Expression of Cytochrome *P*450 1B1 and Catechol-*O*-Methyltransferase in Breast Tissue and Their Associations with Breast Cancer Risk

Wanqing Wen,[1] Zefang Ren,[1] Xiao Ou Shu,[1] Qiuyin Cai,[1] Chuanzhong Ye,[1] Yu-Tang Gao,[2] and Wei Zheng[1]

[1]Vanderbilt Epidemiology Center, Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee and [2]Department of Epidemiology, Shanghai Cancer Institute, Shanghai, China

Abstract

Cytochrome *P*450 1B1 (CYP1B1) and catechol-*O*-methyltransferase (COMT) are important estrogen-metabolizing enzymes that may affect breast cancer risk. Few studies have directly measured the expression of *CYP1B1* and *COMT* genes in breast tissue samples. The subjects in this study were a subgroup of participants of the Shanghai Breast Cancer Study including 64 patients diagnosed with breast cancer and 68 patients diagnosed with benign breast diseases (BBD) who provided samples of tumor tissue and adjacent nontumor tissue to the study. We compared *CYP1B1* and *COMT* mRNA expression in tumor tissue and adjacent nontumor tissue in both breast cancer patients and BBD patients. High levels of *CYP1B1* expression and low levels of *COMT* expression in adjacent nontumor tissue were associated with a significantly increased breast cancer risk in a nonlinear manner. Odds ratios and 95% confidence intervals (in parentheses) for the midpoints of the first, second, fourth, and fifth quintiles of gene expression levels compared with the overall median levels in BBD subjects were 0.21 (0.07-0.67), 0.81 (0.69-0.95), 1.20 (1.05-1.38), and 1.55 (1.12-2.15) for CYP1B1 and 1.72 (1.17-2.55), 1.19 (1.05-1.35), 0.83 (0.73-0.95), and 0.78 (0.65-0.93) for COMT, respectively. These results support the hypothesis that the formation and accumulation of catechol estrogens in breast tissue through increased *CYP1B1* expression and reduced *COMT* expression may play a significant role in breast cancer risk. (Cancer Epidemiol Biomarkers Prev 2007;16(5):917–20)

Introduction

It is well accepted that high-level and prolonged exposure to estrogen plays a central role in breast carcinogenesis. Cytochrome *P*450 1B1 (CYP1B1) and catechol-*O*-methyltransferase (COMT) are important estrogen-metabolizing enzymes. CYP1B1 is the main CYP450 enzyme responsible for the 4-hydroxylation of estradiol to the corresponding catechol, a metabolite shown to be carcinogenic in animal models (1, 2). COMT, on the other hand, is a phase II enzyme that transforms catechol estrogens into nongenotoxic methylethers, thus inactivating them (3). Therefore, it is conceivable that an increase in CYP1B1 activity and a decrease in COMT activity might increase breast cancer risk due to the formation and accumulation of carcinogenic catechol estrogens.

A number of molecular epidemiology studies have been conducted during the past several years to investigate several *CYP1B1* and *COMT* single-nucleotide polymorphisms in relation to breast cancer risk (4-9). The findings from these studies have been inconsistent. A recent meta-analysis indicated no apparent association of breast cancer risk with the single-nucleotide polymorphisms of these estrogen-metabolizing genes (10). However, these genetic variants may not adequately reflect CYP1B1 and COMT enzyme activity in breast tissue because such activity can be regulated by other gene variants and environmental factors, such as dietary flavonoids (11). Because 4-hydroxy catechol estrogens are highly reactive metabolites, it is believed that estrogen hydroxylation and inactivation at the local tissue level are highly relevant to breast carcinogenesis. In other words, it is imperative to directly measure CYP1B1 and COMT activity in breast tissue samples to evaluate their role in the etiology of breast cancer. To address this issue, we measured and compared expression levels of *CYP1B1* and *COMT* genes in breast tissue samples from patients diagnosed with breast cancer and benign breast diseases (BBD) using data and samples collected in the Shanghai Breast Cancer Study and also compared the gene expression levels in tumor tissue with those in adjacent nontumor tissue in both breast cancer and BBD subjects.

