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An Association between the Allele Coding for a Low Activity Variant of Catechol-*O*-methyltransferase and the Risk for Breast Cancer[1]

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Abstract

Mounting evidence suggests that catechol metabolites of estradiol may contribute to the development of estrogen-induced cancers. *O*-Methylation, catalyzed by catechol-*O*-methyltransferase (COMT), inactivates catechol estrogens. COMT is polymorphic in the human population, with 25% of Caucasians being homozygous for a low activity allele of the enzyme (*COMT*^{LL}). We hypothesized that low activity COMT may be a risk factor for human breast cancer and designed a PCR-based RFLP assay to determine *COMT* genotype in a cohort of 112 matched, nested case-control samples. In the total study population, the odds ratios for the association of breast cancer risk with *COMT*^{HL} and *COMT*^{LL} genotypes were 1.30 [confidence interval (CI), 0.66–2.58] and 1.45 (CI, 0.69–3.07), respectively. Postmenopausal *COMT*^{LL} women had a greater than 2-fold increased risk of developing breast cancer [odds ratio (OR), 2.18; CI, 0.93–5.11]. The association of *COMT*^{LL} with the development of postmenopausal breast cancer was stronger and statistically significant in those women with a body mass index >24.47 kg/m^2 (OR, 3.58; CI, 1.07–11.98). When *COMT*^{LL} was combined with either glutathione *S*-transferase (*GST*) *M1* null or with *GSTP1* Ile-105-Val/Val-105-Val (intermediate/low activity, respectively) genotypes, the risk for developing postmenopausal breast cancer was also significantly increased. Our findings suggest that the allele encoding low activity COMT may be an important contributor to the postmenopausal development of breast cancer in certain women.

Introduction

Epidemiological evidence over the last two decades has suggested that cumulative lifetime exposure to estrogen may play an important role in the etiology of breast cancer (1, 2). It has been hypothesized that the mechanism by which estrogen contributes to an elevated breast cancer risk primarily involves estrogen receptor-mediated cell proliferation associated with spontaneous replication errors (2). There is mounting evidence that supports a complementary pathway involving indirect and direct genotoxicity originating from estrogen metabolites, including 16α-hydroxyestrone, estrogen catechols, and the estrogen quinones that result from oxidation of the catechols (3, 4). An individual's ability to eliminate estrogen metabolites may, therefore, be a critical determinant of the level of genetic damage that will accumulate over a lifetime.

In extrahepatic tissues, *O*-methylation catalyzed by COMT[3] is a major contributor to the inactivation of catechol estrogens (4, 5). M-COMT and S-COMT forms of this enzyme are coded for by one gene with two different transcription start sites (6). Quercetin, an inhibitor of COMT, enhanced estrogen carcinogenesis in the Syrian hamster kidney model, and it was proposed that this was due to higher effective concentrations of estrogen catechol metabolites resulting from the loss of COMT-mediated detoxification (7).

COMT has long been known to be polymorphic in the human population. Twenty-five % of Caucasians are homozygous for a low activity allele of the enzyme (8). The low activity enzyme is heat labile and 4–5-fold less effective at methylating catechol substrates *in vitro*. The sequence for human *COMT* from two cDNA sources, placenta and HepG2 hepatoma cells, was published recently, and these sequences differ by 1 bp (9, 10). The HepG2 sequence contains a G to A transition in exon 4, which results in a valine-to-methionine substitution in the protein and the generation of a new *Nla*III site in the *COMT* DNA sequence. This coding polymorphism is associated with both low activity and heat instability in the COMT protein (11).

On the basis of the evidence for a possible role of the estradiol catechol metabolites in experimental estrogen carcinogenesis, we hypothesized that low-activity COMT may be a risk factor for human breast cancer. To test this hypothesis, we designed a PCR-based RFLP assay to test for the *COMT* polymorphism in human DNA and, in a nested case-control study design, determined whether the low-activity *COMT* genotype is associated with the subsequent development of breast cancer.

