

Association of Breast Cancer Risk with a Common Functional Polymorphism (*Asp327Asn*) in the Sex Hormone–Binding Globulin Gene

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Abstract

Sex hormones play a central role in the development of breast cancer. Sex hormone–binding globulin (SHBG) modulates the bioavailability of circulating sex hormones and regulates their signaling system in the breast tissue. We evaluated the association of a common functional polymorphism (*Asp327Asn*) in the *SHBG* gene with breast cancer risk in a population-based case-control study (1,106 cases and 1,180 controls) conducted in Shanghai, China. The variant *Asn* allele was associated with a reduced breast cancer risk in postmenopausal women [odds ratio (OR), 0.73; 95% confidence interval (95% CI), 0.53–0.99], but not in premenopausal women (OR, 1.03; 95% CI, 0.82–1.27). The protective association was much stronger in postmenopausal women with a low body mass index (BMI; OR, 0.46; 95% CI, 0.29–0.75) or

waist-to-hip ratio (OR, 0.51; 95% CI, 0.32–0.83) than those with a high BMI or waist-to-hip ratio (*P* for interaction < 0.05). Furthermore, the association was stronger for estrogen receptor–positive (OR, 0.64; 95% CI, 0.42–0.98) than for estrogen receptor–negative breast cancer (OR, 0.85; 95% CI, 0.50–1.45). Among postmenopausal controls, blood SHBG levels were 10% higher in carriers of the variant *Asn* allele than noncarriers (*P* = 0.06). Postmenopausal control women with the *Asn* allele and low BMI or waist-to-hip ratio had 20% higher SHBG levels (*P* < 0.05). This study suggests that the *Asn* allele in the *SHBG* gene may be related to a reduced risk of breast cancer among postmenopausal women by increasing their blood SHBG levels. (Cancer Epidemiol Biomarkers Prev 2005;14(5):1096–101)

Introduction

Sex steroid hormones play a central role in the development of breast cancer. Most circulating sex hormones are bound to sex hormone–binding globulin (SHBG), which modulates the bioavailability of these hormones to target tissues (1, 2). In addition, SHBG has recently been found to function as an active regulator of the steroid-signaling system in target tissues (3, 4). In breast cancer cells, SHBG, through its specific membrane receptor (SHBG-R) and second messenger system (cyclic AMP and protein kinase A), not only effectively inhibits estradiol-induced cell proliferation but also controls progesterone receptor expression at both the mRNA and protein levels, and influences its function (receptor binding capacity; refs. 5, 6). A number of epidemiologic studies have shown that blood SHBG levels are inversely associated with breast cancer risk (7, 8). Thus, it is conceivable that functional polymorphisms in the *SHBG* gene may be related to the risk of breast cancer.

The human *SHBG* gene is located on the short arm (17p12→p13) of chromosome 17 (9). A single-nucleotide polymorphism (G to A) at nucleotide 5,790 (M31651, GenBank) in exon 8 of this gene results in an amino acid substitution of asparagine for aspartic acid at residue 327 (*Asp327Asn*) in the *SHBG* polypeptide. This change generates an additional *N*-linked carbohydrate chain and introduces an extra consensus site for *N*-glycosylation (Asn-X-Ser or Thr) within the carboxyl-terminal globular domain (10, 11). The variant SHBG with asparagine (Asn) at residue 327 retains its sex hormone–

binding properties (12) and its capability of inhibiting estradiol-induced proliferation of breast cancer cells (13). However, the presence of the additional carbohydrate chain in the variant SHBG may result in a decrease in the clearance rate of this protein. It has been reported that the *Asn* allele of SHBG is associated with an increased half-life of the protein and an elevated blood SHBG levels in an animal model (14) as well as in hirsute women (15). Recently, a large case-control study (16) has shown that the variant *Asn* allele is associated with increased SHBG levels and a reduced estradiol to SHBG ratio in postmenopausal women, suggesting that this genetic variant contributes to the bioavailability of estrogens. Given the biological significance of SHBG in general, and the functional alteration of the *Asp327Asn* polymorphism in particular, we hypothesized that the *Asn* allele of SHBG may modify breast cancer risk and tested this hypothesis in a large population-based case-control study.