Materials and Methods

Subjects and Breast Tissue Samples. The subjects in this study were a subset of patients of the Shanghai Breast Cancer Study. The Shanghai Breast Cancer Study was a population-based case control study. Details of the study have been described elsewhere (12). Briefly, the Shanghai Breast Cancer Study recruited 1,459 breast cancer cases and 410 women with BBD, who were newly diagnosed between 1996 and 1998, and 1,556 controls. Of those, 522 women with breast cancer and 219 women with BBD provided tumor tissue samples and 152 women with breast cancer and 70 women with BBD provided adjacent nontumor tissue samples. The subjects in the current study are randomly selected 64 breast cancer patients and all 68 BBD patients who provided both tumor and adjacent nontumor tissue samples. The breast cancer patients served as the case group and the BBD patients served as the control group for the case-control comparisons.

Tumor tissue samples were removed during surgery from the center of the lesion, whereas adjacent nontumor tissue samples were obtained from the distal edge of the resection. These samples were snap frozen in liquid nitrogen as soon as possible, typically within 10 min. Samples were stored at

Received 12/8/06; revised 2/23/07; accepted 3/2/07.

Grant support: NIH grants RO1CA64277 and R01CA90899.

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.

Requests for reprints: Wanqing Wen, Vanderbilt Epidemiology Center, Vanderbilt University School of Medicine, Nashville, TN 37232. Phone: 615-936-0747; Fax: 615-936-1269. E-mail: wanqing.wen@vanderbilt.edu

doi:10.1158/1055-9965.EPI-06-1032

Table 1. Comparisons of demographic characteristics and known breast cancer risk factors in patients diagnosed with breast cancer or BBD

	All BC cases (n = 1,459)	Selected BC cases (n = 64)	BBD (n = 68)	P*	P †
Age (mean), y	47.9	47.6	43.2	0.70	<0.01
Education ≥high school, %	45.2	50.0	51.5	0.51	0.87
Age at menarche (mean), y	14.5	14.6	14.6	0.52	0.86
Postmenopause, %	34.5	33.3	7.5	0.88	<0.01
Body mass index (mean)	23.5	24.3	23.4	0.06	0.12
Waist-to-hip ratio (mean)	0.81	0.81	0.79	0.67	0.06
Physical activities, %	18.7	18.8	16.2	0.88	0.70
Family history of BC among first-degree relatives	3.7	6.3	1.5	0.45	0.15

Abbreviation: BC, breast cancer.
*The P values were for the comparisons between selected 64 breast cancer cases and the entire 1,459 breast cancer cases in the parent study and derived from the z test.
†The P values were for the comparisons between selected 64 breast cancer cases and 68 BBD subjects and derived from the t test for continuous variables and the χ^2 test for categorical variables.

−70°C until the relevant assays were done. All patients were interviewed at the time of recruitment. Medical charts were reviewed using a standard protocol to obtain information on cancer treatment, clinical stages, and cancer characteristics such as estrogen and progesterone receptor status. Two senior pathologists reviewed all tissue slides to confirm the diagnosis. BBD patients were classified based on the published criteria developed by Dupont and Page (13).

