

Oral Contraceptive Use and Other Risk Factors in Relation to HER-2/*neu* Overexpression in Breast Cancer Among Young Women¹

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Abstract

This study was undertaken to explore whether the incidence of breast tumors that overexpress HER-2/*neu* protein product (HER-2/*neu*+) is more strongly associated with oral contraceptives (OCs) and other factors than is the incidence of tumors that do not (HER-2/*neu*-). In a population-based sample of women <45 years, 42.9% (159 of 371) of *in situ* and invasive breast cancer cases were HER-2/*neu*+ as assessed by immunohistochemistry in archived tissue. Polytomous logistic regression was used to calculate the odds ratios (ORs) and 95% confidence intervals (CIs) for HER-2/*neu*+ and HER-2/*neu*- breast cancer, as compared with 462 population-based controls, in relation to OCs and other factors. The ratio of the ORs (HER-2/*neu*+ versus HER-2/*neu*- tumors) was used as an indicator of heterogeneity in risk. There was little heterogeneity in risk for OC use of 6 months or more by HER-2/*neu* status (age-adjusted ratio of ORs, 1.29; 95% CI, 0.83–2.00). Among early pill users (≤18 years of age) heterogeneity was apparent (2.39; 95% CI, 1.08–5.30), which was attenuated in a multivariate model (1.99; 95% CI, 0.87–4.54); among cases with estrogen receptor-negative tumors, heterogeneity increased to 5-fold. For other risk factors, there was no marked heterogeneity between + and - tumors for HER-2/*neu*. In summary, the incidence of breast cancer among younger women in relation to OC use at an early age varied with HER-2/*neu*

status, with the odds ratio for + tumors twice that for - tumors.

Introduction

Many epidemiological studies (1) have shown no association between breast cancer and OC³ use. Some studies, however, have shown a modest <2-fold increase among young women with breast cancer in relation to long-term OC use, recent use, or use at an early age (1–3). Because the modest increase could be due to uncontrolled or poorly controlled confounding, the etiological significance of the association is unclear.

Some investigators have suggested that OCs may be more strongly associated with pathologically distinct subgroups of breast cancer. However, results of previous studies that have examined the association with cases classified by tumor morphology or estrogen receptor status have been inconsistent (4). Molecular studies indicate that oncogenes, such as HER-2/*neu* and others, are involved with breast cancer pathogenesis (5) and possibly with tumor initiation (6). Thus, classification of tumors by oncogene overexpression or amplification may produce etiologically distinct subgroups. This strategy has been used successfully in a study of occupational exposures and *ras* oncogene activation in acute myeloid leukemia (7).

One previous study (8) has explored the possible association between OCs and HER-2/*neu* status. The adjusted OR in relation to use of OCs at age 20 years or younger was significantly increased 7-fold for HER-2/*neu*-positive breast cancer among young Swedish women, as compared with cases with tumors that lacked oncogene amplification. The study, however, was based on very limited numbers. A consistent association between OCs and HER-2/*neu*-positive tumors would indicate that either HER-2/*neu* is the mechanism by which OCs affect breast cancer, or the oncogene is a cofactor that interacts with OCs in producing the disease.

With regard to other risk factors for breast cancer, another study (9) has addressed the possible interaction between reproductive risk factors and alterations in the HER-2/*neu* oncogene in breast cancer. In this report from the Netherlands (9), the OR was significantly increased 4-fold for HER-2/*neu*-positive breast cancer, as compared with controls, in relation to late age at first birth and ever having breastfed; the corresponding ORs for HER-2/*neu*-negative breast cancer were 2-fold and less than unity, respectively. Thus, classification of breast cancer cases by the presence of a molecular alteration, and thus into etiologically distinct subgroups, may also help to clarify these relationships as well.

We undertook a population-based study to address the hypothesis that the incidence of HER-2/*neu*-positive tumors is more strongly associated with OC use than is the incidence of

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³ The abbreviations used are: OC, oral contraceptive; OR, odds ratio; CI, confidence interval; ER, estrogen receptor.

HER-2/*neu*-negative tumors. The study also explored whether HER-2/*neu*-positive tumors are related to other risk factors for breast cancer.

Materials and Methods

This study included three components: (a) collection of archived paraffin-embedded tissue blocks from a population-based sample of white and black breast cancer cases; (b) laboratory evaluation for evidence of HER-2/*neu* overexpression in the tumor tissue by immunohistochemistry; and (c) combining the laboratory results with risk factor information on the same cases to estimate the ORs for HER-2/*neu*-positive breast cancer in relation to OC use and other established and potential risk factors for breast cancer. This study received approval from the institutional review boards of the participating institutions.

