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

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Abstract

Cytochrome *P*450 1B1 (CYP1B1) and catechol-*O*-methyltransferase (COMT) are important estrogen-metabolizing enzymes and, thus, genetic polymorphisms of these enzymes may affect breast cancer risk. A population-based case-control study was conducted to assess the association of breast cancer risk with *CYP1B1* and *COMT* polymorphisms. A meta-analysis was done to summarize the findings from this and previous studies. Included in this study were 1,135 incident breast cancer cases diagnosed from August 1996 through March 1998 among female residents of Shanghai and 1,235 randomly selected, age frequency-matched controls from the same general population. The common alleles of the *CYP1B1* gene were Arg (79.97%) in codon 48, Ala (80.53%) in codon 119, and Leu (86.57%) in codon 432. The Val allele accounted for 72.46% of the total alleles identified in codon 108/158 of the *COMT* gene. No overall associations of breast cancer risk were found with any of the single nucleotide polymorphisms described above. This finding was supported by a meta-analysis of all previous published studies. No gene-gene interactions were observed between *CYP1B1* and *COMT* genotypes. The associations of breast cancer risk with factors related to endogenous estrogen exposure, such as years of menstruation and body mass index, were not significantly modified by the *CYP1B1* and *COMT* genotypes. We observed, however, that women who carried one copy of the variant allele in *CYP1B1* codons 48 or 119 were less likely to have estrogen receptor–positive breast cancer than those who carried two copies of the corresponding wild-type alleles. The results from this study were consistent with those from most previous studies, indicating no major associations of breast cancer risk with *CYP1B1* and *COMT* polymorphisms. (Cancer Epidemiol Biomarkers Prev 2005;14(2):329–35)

Introduction

Cytochrome *P*450 1B1 (CYP1B1) and catechol-*O*-methyltransferase (COMT) are important estrogen-metabolizing enzymes; thus, genetic polymorphisms in the genes encoding these enzymes may affect breast cancer risk. CYP1B1 has been shown to be the main CYP450 enzyme responsible for catalyzing the formation of 4-hydroxy estrogen, an estrogen metabolite shown to be carcinogenic in animal models (1). Six common polymorphisms of the *CYP1B1* gene have been described, of which four result in amino acid substitutions (2), including Arg→Gly in codon 48, Ala→Ser in codon 119, Leu→Val in codon 432, and Asn→Ser in codon 453. These polymorphic variants of the *CYP1B1* gene have been found to have 2.4- to 3.4-fold higher catalytic activity than the wild-type enzyme (3, 4). COMT, on the other hand, is a phase II enzyme that transforms catechol estrogens into nongenotoxic methylethers, thus inactivating them (5). A single G to A base pair change in the *COMT* gene produces an amino acid change (Val→Met) at codon 108 of soluble COMT and codon 158 of membrane-bound COMT, and this change has been associated with 2- to 3-fold decreased activity of COMT (6, 7). Therefore, it is conceivable that an increase in CYP1B1 activity and a decrease in COMT activity as a result of genetic polymorphisms may increase the formation and accumulation of carcinogenic catechol estrogens and thus increase breast cancer risk.

Several epidemiologic studies have been conducted during the past several years to investigate *CYP1B1* and *COMT* gene polymorphisms in relation to breast cancer risk. However, the findings from these studies have been inconsistent, and no convincing conclusions have been drawn due, at least in part, to the ethnic differences of the study populations, the inherent limitations of study designs, and small sample sizes. For instance, Kocabas et al. (8) reported that carriers of *CYP1B1* codon 432 Val allele (Val/Leu + Val/Val) in Turkish women had a higher risk of breast cancer than those with the Leu/Leu genotype [odds ratio (OR), 2.32; 95% confidence interval (CI), 1.26-4.25]. This association, however, was not observed in other studies (9-12), including a large recent case-control study with 1,521 cases and 1,498 controls conducted in Swedish women (9). Mixed findings have also been reported regarding the association between breast cancer risk and *COMT* genotypes. Yim et al. (13) found that breast cancer risk was increased among carriers of the low-activity *COMT* allele compared with noncarriers (OR, 1.7; 95% CI, 1.04-2.78). Again however, this association was not replicated in other studies (8, 14-18). Herein, we report the results from a large population-based case-control study that has comprehensively evaluated the associations of *CYP1B1* and *COMT* genetic polymorphisms with breast cancer risk, as well as the modifying effects of these polymorphisms on the association between estrogen exposure and breast cancer risk. We also did a meta-analysis to place our findings in the context of previous reports on these associations.

