Genetics and Molecular Research

Genetic polymorphisms and haplotype of hormone-related genes are associated with the risk of breast cancer in Chinese women

Z. Pan[1,2], Z. Fu[3], Q. Song[1], W. Cao[4], W. Cheng[1] and X. Xu[1,2]

[1]Department of Clinical Laboratory, Hangzhou, China
[2]Key Laboratory Diagnosis and Treatment Technology on Thoracic Oncology, Hangzhou, China
[3]Department of Colorectal Cancer Surgery, Hangzhou, China
[4]Department of Medical Oncology, Zhejiang Cancer Hospital, Hangzhou, China

Corresponding author: X. Xu
E-mail: zjhzxxh@163.com

Genet. Mol. Res. 15 (2): gmr.15028640
Received March 23, 2016
Accepted April 11, 2016
Published May 13, 2016
DOI http://dx.doi.org/10.4238/gmr.15028640

ABSTRACT. Sex hormones play important roles in breast cancer (BC) development. This study investigated associations between BC risk and hormone-related gene variants in Chinese women. In a cohort of 336 patients with histopathologically confirmed BC and 390 age-matched controls, we genotyped seven single nucleotide polymorphisms (SNPs) in five hormone-related genes: estrogen receptor-α (*ESR1*), aromatase (*CYP19*), catechol-*O*-methyl transferase (*COMT*), sex hormone-binding globulin (*SHBG*), and glutathione *S*-transferase (*GSTP1*). Among these seven SNPs, the SNPs in *GSTP1* rs1695 [A/G; odds ratio (OR): 1.68; 95% confidence interval (CI): 1.23-2.30] and *ESR1* rs2046210 (C/T; OR: 1.39; 95%CI = 1.02-1.91) were associated with an increased risk among heterozygote carriers. Homozygotes of minor alleles of *CYP19* rs10046 (CC) were associated with a reduced risk of BC with OR: 0.61 (95%CI = 0.39-0.95). In addition, a stratified analysis by menopausal status indicated that the association of the

CYP19 polymorphisms (rs10046 and rs700519) with BC risk was mainly evident in premenopausal women, and the association of *CYP19* rs700519 with BC risk was significant in women less than 50 years old. Haplotype analysis identified 15 common haplotypes (>1%). The haplotype TGGGGTC was significantly associated with BC risk compared with the reference haplotype CGAGGTC (OR > 1000, P < 0.0001). Our data demonstrate that these *ESR1*, *GSTP1*, and *CYP19* polymorphisms are associated with risk of BC, and the risk haplotype TGGGGTC could help to identify populations with high susceptibility to BC in Chinese women.

Key words: Breast cancer; Hormone-related gene; Genetic variants; Haplotype; Association analysis

INTRODUCTION

Endogenous sex steroids have been shown epidemiologically to be associated with breast cancer (BC) development (Chen, 2008; Key et al., 2015). For example, high levels of circulating estradiol and estrone are associated with increased BC in postmenopausal women (Key et al., 2002; Lukanova et al., 2004; Missmer et al., 2004; Kaaks et al., 2005). BC risk is also indirectly linked to premenopausal hormone levels by epidemiological studies (Pike et al., 1983).

Although many epidemiological studies have evaluated the potential role of polymorphisms in estrogen metabolizing genes in BC risk, the overall results remain inconsistent. We selected several genes involved in estrogen synthesis and metabolism, including *COMT* (catechol-*O*-methyl transferase), *CYP19* (aromatase), *ESR1* (estrogen receptor-α), *GSTP1* (glutathione S-transferase P), and *SHBG* (sex hormone-binding globulin). *COMT*, an important estrogen-metabolizing enzyme, has a functional single nucleotide polymorphism (SNP) rs4680 (Val158Met), which results in a valine-to-methionine amino acid substitution and is associated with decreased activity of the COMT enzyme (Dawling et al., 2001). The *CYP19* gene encodes aromatase, which has two SNPs: rs700519, a non-synonymous coding SNP (Arg264Cys) in exon 7, and rs10046, located in the 3'UTR region of the *CYP19* gene. *ESR1* encodes an estrogen receptor with two SNPs: the rs2046210 SNP, which is located 29 kb upstream of the first untranslated exon, and the rs9383951 SNP, which is located in *ESR1* intron 5. The *GSTP1* SNP rs1695 (Ile105Val) G allele has been demonstrated to exhibit abnormal catalytic enzyme activity (Zimniak et al., 1994). A non-synonymous SNP in exon 8 of the *SHBG* gene results in an amino acid substitution of asparagine for aspartic acid, and the asparagine (N) allele is associated with elevated levels of SHBG in postmenopausal women (Haiman et al., 2005). Functionally relevant polymorphisms in these hormone-related genes may alter hormone levels and affect the risk of BC. Candidate polymorphisms selected in our study were associated with BC risk in some, but not all, studies. Therefore, we selected these polymorphisms in hormone-related genes to assess whether these variants are associated with the risk of BC risk in Chinese women.

