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Use of Four Biomarkers to Evaluate the Risk of Breast Cancer Subtypes in the Women's Contraceptive and Reproductive Experiences Study

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Abstract

Epidemiological studies have suggested that some hormone-related breast cancer risk factors differentially influence risk of breast cancer subtypes defined by estrogen receptor (ER) and progesterone receptor (PR) expression status in tumor tissue. However, it remains unclear whether human epidermal growth factor receptor-2 (HER2) and p53 protein (p53) expression status in tumor tissue further differentiate these exposure-risk-group associations. We evaluated the associations of oral contraceptive (OC) use and reproductive factors with incident invasive breast cancer subtypes among 1197 population-based cases and 2015 controls from the Los Angeles County or Detroit components of the Women's Contraceptive and Reproductive Experiences Study. We used multivariable polychotomous unconditional logistic regression methods to conduct case-control comparisons by ER/PR/HER2/p53 status. We found that OC use was not associated with any breast

cancer subtype defined by ER/PR/HER2/p53, except for a 2.9-fold increased risk for triple negative (ER-/PR-/HER2-) tumors among older women (ages 45–64 years) who started OC use before age 18. Parity was associated with decreased risk of luminal A (ER+ or PR+, HER2-), luminal B (ER+ or PR+, HER2+), and ER-/PR-/HER2+ tumors. Age at first full-term pregnancy was positively associated with luminal A tumors among older women. Neither of these reproductive factors was associated with triple negative tumors. Long duration of breastfeeding lowered risk of triple negative and luminal A tumors. No further differential risk patterns were noted when p53 was also considered. These results provide evidence supporting a difference in some hormone-related risk factor profiles between triple negative and other breast cancer subtypes defined by ER/PR/HER2.

Keywords

ER/PR/HER2/p53; breast cancer; hormone-related factors; oral contraceptive; reproductive factors

Introduction

Epidemiological studies have suggested that some hormone-related breast cancer risk factors differentially influence breast cancer risk by estrogen receptor (ER) and progesterone receptor (PR) expression status in tumor tissue (1-3). However, it remains unclear whether these associations differ further by the expression status of human epidermal growth factor receptor-2 (HER2) and p53 protein (p53).

HER2, a transmembrane tyrosine kinase receptor protein, is normally involved in the signal transduction pathways that lead to cell growth and differentiation (4,5). HER2 is overexpressed in approximately 15–25% of breast carcinoma specimens (6-8). A number of epidemiological studies have classified breast cancers as triple negative (ER-/PR-/HER2-), luminal A (ER+ or PR+ plus HER2-), luminal B (ER+ or PR+ plus HER2+), and ER-/PR-/HER2+ subtype (7,9-11). Triple negative breast cancers, which are typically more aggressive and have poorer prognosis than other subtypes, are often characterized by a basal-like molecular profile (12, 13). Basal-like breast cancers, as defined by gene expression microarray analysis, exhibit overexpression of a number of genes in the p21 (*CDKN1A*) pathway that play a critical role in cell proliferation and DNA replication (MCM3, MCM4, MCM7 and MAD2L1), whereas luminal A tumors have been associated with the ER signaling pathway (14,15). Therefore, one might expect that associations with hormone-related breast cancer risk factors would differ between triple negative breast cancer, a proxy for basal-like breast cancer, and other breast cancer subtypes. Thus far, epidemiological data on this topic are inconsistent (9,11,16).

p53, a tumor suppressor gene, inhibits the proliferation of abnormal cells (17). p53 gene mutations that occur in approximately 15–35% of breast carcinoma specimens (18-20) may cause loss of tumor suppressor function and gain of oncogenic activity (21). Overexpression of p53 protein in tumor tissue is associated with the presence of the mutations, especially missense mutations, in the p53 gene (21). It has been unclear whether the overexpression status of p53 protein would modify the associations between hormone-related breast cancer risk factors and breast cancer risk. Three previous epidemiological studies have examined the effects of oral contraceptive (OC) use and reproductive factors by p53 status, but none has concurrently considered the expression status of ER, PR, and HER2 (22-24).

In this study, we examined whether OC use and reproductive factors differentially influenced risk for triple negative, luminal A, luminal B, and ER-/PR-/HER2+ breast cancer. Further, we explored whether any differences in the associations of OC use or reproductive factors with triple negative and luminal A breast cancer, two common subtypes, were confined to younger

(<45 years) or older (≥ 45 years) women. We also evaluated whether p53 expression status modified any observed differences between these two subtypes.

Materials and Methods

Subject Identification

The Women's Contraceptive and Reproductive Experiences (CARE) Study is a population-based case-control study of invasive breast cancer conducted among United States-born White and African-American women ages 35–64 years who resided in one of five areas of the United States (Atlanta, Detroit, Los Angeles [LA] County, Philadelphia or Seattle) (25). This current study is restricted to women who participated in the LA County or the Detroit Metropolitan Area, which includes Wayne, Oakland and MacComb counties.