Materials and Methods

Study Population. The Washington County, Maryland, research specimen bank was established in 1989. It consists of stored plasma, buffy coat, and RBCs, as well as baseline questionnaire data on individuals who participated after signing an informed consent. Incident breast cancer cases that developed between 1989 and 1995 were identified among 25,081 county residents by linkage of participants to the Washington County Cancer Registry. One hundred fifteen women who developed breast cancer were then matched to 115 control women based on age (within 1 year), race (all were Caucasian), time of blood donation, and menopausal status, including days since last menstrual period for premenopausal women and years since last menstrual period for postmenopausal women. At the time of blood donation, all participants completed a brief questionnaire that included information on smoking status, height, weight, and medication use in the previous 48 h. Cases and controls were also sent a self-administered questionnaire to obtain more detailed information about breast cancer risk factors. Response rates were similar for

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[3] The abbreviations used are: COMT, catechol-*O*-methyltransferase; M-COMT, membrane COMT; S-COMT, soluble COMT; *COMT*^{LL}, low-activity COMT genotype; *COMT*^{HH}, high-activity COMT genotype; *COMT*^{HL}, heterozygote COMT genotype; OR, odds ratio; CI, confidence interval; BMI, body mass index; GST, glutathione *S*-transferase; GSH, glutathione.

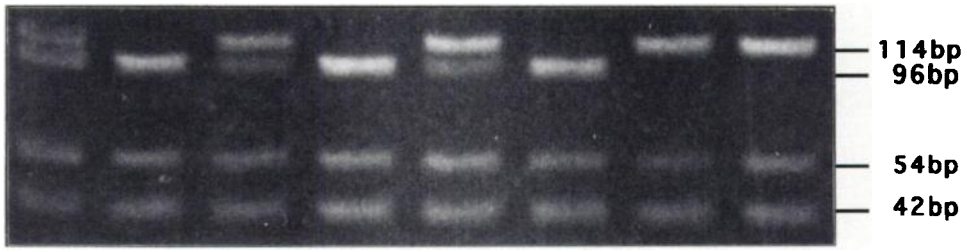

Fig. 1. PCR-based RFLP analysis for the *COMT* polymorphism on samples 144 and 148–153. *Lane 1* contains the combined restriction fragments of DNA amplified from HepG2 and MCF-10A cells, which do and do not contain the *COMT* polymorphism, respectively. *Lanes 2, 4,* and *6* contain DNA from individuals homozygous for the low-activity allele ($COMT^{LL}$), *Lanes 7* and *8* contain DNA from individuals homozygous for the high-activity allele ($COMT^{HH}$), and *Lanes 3* and *5* contain DNA from heterozygotes ($COMT^{HL}$).

cases (90.4%) and controls (89.6%). Exposure to factors such as use of hormone replacement therapy among cases and controls was truncated at the date of diagnosis of the case.

Sample Preparation. Blood was collected in 20-ml heparinized tubes and centrifuged within 6 h of collection. Plasma, buffy coat, and RBCs were separated and stored at −70°C within 24 h of collection. The buffy coat was kept frozen until DNA extraction by high-salt fractionation (12), followed by chloroform:isoamyl alcohol extraction (13). The DNA was then resuspended in Tris-EDTA (pH 7.6) and stored at 4°C until genotype analysis. DNA of sufficient quality for *COMT* genotype analysis was extracted from 112 cases and their matched controls.

