Risk of Breast Cancer According to the Status of HER-2/*neu* Oncogene Amplification[1]

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Abstract

We examined risk factors for breast cancer after subdividing cases based on the presence of HER-2/*neu* oncogene amplification in their tumors. Data were from the Carolina Breast Cancer Study, a population-based, case-control study of 577 invasive breast cancer patients, diagnosed during 1993–1996 and ages 20–74 years, and 790 controls frequency-matched on race and age. Information on breast cancer risk factors was obtained from structured personal interviews. About 20% of paraffin-embedded tissues from the breast cancers of cases were identified as positive for HER-2/*neu* amplification (HER-2/*neu*+) by differential PCR. Early age at menarche, higher waist:hip ratio, and family history of breast or ovarian cancer were associated with elevated odds ratios (ORs) for both HER-2/*neu*+ and HER-2/*neu*− breast cancers. Breastfeeding for at least 1 year was inversely associated with HER-2/*neu*+ breast cancer [OR, 0.3; 95% confidence interval (CI), 0.1–0.7] more so than HER-2/*neu*− breast cancer (OR, 0.8; 95% CI, 0.5–1.2). Most of the remaining risk factors had ORs around 1.0 for both HER-2/*neu*+ and HER-2/*neu*− breast cancers, although a few exhibited possible associations with one disease subtype in analyses stratified by menopausal status. These study results suggest that most recognized breast cancer risk factors do not operate through HER-2/*neu* amplification in breast carcinogenesis. Differential effects of long-term breastfeeding by HER-2/*neu* amplification status have been observed in earlier studies and are provocative; however, the direction and magnitude of the associations have not been consistent.

Introduction

Oncogenes are a class of genes capable of inducing neoplastic change in cells (1, 2). They are derived from normal cellular genes, called proto-oncogenes, by mutation or other types of DNA alteration. This conversion is somatic, as opposed to germ line, occurring only in specific lesions, not in all cells in the body. The HER-2/*neu* proto-oncogene, also known as c-*erb*B-2 or *ERBB2*, is located on chromosome 17q11.2–12 (3). Its encoded protein, a member of the class I receptor tyrosine kinase family (4), shows extensive homology with the receptor for epidermal growth factor (5). Amplification of HER-2/*neu* is the most frequent oncogene amplification found in breast tumors (6) and is present in a wide range of other adenocarcinomas as well (7). HER-2/*neu* gene amplification is associated with protein overexpression and occurs in ~20% of breast tumors (1, 8). Clinical studies have demonstrated that alterations in HER-2/*neu* predict poor prognosis for breast cancer (9–11) and are associated with features of tumor aggressiveness, such as absence of estrogen and progesterone receptors, high rate of cellular proliferation, advanced tumor stage, large tumor size, and young age at diagnosis (12, 13).

Current data support HER-2/*neu* amplification as a potential marker of etiological heterogeneity, rather than solely as a prognostic indicator: (*a*) HER-2/*neu* oncoprotein is not found in benign breast tissue (14, 15), whereas the level of HER-2/*neu* protein overexpression in malignant specimens is apparent at all stages, from intraductal to invasive phases of primary breast cancer and to subsequent metastases (2, 16, 17). Similar findings are observed with gene amplification, suggesting that HER-2/*neu* alterations are fixed markers occurring early in breast cancer evolution (18); and (*b*) it has been proposed that breast cancer positive for HER-2/*neu* amplification (HER-2/*neu*+) develops via a pathway that includes carcinoma *in situ*, whereas other forms of breast cancer may evolve via pathways that bypass the *in situ* phase (1). The hypothesis that HER-2/*neu* alterations may define a subset of breast cancer with a common origin is also suggested by epidemiological studies (13, 19, 20).

Using data from a population-based, case-control study of 577 breast cancer patients with known HER-2/*neu* oncogene amplification status and 790 controls, we examined both case-case comparisons and case-control comparisons among postmenopausal as well as pre/perimenopausal women. In addition to the variables reported by the previous studies, we assessed a variety of other established or suspected risk factors for breast cancer to explore their associations with HER-2/*neu* oncogene amplification in the development of breast cancer.

