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Association of *COMT* Haplotypes and Breast Cancer Risk in Caucasian Women

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Abstract

Catechol-O-methyl transferase (COMT) is an important estrogen-metabolizing enzyme, and common genetic variants in this gene could affect breast cancer risk. We conducted a large population-based case control study in Massachusetts, New Hampshire, and Wisconsin to examine six strategically selected COMT haplotype-tagging (ht) single nucleotide polymorphism (SNPs), including the val158met polymorphism (rs4680), in relation to breast cancer risk. Analyses were based on 1,655 Caucasian women with invasive breast cancer and 1,470 Caucasian controls. None of the six individual SNPs were associated with breast cancer risk. The global test for haplotype associations was nonsignificant (p- value=0.097), although two uncommon haplotypes present in 6% of the study population showed statistically significant inverse associations with risk. These results suggest that genetic variation in COMT has no significant association with breast cancer risk among Caucasian women.

Keywords

Breast neoplasms; genetic polymorphism; epidemiology; estrogens; risk

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The toxic estrogen metabolites, catechol estrogens, are catalyzed into non-toxic methoxyestrogens by the enzyme catechol-*O*-methyl transferase (*COMT*). This detoxification occurs mostly in the liver, but *COMT* is found in variety of tissues including the breast (1). Single nucleotide polymorphisms (SNPs) may affect *COMT* enzymatic activity: a common, functional SNP (Ex4-12 G<A; rs4680), causes a valine to methionine amino acid substitution and is associated with a 2- to 3-fold decrease in *COMT* enzymatic activity (2, 3), which may lead to an accumulation of carcinogenic catechol estrogens. A few studies have shown an association between the rs4680 SNP and breast cancer risk, but most have not (4), and it is possible that other SNPs have an effect on *COMT* enzymatic activity. In this study, we examined the associations between a panel of *COMT* haplotype-tagging (ht) SNPs and invasive breast cancer risk in a large population- based case control study of Caucasian women.

Patients and Methods

Study population

The study population has previously been described (5). Case women were aged 20-69 years and newly diagnosed with invasive breast cancer during 1996-2001. Controls were randomly selected from lists of licensed drivers (if >65 years) and from a roster of Medicare beneficiaries (if 65-74 years). Controls were frequency-matched to cases by age in five-year strata. DNA samples were collected using an oral rinse protocol (6). The current analysis was restricted to Caucasian women who comprised 98% of the study population (1,655 invasive cases and 1,470 controls).

SNP selection and genotyping

A total of six *COMT* SNPs were genotyped (rs1544325, rs174674, rs7290221, rs2239393, rs4680 and rs4646316). Isolation and preparation of the DNA samples have been previously described (5). Genotypes were evaluated using validated Taqman (Applied Biosystems, Forest City, CA, USA) or MGM Eclipse assays (7). Genotyping concordance for the six SNPs among 192 duplicate samples ranged from 94% to 100% (median: 99%). Departure from Hardy-Weinberg equilibrium (HWE) among the controls was examined. Genotypes for five out of the six SNPs were consistent with HWE ($p \geq 0.05$), whereas one SNP (rs4680) showed a statistically borderline departure from HWE ($p=0.01$).

Statistical analysis

For individual SNPs, unconditional logistic regression models were used to obtain age- and state-adjusted odds ratio (OR) estimates and 95% confidence intervals (CIs). Ordinal coding of the genotypes was used in the logistic regression models to evaluate tests for linear trend (SAS Institute, Inc., Cary, NC, USA).

Pairwise linkage disequilibrium (LD) measured by D‘ between the htSNPs was estimated using Haploview (8). Haplotype frequencies and effects were examined with HaploStats (9). A global score test was used to evaluate the overall significance adjusted for participant age and state of residence. Effects of individual haplotypes were also examined by comparing the risk associated with each individual haplotype to the risk associated with the most common haplotype. Rare haplotypes (>1% in cases and controls combined) were pooled into a single category.

Results

None of the genotypes of the individual *COMT* SNPs were associated with risk of invasive breast cancer (Table I). Menopausal status did not modify these associations (results not

shown). Six *COMT* haplotypes with frequency greater than 1% were identified (Table II). There was no overall haplotype effect when comparing cases to controls (p_{global}=0.097). However, when compared to the reference haplotype (AGGAGC, 28.9%), two haplotypes were associated with a decreased risk of breast cancer (AGCGAC: OR=0.61, 95% CI=0.42-0.88; GAGAGC: OR=0.73, 95% CI=0.53-1.00). Data were too sparse to consider haplotype associations according to menopausal status.

Discussion

In this large population-based case control study, we found no significant overall association between breast cancer risk and *COMT* haplotypes, although two individual haplotypes, with frequencies of 3.7% and 2.4% in the controls, were significantly associated with decreased breast cancer risk. No individual SNP was significantly associated with decreased risk, and if the finding is not due to chance, other linked variation(s) may be responsible for these associations. Of note, we found no association of the val158met SNP with breast cancer risk, consistent with results from a recent meta-analysis (4).