MATERIAL AND METHODS

Study population

This study was approved by the Ethics Committee of Zhejiang Cancer Hospital. We

recruited 336 women with pathologically confirmed BC (mean age: 46 years; range: 27-84 years) in the Zhejiang Cancer Hospital during May 2011 and September 2012. We took detailed clinicopathological information, including tumor size, histological grade, and lymph node involvement, as well as estrogen receptor (ER), progesterone receptor, and human epidermal growth factor receptor 2 statuses from patients' medical records (Table 1). As controls, we randomly selected 390 age-matched (± 5 years) cancer-free women from the same residential areas.

Table 1. Clinicopathological characteristics of breast cancer patients.

Characteristics	Cases (%)	Controls (%)
Age (years)		
≤50	200 (59.5%)	236 (60.5%)
>50	136 (40.5%)	154 (39.5%)
Menopausal status		
Premenopausal	203 (60.4%)	
Postmenopausal	133 (39.6%)	
Tumor size (cm)		
≤2	59 (17.6%)	
>2	238 (70.8%)	
Missing	39 (11.6%)	
Histological grade		
I+II	137 (40.8%)	
III	82 (24.4%)	
Missing	117 (34.8%)	
Lymph node status		
Negative	116 (34.5%)	
Positive	213 (63.4%)	
Missing	7 (2.1%)	
ER status		
Negative	114 (33.9%)	
Positive	211 (62.8%)	
Missing	11 (3.3%)	
PR status		
Negative	135 (40.2%)	
Positive	190 (56.5%)	
Missing	11 (3.3%)	
HER2 status		
Negative	201 (59.8%)	
Positive	114 (33.9%)	
Missing	21 (6.3%)	

Missing: the number (%) of cases for which the corresponding information was not available.

DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood samples using a blood genomic DNA extraction kit (Xinjin Genetech Ltd., Hangzhou, China) according to the manufacturer protocol. We selected seven SNPs for genotyping as follows: *ESR1* rs2046210 and rs9383951, *CYP19* rs10046 and rs700519, *COMT* rs4680, *SHBG* rs6259, and *GSTP1* rs1695. Genotyping was performed with the iPLEX MassARRAY® platform (Sequenom, San Diego, CA, USA) with an allele-specific, matrix-assisted, laser desorption/ionization time-of-flight assay (Jurinke et al., 2002). Primers for amplification and extension reactions were designed by Sequenom guidelines using MassARRAY® Assay Design software. Primers for *COMT* rs4680 were 5'-CACCATCGAGATCAACCCCG-3' (F) and 5'-TTCCAGGTCTGACAACGGGT-3' (R); *CYP19* rs10046: 5'-GACTTGTCCTTGCACCCAGA-3' (F) and 5'-GGCCACTGAGTGTTC

ACTGT-3' (R); *CYP19* rs700519: 5'-CAGCAAGGATTTGAAAGATGCCA-3' (F) and 5'-GTTCAGGTCAGTACCTCTGCT-3' (R); *ESR1* rs2046210: 5'-CATCAGGGTGCCTCAA CTGT-3' (F) and 5'-TCCTCACACATACATACAGTCACA-3' (R); *ESR1* rs9383951: 5'-AGT CACAGCCAGCTTACAGAG-3' (F) and 5'-AGGCTCTTTCCTCTCAGGTCA-3' (R); *SHBG* rs6259: 5'-ATGCCACCTTTGCACTACCT-3' (F) and 5'-TTGTGCCCAAAGGCCATTCA-3' (R); *GSTP1* rs1695: 5'-ATCCCCAGTGACTGTGTGTTG-3' (F) and 5'-AAGCCCCTTTCTTT GTTCAGC-3' (R). All procedures were performed following manufacturer instructions. The average genotype call rate for these SNPs was >99%; the concordance rate for all SNPs was 100%.