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Materials and Methods

The Carolina Breast Cancer Study. Data were collected for the Carolina Breast Cancer Study, a population-based, case-control study designed to investigate the etiology of breast cancer, including gene-environment interactions. Details of the study design are presented elsewhere (21). Briefly, participants were recruited from women, ages 20–74 years, residing in 24 contiguous counties of central and eastern North Carolina, an area of suburban, small town, and rural character. Cases were identified through the North Carolina Central Cancer Registry, using a rapid case ascertainment system (22). Controls were selected from records of the North Carolina Division of Motor Vehicles for women <65 years and from records of the United States Health Care Financing Administration for women 65–74 years of age. Women meeting residential and age criteria and first diagnosed with invasive, primary breast cancer from May 1, 1993 through May 31, 1996 were eligible as cases. Sampling took place to obtain roughly equal numbers of cases in four race/age subgroups; therefore, 100% of black women <50, 75% of black women 50 or older, 67% of white women <50, and 20% of white women 50 or older were recruited. Women describing themselves as Native American, Asian, or other races were few (<2% in the underlying population) and were included with whites. A modified, randomized recruitment method (23, 24), applying *a priori* sampling fractions for each race/age subgroup, was used to sample cases as well as controls, who were frequency-matched by race and 5-year age group. The study was conducted in accordance with the principles embodied in the Declaration of Helsinki under the approval of the University of North Carolina School of Medicine Institutional Review Board.

Data Collection. A 1–1.5-h, in-person interview was scheduled to administer a structured questionnaire, to take body measurements, and for cases, to obtain consent for retrieving tumor tissue and medical documentation. Interviews were completed by trained female nurses for 862 cases and 790 controls, corresponding to response rates of 77 and 68%, respectively, calculated among eligible and locatable women (25). Of the interviewed cases, pathology reports were received for 783 (91%) cases to confirm diagnosis and histological characteristics of the breast cancer, and paraffin-embedded tissue blocks were obtained for 577 (67%) cases to conduct molecular assays for HER-2/*neu* amplification.

Molecular Analysis of HER-2/*neu*. Tumor cells were selectively removed from paraffin-embedded tissues using H&E slides on which the tumor areas had been circled by the study pathologist as a guide. DNA was extracted according to standard procedures (26): xylene and ethanol deparaffinization, digestion in lysis buffer containing proteinase K, followed by centrifuged DNA precipitation. Oncogene amplification was detected by differential PCR with two sets of primers in each reaction, one specific for the target gene, *i.e.*, HER-2/*neu*, and the other specific for a diploid reference gene. Specific conditions for differential-PCR are those given previously (26, 27). The ratio of the two PCR products served as a measure of relative gene copy number between the target and the reference genes and was detected by performing acrylamide gel electrophoresis. Each run of PCR reactions includes both positive (*i.e.*, SKBR3 breast cancer cell lines that carried 4–8-fold amplification of HER-2/*neu*) and negative (*i.e.*, normal spleen cell lines that carried HER-2/*neu* nonamplified tissues) controls to compare with DNA samples from Carolina Breast Cancer Study participants because the ratio of target to reference gene PCR products shown on the gel may deviate slightly between reactions. Samples were graded "0" if the ratio of target:reference genes was similar to that observed for the negative control (*i.e.*, assigned a ratio of 1.0), "1" if between the negative and positive controls, "2" if similar to the positive control, and "3" if greater than the positive control. This method detects gene amplification as low as 2–4-fold (28). Data presented in this study combined tumors of grades 1–3 in the HER-2/*neu*+ group; however, results were largely the same using a higher threshold that included only tumors of grades 2–3. All laboratory procedures were conducted by one person (W. Y. H.), and gel pictures were reviewed by a second person (K. C.), both of whom were unaware of clinical characteristics and questionnaire responses at the time. Two sets of reference genes, progesterone receptor and IFN-γ, were tested separately in each DNA sample for dual confirmation, and only samples with amplification of HER-2/*neu* in both reactions, as determined by both reviewers, were considered positive.

