Polymorphic Catechol-*O*-methyltransferase Gene and Breast Cancer Risk[1]

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Abstract

We examined 483 Finnish breast cancer cases and 482 population controls to determine the potential effect of 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/m^2; 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,[3] 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.

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[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 CCG TGA TTC AGG-3′ and 5′-AGG TCT GAC AAC GGG TCA GGC-3′) were digested using the *Nla*III 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 ($\leq$12, 13–14, $\geq$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/ever), and BMI (kg/m^2; weight in kilograms divided by the square of height (meters); $\leq$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-HH* 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,

Table 1 Selected characteristics of the study subjects

Characteristic	Case/control[a]	OR[b] (95% CI)
Menopausal status		
Premenopausal	164/204	1.0
Postmenopausal	319/278	0.73 (0.42–1.27)
Age at menarche		
≤12	98/101	1.0
13–14	219/251	0.82 (0.59–1.16)
≥15	150/127	0.99 (0.68–1.46)
Age at first full-term pregnancy		
Nulliparous	102/57	1.0
≤25	237/263	0.55 (0.38–0.81)
26–30	94/122	0.44 (0.29–0.69)
≥31	47/40	0.64 (0.36–1.12)
Number of full-term pregnancies		
Nulliparous	102/57	1.0
1	68/64	0.59 (0.36–0.98)
2	141/181	0.50 (0.33–0.76)
3+	171/180	0.54 (0.36–0.80)
Use of oral contraceptives		
Never	313/243	1.0
Ever	164/239	0.66 (0.48–0.91)
Postmenopausal use of estrogen		
Never	221/164	1.0
≤30 months	34/57	0.67 (0.41–1.11)
>30 months	56/55	1.09 (0.70–1.71)
WHR		
≤0.91	187/236	1.0
>0.91	291/243	1.38 (1.06–1.81)
BMI		
≤25.4 kg/m^2	208/240	1.0
>25.4 kg/m^2	260/239	1.22 (0.92–1.62)
First-degree family history of breast cancer		
No	424/459	1.0
Yes	54/22	2.53 (1.48–4.31)
History of benign breast disease		
No	296/313	1.0
Yes	180/167	1.33 (1.01–1.75)
Education		
Low	292/277	1.0
Medium	127/135	1.11 (0.81–1.52)
High	62/69	1.19 (0.78–1.80)
Current alcohol intake		
Never	271/206	1.0
Once a month or less	134/187	0.74 (0.54–1.01)
Daily-weekly	75/89	0.87 (0.59–1.29)
Smoking habits		
Never	363/351	1.0
Ex-smoker	52/67	0.94 (0.62–1.41)
Current smoker	64/64	1.34 (0.87–1.93)

[a] Total number of cases and controls does not correspond because of missing values.
[b] Adjusted for age.

37.5–77.2 years) for the controls, yielding slightly fewer cases compared with controls being premenopausal (34 *versus* 42%). The mean age at menarche was similar in cases (13.8 years; SD, 1.6 years) and controls (13.6 years; SD, 1.5 years), as was the age at first full-term pregnancy [24.9 years (SD, 4.6 years) for cases *versus* 24.6 years (SD, 4.1 years) for controls]. The risk of breast cancer was significantly lower for those having had at least one full-term pregnancy (OR, 0.53; 95% CI, 0.37–0.77) or who had ever used oral contraceptives (OR, 0.66; 95% CI, 0.48–0.91). Positive family history of breast cancer posed a 2.53-fold risk (95% CI, 1.48–4.31), and history of benign breast disease posed a 1.33-fold risk (95% CI, 1.01–1.75) of this malignancy compared with absence of these risk factors (Table 1).

