

Differences in Breast Cancer Risk Factors by Tumor Marker Subtypes among Premenopausal Vietnamese and Chinese Women

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Abstract

We evaluated associations between reproductive and lifestyle risk factors with breast cancer tumor marker status in a case-control study. Cases were premenopausal women living in Vietnam and China who were eligible for a clinical trial of oophorectomy and tamoxifen as treatment for breast cancer ($n = 682$). Controls were nonrelative hospital visitors, matched on age to the cases ($n = 649$). Immunohistochemical analysis was used to identify the presence of estrogen receptor (ER) and progesterone receptor and the overexpression of HER-2/*neu* oncogene. Odds ratios (OR) and 95% confidence intervals (95% CI) were estimated using unconditional logistic regression, adjusted for known confounders. Overall, 280 (61%) tumor samples were ER positive and 176 (38%) were ER negative. HER-2/*neu* overexpression was detected in 161 (35%) samples, whereas 286 (26%) samples were HER-2/*neu* negative. We observed an

inverse trend between increasing parity and decreasing breast cancer risk ($P = 0.002$). Women ages ≥ 25 years at first birth had increased breast cancer risk compared with women ages < 25 years at first birth (OR, 1.53; 95% CI, 1.20-1.95). Women who consumed alcohol had increased risk of breast cancer compared with women who did not (OR, 1.85; 95% CI, 1.32-2.61). Compared with controls, OR estimates for breast cancer by parity and age at first birth were significantly associated with ER and/or HER-2/*neu* tumor status by Wald test ($P < 0.05$). Family history, age at menarche, cumulative lactation, body mass index, and education were not significantly related to breast cancer risk. Our findings support the hypothesis that some breast cancer risk factors differ by ER and HER-2/*neu* tumor marker subtypes. (Cancer Epidemiol Biomarkers Prev 2005;14(1):41-7)

Introduction

The effect of breast cancer can be measured on a global scale, with more than one million women diagnosed each year worldwide (1). An estimated 350,000 cases are premenopausal women living in Asia (2, 3). Few studies to date have evaluated breast cancer risk factors specifically in women in Southeast Asia (4-6). In addition to possible differences in risk factors from Western women, breast cancer in Southeast Asia may be characterized by a unique distribution of tumor marker subtypes. Studies in women from developed countries have identified several associations between tumor markers and reproductive factors (7-13).

Hormone receptors, such as the estrogen receptor (ER) and progesterone receptor (PR), are determinants of breast tumor behavior and may suggest etiologic pathways (14, 15). ERs bind estrogen and facilitate protein synthesis, cell division, and breast cell proliferation (16-18). PRs, in turn, are regulated by circulating levels of estrogen (16, 18). The presence of both estrogen and progesterone receptors in breast tumors has been associated with better survival and overall outcome (8, 19-22).

HER-2/*neu*, a proto-oncogene, is the most commonly amplified oncogene in human breast cancer (23). HER-2/*neu* and ER alterations occur early in breast carcinogenesis (14, 24). There is some evidence that HER-2/*neu* overexpression reflects distinct risk factor patterns, such as an association with early use of oral contraceptives

(11, 13, 25). Tumors with HER-2/*neu* overexpression are characterized by poor prognosis and tend to be higher grade and hormone receptor negative (23).

Hormonal and dietary factors such as early age at menarche, late age at first birth, and alcohol consumption are established risk factors for breast cancer (26-28). We designed a case-control study to evaluate potential variation in these and other breast cancer risk factors by ER and HER-2/*neu* status among premenopausal Vietnamese and Chinese women.

Materials and Methods

Subject Identification. A randomized clinical trial of adjuvant surgical oophorectomy and tamoxifen was conducted at six hospitals in Vietnam and one hospital in China from 1993 to 1999 (29). Eligibility for participation in the clinical trial was determined by clinical and cytologic or pathologic evidence of operable breast cancer. Premenopausal women (defined as having had at least one menstrual period in the previous year) who had a new diagnosis of stage IIA, IIB, and IIIA breast tumors and a planned mastectomy within 10 weeks were eligible for enrollment. Additional inclusion criteria required a physical examination to confirm the absence of metastatic cancer and the presence of normal chest X-rays, liver function, and blood calcium levels within 10 weeks of study entry. Women with preoperative radiation remained eligible so long as pretreatment clinical staging fell within the categories described above. This study was evaluated and approved by institutional review boards at the University of Wisconsin Comprehensive Cancer Center, the Office for Protection of Research Risk of the U.S. NIH, the Scientific and Technical Council of the Ministry of Health in Vietnam, and

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institutional review committees in China. Trial conduct and results were periodically reviewed by an independent data monitoring committee. Further details concerning the clinical trial have been published elsewhere (29).