PCR and RFLP Analysis. Genomic DNAs from the cases and controls were analyzed for the presence of the G-to-A transition by a PCR-based RFLP assay. A 237-bp fragment of the *COMT* gene (accession numbers Z26490 and Z26491), including the part of exon 4 that contains the polymorphism, was first amplified by PCR using the forward primer TACTGTGGCTACTCAGCTGTGC (*M-COMT* 1827–1848) and the reverse primer GTGAACGTGGTGTGAACACC (*M-COMT* 2063–2044). A 25-μl PCR reaction containing 50 ng of genomic DNA, 250 μM each deoxynucleotide triphosphates, 300 nM each primer, 1× reaction buffer (Perkin-Elmer, Branchburg, NJ), and 0.63 unit Taq polymerase (Perkin-Elmer) was placed in a Hybaid OmniGene thermocycler. After denaturing for 3 min at 94°C, the DNA was amplified for 35 cycles at 94°C for 30 s, 60°C for 20 s, and 72°C for 30 s, followed by a 5-min extension at 72°C. A control reaction containing everything except DNA was included in every PCR experiment. Four to five μl of each PCR product, including the control, were run on a 1% agarose gel to ensure that the expected 237-bp product was generated. Then, 8 μl of the PCR product were digested for 3 h at 37°C with 10 units of *Nla*III (New England BioLabs, Beverly, MA). The products of the restriction digest were combined with 1 μl of loading buffer (10% Ficoll and 0.25% xylene cyanol) and run on a 10% nondenaturing polyacrylamide gel in 1× Tris-borate-EDTA buffer at 50 V for 2–3 h. Restriction fragments of 27, 42, and 54 bp were present in every digested sample. Although the presence of a G at position 1947 (*M-COMT* codon 158) generates a unique 114-bp fragment, this same fragment is divided into unique 96- and 18-bp fragments when position 1947 contains an A. The 27- and 18-bp fragments run off the gel during electrophoresis. As a control for expected product sizes, HepG2 DNA, which is homozygous for $COMT^{LL}$ (9), and MCF-10A DNA, obtained from the MCF-10A human breast epithelial cell line (14) and found by us to be $COMT^{HH}$ (data not shown), were combined after amplification and *Nla*III digestion and run on every gel. The gels were stained with ethidium bromide and visualized by UV. The identity of the samples as cases or controls was unknown during the PCR/RFLP analysis. These data were provided to the statistician (H-Y. H.), who then performed the statistical analyses.

Statistical Analysis. The association between *COMT* genotype and the subsequent development of breast cancer was examined using conditional and unconditional logistic regression to calculate the ORs and 95% CIs. Individuals homozygous for high-activity COMT were designated as the referent category.

Of interest was the relationship between *COMT* genotypes and the risk of breast cancer within categories of other known risk factors. The variables examined in the stratified analyses included menopausal status of women with breast cancer at the time of diagnosis; family history of breast cancer in mother, sister, or grandmother; smoking history; history of alcohol use; BMI; ever use of hormone replacement therapy or oral contraceptives; a history of oophorectomy; and polymorphisms in GST isoforms M1 and P1.[4] For most of these variables, stratified analyses required breaking the match to preserve adequate numbers in the strata. Therefore, the ORs presented were estimated using unconditional logistic regression, adjusting for the matching factors of age and menopausal status at the time of blood donation. For the analyses where both could be estimated, unmatched analyses produced similar results to the matched analyses.

Results

Our study population included 115 cases and 115 controls matched by age, race, time of blood collection, and menopausal status at time of blood collection. Mean age for cases and controls was 60.4 (±11.7 SD) and 60.2 (±11.5 SD), respectively. Conditional logistic regression analysis revealed no statistically significant differences between the cases and controls for presumed risk factors such as age, age at menarche, age at first birth, use of birth control pills or hormone replacement therapy, BMI >24.47 kg/m^2, and smoking or alcohol drinking. In contrast, a family history of breast cancer in a mother or sister was associated with an increase in breast cancer risk (OR, 2.86; CI, 1.21–6.76), and having two ovaries removed was associated with a decrease in risk (OR, 0.46; CI, 0.21–0.98).

