**Carcinogenesis** vol.24 no.4 pp.681–687, 2003
DOI: 10.1093/carcin/bgg022

# Catechol-*O*-methyltransferase gene polymorphism and post-menopausal breast cancer risk

**Sara Wedrén[1,5], Tove Rylander Rudqvist[2], Fredrik Granath[1], Elisabete Weiderpass[1,3], Magnus Ingelman-Sundberg[2], Ingemar Persson[1,4] and Cecilia Magnusson[1]**

[1]Department of Medical Epidemiology and [2]Division of Molecular Toxicology, Institute of Environmental Medicine, Karolinska Institutet, SE-171 77 Stockholm, Sweden, [3]International Agency for Research on Cancer, Lyon, France and [4]Swedish Medical Products Agency, Uppsala, Sweden

[5]To whom correspondence should be addressed
Email: sara.wedren@mep.ki.se

**Estrogen is involved in breast carcinogenesis. Hypotheses have been raised that its effect is modified by enzymes such as catechol-*O*-methyltransferase (COMT) that deactivate potentially genotoxic estrogen metabolites. We have investigated the association between the functional genetic Val108/158Met polymorphism in *COMT* and breast cancer risk in a large population-based case–control study performed in the genetically homogeneous Swedish population. We determined *COMT* genotype in 1534 women with invasive breast cancer and in 1504 control women and calculated odds ratios (OR) and 95% confidence intervals (CI) from logistic regression models. There was no overall association between *COMT* genotype and breast cancer risk. However, the L allele was associated with an increased risk for lobular breast cancer, with OR 2.0 (95% CI 1.2–3.5) for HL and 1.7 (95% CI 0.9–3.0) for LL. In exploratory subset analyses, we found no statistically significant interaction, but some indication of a positive association between HL and LL genotypes and breast cancer among women with diabetes mellitus and a negative association among nulliparous women. Based on our findings, COMT activity alone does not seem to play a major role in breast carcinogenesis, but may be of importance in certain histotypes or in conjunction with other exposures.**

## Introduction

Estrogens are known to be central in breast carcinogenesis. Estrogens induce proliferation but may also initiate cancer via metabolic activation to potentially carcinogenic catechol estrogen metabolites (1). The impact of this latter mechanism in human breast carcinogenesis remains undefined.

Catechol-*O*-methyltransferase (COMT) is a phase II enzyme that deactivates catechol estrogens into non-genotoxic methyl ethers that can be easily excreted by the body. In *COMT* there is a single nucleotide polymorphism (SNP) that results in an amino acid exchange from valine to methionine at codon 108/158 in the membrane-bound/cytoplasmic protein (2). The Val108/158Met allele encodes a thermolabile variant of the enzyme that in homozygotes confers an about 2- to 4-fold lower catalytic activity (3,4). Heterozygous individuals have intermediate and homozygous wild-type have high enzyme activity. Carrying low activity *COMT* alleles is hypothesized to be associated with an increased risk for breast cancer through accumulation of genotoxic metabolites (5). However, results from epidemiological studies (Table I) have been inconsistent. In an attempt to shed further light on the issue of whether *COMT* genotype has an influence on breast cancer risk we have performed a large case–control study in a genetically homogeneous population with extensive information about lifestyle and tumor characteristics.

**Abbreviations:** BMI, body mass index; CI, confidence interval; COMT, catechol-*O*-methyltransferase; DASH, dynamic allele-specific hybridization; NIDDM, non-insulin-dependent diabetes; OR, odds ratios; SNP, single nucleotide polymorphism.

## Materials and methods

### *Founding study*

This nation wide case–control study encompassed all incident cases of primary breast cancer among women 50 and 74 years of age resident in Sweden between October 1993 and March 1995 (6). Breast cancer patients were identified at diagnosis through a notification system organized within the six Swedish regional cancer registries, to which reporting of all cancers is mandatory. Controls were randomly selected in 5 year age strata (to match the expected age frequency distribution among the cases) from the Swedish Registry of Total Population.

Cases were asked to participate in the study by their respective physicians. Only when patients consented were we notified of their identity and address and they were sent a mailed questionnaire asking for detailed information about intake of menopausal hormones and oral contraceptives, weight, height, reproductive history, medical history and other lifestyle factors. Controls were contacted with the questionnaire directly by us. Eighty-four percent of eligible cases ($n = 3345$) and 82% of the controls ($n = 3454$) subsequently completed the questionnaire. Among these controls, 455 who failed to return the mailed questionnaire were interviewed by telephone. Results from the founding study have been published (6–10). Information about tumor type, size and stage has been collected from medical records in an ongoing follow-up study of all breast cancer cases.

### *Present study*

We randomly selected 1500 women with invasive breast cancer and 1500 age frequency matched controls among post-menopausal participants without any previous malignancy (except cervix carcinoma *in situ* or non-melanoma skin cancer) in the founding study. In order to increase statistical power in subgroup analyses, we additionally selected all remaining women (cases and controls) who had used menopausal hormones (either medium potency estrogen treatment only or medium potency estrogen in combination with progestin) for at least 4 years (191 cases and 108 controls) and all women with self-reported diabetes mellitus (104 cases and 110 controls). In total, 1801 cases and 1712 controls were selected.