Data Analysis. The questionnaire data allowed us to directly assess reproductive and other hormonal factors, such as age at menarche, parity/age at first full-term pregnancy, history of abortion or miscarriage, cumulative duration of breastfeeding, use of oral contraceptives, use of hormone replacement therapy, body mass index (kg/m^2) 1 year prior to interview, and waist:hip ratio (measured during interview). A pregnancy was classified as full-term if it lasted 7 or more months and as abortion or miscarriage otherwise. In addition, information was obtained on family history of breast or ovarian cancer among parents or siblings, medical radiation exposure to the chest (including coronary catheterization, angioplasty, or having axilla, lung, or breast treated or monitored with radiation prior to breast cancer diagnosis for cases or selection for controls), alcohol drinking during the most recent age range (based on the woman's age at diagnosis or selection but categorized as <26, 26–50, or >50 years), smoking more than five packs life long, and education. In analyses, each variable was defined several ways, with definitions derived from quantile distributions among the control population or from general agreement with the literature. The results reported here are for variables defined with the fewest categories that captured the apparent associations (definitions shown in the Tables).

To quantify the associations between risk factors and breast cancer subtyped by HER-2/*neu* status, ORs[3] and 95% CIs comparing each case subgroup to controls were produced. ORs and 95% CIs also were derived from direct case-case comparisons, where the departure of the OR from unity (*i.e.*, 1.0) reflects the presence (and degree) of risk heterogeneity between the two subtypes (HER-2/*neu*+ and HER-2/*neu*−) of disease (29). The intercase OR is a quick, direct measure for comparison between the two case subgroups, whereas the counterpart case-control ORs are necessary for etiological inferences and to reveal the pattern of heterogeneity between case subgroups.

All statistical analyses were weighted according to the sampling fractions applied to subgroups, categorized by disease status, age, and race, to allow inferences to the underlying population from which our sample was obtained. Unconditional binary logistic regression analyses were performed using SAS Proc Genmod (30–32). Using binary, rather than polytomous, logistic regression allowed for the incorporation of an offset term (derived from the ratio of the sampling fractions for cases to controls) to adjust for the sampling design in case-control

[3] The abbreviations used are: OR, odds ratio; CI, confidence interval; ER, estrogen receptor; PR, progesterone receptor.

Table 1 Characteristics of Carolina Breast Cancer Study participants

	Cases $n = 862$ n (%)	Controls $n = 790$ n (%)	OR (95% CI)[a]
Age at selection			
≥50 years	356 (41)	383 (49)	2.7 (2.0–3.5)[b]
<50 years	506 (59)	407 (51)	1.0
Race			
White	527 (61)	458 (58)	0.8 (0.7–1.1)
Black	335 (39)	332 (42)	1.0
Age at menarche			
<12 years	203 (24)	164 (21)	1.2 (0.9–1.6)
≥12 years	658 (76)	623 (79)	1.0
Nulliparity/Age at first full-term pregnancy			
Nulliparous	133 (15)	89 (11)	1.1 (0.8–1.5)
>25 years	187 (22)	162 (21)	1.0 (0.8–1.3)
≤25 years	538 (63)	537 (68)	1.0
Abortion (spontaneous or induced)			
Ever	302 (35)	299 (38)	0.8 (0.7–1.0)
Never	560 (65)	491 (62)	1.0
Breastfeeding			
≥12 months	119 (14)	115 (14)	0.8 (0.6–1.1)
<12 months	159 (18)	187 (24)	0.7 (0.5–0.9)
Never	583 (68)	488 (62)	1.0
Oral contraceptive use			
Ever	552 (64)	470 (60)	1.3 (1.0–1.7)
Never	307 (36)	319 (40)	1.0
Hormone replacement therapy			
Ever (≥3 months)	207 (24)	246 (31)	0.8 (0.6–1.0)[c]
Never	655 (76)	544 (69)	1.0
Body mass index			
>27.3 kg/m^2	389 (45)	356 (46)	1.0 (0.7–1.2)
≤27.3 kg/m^2	467 (55)	419 (54)	1.0
Waist:hip ratio			
>0.8	448 (53)	378 (48)	1.4 (1.1–1.7)
≤0.8	403 (47)	405 (52)	1.0
First-degree family history of breast or ovarian cancer			
Yes	140 (17)	96 (13)	1.5 (1.1–2.0)
No	697 (83)	664 (87)	1.0
Medical radiation to chest area			
Ever	54 (06)	55 (07)	1.1 (0.7–1.6)
Never	807 (94)	735 (93)	1.0
Alcohol drinking (most recent age range)			
Yes	507 (59)	454 (57)	0.9 (0.8–1.2)
No	355 (41)	335 (43)	1.0
Smoking			
Ever (≥5 packs)	418 (49)	367 (47)	1.1 (0.9–1.3)
Never	444 (51)	423 (53)	1.0
Education			
≥College graduate	243 (28)	205 (26)	1.2 (0.8–1.7)
≥High school graduate to <College graduate	460 (53)	420 (53)	1.1 (0.8–1.5)
<High school graduate	159 (19)	165 (21)	1.0