Laboratory Assays. Total RNA was extracted from tissue samples by homogenization in TRIzol solution (Invitrogen), phase separation, precipitation, and wash following the manufacturer's instructions. The quality and quantity of RNA were measured by spectrophotometric analysis. TaqMan Reverse Transcription Reagents (N8080234) were obtained from Applied Biosystems. RNA was reverse transcribed in a final volume of 15 μL containing 0.15-μg RNA and 1× reverse transcription-PCR buffer, 5.5 mmol/L $MgCl_2$, 500 μmol/L each of deoxynucleotide triphosphate, 2.5 μmol/L random hexamers, 0.4 units/μL RNase inhibitor, and 3.125 units/μL multiScribe reverse transcriptase (Applied Biosystem). The mixture was incubated at 25°C for 10 min, 37°C for 120 min, and 95°C for 5 min. The primers and probes for the *COMT* (Hs00241349_m1), *CYP1B1* (Hs00164383_m1), and *β-actin* (Hs99999903) genes were obtained from Applied Biosystems. A pilot trial was done to estimate the amplification efficiency for both target genes (*COMT* and *CYP1B1*) and the endogenous (housekeeping) gene (*β-actin*), and the decision was made to dilute the samples. The cDNA samples were first diluted for the trial as follows: 1:1, 1:10, 1:100, 1:1,000, and 1:10,000. The 1:10 dilution was found to be the optimal concentration and thus was the dilution used in the assay for all samples. Quantitative real-time PCR was done using a 384-well optic tray on a ABI PRISM 7900HT Sequence Detection System (Applied Biosystems). PCR was done in a total volume of 5 μL containing 2.2-μL cDNA template of different dilutions, 1× TaqMan Universal PCR Master Mix (without UNG), and 1× Gene Expressions Assay Mix including the primers, marked probes from ABI Biosystems Assay-on-Demand services. The thermal cycling conditions were as follows: 95°C for 10 min to activate the AmpliTaq Gold enzyme, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Every sample was tested in triplicate. Two control samples were used in each plate to monitor interplate variation, which was found to be smaller than 5%. The threshold cycle (C_t) was determined to be 0.1 based on the amplification linear area of the target genes and the *β-actin* gene (an internal control). The normalized quantity of the target gene was calculated as $2^{-\Delta Ct}$, where ΔC_t was obtained directly by subtracting C_t for the target gene from C_t for the *β-actin* gene. The final result was expressed as $2^{-\Delta Ct} \times 1{,}000$.

Statistical Analysis. The average expression levels of the *CYP1B1* and *COMT* genes were measured with medians (25th, 75th percentiles). Differences in expression levels between tumor tissue and adjacent nontumor tissue from the same individuals were evaluated using the Wilcoxon signed rank test. Differences in expression levels between breast cancer and BBD subjects were evaluated using the Wilcoxon rank-sum test. Associations of *CYP1B1* and *COMT* expression with breast cancer risk compared with BBD were measured with odds ratios (OR) derived from logistic regression models. To evaluate possible nonlinearity in the relation between the outcome and the continuous *CYP1B1* and *COMT* expression levels and to avoid using arbitrary cutoff points to categorize the continuous predictors, we used fractional polynomial functions to look for the best power transformation for the continuous predictors (14-16). The power was chosen from a small preselected set of numbers {−2, −1, −0.5, 0, 0.5, 1, 2, 3}, where power = 0 denotes logarithmic transformation. The model with the best power transformation was the one with the largest log likelihood. The best power transformations for *CYP1B1* and *COMT* expression were evaluated simultaneously in the same model. Other potential confounders were also included in the model for adjustment. The final adjusted model-based OR and 95% confidence intervals (95% CI) were presented for the middle points of each quintile of *CYP1B1* and *COMT* expression levels compared with the overall median levels in BBD subjects. The association shape of the expression levels with breast cancer risk was shown with graphs.

Table 2. Expression levels of *CYP1B1* and *COMT* genes in tumor and adjacent nontumor tissues from patients diagnosed with breast cancer or BBD

Group	Median (25th, 75th)*		
	Tumor tissue	Adjacent tissue	P †
Breast cancer cases			
CYP1B1	35.3 (22.1, 60.3)	48.4 (34.9, 68.9)	0.020
COMT	32.9 (25.1, 60.0)	46.2 (28.2, 64.5)	0.030
BBD subjects			
CYP1B1	37.3 (21.7, 78.4)	35.1 (24.1, 53.5)	0.087
COMT	43.4 (33.8, 64.0)	55.3 (43.9, 85.4)	<0.001

*Median, 25% percentile, and 75% percentile.
†The P values were derived from the Wilcoxon signed rank test.