### *Collection of biological samples*

We contacted all selected living women by mail and those who gave informed consent received a blood sampling kit by mail. Whole blood samples were drawn at a primary health care facility close to the woman's home and sent to us by standard mail. A majority of the samples arrived at our department within 1 day from blood draw. All blood samples were immediately stored at −20°C. Breast cancer cases who did not want to donate a blood sample were asked whether they consented to our use of archived paraffin-embedded tissue samples taken at breast cancer surgery. We also attempted to retrieve archived tissue samples from all deceased breast cancer cases. Samples were anonymized and subsequently transferred to the laboratories. We obtained blood

**Table I.** Review of the case–control studies of COMT polymorphism and breast cancer risk

| Reference | Cases/controls (post-menopausal) | Population | Odds ratios compared with HH[a] | Subgroup analysis |
|---|---|---|---|---|
| Lavigne *et al.*, 1997 (24) | 112/112 (89/89) | American-Caucasian | Post-menopausal<br>HL: 1.70 (0.77–3.75)<br>LL: 2.18 (0.93–5.11) | Tendency for increased risk for post-menopausal L carriers with BMI > 24.47 |
| Thompson *et al.*, 1998 (25) | 281/289 (140/155) | American-Caucasian | Post-menopausal<br>HL: 0.6 (0.4–1.2)<br>LL: 0.4 (0.2–0.7) | Decreased risk for post-menopausal L carriers with BMI ≤ 23 |
| Millikan *et al.*, 1998 (26) | 654/642 (323/344) | American-'white' (389/379) and African-American | 'White'<br>HL: 0.8 (0.6–1.2)<br>LL: 0.7 (0.5–1.1)<br>Post-menopausal<br>HL: 0.9 (0.6–1.3)<br>LL: 0.8 (0.5–1.4) | Decreased risk for post-menopausal physically inactive LL versus active |
| Huang *et al.*, 1999 (33) | 150/150 (75/77) | Taiwanese | LL: 4.02 (1.12–19.08) versus HL and HH | Increased risk for carriers of L in conjunction with high risk CYP17 and CYP1A1 genotypes and prolonged estrogen exposure |
| Yim *et al.*, 2001 (29) | 163/163 (72/72) | Korean | Post-menopausal<br>HL: 2.0 (1.00–3.96)<br>LL: 0.2 (0.02–1.50)<br>LL uncommon | Increased risk among L carrying never-drinkers and never-smokers |
| Hamajima *et al.*, 2001 (28) | 150/165 (68/87) | Japanese | Post-menopausal<br>HL: 1.53 (0.77–3.04)<br>LL: 0.76 (0.27–2.16) | Nothing significant |
| Goodman *et al.*, 2001 (30) | 112/113 (89/89) | American-Caucasian | See (24) | Increased risk with L and less than or equal to median levels of folate or above median levels of homocysteine |
| Bergman-Jungeström and Wingren, 2001 (34) | 126/117 (0/0) | Swedish | HL: 0.85 (0.35–2.1)<br>LL: 0.87 (0.34–2.2) | None performed |
| Mitrunen *et al.*, 2001 (27) | 483/482 (319/278) | Finnish | Post-menopausal<br>HL: 0.63 (0.37–1.09)<br>LL: 0.75 (0.51–1.11) | Decreased risk for local disease in post-menopausal LL<br>Decreased risk for post-menopausal LL women with BMI ≤ 25.4<br>Increased risk for LL long term (>30 months) menopausal hormone users |
| Mitrunen *et al.*, 2002 (31) | 483/482 (319/278) | Finnish | See (27) | Increased risk with LL and GSTP1 Ile/Ile or GSTT1 null among menopausal hormone users that was even more pronounced among long-term users |

[a]HH are homozygous for the high activity COMT allele (Val/Val), HL are heterozygous (Val/Met) and LL are homozygous for the low activity allele (Met/Met).

samples or archived tissue samples for 1322 and 247 breast cancer patients, respectively, and blood samples from 1272 control women. Some controls from the founding study that were not selected for this study donated blood for a parallel study about endometrial cancer. As these women originated from the same source population and fulfilled all inclusion criteria, they could potentially also serve as controls.

We isolated DNA from 3 ml whole blood using a Wizard Genomic DNA Purification Kit (Promega, Madison, WI) according to the manufacturer's instructions. From non-malignant cells in paraffin-embedded tissue, we extracted DNA using a standard phenol/chloroform/isoamyl alcohol protocol (11).

*Genetic analyses*

We used two methods for *COMT* genotyping: multiplex fluorescent solid phase minisequencing (hereafter called minisequencing) (12) and dynamic allele-specific hybridization (DASH) (13). We validated results from the above two methods with a restriction fragment length polymorphism method and through replicates. All PCRs were performed on a Perkin Elmer GeneAmp 9700 system and the presence of amplicons was checked on agarose gels. The multiplex PCR that we used for minisequencing was sensitive to DNA quality. In order to increase genotyping success to include suboptimal samples we switched from minisequencing to DASH. DASH is a robust high throughput method that allows separate PCR reactions with retained efficiency.