Study Subjects and Risk Factor Information. The source of the cases, controls, and the risk factor information is from the New Jersey component of the parent study, which was a multicenter, population-based, case-control study of breast cancer (3). A woman was eligible as a case if she was newly diagnosed with *in situ* or invasive breast cancer between May 1, 1990, and December 31, 1992; was between the ages of 20 to 44 years at diagnosis; and was a resident of a five-county study area in New Jersey (Middlesex, Monmouth, Morris, Somerset, and Union). Potentially eligible case women were ascertained using rapid reporting; field personnel visited hospitals within the five-county study area (as well as those in adjacent counties) on a monthly basis to review pathology reports to identify eligible cases. Physicians of eligible cases were contacted for approval to contact their patients.

A woman was eligible as a control if she was between the age of 20 to 44 years, was a resident of the same five-county area of central New Jersey as cases during the study period, and had access to a residential telephone. Controls were identified by random digit dialing (10) and frequency matched to the expected distribution of cases by 5-year age group. Physician-approved cases and controls were contacted first by letter and then by telephone to seek permission for the in-person interview. Before each interview, the purpose and content of the study was explained, and the informed consent form was signed.

Interviews were completed with 509 cases (83.4% of eligibles) and 462 controls (76.9%). The in-person interview lasted ~70 min and included ascertainment of OC use (using a reproductive and contraceptive calendar along with pictorial memory aids); menstrual and reproductive histories including pregnancies, lactation, and abortions; lifetime alcohol consumption patterns; adolescent diet; body size and development; physical activity; demographic factors; family history of cancer; and medical history including biopsy-proven benign breast disease and gynecological surgery. After completion of the questionnaire, trained interviewers took anthropometric measures such as skinfold thicknesses, circumference measurements, wrist and elbow width, standing and sitting height, and weight. At the conclusion of the interview, respondents completed a comprehensive self-administered food frequency questionnaire that focused on intake of food items over the past year.

Block Retrieval. For this project, retrieval was attempted from the appropriate hospital pathology departments for a representative paraffin-embedded tumor tissue block for each case participant. For the present study, blocks were successfully retrieved for 401 (78.8%) of the interviewed cases. As reported previously (11), the distribution of known and suspected risk factors for breast cancer did not vary significantly between cases with and without tumor tissue available for immunohistochemistry.

Slide Preparation and Laboratory Analyses. HER-2/*neu* overexpression was evaluated in tissue sections by immunohistochemical staining (12, 13) using antibodies with high sensitivity for HER-2/*neu* in paraffin-embedded tissues. The paraffin blocks were used to generate three 5- μ m-thick sections on silane-coated slides. The sections were baked at 60°C for 30 min, deparaffinized in xylene, and hydrated in alcohol and water. One of the sections was stained with H&E. Another was immunohistochemically stained with C-*neu* (Ab-3) mouse monoclonal antibody IgG1 (1:50; Calbiochem, Cambridge MA). The slides were stained using the Ventana ES automated immunostainer (Ventana Medical Systems, Inc., Tucson AZ) and then counterstained using the CAS DNA staining kit, which uses the Feulgen staining (Becton Dickinson, San Jose, CA). The stained DNA was quantified using the CAS200 Image Analyzer. The last of the three sections was used as a negative control for the immunohistochemical staining of C-*neu* and was prepared in identical fashion except that the section lacked the C-*neu* primary antibody. In addition, each batch of staining performed had two controls stained in parallel. This includes a CAS control for DNA content consisting of a cell line of known DNA content. A CAS control cell line of known DNA content and overexpressor of C-*neu* with a known C-*neu* protein content was also used as a control (Becton Dickinson).

The H&E section corresponding to each block was reviewed by the two study pathologists (HH and SB) to confirm the diagnosis of cancer. The corresponding areas were searched for in the C-*neu*-stained sections. Areas of cancer showing predominantly membranous red staining were analyzed by the CAS200, provided that the negative control showed minimal background staining. Using the CAS200 Quantitative Image Analyzer (Becton Dickinson), the C-*neu* protein level was quantitated with the Quantitative Oncogene product program, yielding the average pg protein of C-*neu* per cell.

Levels above 0.1 pg/cell were considered elevated and positive of overexpression. For additional statistical analyses, we also considered an alternative cutpoint for positivity of 0.2 pg. Because results were not substantially different from those based on a cutpoint of 0.1, only the latter are shown.

