COMT genotype, micronutrients in the folate metabolic pathway and breast cancer risk

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Catechol-O-methyltransferase (COMT) catalyzes the O-methylation of catechol estrogens (CEs), using S-adenosylmethionine (SAM) as a methyl donor. Several studies have indicated that the val108met COMT polymorphism, which results in a 3–4-fold decrease in activity, is associated with increased breast cancer risk. Folate, whose intake levels have also been associated with breast cancer risk, and other micronutrients in the folate metabolic pathway influence levels of SAM and S-adenosylhomocysteine (SAH), a COMT inhibitor generated by the demethylation of SAM. Because these micronutrients have been shown to alter SAM and SAH levels, we hypothesized that they could also affect COMT-catalyzed CE methylation. Although measurements of SAM and SAH were not initially collected, a secondary analysis of data from two nested case-control studies was performed to examine whether serum levels of folate, vitamin B12 (B12), pyridoxal 5'-phosphate (PLP), cysteine and homocysteine, in conjunction with COMT genotype, were associated with breast cancer risk. COMT_HH (high activity COMT homozygote) breast cancer cases had statistically significantly lower levels of homocysteine (P = 0.05) and cysteine (P = 0.04) and higher levels of PLP (P = 0.02) than COMT_HH controls. In contrast, COMT_LL (low activity COMT homozygote) cases had higher levels of homocysteine than COMT_HL controls (P = 0.05). No associations were seen between B12, COMT genotype, and breast cancer risk. An increasing number of COMT_L alleles was significantly associated with increased breast cancer risk in women with below median levels of folate (P_trend = 0.05) or above median levels of homocysteine (P_trend = 0.02). These findings are consistent with a role for certain folate pathway micronutrients in mediating the association between COMT genotype and breast cancer risk.

Introduction

The primary mechanism of estrogen carcinogenesis is thought to involve estrogen receptor-mediated cell proliferation associated with spontaneous DNA replication errors (1). However, there is ample evidence that suggests that the catechol metabolites of estrogen can also contribute to the carcinogenic process by additional mechanisms (2). For example, oxidative DNA damage can occur via the participation of catechol estrogens (CEs) in redox cycling (3). Furthermore, CEs can be metabolized to quinones, which can directly adduct DNA (3). As with catecholamines, the methylation of CEs catalyzed by catechol-O-methyltransferase (COMT) prevents quinone formation and redox cycling (4,5). Besides methylation, CEs can also be inactivated by sulfation and glucuronidation (6).

Several of the enzymes that catalyze CE detoxification are polymorphic, including COMT (7). A val to met polymorphism in the COMT gene is present in up to 75% of Caucasians (8). This mutation results in a 3–4-fold decrease in enzyme activity, although the mechanism responsible for the reduced activity is unknown (8). The low activity COMT allele, COMT_H, has been found to be associated with breast cancer risk in four of five published peer-reviewed studies (9–13). For instance, in a nested case-control study, Lavigne et al. (9) found that post-menopausal women with a high body mass index (BMI) were at a greater risk for developing breast cancer if they had at least one COMT_L allele compared with women having two high activity alleles, COMT_HH.

S-adenosylmethionine (SAM) is the necessary methyl donor for COMT catalyzed reactions. Upon the methylation of a COMT substrate, SAM is converted to S-adenosylhomocysteine (SAH), a known COMT inhibitor, representing a negative feedback loop (14,15). The folate metabolic pathway (Figure 1) largely determines SAM and SAH levels. For example, high levels of folate and vitamin B12 (B12) have been associated with high levels of SAM, while increased homocysteine levels in rats and humans are correlated with increased SAH and cysteine levels and decreased pyridoxal 5'-phosphate (PLP) levels (14,16–23). The effects of the folate metabolic pathway on carcinogenesis have been studied intermittently for over 50 years (24).

Wu et al. (12) conducted a nested case-control study, which included participants from the Lavigne et al. study (9), to determine if serum levels of folate, B12, PLP and homocysteine were associated with breast cancer risk (25). In this study, no statistically significant associations with folate, PLP or homocysteine were observed; however, low levels of B12 were associated with an increased risk of breast cancer among post-menopausal women. These results do not exclude a possible role for these micronutrients influencing COMT catalyzed reactions by altering levels of SAM and SAH.

We hypothesized that micronutrients in the folate metabolic pathway that affect levels of SAM and/or SAH (Figure 1), in combination with COMT genotype, could be associated with breast cancer risk by affecting CE methylation. Although SAM and SAH levels were not initially measured, we performed a secondary analysis of the data from the Wu et al. (25) and Lavigne et al. (9) studies to examine whether, and in what
manner, serum levels of folate, B12, PLP, cysteine and homocysteine, in conjunction with COMT genotype, are associated with breast cancer risk.