*Minisequencing*

Five SNPs were analyzed simultaneously, one in the *COMT* gene, reported here, and four in other genes, reported elsewhere. SNPs are detected by specific extension with single fluorescein-labeled (NEN/DuPont, Boston, MA) ddNTPs of a primer that anneals immediately adjacent to the variable site. To minimize pipetting steps, we used an AutoLoad kit (Amersham, Pharmacia Biotech, Uppsala, Sweden) where PCR products are immobilized on streptavidin-coated comb-shaped manifold supports. The primer sequences are shown in Table II. The 25 µl multiplex PCR mix contained 100 ng DNA, 20 mM Tris–HCl pH 8.4, 50 mM KCl, 0.2 mM each dNTP, 1.8 mM $MgCl_2$, 10% DMSO, 0.625 U Platinum® *Taq* polymerase (Life Technologies, Rockville, MD) and 0.25 µM primer. The initial denaturation was performed at 95°C for 2 min, followed by 35 cycles each consisting of denaturation at 95°C for 15 s, annealing at 56°C and extension at 72°C for 1 min, followed by a final extension at 72°C for 7 min. An aliquot of 6 µl biotinylated PCR product was immobilized on streptavidin-coated sequencing combs. The minisequencing procedure was performed as described by Pastinen *et al.* (12), with the following modifications; the four minisequencing reaction mixtures contained 26 mM Tris–HCl pH 9.5, 6.5 mM $MgCl_2$, 2 µM each sequencing primer (Table II), 0.05 µM F-ddGTP or 0.25 µM F-ddATP or 0.5 µM F-ddCTP or 0.5 µM F-ddUTP, 0.5 µM the three other unlabeled ddNTPs and 0.26 U Thermo Sequenase DNA polymerase (Amersham Pharmacia Biotech). The extended primers where analyzed on an ALF DNA-Autosequencer (Amersham, Pharmacia Biotech) and the chromatograms were interpreted by direct visual inspection.

*DASH*

The PCR mix contained 10 ng DNA, 15 mM Tris–HCl pH 8.0, 50 mM KCl, 0.12 µM biotinylated 5′-primer, 0.6 µM 3′-primer (Table II), 0.2 mM each dNTP (HPLC purified; Interactiva GmbH, Ulm, Germany), 3.0 mM $MgCl_2$, 5% DMSO, 0.6 U AmpliTaq GOLD polymerase (Applied Biosystems,

**Table II.** Primers used for COMT genotyping

| | | |
|---|---|---|
| Minisequencing | | |
| PCR | 5′ | 5′-GGCGAGGCTCATCACCATCG-3′ |
| | 3′ (biotinylated) | 5′-CAGGTCTGACAACGGGTCAG-3′ |
| Extension primer | | 5′-ATCACCCAGCGGATGGTGGATTTCGCTGGC-3′ |
| DASH | | |
| PCR | 5′ (biotinylated) | 5′-CCAACGGATGGTGGATTTCG-3′ |
| | 3′ | 5′-TCAGTCATGCACACCTTGTC-3′ |
| DASH probes | Wild-type | 5′-GTCCTTCACGCCAGCGA-3′ |
| | Mutant | 5′-GTCCTTCATGCCAGCGA-3′ |

Foster City, CA) in a total volume of 25 µl. The initial denaturation was performed at 95°C for 10 min, followed by 38 cycles each consisting of denaturation at 95°C for 15 s, annealing at 60°C for 30 s, followed by a final extension at 72°C for 4 min. DASH assays were performed as described by Prince *et al*. (13) and genotypes were scored from fluorescence curves as described by Howell *et al*. (14).

*RFLP analysis*

As reference analysis, the two *COMT* alleles were identified with a PCR-based restriction fragment length polymorphism method according to Lachman *et al*. (2) with the exception that the PCR was performed with the same primers and identical conditions as used in the multiplex PCR (Table II). An aliquot of 8 µl of the PCR product was digested with *Nla*III (New England Biolabs) for 3 h at 37°C. The digested PCR products were visualized on a 4% low melting point agarose gel.

*Statistical analyses*

We checked the assumption of a Hardy–Weinberg equilibrium among the controls using standard $\chi^2$ statistics. As it is known that *COMT* polymorphism has a co-dominant mode of action (15), we considered each genotype to carry its own associated risk. We assessed potential association between genotype and known or suspected risk factors for breast cancer among controls by $\chi^2$ statistics. We calculated odds ratios (OR) and 95% confidence intervals (CI) from conditional logistic regression models using the maximum likelihood method. Models were conditioned on age group and on sampling category to ensure that age-associated non-participation or over-sampling of long-term menopausal hormone users and diabetics did not introduce bias. The potential for confounding by other known breast cancer risk factors is doubtful since these factors may lie in the causal pathway between the studied genetic variation and breast cancer.

We investigated interaction between *COMT* genotype and duration of menopausal estrogen treatment use and diabetes mellitus by performing separate analyses over strata of these exposures. We could not estimate the joint effect of genotype and hormone use or diabetes mellitus due to our sampling scheme. Interactions with these variables were tested through a Wald statistic that evaluates whether the coefficients for genotype effect in successive strata of the risk factor in question are equal to 0. We studied interaction with body mass index (BMI), parity and family history in a model evaluating the joint effects of the risk factor and genotype. To test the interactions formally, these models were compared with models including only the main effects. The likelihood ratio test was used to generate a *P* value. In secondary analyses, we also explored possible interaction between genotype and other exposures.

We performed all analyses using SAS system PHREG procedure release 8.01 (SAS Institute Inc., Cary, NC).

## Results

We successfully genotyped 1534 breast cancer cases and 1252 controls. In the following statistical analysis, we included 252 additional genotyped controls from the same source population that were genotyped for a parallel endometrial cancer study (see above). Thus, the total number of controls included was 1504.