Statistical analysis

Deviation from the Hardy-Weinberg equilibrium was examined in controls using the χ^2 test. Logistic regression analysis was used for odds ratios (OR) and 95% confidence intervals (CIs) for genotype case-control associations and haplotype association analyses. The most common haplotype was selected as the reference. $P < 0.05$ was considered significant. Associations of SNPs and patients' clinicopathological features were assessed using logistic regression analyses restricted to cases (case-only analyses). All statistical analyses were performed with SNPStats software (http://bioinfo.iconcologia.net/SNPstats) (Solé et al., 2006).

RESULTS

Study population characteristics were compared by case-control status, as shown in Table 1. The mean age of controls was similar to that of the BC patients.

Variant allele frequencies and association analysis are shown in Table 2. All SNP frequencies were in Hardy-Weinberg equilibrium among controls (data not shown). Increased BC risk was significantly associated with SNPs in *GSTP1* rs1695 (OR: 1.65; 95%CI = 1.19-2.28) and *ESR1* rs2046210 (OR: 1.43; 95%CI = 1.03-1.99) among heterozygote carriers. Homozygotes of *CYP19* (rs10046) minor alleles (CC) were associated with significantly reduced BC risk (OR: 0.61; 95%CI = 0.39-0.95). The remaining SNPs were not observed to be associated significantly with a risk of BC.

To explore the potential role of these SNP genotypes in BC, we further analyzed the association of these genotypes with clinicopathological features of patients (Table 3). A stratified analysis by menopausal status indicated that rs700519 and rs10046 were mainly evident in premenopausal women (OR: 2.31; 95%CI =1.37-3.91 and OR: 1.68; 95%CI = 1.04-2.70). An analysis by age group showed that the association between SNP rs700519 and BC was stronger in women under 50 years old. Further, the association between the SNPs rs700519 and rs10046 and BC risk according to the ER/progesterone receptor/ human epidermal growth factor receptor 2 (HER-2) status was also explored; no difference was found (data not shown). The *SHBG*, *ESR1*, *COMT*, and *GSTP1* SNPs were not associated with other clinicopathological features (data not shown).

Fifteen haplotypes with a frequency greater than 1% were identified (Table 4). There was an overall haplotype effect when comparing cases to controls ($P = 0.0025$). The haplotype TGGGGTC was associated with higher BC risk than the reference haplotype CGAGGTC (OR > 1000; $P < 0.0001$). Other haplotypes showed no association with BC risk.

Table 2. Genotype frequencies in breast cancer patients and control group.

