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Association between polymorphisms in estrogen metabolism genes and breast cancer development in Chinese women

A prospective case–control study

Juanjuan Qiu, MMD[a], Zhenggui Du, MD[a,b,c], Jingping Liu, MD[d], Yi Zhou, MD[d], Faqing Liang, MMD[a,b], Qing Lü, MD[a,b,*]

Abstract

We comprehensively identified polymorphisms in estrogen-metabolizing genes that may be associated with breast cancer initiation in Chinese women, via an ongoing prospective case–control study.

An ongoing prospective case–control study of 427 female case patients diagnosed with breast cancer from August 2013 to March 2015 and 536 women (case controls) with no prior history of cancer or benign breast tumors was performed. Buccal cell specimens were obtained using the cotton swabbing method. DNA was extracted from the buccal cells using the phenol/chloroform method. Genotype was carried out for 5 single nucleotide polymorphisms (rs4646903, rs1056836, rs1695, rs4970737, and rs4680) using direct sequencing.

The polymorphic genotypes of glutathione *S*-transferase (GSTP1) (P = .044) and catechol-*O*-methyltransferase (COMT) (P=.008) showed significantly different distributions, while that of cytochrome P450 (CYP1B1) (P=.051) showed a slight difference in distribution between healthy women and patients with breast cancer. Individuals with homozygous variant genotypes for GSTP1 or COMT exhibited a higher risk of developing breast cancer than those with wild-type genotypes; however, for CYP1B1, the homozygous variant genotype was associated with a lower risk, and the heterozygous genotype for these 3 genes was not associated with breast cancer development.

An individual's risk of breast cancer is only influenced by the specific combination of risk-associated alleles of COMT and GSTP1, despite the protective effects of the homozygous CYP1B1 genotype revealed by univariate analysis.

Abbreviations: CI = confidence interval, COMT = catechol-*O*-methyltransferase, CVC = cross-validation consistency, CYP1A1 = cytochrome P450 1A1, CYP1B1 = cytochrome P450 1B1, GST = glutathione *S*-transferases, GSTM3 = glutathione *S*-transferases mu enzyme, GSTP1 = glutathione *S*-transferase, HWE = Hardy–Weinberg equilibrium, MDR = multifactor dimensionality reduction, OR = odds ratio, ROS = reactive oxygen species, SNP = single nucleotide polymorphism.

Keywords: breast cancer, estrogen metabolism, polymorphism

1. Introduction

In recent decades, the incidence of breast cancer has been increasing in developed countries as well as in China, making breast cancer by far the most frequent oncological disease in women.[1–3] It has been shown that early onset of menstruation, delayed age at 1st childbirth, and late menopause represent risk factors for breast cancer development,[4,5] implying a correlation between the pathogenesis of breast cancer and cumulative exposure to estrogens across a woman's life span.[6–8] Previous reports have suggested that estrogens exert carcinogenic effects by stimulating cell proliferation, which results in an increase in DNA replication errors.[9–11] However, more recent studies have revealed another important mechanism that the oxidative metabolism of estrogens can form DNA-damaging species, predominantly the 3,4-quinone, which can react with DNA to cause the mutations and therefore leading to the initiation of cancer.[12–15]

It is known that the metabolism of estrogens involves a series of enzymatic steps (Fig. 1).[16] In extrahepatic tissues, endogenous estrogens undergo extensive oxidative metabolism to form 2- and 4-hydroxy estrogens, which are catalyzed by phase I metabolizing enzymes of cytochrome P450 (CYP)1A1 and CYP1B1, respectively.[17–19] The 2- and 4-hydroxy estrogens are then oxidized to form estradiol-2,3-quinone and etradiol-3,4-quinone, respectively, accompanied by generation of the reactive oxygen species (ROS).[19] Estradiol-3,4-quinone reacts with DNA, mainly forming unstable N3-adenine and N7-guanine DNA adducts and generating apurinic sites, which give rise to mutations and initiate breast cancer.[20] Estradiol-2,3-quinone may also react with DNA; however, the 2,3-quinone has a shorter half-life and is apparently less carcinogenic.[19] Hydroxy-estrogens and quinones are detoxified by conjugation reactions catalyzed by phase II metabolizing enzymes such as catechol-O-methyltransferase

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[a] Department of Breast Surgery, [b] Laboratory of Breast Disease, [c] Laboratory of Pathology, West China Hospital, Sichuan University, [d] Sichuan Provincial People's Hospital, Chengdu, China.