The case-control study was designed in conjunction with the ongoing clinical trial to investigate the association of reproductive and lifestyle risk factors and breast cancer tumor marker status among premenopausal Vietnamese and Chinese women. Enrollment for the clinical trial and case-control study was conducted simultaneously at Vietnamese hospitals in Hanoi and Ho Chi Minh City in 1993 and in Danang, Haiphong, Hue, and Nha Trang from 1995 to 1996. In 1997, enrollment was initiated at Haimen City Hospital in the Jiangsu Province of China. All women eligible for the clinical trial of oophorectomy and tamoxifen were eligible for the case-control study ($n = 682$). Control participants were female visitors to noncancer patients in participating hospitals, matched on single year of age to the cases ($n = 649$). All study participants gave written informed consent in Vietnamese or Chinese.

Data Collection. Structured in-person interviews were conducted by local staff to obtain information regarding reproductive experiences, menstrual history, family history of breast cancer, tea and alcohol consumption, and other lifestyle factors. Interview duration was 15 to 30 minutes.

Molecular Analysis. Breast cancer tumor samples from 458 cases (67%) were assessed for the presence of ER and PR proteins and overexpression of HER-2/*neu* proteins by immunocytochemistry 2 to 7 years after diagnosis. Immunohistochemical analysis of formalin-fixed, paraffin-embedded tissue samples was conducted according to previously validated methodology (30-33). These procedures and minor modifications are described in detail elsewhere (29, 34, 35).

Data Analysis. To evaluate the association of known risk factors and breast cancer among premenopausal women living in Vietnam and China we calculated odds ratios (OR) and 95% confidence intervals (95% CI) using unconditional logistic regression. Polytomous logistic regression models were created to analyze ER and HER-2/*neu* case status compared with all controls. Within polytomous models, associations between tumor subtypes were compared with the Wald test using variables parameterized both categorically and continuously. Final models were adjusted for age (5-year groups), parity (defined as the number of full term pregnancies lasting at least 6 months and resulting in a live or still birth, four categories), age at first full term pregnancy (5-year groups), alcohol use (beer or rice vodka, no/yes intake), spouse's education level (primary, middle, secondary, and college), and study site (five hospitals). Body mass index was calculated as weight (kg)/height (m)². To obtain *P*s for trend, we included select variables as continuous linear terms in regression models. Analyses described above were done a second time after restricting the sample to women living in Vietnam only.

Results

Our study population was composed of women living in Vietnam or China. Of the 682 case patients, 637 (93%) were women living in Vietnam and 45 (7%) were women living in China. Among the 649 controls, 609 (94%) were women living in Vietnam and 40 (6%) were women living in China. The mean age of case patients enrolled in the case-control study was 41 years (range, 24-57 years). Mean age among the controls was 42 years (range, 20-57 years).

Table 1 presents the distribution of reproductive risk factors between cases and controls. There was a significant trend between increases in parity and decreasing breast cancer risk ($P = 0.002$). We observed a significant trend between older ages at first full-term pregnancy and elevated breast cancer

risk ($P = 0.0007$). Women who had their first full-term pregnancy at ages ≥ 25 years had 1.5 times the risk of developing breast cancer compared with women who had their first full-term pregnancy before age 25 (95% CI, 1.2-1.95). Family history (based on a diagnosis of breast cancer in a mother or sister), age at menarche, and total months of lactation were not significantly associated with breast cancer risk.

Table 2 illustrates lifestyle risk factors by case and control status. Body mass index, education, and spouse's education level were not significantly associated with increased risk of breast cancer. Women who reported consuming any beer or rice vodka had 1.85 times the risk of developing breast cancer compared with women who did not consume any alcohol (95% CI, 1.32-2.61). We were unable to evaluate levels of alcohol consumption due to small numbers.

