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Original Contribution

Evaluation of Functional Genetic Variants for Breast Cancer Risk: Results From the Shanghai Breast Cancer Study

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In previous studies among 1,144 cases and 1,256 controls recruited in stage 1 of the Shanghai Breast Cancer Study (SBCS I; 1996–1998), 18 known or potentially functional single nucleotide polymorphisms (SNPs) in 16 genes were found to be associated with breast cancer risk. The authors evaluated these associations among 1,918 cases and 1,819 controls recruited in stage 2 of the SBCS (SBCS II; 2002–2005) using genetic effect models and subgroup analyses predetermined from SBCS I results. Five SNPs (*AHR* rs2066853, *ATM* rs1003623, *ESR1* rs2234693, *GSTP1* rs1695, and *SHBG* rs6259) showed generally consistent results in SBCS I and SBCS II and statistically significant associations with breast cancer risk in combined analyses, mostly in subgroups defined by age or menopausal status. Further, the relation between breast cancer risk and *SHBG* rs6259 was found to vary by body mass index (weight (kg)/height $(m)^2$) (P for interaction = 0.003). The strongest reduction in risk associated with *SHBG* rs6259 was found for lean (body mass index <23) postmenopausal minor allele carriers (odds ratio = 0.6, 95% confidence interval: 0.5, 0.8; $P = 4.6 \times 10^{-4}$). This biologically plausible and highly significant finding provides strong evidence for a true association among Asian women. This study also highlights the value of gene-environment interaction analyses in evaluating genetic factors for complex diseases.

breast neoplasms; genetics; polymorphism, genetic; sex hormone-binding globulin

Abbreviations: BMI, body mass index; CI, confidence interval; FPRP, false-positive report probability; OR, odds ratio; SBCS, Shanghai Breast Cancer Study; SHBG, sex hormone-binding globulin; SNP, single nucleotide polymorphism.

Breast cancer is the most common malignancy among women in most parts of the world (1). Heritable factors include germ-line mutations in high-penetrance genes, such as *BRCA1*, *BRCA2*, *PTEN*, and *TP53*, and moderate-penetrance genes, such as *ATM*, *BRIP1*, *CHEK2*, and *PALB2*; however, these genes account for only about 20% of familial breast cancer risk (2). It has been hypothesized that common variants in low-penetrance genes may explain the majority of breast cancer cases (2, 3). Several of these genetic risk variants have been identified and confirmed in recent genome-wide association studies (4–10), including a study we conducted among Chinese women in Shanghai (11). Nevertheless, these genetic risk variants explain only a small fraction of breast cancer heritability, and many genetic risk variants for this common cancer remain to be discovered.

While genome-wide association studies are an important tool with which to search for novel genetic risk variants, their utility is limited by the genomic coverage of arrays available for genome-wide scans. Furthermore, because of the issue of large-scale multiple comparisons in genome-wide association studies, it is difficult to extensively evaluate gene-environment interactions. Over the past 15 years, we and other groups of investigators have examined genetic variants in many candidate genes in relation to breast cancer risk and have identified a number of possible associations, including gene-environment interactions. Using data from 2,400 cases and controls recruited from 1996 to 1998 as part of the first stage of the population-based Shanghai Breast Cancer Study (SBCS I), we previously reported associations with breast cancer risk for known or potentially functional

variants in a number of genes, including *AHR*, *ATM*, *CCND1*, *ESR1*, *GSTP1*, *SHBG*, and *TGFB1* (12–18). In the current study, we systematically reevaluated these associations using 1,918 cases and 1,819 controls recruited in the second stage of the SBCS (SBCS II). We examined both unpublished and previously published results from SBCS I to select single nucleotide polymorphisms (SNPs) that were known or likely to be functional and that had associations with altered breast cancer susceptibility. We chose and analyzed 18 SNPs in 16 genes among SBCS II participants in a manner analogous to the SBCS I results in order to evaluate potential low-penetrance common genetic variants and breast cancer susceptibility.

MATERIALS AND METHODS

Study population

Subjects were participants in the SBCS, a large, 2-stage, population-based case-control study conducted in urban Shanghai, China; the study design and data collection methods have been previously reported in detail (11). Briefly, recruitment for stage 1 (SBCS I) occurred between August 1996 and March 1998. A total of 1,602 eligible breast cancer cases were identified through a rapid case-ascertainment system, supplemented by the population-based Shanghai Cancer Registry. Controls were randomly selected from the general female population using the Shanghai Resident Registry and were frequency-matched to cases by 5-year age interval. A total of 1,724 eligible controls were identified. Stage 2 recruitment (SBCS II) occurred between April 2002 and February 2005, with the same inclusion criteria as SBCS I, except for age, which was expanded from 25–65 years in SBCS I to 20–70 years in SBCS II.

Of eligible participants, 1,459 cases (91.1%) and 1,556 controls (90.3%) in SBCS I and 1,989 cases (83.7%) and 1,989 controls (70.4%) in SBCS II completed in-person interviews with structured questionnaires. Blood or buccal-cell samples were taken, and results were available for 1,193 cases (81.8%) and 1,310 controls (84.2%) from SBCS I and 1,932 cases (97.1%) and 1,857 controls (93.4%) from SBCS II. The cancer diagnoses of the cases were histologically confirmed by 2 senior pathologists; stage of disease for breast cancer cases was determined by medical record abstraction using a standard protocol. All included participants provided informed consent, and approval was granted by relevant review boards in both China and the United States. Genomic DNA was extracted using commercial DNA purification kits.

SNP selection and genotyping

Genetic variants analyzed in the current study are known or potentially functional SNPs for which we had found a significant or marginal association with breast cancer risk, either among all women or in a particular subgroup of participants, in the SBCS I. Eighteen SNPs in 16 genes were included in this analysis (Table 1), of which results from SBCS I have been previously published for 12 (12–23) and preliminary results from SBCS II have been published for 2 (13, 24). Information on SNP function was compiled from the literature, dbSNP (25), Ensembl (26), FASTSNP (27), and F SNP (28) As previously reported, stage 1 genotyping was conducted using a variety of methods, including polymerase chain reaction–restriction fragment length polymorphism for 6 SNPs (rs9344, rs2234693, rs1695, rs2854744, rs6259, and rs1800469) (14–18, 22), TaqMan (Applied Biosystems, Foster City, California) for 4 SNPs (rs10003623, rs2273535, rs11655505, and rs3025039) (13, 19, 20, 23), MassARRAY (Sequenom, San Diego, California) for 1 SNP (rs1256054) (21), and the Masscode array (BioServe Biotechnologies, Laurel, Maryland) for 1 SNP (rs2066853) (12). All remaining stage 1 genotyping and all stage 2 genotyping was conducted using TaqMan allelic discrimination assays. Successful genotyping data for SBCS I participants were obtained from 88.5%–98.7% of cases and 91.2%–98.3% of controls; call rates for SBCS II participants were 96.7%–99.8% for cases and 96.4%–99.9% for controls.