Table 2 Association between *COMT* genotype and development of breast cancer according to menopausal status

Genotype	Case *n* (%)	Control *n* (%)	OR[a] (95% CI)
All			
HH	115 (23.9)	100 (20.8)	1.0
HL	238 (49.5)	237 (49.4)	0.84 (0.59–1.20)
LL	128 (26.6)	143 (29.8)	0.75 (0.51–1.11)
HL or *LL*	366 (76.1)	380 (79.2)	0.81 (0.58–1.13)
Premenopausal			
HH	44 (26.8)	41 (20.3)	1.0
HL	79 (48.2)	112 (55.4)	0.63 (0.37–1.09)
LL	41 (25.0)	49 (24.3)	0.70 (0.37–1.31)
HL or *LL*	120 (73.2)	161 (79.7)	0.65 (0.39–1.09)
Postmenopausal			
HH	71 (22.4)	59 (21.2)	1.0
HL	159 (50.2)	125 (45.0)	1.04 (0.65–1.68)
LL	87 (27.4)	94 (33.8)	0.81 (0.48–1.35)
HL or *LL*	246 (77.6)	219 (78.8)	0.94 (0.61–1.47)

[a] ORs and 95% CIs adjusted for age, age at menarche, age at first full-term pregnancy, number of full-term pregnancies, first-degree family history of breast cancer, history of benign breast disease, postmenopausal use of estrogen, and BMI.

Table 3 Association between *COMT* genotype and the risk of breast cancer according to the stage of the disease

	Premenopausal		Postmenopausal	
	Case[a]/control	OR[b] (95% CI)	Case[a]/control	OR[b] (95% CI)
Local				
HH	21/41	1.0	48/59	1.0
HL	50/112	0.85 (0.44–1.64)	103/125	0.78 (0.46–1.31)
LL	25/49	0.95 (0.45–2.01)	45/94	0.55 (0.31–0.98)[c]
HL or *LL*	75/161	0.88 (0.48–1.64)	148/219	0.68 (0.42–1.11)
Advanced				
HH	23/41	1.0	21/59	1.0
HL	29/112	0.43 (0.21–0.88)	50/125	1.44 (0.67–3.06)
LL	16/49	0.47 (0.20–1.10)[d]	40/94	1.58 (0.72–3.46)
HL or *LL*	45/161	0.44 (0.22–0.87)	90/219	1.50 (0.74–3.05)

[a] The stage of the disease could not be classified for 10 patients because of missing information on lymph node involvement.
[b] ORs and 95% CIs adjusted for age, age at menarche, age at first full-term pregnancy, number of full-term pregnancies, first-degree family history of breast cancer, history of benign breast disease, postmenopausal use of estrogen, and BMI.
[c] P for trend = 0.04.
[d] P for trend = 0.07.

The *COMT* genotype distributions in the control population were in Hardy-Weinberg equilibrium (P = 0.944). No significant associations between *COMT* genotype and breast cancer were observed when all of the cases were considered together, or when they were stratified by menopausal status (Table 2). After stratification by stage of the disease, the *COMT-L* allele-containing genotypes were shown to be inversely associated with premenopausal advanced breast cancer (OR, 0.44; 95% CI, 0.22–0.87; Table 3). Similarly, among postmenopausal women, lower frequency of local carcinoma was observed among women with *COMT-LL* genotype (OR, 0.55; 95% CI, 0.31–0.98).

BMI appeared to modify the *COMT* genotype effects; postmenopausal women with the *COMT-LL* genotype and BMI ≤25.4 kg/m^2 had significantly decreased risk for developing breast cancer (OR, 0.33; 95% CI, 0.13–0.83; Table 4). Similarly, the age of menarche was an important modifier of the risk associated with *COMT* genotypes; a tendency of increased risk

Table 4 Association between *COMT* genotype and the risk for breast cancer according to BMI stratified by menopausal status

	Premenopausal		Postmenopausal	
	Case/control	OR[a] (95% CI)	Case/control	OR[a] (95% CI)
BMI ≤25.4 kg/m^2				
HH	28/28	1.0	25/23	1.0
HL	48/70	0.69 (0.35–1.35)	59/51	0.77 (0.35–1.70)
LL	24/27	0.93 (0.41–2.09)	23/40	0.33 (0.13–0.83)[b]
HL or *LL*	72/97	0.75 (0.40–1.43)	82/91	0.57 (0.27–1.21)
BMI >25.4 kg/m^2				
HH	16/13	1.0	42/36	1.0
HL	31/40	0.48 (0.18–1.26)	91/73	1.22 (0.66–2.26)
LL	17/22	0.38 (0.12–1.17)[c]	62/54	1.26 (0.66–2.42)
HL or *LL*	48/62	0.44 (0.17–1.12)	153/127	1.24 (0.70–2.20)

[a] ORs and 95% CIs adjusted for age, age at menarche, age at first full term pregnancy, number of full term pregnancies, first degree family history of breast cancer, history of benign breast disease, and postmenopausal use of estrogen.
[b] P for trend = 0.01.
[c] P for trend = 0.09.