Materials and Methods

Study Subjects. Cases and controls of this study were participants in the Shanghai Breast Cancer Study, a large population-based case-control study conducted among Chinese women in Shanghai. Details of the Shanghai Breast Cancer Study have been described elsewhere (17). Briefly, eligible cases for the study included all permanent female residents in urban Shanghai who were newly diagnosed with breast cancer from August 1996 to March 1998 and were between the ages of 25 and 64 years. A total of 1,602 eligible cases were identified during the study period through a rapid case-ascertainment system supplemented by the population-based Shanghai Tumor Registry, which has a virtually complete ascertainment of all incident cancer cases in urban Shanghai. Of the patients identified, 1,459 (91.1%) were

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enrolled in the study after completing an in-person interview. The major reasons for nonparticipation were refusal ($n = 109$ cases; 6.8%), death before interview ($n = 17$; 1.1%), and inability to locate ($n = 17$; 1.1%). The initial diagnosis of breast cancer for all patients was confirmed by two senior pathologists through independent review of histologic slides. Clinical information on cancer diagnoses, tumor stage (tumor-node-metastasis stage), cancer treatments, and estrogen receptor/progesterone receptor status was abstracted from medical charts using a standard protocol.

Control subjects were randomly selected from the general female population through the Shanghai Resident Registry, which keeps registry cards for all adult permanent residents of urban Shanghai. Control subjects were frequency matched to case patients by age (5-year intervals). The number of controls in each age-specific stratum was predetermined according to the most recent data on the age distributions of the breast cancer patients available from the Shanghai Tumor Registry. In-person interviews were completed for 1,556 (90.3%) of the 1,724 eligible control subjects identified. Excluded from the study were 168 potential control subjects because of refusal ($n = 166$; 9.6%) and death or a prior cancer diagnosis ($n = 2$; 0.1%).

Exposure information and anthropometrics were taken during an in-person visit by trained interviewers. A structured questionnaire was used to elicit detailed information on demographic factors, menstrual and reproductive history, hormone use, dietary habits, prior disease history, physical activity, tobacco and alcohol use, weight, and family history of cancer. All interviews were tape recorded for quality control purposes. Weight, circumferences of the waist and hips, and sitting and standing heights were measured twice for each study participant using a standard protocol. A third measurement was taken if the difference of the first two measurements was greater than the tolerance limit (1 kg for weight and 1 cm for heights and circumferences). The averaged measurements were used in this analysis.

A fasting morning blood sample (10 mL from each woman) was collected using EDTA or heparin vacutainer tubes from 1,193 (82%) cases and 1,310 (84%) controls who completed the in-person interviews (1,459 cases and 1,556 controls). These samples were processed on the same day, typically within 6 hours after collection, at the Shanghai Cancer Institute, and were stored at -70°C .

Genotyping Method. Genomic DNA was extracted from buffy coat fractions using the Puregene DNA Purification Kit (Gentra Systems, Minneapolis, MN) following the protocol of the manufacturer. DNA concentration was measured using PicoGreen dsDNA Quantitation Kit (Molecular Probes, Eugene, OR).

The genotype assays for the *SHBG* polymorphism were done using the PCR-RFLP method. The primers for the PCR amplification were 5'-TTCTGGATCCGAGCCACCT-3' and 5'-AGTGCCTGGTACATTGCTAG-3'. The PCR reactions were done in a Biometra T Gradient Thermocycler. Each 25 μL of PCR mixture contained 10 ng DNA, $1\times$ PCR buffer, 1.5 mmol/L MgCl_2 , 0.16 mmol/L each of deoxynucleotide triphosphate, 0.4 $\mu\text{mol/L}$ of each primer, and 1 unit of DNA polymerase. The reaction mixture was initially denatured at 94°C for 3 minutes, followed by 35 cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. The PCR was completed by a final extension cycle at 72°C for 7 minutes. Each PCR product (10 μL) was digested with 10 units of *HinfI* (New England Biolabs, Beverly, MA) at 37°C for 3 hours. The DNA fragments were then separated by electrophoresis on 3% agarose gel containing ethidium bromide and visualized under UV light. The PCR product (294 bp) with the Asp allele (G allele) was digested to three fragments (139, 92, and 63 bp), whereas the PCR product with the Asn allele (A allele) was cut to two fragments (155 and 139 bp).