[*] Correspondence: Qing Lü, Department of Breast Surgery, West China Hospital, Sichuan University, 37 Guoxue Street, Chengdu 610041, China (e-mail: lqlq1963@163.com).

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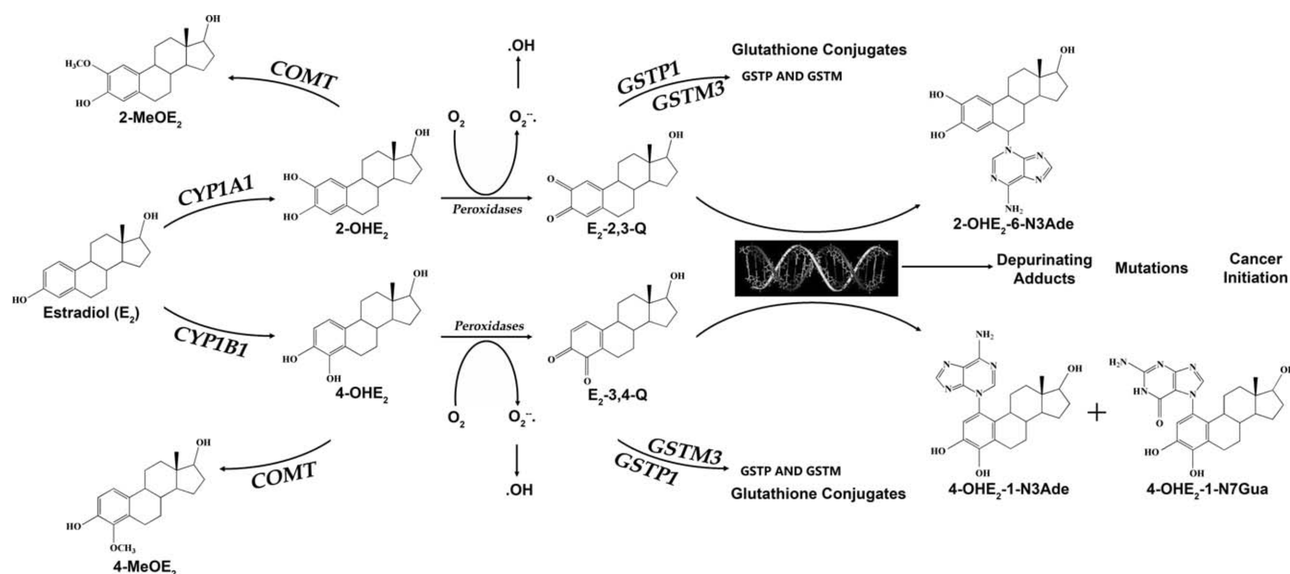

Figure 1. The pathway of estradiol metabolism in extrahepatic tissues such as those of the breast. Estradiol is metabolized into hydroxyl-estradiols, which are then oxidized to form quinones that react with DNA to form depurinating DNA adducts. Catechol-*O*-methyltransferase (COMT), glutathione *S*-transferase 1 (GSTP1), and GSTM3 detoxify hydroxyl-estradiols and quinones via conjugation reactions. Estrogen and other exogenous estrogens are metabolized via the same pathway as estradiol.

(COMT) and glutathione *S*-transferases (GSTs).[12,21,22] COMT inactivates hydroxyl-estrogens by conjugation to the noncarcinogenic methoxy-estrogens, which are even known to act as tumor suppressors (e.g., 2-methoxy-estrogens).[22] GSTs, in particular, the pi-class enzyme GSTP1 and mu enzyme GSTM3, conjugate both hydroxyl-estrogens and estrogen quinones.[21] Conjugated estrogens, which are less active and more water soluble, may be more easily excreted in the bile and urine.[22]