Genotype	Controls (%) N = 392	Cases (%) N = 336	OR (95%CI)	P value
rs2046210				
C/C	146 (37.4%)	97 (30%)	1.00	
C/T	187 (48%)	178 (55.1%)	1.43 (1.03-1.99)	0.031
T/T	57 (14.6%)	48 (14.9%)	1.27 (0.8-2.01)	0.31
C/T+T/T	244 (62.6%)	226 (70%)	1.39 (1.02-1.91)	0.037
Rs9383951				
G/G	305 (78.2%)	272 (83.4%)	1.00	
G/C	75 (19.2%)	50 (15.3%)	0.75 (0.5-1.11)	0.146
C/C	10 (2.6%)	4 (1.3%)	0.45 (0.14-1.45)	0.168
G/C+C/C	85 (21.8%)	54 (16.6%)	0.71 (0.49-1.04)	0.078
Rs1695				
A/A	280 (71.8%)	194 (60.2%)	1.00	
A/G	100 (25.6%)	114 (35.4%)	1.65 (1.19-2.28)	0.0025
G/G	10 (2.6%)	14 (4.3%)	2.02 (0.88-4.64)	0.091
A/G+G/G	110 (28.2%)	128 (39.8)	1.68 (1.23-2.30)	0.0011
Rs6259				
G/G	265 (68%)	221 (68%)	1.00	
G/A	120 (30.8%)	98 (30.1%)	0.98 (0.71-1.35)	0.898
A/A	5 (1.3%)	6 (1.9%)	1.44 (0.43-4.78)	0.550
G/A+A/A	125 (32%)	104 (32%)	1.00 (0.73-1.37)	0.988
Rs4680				
G/G	221 (57.3%)	182 (56.9%)	1.00	
A/G	143 (37%)	119 (37.2%)	1.01 (0.74-1.38)	0.947
A/A	22 (5.7%)	19 (5.9%)	1.05 (0.55-2.00)	0.884
A/G+A/A	165 (42.8%)	138 (43.1%)	1.02 (0.75-1.37)	0.919
Rs10046				
T/T	111 (28.3%)	100 (29.9%)	1.00	
T/C	192 (49%)	185 (55.4%)	1.07 (0.76-1.05)	0.696
C/C	89 (22.7%)	49 (14.7)	0.61 (0.39-0.95)	0.028
T/C+C/C	281 (71.7%)	234 (70.1)	0.92 (0.67-1.27)	0.631
Rs700519				
C/C	289 (74.1%)	225 (70.1%)	1.00	
T/C	96 (24.6%)	87 (27.1%)	1.16 (0.83-1.63)	0.378
T/T	5 (1.3%)	9 (2.8%)	2.31 (0.76-6.99)	0.127
T/C+T/T	101 (25.9%)	96 (29.9%)	1.22 (0.88-1.70)	0.234

Table 3. Subgroups analysis between SNPs and clinicopathological feature of breast cancer patients.

Characteristics	rs700519			
	C/C	T/C	T/T	T/C+T/T
Age onset				
>50/≤50	92/133	24/63	0/9	24/72
OR (95%CI)	1.00	1.82 (1.06-3.12)	NA	2.08 (1.22-3.54)
P value		0.029	0.013	0.006
Menopause				
Post/pre	101/124	25/62	0/9	25/71
OR (95%CI)	1.00	2.02 (1.18-3.44)	NA (0-NA)	2.31 (1.37-3.91)
P value		0.009	0.007	0.001
	rs10046			
	T/T	T/C	C/C	T/C+C/C
Age onset				
>50/≤50	38/62	70/115	13/36	83/151
OR (95%CI)	1.00	1.01 (0.61-1.66)	1.70 (0.80-3.60)	1.12 (0.69-1.81)
P value		0.978	0.165	0.659
Menopause				
Post/pre	48/52	68/117	15/34	83/151
OR (95%CI)	1.00	1.59 (0.97-2.60)	2.09 (1.02-4.31)	1.68 (1.04-2.70)
P value		0.065	0.043	0.031

Table 4. Association analysis of haplotypes with breast cancer patients.

Haplotype	Freq	OR (95%CI)	P value
CGAGGTC	0.135	1.00	-
TGAGGTC	0.105	0.99 (0.51-1.94)	0.99
CGAGGCC	0.087	0.73 (0.37-1.44)	0.36
TGAGGCC	0.062	0.79 (0.39-1.59)	0.51
CGAGGCT	0.054	0.87 (0.42-1.77)	0.69
CGAGATC	0.050	0.81 (0.36-1.83)	0.62
CGAAGTC	0.050	0.90 (0.38-2.13)	0.82
CGGGGTC	0.041	2.02 (0.74-5.51)	0.17
TGAGATC	0.032	1.22 (0.47-3.16)	0.68
TGAGACC	0.026	0.51 (0.17-1.57)	0.24
CGAGACC	0.023	0.46 (0.12-1.69)	0.24
CCAGGTC	0.021	0.15 (0.02-1.10)	0.062
TGGGATC	0.018	1.25 (0.26-5.97)	0.78
TGAGACT	0.017	2.20 (0.71-6.77)	0.17
TGGGGTC	0.017	>1000	<0.0001

Global haplotype association P value: 0.0025.