The laboratory staff was blind to the identity of the subjects. Quality control samples were included in genotyping assays. Each 96-well plate contained one water, two CEPH 1347-02 DNA, two blinded quality control DNA, and two unblinded quality control DNA samples. The blinded and unblinded quality control samples were taken from the samples of randomly selected participants of the study. The consistency rate was 100% for quality control samples. Genotyping data were obtained from 1,106 (92.7%) cases and 1,180 (90.1%) controls who gave blood samples. The major reasons for incomplete genotyping (7.3% of cases and 9.9% of controls) were insufficient DNA and unsuccessful PCR amplification. Thus, the current analysis included 1,106 cases and 1,180 controls with both genotyping data and questionnaire information (75.8% of participants of the Shanghai Breast Cancer Study).

Measurement of Plasma Sex Hormone-Binding Globulin Levels. Plasma levels of SHBG were measured for all postmenopausal breast cancer cases ($n = 195$) who had donated blood samples before any cancer treatment and for 96.3% of postmenopausal controls ($n = 411$). All measurements for SHBG, using an immunoradiometric assay, were done in a reference laboratory at Diagnostic Systems Laboratories, Inc. (DSL, Webster, TX), which is certified by Clinical Laboratory Improvement Amendments and the International Standard ISO 9002. Each sample was tested in duplicate, and two internal quality control samples were included in each run of the assay. The method intra- and interassay precisions expressed as coefficient of variation were 1.1% to 3.7% and 8.7% to 11.5%, respectively.

Statistical Analyses. χ^2 statistics were applied to evaluate the difference in the distribution of *SHBG* allele types and genotypes between cases and controls. SHBG concentrations were presented as geometric means and 95% confidence intervals (CI), and differences between cases and controls and between carriers and noncarriers of the variant *Asn* allele among postmenopausal women were analyzed with the use of *t* test. Unconditional logistic regression was applied to derive odds ratios (OR) and 95% CIs adjusting for age and other potential confounders. The potential modifying effect of major breast cancer risk factors related to endogenous estrogen exposure on the gene-disease association was evaluated by the logistic model. The presence of interaction was assessed using the likelihood ratio test by comparing the model including the main effects only with that including both the main effects and the interaction term. All statistical tests were based on two-tailed probability.

Results

Summarized in Table 1 are comparisons between cases and controls for selected demographic characteristics, nongenetic breast cancer risk factors, distribution of SHBG allele types, and plasma SHBG levels. Cases and controls were well matched on age, with respective mean ages of 47.7 and 47.3 years, and had similar educational attainment. The associations of established risk factors identified in this study were generally consistent with those reported from previous studies conducted in other populations (17). Compared with controls, cases were more likely to have breast fibroadenoma, earlier age at menarche, later age at menopause, later age at first live birth, longer total years of menstruation, higher body mass index (BMI) and waist-to-hip ratio, and were less likely to have regularly engaged in exercise in the past 10 years. Among postmenopausal women, cases also had significantly lower plasma levels of SHBG than controls. There were no significant differences between cases and controls regarding family history of breast cancer among first-degree relatives, hormone

Table 1. Comparison of cases and controls by selected demographic characteristics, major risk factors for breast cancer, SHBG concentration, and SHBG allele type and genotype distribution in the Shanghai Breast Cancer Study