Statistical Analyses. Unordered polytomous logistic regression (14) was used to calculate the ORs and 95% CIs for HER-2/*neu*-positive (+) breast cancer and HER-2/*neu*-negative (-) breast cancer, as compared with the controls, in relation to use of OCs, patterns of OC use, and other factors including age at menarche, age at first birth, parity, lactation, induced abortion, family history of breast cancer, previous breast biopsy, body size, usual alcohol use, race, education, smoking, electric blanket use, physical activity, and caloric intake. The ratio of the ORs (and corresponding CIs; Ref. 15) was used as an indicator of heterogeneity in risk for tumor-positive *versus* tumor-negative cancer. Best fitting models were developed from a saturated model including all known and suspected risk factors for breast cancer and then excluding covariates that did not improve the overall fit of the model as measured by the log likelihood ratio test (14). Cutoff points for the factors that were assessed as continuous variables were based on the distributions observed among the control subjects, with the exception of OCs and cigarette smoking. For these latter variables, cutoff points were used to be consistent with other previous publications (3, 16) of these two controversial topics.

Results

Prevalence of HER-2/*neu* overexpression in the archival tumor tissue was successfully determined for 371 cases with breast can-

Table 1 Characteristics of breast cancer cases by HER-2/*neu* status and controls among young women <45 years of age in New Jersey, 1990–1992

	HER-2/ <i>neu</i> + (n = 159)	HER-2/ <i>neu</i> - (n = 212)	Controls (n = 462)	P ^a
Age at diagnosis				
23–29 years	5 (3.14%)	9 (4.25%)	27 (5.84%)	0.28
30–34 years	25 (15.72%)	31 (14.62%)	83 (17.97%)	
35–39 years	49 (30.82%)	57 (26.89%)	147 (31.82%)	
40–44 years	80 (50.31%)	115 (54.25%)	205 (44.31%)	
Stage at diagnosis (%)				
<i>In situ</i>	11 (6.96%)	27 (13.04%)		0.11
Local	77 (48.73%)	104 (50.24%)		
Regional/Distant	70 (44.30%)	76 (36.71%)		
ER status (%)				0.02
Positive	62 (44.29%)	109 (59.89%)		
Borderline	14 (10.00%)	17 (9.34%)		
Negative	64 (45.71%)	56 (30.77%)		
Progesterone receptor status (%)				0.34
Positive	78 (56.52%)	106 (59.89%)		
Borderline	6 (4.34%)	13 (7.34%)		
Negative	54 (39.13%)	58 (32.77%)		
Race (%)				
White	131 (82.39%)	182 (85.85%)	382 (82.68%)	0.27
Black	16 (10.06%)	24 (11.32%)	48 (10.39%)	
Asian and other	12 (7.55%)	6 (2.83%)	32 (6.93%)	
Religion (%)				
Protestant	52 (32.70%)	70 (33.02%)	154 (33.33%)	0.61
Jewish	14 (8.81%)	23 (10.85%)	46 (9.96%)	
Catholic	86 (54.09%)	115 (54.25%)	238 (51.52%)	
Other/None	7 (4.40%)	4 (1.89%)	24 (5.19%)	

^a P for χ^2 test. *Bold*, statistically significant heterogeneity.

cer. The remaining 7.5% could not be determined because of the lack of tumor tissue in the archived block retrieved from the hospital. In this population-based sample, 42.9% (159/371) of the breast cancer cases showed overexpression of HER-2/*neu*. The prevalence of overexpression did not increase with age among this sample of younger case women newly diagnosed with breast cancer (Table 1). Case women with HER-2/*neu*-negative tumors were more likely than women with HER-2/*neu*-positive tumors to have ER+ tumors ($P = 0.02$) and to be diagnosed with *in situ* disease ($P = 0.11$). There was little association between HER-2/*neu* status and progesterone receptor status or race.

Table 2 shows the age-adjusted ORs and corresponding CIs for HER-2/*neu*+ and HER-2/*neu*- breast cancer in relation to patterns of OC use. There was little heterogeneity in risk for OC use for 6 months or more by HER-2/*neu* status (age adjusted ratio of ORs, 1.29; 95% CI, 0.83–2.00). However, among women who started using the pill at age 18 years or earlier, heterogeneity by HER-2/*neu* status was apparent (2.39; 95% CI, 1.08–5.30). There was little or no heterogeneity of association in relation to duration of OC use, recent use, and recently starting or stopping.