Methods

Study population

The study population has been described in detail previously (9,25,26). All participants were residents of Washington County, Maryland who donated blood and answered a questionnaire in 1989 after signing an informed consent. Incident breast cancer cases between 1989 and 1995 were identified by linkage of the study cohort to the Washington County Cancer Registry. One hundred and fifteen cases were then individually matched to 115 control women by age (within 1 year), race (all were Caucasian), date of blood donation, menopausal status, and, if pre-menopausal, time since last menstrual period. For this study, complete data were available for 112 cases and 113 controls.

Sample preparation and analysis

COMT genotype was determined previously by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) as described by Lavigne et al. (9). Measurements of folate, homocysteine, PLP and B12 were determined previously as described by Wu et al. (25). Briefly, PLP was measured by a radiometric tyrosine assay; cysteine and homocysteine levels were determined simultaneously by an HPLC method; and folate and B12 levels were measured by RIA (25).

Statistical analysis

Unmatched analyses were conducted in order to use all available cases and controls. Because micronutrient levels were not normally distributed, median levels were compared using the Mann–Whitney test in women within the same COMT genotype. The associations between COMT genotype and breast cancer risk were determined in women with low and high nutrient levels (< or > the control median, respectively) by unconditional logistic regression adjusted for age and menopausal status at donation (matching factors). The data were analyzed using STATA Version 6 (Stata Corporation, College Station, TX).

Results

Mean age ± SD of the 112 cases was 60.38 ± 11.75 and that of the 113 controls was 60.17 ± 11.63. Conditional logistic regression revealed that there were no statistically significant differences between cases and controls for potential risk factors including smoking status, age at first full-term birth, oral contraceptive use, hormone replacement therapy and BMI (data not shown). Although also not statistically significant, cases tended to have more years of education, higher alcohol consumption, earlier ages of menarche and less multivitamin and B vitamin intake than controls (data not shown). As expected, cases were more likely than controls to have a family history of breast cancer (OR = 2.58, 95% CI: 1.28–5.19).

In order to determine whether the association between specific micronutrients in the folate pathway and breast cancer risk differed by COMT genotype, micronutrient levels were first compared in cases and controls stratified by COMT genotype (Table I). Breast cancer cases with two high activity COMT alleles (COMTHH) had lower levels of homocysteine (P = 0.05) and cysteine (P = 0.04) and higher levels of PLP (P = 0.02) than COMTHH controls. In contrast, in women homozygous for the low activity COMT allele (COMTL), cases had higher levels of homocysteine than controls (P = 0.05). There were no statistically significant associations found between any analyte and breast cancer risk in COMT heterozygotes (COMTLL) (Table I).

Folate and homocysteine levels have been found to be inversely related in a number of studies (27,28), including this one (data not shown). Furthermore, in several studies, low folate intake has been shown to be associated with increased breast cancer risk (29–32). Therefore, we were interested in determining whether the associations between homocysteine levels and breast cancer risk differed in women with different folate levels according to COMT genotype. In COMTHH women, cases with low folate levels had statistically significantly lower levels of homocysteine (P = 0.04) than controls, while those with high folate levels did not (P = 0.39) (Table II). In contrast, in COMTLL women, cases with high folate levels had statistically significantly higher levels of homocysteine (P = 0.04) than controls, while those with low folate levels did not (P = 0.63) (Table II).

Because COMT genotype has been shown to be associated with breast cancer risk (9,11,12), it was of interest to determine if this association was affected by micronutrient status. We examined this in women with high and low levels of analyte (< control median or > control median). There were no statistically significant associations seen between COMT geno-

| Table I. Median micronutrient concentrations for breast cancer cases and controls stratified by COMT genotype |
|------------------|------------------|------------------|------------------|
|                  | Case             | Control          | Percent difference<sup>a</sup> | P<sup>b</sup> |
| COMTHH           |                  |                  |                                |              |
| n                | 20               | 27               |                                |              |
| Folate (ng/ml)<sup>c</sup> | 8.63            | 6.29             | 37.20                         | 0.23         |
| B12 (pg/ml)      | 450.97           | 500.56           | −9.91                         | 0.33         |
| PLP (pmol/ml)    | 67.52            | 38.72            | 74.38                         | 0.02         |
| Homocysteine (nmol/ml) | 6.81           | 9.66             | −29.50                        | 0.05         |
| Cysteine (nmol/ml) | 236.78          | 284.06           | −16.64                        | 0.04         |
| COMTLL            |                  |                  |                                |              |
| n                | 57               | 55               |                                |              |
| Folate (ng/ml)   | 9.52             | 8.03             | 18.56                         | 0.72         |
| B12 (pg/ml)      | 428.64           | 438.58           | −2.27                         | 0.51         |
| PLP (pmol/ml)    | 42.24            | 51.29            | −17.64                        | 0.34         |
| Homocysteine (nmol/ml) | 8.50           | 8.44             | 0.71                          | 1.00         |
| Cysteine (nmol/ml) | 263.16          | 252.47           | 4.23                          | 0.78         |

<sup>a</sup>Percentage difference was calculated as: (median case – median control)/ median control×100.