Of the 458 case patients with available biomarker information, 280 (61%) were ER positive and 176 (38%) were ER negative. Overall, 169 (37%) samples were PR negative and 285 (62%) were PR positive. When examined by joint ER/PR status, 135 (29%) samples were negative for both, 39 (8.5%) were ER-/PR+, 34 (7%) were ER+/PR-, and 245 (53%) samples were ER and PR positive. HER-2/*neu* overexpression was detected in 161 (35%) tumor samples, whereas 286 (62%) samples were HER-2/*neu* negative. A total of 445 samples were assayable for both ER and HER-2/*neu* status. Of these, 84 (19%) were negative for both, 87 (20%) were positive for HER-2/*neu* only, 201 (45%) were positive for ER only, and 73 (16%) were positive for both.

ER Status. ER status-specific ORs and 95% CIs are presented in Table 3. Parity was associated with a decrease in breast cancer risk in both ER-positive and ER-negative tumors. When evaluated continuously, parity was only associated with ER-positive tumors ($P < 0.0001$) but not ER-negative tumors ($P = 0.6$). The Wald test indicated that the ORs for parity (continuous) were significantly different ($P < 0.01$) for the ER subtypes. Late age at first full-term pregnancy was associated with increased breast cancer risk among ER-positive tumors (OR, 1.76; 95% CI, 1.29-2.42) and ER-negative tumors (OR, 1.49; 95% CI, 1.03-2.15), with both tumor subtypes exhibiting a positive trend between age at first full-term pregnancy as a continuous variable and breast cancer risk ($P < 0.0001$ and $P = 0.01$, respectively). A significant association between alcohol and breast cancer risk was observed in ER-positive tumors (OR, 1.75; 95% CI, 1.11-2.74) but not in ER-negative tumors (OR, 1.31; 95% CI, 0.75-2.28), although the direction of the association remained the same. Family history, age at menarche, lactation, body mass index, and education did not gain significance when evaluated based on tumor marker status.

ER/PR Subtypes. We additionally evaluated tumors that were positive for both ER and PR ($n = 245$, 53%) and tumors that were negative for both ER and PR ($n = 135$, 29%). The results mirrored those for ER status alone (data not shown).

HER-2/*neu* Overexpression. As shown in Table 4, the decreased risk of breast cancer observed among women with high parity was strongly associated with HER-2/*neu*-negative, compared with HER-2/*neu*-positive, tumors ($P < 0.01$ by Wald test). We observed an inverse trend between parity and HER-2/*neu*-negative breast cancer risk ($P < 0.0001$); women with five or more births had 0.17 times the risk of HER-2/*neu*-negative breast cancer (95% CI, 0.07, 0.43) compared with nulliparous women. The increased risk among women with age at first full term pregnancy was also associated with HER-2/*neu*-negative tumors ($P < 0.05$ by Wald test). In this subtype, age at first full-term pregnancy ≥ 25 was associated with twice the risk of breast cancer (OR, 2.04; 95% CI, 1.49-2.80) with a positive trend between age at first full term pregnancy and breast cancer risk ($P < 0.0001$).

Table 1. ORs and 95% CIs for premenopausal breast cancer according to reproductive risk factors, Vietnam and China 1995 to 1999