Table 2 also shows the age-adjusted ORs and corresponding CIs for HER-2/*neu*+ and HER-2/*neu*- breast cancer in relation to reproductive and other risk factors for breast cancer, including family history of breast cancer, body size, alcohol, or cigarette smoking. There was evidence of heterogeneity by HER-2/*neu* status among Asian and other women (ratio of the OR, 2.78; 95% CI, 1.02–7.61); however, the number of Asian and other case and control participants (excluding blacks and whites) in our study was small. There was little or no heterogeneity of effect for other factors examined, including age at first birth (ratio of the OR, 0.96 for each additional year; 95% CI, 0.92–1.01), lactation (ratio of the OR, 0.72 for ever *versus* never; 95% CI, 0.44–1.15), or the other factors listed in Table 2.

In Table 3 are the multivariate-adjusted ORs for breast cancer categorized by HER-2/*neu* status. Table 3 includes a

variable for age at first use of OCs along with those variables that contributed to a best fitting model as described in “Materials and Methods.” The modest heterogeneity in ORs observed for early pill use was no longer statistically significant in a multivariate model (for age 18 or earlier, the ratio of the ORs, 1.99; 95% CI, 0.87–4.54). As shown in Table 3, for other established and suspected breast cancer risk factors, our analyses did not reveal marked heterogeneity in risk between positive and negative HER-2/*neu* tumors.

In Table 4 are the multivariate-adjusted ORs and corresponding CIs for HER-2/*neu*+ and HER-2/*neu*- breast cancer in relation to patterns of OC use with the breast cancer cases further stratified by the ER status of the tumor. Among case women with ER- tumors, the OR for ever use of OCs was 2.58 (95% CI, 1.31–5.10) among HER-2/*neu*+ cases and 0.92 (95% CI, 0.49–1.71) among HER-2/*neu*- cases. The ratio of the ORs for ever use of OCs was significantly elevated (2.81; 95% CI, 1.18–6.67). The heterogeneity was particularly pronounced among women with age at first use before age 18 years (ratio of the OR, 5.37; 95% CI, 1.20–24.01) or after age 22 years (ratio of the OR, 5.92; 95% CI, 1.81–19.36). Little or no heterogeneity, in relation to OC use (Table 4), was noted among case women with ER+ tumors. When cases were stratified by progesterone receptor status, which is highly correlated to ER status, a similar but attenuated pattern of effect was observed; due to sparse cells, however, modification by ER/PR status combined could not be evaluated. Heterogeneity by stage of disease was not apparent (data not shown). Also, there was little variation in the incidence of HER-2/*neu*+ and HER-2/*neu*- breast cancer in relation to other estrogen-related risk factors when cases were stratified by estrogen/progesterone receptor status.

Discussion

The proto-oncogene HER-2/*neu* is the human homologue of the rat *neu* oncogene and is mapped on chromosome 17 at q21. It has

Table 2 Age-adjusted ORs and 95% CIs for HER-2/neu-positive (+) and HER-2/neu-negative (-) breast cancer in relation to known and suspected risk factors among women <45 years of age in New Jersey, 1990–1992