Received 5/25/04; revised 9/13/04; accepted 9/21/04.

Grant support: USPHS grants RO1CA64277 and RO1CA90899 from the National Cancer Institute.

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Materials and Methods

The Shanghai Breast Cancer Study is a population-based case-control study that recruited permanent Shanghai residents between the ages of 25 and 64 years who were newly diagnosed with breast cancer between August 1996 and March 1998. Details of the study have been described elsewhere (19). Briefly, 1,602 eligible cases were identified during the study period through a rapid case-ascertainment system supplemented by the Shanghai Tumor Registry, which has a virtually complete ascertainment of all incident cancer cases diagnosed among residents in urban Shanghai. Of these, 1,459 (91.1%) women participated in the study. The major reasons for nonparticipation were refusal (109 cases, 6.8%), death before interview (17 cases, 1.1%), and inability to locate the subject (17 cases, 1.1%). All cancer diagnoses were confirmed by independent pathologic reviews by two senior pathologists. Information on cancer diagnosis and estrogen receptor (ER) and progesterone receptor (PR) status was abstracted from medical charts using a standard protocol.

Controls were randomly selected from the Shanghai Resident Registry, which covers all permanent residents of urban Shanghai. Controls were frequency-matched to cases by age (5-year interval). The number of controls for each age stratum was predetermined using the age distribution of breast cancer cases reported to the Shanghai Tumor Registry from 1990 to 1993. Of 1,724 eligible controls, 1,556 (90.3%) were interviewed. The major reasons for nonparticipation were refusal (166 controls, 9.6%) and death or a prior cancer diagnosis before the interview date (2 controls, 0.1%).

All participating cases and controls completed a face-to-face interview using a structured questionnaire. The questionnaire included demographic factors, reproductive factors, hormone use, physical activity, tobacco and alcohol use, prior disease history, family history of cancer, and usual dietary habits. Women were measured for current weight, circumference of waist and hips, and sitting and standing heights. All measurements were taken twice by trained interviewers using a standard protocol.

In addition to the in-person interviews, 10-mL blood samples were obtained from 1,193 (82%) cases and 1,310 (84%) controls. The samples were collected in Vacutainer tubes containing EDTA or heparin, and processed on the same day, typically within 6 hours of blood draw, at the Shanghai Cancer Institute. The buffy coat samples were distributed into 2-mL vials and stored at −70°C.

Genomic DNA was extracted from buffy coats (WBC) using a Puregene DNA purification kit (Gentra Systems, Minneapolis, MN) following the manufacturer's protocol. Genotyping assays for the four single nucleotide polymorphisms of the *CYP1B1* gene and the single nucleotide polymorphism of the *COMT* gene were done using PCR-RFLP methods. PCR primers, restriction enzymes, and length of the resulting fragments in each genotype are listed in Appendix 1. The PCR was done in a Biometra T Gradient Thermocycler. Each 25 mL of PCR mixture contained 10 ng DNA, 1× PCR buffer with 1.5 mmol/L $MgCl_2$, 0.16 mmol/L each of deoxynucleotide triphosphate, 0.4 μmol/L of each primer, and 1 unit of HotstarTaq DNA polymerase (Qiagen, Valencia, CA). The reaction mixture was initially denatured at 95°C for 15 minutes followed by 35 cycles of 94°C for 45 seconds, 59°C to 62°C for 45 seconds, and 72°C for 45 seconds. The PCR was completed by a final extension cycle at 72°C for 8 minutes. Each PCR product (10 μL) was digested with restriction enzymes (New England BioLabs, Beverly, MA) at 37°C for 3 hours. The DNA fragments were then separated and visualized by electrophoresis on 1.5% to 3% agarose gel containing ethidium bromide.

The laboratory staff was blind to the identity of the subjects. Quality control samples were included in genotyping assays. Each 96-well plate contained one water, two CEPH 1347-02 DNA, two blinded quality control DNA, and two unblinded quality control DNA samples. The blinded and unblinded quality control samples were taken from the second tube of study samples included in the study. The agreement of the genotypes determined for the quality control samples and for the study samples was 96.2% (200 of 208).