Selected characteristics of the study participants by genotype are shown in Table III. The genotype frequencies among the controls were in accordance with a Hardy–Weinberg equilibrium ($P = 0.83$). Among the controls, there were statistically significant associations between *COMT* genotype and parity (0, 1, 2 or >2 childbirths, $P = 0.04$) and between genotype and intake of brassica vegetables (<1, 1–3 or >3 times per week, $P = 0.03$), but the associations did not follow any dose–response or other plausible pattern. Remaining potential breast cancer risk factors did not co-vary with *COMT* genotype among controls (data not shown). When introduced into the logistic model none of the variables changed the estimates of association between genotype and breast cancer. Thus, the final model contained only *COMT* genotype, but the analyses were conditioned on the variables used for sampling, i.e. use of menopausal estrogen alone (never, <4 years or ≥4 years), use of menopausal estrogen in combination with progestin (never, <4 years or ≥4 years) and diabetes mellitus (yes or no).

We found no relation between *COMT* genotype and breast cancer risk overall (Table IV). Yet, the low activity allele appeared associated with an increased risk for lobular type breast cancer; OR 1.8 (95% CI 1.1–3.1) for HL and 1.6 for LL (95% CI 0.9–2.8) (Table IV). There were no associations between *COMT* genotype and tumor size or tumor stage at diagnosis (data not shown).

Results of exploratory stratified analyses are shown in Tables V and VI. In Table V we present analyses stratified by menopausal hormone use and diabetes mellitus using women with the HH genotype as reference. Our data do not speak in favor of an interaction between genotype and menopausal hormone use. Among HL women, short-term use of any menopausal hormones was negatively associated and long-term use was positively (and non-significantly) associated with breast cancer (Table V). The contrast between short- and long-term hormone users with the HL genotype conferred a *P* for interaction of 0.06, but no difference in the effect of genotype by duration of use was seen among LL women. There was a suggestion that *COMT* genotype was associated with breast cancer risk among women with diabetes mellitus but not among those without diabetes (*P* for interaction = 0.07) (Table V).

In Table VI we present associations between breast cancer and jointly genotype and either BMI, parity or first degree family history of breast cancer. Established associations between breast cancer and BMI, parity and family history did not appear to vary substantially with *COMT* genotype (Table VI). It could be noted, however, that among nulliparous women, HL and LL genotypes seemed to be negatively associated with breast cancer. No suggestions of a gene–environment interaction emerged in secondary analyses stratified by other known or suspected risk factors (data not shown).

## Discussion

There was no overall association between *COMT* genotype and breast cancer risk. However, genotypes conferring lowered

**Table III.** Characteristics among breast cancer cases and controls by COMT genotype

| | COMT genotype HH[a] | | | COMT genotype HL[a] | | | COMT genotype LL[a] | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. cases/ controls | Cases | Controls | No. cases/ controls | Cases | Controls | No. cases/ controls | Cases | Controls |
| Crude genotype percentage | 290/289 | 18.9 | 19.2 | 790/736 | 51.5 | 48.9 | 453/479 | 29.6 | 31.9 |
| Means | | | | | | | | | |
| Age (years) | 290/289 | 63.3 | 62.9 | 790/736 | 63.4 | 63.2 | 453/479 | 63.3 | 63.3 |
| Age at menarche (years) | 253/261 | 13.5 | 13.6 | 728/671 | 13.5 | 13.6 | 412/444 | 13.6 | 13.5 |
| Age at menopause (years) | 290/289 | 50.5 | 49.9 | 790/736 | 50.7 | 49.9 | 453/479 | 50.5 | 50.4 |
| Parity | 290/289 | 1.9 | 2.3 | 790/736 | 1.9 | 2.1 | 453/479 | 1.8 | 2.2 |
| Age at first full term pregnancy (years) | 254/274 | 25.9 | 25.3 | 670/650 | 25.2 | 24.8 | 380/435 | 25.4 | 24.4 |
| Weight gain during adult life (kg) | 236/218 | 12.5 | 10.9 | 656/591 | 13.9 | 11.5 | 374/373 | 12.8 | 12.9 |
| Body mass index (kg/m$^2$) | 289/283 | 25.7 | 25.4 | 785/725 | 25.9 | 25.4 | 450/475 | 25.8 | 25.9 |
| Height (cm) | 290/287 | 164.5 | 163.7 | 786/730 | 164.1 | 163.7 | 452/477 | 164.5 | 163.8 |
| Alcohol intake (g/week) | 261/234 | 2.6 | 2.5 | 698/625 | 2.4 | 2.3 | 406/403 | 2.6 | 2.2 |
| Brassica intake (times/week) | 290/256 | 3.7 | 3.5 | 789/689 | 3.4 | 3.8 | 453/445 | 3.4 | 4.2 |
| Percentages | | | | | | | | | |
| Duration of menopausal hormone use (years) | | | | | | | | | |
| 0 | 189/204 | 66 | 72 | 545/525 | 70 | 72 | 287/346 | 64 | 74 |
| $<4$ | 51/40 | 18 | 14 | 101/122 | 13 | 17 | 81/65 | 18 | 14 |
| $\geq 4$ | 47/40 | 16 | 14 | 138/78 | 18 | 11 | 82/59 | 18 | 13 |
| Oral contraceptive use | 279/272 | 31 | 35 | 758/710 | 31 | 35 | 439/459 | 32 | 34 |
| First degree family history of breast cancer | 283/252 | 18 | 12 | 770/678 | 17 | 8 | 443/437 | 14 | 9 |
| Previous benign breast disease | 290/289 | 14 | 11 | 790/736 | 14 | 10 | 453/479 | 14 | 10 |
| Smoker | 290/289 | 41 | 46 | 790/736 | 42 | 43 | 453/479 | 46 | 41 |
| Diabetes mellitus | 290/256 | 6 | 9 | 788/688 | 10 | 7 | 453/445 | 9 | 9 |
| $>2$ h physical activity/week | | | | | | | | | |
| At age $<18$ | 289/272 | 37 | 45 | 784/708 | 44 | 43 | 450/466 | 46 | 46 |
| At age 18–30 | 288/274 | 31 | 34 | 785/712 | 35 | 35 | 449/464 | 33 | 37 |
| In recent years | 288/273 | 29 | 35 | 786/712 | 33 | 36 | 445/464 | 33 | 36 |

[a]HH are homozygous for the high activity COMT allele (Val/Val), HL are heterozygous (Val/Met) and LL are homozygous for the low activity allele (Met/Met).