Consistency rates for quality control samples genotyped by TaqMan assays ranged from 97% to 100%. Stringent quality control measures were also employed for all other genotyping methods included, and laboratory staff were blinded to the case-control status of all samples.

Statistical analysis

All statistical tests were 2-tailed, and statistical significance was defined as $P \leq 0.05$ unless stated otherwise. Differences between cases and controls in the distributions of categorical variables were evaluated using chi-squared tests; continuous variables were evaluated with *t* tests. Hardy-Weinberg equilibrium among controls was evaluated using Fisher's exact test. Allelic odds ratios and corresponding 95% confidence intervals were calculated by unconditional logistic regression that included adjustment for age. Heterogeneity between SBCS I and SBCS II results was evaluated with Cochran's *Q* statistic (29); when the *P* value was greater than 0.1, results were pooled, and when the *P* was less than or equal to 0.1, results were combined using a random-effects method (30).

Specific subgroup analyses for SBCS II data, including the appropriate genetic model (additive, dominant, or recessive), were predetermined on the basis of SBCS I results. Subgroup analyses included adjustment for age, although additional adjustment for education, menopausal status, body mass index (BMI; weight (kg)/height $(m)^2$), and waist-to-hip ratio was also considered. Interactions were also evaluated on the basis of results from SBCS I. Multiplicative interactions between genetic variants and demographic variables or breast cancer risk factors were evaluated using the likelihood ratio test when interaction terms were added to logistic regression models. BMI was dichotomized at 23, the proposed cutoff point for overweight in Asian populations (31).

All analyses were implemented using SAS 9.2 (SAS Institute Inc., Cary, North Carolina) and Stata 9.2 (Stata Corporation, College Station, Texas).

Table 1. Single Nucleotide Polymorphisms Evaluated for Replication, Including Functional Information, in the Shanghai Breast Cancer Study, 1996–2005

Gene	Variant	Alleles (Major/Minor)	Minor Allele Frequency[a]	Functional Information[b]	SBCS Publication (First Author, Year, Reference No.)	Previous Denotation (If Applicable)
AHR	rs2066853	G/A	0.36	Nonsynonymous, exon 10, codon 554, Lys→Arg	Long, 2006 (12)	$Lys^{554}Arg$
ATM	rs1003623	C/T	0.39	Intron 24; possible intronic enhancer	Ye, 2007 (13)	
AURKA (*STK15*)	rs2273535	A/T	0.34	Nonsynonymous, exon 5, codon 31, Phe→Ile	Dai, 2004 (19)	$Phe^{31}Ile$
BRCA1	rs11655505	C/T	0.38	Promoter; T allele shown to enhance promoter activity	Chan, 2009 (20)	−2,265C > T
CCND1	rs9344	A/G	0.44	Splice site, exon 4; G allele results in alternative transcript	Shu, 2005 (14)	A870G
ESR1	rs2234693	T/C	0.26	Intron 1; may influence transcriptional regulation	Cai, 2003 (15)	*Pvu*II
ESR2	rs1256054	G/C	0.03	Exon 7 (synonymous); may influence splicing regulation	Zheng, 2003 (21)	C(33,390)G
GSTP1	rs1695	A/G	0.18	Nonsynonymous, exon 5, codon 105, Ile→Val	Egan, 2004 (16)	$Ile^{105}Val$
IGF1R	rs2593053	G/A	0.26	Intron 20; may influence transcriptional regulation	Unpublished data	
IGFALS	rs3764349	G/A	0.19	Promoter	Unpublished data	
IGFALS	rs35587190	C/T	0.16	Promoter	Unpublished data	
IGFBP3	rs2854744	A/C	0.23	Intron 1; may influence splicing	Ren, 2004 (22)	A-202C
				C allele associated with lower circulating levels of insulin-like growth factor-binding protein 3		
SHBG	rs6259	G/A	0.18	Nonsynonymous, exon 8, codon 356, Asp→Asn	Cui, 2005 (18)	$Asp^{327}Asn$
				A allele associated with higher postmenopausal circulating SHBG levels		
STS	rs727519	G/C	0.31	Promoter; may influence transcriptional regulation	Unpublished data	
STS	rs1131289	G/A	0.36	Exon 10, 3′-UTR	Unpublished data	
SULT1E1	rs4149525	T/C	0.29	Promoter	Unpublished data	
TGFB1	rs1800469	T/C	0.48	Promoter; may influence transcriptional regulation	Shin, 2005 (17)	C-509T
VEGFA	rs3025039	C/T	0.19	Exon 8, 3′-UTR	Kataoka, 2006 (23)	T936C

Abbreviations: Arg, arginine; Asn, asparagine; Asp, aspartic acid; Ile, isoleucine; Lys, lysine; Phe, phenylalanine; Pro, proline; PvuII, *Proteus vulgaris* II [restriction enzyme]; SBCS, Shanghai Breast Cancer Study; SHBG, sex hormone-binding globulin; UTR, untranslated region; Val, valine.

[a] Frequency of the minor allele among controls in stage 1 of the SBCS.

[b] Functional information compiled from the literature, as well as from the dbSNP, Ensemble, FASTSNP, and F SNP Web sites.

RESULTS

In total, 1,144 breast cancer cases and 1,256 controls from SBCS I and 1,918 breast cancer cases and 1,819 controls from SBCS II were genotyped and included in the current study (Table 2). Participants from the 2 stages were generally comparable, although women in SBCS II were slightly older than those in SBCS I, because of the expansion in eligibility criteria. Established breast cancer risk factors, including early age at menarche, late age at menopause, late age at first livebirth, number of livebirths, family history of breast cancer, prior history of fibroadenoma, high BMI or waist-to-hip ratio, and low physical activity, were found to be associated with breast cancer among SBCS participants.

Eighteen known or potentially functional SNPs that showed a significant or marginally significant association with breast cancer risk among SBCS I participants were evaluated among SBCS II participants in the current study (Table 1). Genotype frequencies for all SNPs were in Hardy-Weinberg equilibrium among both SBCS I and SBCS II controls, and minor allele frequencies were similar across the SBCS I and SBCS II study populations (data not shown).

