Polymorphic Catechol-O-methyltransferase Gene and Breast Cancer Risk

Katja Mitrunen, Nadejda Jourenkova, Vesa Kataja, Matti Eskelinen, Yeli-Matti Kosma, Simone Benhamou, Daechee Kang, Harri Vainio, Matti Uusitupa, and Ari Hirvonen

Department of Industrial Hygiene and Toxicology, Finnish Institute of Occupational Health, 00250 Helsinki, Finland [K. M., A. H.]; Unit of Cancer Epidemiology, Gustave-Roussy Institute, 54805 Villejuif, France [N. I., S. B.]; Departments of Oncology [V. K.] and Surgery [M. E.], Kuopio University Hospital, 70211 Kuopio, Finland; Departments of Clinical Pathology and Forensic Medicine [V-M. K.] and Clinical Nutrition [M. U.], University of Kuopio, 70211 Kuopio, Finland; Department of Preventive Medicine, Seoul National University College of Medicine, Institute of Environmental Medicine, SNUMRC, Seoul 110-799, Korea [D. K.]; and International Agency for Research on Cancer, 69372 Lyon, France [H. V.]

Abstract

We examined 483 Finnish breast cancer cases and 482 population controls to determine the potential effect of polymorphic catechol-O-methyltransferase (COMT) genotype in individual susceptibility to breast cancer. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated by unconditional logistic regression after adjustment for known or suspected risk factors for breast cancer. When studied separately by menopausal status, the COMT-L allele-containing genotypes were inversely associated with premenopausal breast cancer, especially with advanced stage of the disease (OR, 0.44; 95% CI, 0.22–0.87). Among postmenopausal women a similar decreased risk was seen for local carcinoma associated with the COMT-LL genotype (OR, 0.55; 95% CI, 0.31–0.98). The lowest breast cancer risk was seen in the postmenopausal women with the COMT-LL genotype and low body-mass index (≤25.4 kg/m²); OR, 0.33; 95% CI, 0.13–0.83). Significantly increased risk, on the other hand, was seen for postmenopausal women with the COMT-LL genotype and long-term (>30 months) use of estrogen (OR, 4.02; 95% CI, 1.13–14.3), or with the COMT-L allele-containing genotypes and early age (≤12 years) at menarche (OR, 8.59; 95% CI, 1.85–39.8). Our study, therefore, suggests that the COMT genotype may define a portion of the individual breast cancer susceptibility that is associated with reproductive events and hormone exposure even if it does not seem to be a major overall risk factor for this malignancy.

Introduction

It is widely accepted that estrogens are involved in the development of breast tumors, and that elevated lifetime exposure to endo- and exogenous estrogens increase the risk of breast cancer (1–3). In supporting these views, early age at menarche, late menopause, and hormone replacement therapy have been shown to increase the breast cancer risk. Because the primary source of estrogens in postmenopausal women is from the conversion of androstenedione to estrone in adipose tissue, postmenopausal obesity is also associated with elevated risk of this malignancy. Because of the important role of steroid hormones in the etiology of breast cancer, genes involved in their metabolism and transport have also been intensively studied during recent years (4).

One possible mechanism for estrogen carcinogenicity is the hydroxylation of estradiol or estrone to form chemically reactive catechol estrogens that are known to have a role in estrogen-linked carcinogenesis (5). They are mainly inactivated by COMT, a Phase II enzyme that methylates catechol estrogens to less polar monomethyl ethers, which can then be excreted. If production of these conjugates is incomplete, catechol estrogens may be oxidated to reactive quinone/semiquinone intermediates capable of free radical formation, or direct formation of DNA adducts (6).

A 3- to 4-fold decreased methylation activity of COMT has been linked to a G to A transition in COMT gene, differentiating the COMT-H and COMT-L alleles, respectively, and resulting in valine to methionine amino acid change in codon 108/158 in the cytosolic/membrane-bound form of the protein (7). Around 25% of Caucasians are homozygous for the COMT-L allele (8), which has been hypothesized to increase the risk of breast cancer.