DISCUSSION

In this case-control study, we investigated associations of hormone-related gene variants and BC risk in a Chinese population. Of the seven SNPs, *CYP19* rs10046, *ESR1* rs2046210, and *GSTP1* rs1695 were significantly associated with BC risk in this population; one haplotype comprising seven SNPs significantly increased the risk of BC in Chinese women.

CYP19, which converts androgens to estrogens, contributes to variations in circulating hormone levels, and polymorphisms in this gene potentially affect BC risk (Dunning et al., 2004). Association analysis between rs10046 and BC in different populations showed inconsistent results (Zhang et al., 2009; Pineda et al., 2013). Karin et al. (Zins et al., 2014) reported that the TT genotype of this polymorphism was associated with an increased BC risk. Our data contradicted two earlier studies in which the CC genotype had a reduced risk of BC. In a stratified analysis, Karin et al. found that rs10046 may affect BC susceptibility in women below the age of 50 years in an Austrian population. We found that the rs10046 CC genotype occurred in 16.9% of women under 50 years of age and 10.7% in women above 50 years of age in subgroup analysis; however, this difference was not significant. In a stratified analysis by menopausal status, the association between the rs10046 C allele and BC was more evident in premenopausal women than postmenopausal women.

Chattopadhyay et al. (2014) previously observed that rs700519 had a significant association with BC risk in North Indian women, which was strongly affected by menopausal status. Sun et al. (2015) also reported that rs700519 is associated with susceptibility to BC among the Han Chinese population. However, Khvostova et al. (2012) and Sangrajrang et al. (2009) found that rs700519 was not associated with BC risk in Siberian and Thai women. Our findings also found no association between rs700519 and BC risk, similar to those of Khvostova and Sangrajrang. However, stratified analysis by age and menopausal status showed the rs700519 T allele to be more strongly associated with premenopausal women or women under 50 years of age. It suggested that the association between rs700519 and BC was evident in premenopausal or women under the age of 50 years.

The *SHBG* rs6259 influences estrogen bioavailability, but its association with BC is uncertain. In a large study of Chinese women, Zhang et al. (2011) reported that *SHBG* rs6259 was significantly associated with BC risk in stage I and II combined analyses, in which

the minor allele (A) produced a reduced risk of disease. However, Clendenen et al. (2013) and Diergaarde et al. (2008) found no association between rs6259 and BC risk, which is in agreement with our findings.

COMT encodes an estrogen-metabolizing enzyme, and the *COMT* Val108/158Met (rs4680) is thought to reduce enzymatic activity, which has been widely studied as a potential risk factor for BC. Several studies showed no association between rs4680 and BC risk (20, 24-27). We also found no association between the *COMT* rs4680 and BC risk in Chinese women.

The glutathione S-transferase family of proteins is thought to affect conjugation of catechol estrogen quinones; the *GSTP1* Val105 allele has been shown to disrupt normal catalytic enzyme activity. Reding et al. (2009) showed that the *GSTP1* rs1695 A allele was a protective factor for BC compared with the G allele, but several other studies found no association between rs1695 and BC risk (Ramalhinho et al., 2011; Liu et al., 2013). Zhang et al. (2011) reported that the minor allele homozygotes of rs1695 (GG) were associated with an increased risk of developing BC, which is consistent with three other studies in Chinese women (Chang et al., 2006; Lee et al., 2008; Sakoda et al., 2008). Liu et al. (2013) found that *GSTP1* rs1695 was associated with BC risk in an Asian population, but not in Caucasians or Africans. In our data, the SNP in rs1695 was found to be associated with an increased BC risk among heterozygote carriers, which is similar to the results of Zhang et al. (2011). The differences in association between rs1695 and BC among Caucasians, Africans, and Asians could be attributed to the different ethnicities of the various populations.

