COMT* genotypes among white controls in our dataset (Table I) was similar to the distributions among controls reported by Lavigne *et al*. (19) (24% for *COMT-HH*, 49% for *COMT-HL* and 27% for *COMT-LL*) and Thompson *et al*. (20) (27% for *COMT-HH*, 48% for *COMT-HL* and 25% for *COMT-LL*). We observed a slightly higher frequency of the *COMT-HH* genotype and a lower frequency of *COMT-LL* among white and African-American cases compared with controls (Table I). Lavigne *et al*. (19) reported a higher frequency of *COMT-LL* (31%) and *COMT-HL* (50%) genotypes and a lower frequency of *COMT-HH* (19%) among cases compared with controls, while Thompson *et al*. (20) reported a lower frequency of *COMT-LL* (19%), a higher frequency of *COMT-HL* (56%) and a lower frequency of *COMT-HH* (25%).

Table II. RRs and 95% CIs for breast cancer according to *COMT* genotype, *COMT* genotype and current BMI and *COMT* genotype and recent physical activity, stratified by menopausal status

Genotype	Pre-menopausal			Post-menopausal		
	Cases (n = 331)	Controls (n = 297)	RR (95% CI)	Cases (n = 323)	Controls (n = 344)	RR (95% CI)
All subjects[a]						
COMT-HH	116	88	1.0 (ref)	116	108	1.0 (ref)
COMT-HL	148	142	0.8 (0.5–1.2)	142	164	0.9 (0.6–1.3)
COMT-LL	67	67	0.7 (0.4–1.2)	65	72	0.8 (0.5–1.4)
BMI $\leqslant$ 27.8[b]						
COMT-HH	60	39	1.0 (ref)	49	38	1.0 (ref)
COMT-HL	86	70	0.9 (0.5–1.5)	80	87	0.7 (0.4–1.2)
COMT-LL	47	39	0.8 (0.4–1.4)	40	45	0.7 (0.4–1.3)
BMI $>$ 27.8[b]						
COMT-HH	54	46	0.8 (0.4–1.4)	65	69	0.6 (0.3–1.1)
COMT-HL	58	71	0.6 (0.3–1.0)	59	76	0.6 (0.3–1.1)
COMT-LL	20	27	0.5 (0.3–1.1)	23	27	0.6 (0.3–1.2)
Physically active[c]						
COMT-HH	50	37	1.0 (ref)	66	53	1.0 (ref)
COMT-HL	73	73	0.7 (0.4–1.2)	78	87	0.7 (0.4–1.1)
COMT-LL	41	32	0.9 (0.5–1.8)	35	32	0.9 (0.5–1.7)
Physically inactive[c]						
COMT-HH	66	51	0.9 (0.5–1.6)	50	55	0.6 (0.3–1.0)
COMT-HL	75	69	0.8 (0.4–1.4)	64	77	0.7 (0.4–1.2)
COMT-LL	26	35	0.5 (0.2–0.9)	30	40	0.5 (0.3–0.9)

Models included offset terms to control for sampling probabilities as described in Materials and methods.
[a]Adjusted for age, race, age at menarche, parity, breast feeding, breast cancer in first-degree relative, breast biopsy, alcohol consumption, HRT use, OC use, BMI, smoking and recent physical activity.
[b]Adjusted for age, race, age at menarche, parity, breast feeding, breast cancer in first-degree relative, breast biopsy, alcohol consumption, HRT use, OC use, smoking and recent physical activity.
[c]Adjusted for age, race, age at menarche, parity, breast feeding, breast cancer in first-degree relative, breast biopsy, alcohol consumption, HRT use, OC use, BMI and smoking.

Our study was considerably larger than Lavigne *et al*. (19) (113 cases and 114 controls) and Thompson *et al*. (20) (281 cases and 289 controls). In addition, we examined associations among African-American women and had sufficient statistical power to explore modification of RRs by a variety of covariates (33).

The observed relationship between *COMT* genotype, physical activity and breast cancer risk in our data was the opposite of that expected: women with the *COMT-LL* genotype who were physically inactive showed a slightly stronger inverse association with breast cancer risk (Table II). Catechol estrogens are hypothesized to mediate estrogen-induced carcinogenesis in breast tissue (17). Based upon the results of Cree *et al*. (21), showing that exercise increased the proportion of catechol estrogens which undergo *O*-methylation by COMT, we hypothesized that women with low physical activity and low COMT activity would accumulate more catechol estrogens and therefore be at increased risk of breast cancer. Our data on adult physical activity was limited to the period immediately preceding diagnosis in cases or enrollment in controls. Thus, our analysis of joint effects must be interpreted with caution. To fully examine the relationship of physical activity and breast cancer, information on recreational and occupational activity is required over a longer period of time (5). An additional shortcoming of our study is the lower response rate among controls compared with cases, but this should not affect the primary analyses of *COMT*, since participation is unlikely to be related to genotype.

In order to interpret association studies of estrogen metabolism genes and breast cancer, greater knowledge is needed of the role of estrogen-induced carcinogenesis in the breast (10,11,34,35). With respect to *COMT*, there may be no overall association of genotype with breast cancer, but a strong association could be observed in the presence of relevant environmental exposures (36). We examined several of the most obvious candidates for gene–environment interaction, but cannot exclude additional exposures which might be of interest. Since recent epidemiological studies of physical activity and breast cancer yield inconsistent results (6,37), future studies might benefit from incorporating information on *COMT* and other genes involved in estrogen metabolism. The possibility of gene–gene interaction should also be addressed. Lavigne *et al*. (19) reported interaction between *COMT* and glutathione *S*-transferase M1 (*GSTM1*) and P1 (*GSTP1*) genotypes in risk of breast cancer. We did not examine gene–gene interaction because information on *GSTM1* and *GSTP1* was unavailable. In conclusion, our results suggest that *COMT* genotype is not related to breast cancer risk, however, further studies are needed to more fully investigate this relationship.

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