	Controls (n = 462)	HER-2/neu+ cases (n = 159)	HER-2/neu- cases (n = 212)	Age-adjusted OR (95% CI)		Ratio of the ORs (95% CI)
				HER-2/neu+	HER-2/neu-	
Oral contraceptives						
OC use						
Never	168	48	76	1.0	1.0	
Ever	294	111	136	1.33 (0.90–1.96)	1.03 (0.73–1.44)	1.29 (0.83–2.00)
OC duration (years)^a						
<5	37	18	18	1.30 (0.85–2.00)	1.00 (0.69–1.47)	1.30 (0.80–2.11)
5–9	81	27	37	1.19 (0.69–2.05)	1.03 (0.64–1.66)	1.16 (0.63–2.14)
≥10	176	66	81	1.75 (0.91–3.36)	1.13 (0.60–2.13)	1.55 (0.73–3.27)
Age at first use of OCs (in years)^a						
<18	40	20	13	1.91 (1.01–3.59)	0.80 (0.40–1.59)	2.39 (1.08–5.30)
18–21	152	48	81	1.10 (0.70–1.74)	1.18 (0.80–1.73)	0.94 (0.56–1.56)
≥22	102	43	42	1.45 (0.90–2.35)	0.89 (0.56–1.39)	1.64 (0.94–2.87)
Number of years since first use^a						
<15	86	29	32	1.37 (0.77–2.44)	0.96 (0.56–1.63)	1.43 (0.73–2.81)
15–19	113	39	41	1.21 (0.74–1.97)	0.83 (0.53–1.32)	1.45 (0.81–2.57)
≥20	95	43	63	1.44 (0.86–2.41)	1.28 (0.81–2.00)	1.13 (0.64–1.98)
Number of years since last use^a						
<1	43	13	19	1.25 (0.60–2.59)	1.29 (0.68–2.45)	0.97 (0.42–2.22)
1–4	36	15	13	1.66 (0.68–2.45)	0.99 (0.49–2.01)	1.67 (0.72–3.90)
5–9	41	15	25	1.37 (0.70–2.70)	1.50 (0.84–2.66)	0.92 (0.44–1.92)
≥10	174	68	79	1.27 (0.82–1.96)	0.89 (0.60–1.31)	1.42 (0.87–2.34)
Reproductive factors						
Parous						
Ever	361	125	164	1.0	1.0	
Never	101	34	48	1.06 (0.67–1.66)	1.21 (0.81–1.82)	0.87 (0.52–1.46)
Age at first birth (each additional year)						
Children (number, among parous only)						
1	92	28	43	1.0	1.0	
2	161	59	82	1.18 (0.71–2.00)	1.05 (0.67–1.65)	1.03 (0.63–2.02)
≥3	108	38	39	1.13 (0.64–2.00)	0.73 (0.43–1.23)	1.55 (0.80–2.99)
Lactation (among parous women)						
Never	179	68	77	1.0	1.0	
Ever	177	57	86	0.92 (0.61–1.40)	1.29 (0.88–1.88)	0.72 (0.44–1.15)
Age at menarche (years)						
8–12	230	88	121	1.0	1.0	
≥13	232	71	91	0.80 (0.56–1.16)	0.73 (0.52–1.10)	1.10 (0.72–1.67)
Other factors						
Family history of breast cancer						
None	431	136	179	1.0	1.0	
First degree	31	23	33	2.27 (1.28–4.03)	2.44 (1.44–4.12)	0.93 (0.52–1.67)
Previous biopsy						
None	440	145	187	1.0	1.0	
≥1	22	14	25	1.89 (0.94–3.80)	2.52 (1.38–4.60)	0.75 (0.37–1.50)
Body size (body mass index)						
<23	171	64	87	1.0	1.0	
23–26	149	49	58	0.82 (0.53–1.29)	0.68 (0.45–1.02)	1.21 (0.73–2.01)
≥27	142	46	67	0.80 (0.51–1.26)	0.80 (0.54–1.19)	1.00 (0.61–1.66)
Physical activity (average of three time periods, relative units in quartiles)						
1	113	40	50	1.0	1.0	
2	119	41	55	1.00 (0.60–1.67)	1.09 (0.69–1.74)	0.92 (0.51–1.65)
3	115	36	54	0.92 (0.54–1.55)	1.13 (0.71–1.80)	0.81 (0.45–1.48)
4	115	42	53	1.08 (0.65–1.79)	1.12 (0.70–1.78)	0.97 (0.54–1.73)
Caloric intake (kilocalories, in quartiles)						
<1100	125	32	48	1.0	1.0	
1100–1450	113	41	59	1.42 (0.82–2.44)	1.53 (0.94–2.47)	0.93 (0.50–1.72)
1450–1830	112	32	51	1.12 (0.64–1.98)	1.34 (0.82–2.19)	0.84 (0.44–1.61)
≥1830	112	54	54	1.94 (1.15–3.28)	1.45 (0.89–2.37)	1.34 (0.73–2.46)
Education						
High school/Technical class	160	45	74	1.0	1.0	
Some college	116	42	55	1.32 (0.81–2.14)	1.06 (0.69–1.62)	1.24 (0.72–2.15)
College graduate	186	72	83	1.41 (0.92–2.17)	1.01 (0.69–1.49)	1.39 (0.85–2.27)
Race						
Whites	382	131	182	1.0	1.0	
Blacks	48	16	24	0.97 (0.53–1.77)	1.06 (0.63–1.78)	0.92 (0.47–1.80)
Asians and others	32	12	6	1.13 (0.56–2.26)	0.40 (0.17–0.99)	2.78 (1.02–7.61)