Subject characteristics*	Cases (n = 1,106)	Controls (n = 1,180)	P
Demographic factors			
Age (y)	47.7 ± 8.0	47.3 ± 8.8	0.22
Education (% high school or more)	43.3	42.5	0.71
Reproductive risk factors			
Age at menarche	14.5 ± 1.6	14.7 ± 1.7	<0.01
Age at menopause [†]	48.2 ± 4.6	47.4 ± 5.0	0.03
Age at first live birth [‡]	26.8 ± 4.1	26.1 ± 3.8	<0.01
Total years of menstruation	30.4 ± 5.4	29.2 ± 5.8	<0.01
Other risk factors			
Breast cancer among first-degree relatives (%)	3.3	2.4	0.20
Ever had breast fibroadenoma (%)	9.5	5.2	<0.01
Ever used hormone replacement therapy (%)	2.5	2.5	0.99
BMI (kg/m ²)	23.5 ± 3.4	23.3 ± 3.5	0.09
Waist-to-hip ratio	0.81 ± 0.06	0.80 ± 0.06	<0.01
Alcohol consumption (% ever)	3.6	3.9	0.72
Physically active during past 10 y (%)	19.4	25.6	<0.01
Plasma SHBG concentration (nmol/L) [†]	71.8 (16.4-313.6) [§]	90.6 (27.4-299.4) [§]	<0.01
SHBG allele frequency			
G (<i>Asp</i>)	0.83	0.82	0.49
A (<i>Asn</i>)	0.17	0.18	
SHBG genotype (%)			
GG (<i>Asp/Asp</i>)	768 (69.4)	795 (67.4)	
GA/ <i>AA</i>	338 (30.6)	385 (32.6)	0.32
GA (<i>Asp/Asn</i>)	302 (27.3)	353 (29.9)	
AA (<i>Asn/Asn</i>)	36 (3.3)	32 (2.7)	

*Values are presented as means ± SD among cases and controls unless otherwise noted.

[†] Among postmenopausal women.

[‡] Among parous women.

[§] Values represent geometric means (95% CI).

replacement therapy, and alcohol consumption. The number of subjects in the exposure group for these variables, however, was small.

The distribution of SHBG genotype was consistent with Hardy-Weinberg equilibrium for both cases ($P = 0.35$) and controls ($P = 0.33$). The frequencies of the variant *Asn* allele were similar in cases (17%) and controls (18%). Because only a small proportion of women were homozygous for the *Asn* allele, they were combined with the heterozygotes subsequent analysis.

Overall, 30.6% of cases and 32.6% of controls possessed the *Asn* allele with an OR of 0.91 (95% CI, 0.76-1.09; Table 2). When stratified by menopausal status, the 327*Asn* variant (GA/*AA* genotypes) was associated with a significantly reduced breast cancer risk among the postmenopausal women (OR, 0.73; 95% CI, 0.53-0.99), but not in premenopausal women (OR, 1.03; 95% CI, 0.83-1.29). These results remained essentially unchanged after adjusting for all major breast cancer risk factors.

Table 3 presents comparisons of plasma SHBG levels among postmenopausal women in the control group by SHBG

genotypes and further stratified by selected hormone-related conditions (BMI, waist-to-hip ratio, and years of menstruation). Compared with noncarriers, postmenopausal women carrying the 327*Asn* variant had higher plasma levels of SHBG with an 11.9% difference ($P = 0.06$). There were significant differences in SHBG level by genotype for postmenopausal women who had a BMI < 24 kg/m² ($P = 0.03$) or waist-to-hip ratio < 0.81 ($P = 0.01$). SHBG level was unrelated to SHBG *Asp327Asn* polymorphism among premenopausal women (data not shown).

Additional analyses were carried out to evaluate whether the associations between the *Asp327Asn* polymorphism and breast cancer risk in postmenopausal women could be modified by hormone-related factors. As shown in Table 4, compared with noncarriers, postmenopausal women carrying the 327*Asn* variant had a reduced risk of more than 50% when their BMI was less than 24 kg/m² (OR, 0.46; 95% CI, 0.29-0.75) or their waist-to-hip ratio was less than 0.81 (OR, 0.51; 95% CI, 0.32-0.83), and had a more than 35% reduction of risk when years of menstruation was less than 33 years (OR, 0.63; 95% CI,

Table 2. Association of the Asp327Asn polymorphism of SHBG with breast cancer risk by menopausal status in the Shanghai Breast Cancer Study