Associations with breast cancer risk are shown in Table 3, including allelic associations among all women and specific subgroup analyses and genetic models as predetermined by the results from SBCS I. Fourteen of the 18 variants selected had significant SBCS I results ($P \leq 0.05$), either among all women or in subgroup analyses; the remaining variants

Table 2. Demographic Characteristics and Distribution of Risk Factors for Breast Cancer in Participants, by Study Stage, Shanghai Breast Cancer Study, 1996–2005

Characteristic	Stage 1 (SBCS I; 1996–1998)					Stage 2 (SBCS II; 2002–2005)					SBCS I and SBCS II Combined				
	Cases (n = 1,144)		Controls (n = 1,256)		*P* Value	Cases (n = 1,918)		Controls (n = 1,819)		*P* Value	Cases (n = 3,062)		Controls (n = 3,075)		*P* Value
	Mean (SD)	%	Mean (SD)	%		Mean (SD)	%	Mean (SD)	%		Mean (SD)	%	Mean (SD)	%	
Demographic factors															
Age, years	47.6 (8.0)		47.2 (8.7)		0.227	50.9 (8.3)		51.7 (8.3)		0.002	49.7 (8.3)		49.9 (8.8)		0.317
Educational level of high school or more		43.4		42.8	0.763		57.4		47.7	<0.001		52.2		47.6	<0.001
Postmenopausal		33.0		36.0	0.132		43.6		49.7	<0.001		39.7		44.1	<0.001
Reproductive risk factors for breast cancer															
Age at menarche, years	14.5 (1.6)		14.7 (1.7)		<0.001	14.4 (1.7)		14.7 (1.8)		<0.001	14.4 (1.7)		14.7 (1.8)		<0.001
Age at menopause[a], years	48.2 (4.6)		47.5 (4.9)		0.038	48.5 (4.4)		48.3 (4.6)		0.231	48.4 (4.4)		48.0 (4.7)		0.023
Age at first livebirth, years	26.8 (4.1)		26.2 (3.8)		<0.001	26.3 (3.6)		25.7 (3.8)		<0.001	26.5 (3.8)		25.9 (3.8)		<0.001
No. of livebirths[b]	1.5 (0.8)		1.5 (0.9)		0.191	1.2 (0.7)		1.4 (0.8)		<0.001	1.3 (0.7)		1.4 (0.8)		<0.001
Prior hormone replacement therapy		2.5		2.6	0.989		4.3		3.1	0.053		3.6		2.9	0.070
Other risk factors breast cancer															
Family history of breast cancer		3.3		2.4	0.177		5.4		3.1	<0.001		4.6		2.8	<0.001
History of fibroadenoma		9.4		5.2	<0.001		10.1		5.6	<0.001		9.9		5.4	<0.001
Body mass index[c]	23.6 (3.4)		23.2 (3.4)		0.013	23.7 (3.3)		23.4 (3.2)		0.004	23.7 (3.3)		23.3 (3.3)		<0.001
Waist-to-hip ratio	0.81 (0.1)		0.80 (0.1)		<0.001	0.83 (0.1)		0.82 (0.1)		<0.001	0.82 (0.1)		0.81 (0.1)		<0.001
Physical exercise[d]		19.3		26.1	<0.001		29.3		34.1	0.002		25.6		30.8	<0.001

Abbreviations: SBCS, Shanghai Breast Cancer Study; SD, standard deviation.

[a] Among postmenopausal women.

[b] Among parous women.

[c] Weight (kg)/height $(m)^2$.

[d] Self-reported regular leisure-time physical activity (any vs. none).

selected had marginal associations in subgroup analyses ($P \leq 0.06$), with the exception of *CCND1* rs9344. This variant does not appear to have a significant or marginal association in SBCS I in the current analysis, since we present results from allelic and recessive tests using major allele homozygotes or major allele carriers as the respective reference groups; however, in the initial paper, Shu et al. (14) reported a marginal effect among young heterozygotic women compared with minor allele homozygotes, so this variant was selected for inclusion in the current analysis.

Five SNPs (*AHR* rs2066853, *ATM* rs1003623, *ESR1* rs2234693, *GSTP1* rs1695, and *SHBG* rs6259) were found to have generally consistent results in SBCS I and SBCS II and significant associations ($P \leq 0.05$) with breast cancer risk in combined analyses of subjects from both stages; these associations were primarily observed in the subgroup analyses based on models established in SBCS I. Among all women, the *GSTP1* rs1695 minor allele homozygotic genotype (GG) was associated with an increased risk of breast cancer in comparison with major allele carriers (recessive $P = 0.007$). Among premenopausal women, the minor allele (A) of *AHR* rs2066853 showed an association with reduced risk in a dose-response manner (P for trend $= 0.029$). Among younger women (age $\leq$45 years), the minor allele (C) of *ESR1* rs2234693 was associated with reduced risk in an additive fashion (P for trend $= 0.036$). Among postmenopausal women, carriers of the minor allele (A) of *SHBG* rs6259 had a reduced risk of breast cancer (dominant $P = 0.028$). Among older women (age >45 years), carriers of the minor allele (T) of *ATM* rs1003623 had an increased risk of breast cancer (dominant $P = 0.001$). In addition, 2 SNPs were found to have fairly consistent results from SBCS I and SBCS II, of which 1 (*TGFB1* rs1800469) had a marginally significant additive trend ($P = 0.068$) in the combined analysis, while 1 (*CCND1* rs9344) did not. Results from SBCS I and SBCS II were not in agreement for the 11 remaining SNPs. Current results for SBCS I may differ from those of previously published reports because of additional genotyping, updated participant exclusions, or the use of either major allele homozygotes or major allele carriers as the reference group. Associations shown included adjustment for age; additional adjustment for education, menopausal status, BMI, or waist-to-hip ratio did not materially alter our estimates of effect (data not shown).

SNPs that showed consistent or generally consistent associations with breast cancer risk were further evaluated for interactions with potential effect modifiers, as reported for SBCS I (14, 18). Significant interactions between *CCND1* rs9344 and BMI and waist-to-hip ratio were reported among postmenopausal women in the original SBCS I publication (14); these interactions were evident neither in the SBCS II data nor in the pooled analyses (data not shown). On the contrary, a significant interaction between *SHBG* rs6259 and BMI was found among both SBCS I participants and SBCS II participants (Table 4). *SHBG* rs6259 minor allele carriers (AG or AA) who were lean (BMI <23) had a reduced risk of breast cancer (odds ratio (OR) = 0.8, 95% confidence interval (CI): 0.7, 0.9), whereas heavier (BMI $\geq$23) minor allele carriers had an increased risk (OR = 1.2, 95% CI: 1.0, 1.4) (P for interaction = 0.003). This effect modification was evident regardless of whether BMI was dichotomized at 23 or 25 (data not shown), and results did not differ by menopausal status. The strongest reduction in risk associated with *SHBG* rs6259 was found for lean postmenopausal minor allele carriers (OR = 0.6, 95% CI: 0.5, 0.8; $P = 4.6 \times 10^{-4}$).

DISCUSSION

In this large, population-based case-control study, we systematically evaluated promising associations of known or potentially functional genetic variants with breast cancer risk. We found that results for 5 SNPs were generally consistent between SBCS I and SBCS II and statistically significant in combined analyses, including *GSTP1* rs1695, *AHR* rs2066853, *ESR1* rs2234693, *SHBG* rs6259, and *ATM* rs1003623. Intriguingly, most of these significant associations were found in subgroup analyses stratified by age or menopausal status; these associations would have been missed in genome-wide association studies, in which the current focus is to evaluate main effects of genetic variants. Therefore, our study has not only confirmed previously identified associations with breast cancer risk but also demonstrated the value of candidate-gene association studies with a more focused and detailed evaluation of specific genetic variants with a strong biologic rationale.