Characteristic	Cases, <i>n</i> = 682 (%)	Controls, <i>n</i> = 649 (%)	Age-adjusted* OR (95% CI)	Multivariate† OR (95% CI)
Family history of breast cancer				
No	654 (95.9)	628 (96.8)	1	1
Yes	11 (1.6)	12 (1.8)	0.84 (0.37-1.93)	0.75 (0.32-1.75)
Unknown	17 (2.5)	9 (1.4)		
Age at menarche				
<15	233 (34.2)	208 (32.0)	1	1
15	93 (13.6)	112 (17.3)	0.74 (0.53-1.03)	0.74 (0.53-1.04)
16	133 (19.5)	116 (17.9)	1.04 (0.76-1.43)	1.11 (0.80-1.53)
>17	215 (31.5)	197 (30.4)	0.98 (0.75-1.29)	1.09 (0.82-1.45)
Missing	8 (1.2)	16 (2.5)		
<i>P</i>			0.9	0.5
Parity				
Nulliparous	51 (7.5)	39 (6.0)	1	1
1-2	341 (50.0)	275 (42.4)	1.01 (0.64-1.58)	0.83 (0.46-1.51)
3-4	227 (33.3)	252 (38.8)	0.68 (0.43-1.08)	0.58 (0.32-1.07)
>5	57 (8.4)	75 (11.6)	0.55 (0.32-0.95)	0.45 (0.23-0.89)
Missing	6 (0.9)	8 (1.2)		
<i>P</i>			0.0005	0.002
Age at first full-term pregnancy‡				
<25	318 (50.9)	374 (62.1)	1	1
≥25	286 (45.8)	210 (34.9)	1.59 (1.26-2.02)	1.53 (1.20-1.95)
Missing	21 (3.4)	18 (3.0)		
<i>P</i>			0.0002	0.0007
Cumulative lactation (mos)‡				
0-11	50 (8.0)	44 (7.3)	1	1
12-35	276 (44.2)	239 (39.7)	0.96 (0.63-1.44)	0.96 (0.62-1.48)
36-59	192 (30.7)	194 (32.2)	0.80 (0.52-1.23)	0.95 (0.58-1.55)
>60	95 (15.2)	119 (19.8)	0.63 (0.40-1.01)	0.82 (0.46-1.44)
Missing	12 (1.9)	6 (1.0)		
<i>P</i>			0.1	0.8

*ORs adjusted for age only.

†ORs adjusted for age, hospital, parity, age at first birth, alcohol use, and spouse's education.

‡Parous women only (parity and age at first birth are not adjusted for simultaneously when evaluating the effect of either variable).

Table 2. ORs and 95% CIs for premenopausal breast cancer according to lifestyle factors, Vietnam and China 1995 to 1999

Characteristic	Cases (<i>N</i> = 682) <i>n</i> (%)	Controls (<i>N</i> = 649) <i>n</i> (%)	Age-Adjusted* OR (95% CI)	Multivariate† OR (95% CI)
Body mass index quartile (kg/m ²)				
1 (13.2-18.5)	195 (28.6)	159 (24.5)	1	1
2 (18.6-20.0)	156 (22.9)	160 (24.7)	0.80 (0.59-1.09)	0.79 (0.58-1.08)
3 (20.1-21.6)	131 (19.2)	155 (23.9)	0.69 (0.50-0.94)	0.67 (0.49-0.93)
4 (21.7-40.8)	169 (24.8)	156 (24.0)	0.86 (0.63-1.17)	0.85 (0.62-1.16)
Missing	31 (4.5)	19 (2.9)		
<i>P</i>			0.2	0.2
Alcohol (beer or rice vodka)				
None	504 (73.9)	533 (82.1)	1	1
Any	109 (16.0)	63 (9.7)	1.89 (1.35-2.65)	1.85 (1.32-2.61)
Missing‡	69 (10.1)	53 (8.2)		
Education‡				
Primary	146 (21.4)	158 (24.3)	1	1
Middle	251 (36.8)	238 (36.7)	1.19 (0.89-1.60)	1.13 (0.83-1.55)
Secondary	166 (24.3)	159 (24.5)	1.20 (0.87-1.66)	1.01 (0.71-1.44)
College	91 (13.3)	76 (11.7)	1.33 (0.90-1.95)	1.09 (0.71-1.67)
Unknown or none	18 (2.6)	14 (2.2)		
Missing	10 (1.5)	4 (0.6)		
<i>P</i>			0.2	0.9
Spouse's education				
Primary	86 (12.6)	97 (14.9)	1	1
Middle	230 (33.7)	233 (35.9)	1.15 (0.81-1.62)	1.08 (0.75-1.55)
Secondary	189 (27.7)	181 (27.9)	1.21 (0.85-1.74)	1.10 (0.75-1.61)
College	121 (17.7)	86 (13.3)	1.59 (1.06-2.38)	1.33 (0.85-2.08)
Unknown or none	5 (0.7)	2 (0.3)		
Missing	52 (7.6)	50 (7.7)		
<i>P</i>			0.02	0.2

*ORs adjusted for age only.

†ORs adjusted for age, hospital, parity, age at first birth, alcohol use, and spouse's education.