Results

As shown in Table 1, the breast cancer cases included in the current study are similar to the entire case group in the parent study in demographic characteristics. The demographic characteristics of the breast cancer cases and BBD subjects are also compared in Table 1. Breast cancer cases, compared with BBD subjects, were significantly older at interview, were more likely to be postmenopausal, and have a higher

Table 3. Model-based ORs and 95% CIs for the association of CYP1B1/COMT expression in adjacent nontumor tissue with breast cancer risk

Expression levels*	Reference points†	Number‡		Model-based OR (95% CI)
		BC	BBD	
CYP1B1				
<18.9	10.6	5	14	0.21 (0.07-0.67)
18.9-30.0	26.6	7	13	0.81 (0.69-0.95)
30.1-42.5	35.1	13	13	1.00 (reference)
42.6-66.4	48.5	20	13	1.20 (1.05-1.38)
>66.4	100.7	18	14	1.55 (1.12-2.15)
COMT				
<40.8	35.0	28	13	1.72 (1.17-2.55)
40.8-49.3	45.5	6	13	1.19 (1.05-1.35)
49.4-68.3	55.3	13	13	1.00 (reference)
68.4-86.4	78.7	4	13	0.83 (0.73-0.95)
>86.4	99.9	12	13	0.78 (0.65-0.93)

*Gene expression levels were categorized by quintiles of CYP1B1 or COMT levels in BBD subjects.
†The middle value in each quintile was assigned as the reference point.
‡Number of subjects for breast cancer and BBD.

waist-to-hip ratio. These variables were considered as potential confounders in later analysis.

Table 2 compares gene expression levels in tumor tissue and adjacent nontumor tissue. In breast cancer subjects, both CYP1B1 and COMT levels were significantly lower in tumor tissue than in adjacent nontumor tissue (median 35.3 versus 48.4, $P = 0.020$ for CYP1B1 and median 32.9 versus 46.2, $P = 0.030$ for COMT). In BBD subjects, only COMT levels were significantly lower in tumor tissue compared with adjacent nontumor tissue (median, 43.4 versus 55.3; $P < 0.001$).

The differences in expression levels in adjacent nontumor tissue between breast cancer cases and BBD subjects were also examined (Table 2). The median level of CYP1B1 in breast cancer cases was found to be significantly higher than in BBD subjects (48.4 versus 35.1; $P = 0.005$, Wilcoxon rank-sum test), whereas the median level of COMT in breast cancer patients was significantly lower than in BBD subjects (46.2 versus 55.3; $P = 0.016$, Wilcoxon rank-sum test). Using fractional polynomial functions in logistic regression models including both CYP1B1 and COMT and potential confounders (age, waist-to-hip ratio, and menopausal status), we found that the best power transformation was -1 for CYP1B1 and -2 for COMT, whereas no power transformation was needed for age or waist-to-hip ratio. Whereas the improvement of the model with the power transformation over the linear model was significant ($P = 0.019$ for CYP1B1 and $P = 0.008$ for COMT), the model additionally adjusted for age and waist-to-hip ratio produced very similar results as the model only adjusted for menopausal status. Thus, the final model was only adjusted for menopausal status. Consistent with the Wilcoxon rank-sum test above, we found that both CYP1B1 and COMT were significant predictors ($P < 0.01$ for both). Using the median expression levels in the BBD subjects as the reference (35.1 for CYP1B1 and 55.3 for COMT), the fitted model for CYP1B1 was

$$\mathrm{OR} = \exp[(-23.664 \pm 1.96 \times 8.995) \times (\mathrm{CYP1B1}^{-1} - 35.1^{-1})]$$

and the fitted model for COMT was

$$\mathrm{OR} = \exp[(1113.2 \pm 1.96 \times 408.7) \times (\mathrm{COMT}^{-2} - 55.3^{-2})]$$

As compared with the midpoint of the third quintile (i.e., the overall median) levels in BBD subjects, the model-based ORs (95% CIs) for the midpoints of the first, second, fourth, and fifth quintiles of gene expression levels were 0.21 (0.07-0.67), 0.81 (0.69-0.95), 1.20 (1.05-1.38), and 1.55 (1.12-2.15) for CYP1B1 and 1.72 (1.17-2.55), 1.19 (1.05-1.35), 0.83 (0.73-0.95), and 0.78 (0.65-0.93) for COMT, respectively (Table 3).