The SNP rs2046210 is located 180 kb upstream of the transcription initiation site of the *ESR1* first coding exon and was first shown to be associated with increased BC risk by Zheng et al. (2009). Since then, several studies on *ESR1* rs2046210 have yielded inconsistent results. Guo et al. (2012) reported that the rs2046210 A allele was significantly associated with BC risk in a case-control study and then confirmed these findings in a meta-analysis of the overall population. A meta-analysis by Yang et al. (2013) reported that the A allele of rs2046210 was associated with significantly increased BC risk in the overall population. When stratified by ethnicity, this significance was lost in those of African descent, but it was maintained in European and Asian populations. Mizoo et al. (2013) performed a case-control study that associated rs2046210 with higher BC risk in Japanese women. Our results also suggest that rs2046210 T allele carriers (TC and TT genotypes) have a significantly elevated BC risk compared with those with CC genotypes. However, we found no evidence that this association was stronger in pre- or postmenopausal women.

The SNP rs9383951 is located in *ESR1* intron 5. Long et al. suggested that it was a susceptibility locus for BC, and this allele was associated with decreased BC risk in East Asians (Long et al., 2012). In our study, rs9383951 was not associated with BC risk in Chinese women.

Haplotype analysis can detect cis-acting causal variants that are potentially associated with disease risk, and the haplotype-specific risks are interesting to consider. In our data, haplotype analyses encompassing all seven SNPs identified 15 haplotypes with frequencies >0.01. A haplotype with frequency 0.017 was associated with extremely increased BC risk (TGGGGTC; OR: >1000) compared with the reference haplotype (CGAGGTC, 13.5%). Although not all seven SNPs in hormone-related genes are associated with BC risk, the haplotype TGGGGTC composed of seven SNPs significantly increased the risk of BC.

In summary, we reported associations between BC risk and *ESR1* rs2046210, *GSTP1* rs1695, and *CYP19* rs10046 SNPs and identified a haplotype TGGGGTC that was associated with a highly increased risk of BC in Chinese women. These findings suggest that the *ESR1*,

GSTP1, and *CYP19* polymorphisms are likely to play an important role in BC among Chinese women, and the TGGGGTC haplotype could be a genetic marker for BC risk.

ACKNOWLEDGMENTS

Research supported by the National Natural Science Foundation (#81372210), the Talents Project of Zhejiang Province Medical Health Platform (#2011RCA011), and the Medical and Health Science Project of Zhejiang Province (#2015103934).

REFERENCES

Chang TW, Wang SM, Guo YL, Tsai PC, et al. (2006). Glutathione S-transferase polymorphisms associated with risk of breast cancer in southern Taiwan. *Breast* 15: 754-761. http://dx.doi.org/10.1016/j.breast.2006.03.008

Chattopadhyay S, Siddiqui S, Akhtar MS, Najm MZ, et al. (2014). Genetic polymorphisms of ESR1, ESR2, CYP17A1, and CYP19A1 and the risk of breast cancer: a case control study from North India. *Tumour Biol.* 35: 4517-4527. http://dx.doi.org/10.1007/s13277-013-1594-1

Chen WY (2008). Exogenous and endogenous hormones and breast cancer. *Best Pract. Res. Clin. Endocrinol. Metab.* 22: 573-585. http://dx.doi.org/10.1016/j.beem.2008.08.001

Clendenen T, Zeleniuch-Jacquotte A, Wirgin I, Koenig KL, et al. (2013). Genetic variants in hormone-related genes and risk of breast cancer. *PLoS One* 8: e69367. http://dx.doi.org/10.1371/journal.pone.0069367

Dawling S, Roodi N, Mernaugh RL, Wang X, et al. (2001). Catechol-O-methyltransferase (COMT)-mediated metabolism of catechol estrogens: comparison of wild-type and variant COMT isoforms. *Cancer Res.* 61: 6716-6722.