[a] Adjusted for all the 13 primary exposure variables assessed in the study, as well as race, age at diagnosis/selection (5-year age group), and the offset term.
[b] Adjusted for race, as well as all the 13 primary exposure variables assessed in the study and the offset term.
[c] Additionally adjusted for menopausal status.

comparisons. To control for potential confounding effects, all of the 13 primary variables assessed in the study as well as the matching factors, age and race, were included in the models. Hormone replacement therapy was further adjusted for menopausal status. Individuals with missing values for one or more of the variables in the models were eliminated from analyses.

Additional analyses stratified women on menopausal status. "Postmenopausal" was defined as natural menopause, cessation of cycling attributable to radiation treatment (prior to current diagnosis for cases), hysterectomy with bilateral oophorectomy, or hysterectomy with at least one ovary intact but age at diagnosis/selection >55 years (*i.e.*, age beyond which 95% of women in the control population reported reaching menopause). Women who reported experiencing menopausal symptoms after surgery or continuing to have menstrual periods while taking hormone replacement therapy and being >55 years were also considered postmenopausal. The remaining women who reported not having menstrual cycles were classified as perimenopausal, whereas women reporting that they were still cycling at the time of diagnosis or selection were classified as premenopausal.

Table 2 Basic characteristics of breast cancer patients by status of HER-2/*neu* oncogene amplification

	HER-2/*neu*+ $n = 115$ Weighted %[a]	HER-2/*neu*− $n = 462$ Weighted %[a]	Unknown status $n = 285$ Weighted %[a]
Age at selection			
≥50 years	55	66	64
<50 years	45	34	36
Race			
White	80	79	80
Black	20	21	20
Menopausal status			
Postmenopausal	62	68	62
Pre/Perimenopausal	38	32	38
First-degree family history of breast or ovarian cancer			
Yes	20	16	20
No	80	84	80
Stage			
I	36	48	57
II	49	46	32
III	11	5	7
IV	4	1	4

[a] Weighted by the probabilities used in the sampling design to allow inferences to the underlying population.

Results

Characteristics of our study population are displayed in Table 1. Although we intended to recruit similar numbers of women in each race/age level, we had somewhat fewer older and black women. After taking into account potential confounding effects and the sampling design, older age, higher waist:hip ratio, and a family history of breast or ovarian cancer were associated with increased risks of breast cancer.

About 20% of cases had tumors with evidence of HER-2/*neu* oncogene amplification, which corresponded to 19% of breast cancer patients in the general population after adjusting for the sampling design. Discordant results for the two reference genes occurred infrequently (for ~1.5% of samples), and these women were classified as HER-2/*neu*− for analytic purposes. HER-2/*neu*+ breast cancer, compared with the HER-2/*neu*− subtype, was more common among patients who were younger (Mantel-Haenszel χ^2; $P = 0.05$) and had a more advanced stage of disease at diagnosis ($P = 0.01$), whereas the distributions of race, menopausal status, and family history of breast or ovarian cancer were similar between subtypes of cases ($P = 0.3$–0.6; Table 2). Women with unknown HER-2/*neu* status (33% of all cases) were more similar to the negative group in terms of age and race but shared a closer pattern with the positive group for menopausal status, family history, and stage. However, cases with known HER-2/*neu* status were not

Table 3 Associations between various factors and breast cancer characterized by HER-2/*neu* amplification status