The few studies reported thus far on COMT genotypes and breast cancer risk have given discrepant results (9–13). Thompson et al. (13) reported an increased risk with the COMT-L allele in premenopausal women and a decreased risk among postmenopausal women, whereas Huang et al. (9) found an increased risk to be mainly attributable to postmenopausal breast cancer in Taiwanese women. Lavigne et al. (10) found significantly increased risk only among obese postmenopausal women carrying the COMT-LL genotype. In a recent study by Matsui et al. (12), the COMT-L allele was found to be associated with progression and lymph node metastasis of breast cancer in Japanese women. In contrast, Millikan et al. (11) found no significant associations in a large population-based case-control study.

Received 11/2/00; revised 3/8/01; accepted 3/15/01.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The work was supported by the Academy of Finland, the Finnish Konkordia Foundation, and EVO funds from Kuopio University Hospital.

2 To whom requests for reprints should be addressed, at Molecular Epidemiology Group, Department of Industrial Hygiene and Toxicology, Finnish Institute of Occupational Health, Topeliuksenkatu 41 a A, FIN-00250 Helsinki, Finland. Phone: 358-9-47472204; Fax: 358-9-47472110; E-mail: Ari.Hirvonen@occuphealth.fi.

3 The abbreviations used are: COMT, catechol-O-methyltransferase; OR, odds ratio; CI, confidence interval; BMI, body-mass index; WHR, waist:hip ratio.
We examined this issue further in a Finnish Caucasian study population. The potential modifying role of body size indicators and other factors known to contribute to the lifetime estrogen exposure, were also studied.

Materials and Methods

Study Subjects. This case-control study is an extension of Kuopio Breast Cancer Study that follows the protocol of the International Collaborative Study of Breast and Colorectal Cancer coordinated by the European Institute of Oncology in Milan. The study was approved by the Joint Ethics Committee of the University of Kuopio and Kuopio University Hospital. Participation was based on written consent. All women who had a suspicious breast lump or breast symptoms and lived in the catchment area of the Kuopio University Hospital during the study period from 1990 to 1995 were referred by a physician for further examination. Subjects with previous history of breast cancer in the past 5 years were ineligible. Thus, the final study population included a total of 516 women with newly diagnosed histologically confirmed breast cancer. Women were asked to participate in the study at the first hospital examination and were interviewed before any diagnostic procedures. The recruitment protocol missed 51 women within the hospital, all private patients who did not enter the hospital by the standard procedure. Furthermore, 11 cases were missed during the nurses’ 1-month strike in 1995. According to the comparison with the Finnish Cancer Registry, only 26 breast cancer cases were treated elsewhere. The contact rate for cases, calculated as described by Slattery et al. (14), was therefore 86%. Only 12 women later diagnosed with breast cancer refused to participate in the study. Because all of the interviewed women agreed to donate blood samples, the cooperation rate was 98%, and the overall response rate 84%.

Detailed data concerning socio-economic background, reproductive history, medical history, family history of breast cancer, current alcohol intake, smoking, and body-size indicators were recorded (15). At the time of the present study, lymphocyte DNA was available for 486 women. The DNA, however, was of poor quality in three of them, which excluded them from the study. Thus, the present study population included 483 incident breast cancer cases.

Malignant breast tumors were classified based on UICC TNM classification (16). For this study, women having axillary lymph node positive disease ($n = 164$) or metastatic (stage IV; $n = 16$) breast cancer at diagnosis were here considered as advanced cases. The cases diagnosed with a tumor confined to the breast, whether in situ ($n = 40$) or invasive ($n = 253$), were here designated as local. This categorization could not be performed for 10 patients because of missing information on lymph node involvement.

Healthy population control subjects with no breast symptoms or previous breast problems, and living in the catchment area of the cases, were drawn from the Finnish National Population Register. They were initially contacted by a letter explaining the study protocol, and later called by the research nurse. Data on the exact contact rate of the controls are not available. However, because of the exact individual social security number system used in Finland, the majority of the eligible controls are anticipated to have been contacted. In all, 514 women were interviewed in parallel with the cases, all of whom agreed to participate in a genetic study. The cooperation rate of the controls was 72%; the major reason for nonparticipation among controls was refusal. Of the women who agreed to participate in the study, the lymphocyte DNA was still available for 492 subjects. Four population controls who had received an earlier breast cancer diagnosis, two controls of non-Finnish origin, and four controls whose DNA was of poor quality were excluded from the study. Therefore, the final study population included 482 controls.