<sup>b</sup>The Mann–Whitney test was used to assess differences between analyte concentrations in cases and controls in each COMT strata.

<sup>c</sup>Micronutrients levels are per ml plasma.
type and breast cancer risk in any subgroup of B12, PLP or cysteine (Table III). An increasing number of COMTI alleles were significantly associated with increased breast cancer risk in women with low levels of folate (Ptrend = 0.05) or high levels of homocysteine (Ptrend = 0.02) (Table III). This association was even more pronounced in women with both low folate and high homocysteine levels. Although there were small numbers in each category, in these women, the OR for breast cancer risk for COMTHH versus COMTHL was 5.10 (95% CI: 1.15–22.53) and that for COMTHL versus COMTHH was 6.18 (95% CI: 1.30–29.37) (Ptrend = 0.03).

### Discussion

Several studies have indicated that COMTI is associated with increased breast cancer risk; however, the biological mechanism is currently unknown. It has been proposed that compared with COMTH, COMTI is less active in methylating, and hence inactivating, CEs (2). We tested the hypothesis that micronutrients in the folate metabolic pathway that can regulate levels of SAM and/or SAH, in combination with COMT genotype, are associated with breast cancer risk. Our results are the first to indicate that an increasing number of COMTI alleles, in conjunction with high levels of homocysteine and/or low levels of folate, is associated with breast cancer risk.

The association between dietary folate and breast cancer risk has been addressed in several epidemiological studies (25,30,31,33,34). Two case-control studies have found an inverse association between dietary folate and breast cancer risk (31,32). Three large studies have recently examined the association between folate, alcohol (a known folate antagonist) and breast cancer risk (30,33,34). All found that with high levels of alcohol consumption, dietary folate intake could be protective against breast cancer risk. Wu et al. (25) found no association between serum levels of folate and breast cancer risk. However, using a subset of their study population, we found that in women with low folate levels, breast cancer risk increased with an increasing number of COMTI alleles (Table III).

It is well established that folate and homocysteine levels are inversely related (20–22,28,35). Thus, our finding of increasing breast cancer risk with an increasing number of...
**COMT** alleles in women with low folate levels was strengthened by the fact that an increase in **COMT** alleles was also positively associated with breast cancer risk in women with high serum levels of homocysteine. This finding was further strengthened in that this association was even more pronounced in women with both low levels of folate and high levels of homocysteine. Furthermore, in examinations of women stratified by **COMT** genotype, associations between homocysteine levels and breast cancer risk were dependent on the folate levels (Table II).

The correlation among homocysteine, SAM, SAH and SAH:SAM ratios have been examined in both humans and animal models (20–22,36,37). Most have found that an increase in plasma homocysteine is associated with an increase in SAH but has no effect on SAM levels (20–22). Therefore, the results of our study indicate that **COMT** and **COMT** are likely to be differentially affected by changes in SAH. Perhaps increases in SAH have larger inhibitory effects on **COMT** than **COMT**, rendering **COMT** less able to methylate CEs. We also found that low levels of homocysteine were associated with an increased breast cancer risk in **COMT** women; however, additional studies are needed to elucidate the biochemical mechanism of this observation.

B12 is a necessary co-factor for the methionine synthetase catalyzed metabolism of methyltetrahydrofolate and homocysteine to folic acid and methionine, respectively (Figure 1). Because methionine is the direct precursor to SAM, an increase in B12 levels could cause an increase in SAM levels. In the total study population, low levels of B12 were found to be associated with an increased breast cancer risk in post-menopausal women (25). Although this relationship did not have statistical significance in any subgroup of this study, median B12 levels were lower in cases within each genotype (Table I), indicating that a decrease in SAM could lead to a decrease in both **COMT** and **COMT** activity.

We found that higher levels of PLP, the active form of vitamin B6, were associated with an increased breast cancer risk in **COMT** women. However, in these women, breast cancer cases had a lower median level of cysteine than controls (Table I). While PLP is necessary for the metabolism of homocysteine to cysteine (Figure 1), it is also involved in many other biological processes. For example, uterine slices from vitamin B6-deficient rats accumulated more [3H]estradiol than did tissue from B6 repleted animals, suggesting that PLP could be involved with sensitivity to steroid hormones (38). Therefore, increased PLP may increase breast cancer risk via a mechanism unrelated to the folate metabolic pathway.

We found that high homocysteine levels and/or low folate levels in combination with an increasing number of **COMT** alleles is associated with an increased breast cancer risk. The major advantages of this study are its prospective design and its use of serum, rather than dietary, measurements. A major disadvantage of this study is a relatively small sample size. For example, it would have been desirable to stratify the population by menopausal status, given that the association between **COMT** genotype and breast cancer risk differed for pre- and post-menopausal women in several studies (9,11–13). Therefore, larger studies need to be conducted to confirm our findings. Furthermore, serum levels of SAM and SAH should be measured in order to understand better the direct effects of folate and homocysteine levels on breast cancer risk in women with specific **COMT** genotypes.

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**References**


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