Diergaarde B, Potter JD, Jupe ER, Manjeshwar S, et al. (2008). Polymorphisms in genes involved in sex hormone metabolism, estrogen plus progestin hormone therapy use, and risk of postmenopausal breast cancer. *Cancer Epidemiol. Biomarkers Prev.* 17: 1751-1759. http://dx.doi.org/10.1158/1055-9965.EPI-08-0168

Dunning AM, Dowsett M, Healey CS, Tee L, et al. (2004). Polymorphisms associated with circulating sex hormone levels in postmenopausal women. *J. Natl. Cancer Inst.* 96: 936-945. http://dx.doi.org/10.1093/jnci/djh167

Guo H, Ming J, Liu C, Li Z, et al. (2012). A common polymorphism near the ESR1 gene is associated with risk of breast cancer: evidence from a case-control study and a meta-analysis. *PLoS One* 7: e52445. http://dx.doi.org/10.1371/journal.pone.0052445

Haiman CA, Riley SE, Freedman ML, Setiawan VW, et al. (2005). Common genetic variation in the sex steroid hormone-binding globulin (SHBG) gene and circulating shbg levels among postmenopausal women: the Multiethnic Cohort. *J. Clin. Endocrinol. Metab.* 90: 2198-2204. http://dx.doi.org/10.1210/jc.2004-1417

Jurinke C, van den Boom D, Cantor CR and Köster H (2002). Automated genotyping using the DNA MassArray technology. *Methods Mol. Biol.* 187: 179-192.

Kaaks R, Rinaldi S, Key TJ, Berrino F, et al. (2005). Postmenopausal serum androgens, oestrogens and breast cancer risk: the European prospective investigation into cancer and nutrition. *Endocr. Relat. Cancer* 12: 1071-1082. http://dx.doi.org/10.1677/erc.1.01038

Key T, Appleby P, Barnes I and Reeves G; Endogenous Hormones and Breast Cancer Collaborative Group (2002). Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. *J. Natl. Cancer Inst.* 94: 606-616. http://dx.doi.org/10.1093/jnci/94.8.606

Key TJ, Appleby PN, Reeves GK, Travis RC, et al.; Endogenous Hormones and Breast Cancer Collaborative Group (2015). Steroid hormone measurements from different types of assays in relation to body mass index and breast cancer risk in postmenopausal women: Reanalysis of eighteen prospective studies. *Steroids* 99 (Pt A): 49-55. http://dx.doi.org/10.1016/j.steroids.2014.09.001

Khvostova EP, Pustylnyak VO and Gulyaeva LF (2012). Genetic polymorphism of estrogen metabolizing enzymes in Siberian women with breast cancer. *Genet. Test. Mol. Biomarkers* 16: 167-173. http://dx.doi.org/10.1089/gtmb.2011.0131

Lee SA, Fowke JH, Lu W, Ye C, et al. (2008). Cruciferous vegetables, the GSTP1 Ile105Val genetic polymorphism, and breast cancer risk. *Am. J. Clin. Nutr.* 87: 753-760.

Liu JJ, Liu JL, Zhang X, Xie L, et al. (2013). A meta-analysis of the association of glutathione S-transferase P1 gene polymorphism with the susceptibility of breast cancer. *Mol. Biol. Rep.* 40: 3203-3212. http://dx.doi.org/10.1007/s11033-012-2396-z

Long J, Cai Q, Sung H, Shi J, et al. (2012). Genome-wide association study in east Asians identifies novel susceptibility loci for breast cancer. *PLoS Genet.* 8: e1002532. http://dx.doi.org/10.1371/journal.pgen.1002532

Lukanova A, Lundin E, Micheli A, Arslan A, et al. (2004). Circulating levels of sex steroid hormones and risk of endometrial cancer in postmenopausal women. *Int. J. Cancer* 108: 425-432. http://dx.doi.org/10.1002/ijc.11529

Missmer SA, Eliassen AH, Barbieri RL and Hankinson SE (2004). Endogenous estrogen, androgen, and progesterone concentrations and breast cancer risk among postmenopausal women. *J. Natl. Cancer Inst.* 96: 1856-1865. http://dx.doi.org/10.1093/jnci/djh336

Mizoo T, Taira N, Nishiyama K, Nogami T, et al. (2013). Effects of lifestyle and single nucleotide polymorphisms on breast cancer risk: a case-control study in Japanese women. *BMC Cancer* 13: 565. http://dx.doi.org/10.1186/1471-2407-13-565