Genotyping Analyses. One hundred ng of lymphocyte DNA, extracted by standard techniques, were used as a template in a PCR-based assay that was performed essentially as described earlier (17). Briefly, the 217-bp PCR products amplified using specific primers (5′-TCG TGG ACG CGG TGA TTC AGG-3′ and 5′-AGG TCT GAC AAC GGG TCA GGC-3′) were digested using the NlaIII enzyme (New England BioLabs, Beverly, MA). The presence of an additional cleavage site differentiated the variant COMT-L allele from the wild-type COMT-H allele.

Positive and negative controls were used within each batch of PCR amplification, performed unaware of the case-control status. All of the samples with ambiguous results, as well as a random selection of 10% of all samples, were repeated to ensure laboratory quality control. Because the COMT genotype could not be achieved for 2 cases and 2 controls, results are presented for 481 cases and 480 controls.

Statistical Methods. Adjusted ORs and 95% CIs were calculated by unconditional logistic regression. Multivariate logistic models were used to adjust for known or suspected risk factors for breast cancer. Covariates included in the model were: age (5-year intervals), age at menarche (≤12, 13–14, ≥15 years), age at first full-term pregnancy (nulliparous, <25, 25–30, 30 years), number of full-term pregnancies (continuous), history of benign breast disease (no/yes), first-degree (mother, sister, daughter) family history of breast cancer (no/yes), postmenopausal use of estrogen (never/every), and BMI (kg/m$^2$; weight in kilograms divided by the square of height (meters); ≤25.4 kg/m$^2$, ≥25.4 kg/m$^2$). If data on any of the adjusting variables were missing, subjects were excluded from the logistic regression.

The median value for population controls was used to dichotomize WHR (0.91), BMI (25.4 kg/m$^2$), age at menarche (13 years), and length of postmenopausal use of estrogen (30 months) among ever users (at least 1 month). All of the results are shown stratified by menopausal status (at the time of the diagnosis of the case patient). Women who reported natural menopause or had undergone bilateral oophorectomy were classified postmenopausal. Hysterectomized women with intact ovaries (ovary; 40 cases and 41 controls) and women for whom the details of the operation were unknown (6 cases and 2 controls) were also classified postmenopausal if they were no longer menstruating and were older than 51 years (median for menopause in Finnish women). All of the others were classified premenopausal.

The relationship between COMT genotype and other known or suspected risk factors for breast cancer were studied by stratified analysis. Variables of interest were estrogen receptor status, smoking history, current alcohol use, BMI, WHR, postmenopausal use of estrogen, use of oral contraceptives, age at menarche, age at first full-term pregnancy, and parity.

Individuals carrying the COMT-HHI genotype, presumed as a protective factor based on previous reports, served as a referent category for all of the separate analyses of the COMT genotype.

Results

The mean age was 58.9 years (SD, 14 years; range, 44.3–91.6 years) for the cases and 53.5 years (SD, 11 years; range,
values.

(95% CI).

Postmenopausal.

Premenopausal.

Premenopausal.

Premenopausal.

Premenopausal.

Premenopausal.

Premenopausal.

Premenopausal.

Premenopausal.

Premenopausal.

Premenopausal.

Premenopausal.

Premenopausal.

Premenopausal.

Premenopausal.

Premenopausal.
was found for postmenopausal women with earlier than median age (≤13 years) at menarche and carrying COMT-L allele-containing genotypes with respective ORs of 2.13 (95% CI, 0.99–4.59) and 1.23 (95% CI, 0.55–2.73; data not shown).

Moreover, when the cutoff point was set to 12 years, substantially high increase in the risk was seen among postmenopausal women with ≤12 years at menarche and COMT-L allele-containing genotypes (OR, 8.59; 95% CI, 1.85–39.8; P for trend = 0.01). No difference was seen when the cutoff point was set to 12 years at menarche and carrying COMT-L allele-containing genotypes to be associated with increased risk for premenopausal women, although the results were based on a rather small number of subjects (24 cases and 25 controls). Thompson et al. (13), on the other hand, saw the opposite; they observed increased risk for premenopausal women carrying the COMT-L allele-containing genotypes and decreased risk for postmenopausal women with these genotypes.