Case participants in the Women's CARE Study had no prior diagnosis of invasive or *in situ* breast cancer and were diagnosed with their first primary invasive breast cancer between June 1994 and August 1998. Control participants were women with no history of invasive or *in situ* breast cancer who were identified by random digit dialing. Control participants were frequency matched to the expected distribution of cases in strata defined by 5 year age groups, ethnicity (White or African American) and residence located in the same geographic (study) region. The Women's CARE Study recruited 1921 cases (1072 White and 849 African American) and 2034 control participants (1161 White and 873 African American) from LA and Detroit. The interview response rates were 73.3% for cases in LA, 73.7% for controls in LA, 74.7% for cases in Detroit, and 74.1% for controls in Detroit.

The Women's CARE Study collected demographic characteristics, detailed information about OC use, complete histories of menstrual and reproductive factors, family history of breast cancer, and information pertaining to other factors from each participant during an in-person interview. Information was recorded up to a predetermined reference date for each participant, the date of diagnosis for case patients and the date of initial telephone screening of the household for control subjects. The detailed information on OC use was collected by using a mixture of recall and recognition techniques (structured questionnaire, response cards, color pictures of all OC preparations marketed in the United States, and a life-events calendar). The detailed data regarding OC use and breast cancer risk (26), and combined effect of OC use and hormone replacement therapy on breast cancer risk (27) appear elsewhere. All participants provided written informed consent. The study protocol was approved by the federally approved Institutional Review Boards at participating institutions.

Assessment of Biomarkers

Paraffin-embedded tumor blocks were obtained for 1333 cases (LA: 919, Detroit: 414), approximately 80% of those requested. Tumor blocks were carefully reviewed and evaluated in Dr. Press' laboratory at the University of Southern California (referred to as the centralized laboratory). This laboratory was among the first to validate use of monoclonal estrogen receptor antibodies (28-32) and monoclonal progesterone receptor antibodies (33-35) for localization of ER and PR in tissue sections by immunohistochemistry (IHC), and to subsequently adapt these methods to analysis of archival tissues (36,37).

We excluded 127 case samples because tumor blocks contained only carcinoma *in situ* ($n = 56$) or no tumor tissue ($n = 46$); were hematoxylin-and-eosin stained tissue sections ($n = 8$), had insufficient tissue for assay ($n = 3$); or had other problems ($n = 14$). We successfully determined ER, PR, HER2, and p53 expression status for 1206 case subjects (LA: 839, Detroit: 367) in the centralized laboratory. In one previous study of the association between percent mammographic density and subtypes of breast cancer, we reported on 352 of the LA cases for

whom we also had mammograms (7). In a prior report comparing ER/PR status from the centralized pathology laboratory to the classification obtained from a population-based cancer surveillance registry, we reported on concordance for ER and PR status between the two sources for 919 cases (38).

The status of ER and PR was determined using previously published IHC methods (31,36). Immunostaining results for ER and PR expression were interpreted in a blinded fashion and scored semiquantitatively on the basis of the visually estimated percentage of positively stained tumor cell nuclei. The intensity of nuclear staining was scored for individual tumor cell nuclei as negative (–)/no staining, plus one (+1)/weak intensity, plus two (+2)/intermediate intensity, or plus three (+3)/strong intensity. A minimum of 100 tumor cells were scored with the percentage of tumor cell nuclei in each category recorded. In this paper, an overall score of $\geq 1\%$ immunostained tumor cell nuclei was considered positive for ER or PR status.

HER2 expression status was determined by IHC using the 10H8 monoclonal antibody (8,39) to assess HER2 membrane protein immunostaining. No immunostaining (0) or weak (1+) membrane immunostaining was considered low HER2 expression (HER2–). Moderate (2+) or strong membrane immunostaining (3+) was considered HER2 overexpression (HER2+).

The expression status of p53 was determined by IHC using the monoclonal mouse antibodies DO7 (Oncogene Science, Inc. Cambridge, MA) and BP 53–12–1 (Biogenex) to measure p53 nuclear protein immunostaining. Based on previous study findings comparing p53 mutations in exons 2–11 with p53 protein expression levels (40,41) any nuclear staining for p53 protein was deemed positive (42).

Statistical Analysis

We used *t* tests to evaluate differences in continuous variables and Pearson χ^2 tests to evaluate differences in the frequency distributions of categorical variables comparing case patients with control subjects.