SHBG genotype	Cases	Controls	Age-adjusted OR (95% CI)	Multiaadjusted* OR (95% CI)
	n (%)	n (%)		
All participants				
<i>Asp/Asp</i>	768 (69.4)	795 (67.4)	1.00 (reference)	1.0 (reference)
<i>Asp/Asn</i> or <i>Asn/Asn</i>	338 (30.6)	385 (32.6)	0.90 (0.75-1.07)	0.91 (0.76-1.09)
Premenopausal women				
<i>Asp/Asp</i>	499 (67.7)	514 (68.6)	1.0 (reference)	1.0 (reference)
<i>Asp/Asn</i> or <i>Asn/Asn</i>	238 (32.3)	235 (31.4)	1.02 (0.82-1.27)	1.03 (0.83-1.29)
Postmenopausal women				
<i>Asp/Asp</i>	265 (72.8)	279 (65.3)	1.0 (reference)	1.0 (reference)
<i>Asp/Asn</i> or <i>Asn/Asn</i>	99	27.2	0.71 (0.52-0.96)	0.73 (0.53-0.99)

*Adjusted for age, education, ever had breast fibroadenoma, age at first live birth, age at menopause, BMI, and physical activity in the past 10 years.

Table 3. Comparison of SHBG concentration between carriers and noncarriers of the variant *Asn* allele among postmenopausal controls by BMI, waist-to-hip ratio, and years of menstruation in the Shanghai Breast Cancer Study

Variables*	Plasma SHBG concentration (nmol/L) [†]			
	Asp/Asp (<i>n</i> = 268)	Asp/Asn or Asn/Asn (<i>n</i> = 143)	% Difference	<i>P</i>
Total SHBG concentration (nmol/L)	87.1 (24.7-307)	97.5 (34-279.6)	11.9	0.06
BMI (kg/m ²)				
<24	98 (23.3-410.9)	117.8 (43.4-319.7)	20.2	0.03
≥24	77.9 (27.8-218.2)	78.6 (30.2-204.9)	0.9	0.91
Waist-to-hip ratio				
<0.81	96.1 (23.6-391.1)	117.3 (51.3-267.9)	22.1	0.01
≥0.81	79.2 (26.9-233)	81.2 (26.1-252.5)	2.5	0.76
Years of menstruation				
<33	88.8 (24.9-316.3)	101.8 (33.7-307.3)	14.6	0.15
≥33	85.8 (24.5-301.2)	94 (34.2-258.3)	9.6	0.26

*Cut points were chosen by medians of BMI, waist-to-hip ratio, and years of menstruation among postmenopausal controls.

[†]Values of SHBG concentration are presented as geometric means (95% CI).

0.39-1.03); whereas these inverse associations were attenuated among women with a higher BMI or waist-to-hip ratio, or longer duration of menstruation. Tests for multiplicative interaction were significant for SHBG genotype with BMI or waist-to-hip ratio (*P* = 0.01 and 0.04, respectively), but not with years of menstruation.

We further examined the association of the *Asp327Asn* polymorphism with breast cancer risk among postmenopausal women stratified by plasma SHBG level (see Table 4). The *327Asn* variant was associated with a significantly reduced risk of breast cancer among women with higher plasma SHBG concentrations (OR, 0.50; 95% CI, 0.28-0.90), but not in those with lower plasma SHBG levels (OR, 0.83; 95% CI, 0.49-1.40). However, a test for multiplicative interaction was not statistically significant. Similar analyses among premenopausal women did not show any significant gene-disease association (data not shown).

We also examined the association of the *Asp327Asn* polymorphism with postmenopausal breast cancer risk by estrogen receptor status of the tumor. Compared with the entire control group (*n* = 279 for GG genotype and *n* = 148 for GA/AA genotypes), the *327Asn* variant was related to a

significantly reduced risk of estrogen receptor-positive breast cancer (OR, 0.64; 95% CI, 0.42-0.98, based on 117 and 38 breast cancer cases with the GG and GA/AA genotypes, respectively) and a nonsignificantly reduced risk of estrogen receptor-negative breast cancer (OR, 0.85; 95% CI, 0.50-1.45, based on 56 and 24 breast cancer patients with the GG and GA/AA genotypes, respectively; data not shown in tables). Additional analyses by both estrogen receptor and progesterone receptor status showed that among postmenopausal women carrying the *327Asn* variant, ORs (95% CIs) were 0.61 (0.4-0.92), 0.57 (0.21-1.60), 0.78 (0.42-1.44), and 0.90 (0.38-2.16) for estrogen receptor (+)/progesterone receptor (+), estrogen receptor (+)/progesterone receptor (-), estrogen receptor (-)/progesterone receptor (-), and estrogen receptor (-)/progesterone receptor (+) tumors, respectively (data not shown in tables).