Polymorphisms in genes encoding phase I and phase II metabolizing enzymes are reported to be associated with differences in the enzyme activities, which may alter the levels of DNA-damaging species, such as 4-hydroxy-estrogens, estradiol-3,4-quinone, ROS, and depurinating DNA adducts, in breast cells, ultimately influencing the individual's susceptibility to breast cancer.[18,21,22] Genetic epidemiology studies have proposed correlations between polymorphisms of estrogen metabolism genes and breast cancer risk; however, the results of these studies have been inconsistent, and none have focused on all the five genes known to encode estrogen-metabolizing enzymes.[13,21,22] The present study, which was based on the hypothesis that the risk of developing breast cancer varies according to the genotype for genes involved in estrogen metabolism, aimed to determine whether polymorphisms of genes encoding phase I and phase II estrogen-metabolizing enzymes, such as *CYP1A1*, *CYP1B1*, *GSTM3*, *GSTP1*, and *COMT*, are related to breast cancer development. We additionally evaluated the association between higher order gene–gene interactions of these polymorphisms and the risk of breast cancer.

2. Methods

2.1. Study population

An ongoing prospective case–control study of 427 female case patients diagnosed with breast cancer from August 2014 to March 2017 and 536 women (case controls) with no prior history of cancer or benign breast tumors was performed. Cancer diagnoses for all patients were confirmed by 2 senior study pathologists via a review of tumor slides. Control subjects were recruited from a group of healthy female volunteers and frequency matched to case patients by age (5-year intervals). The study protocol was approved by the Ethics Committee of Sichuan University and relevant institutions for the use of human subjects in research (no: 186). All study participants were interviewed in-person by trained interviewers and informed consent for participation in the study was obtained.

2.2. DNA extraction and genotyping

Buccal cell specimens were obtained using the cotton swabbing method. DNA was extracted from the buccal cells using the phenol/chloroform method.[23]*CYP1A1* (rs4646903), *CYP1B1* (rs1056836), *GSTP1* (rs1695), *GSTM3* (rs4970737), and *COMT* (rs4680) were identified by direct sequencing. The PCR primers used are listed in supplementary Table 1, http://links.lww.com/MD/C652. The assay results were verified by 2 independent research staff who were blind to the case–control status. Ten percent of the samples from patients, including samples of each genotype, were regenotyped by independent laboratory personnel. No discrepancy was found on sequencing a randomly selected 5% of the samples.

2.3. Statistical analysis

Descriptive statistics for continuous variables were compared using the unpaired Student *t* test. Differences between proportions of categorical data were compared using Pearson Chi-squared test or Fisher exact test, including Hardy–Weinberg equilibrium (HWE) assumption assessment, and the correlation between various genotypes and breast cancer development. All statistical evaluations were performed using the SPSS for Windows package (SPSS 18.0; IBM, Armonk, NY). Multifactor dimensionality reduction (MDR) analysis was performed for detection and characterization of gene–gene interactions. The

most suitable gene–gene interaction model was selected on the basis of maximum testing accuracy and cross-validation consistency (CVC). Permutation results were considered statistically significant at the 0.05 level.

3. Results

3.1. Patient characteristics and Hardy–Weinberg equilibrium testing

A total of 427 patients with breast cancer and 536 case–control healthy women from western China were enrolled in this study. Mean ages at diagnosis (for patients with cancer) and at the time of enrollment (for case–control healthy women) were 46.5 years (range: 20–75 years) and 47.2 (range: 20–78 years) years, respectively. Tumor histology data for patients with cancer is shown in supplementary Table 2, http://links.lww.com/MD/C652. Additionally, among the 536 case–control healthy subjects, 324 did not suffer from cancer or any kind of breast-related disease, whereas 212 were diagnosed with benign cystic hyperplastic diseases; however, solid nodules were not present in any of the healthy case controls.

Table 1 shows the genotype distribution of *CYP1A1*, *CYP1B1*, *GSTP1*, *GSTM3*, and *COMT* polymorphisms in patients with breast cancer and healthy case-controls. HWE was tested for both groups using a chi square test, with $P=.05$ as the threshold. All polymorphisms were found to be in genetic equilibrium, indicating that the observed genotype frequencies of the 2 groups were constant and representative of the respective group.