Of the SNPs that did not have consistent results between SBCS I and SBCS II, the most surprising was *BRCA1* rs11655505, a promoter polymorphism which was previously demonstrated to influence nuclear protein-binding and transcriptional activity and to be associated with a dominant decreased risk of breast cancer among 743 Hong Kong Chinese and 2,294 SBCS I participants (20). However, in the current study, there was no association with breast cancer risk among SBCS II participants for *BRCA1* rs11655505 minor allele carriers, even when the analysis was limited to older women (age $\geq$45 years) without a family history of breast cancer, who had the strongest association in the previous report (20). Reasons for these and other discordant results include potential false-positive findings in the original analyses or potential false-negative findings in the current study, especially for associations of small magnitude. Given our sample size for SBCS II, we had greater than 84.6% power to detect an additive odds ratio of 1.25 for a SNP with a minor allele frequency of 0.1 and greater than 89.1% power to detect an additive odds ratio of 1.20 for a SNP with a minor allele frequency of 0.2. For SBCS I and SBCS II combined, we had greater than 88.0% power to detect an additive odds ratio of 1.20 for a SNP with a minor allele frequency of 0.1 and greater than 96.0% power to detect an odds ratio of 1.2 for a SNP with a minor allele frequency of 0.15. Notably, SNPs that did not show consistent results between SBCS I and SBCS II were more likely to have recessive effects or lower minor allele frequencies than SNPs that did show consistent results. These findings highlight the necessity of including large study populations and the importance of replication across studies to identify true markers of disease susceptibility.

The most striking and consistent association identified in this study was the interaction between *SHBG* rs6259 and

Table 3. Results[a] From 2-Stage Analysis of the Relation of Known and Potentially Functional Single Nucleotide Polymorphisms With Breast Cancer Risk, Shanghai Breast Cancer Study, 1996–2005

Gene, SNP (Major/Minor Allele), Model, and Analysis	Stage 1 (SBCS I; 1996–1998)			Stage 2 (SBCS II; 2002–2005)			SBCS I and SBCS II Combined		
	OR[b]	95% CI[b]	*P* Value	OR	95% CI	*P* Value	OR	95% CI	*P* Value
AHR rs2066853 (G/A)									
Allelic association among all women	0.85*	0.75, 0.97	0.012	0.95	0.87, 1.05	0.334	0.92*	0.85, 0.99	0.024
Additive effect among premenopausal women			0.022			0.317			0.029
Heterozygotes	0.85	0.68, 1.05		0.87	0.72, 1.05		0.86*	0.75, 0.99	
Homozygotes	0.68*	0.49, 0.97		0.93	0.69, 1.24		0.83	0.66, 1.03	
ATM rs1003623 (C/T)									
Allelic association among all women	1.09	0.97, 1.23	0.159	1.06	0.97, 1.17	0.167	1.08	1.00, 1.16	0.055
Dominant effect among older women (age >45 years)	1.43*	1.12, 1.82	0.004	1.17*	1.00, 1.36	0.047	1.24*	1.09, 1.41	0.001
AURKA (*STK15*) rs2273535 (A/T)									
Allelic association among all women	0.98	0.86, 1.10	0.691	1.15*	1.05, 1.27	0.004	1.07	0.90, 1.26	0.450
Additive effect among postmenopausal women with body mass index[c] ≥25			0.013			0.717			0.232
Heterozygotes	0.92	0.57, 1.47		1.18	0.84, 1.64		1.08	0.82, 1.43	
Homozygotes	0.30*	0.13, 0.66		0.96	0.55, 1.66		0.64	0.41, 1.00	
BRCA1 rs11655505 (C/T)									
Allelic association among all women	0.91	0.80, 1.02	0.110	1.08	0.98, 1.19	0.122	1.01	0.94, 1.09	0.932
Dominant effect among older women (age ≥45 years) without a family history of cancer	0.81	0.65, 1.01	0.062	1.04	0.89, 1.21	0.632	0.96	0.85, 1.09	0.530
CCND1 rs9344 (A/G)									
Allelic association among all women	1.00	0.89, 1.13	0.950	0.98	0.90, 1.08	0.693	0.99	0.92, 1.07	0.817
Recessive effect among younger women (age <45 years)	0.77	0.55, 1.07	0.123	0.93	0.63, 1.36	0.707	0.84	0.65, 1.07	0.160
ESR1 rs2234693 (T/C)									
Allelic association among all women	0.89	0.79, 1.01	0.064	0.98	0.89, 1.07	0.622	0.94	0.88, 1.02	0.131
Additive effect among younger women (age ≤45 years)			0.046			0.287			0.036
Heterozygotes	0.86	0.65, 1.12		0.75	0.56, 1.00		0.82*	0.68, 1.00	
Homozygotes	0.67	0.46, 1.00		0.90	0.60, 1.36		0.78	0.59, 1.03	
ESR2 rs1256054 (C/G)									
Allelic association among all women	1.28	0.91, 1.79	0.155	1.05	0.80, 1.36	0.738	1.13	0.92, 1.39	0.253
Dominant effect among women with longer duration of menstruation (≥34 years)	2.15*	1.07, 4.35	0.033	1.03	0.70, 1.53	0.870	1.24	0.88, 1.74	0.220
GSTP1 rs1695 (A/G)									
Allelic association among all women	1.19*	1.03, 1.39	0.017	1.04	0.92, 1.16	0.560	1.09	1.00, 1.20	0.052
Recessive effect among all women	1.71*	1.10, 2.65	0.017	1.33	0.93, 1.92	0.122	1.47*	1.11, 1.95	0.007
IGF1R rs2593053 (G/A)									
Allelic association among all women	0.90	0.79, 1.03	0.120	1.02	0.91, 1.13	0.771	0.97	0.89, 1.05	0.435
Additive effect among overweight women (body mass index ≥23)			0.009			0.057			0.914