‡Spouse's education is not adjusted for when evaluating the effect of education.

Table 3. Association of reproductive and lifestyle characteristics with risk of ER status-specific breast cancer among Vietnamese and Chinese women, 1995 to 1999

Characteristic	ER positive		ER negative	
	<i>n</i> (%)	OR (95% CI)*	<i>n</i> (%)	OR (95% CI)*
Family history of breast cancer				
No	269 (96.1)	1	164 (93.2)	1
Yes	6 (2.1)	0.94 (0.33-2.61)	5 (2.8)	1.37 (0.46-4.06)
Age at menarche				
<15	90 (32.1)	1	69 (39.2)	1
15	46 (16.4)	0.95 (0.61-1.47)	19 (10.8)	0.51 (0.29-0.90)
16	51 (18.2)	1.15 (0.75-1.77)	36 (20.5)	1.04 (0.64-1.69)
>17	89 (31.8)	1.22 (0.84-1.77)	51 (29.0)	0.87 (0.57-1.35)
<i>P</i>		0.7		1
Parity [†]				
Nulliparous	26 (9.3)	1	13 (7.4)	1
1-2	152 (54.3)	0.58 (0.27-1.28)	85 (48.3)	0.58 (0.22-1.54)
3-4	86 (30.7)	0.34 (0.15-0.76)	56 (31.8)	0.43 (0.16-1.17)
>5	14 (5.0)	0.17 (0.07-0.46)	21 (11.9)	0.53 (0.18-1.56)
<i>P</i>		<0.0001		0.6
Age at first full-term pregnancy [‡]				
<25	121 (48.0)	1	82 (50.6)	1
>25	124 (49.2)	1.76 (1.29-2.42)	75 (46.3)	1.49 (1.03-2.15)
<i>P</i>		<0.0001		0.01
Cumulative lactation (mos) [‡]				
0-11	19 (7.5)	1	17 (10.5)	1
12-35	121 (48.0)	1.23 (0.69-2.19)	70 (43.2)	0.77 (0.40-1.47)
36-59	80 (31.7)	1.29 (0.67-2.47)	48 (29.6)	0.68 (0.32-1.41)
>60	27 (10.7)	0.78 (0.36-1.72)	26 (16.0)	0.57 (0.24-1.37)
<i>P</i>		0.46		0.32
Body mass index, quartile (kg/m ²)				
1 (13.2-18.5)	78 (27.9)	1	48 (27.3)	1
2 (18.6-20.0)	64 (22.9)	0.84 (0.56-1.26)	39 (22.2)	0.78 (0.48-1.28)
3 (20.1-21.6)	61 (21.8)	0.81 (0.53-1.23)	37 (21.0)	0.71 (0.43-1.18)
4 (21.7-40.8)	68 (24.3)	0.89 (0.59-1.35)	44 (25.0)	0.88 (0.54-1.42)
<i>P</i>		0.3		0.4
Alcohol (beer or rice vodka)				
None	203 (72.5)	1	131 (74.4)	1
Any	39 (13.9)	1.75 (1.11-2.74)	20 (11.4)	1.31 (0.75-2.28)
Education				
Primary	46 (16.4)	1	45 (25.6)	1
Middle	111 (39.6)	1.59 (1.03-2.44)	59 (33.5)	0.92 (0.57-1.47)
Secondary	77 (27.5)	1.52 (0.94-2.47)	43 (24.4)	0.98 (0.57-1.69)
College	36 (12.9)	1.40 (0.79-2.49)	21 (11.9)	0.95 (0.49-1.83)
Spouse's education				
Primary	30 (10.7)	1	26 (14.8)	1
Middle	102 (36.4)	1.27 (0.77-2.08)	51 (29.0)	0.84 (0.48-1.45)
Secondary	78 (27.9)	1.18 (0.70-2.00)	51 (29.0)	1.09 (0.61-1.93)
College	46 (16.4)	1.28 (0.70-2.32)	35 (19.9)	1.55 (0.81-2.96)

*ORs adjusted for age, parity, age at first birth, alcohol use, and spouse's education (parity and age at first birth are not adjusted for simultaneously when evaluating the effect of either variable).

[†]*P* < 0.01 for the Wald test comparing ER+ and ER- associations evaluated continuously; all other Wald tests for characteristics in this table have *P* > 0.05.