	HER-2/*neu*+ cases/Controls n = 115/790 OR (95% CI)[a]	HER-2/*neu*− cases/Controls n = 462/790 OR (95% CI)[a]	HER-2/*neu*+ cases/HER-2/*neu*− cases n = 115/462 OR (95% CI)[b]
Age at menarche			
<12 years	1.4 (0.8–2.2)	1.3 (1.0–1.8)	1.1 (0.7–1.9)
≥12 years	1.0	1.0	1.0
Nulliparity/Age at first full-term pregnancy			
Nulliparous	1.1 (0.6–2.0)	0.9 (0.6–1.4)	1.2 (0.7–2.3)
>25 years	0.9 (0.5–1.6)	1.0 (0.7–1.4)	0.9 (0.5–1.7)
≤25 years	1.0	1.0	1.0
Abortion or miscarriage			
Ever	0.9 (0.6–1.4)	0.9 (0.7–1.2)	1.0 (0.6–1.6)
Never	1.0	1.0	1.0
Breastfeeding			
≥12 months	0.3 (0.1–0.7)	0.8 (0.5–1.2)	0.3 (0.1–0.9)
<12 months	0.7 (0.4–1.1)	0.6 (0.4–0.9)	1.1 (0.6–2.0)
Never	1.0	1.0	1.0
Oral contraceptive use			
Ever	0.9 (0.5–1.5)	1.2 (0.9–1.7)	0.7 (0.4–1.2)
Never	1.0	1.0	1.0
Hormone replacement therapy[c]			
Ever (≥3 months)	0.6 (0.3–1.0)	0.8 (0.6–1.1)	0.6 (0.3–1.2)
Never	1.0	1.0	1.0
Body mass index			
>27.3 kg/m^2	0.6 (0.4–1.0)	1.0 (0.7–1.3)	0.8 (0.5–1.2)
≤27.3 kg/m^2	1.0	1.0	1.0
Waist:hip ratio			
>0.8	1.4 (0.9–2.3)	1.4 (1.0–1.8)	1.0 (0.7–1.7)
≤0.8	1.0	1.0	1.0
First-degree family history of breast or ovarian cancer			
Yes	1.7 (1.0–3.0)	1.4 (1.0–2.0)	1.2 (0.7–2.1)
No	1.0	1.0	1.0
Medical radiation to the chest			
Ever	1.2 (0.5–2.8)	1.1 (0.7–1.8)	1.0 (0.4–2.5)
Never	1.0	1.0	1.0
Alcohol drinking during most recent age range			
Yes	1.0 (0.6–1.5)	0.9 (0.7–1.2)	1.1 (0.7–1.8)
No	1.0	1.0	1.0
Smoking			
Ever (≥5 packs)	1.1 (0.7–1.7)	1.0 (0.8–1.3)	1.1 (0.7–1.8)
Never	1.0	1.0	1.0
Education			
≥College graduate	1.1 (0.5–2.3)	1.0 (0.7–1.6)	1.2 (0.6–2.6)
≥High school graduate to College graduate	1.1 (0.6–2.0)	1.1 (0.7–1.5)	1.2 (0.6–2.2)
<High school graduate	1.0	1.0	1.0

[a] Adjusted for all the 13 exposure variables simultaneously, as well as race, age at diagnosis/selection (5-year age group), and the offset term.
[b] Case-case ORs adjusted for all the 13 exposure variables simultaneously, as well as race and age at diagnosis/selection (two levels to reflect the sampling design for cases).
[c] Additionally adjusted for menopausal status.

significantly different from cases with unknown status for these factors (*P*s ranged from 0.7–1.0).