Table continues

Table 3. Continued

Gene, SNP (Major/Minor Allele), Model, and Analysis	Stage 1 (SBCS I; 1996–1998)			Stage 2 (SBCS II; 2002–2005)			SBCS I and SBCS II Combined		
	OR[b]	95% CI[b]	*P* Value	OR	95% CI	*P* Value	OR	95% CI	*P* Value
Heterozygotes	0.81	0.64, 1.04		1.15	0.95, 1.38		1.00	0.86, 1.16	
Homozygotes	0.55*	0.33, 0.92		1.34	0.91, 1.96		0.96	0.71, 1.30	
IGFALS rs3764349 (G/A)									
Allelic association among all women	0.86	0.74, 1.01	0.058	1.02	0.91, 1.14	0.778	0.96	0.88, 1.05	0.390
Recessive effect among premenopausal women	0.47*	0.25, 0.88	0.019	1.07	0.66, 1.72	0.794	0.77	0.54, 1.12	0.174
IGFALS rs35587190 (C/T)									
Allelic association among all women	0.89	0.76, 1.05	0.171	1.04	0.92, 1.18	0.563	0.99	0.89, 1.09	0.764
Recessive effect among premenopausal women	0.29*	0.12, 0.67	0.004	1.17	0.66, 2.06	0.592	0.72	0.46, 1.12	0.144
IGFBP3 rs2854744 (A/C)									
Allelic association among all women	1.09	0.95, 1.25	0.231	1.02	0.91, 1.13	0.757	1.04	0.96, 1.14	0.327
Recessive effect among all women	1.60*	1.08, 2.37	0.020	0.98	0.74, 1.30	0.900	1.17	0.93, 1.47	0.167
SHBG rs6259 (G/A)									
Allelic association among all women	0.95	0.82, 1.11	0.524	0.97	0.86, 1.10	0.666	0.96	0.88, 1.06	0.450
Dominant effect among postmenopausal women	0.71*	0.52, 0.96	0.026	0.89	0.72, 1.09	0.253	0.83*	0.70, 0.98	0.028
STS rs727519 (G/C)									
Allelic association among all women	0.98	0.87, 1.12	0.809	1.07	0.97, 1.18	0.149	1.04	0.97, 1.13	0.134
Recessive effect among premenopausal women	0.73	0.52, 1.02	0.061	1.07	0.81, 1.41	0.625	0.92	0.74, 1.13	0.423
STS rs1131289 (G/A)									
Allelic association among all women	0.92	0.81, 1.04	0.186	1.02	0.93, 1.23	0.615	0.99	0.92, 1.07	0.776
Recessive effect among premenopausal women	0.66*	0.49, 0.91	0.010	1.01	0.79, 1.30	0.922	0.86	0.71, 1.04	0.130
SULT1E1 rs4149525 (T/C)									
Allelic association among all women	1.13	0.99, 1.28	0.064	1.03	0.93, 1.14	0.525	1.07	0.99, 1.15	0.103
Additive effect among postmenopausal women			0.037			0.481			0.572
Heterozygotes	1.19	0.89, 1.59		1.09	0.89, 1.33		1.11	0.95, 1.31	
Homozygotes	1.69*	1.03, 2.77		0.75	0.53, 1.06		0.97	0.73, 1.29	
TGFB1 rs1800469 (T/C)									
Allelic association among all women	1.03	0.92, 1.15	0.653	0.98	0.90, 1.08	0.698	1.00	0.93, 1.07	0.994
Recessive effect for early-stage (stages 0 and 1) breast cancer	1.34	0.99, 1.80	0.056	1.11	0.88, 1.39	0.383	1.18	0.99, 1.42	0.068
VEGF rs3025039 (C/T)									
Allelic association among all women	0.83	0.80, 1.08	0.338	0.99	0.88, 1.12	0.885	0.97	0.88, 1.06	0.459
Additive effect among premenopausal women			0.033			0.838			0.179
Heterozygotes	0.93	0.74, 1.17		0.95	0.78, 1.16		0.95	0.82, 1.10	
Homozygotes	0.45*	0.25, 0.79		1.34	0.78, 2.30		0.77	0.53, 1.12	

Abbreviations: CI, confidence interval; OR, odds ratio; SBCS, Shanghai Breast Cancer Study; SNP, single nucleotide polymorphism.

*$P \leq 0.05$.

[a] Allelic associations among all women for all variants and specific subgroup analyses (genetic model and population) based on SBCS I results.

[b] Odds ratio and 95% confidence interval for the allelic test among all women or the specific model (additive, dominant, or recessive) in subgroup analysis. Additive models included effects for both heterozygotes and homozygotes. All effects were adjusted for age.

[c] Weight (kg)/height $(m)^2$.

Table 4. Association[a] Between the Sex Hormone-Binding Globulin (*SHBG*) rs6259 Polymorphism and Breast Cancer Risk According to Body Mass Index, by Study Stage, Shanghai Breast Cancer Study, 1996–2005

Study and Genotype	Premenopausal Women						Postmenopausal Women						All Women[b]					
	BMI[b,c] <23			BMI ≥23			BMI <23			BMI ≥23			BMI <23			BMI ≥23		
	Cases/Controls[d]	OR	95% CI	Cases/Controls	OR	95% CI	Cases/Controls	OR	95% CI	Cases/Controls	OR	95% CI	Cases/Controls	OR	95% CI	Cases/Controls	OR	95% CI
Stage 1 (SBCS I; 1996–1998)																		
GG	282/301	1.0	Reference	215/212	1.0	0.8, 1.3	101/100	1.0	Reference	164/178	0.9	0.7, 1.3	384/402	1.0	Reference	380/392	1.0	0.8, 1.2
AG/AA	115/137	0.9	0.7, 1.2	123/98	1.2	0.9, 1.7	24/63	0.4*	0.2, 0.7	75/84	0.9	0.6, 1.4	140/200	0.7*	0.6, 1.0	198/185	1.1	0.9, 1.4
P for interaction			0.160						0.005						0.024			
Stage 2 (SBCS II; 2002–2005)																		
GG	390/353	1.0	Reference	359/282	1.2	0.9, 1.4	208/234	1.0	Reference	381/380	1.1	0.9, 1.4	598/588	1.0	Reference	740/662	1.2	1.0, 1.4
AG/AA	148/154	0.9	0.7, 1.1	182/123	1.4*	1.0, 1.8	85/128	0.8	0.5, 1.0	161/161	1.1	0.9, 1.5	233/282	0.8	0.7, 1.0	343/284	1.2*	1.0, 1.5
P for interaction			0.135						0.144						0.046			
SBCS I and SBCS II combined																		
GG	672/654	1.0	Reference	574/494	1.1	0.9, 1.3	309/334	1.0	Reference	545/558	1.1	0.9, 1.3	982/990	1.0	Reference	1,120/1,054	1.1	1.0, 1.2
AG/AA	263/291	0.9	0.7, 1.1	305/221	1.3*	1.1, 1.6	109/191	0.6*	0.5, 0.8	236/245	1.1	0.8, 1.3	373/482	0.8*	0.7, 0.9	541/469	1.2*	1.0, 1.4
P for interaction			0.044						0.007						0.003			

Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratio; SBCS, Shanghai Breast Cancer Study.

*$P \leq 0.05$.

[a] Odds ratios and 95% confidence intervals from dominant effect models, adjusted for age.

[b] Numbers of premenopausal and postmenopausal women do not sum to those for all woman because some participants were missing information on menopausal status.

[c] Weight (kg)/height $(m)^2$.

[d] No. of cases/no. of controls.