[‡]Parous women only: ER+ (*n* = 252) and ER- (*n* = 162).

When we restricted our analyses to Vietnamese women only, the patterns of effect remained unchanged (data not shown).

Discussion

We observed significant relationships between parity, age at first full-term pregnancy, and alcohol consumption with breast cancer risk among young and middle-aged Vietnamese and Chinese women.

The distributions of age at menarche and body mass index in our study were very different than those commonly observed in epidemiologic studies of Caucasian women. Similarly, within our study population, a very high percentage (93%) of women had breast-fed for a year or more cumulatively. The distributions of these risk factors are consistent with the relatively low incidence rates of breast cancer in Vietnam and China as compared with the United States (1).

Our study did not observe some of the known associations of breast cancer risk factors in the United States. For example, our results did not indicate significant associations with risk

for family history, age at menarche, lactation history, or education level. As referenced above, the distribution of age at menarche and lactation history variables were unique to our study population of premenopausal Southeast Asian women; therefore, we would not necessarily expect to reproduce the associations between these factors and breast cancer risk observed in U.S. samples. Given that very few women reported breast cancer in close relatives, the frequency of major breast cancer genes (*BRCA1* and *BRCA2*) in our study population was considered likely to be low relative to women of European descent. The lack of association between personal and/or spousal education level and breast cancer risk may indicate that these variables are not suitable proxies for socioeconomic status in Southeast Asia. Alternatively, patterns of risk may not fall along social class lines in Vietnam and China as they do in the United States (4-6). We are unable to disentangle the contributions of these risk factors from the putative underlying genetic predisposition of these premenopausal Asian women in relation to breast cancer risk.

Epidemiologic studies have repeatedly shown an increased risk of breast cancer associated with alcohol consumption

Table 4. Association of reproductive and lifestyle characteristics with risk of HER-2/*neu* status-specific breast cancer among Vietnamese and Chinese women, 1995 to 1999

Characteristic	HER-2/ <i>neu</i> Positive		HER-2/ <i>neu</i> Negative	
	<i>n</i> (%)	OR (95% CI)*	<i>n</i> (%)	OR (95% CI)*
Family history of breast cancer				
No	152 (94.4)	1	272 (95.1)	1
Yes	4 (2.5)	1.12 (0.35-3.61)	7 (2.4)	1.14 (0.43-3.07)
Age at menarche				
<15	48 (29.8)	1	109 (38.1)	1
15	24 (14.9)	0.91 (0.53-1.58)	39 (13.6)	0.66 (0.42-1.03)
16	26 (16.1)	1.08 (0.62-1.86)	57 (19.9)	1.06 (0.69-1.86)
>17 [†]	61 (37.9)	1.49 (0.95-2.35)	79 (27.6)	0.88 (0.60-1.28)
<i>P</i>		0.2		0.7
Parity [‡]				
Nulliparous	7 (4.3)	1	32 (11.2)	1
1-2	83 (51.6)	0.69 (0.23-2.11)	152 (53.1)	0.56 (0.26-1.19)
3-4	51 (31.7)	0.46 (0.15-1.43)	85 (29.7)	0.33 (0.15-0.72)
>5 [‡]	20 (12.4)	0.62 (0.19-2.04)	14 (4.9)	0.17 (0.07-0.43)
<i>P</i>		0.5		<0.0001
Age at first full-term pregnancy [§]				
<25	85 (55.2)	1	111 (44.2)	1
>25 [†]	62 (40.3)	1.24 (0.84-1.81)	135 (53.8)	2.04 (1.49-2.80)
<i>P</i>		0.08		<0.0001
Cumulative lactation (mos) [§]				
0-11	11 (7.1)	1	25 (10.0)	1
12-35	66 (42.9)	1.25 (0.60-2.59)	121 (48.2)	0.90 (0.52-1.56)
36-59	52 (33.8)	1.31 (0.58-2.95)	73 (29.1)	0.85 (0.46-1.60)
>60	24 (15.6)	0.93 (0.36-2.38)	27 (10.8)	0.60 (0.28-1.29)
<i>P</i>		0.3		0.6
Body mass index, quartile (kg/m ²)				
1 (13.2-18.5)	48 (29.8)	1	74 (25.9)	1
2 (18.6-20.0)	40 (24.8)	0.79 (0.49-1.28)	64 (22.4)	0.89 (0.59-1.36)
3 (20.1-21.6)	32 (19.9)	0.62 (0.37-1.04)	65 (22.7)	0.92 (0.60-1.40)
4 (21.7-40.8)	32 (19.9)	0.64 (0.38-1.07)	76 (26.6)	1.08 (0.71-1.62)
<i>P</i>		0.2		0.6
Alcohol (beer or rice vodka)				
None	122 (75.8)	1	205 (71.7)	1
Any	16 (9.9)	1.17 (0.64-2.12)	41 (14.3)	1.77 (1.13-2.77)
Education				
Primary	36 (22.4)	1	54 (18.9)	1
Middle	59 (36.6)	1.13 (0.69-1.85)	108 (37.8)	1.30 (0.86-1.99)
Secondary	40 (24.8)	1.15 (0.65-2.03)	76 (26.6)	1.26 (0.78-2.02)
College	16 (9.9)	0.96 (0.47-1.97)	41 (14.3)	1.31 (0.75-2.29)
Spouse's education				
Primary	24 (14.9)	1	29 (10.1)	1
Middle	54 (33.5)	0.94 (0.54-1.65)	99 (34.6)	1.27 (0.77-2.11)
Secondary	51 (31.7)	1.17 (0.65-2.10)	74 (25.9)	1.14 (0.66-1.94)
College	27 (16.8)	1.30 (0.66-2.58)	52 (18.2)	1.46 (0.81-2.65)