**Table IV.** Overall analyses of the association between COMT genotype and breast cancer risk with OR and 95% CI

| | COMT genotype HH[a] | | | COMT genotype HL[a] | | | COMT genotype LL[a] | | | $P$[b] |
|---|---|---|---|---|---|---|---|---|---|---|
| | Cases/controls | OR | CI | Cases/controls | OR | CI | Cases/controls | OR | CI | |
| Overall association[c] | | | | | | | | | | |
| All cancers | 281/245 | 1 | Reference | 767/662 | 1.0 | 0.8–1.2 | 442/433 | 0.9 | 0.7–1.1 | 0.31 |
| Ductal cancers | 216/245 | 1 | Reference | 570/662 | 1.0 | 0.8–1.2 | 315/433 | 0.8 | 0.6–1.0 | 0.14 |
| Lobular cancers | 19/245 | 1 | Reference | 97/662 | 2.0 | 1.2–3.5 | 55/433 | 1.7 | 0.9–3.0 | 0.05 |

[a]HH are homozygous for the high activity COMT allele (Val/Val), HL are heterozygous (Val/Met) and LL are homozygous for the low activity allele (Met/Met).
[b]$P$ value from $\chi^2$ test of difference in genotype distribution between cases and controls.
[c]Long-term users of menopausal hormones and diabetes mellitus patient were over-sampled. Thus, analyses were conditioned on 5 year age category, use of menopausal estrogen alone (3 categories), use of menopausal estrogen in combination with progestin (3 categories) and diabetes mellitus.

COMT activity were associated with an increased risk for lobular breast cancer. Similarly, HL or LL women with diabetes mellitus had a 2-fold increased risk for breast cancer, whereas there was no association among non-diabetics.

There is evidence that the connection between estrogen and breast cancer is stronger for the lobular histotype (16,17). Since low COMT activity could entail relatively higher levels of estrogens, the finding of an effect of COMT genotype on risk for lobular but not ductal breast cancer is biologically plausible. We did not find any support for our *a priori* hypothesis that *COMT* genotype should be more important among long-term users of menopausal hormones. Our finding of an apparently halved breast cancer risk among L allele heterozygotes with short-term use of combined estrogen/progestin treatment is difficult to interpret biologically and may be due to chance. We found an increased risk among women with the HL or LL genotypes and diabetes mellitus (most commonly non-insulin-dependent diabetes, NIDDM). NIDDM involves hyperinsulinemia and higher levels of insulin-like growth factors, which may stimulate proliferation of the breast epithelium (18) and thus render it more susceptible to DNA damage. Our finding of an increased risk for breast cancer among diabetics is also consistent with a recent finding that hyperglycemia reduces COMT activity (19), which would further enhance exposure of the breast epithelium to estrogens and their genotoxic metabolites. Lastly, our data suggested that the *COMT* L allele is protective among nulliparous women, a finding that needs corroboration and biological explanation.

**Table V.** Analyses of the association between COMT genotype and breast cancer risk stratified by use of menopausal hormones and diabetes mellitus with OR and 95% CI

| | COMT genotype HH[a] | | | COMT genotype HL[a] | | | COMT genotype LL[a] | | | *P* for interaction |
|---|---|---|---|---|---|---|---|---|---|---|
| | Cases/controls | OR[b] | CI | Cases/controls | OR[b] | CI | Cases/controls | OR[b] | CI | |
| Duration of menopausal hormone use | | | | | | | | | | |
| Never | 189/180 | 1 | Reference | 543/491 | 1.0 | 0.8–1.3 | 287/325 | 0.8 | 0.6–1.1 | |
| Any kind | | | | | | | | | | 0.06 |
| <4 years use | 39/24 | 1 | Reference | 78/86 | 0.5 | 0.3–0.9 | 63/41 | 0.9 | 0.5–1.8 | |
| ≥4 years use | 53/41 | 1 | Reference | 146/85 | 1.2 | 0.7–2.0 | 92/67 | 0.9 | 0.6–1.6 | |
| Estrogen only | | | | | | | | | | 0.69 |
| <4 years use | 17/15 | 1 | Reference | 38/33 | 0.9 | 0.4–2.3 | 23/18 | 1.3 | 0.5–3.6 | |
| ≤4 years use | 16/16 | 1 | Reference | 41/27 | 1.3 | 0.5–3.2 | 37/24 | 1.3 | 0.5–3.1 | |
| Estrogen + progestin | | | | | | | | | | 0.19 |
| <4 years use | 28/22 | 1 | Reference | 58/68 | 0.6 | 0.3–1.2 | 53/40 | 1.0 | 0.5–2.0 | |
| ≥4 years use | 39/24 | 1 | Reference | 108/63 | 1.1 | 0.6–2.1 | 58/42 | 0.8 | 0.4–1.6 | |
| Diabetes mellitus | | | | | | | | | | 0.07 |
| No | 267/223 | 1 | Reference | 687/615 | 0.9 | 0.8–1.2 | 401/398 | 0.8 | 0.7–1.1 | |
| Yes | 14/22 | 1 | Reference | 80/47 | 2.5 | 1.1–5.6 | 41/35 | 1.8 | 0.8–4.2 | |

[a]HH are homozygous for the high activity COMT allele (Val/Val), HL are heterozygous (Val/Met) and LL are homozygous for the low activity allele (Met/Met).
[b]Long-term users of menopausal hormones and diabetes mellitus patients were over-sampled. Thus, analyses were conditioned on 5 year age category, use of menopausal estrogen alone (3 categories), use of menopausal estrogen in combination with progestin (3 categories) and diabetes mellitus.