Table 3 presents the ORs and 95% CIs for HER-2/*neu*+ cases compared with HER-2/*neu*− cases, as well as for each subtype of cases to controls. Age at menarche <12 years (the 1st quartile), waist:hip ratio >0.8 (the median), and family history of breast or ovarian cancer in a first-degree relative were associated with increased risks of both HER-2/*neu*+ and HER-2/*neu*− breast cancers. Breastfeeding ≥12 months was related to HER-2/*neu* status (case-case OR, 0.3), revealing a much stronger inverse association with HER-2/*neu*+, compared with HER-2/*neu*− breast cancer. The associations with HER-2/*neu* status appeared weaker as duration of breastfeeding decreased [case-case ORs (95% CIs): 0.5 (0.2–1.1) for ≥9 months, 0.7 (0.4–1.3) for ≥6 months, and 0.8 (0.5–1.3) for ever breastfeeding]. In addition, there was some evidence that use of hormone replacement therapy and body mass index >27.3 kg/m (the median) was associated with decreased risks of HER-2/*neu*+ breast cancer, and oral contraceptive use was associated with slightly increased risk of HER-2/*neu*− breast cancer. For the remaining risk factors, ORs were ~1.0 (generally 0.9–1.1) for both HER-2/*neu*+ and HER-2/*neu*− breast cancers. Adjustment for the stage of breast cancer and/or hormone receptor status in the case-case comparisons made essentially no difference in the results (data not shown).

Analyses were repeated after women were stratified by menopausal status (Table 4). Again, age at menarche <12 years, waist:hip ratio >0.8, and first-degree family history of breast or ovarian cancer showed a trend toward positive associations with breast cancer, regardless of HER-2/*neu* status, for both postmenopausal and pre/perimenopausal women. The pattern observed for breastfeeding ≥12 months, in which the inverse association was stronger for HER-2/*neu*+ (than HER-2/*neu*−) breast cancer, was more pronounced among post-

Table 4 Association between various factors and HER-2/*neu*+ and HER-2/*neu*− breast cancer among postmenopausal and pre- or perimenopausal women

	Postmenopausal		Pre/Perimenopausal	
	HER-2/*neu*+ cases/Controls n = 53/436 OR (95% CI)[a]	HER-2/*neu*− cases/Controls n = 233/436 OR (95% CI)[a]	HER-2/*neu*+ cases/Controls n = 62/354 OR (95% CI)[a]	HER-2/*neu*− cases/Controls n = 229/354 OR (95% CI)[a]
Age at menarche				
<12 years	1.3 (0.6–2.7)	1.4 (0.9–2.1)	1.4 (0.7–2.7)	1.2 (0.8–1.9)
≥12 years	1.0	1.0	1.0	1.0
Nulliparity/Age at first full-term pregnancy				
Nulliparous	2.3 (0.9–5.8)	1.4 (0.8–2.6)	0.7 (0.3–1.6)	0.7 (0.4–1.2)
>25 years	1.7 (0.7–4.1)	1.3 (0.8–2.1)	0.5 (0.2–1.2)	0.9 (0.6–1.4)
≤25 years	1.0	1.0	1.0	1.0
Abortion or miscarriage				
Ever	0.8 (0.4–1.5)	1.0 (0.7–1.5)	1.2 (0.7–2.2)	0.8 (0.6–1.2)
Never	1.0	1.0	1.0	1.0
Breastfeeding				
≥12 months	0.1 (0.0–1.1)	1.1 (0.6–1.8)	0.4 (0.1–1.4)	0.7 (0.4–1.2)
<12 months	0.9 (0.4–1.9)	0.7 (0.5–1.1)	0.6 (0.3–1.4)	0.6 (0.4–1.0)
Never	1.0	1.0	1.0	1.0
Oral contraceptive use				
Ever	1.1 (0.5–2.2)	1.1 (0.7–1.7)	0.7 (0.3–1.6)	1.3 (0.8–2.1)
Never	1.0	1.0	1.0	1.0
Hormone replacement therapy				
Ever (≥3 months)	0.5 (0.3–1.0)[b]	0.7 (0.5–1.0)[b]		
Never	1.0	1.0		
Body mass index				
>27.3 kg/m^2	0.6 (0.3–1.3)	1.1 (0.7–1.6)	0.7 (0.3–1.3)	0.8 (0.5–1.2)
≤27.3 kg/m^2	1.0	1.0	1.0	1.0
Waist:hip ratio				
>0.8	1.5 (0.8–3.1)	1.2 (0.8–1.8)	1.4 (0.7–2.8)	1.7 (1.1–2.6)
≤0.8	1.0	1.0	1.0	1.0
First-degree family history of breast or ovarian cancer				
Yes	1.8 (0.8–3.9)	1.3 (0.8–2.0)	1.6 (0.7–3.7)	1.6 (1.0–2.8)
No	1.0	1.0	1.0	1.0
Medical radiation to the chest				
Ever	1.1 (0.4–3.1)	1.1 (0.6–1.9)	1.7 (0.3–9.3)	1.1 (0.3–3.6)
Never	1.0	1.0	1.0	1.0
Alcohol drinking during most recent age range				
Yes	0.6 (0.3–1.1)	1.0 (0.7–1.4)	2.0 (0.9–4.6)	0.7 (0.5–1.1)
No	1.0	1.0	1.0	1.0
Smoking				
Ever (≥5 packs)	1.1 (0.6–2.0)	1.0 (0.7–1.4)	1.0 (0.6–1.9)	1.0 (0.7–1.5)
Never	1.0	1.0	1.0	1.0
Education				
≥College graduate	1.0 (0.3–2.8)	0.9 (0.5–1.6)	1.2 (0.4–3.4)	1.3 (0.6–2.6)
≥High school graduate to College graduate	1.0 (0.4–2.3)	1.0 (0.6–1.5)	1.0 (0.4–2.7)	1.2 (0.6–2.3)
<High school graduate	1.0	1.0	1.0	1.0