Genotyping data were obtained from 1,135 (95.1%) cases and 1,235 (94.3%) controls who provided blood samples, representing 70.8% (1,135 of 1,602) of eligible case patients and 71.6% (1,235 of 1,724) of eligible control subjects. The major reasons for incomplete genotyping were insufficient DNA used for the particular assay and unsuccessful PCR amplification. We genotyped 200 samples and found no $Asn^{453}Ser$ polymorphism of the *CYP1B1* gene in our study population. This polymorphism was not included in the final analysis.

Variables used to measure endogenous estrogen exposure in this study included total years of menstruation, which was defined as the interval from menarche age to current age (for premenopausal women) or menopausal age (for postmenopausal women) excluding total pregnancy time, years of menstruation before first full-term pregnancy, body mass index, and waist-to-hip ratio.

The χ^2 test was used to compare the distributions of *CYP1B1* and *COMT* alleles and genotypes in cases and controls. The χ^2 goodness-of-fit test was used for testing Hardy-Weinberg equilibrium. Haplotype frequencies were estimated via the expectation-maximization algorithm (20). Haplotype-trait association was tested using an exact test (21). Logistic regression models were used to estimate ORs and 95% CIs for *CYP1B1* and *COMT* genotypes and to evaluate the gene-gene interaction and interaction of these genotypes with estrogen exposures. The potential confounding effect of major demographic factors and all known breast cancer risk factors, such as age, education, age at menopause, parity, and age at the first live birth was adjusted for using logistic models in the estimation of ORs. Adjustments for these factors did not produce substantial changes in the results. We report the results without adjustments for these factors.

The studies that reported associations of *CYP1B1* and *COMT* genes with breast cancer risk were identified by searching Medline for articles published through July 2004 using the key words CYP1B1, COMT, and breast cancer for a meta-analysis. We cross-referenced literature cited in relevant research and review articles for studies not otherwise identified. To summarize the current findings and findings from published data, we used the random effect methods of DerSimonian and Laird (22), using the STATA routine ''meta'' (23) in which the assumption of a common effect is relaxed. Thus, we did not assume that the studies represented the same effect. Rather, the effect sizes came from a normal distribution. We also used additional subgroup analyses to examine the possible impact of menopausal status and population ethnicity on the meta-analysis. The publication bias was examined using the method of Begg and Mazumdar (24), which evaluates whether there is correlation between effect estimates and study variances in the published literature.

Results

The distributions of selected demographic characteristics and major risk factors for breast cancer and the representativeness of the subjects with genotyping data relative to the parent study have been reported elsewhere (25, 26). Briefly, cases and controls had similar ages and education levels and the major risk factors identified in this study are consistent with those reported in previous studies conducted in other populations (19, 25, 26). Subjects with genotyping data were good representatives of those in the parent study with regard to the distribution of major demographic and risk factors.

Table 1 presents *CYP1B1* and *COMT* allele frequencies and estimated frequencies of the *CYP1B1* haplotypes for cases and controls. All *CYP1B1* and *COMT* single nucleotide polymorphisms were in Hardy-Weinberg equilibrium among both cases and controls. The common allele in *CYP1B1* codons 48, 119, and 432 among controls were Arg, Ala, and Leu with the frequencies 79.97%, 80.53%, and 86.57, respectively. The frequency of the common *COMT* allele (Val) was 72.46% among controls. Variant alleles at *CYP1B1* codons 48 and 119 were in strong linkage disequilibrium (Lewontin's $D' = 0.98$; correlation, $r = 0.97$). The most common haplotype for *CYP1B1* codons 48, 119, and 432 was Arg-Ala-Leu with the estimated frequencies 68.9% among cases and 67.8% among controls. Overall, neither the frequency of the *CYP1B1* and *COMT* alleles nor the estimated frequencies of *CYP1B1* haplotypes were significantly different between cases and controls.

Overall, we found that the *CYP1B1* and *COMT* genotypes were not significantly associated with breast cancer risk (Table 2). Further analysis stratified by menopausal status showed little change to these results. The interactions between menopausal status and the genotypes was not significant (all *P* values for interaction test >0.74; Table 2), nor was the gene-gene interaction between *CYP1B1* and *COMT* genotypes (all *P* values for interaction test >0.39; data not shown).