The stage of the disease has been considered in only two studies on breast cancer and COMT genotype. Millikan et al. (11) reported that stratification by the stage at diagnosis did not change the results. In contrast, Matsui et al. (12) found the COMT-L allele-containing genotypes to be associated with highly advanced stage and elevated frequency of lymph node metastasis in a Japanese study population. However, the size of the study was relatively small (n = 140), and it did not contain any controls.

Because the conversion of androgens to estrogen takes place in adipose tissue, obese women are reasoned to have higher levels of circulating estrogen and, thus, increased risk especially for postmenopausal breast cancer (18). In contrast to...
Lavigne et al. (10), we did not see significantly increased risk for obese postmenopausal women carrying the COMT-L allele-containing genotypes, but similarly to the findings of Thompson et al. (13), the lowest risk was seen among lean (BMI $\leq 25.4 \text{ kg/m}^2$) postmenopausal women carrying these genotypes. However, the obese premenopausal women with this allele were also at increased risk, whereas in the present study a tendency of decreased risk was seen for them. Premenopausal obesity has been suggested to result in inverse association with breast cancer risk probably because of a higher degree of anovulation (19). It should be noted that in the study by Lavigne et al. (10), which reported a high increase in the risk of breast cancer among postmenopausal obese women carrying the COMT-LL genotype, the BMI was measured prospectively, whereas in the present study and the study by Thompson et al. (13), it was measured around the time of diagnosis. Taking into account both the overall long process from cancer initiation to its detection and the possible effect of the prevailing cancer on body weight, this might at least partly explain the differences in the outcomes of the studies.

A somewhat higher risk was seen among postmenopausal women with earlier than median age at menarche (13 years) and the COMT-L allele-containing genotype. In a study with Finnish schoolgirls, those with early menarche (before 12 years) were shown to be an endocrinologically distinct cohort establishing regular ovulatory cycles significantly more quickly, and have higher serum estradiol concentration (20). If they simultaneously carry the putative high risk COMT allele they can be exposed to increasing number of catechol estrogens, known to have an important modifying role in carcinogenesis. When the cutoff point of 12 years was used in this study, postmenopausal women with early age at menarche ($\leq 12$ years) and with the COMT-L allele-containing genotypes were indeed found to be at a remarkably increased risk of breast cancer. No genotype effect was seen when the age at menopause and the total length of reproductive years was considered. This supports the idea that early exposure to the hormonal milieu is critical in establishing risk of breast carcinoma (21).

Apart from the above mentioned differences in individual endogenous estrogen burden associated with increased breast cancer risk, there is a growing number of studies supporting the role of postmenopausal hormone replacement therapy as a risk factor (3). The increase in risk is predominantly for small localized carcinomas of the breast (3). Our findings suggest that this modest increase in risk could be explained, at least partly, by interindividual differences in the metabolism of estrogen. Whereas no overall increase in the breast cancer risk was seen among long-term estrogen users, postmenopausal women carrying two COMT-L alleles had a 4-fold risk for breast cancer compared with those with the COMT-HH genotype if they had undergone long-term estrogen replacement therapy. Our study, therefore, suggests that the risk associated with hormone replacement therapy may be higher than previously estimated, especially in certain subgroups. Furthermore, the lack of information of the use of postmenopausal estrogen may have been one important factor leading to varying results of the importance of COMT alleles in the past studies. Because the participation rate for the cases was higher than that for controls, a possibility of selection bias must be considered. However, because all of the subgroup analyses were performed within exposure groups (e.g., among users of estrogen replacement therapy), this potential source of bias should not have affected our risk estimates. Nevertheless, because the results in subgroup analyses were all based on small number of subjects leading to risk estimates with wide CIs, these findings remain to be confirmed in future studies with even larger sample sizes.

In summary, our study suggests that individual variation in the COMT genotype may define a portion of the breast cancer susceptibility associated with reproductive events and hormone exposure even if it does not seem to be a major overall risk factor for this malignancy.

Acknowledgments

We thank our colleagues at the Kuopio Cancer Research Center and A. K. Lyytinen for data collection.

References

10. 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. An association between the allele coding for a low activity variant of catechol-O-