Table 5 Association between the *COMT* genotypes and the risk for breast cancer according to the age at menarche stratified by menopausal status

	Premenopausal		Postmenopausal	
	Case/control	OR[a] (95% CI)	Case/control	OR[a] (95% CI)
Age at menarche ≤12 years				
HH	14/10	1.0	4/15	1.0
HL	21/21	0.40 (0.11–1.36)	29/21	16.7 (3.02–92.7)
LL	14/13	0.59 (0.15–2.27)	16/20	4.76 (0.92–24.7)
HL or *LL*	35/34	0.46 (0.15–1.45)	45/41	8.59 (1.85–39.8)
Age at menarche >12 years				
HH	30/31	1.0	62/43	1.0
HL	58/91	0.59 (0.31–1.13)	123/104	0.78 (0.47–1.32)
LL	27/36	0.72 (0.34–1.54)	67/72	0.65 (0.37–1.14)
HL or *LL*	85/127	0.63 (0.34–1.16)	190/176	0.73 (0.45–1.19)

[a] ORs and 95% CIs adjusted for age, age at first full-term pregnancy, number of full-term pregnancies, first-degree family history of breast cancer, history of benign breast disease, postmenopausal use of estrogen, and BMI.

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 interaction = 0.008; Table 5). No difference was seen when the age at menopause or the total length of reproductive years was considered (data not shown). Increased risk was also seen for postmenopausal women who had the *COMT-LL* genotype and who had undergone estrogen replacement therapy for longer than 30 months (OR, 4.02; 95% CI, 1.13–14.3; Table 6). Estrogen receptor status, family history of breast cancer, WHR, use of oral contraceptives, history of smoking, or current alcohol use did not affect the risk associated with the *COMT* genotypes (data not shown).

Discussion

In this study, the subjects with the *COMT-L* allele-containing genotypes tended to be at decreased risk of developing breast cancer, especially advanced stage of the disease in premenopausal women and local carcinoma in postmenopausal women. A tendency of decreasing risk can also be seen for both pre- and postmenopausal women in the study of Millikan *et al.* (11), including 654 cases and 642 controls of whom they classified 389 cases and 379 controls as white. The frequencies of *COMT-HH*, *-HL*, and *-LL* genotypes were 42, 45, and 13%, respectively, for African-American controls, whereas among the white controls the respective percentages were 22, 50, and 28%, respectively. The latter are in good agreement with those found in the present study (21, 49, and 30%). Similarly, in the study of Lavigne *et al.* (10) with 112 matched case-control pairs, a tendency of decreasing risk was seen among 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

Table 6 Association between the *COMT* genotypes and the risk for breast cancer stratified by postmenopausal estrogen use

	Case/control	OR[a]
Never users		
HH	55/35	1.0
HL	111/74	0.88 (0.48–1.63)
LL	54/55	0.56 (0.29–1.10)[b]
HL or *LL*	165/129	0.74 (0.42–1.31)
Ever users ≤30 months		
HH	7/7	1.0
HL	16/26	0.76 (0.17–3.38)
LL	10/24	0.47 (0.10–2.23)[c]
HL or *LL*	26/50	0.61 (0.15–2.48)
Ever users >30 months		
HH	7/17	1.0
HL	27/25	2.51 (0.76–8.24)
LL	22/13	4.02 (1.13–14.3)[d]
HL or *LL*	49/38	3.07 (1.02–9.23)

[a] ORs and 95% CIs adjusted for age, age at first full-term pregnancy, number of full-term pregnancies, first-degree family history of breast cancer, history of benign breast disease, and BMI.
[b] P for trend = 0.08.
[c] P for trend = 0.30.
[d] P for trend = 0.03.

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 ≤25.4 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-L* 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 (≤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.

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