Pike MC, Krailo MD, Henderson BE, Casagrande JT, et al. (1983). 'Hormonal' risk factors, 'breast tissue age' and the age-incidence of breast cancer. *Nature* 303: 767-770. http://dx.doi.org/10.1038/303767a0

Pineda B, García-Pérez MA, Cano A, Lluch A, et al. (2013). Associations between aromatase CYP19 rs10046 polymorphism and breast cancer risk: from a case-control to a meta-analysis of 20,098 subjects. *PLoS One* 8: e53902. http://dx.doi.org/10.1371/journal.pone.0053902

Ramalhinho AC, Fonseca-Moutinho JA and Breitenfeld L (2011). Glutathione S-transferase M1, T1, and P1 genotypes and breast cancer risk: a study in a Portuguese population. *Mol. Cell. Biochem.* 355: 265-271. http://dx.doi.org/10.1007/s11010-011-0863-9

Reding KW, Weiss NS, Chen C, Li CI, et al. (2009). Genetic polymorphisms in the catechol estrogen metabolism pathway and breast cancer risk. *Cancer Epidemiol. Biomarkers Prev.* 18: 1461-1467.

Sakoda LC, Blackston CR, Xue K, Doherty JA, et al. (2008). Glutathione S-transferase M1 and P1 polymorphisms and risk of breast cancer and fibrocystic breast conditions in Chinese women. *Breast Cancer Res. Treat.* 109: 143-155. http://dx.doi.org/10.1007/s10549-007-9633-5

Sangrajrang S, Sato Y, Sakamoto H, Ohnami S, et al. (2009). Genetic polymorphisms of estrogen metabolizing enzyme and breast cancer risk in Thai women. *Int. J. Cancer* 125: 837-843. http://dx.doi.org/10.1002/ijc.24434

Solé X, Guinó E, Valls J, Iniesta R, et al. (2006). SNPStats: a web tool for the analysis of association studies. *Bioinformatics* 22: 1928-1929. http://dx.doi.org/10.1093/bioinformatics/btl268

Sun MY, Du HY, Zhu AN, Liang HY, et al. (2015). Genetic polymorphisms in estrogen-related genes and the risk of breast cancer among Han Chinese women. *Int. J. Mol. Sci.* 16: 4121-4135. http://dx.doi.org/10.3390/ijms16024121

Yang Z, Shen J, Cao Z and Wang B (2013). Association between a novel polymorphism (rs2046210) of the 6q25.1 locus and breast cancer risk. *Breast Cancer Res. Treat.* 139: 267-275. http://dx.doi.org/10.1007/s10549-013-2494-1

Zhang B, Beeghly-Fadiel A, Lu W, Cai Q, et al. (2011). Evaluation of functional genetic variants for breast cancer risk: results from the Shanghai breast cancer study. *Am. J. Epidemiol.* 173: 1159-1170. http://dx.doi.org/10.1093/aje/kwr004

Zhang L, Gu L, Qian B, Hao X, et al. (2009). Association of genetic polymorphisms of ER-alpha and the estradiol-synthesizing enzyme genes CYP17 and CYP19 with breast cancer risk in Chinese women. *Breast Cancer Res. Treat.* 114: 327-338. http://dx.doi.org/10.1007/s10549-008-9998-0

Zheng W, Long J, Gao YT, Li C, et al. (2009). Genome-wide association study identifies a new breast cancer susceptibility locus at 6q25.1. *Nat. Genet.* 41: 324-328. http://dx.doi.org/10.1038/ng.318

Zimniak P, Nanduri B, Pikuła S, Bandorowicz-Pikuła J, et al. (1994). Naturally occurring human glutathione S-transferase GSTP1-1 isoforms with isoleucine and valine in position 104 differ in enzymic properties. *Eur. J. Biochem.* 224: 893-899. http://dx.doi.org/10.1111/j.1432-1033.1994.00893.x

Zins K, Mogg M, Schneeberger C, Abraham D, et al. (2014). Analysis of the rs10046 polymorphism of aromatase (CYP19) in premenopausal onset of human breast cancer. *Int. J. Mol. Sci.* 15: 712-724. http://dx.doi.org/10.3390/ijms15010712