The association shape is presented in Fig. 1 for CYP1B1 and in Fig. 2 for COMT. As shown in Figs. 1 and 2, higher levels of CYP1B1 were associated with increased breast cancer risk, whereas the opposite is true for COMT. The nonlinear association trend shown in both graphs is apparent (nonlinearity test, $P = 0.019$ for CYP1B1 and $P = 0.008$ for COMT).

We also analyzed the correlation and interaction between CYP1B1/COMT levels and CYP1B1/COMT genotypes that we previously reported (10) and found that they were not significant (data not shown).

Discussion

Several recent studies (17-19), all with very small sample sizes, the biggest one having only 29 subjects (18), have agreed that *CYP1B1* is expressed in both breast tumor tissue and nontumor tissue. However, the conclusions of these studies about specific expression levels in each type of tissue were not consistent. One study found no qualitative difference in *CYP1B1* expression between breast tumor tissue and adjacent nontumor tissue (17), whereas two other studies reported opposite findings with one study finding higher levels of CYP1B1 in nontumor tissue (18) and the other finding higher levels in tumor tissue (19). Few studies have examined *COMT* expression in breast tumor tissue versus nontumor tissue. Only one small study reported that COMT levels in the breast tissue of four women without breast cancer were higher compared with five women with breast cancer (19). Our study, thus far the largest study on the topic, found that *CYP1B1* and *COMT* were expressed in both tumor and nontumor tissue among breast cancer and BBD subjects and that CYP1B1 levels were lower in breast cancer tumor tissue than in the adjacent nontumor tissue, and that COMT levels were lower in tumor tissue than in the adjacent nontumor tissue in both breast cancer and BBD subjects. Although the findings on expression levels of these genes in breast tumor and nontumor tissue are not consistent across studies, it is noteworthy that all the studies reported that these estrogen-metabolizing enzymes were present in breast tissue, suggesting a potential role for them in breast cancer carcinogenesis.

We found no previous epidemiologic studies that specifically investigated the association of *CYP1B1* and *COMT*

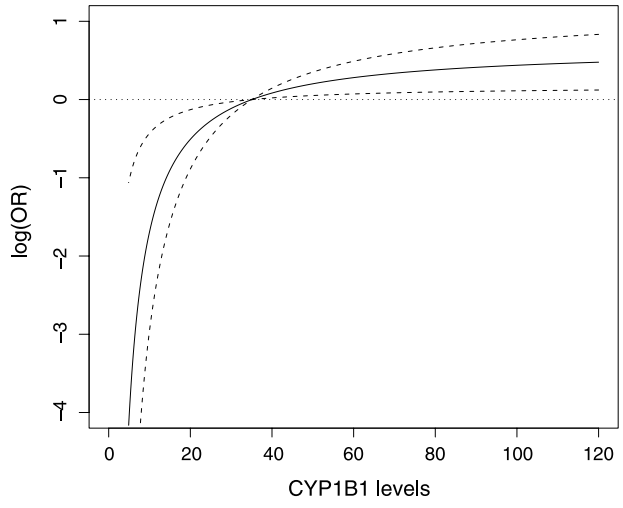

Figure 1. The log (OR) (*solid line*) and 95% CI (*dashed lines*) for different CYP1B1 levels versus the median CYP1B1 level (in BBD) in adjacent nontumor tissue.