Two previous studies found that haplotypes encompassing the 3‘ UTR of *COMT* were associated with increased breast cancer risk (10, 11). A case control study of Polish women used a comprehensive two-step approach developed by the Breast and Prostate Cohort Consortium to assess common genetic variation in European Americans and identified a set of 11 htSNPs (10). Two linkage disequilibrium (LD) blocks were defined: one LD block containing seven htSNPs (three of which were included in our analysis: rs7290221, rs2239393, rs4680) was not associated with breast cancer risk. The other LD block including the 3‘ UTR region of *COMT* and armadillo repeat deleted in velocardiofacial syndrome (*ARVCF*), a human catenin gene adjacent to *COMT* whose border is difficult to separate from *COMT* (10), contained a haplotype that was significantly associated with breast cancer risk. A second study conducted among women in Long Island, New York found a significant association with breast cancer risk for a specific haplotype encompassing four *COMT-ARVCF* SNPs that had previously been associated with schizophrenia (11). None of the haplotypes in our study spanned the 3‘UTR region of *COMT*. One genome-wide association study based on the Cancer Genetic Markers of Susceptibility (CGEMS) Project failed to identify breast cancer associations with markers at chromosome 22q11 where *COMT* resides (12).

In summary, we found no significant overall association of *COMT* haplotypes with invasive breast cancer risk. A denser panel of htSNPs and coverage of the *ARVCF* region may be needed to identify associations not evident in the current analysis.

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Conflict of Interest: None for all authors

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References

1. Zhu BT. Catechol-*O*-methyltransferase (*COMT*)-mediated methylation metabolism of endogenous bioactive catechols and modulation by endobiotics and xenobiotics: importance in pathophysiology and pathogenesis. Curr Drug Metab. 2002; 3:321–349. [PubMed: 12083324]
2. Dawling S, Roodi N, Mernaugh RL, Wang X, FF Parl. Catechol-*O*-methyltransferase (COMT)-mediated metabolism of catechol estrogens: comparison of wild-type and variant *COMT* isoforms. Cancer Res. 2001; 61:6716–6722. [PubMed: 11559542]
3. Lachman HM, Papolos DF, Saito T, Yu YM, Szumlanski CL, Weinshilboum RM. Human catechol-*O*-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. Pharmacogenetics. 1996; 6:243–250. [PubMed: 8807664]
4. Wen W, Cai Q, Shu XO, Cheng JR, Parl F, Pierce L, Gao YT, Zheng W. Cytochrome P450 1B1 and catechol-*O*-methyltransferase genetic polymorphisms and breast cancer risk in Chinese women: results from the shanghai breast cancer study and a meta-analysis. Cancer Epidemiol Biomarkers Prev. 2005; 14:329–335. [PubMed: 15734954]
5. Garcia-Closas M, Egan KM, Newcomb PA, Brinton LA, Titus-Ernstoff L, Chanock S, Welch R, Lissowska J, Peplonska B, Szeszenia-Dabrowska N, Zatonski W, Bardin-Mikolajczak A, Struewing JP. Polymorphisms in DNA double-strand break repair genes and risk of breast cancer: two population-based studies in USA and Poland, and meta-analyses. Hum Genet. 2006; 119:376–388. [PubMed: 16485136]
6. Garcia-Closas M, Egan KM, Abruzzo J, Newcomb PA, Titus-Ernstoff L, Franklin T, Bender PK, Beck JC, Le Marchand L, Lum A, Alavanja M, Hayes RB, Rutter J, Buetow K, Brinton LA, Rothman N. Collection of genomic DNA from adults in epidemiological studies by buccal cytobrush and mouthwash. Cancer Epidemiol Biomarkers Prev. 2001; 10:687–696. [PubMed: 11401920]
7. Packer BR, Yeager M, Burdett L, Welch R, Beerman M, Qi L, Sicotte H, Staats B, Acharya M, Crenshaw A, Eckert A, Puri V, Gerhard DS, Chanock SJ. SNP500Cancer: a public resource for sequence validation, assay development, and frequency analysis for genetic variation in candidate genes. Nucleic Acids Res. 2006; 34:D617–D621. [PubMed: 16381944]
8. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21:263–265. [PubMed: 15297300]
9. Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA. Score tests for association between traits and haplotypes when linkage phase is ambiguous. Am J Hum Genet. 2002; 70:425–434. [PubMed: 11791212]
10. Gaudet MM, Chanock S, Lissowska J, Berndt SI, Peplonska B, Brinton LA, Welch R, Yeager M, Bardin-Mikolajczak A, Garcia-Closas M. Comprehensive assessment of genetic variation of catechol-*O*-methyltransferase and breast cancer risk. Cancer Res. 2006; 66:9781–9785. [PubMed: 17018638]
11. Gaudet MM, Bensen JT, Schroeder J, Olshan AF, Terry MB, Eng SM, Teitelbaum SL, Britton JA, Lehman TA, Neugut AI, Ambrosone CB, Santella RM, Gammon MD. Catechol-*O*-methyltransferase haplotypes and breast cancer among women on Long Island, New York. Breast Cancer Res Treat. 2006; 99:235–240. [PubMed: 16596327]
12. Hunter DJ, Kraft P, Jacobs KB, Cox DG, Yeager M, Hankinson SE, Wacholder S, Wang Z, Welch R, Hutchinson A, Wang J, Yu K, Chatterjee N, Orr N, Willett WC, Colditz GA, Ziegler RG, Berg CD, Buys SS, McCarty CA, Feigelson HS, Calle EE, Thun MJ, Hayes RB, Tucker M, Gerhard DS, Fraumeni JF Jr, Hoover RN, Thomas G, Chanock SJ. A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. Nat Genet. 2007; 39:870–874. [PubMed: 17529973]