RFLP identification of *COMT* genotype was determined on all but two of the cases and one of the controls, leaving 112 matched pairs for the genotype data analysis. Fig. 1 illustrates the results of the PCR-based RFLP analysis for the *COMT* polymorphism on seven representative samples. It shows that the $COMT^{HH}$, $COMT^{LL}$, and $COMT^{HL}$ genotypes are easily distinguishable using this technique.

In this study population, the distribution of the $COMT^{HH}$, $COMT^{LL}$, and $COMT^{HL}$ genotypes in the total population controls and in the postmenopausal controls was 24, 49, and 27% and 26, 47, and 27%, respectively (calculated from data in Table 1). This is in close agreement with the approximately 25, 50, and 25%, distribution reported for Caucasians (8). This distribution was somewhat skewed in the premenopausal controls (16, 56, and 28%), perhaps because of the small numbers of subjects in this group of women (Table 1).

The association between *COMT* genotype and breast cancer was first examined in the total study population. Compared to women with the $COMT^{HH}$ genotype, the ORs for the association of breast cancer with $COMT^{HL}$ and $COMT^{LL}$ were 1.30 and 1.45, respectively (Table 1).

Because different risk factors may contribute to breast cancer development in pre- and postmenopausal women, the association between *COMT* genotype and breast cancer risk was further analyzed by stratifying for menopausal status at the time of diagnosis (Table 1). This analysis suggested that, compared to the $COMT^{HH}$ subjects, the presence of the low-activity *COMT* allele in women with premenopausal breast cancer was associated with a decreased risk. However, the number of individuals in each strata were quite small, and these results do not approach statistical significance. In contrast, this analysis suggested a stronger association between the risk for developing postmenopausal breast cancer and the presence of the low-activity *COMT* allele. In this group, the OR increased to 1.7 for cases with the $COMT^{HL}$ genotype and to 2.2 for cases with the $COMT^{LL}$ genotype.

[4] K. J. Helzlsouer, O. Selmin, H-Y. Huang, P. T. Strickland, S. Hoffman, A. J. Alberg, M. A. Watson, G. W. Comstock, and D. A. Bell. A study of the association between glutathione *S*-transferase M1 (GSTM1), P1 (GSTP1), and T1 (GSTT1) genetic polymorphisms and the development of breast cancer, submitted for publication.

The latter OR showed a borderline statistical significance ($P = 0.07$), as did an analysis for trend ($COMT^{HH} \rightarrow COMT^{HL} \rightarrow COMT^{LL}$, $P_{trend} = 0.08$), suggesting a possible increased risk for developing postmenopausal breast cancer with decreasing COMT activity.

Additional factors were analyzed to determine whether they affected the relationship between *COMT* genotype and the risk for developing postmenopausal breast cancer. The association of $COMT^{LL}$ genotype with enhanced susceptibility for postmenopausal breast cancer did not vary significantly with family history of breast cancer, history of alcohol use, ever use of hormone replacement therapy, use of birth control pills, or history of oophorectomy (data not shown). Upon stratification by smoking, the association of developing postmenopausal breast cancer with the presence of the low-activity *COMT* allele was stronger in nonsmokers. Compared to $COMT^{HH}$, the ORs for $COMT^{HL}$ and $COMT^{LL}$ were 1.90 (CI, 0.73–4.96) and 2.81 (CI, 0.98–8.01), respectively, with $P_{trend} = 0.06$. For smokers, the ORs for $COMT^{HL}$ and $COMT^{LL}$ were 1.87 (CI, 0.42–8.43) and 1.79 (CI, 0.37–8.59), respectively.

Others have shown that obesity in postmenopausal women is positively correlated with breast cancer risk, possibly due to higher rates of conversion of androstenedione to estrogen in adipose tissue (1). Upon stratifying by median BMI (Table 2), the data showed that women with the $COMT^{LL}$ genotype and a BMI >24.47 kg/m^2 had a statistically significant, 3.6-fold increased risk for developing postmenopausal breast cancer. In addition, there was a statistically significant trend for increased risk with variant allele dosage.