We assessed the association of triple negative, ER–/PR–/HER2+, luminal A, and luminal B breast cancer, with the following factors: OC use (never, ever), duration of OC use, age at first OC use, duration between menarche and first OC use, time since last OC use to reference date, number of full-term (greater than 26-week gestation) pregnancies, age at first full-term pregnancy (defined for each woman as the age at which that pregnancy ended), and duration of breastfeeding. We estimated odd ratios (ORs) and corresponding 95% confidence intervals (95% CIs) using multivariable polychotomous unconditional logistic regression for case-control comparisons.

Tests for trend were conducted by fitting ordinal values corresponding to exposure categories and testing whether the slope coefficient differed from zero. We also conducted Wald chi-square tests for homogeneity of the associations with OC use or reproductive factors across breast cancer subtypes by fitting a multivariable polychotomous unconditional logistic regression model for dichotomous or ordinal variables.

We included the following factors, selected *a priori*, as potential confounders in all multivariable models: study site (LA or Detroit), race (white or African American), education as a proxy for social economic status (high school or lower level of education, technical school or some college, or college graduate), age (35–39, 40–44, 45–49, 50–54, 55–59, or 60–64 years), family history of breast cancer (first-degree [mother, sister, or daughter]; no first-degree family history including 4% of participants with uncertain answers), age at menarche (≤ 11 , 12, 13, >13), menopausal status (premenopausal, postmenopausal, unknown), and body mass index (BMI) 5 years before the reference date (continuous variable, kg/m²). We also included

parity (nulligravid, pregnant but no full-term pregnancy, or parity 1, 2, 3, ≥ 4) in models as a potential confounder when it was not the exposure of interest. When parity was the exposure of interest, we chose women who had never been pregnant as our reference group and treated women who had been pregnant but had never carried to term as a separate group that was excluded when testing for trend across categories of parity. When restricting analyses to parous women, a single model was fit to assess the joint effects of age at first full-term pregnancy and breastfeeding duration.

Using two major subtypes - triple negative and luminal A breast cancer, we explored whether any differences in the associations of OC use or reproductive factors were confined to younger (<45 years) or older (≥ 45 years) women. We also evaluated whether p53 expression status modified any observed differences between these two subtypes.

We excluded 19 control subjects and 9 case patients from the analyses who were missing information on OC use (6 controls, 4 cases), BMI (9 controls, 4 cases), or parity (4 controls, 1 case). This resulted in 2015 controls and 1197 cases available for the current analysis. Of the 1197 cases, 28.0% were triple negative; 8.1% were ER-/PR-/HER2+, 53.9% were luminal A, and 10.0% were luminal B breast cancer. Among 335 cases with triple negative breast cancer, 45.1% were p53+ while among 645 cases with luminal A breast cancer, 29.2% were p53+ breast cancer.

In reporting the results of trend tests or homogeneity tests, we considered a two-sided P value less than 0.05 as statistically significant. All analyses were performed using the SAS statistical package (Version 9.2, SAS Institute, Cary, NC).

Results

Characteristics of Controls and Cases

As previously reported among all participants of the Women's CARE Study (26,43,44), there were no differences in the OC use characteristics between case and control patients; in contrast, case participants were more likely to have a first-degree breast cancer family history ($P_{\chi^2} < 0.0001$), to have fewer full-term pregnancies ($P_{t-test} = 0.008$), and to have never breastfed ($P_{\chi^2} = 0.02$) compared to control participants (Supplementary Table 1).

Associations with Subtypes Defined by ER/PR/HER2

Examination of multiple aspects of OC use (ever use, duration of use, age at first use, interval between age at menarche and age at first OC use, time since last OC use to reference date) showed that OC use was not associated with any subtype defined by ER, PR, and HER2 (Table 1).

Number of full-term pregnancies was not associated with risk of triple negative breast cancer ($P_{trend} = 0.84$), but was inversely associated with risk of the other three subtypes (all $P_{trend} < 0.05$, Table 2). The difference in trends across the four subtypes of breast cancer was marginally statistically significant (homogeneity test: $P = 0.06$).

Among parous women, older age at first full-term pregnancy was not associated with any subtype defined by ER, PR, and HER2 (Table 2). A statistically significant protective effect of longer duration of breastfeeding was observed for the two major subtypes, triple negative ($P_{trend} = 0.03$) and luminal A ($P_{trend} = 0.004$).

Associations with Triple Negative and Luminal A Subtype by Age

Analyses of triple negative and luminal A subtype within age strata (younger women: 35–44 years and older women: 45–64 years) showed that older women who initiated OC use before age 18 years had a 2.9-fold increased risk for triple negative tumors compared to women in the same age group who had never used OCs (OR = 2.87, 95% CI = 1.44–5.74); no such association for luminal A cancers was observed among older women (OR = 1.36, 95% CI = 0.75–2.48) or for either subtype among younger women (Table 3).