Discussion

In the present study, we observed that the variant *Asn* allele in the *SHBG* gene was associated with elevated plasma SHBG levels and a reduced breast cancer risk in postmenopausal

Table 4. Association between the *Asp327Asn* polymorphism in the *SHBG* gene and breast cancer risk by BMI, waist-to-hip ratio, years of menstruation, and plasma SHBG concentration among postmenopausal women in the Shanghai Breast Cancer Study

Variables*	<i>Asp/Asp</i>		<i>Asp/Asn</i> or <i>Asn/Asn</i>	
	Case/control	OR (reference)	Case/control	OR (95% CI) [†]
BMI (kg/m ²)				
<24	132/134	1.00 (reference)	34/78	0.46 (0.29-0.75)
≥24	133/145	1.01 (0.71-1.43)	65/70	1.07 (0.70-1.64)
		<i>P</i> for interaction = 0.01		
Waist-to-hip ratio				
<0.81	129/136	1.00 (reference)	35/75	0.51 (0.32-0.83)
≥0.81	136/143	1.04 (0.73-1.48)	64/73	0.99 (0.64-1.53)
		<i>P</i> for interaction = 0.04		
Years of menstruation				
<33	104/117	1.00 (reference)	37/69	0.63 (0.39-1.03)
≥33	161/162	1.21 (0.83-1.77)	62/79	1.00 (0.53-1.59)
		<i>P</i> for interaction = 0.52		
SHBG concentration				
<Median	80/140	1.00 (reference)	31/65	0.83 (0.49-1.40)
≥Median	64/128	0.92 (0.61-1.41)	20/78	0.50 (0.28-0.90)
		<i>P</i> for interaction = 0.25		

*Cut points were chosen by medians of BMI, waist-to-hip ratio, and years of menstruation among postmenopausal controls.

[†]Adjusted for age, education, ever had breast fibroadenoma, age at first live birth, age at menopause, BMI, and physical activity in the past 10 years.

women. The protective effect of the *Asn* allele was much stronger among postmenopausal women with low adiposity (BMI < 24 kg/m² or waist-to-hip ratio < 0.81) than those with high adiposity, and the interactions were statistically significant. The inverse association between the *Asn* allele and breast cancer risk was also stronger for estrogen receptor–positive cancer than estrogen receptor–negative cancer. These findings are new and consistent with the biological functions of SHBG and the polymorphism under study.

The functionality of the *Asp327Asn* polymorphism in the *SHBG* gene has been investigated in both animal models and human subjects. In an interesting experiment conducted by Cousin et al. (14), SHBG was purified separately from human subjects who carried *Asp/Asp*, *Asp/Asn*, or *Asn/Asn* genotypes. These three SHBG isoforms were labeled with biotin and injected into rabbits. It was found that the biological half-life was significantly higher for SHBG purified from subjects homozygous for the variant *Asn* allele than for that from subjects homozygous for the wild-type *Asp* allele. The association of the *Asn* allele with higher serum SHBG concentration was also shown in a study conducted in hirsute women (15). More recently, Dunning et al. (16) reported that the *Asn* allele was significantly related to an increased circulating SHBG and a reduced level of estradiol to SHBG ratio in postmenopausal women. These data are consistent with our findings and support the notion that *Asp327Asn* polymorphism is likely to be functionally significant.