**Table VI.** Analyses of the joint effect of COMT genotype and BMI, parity and family history on breast cancer risk with odds ratios (OR) and 95% confidence intervals (CI)

| | COMT genotype HH[a] | | | COMT genotype HL[a] | | | COMT genotype LL[a] | | | *P* for interaction |
|---|---|---|---|---|---|---|---|---|---|---|
| | Cases/controls | OR[b] | CI | Cases/controls | OR[b] | CI | Cases/controls | OR[b] | CI | |
| Body mass index | | | | | | | | | | 0.53 |
| <25 | 147/123 | 1 | Reference | 361/344 | 0.9 | 0.7–1.2 | 206/204 | 0.8 | 0.6–1.1 | |
| 25–<28 | 69/68 | 0.9 | 0.6–1.4 | 176/175 | 0.9 | 0.6–1.2 | 123/134 | 0.8 | 0.6–1.1 | |
| ≥28 | 64/50 | 1.1 | 0.7–1.7 | 225/136 | 1.4 | 1.0–2.0 | 110/92 | 1.0 | 0.7–1.5 | |
| Parity | | | | | | | | | | 0.13 |
| Nullipara | 36/11 | 1 | Reference | 117/74 | 0.4 | 0.2–1.0 | 69/39 | 0.5 | 0.2–1.1 | |
| 1 childbirth | 61/50 | 0.3 | 0.1–0.7 | 147/117 | 0.3 | 0.2–0.7 | 109/74 | 0.4 | 0.2–0.9 | |
| 2 childbirths | 117/93 | 0.3 | 0.2–0.7 | 296/257 | 0.3 | 0.1–0.6 | 156/166 | 0.3 | 0.1–0.5 | |
| >2 childbirths | 67/91 | 0.2 | 0.1–0.5 | 207/214 | 0.3 | 0.1–0.6 | 108/154 | 0.2 | 0.1–0.4 | |
| Family history | | | | | | | | | | 0.90 |
| No | 231/223 | 1 | Reference | 642/621 | 1.0 | 0.8–1.2 | 380/396 | 0.9 | 0.7–1.1 | |
| Yes | 52/29 | 1.6 | 1.0–2.6 | 128/57 | 2.1 | 1.5–3.1 | 64/41 | 1.4 | 0.9–2.2 | |

[a]HH are homozygous for the high activity COMT allele (Val/Val), HL are heterozygous (Val/Met) and LL are homozygous for the low activity allele (Met/Met).
[b]Long-term users of menopausal hormones and diabetes mellitus patients were over-sampled. Thus, analyses were conditioned on 5 year age category, use of menopausal estrogen alone (3 categories), use of menopausal estrogen in combination with progestin (3 categories) and diabetes mellitus.

This is the largest study of the relation between *COMT* genotype and breast cancer published to date. Given our sample size and 5% significance level, we had 83% statistical power to detect a relative risk for breast cancer of 1.4 or higher for the LL genotype in overall analyses. Previously collected information about lifestyle factors and medication allowed us to explore their possible interaction with genotype. This may be vital since it is conceivable that an effect of genotype does not manifest itself in the absence of other exposures. Exposure information was collected retrospectively. However, in order for differential recall to influence our results, women would systematically have to answer according to genotype. *COMT* genotype has been linked to personality aspects such as schizophrenia (20) and prefrontal cognition (21), but these associations are weak and we believe that genotype guided recall or non-participation is unlikely. We did not have access to tumor biomarker characteristics and thus could not further define, beyond histologic type, distinct sub-types of breast cancer that may have distinct etiologies, as has been suggested by, among others, Thompson and Ambrosone (22). Most of our subgroup findings were associations between HL genotype and breast cancer. There are no data to indicate that those with intermediate COMT activity (i.e. the heterozygotes) should biochemically be at higher risk for disease. Rather, one would expect clear allele dose–response relations in the results. In the LL group, however, smaller sample size leads to more chance fluctuation and decreased power. Obviously, our results should be interpreted with caution because of the multiple tests performed and the lack of persuasive allele dose–response patterns.

Haiman and colleagues recently showed that, compared with *COMT* HH women, those with the LL genotype had higher mammographic density, an established risk factor for breast cancer (23). Case–control studies of the association between

*COMT* genotype and breast cancer have, all the same, been inconsistent (Table I). Initially, Lavigne and colleagues found indications of a moderately increased risk, with an allele dose–response pattern, for post-menopausal breast cancer for women with the HL or LL genotypes (24). Subsequently, investigators have found the L allele to confer protection against post-menopausal breast cancer (25), no altered risk (26,27) or increased risk among heterozygotes and protection among homozygotes (28,29). The conflicting previous results may indicate that *COMT* polymorphism has no influence on breast cancer risk and may represent chance findings. There are, however, alternative explanations. If *COMT* variation only reveals itself phenotypically in the presence of other exposures, diverging results may be due to varying prevalence of these exposures across different populations.