The associations of breast cancer risk with four variables related to endogenous estrogen exposure, including total years of menstruation, years of menstruation before first full-term pregnancy, body mass index, and waist-to-hip ratio, were examined for the whole group and stratified by the *CYP1B1* and *COMT* genotypes. An increased breast cancer risk was observed in all strata defined by *CYP1B1* and *COMT* genotypes. None of the interactions of the four estrogen-related variables with the *CYP1B1* or *COMT* genotypes was statistically significant. Analyses stratifying by menopausal status did not change this pattern (data not shown).

Table 3 shows that the women who carried one copy of the variant allele in *CYP1B1* codons 48 (OR, 0.71; $P = 0.033$) or 119 (OR, 0.67; $P = 0.012$) were less likely to have ER-positive breast cancer than those who were homozygous for the corresponding wild-type alleles. The relation between ER and the *CYP1B1* codon 432 and the *COMT* was not significant, nor was the relation between PR and the *CYP1B1* and *COMT* genotypes.

Finally, Table 4 presents the results of the meta-analysis. We found 3, 8, 3, and 13 published studies (8-18, 27-34) that reported data on associations of breast cancer risk with *CYP1B1* codons 119, 432, 453 and *COMT* genotypes, with the totals of patients with breast cancer, including the current study, being 3,969, 5,712, 2,165, and 8,286, respectively. Here, we did not include the results from a previous report based on a subset of the Shanghai Breast Cancer Study, as those subjects were included in the current study and there were errors in determining genotypes in the earlier study (35). Overall, none of the summary ORs were statistically significant for any genotype regardless of the inclusion or exclusion of the current study. Although a test for heterogeneity indicated substantial variability among different studies for heterozygous genotypes of *CYP1B1* codon 432 and *COMT* genotypes, the associations from most studies listed in Table 4 were not statistically significant. We only obtained enough information on menopausal status and population ethnicity for studies on *COMT*. Stratified analysis indicated that the summary OR of *COMT* for either premenopausal or postmenopausal women was not significant, nor was the summary OR for either Caucasian or Asian women. Publication bias was examined and found to be nonsignificant.

Table 1. Allele frequencies (%) of the *CYP1B1* and *COMT* genes and haplotype distribution of *CYP1B1* polymorphisms, the Shanghai Breast Cancer Study

		Case (n = 1,135)	Control (n = 1,235)	P*
Alleles				
CYP1B1†				
Codon 48	Arg	81.30	79.97	
	Gly	18.70	20.03	0.25
	HWE	0.66	0.85	
Codon 119	Ala	81.27	80.53	
	Ser	18.73	19.47	0.52
	HWE	0.50	0.46	
Codon 432	Val	13.24	13.43	
	Leu	86.76	86.57	0.85
	HWE	0.36	0.18	
COMT				
	Val	73.62	72.46	
	Met	26.38	27.54	0.38
	HWE	0.44	0.70	
Estimated frequency of *CYP1B1* haplotypes (codons 48-119-432)				
Arg-Ala-Val		11.77	11.40	0.72
Arg-Ala-Leu		68.90	67.82	0.41
Arg-Ser-Val		0.08	0.00	0.13
Arg-Ser-Leu		0.33	0.38	0.80
Gly-Ala-Val		0.33	0.29	0.78
Gly-Ala-Leu		0.20	0.47	0.19
Gly-Ser-Val		1.04	1.66	0.19
Gly-Ser-Leu		17.18	17.34	0.89
Overall χ^2 test			$P = 0.59$	

Abbreviation: HWE, Hardy-Weinberg equilibrium.
*P values for alleles are from χ^2 test, for haplotypes from the exact test.
†We genotyped 200 samples and found no $Asn^{453}Ser$ polymorphism in this study.

Discussion

In this large population-based case-control study, we found no overall associations of breast cancer risk with *CYP1B1* and *COMT* genotypes. These findings were consistent with most previous studies as summarized in our meta-analysis.