Epidemiologic studies have consistently shown that high blood SHBG levels are associated with a reduced breast cancer risk in postmenopausal women (7, 8). SHBG binds to circulating sex hormones, including estrogens, and reduces the bioavailability of these hormones to target tissues (1, 2, 18). It has also been reported that in breast cancer cells, the binding of SHBG to its specific membrane receptor can activate second messenger systems (cyclic AMP and protein kinase A), and result in inhibition of estradiol-induced cell proliferation (5, 6). Given the role of SHBG in reducing the risk of breast cancer and the functionality of the *Asp327Asn* polymorphism in this gene, it is conceivable that women carrying the *Asn* allele may be at a reduced risk of breast cancer. The hypothesis, however, was tested in only a few epidemiologic studies (16, 19, 20), and the results from these studies have been inconsistent. In a study involving both familial ($n = 166$) and sporadic ($n = 223$) breast cancer in Polish and Nordic populations (19), the authors reported an overall reduced, but not statistically significant, OR of breast cancer among 327*Asn* carriers (OR, 0.81; 95% CI, 0.58–1.15). It is intriguing that healthy male blood donors ($n = 1,066$) were used as the control group in that study. In contrast, Becchis et al. (20) reported, from a hospital-based study including 255 breast cancer cases and 120 healthy women in Italy, that breast cancer patients had a higher frequency of the 327*Asn* variant (21.2%) than the controls (11.6%). The difference, however, was not statistically significant ($P = 0.08$). From the Anglian Breast Cancer Study, Dunning et al. (16) recently reported no significant association between the *Asn* variant and breast cancer risk, which was unexpected because this allele was related to an increased circulating SHBG and a reduced estradiol to SHBG ratio in that study. The authors argued that the reduction of blood SHBG levels by this polymorphism may be too small to exhibit a significant effect on the risk of breast cancer. In our study, we found the inverse association of *Asn* allele with breast cancer risk was only present in postmenopausal women, particularly among those with a low BMI or waist-to-hip ratio, or in tumors positive for estrogen receptor. Had we not done stratified analyses by these factors, we might have missed these associations as well. The findings from our study point to the need to consider the potential modifying effects of other factors in the evaluation of the association of genetic factors with cancer risk.

The conversion of androgen to estrone by aromatase in adipose tissue is the major source of estrogens in postmenopausal women (21, 22). It has been consistently documented that BMI is positively associated with blood estrogen level in postmenopausal women (23–25). We found in this study that the inverse association of *Asn* allele with breast cancer risk was primarily observed in postmenopausal women with a low BMI or waist-to-hip ratio, a condition with a lower blood estrogen level. This finding seems to support the hypothesis that the effect of genetic factors may be stronger in subjects with lower environmental exposure than those with high exposure (26). In other words, certain environmental exposures at a high level may overwhelm the possible effect of genetic factors. Although not statistically significant in interaction tests, we also found in this study that the inverse association of the *Asn* allele with breast cancer risk was stronger in women with a short duration of menstruation or with a higher blood SHBG level, which provides additional support to the notion that the effects of SHBG polymorphism may exist predominantly in women with a low estrogen concentration. Previous studies were conducted predominantly in Caucasian women whose levels of blood estrogens were reported to be more than 20% higher than Asian women (27). This may have contributed to the null association reported in these earlier studies.

Five polymorphisms, in exon 1, 4, 7, or 8, of the *SHBG* gene have been reported (28). With the exception of the *Asp327Asn* polymorphism in exon 8, the variant alleles of the remaining four polymorphisms have very low frequencies in Asians (allele frequency < 0.01). The *Asp327Asn* polymorphism has been found in various ethnic populations (10, 29). The *Asn* allele frequency varied from 4.5% to 12.5% in Caucasian populations (15, 16, 19, 20). Noticeably, we found that Chinese women carried a much higher allele frequency of the *Asn* variant (18%) than their Caucasian counterparts, consistent with a low risk of breast cancer in the Chinese population.

There are several noticeable methodologic strengths in this study. Chinese women living in Shanghai are relatively homogeneous in ethnic background; over 98% of them are classified into a single ethnic group (Han Chinese). Thus, the potential confounding effect of ethnicity and genetic background is not a major concern in the present study. Our study also benefits from the population-based study design, a high participation rate, and strictly implemented quality control procedures in data collection that substantially minimized selection and measurement biases. Furthermore, the large sample size and the wealth of data collected on lifestyle factors allowed us to evaluate potential modifying effects and to control for confounding factors.

In summary, in this large population-based case-control study, we found that the *Asn* variant of SHBG was significantly associated with a reduced risk of breast cancer among postmenopausal women, and the association was modified by body adiposity, and possibly by the estrogen receptor status of breast cancer. Our findings suggest that the variant *Asn* allele may be an important genetic susceptibility factor for breast cancer among postmenopausal Chinese women. More studies, particularly from populations that have a different prevalence of obesity and estrogen receptor–positive breast cancer than Chinese populations, are needed to confirm our findings and to investigate their generalizability.

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