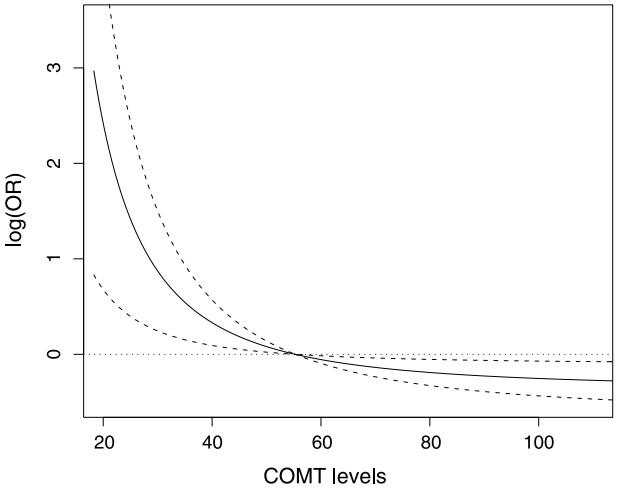

Figure 2. The log (OR) (*solid line*) and 95% CI (*dashed lines*) for different COMT levels versus the median COMT level (in BBD) in adjacent nontumor tissue.

expression with breast cancer risk using adjacent nontumor tissues. In this study, using BBD subjects as controls, we found that high levels of CYP1B1 and low levels of COMT were associated with increased breast cancer risk. These findings are consistent with the hypothesis that the formation and accumulation of carcinogenic catechol estrogens in breast tissue may increase the risk of breast cancer. A recent study reported that CYP1B1 levels were elevated in the peripheral leukocytes of lung cancer patients compared with subjects without cancer (20). Another recent study showed that *CYP1B1* was overexpressed in prostate cancer tissue compared with benign prostatic hyperplasia samples (21). These studies suggest that CYP1B1 may play an important role in tumor development due to the metabolic activation of endogenous and exogenous carcinogens.

Several limitations of this study should be noted. First, the use of BBD subjects as controls is less than optimal, although it is extremely difficult to obtain breast tissues from women without any breast diseases. Previous studies (13, 22, 23) have indicated that women with proliferative BBD are at an elevated risk of breast cancer as compared with women without BBD, whereas women with nonproliferative BBD are not. The BBD subjects in this study consisted of 42.6% proliferative cases and 57.4% nonproliferative cases. The findings of this study could be biased towards the null if the elevated breast cancer risk related to proliferative BBD did result from CYP1B1 and COMT expression. Second, it is possible that the tumor samples contained some normal tissue and vice versa. However, the adjacent nontumor tissue was obtained from regions as far as possible from the tumor in our study. It is noteworthy that the expression levels of CYP1B1 in breast cancer tissue were lower than the levels in the adjacent nontumor tissues. Therefore, any contamination from cancer tissue in the adjacent nontumor tissue would attenuate the true association. Third, the sample size in this study is still small although it is bigger than any other studies on the topic; both type I and type II errors may be of concern.

In conclusion, we found that *CYP1B1* and *COMT* were expressed in breast cancer tumor tissue and adjacent nontumor tissue and high levels of *CYP1B1* expression and low levels of *COMT* expression were associated with increased breast cancer risk. These findings support the hypothesis that the formation and accumulation of catechol estrogens in breast tissue through increased CYP1B1 expression and reduced COMT expression may play a significant role in breast cancer risk.