*ORs adjusted for age, parity, age at first birth, alcohol use, and spouse's education (parity and age at first birth are not adjusted for simultaneously when evaluating the effect of either variable).

[†]*P* < 0.05 for the Wald test comparing HER-2/*neu* positive and negative associations categorically, all other Wald tests for categorical characteristics have *P* > 0.05.

[‡]*P* < 0.01 for the Wald test comparing HER-2/*neu* positive and negative associations continuously, all other Wald tests for continuous characteristics have *P* > 0.05.

[§]Parous women only: HER-2/*neu* positive (*n* = 154) and HER-2/*neu* negative (*n* = 251).

(26, 27). Studies showing higher estrogen levels among women who consume alcohol have been cited as evidence of an operating biological mechanism (36, 37). Other mechanisms suggested include a direct carcinogenic effect of alcohol or its congeners on breast cancer risk (26, 27).

Within our study population of premenopausal women living in Vietnam and China, 61% of analyzed tumor samples were ER positive. This prevalence is similar to studies of young Caucasian women (7, 8, 19, 38). In a prospective investigation of *postmenopausal* American women, Sellers et al. (39) found that the increased risk of breast cancer associated with alcohol was limited to ER-negative tumors. Conversely, a case-control study of American women ages 20 to 79 years, conducted by Nasca et al. (40), found an association between ER-positive breast cancer and alcohol consumption and failed to detect any association with ER-negative tumors. In our polytomous regression models, the increased breast cancer risk associated with alcohol consumption did not differ significantly between ER-positive and ER-negative tumors.

Overexpression of the HER-2/*neu* protein has been identified in 10% to 34% of breast cancer cases in other

studies (10, 41); in our study, 35% of cases had HER-2/*neu*-positive tumors. Tumors that overexpress HER-2/*neu* have been associated with the absence of hormone expression (10, 11, 42, 43). For this reason, it has been speculated that hormonal risk factors should only influence HER-2/*neu*-negative tumors (10, 42, 43).

An Italian study by Balsari et al. evaluated the risk associated with parity, age at menarche, and age at menopause by HER-2/*neu* status in two series of breast cancer patients in Milan (1st series: *n* = 1,211 patients and 2nd series: *n* = 717 patients). Their results were consistent with the hypothesis presented above; parity, age at menarche, and menopause were only associated with changes in breast cancer risk among HER-2/*neu*-negative cases but not HER-2/*neu*-positive cases (10). Our results lend support to this hypothesis in that we observed a relatively stronger association between parity and age at first birth on breast cancer risk among HER-2/*neu*-negative compared with HER-2/*neu*-positive tumors. However, unlike studies of women of Asian (44-46) and European (7, 12, 28) descent, we did not find an association between age at

menarche and breast cancer risk, either overall or by tumor marker status.