3.2. Association of genetic variants with breast cancer risk

Table 2 shows univariate analysis and odds ratio (ORs) associated with each polymorphism. The polymorphic genotypes of *GSTP1* ($P=.04$) and *COMT* ($P=.008$) showed significantly different distributions, whereas the *CYP1B1* genotype showed a slightly different distribution in healthy women and patients with breast cancer ($P=.05$). Compared with the wild-type genotypes of *GSTP1* (AA) or *COMT* (GG), a significantly higher risk of breast cancer was associated with the homozygous variant genotypes of *GSTP1* (GG) or *COMT* (AA), yielding an OR of 2.230 (95% confidence interval [CI]=1.127–4.412) and 2.431 (95% CI=1.368–4.323), respectively. However, women with homozygous variant genotype for *CYP1B1* (GG) exhibited a significantly reduced risk of breast cancer (OR=0.389, 95% CI=0.152–0.990) compared with those with the wild-type genotype. However, the heterozygous genotype for these 3 genes was not found to be associated with breast cancer development. When the dominant model was considered, only *CYP1B1* was associated with a significantly reduced risk of breast cancer ($P=.049$; OR=0.743, 95% CI=0.553–0.999), whereas only marginally increased risks were observed for *GSTP1* and *COMT*, with ORs of 1.273 (95% CI=0.970–1.669) and 1.258 (95% CI=0.974–1.625), respectively; however, the differences were not statistically significant ($P=.08$ and .07, respectively). In addition, no associations with breast cancer risk were observed for polymorphisms in *CYP1A1* and *GSTM3*, with *P*-values ranging from .478 to .888.

3.3. Evaluation of higher order gene–gene interaction by multifactor dimensionality reduction analysis

The MDR analysis was performed for evaluation of higher order gene–gene interaction models, to discover the best model for the prediction of breast cancer development. As shown in Table 3, the best prediction model was based on a combination of *COMT* and *GSTP1* polymorphisms (testing accuracy=0.5562, CVC=10/10, permutation $P<.0001$). As described above, heterozygous genotypes for *GSTP1* and *COMT* showed no association with breast cancer development. Figure 2A additionally shows that patients who were heterozygous for either *GSTP1* or *COMT* exhibited a lower risk of breast cancer development; however, the present model indicated that those heterozygous for both *GSTP1* and *COMT* exhibited a higher risk. Furthermore, Table 4 and Figure 2B show that the association between variant alleles of *GSTP1* and *COMT* and the risk of breast cancer development is gene dosage dependent; patients with a larger number of variant alleles of *COMT* and *GSTP1*, except those with only 1 variant allele of either gene, exhibit a higher risk of breast cancer development.

Table 1

The genotype distribution for CYP1A1, CYP1B1, GSTP1, GSTM3, and COMT polymorphisms in the cases and controls and HWE testing.

Gene and rs number	Genotype (n)			Allele frequency, %			
	wt/wt (%)	wt/vt (%)	vt/vt (%)	Wt	vt	HWE χ^2	*P*-value
CYP1A1 rs4646903							
Case	136 (31.9%)	216 (50.6%)	75 (17.6%)	57.1	42.9	0.459	.498
Control	173 (32.3%)	280 (52.2%)	83 (15.5%)	58.4	41.6	3.022	.082
CYP1B1 rs1056836							
Case	332 (77.8%)	89 (20.8%)	6 (1.8%)	88.2	11.8	0.0001	.990
Control	387 (72.2%)	131 (24.4%)	18 (4.4%)	84.4	15.6	2.688	.101
GSTP1 rs1695							
Case	277 (64.9%)	127 (29.7%)	23 (5.4%)	79.7	20.3	2.692	.101
Control	376 (70.1%)	146 (27.2%)	14 (2.6%)	83.8	16.2	0.001	.969
GSTM3 rs4970737							
Case	229 (53.6%)	164 (38.4%)	34 (8.0%)	72.8	27.2	0.370	.543
Control	293 (54.7%)	205 (38.2%)	38 (7.1%)	73.8	26.2	0.068	79.4
COMT rs4680							
Case	226 (52.9%)	166 (38.9%)	35 (8.2%)	72.4	27.6	0.335	.563
Control	314 (58.6%)	202 (37.7%)	20 (3.7%)	77.4	22.6	3.268	.071

COMT = catechol-*O*-methyltransferase, HWE=Hardy–Weinberg equilibrium, SNP=single nucleotide polymorphism, wt=wild type, vt=variant type.