BMI in relation to breast cancer risk, particularly among postmenopausal women. A similar pattern of association was found in a population-based endometrial cancer case-control study that we conducted in Shanghai, where the protective dominant effect associated with *SHBG* rs6259 was strongest among lean postmenopausal women (32). When data from both the breast cancer study and the endometrial cancer study were combined, highly significant associations were found for *SHBG* rs6259 and these hormone-related cancers in postmenopausal women ($P = 4 \times 10^{-3}$), lean (BMI <23) women ($P = 5 \times 10^{-4}$), and lean postmenopausal women ($P = 4 \times 10^{-5}$). The consistent findings for breast and endometrial cancer are expected, since endogenous estrogens play a central role in the etiology of these cancers and sex hormone-binding globulin (SHBG) reduces the level of bioavailable estrogens. Located in exon 8, the variant allele of *SHBG* rs6259 causes a substitution of aspartic acid for asparagine at codon 356, which has been shown to increase the half-life of the protein in an animal model (33). In humans, the rs6259 variant allele has been shown to be associated with higher circulating SHBG levels among 303 hirsute women (34) and 4,467 healthy postmenopausal women (35). We previously reported that plasma SHBG levels were significantly higher among lean postmenopausal rs6259 minor allele carriers (18). Although results were not statistically significant, Haiman et al. (36) also found that postmenopausal carriers of the variant allele in the Multiethnic Cohort had higher plasma SHBG levels. Postmenopausal women with high circulating SHBG levels had a lower risk of breast cancer in a recent meta-analysis of 9 prospective studies (37). SNP rs6259 has been evaluated in terms of breast cancer risk in 2 other studies; reduced risks were consistently observed for the variant allele, although estimates did not reach statistical significance (35, 38). However, neither study stratified results by BMI, and only 1 study was limited to postmenopausal women. In the current study, the effect associated with *SHBG* rs6259 was most evident among lean postmenopausal women, who have lower endogenous estrogen levels than premenopausal women or postmenopausal women with higher BMIs. Among women with low estrogen levels, sequestering by SHBG would have the greatest effect on breast cancer risk. Alternatively, the protective effect of rs6259 may be masked by the adverse effect of high estrogen levels among premenopausal women or overweight postmenopausal women.

Located in intron 1 of the estrogen receptor alpha gene (*ESR1*), rs2234693 is predicted to influence transcriptional regulation and isoform formation (39, 40). Among younger (≤45 years) SBCS participants, rs2234693 minor allele carriers had an additive significantly decreased risk of breast cancer. Our findings are supported by a recent meta-analysis of 17 studies that included 10,300 cases and 16,620 controls and found an additive decreased risk associated with the variant allele (39). In addition, the minor allele of this SNP has also been found to be associated with a significantly decreased risk of endometrial cancer, both in a smaller study of Australian women (41) and in a large study of Swedish women (42). Thus, a possible protective effect for this *ESR1* variant allele in breast and other hormone-related cancers is indicated.

Other SNPs with consistent results between SBCS I and SBCS II include glutathione *S*-transferase π-1 (*GSTP1*) rs1695, where an isoleucine-to-valine substitution at codon 105 in the substrate binding pocket alters the properties of this phase II detoxification enzyme (43). We found an increased risk of breast cancer for minor allele homozygotes, a finding that is consistent with 4 other studies among Chinese women that were included in a recent meta-analysis (44). On the other hand, the meta-analysis found no association among Caucasian women. Reasons for the racial difference in this association are unknown. We recently reported that *GSTP1* rs1695 may modify the association between cruciferous vegetable intake and breast cancer risk (24). It is possible that differences in certain lifestyle factors and environmental exposures between Chinese and Caucasian populations contribute to this difference in association.

Also consistent between SBCS I and SBCS II was rs2066853, a nonsynonymous SNP in the transactivation domain of the aryl hydrocarbon receptor gene (*AHR*) (45); a decreased breast cancer risk was found for premenopausal SBCS minor allele carriers. On the contrary, a significantly increased risk was found among Thai women who were heterozygous for rs2066853 (46), while no effect was seen among participants in the Multiethnic Cohort (47). However, neither study showed results stratified by menopausal status, and the samples in both studies were small. Further, the *AHR* rs2066853 genotype was found to deviate from Hardy-Weinberg equilibrium among Caucasians and Latinos in the Multiethnic Cohort (47).

We previously reported an increased risk for SBCS I postmenopausal minor allele carriers of *ATM* rs1003623, which was not replicated among SBCS II participants (13). Here, we update this analysis with a consistent association that was found among older (age >45 years) SBCS participants, such that minor allele carriers had an increased risk of breast cancer. While rare mutations in the *ATM* gene have been consistently linked to an increased risk of breast cancer (48, 49), associations for common variation have been sparse. Four studies carried out among Caucasians found no associations with *ATM* SNPs and breast cancer risk (50–53), while 1 study in Koreans did find evidence of associations with altered breast cancer susceptibility (54). However, rs1003623 was evaluated in only 1 of these studies, which found no association among Caucasian women (51).

Finally, 2 polymorphisms were found to have associations that were generally consistent among SBCS I and SBCS II participants, although results did not reach statistical significance in combined analyses. *CCND1* rs9344 (also known as *CCND1* G870A or rs603965) is a functional SNP that results in an alternate transcript with a longer protein half-life cyclin D1 protein (55). Among SBCS participants, younger women with 2 G alleles tended have a lower risk of breast cancer. This is in agreement with a recent meta-analysis of 5,371 cases and 5,336 controls which found a small increased risk for carriers of the A allele (55); SBCS I participants comprised 20.2% of the meta-analysis study population. Transforming growth factor 1 (*TGFB1*) rs1800469 is a functional promoter SNP with alleles that differentially bind transcription factor AP-1,

resulting in expression differences (56). *TGFB1* has been shown to play a dual role in cancer, inhibiting growth in early stages and promoting growth in later stages (57). Among SBCS participants, minor allele homozygotes had a marginally increased risk of early-stage breast cancer. In a recent meta-analysis of 10,197 breast cancer cases and 13,832 controls, Niu et al. (58) found no significant association with breast cancer risk but did not stratify results by cancer stage.