Although most previous studies listed in Table 4 showed no association, some studies did find significant associations. One possible explanation for the discrepancy in previously reported findings may be the relatively small sample sizes of some studies. As Thompson and Ambrosone pointed out (36), studies with small sample sizes are prone to result in both type I and type II errors, although we did not find a significant publication bias in the meta-analysis. The results from the Kocabas et al. study (8) on heterozygous genotypes of *CYP1B1* codon 432 and the Yim et al. (13) and Comings et al. (34) studies on *COMT* genotypes seemed to be very different from other studies. All three of these studies had small sample sizes. On the other hand, the three studies with the largest samples sizes, ours, the Swedish studies (9, 18), and the Dunning et al. study (28) found no significant association of breast cancer risk with *CYP1B1* and *COMT* genotypes. A recently published family-based genetic association study also found a lack of association between the *CYP1B1* and *COMT* genotypes and breast cancer risk (37). The meta-analysis provided a global impression on these genotypes in relation to breast cancer. The results from the meta-analysis indicated that these genotypes were not associated with the risk of breast cancer, and the subgroup meta-analysis on *COMT* showed that the conclusion for a null association held for both Caucasian and Asian women and both pre- and postmenopausal women.

Laboratory studies have shown that all enzymes encoded by the variant *CYP1B1* gene have higher catalytic activity than the wild-type enzyme in converting estrogen to 4-hydroxy estrogens and inducing DNA damage (3, 4). The *COMT* variant allele, on the other hand, is associated with decreased activity of COMT that inactivates catechol estrogens into nongenotoxic methylethers (5). Whereas it is biologically reasonable to hypothesize that women who carry variant *CYP1B1* and *COMT* alleles should have higher breast cancer risk, the

Table 2. Breast cancer risk associated with *CYP1B1* and *COMT* genotypes, the Shanghai Breast Cancer Study

	All subjects			Premenopausal women			Postmenopausal women		
	Case	Control	OR (95% CI)*	Case	Control	OR (95% CI)	Case	Control	OR (95% CI)
CYP1B1									
Codon 48									
Arg/Arg	740	765	1.00 (reference)	494	481	1.00 (reference)	243	281	1.00 (reference)
Arg/Gly	346	386	0.93 (0.78-1.11)	230	252	0.89 (0.71-1.11)	114	133	0.99 (0.73-1.34)
Gly/Gly	37	47	0.81 (0.52-1.27)	25	30	0.81 (0.47-1.40)	12	17	0.82 (0.38-1.74)
						Interaction,* P = 0.848			
Codon 119									
Ala/Ala	739	790	1.00 (reference)	492	501	1.00 (reference)	244	286	1.00 (reference)
Ala/Ser	349	372	1.00 (0.84-1.20)	233	241	0.98 (0.79-1.23)	114	130	1.03 (0.76-1.39)
Ser/Ser	36	50	0.77 (0.50-1.20)	25	32	0.80 (0.47-1.36)	11	18	0.72 (0.33-1.55)
						Interaction, P = 0.945			
Codon 432									
Leu/Leu	839	901	1.00 (reference)	555	567	1.00 (reference)	280	330	1.00 (reference)
Val/Leu	248	267	1.09 (0.61-1.95)	171	177	1.02 (0.52-2.01)	76	90	1.35 (0.42-4.30)
Val/Val	23	27	1.09 (0.62-1.92)	18	19	1.03 (0.54-1.99)	5	8	1.36 (0.44-4.20)
						Interaction, P = 0.916			
COMT									
Val/Val	612	628	1.00 (reference)	414	406	1.00 (reference)	195	220	1.00 (reference)
Val/Met	425	470	0.93 (0.78-1.10)	276	298	0.91 (0.73-1.12)	147	171	0.97 (0.72-1.30)
Met/Met	83	93	0.92 (0.67-1.26)	56	55	1.00 (0.67-1.48)	27	37	0.82 (0.48-1.40)
						Interaction, P = 0.749			

*The ORs (95% CIs) and P values for the interaction test were derived from logistic models.

evidence summarized in Table 4 from this study and previous ones does not seem to support this hypothesis.

Consistent with previous studies (19, 38, 39), all variables selected to measure endogenous estrogen exposure in this analysis were associated with an increased risk of breast cancer. However, these associations were not significantly modified by the *CYP1B1* and *COMT* genotypes. Many genes are involved in the estrogen biosynthesis/metabolism pathways (1, 2). We cannot exclude the confounding and modifying effects of other genes. Furthermore, the effects of the relevant genes on the carcinogenesis of breast cancer may only be triggered by endogenous estrogen exposure or environmental exposures over a certain period. It is difficult, however, for epidemiologic studies to detect potential modifying effects according to the timing of exposure.