Table 2 Continued

	Controls (n = 462)	HER-2/neu+ cases (n = 159)	HER-2/neu- cases (n = 212)	Age-adjusted OR (95% CI)		Ratio of the ORs (95% CI)
				HER-2/neu+	HER-2/neu-	
Environmental factors						
Cigarette smoking						
Never	248	81	103	1.0	1.0	
Former	100	43	58	1.31 (0.84–2.02)	1.37 (0.92–2.05)	0.95 (0.58–1.55)
Current	113	35	51	0.95 (0.60–1.50)	1.11 (0.74–1.66)	0.86 (0.51–1.44)
Duration of smoking ^b (pack-years)						
<5	69	27	36	1.23 (0.74–2.06)	1.30 (0.81–2.07)	0.95 (0.53–1.70)
5–15	73	24	36	1.03 (0.60–1.72)	1.20 (0.76–1.91)	0.84 (0.47–1.53)
≥16	71	27	37	1.12 (0.67–1.87)	1.21 (0.76–1.92)	0.93 (0.52–1.65)
Age started smoking ^b (in years)						
8–16	66	15	21	0.71 (0.38–1.31)	0.78 (0.45–1.34)	0.91 (0.44–1.87)
16–17	55	21	33	1.18 (0.67–2.07)	1.48 (0.90–2.42)	0.80 (0.43–1.48)
≥18	92	42	55	1.37 (0.88–2.14)	1.41 (0.94–2.13)	0.97 (0.59–1.60)
Alcohol use (drinks/week)						
None	197	8	72	1.0	1.0	
<7	227	75	119	0.95 (0.65–1.40)	1.43 (1.0–2.04)	0.67 (0.43–1.03)
≥7	38	16	21	1.24 (0.65–2.36)	1.54 (0.84–2.80)	0.81 (0.39–1.67)
Electric blanket and mattress pad use						
Never	325	100	141	1.0	1.0	
Ever	137	59	71	1.38 (0.95–2.03)	1.17 (0.83–1.66)	1.18 (0.77–1.82)
Electric blanket and mattress pad use (in months)						
Never	325	100	141	1.0	1.0	
1–9	41	21	19	1.65 (0.93–2.92)	1.04 (0.58–1.86)	1.58 (0.81–3.10)
10–29	46	13	31	0.92 (0.48–1.77)	1.54 (0.94–2.54)	0.59 (0.30–1.19)
≥30	50	25	21	1.60 (0.94–2.73)	0.94 (0.54–1.63)	1.70 (0.90–3.21)

^a Relative to never users.^b Relative to never smokers.Table 3 Multivariate-adjusted^a ORs and 95% CIs for HER-2/neu-positive (+) and HER-2/neu-negative (–) breast cancer among women <45 years of age in New Jersey, 1990–1992

	HER-2/neu+ OR (95% CI)	HER-2/neu- OR (95% CI)	Ratio of the ORs (95% CI)
Age at first use of OCs (in years)			
Never users	1.0	1.0	
<18	1.89 (0.97–3.85)	0.97 (0.47–2.00)	1.99 (0.87–4.54)
18–21	1.09 (0.68–1.77)	1.24 (0.82–1.87)	0.88 (0.52–1.50)
22+	1.46 (0.88–2.42)	0.84 (0.52–1.36)	1.75 (0.98–3.12)
Body mass index			
<23	1.0	1.0	
23–26	0.80 (0.50–1.26)	0.71 (0.46–1.08)	1.13 (0.67–1.90)
27+	0.76 (0.47–1.23)	0.80 (0.52–1.22)	0.96 (0.56–1.62)
Age at first birth (for each additional year)	1.02 (0.97–1.06)	1.06 (1.02–1.10)	0.96 (0.91–1.01)
Parous			
Ever	1.0	1.0	
Never	1.03 (0.63–1.68)	1.27 (0.82–1.96)	0.81 (0.47–1.40)
Age at menarche			
8–12	1.0	1.0	
13+	0.73 (0.49–1.07)	0.64 (0.45–0.91)	1.14 (0.74–1.77)
Family history			
None	1.0	1.0	
First degree	2.13 (1.16–3.91)	2.25 (1.29–3.91)	0.95 (0.52–1.75)
Prior breast biopsy			
No	1.0	1.0	
Yes	2.08 (0.98–4.42)	2.65 (1.36–5.17)	0.78 (0.37–1.65)
Caloric intake (kilocalories, in quartiles)			
<1100	1.0	1.0	
1100–1450	1.44 (0.82–2.51)	1.52 (0.92–2.51)	0.95 (0.50–1.78)
1450–1830	1.02 (0.56–1.85)	1.32 (0.79–2.22)	0.77 (0.39–1.51)
≥1830	2.04 (1.19–3.52)	1.57 (0.95–2.64)	1.29 (0.69–2.42)

^a Adjusted for age and all other variables in the Table.