Table 2

Univariate analysis and ORs associated with each polymorphism.

Gene	Genotype	Case n (%)	Control n (%)	P-value*	OR (95% CI)	P-value†
CYP1A1	TT 309 (32.1%)	136 (31.9%)	173 (32.3%)	.682	1	–
	TC 496 (51.5%)	216 (50.6%)	280 (52.2%)		0.981 (0.737–1.307)	.897
	CC 158 (16.4%)	75 (17.6%)	83 (15.5%)		1.149 (0.782–1.689)	.478
	TC + CC 654 (67.9%)	291 (68.1%)	363 (67.7%)		1.020 (0.777–1.339)	.888
CYP1B1	CC 719 (74.7%)	332 (77.8%)	387 (72.2%)	.051	1	–
	CG 220 (22.8%)	89 (20.8%)	131 (24.4%)		0.792 (0.583–1.076)	.135
	GG 24 (2.5%)	6 (1.8%)	18 (4.4%)		0.389 (0.152–0.990)	.040
	CG + GG 244 (24.6%)	95 (22.2%)	149 (27.8%)		0.743 (0.553–0.999)	.049
GSTP1	AA 653 (67.8%)	277 (64.9%)	376 (70.1%)	.044	1	–
	AG 273 (28.3%)	127 (29.7%)	146 (27.2%)		1.181 (0.889–1.569)	.251
	GG 37 (3.8%)	23 (5.4%)	14 (2.6%)		2.230 (1.127–4.412)	.018
	AG + GG 310 (32.2%)	150 (35.1%)	160 (29.9%)		1.273 (0.970–1.669)	.082
GSTM3	GG 522 (54.2%)	229 (53.6%)	293 (54.7%)	.865	1	–
	GC 369 (38.3%)	164 (38.4%)	205 (38.2%)		1.024 (0.783–1.339)	.865
	CC 72 (7.5%)	34 (8.0%)	38 (7.1%)		1.145 (0.699–1.876)	.591
	GC + CC 441 (45.8%)	198 (46.4%)	243 (45.3%)		1.043 (0.808–1.346)	.749
COMT	GG 540 (56.1%)	226 (52.9%)	314 (58.6%)	.008	1	–
	GA 370 (38.4%)	166 (38.9%)	202 (37.7%)		1.142 (0.874–1.491)	.331
	AA 53 (5.5%)	35 (8.2%)	20 (3.7%)		2.431 (1.368–4.323)	.002
	GA + AA 423 (43.9%)	201 (47.1%)	222 (41.4%)		1.258 (0.974–1.625)	.079

* Comparison of wild-type genotypes with heterozygous genotypes, homozygous variant genotypes, and dominant model genotypes, respectively.

† Comparison of polymorphic genotype distributions in patients with breast cancer and healthy case-controls.

4. Discussion

A large number of studies have confirmed the hereditary nature of breast cancer.[24,25] However, genes with a high rate of penetrance, such as *BRCA1* and *BRCA2*, account for <15% of cases of breast cancer,[26,27] suggesting that the hereditary nature of breast cancer may be attributed to a large number of low-penetrance genes whose polymorphisms result in differences in susceptibility to this disease.[21,22,26] In this study, we focused on the evaluation of the relationship between polymorphisms in estrogen-metabolizing genes and breast cancer initiation. Univariate analysis identified that genotypes for *GSTP1*, *COMT*, and *CYP1B1* were associated with breast cancer development; however, MDR analysis revealed that the best prediction model was based only on a combination of *COMT* and *GSTP1* polymorphisms.