Other investigations of HER-2/*neu* status and breast cancer risk factor associations have not been in agreement. Variation in the strength of associations by HER-2/*neu* status for reproductive risk factors is notable. In a large population-based case-control study in North Carolina, Huang et al. (9) observed similar associations for age at menarche, parity, and age at first birth between HER-2/*neu*-positive and HER-2/*neu*-negative tumors. An investigation in Sweden also failed to detect an association between HER-2/*neu* status and parity and age at first birth in their case-to-case analysis (25). However, Treurniet et al.'s (13) case-control analysis identified a 2- to 4-fold increase in both the protective effect of breast-feeding and the increased risk associated with late age at first full-term pregnancy among women with HER-2/*neu*-positive tumors.

Of note, when Huang et al. (9) adjusted their HER-2/*neu* results for ER status there was no change in the observed associations. However, Gammon et al. (11) found stark differences in ORs calculated from their case-control study of HER-2/*neu* and oral contraceptive use when they stratified by ER status. Among women with ER-positive breast tumors, the association between ever use of oral contraceptives and HER-2/*neu*-positive and HER-2/*neu*-negative breast cancer was null (11). However, among women with ER-negative tumors, ever use of oral contraceptives was significantly associated with HER-2/*neu*-positive breast cancer (OR, 2.58; 95% CI, 1.31-5.10), but not HER-2/*neu*-negative breast cancer (OR, 0.92; 95% CI, 0.49-1.71; ref. 11). Our study did not have sufficient sample size to investigate the possible interaction of ER and HER-2/*neu* status.

Biological evidence supports HER-2/*neu* and ER alterations early in breast carcinogenesis (14, 24). HER-2/*neu* protein is not found in benign breast tissue but may be present in all stages of malignant disease (47, 48). The hypothesis that hormone receptor and HER-2/*neu* overexpression are markers of etiologic heterogeneity has been supported by evidence of interaction between biomarkers and known risk factors for breast cancer in other epidemiologic studies (8, 11-13, 25, 49).

Several limitations should be considered in interpreting our study results. Due to inadequate record keeping, participation proportions for cases and controls are not available. Additionally, we were unable to complete immunohistochemical analysis on all cases. Of the 682 breast cancer patients enrolled in conjunction with the clinical trial of adjuvant surgical oophorectomy and tamoxifen, 224 case patients did not have assayable pathology samples. However, cases with samples and cases without samples were similar with respect to age, body mass index, education, and general risk factors of interest (data not shown) and are unlikely to introduce systematic bias. Immunohistochemistry offers a cost-efficient means of assessing tumor marker status for epidemiologic research; however, some misclassification of ER and/or HER-2/*neu* status may have occurred by this method (8, 11, 30, 32).

Our study relied upon self-reported data. As in all case-control studies, recall bias is a concern. In general, reproductive histories and lifestyle factors are validly and reliably recalled (50-52). However, there is little data on this issue in Asian epidemiologic studies. In the Shanghai Breast Self-Examination trial, women reliably reported abortion history in two interviews (53). The composition of our subject population may limit the generalizability of our findings. Cases were restricted to women with regionally advanced disease, so not all women with incident breast cancer were eligible for the parent clinical trial or this case-control study. The most appropriate control group for these cases is difficult to readily define. Our observed results may not apply to women diagnosed with stage I or stage IV breast cancer, postmenopausal women, or to women living in other countries with different genetic backgrounds.

Prior investigations have focused on the role of biomarker status in predicting tumor size, type, and grade, aggressive-

ness, lymph node status, recurrence, disease-free and overall survival, and treatment response (16, 18, 20-23, 49). The primary strength of our study is our ability to provide much-needed information on the differential relation between reproductive and lifestyle risk factors and breast cancer risk by two highly prevalent tumor markers. In our study, the potential for confounding by ethnicity, menopausal status and cancer stage was extremely limited.

This study of women living in Vietnam and China suggests that reproductive patterns and emerging lifestyle behaviors such as alcohol consumption are strongly associated with premenopausal breast cancer risk. Interactions between reproductive factors with breast cancer tumor markers may suggest differences in etiologic pathways.

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