References

1. Hayes CL, Spink DC, Spink BC, Cao JQ, Walker NJ, Sutter TR. 17β-Estradiol hydroxylation catalyzed by human cytochrome *P*450 1B1. Proc Natl Acad Sci U S A 1996;93:9776–81.
2. Liehr JG, Ricci MJ. 4-Hydroxylation of estrogens as marker of human mammary tumors. Proc Natl Acad Sci U S A 1996;93:3294–6.
3. Guldberg HC, Marsden CA. Catechol-*O*-methyl transferase: pharmacological aspects and physiological role. Pharmacol Rev 1975;7:419–4.
4. Kocabas NA, Sardas S, Cholerton S, Daly AK, Karakaya AE. Cytochrome *P*450 CYP1B1 and catechol *O*-methyltransferase (COMT) genetic polymorphisms and breast cancer susceptibility in a Turkish population. Arch Toxicol 2002;76:643–9.
5. Rylander-Rudqvist T, Wedren S, Granath F, et al. Cytochrome *P*450 1B1 gene polymorphisms and postmenopausal breast cancer risk. Carcinogenesis 2003;24:1533–9.
6. Yim DS, Parkb SK, Yoo KY, et al. Relationship between the Val158Met polymorphism of catechol *O*-methyl transferase and breast cancer. Pharmacogenetics 2000;11:279–86.
7. Wedren S, Rudqvist TR, Granath F, et al. Catechol-*O*-methyltransferase gene polymorphism and post-menopausal breast cancer risk. Carcinogenesis 2003;24:681–7.
8. Ahsan H, Chen Y, Whittemore AS, et al. A family-based genetic association study of variants in estrogen-metabolism genes COMT and CYP1B1 and breast cancer risk. Breast Cancer Res Treat 2004;85:121–31.
9. Thyagarajan B, Brott M, Mink P, et al. CYP1B1 and CYP19 gene polymorphisms and breast cancer incidence: no association in the ARIC study. Cancer Lett 2004;207:183–9.
10. Wen WQ, Cai Q, Shu XO, et al. Cytochrome *P*450 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;14:329–35.
11. Denison MS, Nagy SR. Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. Annu Rev Pharmacol Toxicol 2003;43:309–34.
12. Gao YT, Shu XO, Dai Q, et al. Association of menstrual and reproductive factors with breast cancer risk: results from the Shanghai Breast Cancer Study. Int J Cancer 2000;87:295–300.
13. Dupont WD, Page DL. Risk factors for breast cancer in women with proliferative breast cancer disease. N Engl J Med 1985;312:146–51.
14. Royston P, Ambler G, Sauerbrei W. The use of fractional polynomials to model continuous risk variables in epidemiology. Int J Epidemiol 1999;28:964–74.
15. Royston P, Altman DG. Regression using fractional polynomials of continuous covariates: parsimonious parametric modelling (with discussion). Appl Stat 1994;43:429–67.
16. Sauerbrei W, Royston P. Building multivariable prognostic and diagnostic models: transformation of the predictors using fractional polynomials. J R Stat Soc Ser A 1999;162:71–94.
17. Iscan M, Klaavuniemi T, Coban T, Kapucuoglu N, Pelkonen O, Raunio H. The expression of cytochrome *P*450 enzymes in human breast tumours and normal breast tissue. Breast Cancer Res Treat 2001;70:47–54.
18. Modugno F, Knoll C, Kanbour-Shakir A, Romkes M. A potential role for the estrogen-metabolizing cytochrome *P*450 enzymes in human breast carcinogenesis. Breast Cancer Res Treat 2003;82:191–7.
19. Singh S, Chakravarti D, Edney JA, et al. Relative imbalances in the expression of estrogen-metabolizing enzymes in the breast tissue of women with breast carcinoma. Oncol Rep 2005;14:1091–6.
20. Wu MF, Wu WJ, Chang GC, et al. Increased expression of cytochrome *P*4501B1 in peripheral leukocytes from lung cancer patients. Toxicol Lett 2004;150:211–9.
21. Tokizane T, Shiina H, Igawa M, et al. Cytochrome *P*450 1B1 is overexpressed and regulated by hypomethylation in prostate cancer. Clin Cancer Res 2005;11:5793–801.
22. Fitzgibbons PL, Henson DE, Hutter RV; Cancer Committee of the College of American Pathologists. Benign breast changes and the risk for subsequent breast cancer: an update of the 1985 consensus statement. Arch Pathol Lab Med 1998;122:1053–5.
23. Hartmann LC, Sellers TA, Frost MH, et al. Benign breast disease and the risk of breast cancer. N Engl J Med 2005;353:229–37.