Several of the above studies found increased or decreased risks with *COMT* genotype in subgroups defined by one or combinations of other risk factors, such as menopausal status (24–27), BMI (24,25,27), physical activity (26), folate or homocysteine levels (30), hormone use (27,31) and glutathione *S*-transferase activity (31). However, since the power for subgroup analyses was generally low, the patterns inconsistent and the multiple comparisons problem evident, these results have to be carefully interpreted. We did not replicate the finding by Matsui and colleagues (32) of an association between the L allele and more advanced clinical stage of breast cancer or a higher degree of lymph node metastasis. To our knowledge, a connection between *COMT* polymorphism and lobular breast cancer, as indicated in this study, has not been reported before.

Based on our findings, COMT activity alone does not seem to play a major role in breast carcinogenesis but may be of importance in certain histotypes or in conjunction with other exposures.

## Acknowledgements

We are grateful to all women who have participated in this study, in particular those who took the effort to supply us with a blood sample. We are also indebted to Anna Christensson and Boel Bissmarck for invaluable assistance in blood sample collection, to Susanne Ahlberg for skillful technical assistance with the genotyping, and to Hans-Olov Adami for all-embracing guidance. Furthermore, we wish to express our gratitude to primary health care centers and pathology departments all over Sweden for their cooperation. This study was supported by the National Institutes of Health, grant number R03 CA83114, and by the Swedish Cancer Society, grant number 4112-B99-02XBB.

## References

1. Yager,J.D. and Liehr,J.G. (1996) Molecular mechanisms of estrogen carcinogenesis. *Annu. Rev. Pharmacol. Toxicol.*, **36**, 203–232.
2. Lachman,H.M., Papolos,D.F., Saito,T., Yu,Y.M., Szumlanski,C.L. and Weinshilboum,R.M. (1996) Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. *Pharmacogenetics*, **6**, 243–250.
3. Dawling,S., Roodi,N., Mernaugh,R.L., Wang,X. and Parl,F.F. (2001) Catechol-O-methyltransferase (COMT)-mediated metabolism of catechol estrogens: comparison of wild-type and variant COMT isoforms. *Cancer Res.*, **61**, 6716–6722.
4. Syvanen,A.C., Tilgmann,C., Rinne,J. and Ulmanen,I. (1997) Genetic polymorphism of catechol-O-methyltransferase (COMT): correlation of genotype with individual variation of S-COMT activity and comparison of the allele frequencies in the normal population and parkinsonian patients in Finland. *Pharmacogenetics*, **7**, 65–71.
5. Lavigne,J.A., Goodman,J.E., Fonong,T., Odwin,S., He,P., Roberts,D.W. and Yager,J.D. (2001) The effects of catechol-O-methyltransferase inhibition on estrogen metabolite and oxidative DNA damage levels in estradiol-treated MCF-7 cells. *Cancer Res.*, **61**, 7488–7494.
6. Magnusson,C.M., Persson,I.R., Baron,J.A., Ekbom,A., Bergstrom,R. and Adami,H.O. (1999) The role of reproductive factors and use of oral contraceptives in the aetiology of breast cancer in women aged 50 to 74 years. *Int. J. Cancer*, **80**, 231–236.
7. Magnusson,C., Colditz,G., Rosner,B., Bergstrom,R. and Persson,I. (1998) Association of family history and other risk factors with breast cancer risk (Sweden). *Cancer Causes Control*, **9**, 259–267.
8. Magnusson,C., Baron,J.A., Correia,N., Bergstrom,R., Adami,H.O. and Persson,I. (1999) Breast-cancer risk following long-term oestrogen- and oestrogen-progestin-replacement therapy. *Int. J. Cancer*, **81**, 339–344.
9. Terry,P., Wolk,A., Persson,I. and Magnusson,C. (2001) Brassica vegetables and breast cancer risk. *J. Am. Med. Assoc.*, **285**, 2975–2977.
10. Moradi,T., Nyren,O., Zack,M., Magnusson,C., Persson,I. and Adami,H.O. (2000) Breast cancer risk and lifetime leisure-time and occupational physical activity (Sweden). *Cancer Causes Control*, **11**, 523–531.
11. Isola,J., DeVries,S., Chu,L., Ghazvini,S. and Waldman,F. (1994) Analysis of changes in DNA sequence copy number by comparative genomic hybridization in archival paraffin-embedded tumor samples. *Am. J. Pathol.*, **145**, 1301–1308.
12. Pastinen,T., Partanen,J. and Syvanen,A.C. (1996) Multiplex, fluorescent, solid-phase minisequencing for efficient screening of DNA sequence variation. *Clin. Chem.*, **42**, 1391–1397.
13. Prince,J.A., Feuk,L., Howell,W.M., Jobs,M., Emahazion,T., Blennow,K. and Brookes,A.J. (2001) Robust and accurate single nucleotide polymorphism genotyping by dynamic allele-specific hybridization (DASH): design criteria and assay validation. *Genome Res.*, **11**, 152–162.
14. Howell,W.M., Jobs,M., Gyllensten,U. and Brookes,A.J. (1999) Dynamic allele-specific hybridization. A new method for scoring single nucleotide polymorphisms. *Nature Biotechnol.*, **17**, 87–88.
15. Spielman,R.S. and Weinshilboum,R.M. (1981) Genetics of red cell COMT activity: analysis of thermal stability and family data. *Am. J. Med. Genet.*, **10**, 279–290.
16. Chen,C.L., Weiss,N.S., Newcomb,P., Barlow,W. and White,E. (2002) Hormone replacement therapy in relation to breast cancer. *J. Am. Med. Assoc.*, **287**, 734–741.
17. Newcomb,P.A., Titus-Ernstoff,L., Egan,K.M., Trentham-Dietz,A., Baron,J.A., Storer,B.E., Willett,W.C. and Stampfer,M.J. (2002) Postmenopausal estrogen and progestin use in relation to breast cancer risk. *Cancer Epidemiol. Biomarkers Prev.*, **11**, 593–600.
18. Kaaks,R. and Lukanova,A. (2001) Energy balance and cancer: the role of insulin and insulin-like growth factor-I. *Proc. Nutr. Soc.*, **60**, 91–106.
19. Wang,J.P., Liu,I.M., Tzeng,T.F. and Cheng,J.T. (2002) Decrease in catechol-O-methyltransferase activity in the liver of streptozotocin-induced diabetic rats. *Clin. Exp. Pharmacol. Physiol.*, **29**, 419–422.
20. Park,T.W., Yoon,K.S., Kim,J.H., Park,W.Y., Hirvonen,A. and Kang,D. (2002) Functional catechol-O-methyltransferase gene polymorphism and susceptibility to schizophrenia. *Eur. Neuropsychopharmacol.*, **12**, 299–303.
21. Malhotra,A.K., Kestler,L.J., Mazzanti,C., Bates,J.A., Goldberg,T. and Goldman,D. (2002) A functional polymorphism in the COMT gene and performance on a test of prefrontal cognition. *Am. J. Psychiatry*, **159**, 652–654.
22. Thompson,P.A. and Ambrosone,C. (2000) Molecular epidemiology of genetic polymorphisms in estrogen metabolizing enzymes in human breast cancer. *J. Natl Cancer Inst. Monogr.*, 125–134.
23. Haiman,C.A., Bernstein,L., Berg,D., Ingles,S.A., Salane,M. and Ursin,G. (2002) Genetic determinants of mammographic density. *Breast Cancer Res.*, **4**, R5.
24. Lavigne,J.A., Helzlsouer,K.J., Huang,H.Y., Strickland,P.T., Bell,D.A., Selmin,O., Watson,M.A., Hoffman,S., Comstock,G.W. and Yager,J.D. (1997) An association between the allele coding for a low activity variant of catechol-O-methyltransferase and the risk for breast cancer. *Cancer Res.*, **57**, 5493–5497.
25. Thompson,P.A., Shields,P.G., Freudenheim,J.L. *et al.* (1998) Genetic polymorphisms in catechol-O-methyltransferase, menopausal status and breast cancer risk. *Cancer Res.*, **58**, 2107–2110.
26. Millikan,R.C., Pittman,G.S., Tse,C.K., Duell,E., Newman,B., Savitz,D., Moorman,P.G., Boissy,R.J. and Bell,D.A. (1998) Catechol-O-methyltransferase and breast cancer risk. *Carcinogenesis*, **19**, 1943–1947.
27. Mitrunen,K., Jourenkova,N., Kataja,V., Eskelinen,M., Kosma,V.M., Benhamou,S., Kang,D., Vainio,H., Uusitupa,M. and Hirvonen,A. (2001) Polymorphic catechol-O-methyltransferase gene and breast cancer risk. *Cancer Epidemiol. Biomarkers Prev.*, **10**, 635–640.
28. Hamajima,N., Matsuo,K., Tajima,K., Mizutani,M., Iwata,H., Iwase,T., Miura,S., Oya,H. and Obata,Y. (2001) Limited association between a catechol-O-methyltransferase (COMT) polymorphism and breast cancer risk in Japan. *Int. J. Clin. Oncol.*, **6**, 13–18.