In a parallel investigation in this study population on the association of GST polymorphisms with breast cancer risk, Helzlsouer *et al.*[4] found that the *GSTM1* null and the Val-105-Val *GSTP1* (low-activity) genotypes were associated with a 2-fold increased breast cancer risk. Because various GST isoforms are involved in the inactivation of free radicals, the formation of which is caused by oxidative damage (15, 16), we performed an analysis of the risk of postmenopausal breast cancer associated with the low-activity *COMT* allele stratified by these *GST* genotypes. When $COMT^{LL}$ was combined with either *GST M1* null or with *GSTP1* Ile-105-Val/Val-105-Val (intermediate/low activity, respectively) genotypes, the risk for developing postmenopausal breast cancer was significantly increased 3–4-fold (*GSTM1* null: OR, 4.10; CI, 1.17–14.27; 25 cases, 9 controls, and *GSTP1* Ile-105-Val + *GSTP1* Val-105-Val: OR, 3.40; CI, 1.17–12.33; 18 cases, 11 controls). Furthermore, there was a statistically significant trend for increased risk with $COMT^{L}$ dosage (*GSTM1* null, $P_{trend} = 0.02$; *GSTP1* Ile-105-Val + *GSTP1* Val-105-Val, $P_{trend} = 0.04$).

Table 1 *Association between COMT genotype and development of breast cancer according to menopausal status at time of diagnosis of cancer*

COMT	Case	Control	OR (95% CI)	P
Total population				
HH	21	27	1.0[a]	
HL	57	56	1.34 (0.67, 2.69)[a]	0.41
LL	35	31	1.42 (0.69, 2.94)[a]	0.34
			$P_{trend} = 0.37$	
Premenopausal at diagnosis				
HH	7	4	1.0[b]	
HL	14	14	0.57 (0.14, 2.40)[b]	0.45
LL	3	7	0.24 (0.04, 1.51)[b]	0.13
			$P_{trend} = 0.13$	
Postmenopausal at diagnosis				
HH	14	23	1.0[b]	
HL	43	42	1.70 (0.77, 3.75)[b]	0.19
LL	32	24	2.18 (0.93, 5.11)[b]	0.07
			$P_{trend} = 0.08$	

[a] Matched OR from conditional logistic regression.
[b] Unmatched OR from unconditional logistic regression, adjusted for matching factor of age.

Table 2 *Association between COMT genotype and development of postmenopausal breast cancer, stratified by BMI*

COMT	Case	Control	OR[a] (95% CI)	P
BMI ≤ 24.47 kg/m^2				
HH	8	12	1.0	
HL	20	19	1.55 (0.51, 4.77)	0.44
LL	7	11	0.92 (0.25, 3.40)	0.90
			$P_{trend} = 0.93$	
BMI > 24.47 kg/m^2				
HH	6	11	1.0	
HL	23	23	1.77 (0.56, 5.64)	0.33
LL	25	13	3.58 (1.07, 11.98)	0.04
			$P_{trend} = 0.03$	

[a] Unmatched OR from unconditional logistic regression, adjusted for age and menopausal status at baseline.

Discussion

The objective of this prospective molecular epidemiology study was to test the hypothesis that the low-activity form of COMT is a risk factor for breast cancer. This hypothesis arose from the mounting evidence that catechol metabolites of estradiol, particularly 4-hydroxy estradiol, may contribute to estrogen carcinogenesis and that COMT has an important role in the inactivation of estrogen catechol metabolites in extrahepatic tissues (4). The results of this prospective study suggest that the low activity form of COMT is associated with an increased risk of developing postmenopausal breast cancer in certain women. Thus, postmenopausal women homozygous for the low activity allele of *COMT* showed an increased risk of breast cancer, although the increased OR was only of borderline statistical significance. However, the increase in risk was stronger and statistically significant in postmenopausal women who had a BMI >24.47 kg/m^2 or those who were *GSTM1* null or those with at least one low-activity *GSTP1* allele. Because catechol estrogens are known substrates for COMT, this implies that modulation of the levels of catechols over an individual's lifetime may directly modify the risk of developing breast cancer: the longer and/or greater the exposure, the greater the risk.