Table 4 Multivariate-adjusted^a ORs and 95% CIs for HER2/neu-positive (+) and HER2/neu-negative (-) breast cancer in relation to patterns of OC use by estrogen receptor status

	Controls (n = 462)	HER2/neu+ (n = 62)	HER2/neu- (n = 109)	HER2/neu+ OR (95% CI)	HER2/neu- OR (95% CI)	Ratio of the ORs (95% CI)
Among cases with ER+ tumors						
OC use						
Never	168	21	40	1.0	1.0	
Ever	294	41	69	0.99 (0.55–1.80)	0.93 (0.58–1.47)	1.07 (0.54–2.12)
OC duration (years) ^b						
<5	37	22	41	0.88 (0.44–1.74)	0.89 (0.53–1.50)	0.99 (0.45–2.16)
5–9	81	11	18	0.97 (0.42–2.25)	0.90 (0.47–1.73)	1.08 (0.41–2.82)
≥10	176	8	10	1.56 (0.59–4.13)	1.17 (0.52–2.67)	1.33 (0.43–4.13)
Age at first use of OC (in years) ^b						
<18	40	6	7	1.32 (0.44–3.99)	1.12 (0.44–2.84)	1.18 (0.32–4.35)
18–21	152	18	39	0.79 (0.39–1.62)	0.95 (0.56–1.62)	0.83 (0.37–1.86)
≥22	102	17	23	1.20 (0.58–2.51)	0.84 (0.46–1.53)	1.44 (0.61–3.39)
Number of years since first use ^b						
<15	86	10	13	0.91 (0.36–2.34)	0.78 (0.36–1.69)	1.17 (0.39–3.57)
15–19	113	15	20	0.77 (0.35–1.67)	0.67 (0.36–1.26)	1.14 (0.46–2.84)
≥20	95	16	36	1.39 (0.62–3.11)	1.36 (0.74–2.47)	1.03 (0.42–2.50)
Number of years since last use ^b						
<5	79	13	15	1.71 (0.74–3.95)	1.09 (0.52–2.26)	1.57 (0.58–4.28)
5–9	41	4	9	0.84 (0.26–2.74)	0.95 (0.40–2.23)	0.89 (0.24–3.34)
≥10	174	42	45	0.79 (0.39–1.57)	0.87 (0.51–1.46)	0.91 (0.42–1.99)
Among cases with ER- tumors						
OC use						
Never	168	13	22	1.0	1.0	
Ever	294	51	34	2.58 (1.31–5.10)	0.92 (0.49–1.71)	2.81 (1.18–6.67)
OC duration (years) ^b						
<5	37	32	20	2.89 (1.40–5.97)	0.95 (0.47–1.92)	3.04 (1.18–7.82)
5–9	81	12	9	1.90 (0.78–4.58)	0.82 (0.34–1.99)	2.31 (0.72–7.46)
≥10	76	7	5	2.92 (1.03–8.29)	1.02 (0.31–3.29)	2.87 (0.66–12.45)
Age at first use of OC (in years) ^b						
<18	40	11	3	3.72 (1.44–9.63)	0.69 (0.19–2.53)	5.37 (1.20–24.01)
18–21	152	20	23	2.01 (0.93–4.34)	1.29 (0.65–2.55)	1.55 (0.59–4.08)
≥22	102	20	8	2.95 (1.34–6.49)	0.50 (0.19–1.32)	5.92 (1.81–19.36)
Number of years since first use ^b						
<15	86	17	12	2.96 (1.27–6.92)	1.21 (0.51–2.87)	2.44 (0.80–7.46)
15–19	113	18	10	2.15 (0.95–4.85)	0.64 (0.26–1.55)	3.38 (1.08–10.56)
≥20	95	16	12	2.76 (1.12–6.77)	0.98 (0.41–2.35)	2.81 (0.87–9.11)
Number of years since last use ^b						
<5	79	13	10	2.76 (1.13–6.73)	1.35 (0.54–3.38)	2.05 (0.62–6.77)
5–9	41	10	9	3.36 (1.31–8.63)	1.52 (0.58–3.98)	2.21 (0.64–7.61)
≥10	174	28	5	2.29 (1.07–4.89)	0.64 (0.30–1.37)	3.58 (1.30–9.83)

^a Adjusted for age, body mass index, age at first birth, parity status, age at menarche, first-degree family history of breast cancer, prior breast biopsy, and caloric intake.

^b Relative to never user.

been clinically demonstrated that gene protein overexpression assessed by immunohistochemistry, which has been shown to be associated with gene amplification, is related to worse prognosis and differential treatment responsiveness and is correlated with high tumor grade, large size, positive nodal status, ductal infiltration, histological type, and low values of estrogen and progesterone receptors (5, 17, 18). Whether HER-2/neu status can help to identify etiologically distinct subgroups of breast cancer cases has received only limited attention (8, 9, 19).