In this report, we not only presented a summary of our previous candidate-gene studies of known or potentially functional SNPs and breast cancer risk but also sought to replicate these putative genotype-phenotype associations among additional breast cancer and endometrial cancer study populations. To our knowledge, it is one of the largest 2-stage candidate-gene association studies of low-penetrance variants and breast cancer susceptibility. We found that known or potentially functional SNPs in *GSTP1*, *AHR*, *ESR1*, *SHBG*, and *ATM* were significantly associated with breast cancer risk in analyses of our 2 study stages combined. However, some of these associations could have been due to chance, since results for these SNPs were not strictly replicated at $P \leq 0.05$ in the stage 2 analysis. To address this, we employed the false-positive report probability (FPRP) tool (59), using prior probabilities of 0.10 and 0.05 for true associations between tested variants and breast cancer risk. Associations for *ATM* rs1003623 among older women and *GSTP1* rs1695 among all women had FPRP values below 0.2 for both prior probabilities selected. Further, the association for *AHR* rs2066853 among premenopausal women had an FPRP value below 0.4, regardless of the prior probability used, and associations for *ESR1* rs2234693 among younger women and *SHBG* rs6259 among postmenopausal women had FPRP values below 0.4 for prior probabilities of 0.10 but not for prior probabilities of 0.05. Therefore, assuming probabilities of associations between these known or potentially functional variants and breast cancer of at least 10%, the probability that these findings are false-positive is less than 40%. Further, a highly significant interaction between a functional polymorphism in the *SHBG* gene and BMI was identified in relation to breast cancer. The effect for *SHBG* rs6259 among lean postmenopausal women, as identified in our interaction analysis, was found to have an FPRP value of 0.016 or 0.034, depending on whether a prior probability of 0.10 or 0.05 was used; this indicates that the effect of *SHBG* rs6259 among lean postmenopausal women is unlikely to be a false-positive finding.

In conclusion, these findings support a role for common genetic variation in low-penetrance genes in breast cancer susceptibility and highlight the utility of candidate-gene association studies in evaluating gene-environment interactions.

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REFERENCES

1. Parkin DM, Bray F, Ferlay J, et al. Global cancer statistics, 2002. *CA Cancer J Clin*. 2005;55(2):74–108.
2. Stratton MR, Rahman N. The emerging landscape of breast cancer susceptibility. *Nat Genet.* 2008;40(1):17–22.
3. Pharoah PD, Antoniou A, Bobrow M, et al. Polygenic susceptibility to breast cancer and implications for prevention. *Nat Genet.* 2002;31(1):33–36.
4. Easton DF, Pooley KA, Dunning AM, et al. Genome-wide association study identifies novel breast cancer susceptibility loci. *Nature*. 2007;447(7148):1087–1093.
5. Hunter DJ, Kraft P, Jacobs KB, et al. A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. *Nat Genet.* 2007;39(7):870–874.
6. Stacey SN, Manolescu A, Sulem P, et al. Common variants on chromosomes 2q35 and 16q12 confer susceptibility to estrogen receptor-positive breast cancer. *Nat Genet.* 2007; 39(7):865–869.
7. Stacey SN, Manolescu A, Sulem P, et al. Common variants on chromosome 5p12 confer susceptibility to estrogen receptor-positive breast cancer. *Nat Genet.* 2008;40(6): 703–706.
8. Ahmed S, Thomas G, Ghoussaini M, et al. Newly discovered breast cancer susceptibility loci on 3p24 and 17q23.2. *Nat Genet.* 2009;41(5):585–590.
9. Thomas G, Jacobs KB, Kraft P, et al. A multistage genome-wide association study in breast cancer identifies two new risk alleles at 1p11.2 and 14q24.1 (RAD51L1). *Nat Genet.* 2009; 41(5):579–584.
10. Turnbull C, Ahmed S, Morrison J, et al. Genome-wide association study identifies five new breast cancer susceptibility loci. *Nat Genet.* 2010;42(6):504–507.
11. Zheng W, Long J, Gao YT, et al. Genome-wide association study identifies a new breast cancer susceptibility locus at 6q25.1. *Nat Genet.* 2009;41(3):324–328.
12. Long JR, Egan KM, Dunning L, et al. Population-based case-control study of AhR (aryl hydrocarbon receptor) and CYP1A2 polymorphisms and breast cancer risk. *Pharmacogenet Genomics*. 2006;16(4):237–243.