Although the role of estrogen metabolites in breast cancer has been controversial (4), there has been much evidence for a role for the catechol metabolites in cancer development. Estrogen catechols are formed by oxidation of the steroid ring by specific cytochrome P-450 isoforms at either the C-2 or C-4 positions, forming 2-OH and 4-OH estrogen catechols, respectively (4). It has been found that these metabolites can directly and indirectly cause oxidative DNA damage (4, 17–19), lipid peroxidation (20), and through their quinone metabolites, DNA adducts (21). Reactive oxygen species formation by catechol estrogen-mediated redox cycling *in vivo* likely contributes to estrogen carcinogenicity observed in several model systems through increased oxidative DNA damage (4). In fact, increased oxidative DNA damage has been detected during estrogen carcinogenesis in Syrian hamster kidney and rat liver (19, 22).[5] In the Syrian hamster kidney model of estrogen-induced carcinogenesis, 2-OH and 4-OH catechols of estrogens are formed (23), and the 4-OH catechol appears to contribute most significantly to carcinogenesis (24).

Malins *et al.* (25) have shown elevated oxidative DNA damage in human breast tumor tissue as compared to normal breast tissue. In a recent study, Liehr and Ricci (26) reported that microsomes from human breast tumor tissue metabolized estradiol predominantly to its carcinogenic 4-OH catechol at higher levels than normal breast tissue microsomes. This finding provides a possible mechanism for generation of the increased oxidative DNA damage observed by Malins *et al.* (25).

[5] A. M. Seacat, M. A. O'Gorman, P. F. Scholl, T. A. Young, J. D. Groopman, and J. D. Yager, unpublished observations.

On the basis of evidence that abnormal accumulation of 4-OH catechol can produce local cellular damage due to oxidative stress, Creveling (5) hypothesized that the ability of COMT to methylate estrogen catechols might play a critical role in preventing estrogen-induced carcinogenesis. Creveling suggested that low levels of *O*-methylation might cause an inappropriate accumulation of catechol estrogens in mammary epithelium. At that time, it was already known that in humans, COMT activity in RBCs, which correlates with its activity in other tissues, exhibits differences among individuals of up to 5-fold (8). Based on these activity differences, it was proposed that the enzyme is polymorphic. It was also demonstrated in Caucasians that there is a Mendelian inheritance of high-, intermediate-, and low-activity phenotypes in the ratio of approximately 25, 50, and 25%, respectively; however, the genetic cause was not yet known (8). Molecular genetic studies subsequently showed that a coding polymorphism in *COMT*, which can be detected by RFLP analysis, is responsible for the low enzyme-activity phenotype (11).

In our analysis of risk associated with *COMT* genotype in women who developed pre- *versus* postmenopausal breast cancer, we observed that the trend toward increased risk with $COMT^{LL}$ associated with the postmenopausal onset of breast cancer was reversed in women with premenopausal onset. According to our hypothesis, this should not be the case, because low-activity COMT would presumably increase catechol levels both pre- and postmenopausally. However, there were fewer cases and controls in this premenopausal group, and the differences did not approach statistical significance. The relationship between *COMT* genotype and breast cancer risk in premenopausal women will, therefore, have to be tested in a larger study.

Stratifying individuals with postmenopausal breast cancer by smoking status showed that an increased risk was associated with $COMT^{LL}$ in both never- and ever-smokers, although the association was stronger and approached borderline statistical significance in nonsmokers. The weaker association in the ever-smokers could be due to effects of smoking on the levels of multiple enzymes, including some of those that metabolize estradiol.