In the study reported here, the OR for breast cancer in relation to OC use before age 18 was elevated among women with HER-2/neu-positive tumors and decreased among women with HER-2/neu-negative tumors. The 2-fold heterogeneity in the ORs was statistically significant in age-adjusted models but not in multivariate-adjusted models. With further stratification by ER status, the ratio of the OR increased to 5-fold among women with tumors that were ER-, which reflects over a 3-fold increase in risk among women with HER-2/neu-positive

tumors and a 31% decrease among women with HER-2/neu-negative tumors. There was little or no heterogeneity in relation to other risk factors, including age at first birth.

Interpretation of these results must be considered in light of the limitations and strengths of our study. The study sample was population based, which would decrease the likelihood of ascertainment bias. Also, there was little difference in the distribution of known and suspected risk factors between cases with and without archived tumor tissue available for our laboratory assays (11). In addition, the structured interview was developed to specifically assess OC use among young women and was administered by trained interviewers using a reproductive calendar to enhance recall (3).

Drawbacks to consider include the possibility that chance may account for some of the pattern of findings in our study. However, the variable for which our results are strongest is the one for which there is empirical support from previous research. Thus, our data confirm and expand upon an earlier observation of a large

increase in breast cancer risk in relation to OC use at an early age and HER-2/*neu* status that was reported previously by Olsson *et al.* (8) in 1991. However, we did not corroborate the earlier finding by Treurniet *et al.* (9) in which a 4-fold increase in risk in relation to age at first birth or breastfeeding was noted among women with HER-2/*neu*-positive tumors. A third study (19) found no association between HER-2/*neu* status in women with node-negative breast cancer and four risk factors examined, menstrual status, family history of breast cancer, age at first pregnancy, and number of pregnancies. Generalizability for all of these studies was hindered by a very select group of study subjects. In our study, our larger, population-based sample size permitted a more thorough and generalizable exploration of reproductive factors, as well as other risk factors for breast cancer, in relation to HER-2/*neu* status.

For a large epidemiological study, assessment of HER-2/*neu* protein overexpression by immunohistochemistry is a more cost-efficient method than assessing amplification or specific mutations. However, use of immunohistochemistry may have resulted in some misclassification of HER-2/*neu* status, although the correlation between amplification and overexpression is high (5, 17, 20). Olsson *et al.* (8) determined gene amplification and reported similar findings to those shown here. Also, in our population-based sample of young women <45 years of age, 43.9% of breast cancer cases showed evidence of HER-2/*neu* overexpression in the archived tumor tissue, which is within the 18–50% range reported by others (6, 9, 19, 21, 22).

In a recent large pooled analysis, the risk of breast cancer was found to be modestly elevated in relation to OC use (1), particularly long-term use, recent use, or use at an early age. However, there appears to be some heterogeneity in risk among certain subgroups, with the magnitude of risk higher among black women or among women with a family history of breast cancer (2, 3). Our study, however, had few nonwhite subjects to explore possible heterogeneity in the association between OC use and breast cancer risk stratified by race and with the cases categorized by HER-2/*neu* status.

Olsson (23) hypothesized that because both early age at first use of OCs and HER-2/*neu* amplification were associated with a shared tumor biology (larger tumor size, advanced tumor stage, absence of steroid receptors, a higher rate of proliferation, and high tumor grade), it is possible that the exposure and gene amplification were related. In addition, the strong association between patterns of OC use and HER-2/*neu* positivity among women with ER-negative tumors noted in our study may be biologically plausible. Because antiestrogens lower HER-2/*neu* levels in ER-tumors (24), it is plausible that estrogens stimulate HER-2/*neu* in these tumors. Thus, variation in the distribution of ER status in populations could result in heterogeneous results when examining the relation between OCs and HER-2/*neu*+ breast cancer. Thus, failure to consider HER-2/*neu* and ER status could mask any strong, underlying association between OCs and breast cancer risk.

In summary, this study of young women confirms the association first noted by Olsson *et al.* (8) of a heterogeneity of effect for breast cancer in relation to OCs when cases are stratified by HER-2/*neu* status. This study is the first to report a significant 3-fold increase in risk associated with oral contraceptive use among young women with tumors that are HER-2/*neu* positive and ER negative. Further corroboration by others is needed to examine these provocative associations among younger and older women with breast cancer.

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