13. Ye C, Dai Q, Lu W, et al. Two-stage case-control study of common ATM gene variants in relation to breast cancer risk. *Breast Cancer Res Treat*. 2007;106(1):121–126.
14. Shu XO, Moore DB, Cai Q, et al. Association of cyclin D1 genotype with breast cancer risk and survival. *Cancer Epidemiol Biomarkers Prev.* 2005;14(1):91–97.
15. Cai Q, Shu XO, Jin F, et al. Genetic polymorphisms in the estrogen receptor alpha gene and risk of breast cancer: results from the Shanghai Breast Cancer Study. *Cancer Epidemiol Biomarkers Prev.* 2003;12(9):853–859.
16. Egan KM, Cai Q, Shu XO, et al. Genetic polymorphisms in GSTM1, GSTP1, and GSTT1 and the risk for breast cancer: results from the Shanghai Breast Cancer Study and meta-analysis. *Cancer Epidemiol Biomarkers Prev.* 2004;13(2): 197–204.
17. Shin A, Shu XO, Cai Q, et al. Genetic polymorphisms of the transforming growth factor-β1 gene and breast cancer risk: a possible dual role at different cancer stages. *Cancer Epidemiol Biomarkers Prev.* 2005;14(6):1567–1570.
18. Cui Y, Shu XO, Cai Q, et al. Association of breast cancer risk with a common functional polymorphism (Asp327Asn) in the sex hormone-binding globulin gene. *Cancer Epidemiol Biomarkers Prev.* 2005;14(5):1096–1101.
19. Dai Q, Cai QY, Shu XO, et al. Synergistic effects of STK15 gene polymorphisms and endogenous estrogen exposure in the risk of breast cancer. *Cancer Epidemiol Biomarkers Prev.* 2004;13(12):2065–2070.
20. Chan KY, Liu W, Long JR, et al. Functional polymorphisms in the BRCA1 promoter influence transcription and are associated with decreased risk for breast cancer in Chinese women. *J Med Genet.* 2009;46(1):32–39.
21. Zheng SL, Zheng W, Chang BL, et al. Joint effect of estrogen receptor beta sequence variants and endogenous estrogen exposure on breast cancer risk in Chinese women. *Cancer Res.* 2003;63(22):7624–7629.
22. Ren Z, Cai Q, Shu XO, et al. Genetic polymorphisms in the IGFBP3 gene: association with breast cancer risk and blood IGFBP-3 protein levels among Chinese women. *Cancer Epidemiol Biomarkers Prev.* 2004;13(8): 1290–1295.
23. Kataoka N, Cai Q, Wen W, et al. Population-based case-control study of VEGF gene polymorphisms and breast cancer risk among Chinese women. *Cancer Epidemiol Biomarkers Prev.* 2006;15(6):1148–1152.
24. Lee SA, Fowke JH, Lu W, et al. Cruciferous vegetables, the GSTP1 Ile105Val genetic polymorphism, and breast cancer risk. *Am J Clin Nutr.* 2008;87(3):753–760.
25. Sherry ST, Ward MH, Kholodov M, et al. dbSNP: the NCBI database of genetic variation. *Nucleic Acids Res.* 2001;29(1): 308–311.
26. Hubbard TJ, Aken BL, Beal K, et al. Ensembl 2007. *Nucleic Acids Res.* 2007;35(database issue):D610–D617.
27. Yuan HY, Chiou JJ, Tseng WH, et al. FASTSNP: an always up-to-date and extendable service for SNP function analysis and prioritization. *Nucleic Acids Res.* 2006;34(web server issue):W635–W641.
28. Lee PH, Shatkay H. F-SNP: computationally predicted functional SNPs for disease association studies. *Nucleic Acids Res.* 2008;36(database issue):D820–D824.
29. Lau J, Ioannidis JP, Schmid CH. Quantitative synthesis in systematic reviews. *Ann Intern Med.* 1997;127(9):820–826.
30. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials*. 1986;7(3):177–188.
31. WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet*. 2004;363(9403):157–163.
32. Kataoka N, Cai Q, Xu WH, et al. Association of endometrial cancer risk with a functional polymorphism (Asp(327)Asn) in the sex hormone-binding globulin gene. *Cancer.* 2007;109(7): 1296–1302.
33. Cousin P, Déchaud H, Grenot C, et al. Human variant sex hormone-binding globulin (SHBG) with an additional carbohydrate chain has a reduced clearance rate in rabbit. *J Clin Endocrinol Metab.* 1998;83(1):235–240.
34. Cousin P, Calemard-Michel L, Lejeune H, et al. Influence of SHBG gene pentanucleotide TAAAA repeat and D327N polymorphism on serum sex hormone-binding globulin concentration in hirsute women. *J Clin Endocrinol Metab.* 2004;89(2):917–924.
35. Thompson DJ, Healey CS, Baynes C, et al. Identification of common variants in the SHBG gene affecting sex hormone-binding globulin levels and breast cancer risk in postmenopausal women. *Cancer Epidemiol Biomarkers Prev.* 2008;17(12):3490–3498.
36. Haiman CA, Riley SE, Freedman ML, et al. 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.* 2005;90(4):2198–2204.
37. Key T, Appleby P, Barnes I, et al. Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. *J Natl Cancer Inst*. 2002;94(8): 606–616.
38. Foulkes WD. Inherited susceptibility to common cancers. *N Engl J Med*. 2008;359(20):2143–2153.
39. Li N, Dong J, Hu Z, et al. Potentially functional polymorphisms in ESR1 and breast cancer risk: a meta-analysis. *Breast Cancer Res Treat*. 2010;121(1):177–184.
40. Herrington DM, Howard TD, Brosnihan KB, et al. Common estrogen receptor polymorphism augments effects of hormone replacement therapy on E-selectin but not C-reactive protein. *Circulation*. 2002;105(16):1879–1882.
41. Ashton KA, Proietto A, Otton G, et al. Estrogen receptor polymorphisms and the risk of endometrial cancer. *BJOG*. 2009;116(8):1053–1061.
42. Wedrén S, Lovmar L, Humphreys K, et al. Estrogen receptor alpha gene polymorphism and endometrial cancer risk—a case-control study. *BMC Cancer.* 2008;8:322. (doi: 10.1186/1471-2407-8-322).
43. Katoh T, Yamano Y, Tsuji M, et al. Genetic polymorphisms of human cytosol glutathione *S*-transferases and prostate cancer. *Pharmacogenomics*. 2008;9(1):93–104.
44. Sergentanis TN, Economopoulos KP. GSTT1 and GSTP1 polymorphisms and breast cancer risk: a meta-analysis. *Breast Cancer Res Treat*. 2010;121(1):195–202.
45. Harper PA, Wong JY, Lam MS, et al. Polymorphisms in the human AH receptor. *Chem Biol Interact*. 2002;141(1-2): 161–187.
46. Sangrajrang S, Sato Y, Sakamoto H, et al. Genetic polymorphisms of estrogen metabolizing enzyme and breast cancer risk in Thai women. *Int J Cancer.* 2009;125(4):837–843.
47. Le Marchand L, Donlon T, Kolonel LN, et al. Estrogen metabolism-related genes and breast cancer risk: the Multiethnic Cohort Study. *Cancer Epidemiol Biomarkers Prev.* 2005;14(8):1998–2003.

48. Renwick A, Thompson D, Seal S, et al. ATM mutations that cause ataxia-telangiectasia are breast cancer susceptibility alleles. *Nat Genet.* 2006;38(8):873–875.
49. Milne RL. Variants in the ATM gene and breast cancer susceptibility. *Genome Med*. 2009;1(1):12. (doi: 10.1186/gm12).
50. Tamimi RM, Hankinson SE, Spiegelman D, et al. Common ataxia telangiectasia mutated haplotypes and risk of breast cancer: a nested case-control study. *Breast Cancer Res.* 2004;6(4):R416–R422.
51. Einarsdóttir K, Rosenberg LU, Humphreys K, et al. Comprehensive analysis of the ATM, CHEK2 and ERBB2 genes in relation to breast tumour characteristics and survival: a population-based case-control and follow-up study. *Breast Cancer Res.* 2006;8(6):R67. (doi: 10.1186/bcr1623).
52. Tommiska J, Jansen L, Kilpivaara O, et al. ATM variants and cancer risk in breast cancer patients from Southern Finland. *BMC Cancer.* 2006;6:209. (doi: 10.1186/1471-2407-6-209).
53. Baynes C, Healey CS, Pooley KA, et al. Common variants in the ATM, BRCA1, BRCA2, CHEK2 and TP53 cancer susceptibility genes are unlikely to increase breast cancer risk. *Breast Cancer Res.* 2007;9(2):R27. (doi: 10.1186/bcr1669).
54. Lee KM, Choi JY, Park SK, et al. Genetic polymorphisms of ataxia telangiectasia mutated and breast cancer risk. *Cancer Epidemiol Biomarkers Prev.* 2005;14(4):821–825.
55. Lu C, Dong J, Ma H, et al. CCND1 G870A polymorphism contributes to breast cancer susceptibility: a meta-analysis. *Breast Cancer Res Treat*. 2009;116(3):571–575.
56. Shah R, Hurley CK, Posch PE. A molecular mechanism for the differential regulation of TGF-β1 expression due to the common SNP −509C-T (c. −1347C>T). *Hum Genet.* 2006;120(4):461–469.
57. Zheng W. Genetic polymorphisms in the transforming growth factor-beta signaling pathways and breast cancer risk and survival. *Methods Mol Biol.* 2009;472(1):265–277.
58. Niu W, Qi Y, Gao P, et al. Association of TGFB1 −509 C>T polymorphism with breast cancer: evidence from a meta-analysis involving 23,579 subjects. *Breast Cancer Res Treat*. 2010;124(1):243–249.
59. Wacholder S, Chanock S, Garcia-Closas M, et al. Assessing the probability that a positive report is false: an approach for molecular epidemiology studies. *J Natl Cancer Inst*. 2004; 96(6):434–442.