29. Yim,D.S., Park,S.K., Yoo,K.Y. *et al*. (2001) Relationship between the Val158Met polymorphism of catechol O-methyl transferase and breast cancer. *Pharmacogenetics*, **11**, 279–286.
30. Goodman,J.E., Lavigne,J.A., Wu,K., Helzlsouer,K.J., Strickland,P.T., Selhub,J. and Yager,J.D. (2001) COMT genotype, micronutrients in the folate metabolic pathway and breast cancer risk. *Carcinogenesis*, **22**, 1661–1665.
31. Mitrunen,K., Kataja,V., Eskelinen,M., Kosma,V.M., Kang,D., Benhamou,S., Vainio,H., Uusitupa,M. and Hirvonen,A. (2002) Combined COMT and GST genotypes and hormone replacement therapy associated breast cancer risk. *Pharmacogenetics*, **12**, 67–72.
32. Matsui,A., Ikeda,T., Enomoto,K., Nakashima,H., Omae,K., Watanabe,M., Hibi,T. and Kitajima,M. (2000) Progression of human breast cancers to the metastatic state is linked to genotypes of catechol-O-methyltransferase. *Cancer Lett*., **150**, 23–31.
33. Huang,C.S., Chern,H.D., Chang,K.J., Cheng,C.W., Hsu,S.M. and Shen,C.Y. (1999) Breast cancer risk associated with genotype polymorphism of the estrogen-metabolizing genes CYP17, CYP1A1 and COMT: a multigenic study on cancer susceptibility. *Cancer Res*., **59**, 4870–4875.
34. Bergman-Jungestrom,M. and Wingren,S. (2001) Catechol-O-methyltransferase (COMT) gene polymorphism and breast cancer risk in young women. *Br. J. Cancer*, **85**, 859–862.

*Received August 30, 2002; revised January 13, 2003; accepted February 11, 2003*