Table I

Polymorphisms in six COMT SNPs and odds ratios for breast cancer in a case control study of Caucasian women.

SNP	Location	No. cases (%) n=1.655*	No. controls (%) n=1.470*	OR†	95% CI†	*P*trend‡
rs1544325	IVS1+2248A>G					
GG		509 (30.9)	484 (33.2)	1.00	Ref	
AG		811 (49.3)	686 (47.1)	1.12	0.95-1.31	
AA		287 (17.5)	264 (18.1)	1.02	0.83-1.26	0.63
rs174674	IVS1+4605A>G					
GG		847 (51.4)	703 (48.1)	1.00	Ref	
AG		615 (38.4)	586 (40.1)	0.87	0.75-1.01	
AA		140 (8.5)	122 (8.3)	0.95	0.73-1.24	0.21
rs7290221	IVS1-6042C>G					
GG		398 (24.2)	390 (26.8)	1.00	Ref	
CG		832 (50.6)	704 (48.3)	1.15	0.97-1.37	
CC		376 (22.9)	334 (22.9)	1.09	0.89-1.34	0.36
rs2239393	IVS3+90A>G					
AA		593 (36.1)	511 (35.1)	1.00	Ref	
AG		761 (46.3)	673 (46.2)	0.98	0.83-1.14	
GG		235 (14.3)	238 (16.3)	0.87	0.70-1.08	0.24
rs4680	Ex4-12G>A					
AA		420 (25.5)	403 (27.7)	1.00	Ref	
AG		794 (48.3)	665 (45.6)	1.15	0.96-1.36	
GG		370 (22.5)	348 (23.9)	1.04	0.85-1.27	0.65
rs4646316	IVS5+310C>T					
CC		957 (58.1)	844 (57.7)	1.00	Ref	
CT		554 (33.6)	499 (34.1)	0.98	0.84-1.15	
TT		88 (5.2)	71 (4.9)	1.13	0.82-1.58	0.74

OR, odds ratio; CI, confidence interval.

*Differences between the total numbers of cases and controls and frequencies shown in the table are due to missing genotype data.

†Adjusted for reference age and state of residence.

‡Tests for trend in breast cancer risk for each SNP included an indicator term representing the number of minor alleles (0, 1, 2).

Table II

Haplotypes of COMT polymorphisms and risk of invasive breast cancer.

COMT haplotype*	Invasive breast cancer		
	Frequency		OR (95% CI)†
	Cases n=1.655	Controls n=1.470	
AGGAGC	30.2	28.9	1.00 (ref)
GGCGAC	14.2	14.9	0.93 (0.79-1.10)
GACAGC	11.1	12.0	0.88 (0.73-1.07)
GGCGAT	10.2	9.8	1.01 (0.83-1.23)
AGGGAT	5.1	5.2	0.94 (0.71-1.24)
GACAAC	4.1	3.2	1.29 (0.95-1.76)
GACGAT	3.9	4.0	0.94 (0.69-1.28)
GAGAGC	3.2	4.2	**0.73 (0.53-1.00)**
GAGAAC	3.1	3.2	0.95 (0.69-1.30)
GGCAGC	2.5	2.2	1.09 (0.74-1.62)
AGGAGT	2.2	2.3	0.91 (0.61-1.37)
AGCGAC	1.9	3.0	**0.61 (0.42-0.88)**
GGGAGC	1.3	1.2	1.07 (0.62-1.85)
GACGAC	0.9	1.3	0.71 (0.38-1.33)
AGGAAC	1.4	0.9	1.51 (0.84-2.70)
Rare haplotypes‡	4.7	3.7	1.18 (0.83-1.68)
			Global *p*=0.097

OR, odds ratio; CI, confidence interval.

*Polymorphic bases include rs1544325, rs174674, rs7290221, rs2239393, rs4680, and rs4646316.

†ORs based on an additive effect model with adjustment for reference age (categorical) and state of residence.

‡Haplotypes with <1% frequency.