During the 1st phase of estrogen metabolism, catalyzed by CYP1A1 and CYP1B1, the polarity of estrogens increases, which may be associated with an increased risk of the breast cancer.[17,19] In this study, we found that the variant allele of *CYP1B1* is associated with a lower risk of breast cancer, whereas the genotypes of *CYP1A1* are not associated with breast cancer development. The precise mechanism underlying the protective effects of the variant allele of *CYP1B1* remain unknown[28]; however, we hypothesize that heterozygous or homozygous variant genotypes for *CYP1B1* may exhibit significantly reduced enzyme function, which results in the generation of lower

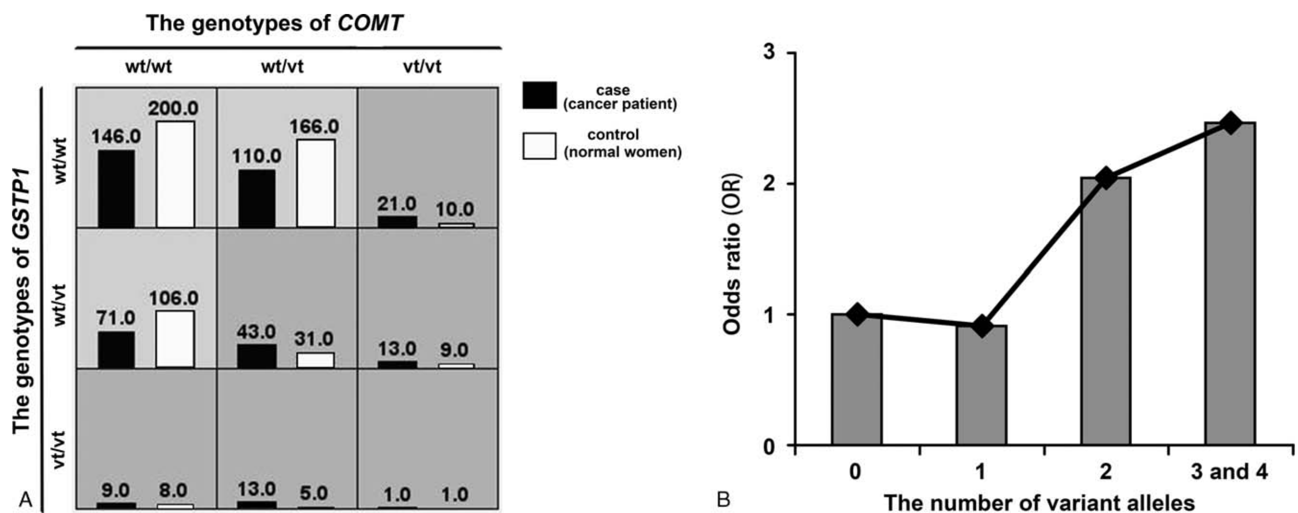

Figure 2. (A) The patients were divided into 2 groups according to the risk of developing breast cancer by multifactor dimensionality reduction analysis; the 3 pale gray cells in the upper left part of (A) shows patients with a lower breast cancer risk as well as a smaller number of risk-associated alleles, while the 6 dark gray cells in the bottom right shows patients with both a higher breast cancer risk and a bigger number of risk-associated alleles. (B) The relationship between odds ratio and the number of risk-associated alleles of glutathione *S*-transferase 1 (GSTP1) and catechol-*O*-methyltransferase (COMT) is shown.

Table 3

Evaluation of higher order gene–gene interaction models by MDR analysis.

Model	CV training	CV testing	CVC	*P*-value
COMT	0.5318	0.4904	4/10	.0617
COMT, GSTP1	0.5575	0.5562	10/10	<.0001
COMT, GSTP1, CYP1B1	0.5702	0.5390	5/10	<.0001

CV = cross-validation, CVC = cross-validation consistency, MDR = multifactor dimensionality reduction.

Table 4

The association between breast cancer development risk and variant alleles of *GSTP1* and *COMT*.

The number of variant alleles	Case n (%)	Control n (%)	χ^2	*P*-value	OR (95% CI)
0	146 (42.2%)	200 (57.8%)	–	–	1
1	181 (40.0%)	272 (60.0%)	0.407	.523	0.912 (0.686–1.211)
2	73 (59.8%)	49 (40.2%)	11.272	.001	2.041 (1.341–3.107)
3 and 4	27 (64.3%)	15 (35.7%)	7.397	.007	2.466 (1.266–4.801)
0 and 1, 2, 3, and 4	327 (40.9%) 100 (61.0%)	472 (59.1%) 64 (39%)	22.163	.000	1 2.255 (1.599–3.181)