[a] Adjusted for all the 13 primary variables simultaneously, as well as race, age at diagnosis/selection (5-year age group), and the offset term.
[b] Including perimenopausal women with postmenopausal women.

menopausal women than among pre/perimenopausal women. The slight increase of HER-2/*neu*− (but not HER-2/*neu*+) breast cancer risk for oral contraceptive use was restricted to pre/perimenopausal women, whereas the modest decrease of HER-2/*neu*+ (but not HER-2/*neu*−) breast cancer risk for high body mass index was observed only among postmenopausal women. In addition, there was some evidence that nulliparity and age at first full-term pregnancy >25 years were associated with more elevated risks for HER-2/*neu*+ breast cancer among postmenopausal women. In contrast, medical radiation to the chest area and recent alcohol drinking showed possible elevated risks for HER-2/*neu*+ breast cancer among pre/perimenopausal women only. For the remaining risk factors of hormone replacement therapy, smoking, and education, ORs were similar across all subgroups. Because of limited sample size in analyses stratified by menopausal status, CIs for all of these results were wide, frequently overlapping, and almost always contained 1.0.

Discussion

In this population-based, case-control study among North Carolina women, using HER-2/*neu* oncogene amplification to subdivide breast cancer did not help discriminate associations between subgroups of disease for the risk factors studied, with the possible exception of breastfeeding. Breastfeeding for at least 1 year was associated with stronger decreased risks for HER-2/*neu*+, compared with HER-2/*neu*−, breast cancers. The distinction between HER-2/*neu*+ and HER-2/*neu*− breast cancers became less apparent as duration of breastfeeding decreased. In addition, some hint of differential patterns between

HER-2/*neu*+ and HER-2/*neu*− breast cancer emerged for nulliparity, age at first full-term pregnancy, oral contraceptive use, body mass index, first-degree family history of breast or ovarian cancer, medical radiation to the chest, and recent alcohol drinking when women were stratified by menopausal status. However, these measures had wide, overlapping CIs and, therefore, may reflect fluctuations generated by stratification.

In aggregate, these results are not consistent with those previously published (13, 19, 20). On the basis of case-case comparisons of 72 Swedish, premenopausal breast cancer patients, Olsson *et al.* (13) reported that breast cancer with HER-2/*neu* amplification (31% of all cases) was positively associated with early oral contraceptive use (<20 years) and nulliparity but inversely associated with miscarriage or abortion after the first full-term pregnancy. No data on breastfeeding were presented. Treurniet *et al.* (19), in making population-based, case-control comparisons (296 cases and 737 controls) among women ages 20–54 years in the Netherlands, found that breastfeeding was associated with a 4-fold increased risk only for breast cancer that overexpressed HER-2/*neu*(18% of all cases), and that early age at first full-term pregnancy exhibited a stronger increased risk for HER-2/*neu*+ than HER-2/*neu*− breast cancer. Recently, from a population-based study in New Jersey of 371 breast cancer cases and 462 control women of ages <45 years, Gammon *et al.* (20) reported an almost 2-fold increased risk of breast cancer associated with oral contraceptive use prior to age 18 but only for breast tumors that overexpressed HER-2/*neu* protein (43% of all cases). The OR increased to over three for HER-2/*neu*+ breast cancer that was negative for estrogen receptors.