Chinese women, in general, have lower levels of estrogen than Caucasian women such as the populations in the Swedish studies (9, 18) and Dunning et al. study (28). It is interesting to note that all of these studies with large sample sizes consistently suggest that *CYP1B1* and *COMT* genotypes do not play an important role in breast cancer risk. This finding also confirms that there is no important interaction between *CYP1B1* and *COMT* genotypes and endogenous hormone levels.

Whereas our analysis above points to a lack of association of breast cancer risk with *CYP1B1* and *COMT* polymorphisms, there is some evidence for a link with the ER status of breast cancer. Bailey et al. (10) and De Vivo et al. (11) observed that the percentage of ER-positive breast cancer patients was significantly higher among carriers of *CYP1B1* codon 432 Val/Val genotype. We did not observe this relation, but we found that women who carried one copy of the variant allele in the *CYP1B1* codons 48 or 119 were less likely to have ER-positive breast cancer. This finding indicates an interaction between ER status and the *CYP1B1* codons 48 or 119, but its biological significance needs to be investigated in future studies.

The inherent limitations of a case-control study such as recall bias of environmental exposures and selection bias may not be major concerns in this study because we mainly dealt with genotypes and the subjects with genotyping data were good representatives of those in the parent study. The limitations in the meta-analysis were that we selected only published, peer-reviewed studies and we did not take the quality weighting of studies into consideration. However, we

Table 3. The association of *CYP1B1* and *COMT* genotypes with ER/PR status among breast cancer cases, the Shanghai Breast Cancer Study

	ER−/ER+*	OR (95% CI)†	P	PR−/PR+*	OR (95% CI)†	P
CYP1B1						
Codon 48						
Arg/Arg	163/330	1.00 (reference)		169/318	1.00 (reference)	
Other‡	101/145	0.71 (0.52-0.97)	0.033	90/154	0.91 (0.66-1.25)	0.561
Codon 119						
Ala/Ala	160/332	1.00 (reference)		165/321	1.00 (reference)	
Other‡	104/144	0.67 (0.49-0.91)	0.012	92/154	0.86 (0.63-1.18)	0.356
Codon 432						
Leu/Leu	205/347	1.00 (reference)		194/352	1.00 (reference)	
Other‡	60/121	1.19 (0.84-1.70)	0.333	64/115	0.99 (0.70-1.41)	0.957
COMT						
Val/Val	149/246	1.00 (reference)		145/245	1.00 (reference)	
Other‡	114/231	1.23 (0.91-1.66)	0.185	113/229	1.20 (0.88-1.63)	0.242

*The number of patients with ER−/ER+ or PR−/PR+.
†The ORs (95% CIs) estimating the probability of ER+ or PR+.
‡Combining the other two genotypes for each single nucleotide polymorphism.

did not detect a significant publication bias and we believed that unpublished studies would represent lower quality and thus did not include them. We treated all published studies as equal quality because virtually all studies identified were case-control studies. Although most previous studies did not find an overall association of breast cancer with CYP1B1 or COMT genotypes, some of them reported significant associations in several specific subgroups, such as associations

Table 4. Results of meta-analysis examining breast cancer risk associated with *CYP1B1* and *COMT* gene polymorphisms