amounts of 4-hydroxy estrogens, 3,4-quinones, and depurinating adducts, and consequently, a lower risk of developing breast cancer. Although the precise relationship between *CYP1B1* genotypes and enzyme function has not been elucidated, it has been reported that heterozygous or homozygous variant genotypes for *CYP1A1* exhibit significantly reduced enzyme function,[29] which may result in alterations of the levels of 2-hydroxy estrogens and estradiol-2,3-quinone. However, the 2,3-quinone has a shorter half life and is less carcinogenic,[16] likely explaining why the distribution of *CYP1A1* polymorphisms did not differ between healthy women and patients with breast cancer. Thus, we suggest that the rs4646903 polymorphism of the *CYP1A1* gene does not represent a candidate polymorphism for low-penetrance breast cancer susceptibility in the Chinese population; therefore, the use of this polymorphism in clinical genetic testing to evaluate susceptibility to breast cancer is not recommended.

The second phase of estrogen metabolism involves the conjugation of catechol estrogens or estrogen quinines. Conjugated estrogens may be excreted in the bile and urine more easily.[22] COMT, the mu class (GSTM3), and pi class (GSTP1) enzymes are considered key enzymes in this process.[21,30,31] In this study, we found that the polymorphic genotypes for *GSTM3* showed no association with breast cancer development, which is consistent with the findings of Jaramillo-Rangel et al.[21] Therefore, the *GSTM3* gene polymorphism does not represent a candidate locus for low-penetrance breast cancer susceptibility in the Chinese population. In addition, we believe that individuals with heterozygous for *COMT* or *GSTP1* exhibit reduced enzymatic activity and impaired ability to detoxify substrates; however, the activity of the other enzyme may increase as a compensatory mechanism so that individuals who were heterozygous for either *GSTP1* or *COMT* showed no increased risk of breast cancer development. Besides, MDR analysis also showed that double-heterozygous genotypes for *COMT* and *GSTP1* are at a higher risk of developing breast cancer. This was attributed to that the compensatory mechanism may be impaired for individuals who were double-heterozygous for both *COMT* and *GSTP1*. A previous study also reported the phenomena that double-heterozygous genotypes led to significant changes in some phenotypes; however, there was no significant change in single heterozygote genotype and wild-type genotype.[32] Furthermore, we found that the association between breast cancer development and variant alleles of *GSTP1* and *COMT* is dependent on gene dosage: the larger the number of variant alleles, the more greatly reduced the ability to detoxify substrates, resulting in a higher risk of breast cancer development. We suggest that, in such high-risk individuals, the inter-examination periods are shortened to effectively monitor the onset of breast cancer, or that chemical or surgical prevention is made available to prevent the development of this disease.

We additionally found that the protective effect of the homozygous variant genotype of *CYP1B1*, as determined by univariate analysis, was not confirmed by MDR analysis. This was attributed to the large degree of overlap between the protective genotypes of *COMT* and *GSTP1* (double-homozygotes of the wild-type genotype for *COMT* and *GSTP1*) and *CYP1B1*. Individuals with the double-homozygous wild-type genotype for *COMT* and *GSTP1* were protected against breast cancer to a higher degree than those with the homozygous variant genotype for *CYP1B1*. Therefore, the best gene–gene interaction model for predicting breast cancer development was based on a combination of *COMT* and *GSTP1* polymorphisms, but not those of *CYP1B1*. Therefore, despite the rs1056836 polymorphism of the *CYP1B1* gene representing a candidate polymorphism for low-penetrance breast cancer susceptibility in Chinese population, the use of this polymorphism in clinical genetic testing is not recommended.

In conclusion, our findings indicate that the homozygous genotype for *CYP1B1* may confer protection against the development of breast cancer. However, an individual's risk of developing breast cancer appeared to be influenced only by a combination of risk-associated alleles of *COMT* and *GSTP1*.

Author contributions

Data curation: Zhenggui Du, Jingping Liu, Yi Zhou.
Formal analysis: juanjuan Qiu, Faqing Liang.
Investigation: Jingping Liu.
Methodology: Zhenggui Du, Yi Zhou.
Resources: Jingping Liu, Faqing Liang.
Software: Zhenggui Du, Yi Zhou.
Supervision: Faqing Liang.
Writing – original draft: juanjuan Qiu.
Writing – review & editing: Qing lü.

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