The lack of agreement across studies is difficult to explain. Although HER-2/*neu* status was determined by three different methods, this alone is unlikely to account for the observed differences in results. The differential-PCR method (this study) and dot-blot procedures (13) used to detect HER-2/*neu* amplification are considered comparable (27, 28). In contrast, there is a recognized lack of concordance between the protein and DNA assays, because overexpression can occur in the absence of amplification (33–35). However, the prevalences of HER-2/*neu* alterations and the patterns of results observed in the various studies do not correlate with the type of HER-2/*neu* measurement used. Additionally, we repeated analyses using the same variable definitions used in the other studies, including assessment of early oral contraceptive use and stratification by estrogen receptor status, but this was not sufficient to replicate the findings reported by others (data not shown). Restriction of our study to the ages represented in the other studies also failed to produce similar results (*e.g.*, analyses stratified by menopausal status). We cannot dismiss the possibility of differences between populations in the risk factors being analyzed, *e.g.*, the proportion of women breastfeeding for at least 1 year and breastfeeding practices (36) or the types of oral contraceptives used and changes in formulations over time (37), which may contribute to variability across studies.

Selection bias in our study is a potential concern because HER-2/*neu* amplification status was missing for 33% of cases. However, data availability was not statistically significantly associated with tumor stage ($P = 0.7$) nor with other characteristics listed in Table 3 (*P*s ranging from 0.8–1.0). Although refusal rates differed by disease status and nonparticipation may be related to risk factor status, substantial selection bias is not expected, based on the relatively high response rates in our study (reaching 70–80% for most subgroups) and our assessment of a mini-survey conducted on a portion of the nonparticipants (25).

Other than long-term breastfeeding, none of the hormone-related breast cancer risk factors under study showed sufficient evidence of associations with HER-2/*neu* status in our data. This contradicts our original hypothesis, which was derived from two key observations: (*a*) Matsuda *et al.* (38) demonstrated that estrogens can bind to HER-2/*neu* protein and activate its kinase activity; thus, estrogen-induced HER-2/*neu* kinase activity could represent an important pathway in breast carcinogenesis; and (*b*) the activation of oncogenes, including HER-2/*neu* amplification, requires cell division (39), which is influenced by ovarian hormones (39, 40). Interestingly, in another set of analyses that subdivided breast cancer by ER and PR status, several hormone-related risk factors were associated with increased risks of breast cancer positive for ER and PR and not for breast cancer lacking ER and PR (41). Breastfeeding, however, was an exception, showing no association with ER and PR status. The contrast between these ER/PR results and the HER-2/*neu* results reported here and the fact that adjustment for ER and/or PR status (with or without tumor stage) did not alter the associations observed between HER-2/*neu* and the various risk factors in our data are suggestive that independent pathways may exist. This is further supported by biological evidence that HER-2/*neu* amplification and ER/PR alterations are early events in breast carcinogenesis (18, 42). These findings require confirmation and expansion by other studies.

Results from this population-based study of relatively large size suggest that DNA amplification of HER-2/*neu* is not related to most of the commonly recognized risk factors in the development of breast cancer. Differential effects of long-term breastfeeding between HER-2/*neu*+ and HER-2/*neu*− breast cancer have been observed in an earlier study and are provocative; however, the direction and magnitude of the associations have not been consistent. This lack of agreement within the modest literature available is puzzling. Future insight into breast cancer causality, therefore, requires additional accumulation of biological knowledge as well as further epidemiological observations, using standardized assays for determining biomarker status. The importance of larger sample sizes when attempting to identify more homogeneous subgroups of breast cancer is also apparent.

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Risk of Breast Cancer According to the Status of HER-2/*neu* Oncogene Amplification

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