Study	Population	No. of subjects		OR (95% CI)*		References
		Case	Control			
CYP1B1 codon 119 (reference: Ala/Ala)				Ala/Ser	Ser/Ser	
Watanabe et al.	Japanese	339	361	1.62 (1.15-2.29)	0.60 (0.11-3.31)	(27)
Rylander-Rudqvist et al.	Swedish	1,499	1,338	1.00 (0.90-1.20)	1.00 (0.80-1.30)	(9)
Dunning et al.	Caucasian, United Kingdom	997	812	0.99 (0.81-1.20)	1.12 (0.78-1.60)	(28)
Overall†		2,835	2,511	1.12 (0.88-1.41)	1.04 (0.84-1.28)	(9, 27, 28)
This study	Chinese	1,134	1,212	1.00 (0.83-1.19)	0.75 (0.48-1.17)	
Overall‡		3,969	3,723	1.07 (0.91-1.25)	0.98 (0.81-1.19)	
Test for heterogeneity				$P = 0.065$	$P = 0.560$	
CYP1B1 codon 432 (reference: Leu/Leu)				Leu/Val	Val/Val	
Bailey et al.	Caucasian Americans, African Americans	223	223	0.81 (0.52-1.28)	1.09 (0.63-1.89)	(10)
Watanabe et al.	Japanese	336	324	0.90 (0.63-1.27)	1.10 (0.36-3.31)	(27)
De Vivo et al.	Caucasian Americans	453	453	1.46 (1.08-1.96)	1.27 (0.88-1.84)	(11)
Kocabas et al.	Turkish	84	103	2.68 (1.43-5.02)	1.27 (0.47-3.43)	(8)
Rylander-Rudqvist et al.	Swedish	1,484	1,336	1.00 (0.90-1.20)	1.00 (0.80-1.30)	(9)
Lee et al.	Korean	241	290	1.00 (0.70-1.60)	1.10 (0.30-4.70)	(12)
Dunning et al.	Caucasian, United Kingdom	1,617	845	0.78 (0.62-0.98)	0.85 (0.67-1.08)	(28)
Thyagarajan et al.	Caucasian Americans, African Americans	164	338	1.16 (0.66-2.06)	1.27 (0.78-2.07)	(29)
Overall†		4,602	3,912	1.09 (0.88-1.34)	1.01 (0.88-1.16)	(8–12,27–29)
This study	Chinese	1,110	1,195	1.00 (0.82-1.22)	0.86 (0.49-1.52)	
Overall‡		5,712	5,107	1.06 (0.90-1.26)	1.01 (0.88-1.15)	
Test for heterogeneity				$P = 0.005$	$P = 0.832$	
CYP1B1 codon 453 (reference: Asn/Asn)				Asn/Ser	Ser/Ser	
Bailey et al.	Caucasian Americans, African Americans	223	223	0.96 (0.62-1.49)	1.24 (0.33-4.71)	(10)
De Vivo et al.	Caucasian Americans	453	453	0.88 (0.64-1.20)	0.57 (0.23-1.42)	(11)
Rylander-Rudqvist et al.	Swedish	1,489	1,334	0.90 (0.80-1.10)	0.90 (0.60-1.50)	(9)
Overall†		2,165	2,010	0.91 (0.79-1.04)	0.85 (0.54-1.34)	(9–11)
Test for heterogeneity				$P = 0.920$	$P = 0.306$	
COMT (reference: Val/Val)				Val/Met	Met/Met	
Lavigne et al.	Caucasian Americans	113	114	1.34 (0.67-2.69)	1.42 (0.69-2.94)	(30)
Millikan et al.	Caucasian Americans, African Americans	654	642	0.80 (0.62-1.02)	0.80 (0.59-1.09)	(14)
Thompson et al.	Caucasian Americans	281	289	1.30 (0.90-1.90)	0.80 (0.50-1.40)	(31)
Huang et al.	Chinese	118	125	0.65 (0.37-1.16)	3.15 (0.89-12.1)	(32)
Mitrunen et al.	Finnish	481	480	0.84 (0.59-1.20)	0.75 (0.51-1.11)	(15)
Yim et al.	Korean	163	163	2.30 (1.35-3.85)	0.20 (0.07-0.92)	(13)
Hamajima et al.	Japanese	150	165	1.46 (0.90-2.36)	0.99 (0.49-2.02)	(16)
Bergman-Jungestrom and Wingren	Swedish	126	117	0.85 (0.35-2.10)	0.87 (0.34-2.20)	(17)
Kocabas et al.	Turkish	84	103	0.95 (0.50-1.81)	1.35 (0.55-3.32)	(8)
Wedren et al.	Swedish	1,490	1,340	1.00 (0.80-1.20)	0.90 (0.70-1.10)	(18)
Wu et al.	Asian§	589	562	0.82 (0.64-1.06)	0.84 (0.54-1.30)	(33)
Comings et al.	Californian	67	145	0.29 (0.15-0.57)	0.30 (0.13-0.67)	(34)
Dunning et al.	Caucasian, United Kingdom	2,850	1,908	1.02 (0.88-1.18)	10 (0.93-1.29)	(28)
Overall†		7,166	6,153	0.97 (0.81-1.15)	0.89 (0.74-1.07)	(8, 13–18, 28, 30–34)
This study	Chinese	1,120	1,191	0.93 (0.78-1.11)	0.93 (0.68-1.27)	
Overall‡		8,286	7,344	0.96 (0.83-1.12)	0.89 (0.76-1.05)	
Test for heterogeneity				$P < 0.001$	$P = 0.023$	
Caucasian women (from refs. 14, 15, 17, 18, 28, 30, 31, 34)		5,797	4,772	0.93 (0.77-1.12)	0.88 (0.72-1.07)	
Test for heterogeneity				$P = 0.018$	$P = 0.042$	
Asian women (from refs. 13, 16, 32, 33, and this study)		2,140	2,206	1.07 (0.77-1.48)	0.91 (0.59-1.38)	
Test for heterogeneity				$P = 0.001$	$P = 0.057$	
Premenopausal women (from refs. 8, 13–17, 30–32, and this study)		1,784	1,802	1.11 (0.82-1.50)	0.97 (0.72-1.31)	
Test for heterogeneity				$P = 0.004$	$P = 0.168$	
Postmenopausal women (from refs. 8, 13–16, 18, 28, 30–32, and this study)		5,836	4,829	0.98 (0.85-1.13)	0.89 (0.71-1.10)	
Test for heterogeneity				$P = 0.124$	$P = 0.027$	