A statistically significant increase in risk was seen in $COMT^{LL}$ postmenopausal women with a BMI >24.47 kg/m^2. Because postmenopausal obesity is thought to be associated with higher estrogen levels (2), estrogen catechols in turn may be elevated in obese women, particularly in those with low activity COMT, predisposing them to the development of breast cancer.

The association of genetic polymorphisms in three GST isozymes with breast cancer was determined in the same population in a parallel study.[4] Because GSTM1 and GSTP1 isozymes are involved in the deactivation of products of oxidative damage (15), we examined them in combination with $COMT^{LL}$. When $COMT^{LL}$ was stratified by *GSTM1* null and Ile-105-Val or Val-105-Val *GSTP1* genotypes, the risk for postmenopausal breast cancer in $COMT^{LL}$ women was approximately 4-fold greater than in the $COMT^{HH}$ subjects. The glutathione transferases are a family of enzymes that catalyze the conjugation of reduced GSH with various electrophilic compounds (15, 16). Compared to other GST isozymes, GSTM1 has been shown to have greater activity with several 4-hydroxyalkenals, which are genotoxic α,β-unsaturated aldehydes produced as a result of free radical-initiated lipid peroxidation. As mentioned above, increased lipid peroxidation has been observed in kidneys of Syrian hamsters treated with estradiol (20). The *GSTM1* null genotype could, therefore, contribute to increased risk of breast cancer due to a loss of the ability to detoxify these reactive aldehydes resulting from estradiol-mediated lipid peroxidation. Another group of genotoxic α,β-unsaturated aldehydes are the base propenals, DNA degradation products whose generation is initiated by reactive oxygen species (15). The GSTP1 isozyme was found to have activity for several base propenals, particularly adenine propenal. This finding suggests that GSTP1 is also important in the cellular response to oxidative stress and in this case could increase susceptibility to estrogen-induced damage due to the low-activity GSTP1 variant. As suggested by Helzlsouer *et al.*,[4] the high-risk genotypes of these isozymes of glutathione transferase may also be contributing to an increase in breast cancer risk due to their involvement in the metabolism of several other carcinogenic compounds, such as polycyclic aromatic hydrocarbons and benzo-(*a*)pyrene (16), which could also contribute to breast carcinogenesis.

In summary, our results suggest an increased susceptibility to postmenopausal onset of breast cancer that is related to individual genetic differences in the ability to detoxify estrogen catechol metabolites. Others have found an association between increased risk for breast cancer in African-American and Caucasian women and polymorphisms in cytochrome P-450 1A1, the enzyme responsible for metabolism of estradiol to its 2-hydroxy catechol metabolite (27, 28). Recently, Feigelson *et al.* (29) reported that a polymorphism in the cytochrome P-450 gene *CYP17* is associated with an increased risk for developing breast cancer. This polymorphism would create a new Sp1-type site in the promoter region of this gene, which theoretically would increase CYP17 expression with the consequences of increased production of estradiol. In this report, the authors hypothesize the existence of other breast cancer susceptibility genes that may carry moderate associated risk but potentially high population risk due to their relatively common presence in the general population (29). *COMT* may exemplify this type of gene. The $COMT^{LL}$ genotype is present in about 25% of United States Caucasians and, when considered in combination with other common risk factors such as BMI >24.47 kg/m^2 or GST high-risk genotypes,[4] could contribute significantly to the development of postmenopausal breast cancer. It would be of great interest to examine the effects of the *COMT* low-activity allele in combination with other relevant polymorphic genotypes, such as *CYP17* and cytochrome P-450 1A1 in light of these findings. Because our report is the first to show an association between the allele for low-activity COMT and the postmenopausal development of breast cancer, additional molecular epidemiology studies need to be performed to test these findings and to determine the mechanism by which low-activity COMT contributes to breast cancer.

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