*ORs (95% CIs) shown in italic were calculated by authors of this study.
†Overall estimates for studies not including this study.
‡Overall estimates for studies including this study.

between COMT genotypes and breast cancer risk among postmenopausal women who had a low body mass index (15, 31), a high body mass index (30), or a young age at menarche (15). It is difficult for a meta-analysis to capture such a stratum-specific associations when results from previous studies were not presented in a uniform manner. In our study in Shanghai, we did not find any significant association of breast cancer with CYP1B1 or COMT genotypes in stratified analyses by menopausal status and major risk factors for breast cancer. The statistical power, however, might not be adequate for evaluating some stratum-specific associations in this study.

There are several notable strengths of this study. The study was population based and had a high participation rate, minimizing the potential selection bias. The sample size was bigger than most previous studies, producing more stable results. Both *CYP1B1* and *COMT* genotype frequencies were consistent with Hardy-Weinberg equilibrium in both cases and controls and the allele frequencies of the *CYP1B1* single nucleotide polymorphisms and *COMT* single nucleotide polymorphism assayed were consistent with those reported for other Asian populations (12, 13, 16, 27, 32, 33). Taking into consideration the findings from this and previous studies, we conclude that *CYP1B1* and *COMT* polymorphisms alone may not be important independent risk factors for breast cancer.

Acknowledgments

We thank Drs. Qi Dai and Fan Jin for their contributions in coordinating data and specimen collection in Shanghai, Bethanie Hull for technical assistance in the preparation of this article, and all of the study participants and research staff of the Shanghai Breast Cancer Study.

Appendix A Summary of Genotyping Methods of *CYP1B1* and *COMT* Polymorphisms

Polymorphisms	PCR primers	Restriction enzyme	Alleles	Restriction fragments (bp)
CYP1B1				
Arg^{48}Gly	5′-TAAACCCGC TGTCCATCCA-3′, 5′-GAGTAGTGGC CGAAAGCCAT-3′	*Rsr*II	Arg (C)	270, 106
			Gly (G)	376
Ala^{119}Ser	Same as Arg^{48}Gly	*Ngo*MIV	Ala (G)	314, 62
			Ser (T)	376
Val^{432}Leu	5′-CACTGCCAA CACCTCTGTCT-3′, 5′-GCAGGCTCA TTTGGGTTG-3′	*Eco*57I	Leu (C)	187, 107
			Val (G)	294
Asn^{453}Ser	5′-CACTGCCAAC ACCTCTGTCT-3′, 5′-GCAGGCTCA TTTGGGTTG-3′	*Mwo*I	Ser (G)	147, 147
			Asn (A)	294
COMT				
Val^{158}Met	5′-ACTGTGGCTA CTCAGCTGTG-3′, 5′-CCTTTTTCCAG GTCTGACAAC-3′	*Nla*III	Val (G)	114, 29, 26
			Met (A)	96, 29, 26, 18

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Cancer Epidemiol Biomarkers Prev 2005;14:329-335.