Among older women, those who had their first full-term pregnancies at or after age 25 years appeared to have a higher risk of luminal A breast cancer than those who had their first full-term pregnancies prior to age 20 years, ($P_{trend} = 0.04$); however, the risk estimates declined in the oldest age group and the CI for the risk estimate for women with a first full-term pregnancy at or after age 30 years included 1.0 (OR = 1.22, 95% CI = 0.71–2.09, Table 4).

Similar to the analyses across all age groups, risk of luminal A cancers decreased with an increasing number of full-term pregnancies for both younger and older women ($P_{trend} = 0.09$ and $P_{trend} = 0.006$, respectively, Table 4). Increasing duration of breastfeeding was inversely associated with risk for both subtypes in younger and older women.

Associations with Triple Negative and Luminal A Subtype by p53

We evaluated whether any associations between OC use or reproductive factors and breast cancer risk differed by p53 status alone and did not observe any statistically significant differences in these associations (results not shown). Subclassification of the triple negative and luminal A subtypes by p53 status did not further differentiate the associations of these subtypes with OC use (Table 5) or reproductive history (Table 6). Although we found that number of full-term pregnancies was statistically significantly associated with luminal A tumors without p53 overexpression ($P_{trend} = 0.002$) and that duration of breastfeeding was statistically significantly associated with both triple negative ($P_{trend} = 0.03$) and luminal A tumors ($P_{trend} = 0.008$) without p53 overexpression, these associations did not differ statistically from those for the corresponding subtypes where p53 was overexpressed (all homogeneity tests $P > 0.20$, Table 6).

Discussion

A recent study reported that, among women ages 20 to 45 years, use of OCs for at least one year was associated with a 2.5-fold increased risk for triple-negative breast cancer (45); this association was not observed in another population-based case-control study that included women ages 20–74 years (16). We previously reported that OC use was not associated with breast cancer risk among participants in the Women's CARE Study (26). In the current analysis, OC use was not associated with any subtype except for a 2.9-fold increase in risk for triple negative breast cancer among older women (ages 45–64 years) who started OC use before age 18. All of these older triple negative breast cancer patients began OC use before 1971 when most OCs contained high doses of synthetic estrogen. In contrast, exposure to OCs at an early age in older women did not significantly increase the risk of luminal A breast cancer in our data.

The association with OC use at a young age among older women that is restricted to triple negative breast cancer can be viewed as inconsistent with the hypothesis that hormone-related risk factors act through estrogen mediated by its receptor (46). However, there is evidence that ER+ progenitor cells produce paracrine signals when exposed to estrogen, which cause the proliferation of nearby ER– cells (47). Furthermore, the Breast and Prostate Cancer Cohort Consortium showed that 2 common haplotypes of the 17 β -hydroxysteroid dehydrogenase 1

gene (*HSD17B1*) are associated with risk of ER– but not ER+ breast cancer (48). This gene encodes 17HSD1, which affects the conversion of estrone to estradiol. The increase in risk for triple negative invasive breast cancer suggests that early high dose OC initiation may increase tumor aggressiveness.

We previously reported that multiparity and early age at first birth were associated with reduced relative risk of ER+/PR+, but not of ER–/PR– tumors, whereas duration of breastfeeding was associated with lower relative risk of both receptor-positive and receptor-negative breast cancer among all cases (who had ER/PR status from SEER) and control participants of the Women's CARE Study (49). Consistent with our previous findings, in the current analysis we found that parity was inversely associated with risk for luminal subtypes. Age at first full-term pregnancy was positively associated with luminal A tumors among older women (ages 45–64 years). Neither parity nor age at first full-term pregnancy was associated with triple negative tumors. These findings, and particularly the inverse association of parity with all subtypes except triple negative breast cancer suggest that some hormone-related risk factor profiles differ between triple negative and other breast cancer subtypes. In addition, the lack of heterogeneity in the effect of breastfeeding in our data suggests that there are different pathways involving in the association between breastfeeding and the risk of breast cancer, such as paracrine signals produced by ER+ progenitors described previously (47).

Consistent with our findings, a pooled analysis of two population-based case-control studies conducted in Western Washington State found that nulliparity was marginally associated with an increased risk of ER+ breast cancer, but was not associated with triple-negative breast cancer, while breastfeeding protected against both ER+ and triple-negative subtypes (9). Our results are also consistent with those from a population-based case-control study conducted in Poland which found that parity was inversely associated with the luminal A subtype, but not with the basal-like subtype (triple-negative plus the expression of HER-1 and/or cytokeratin 5) (11). Our results are not consistent with those from a population-based case-control study conducted in North Carolina in which parity and early age at first full-term pregnancy were associated with an increased risk of basal-like (ER–, PR–, HER2–, HER-1+ and/or cytokeratin 5/6 positive) breast cancer and long duration of breastfeeding was associated with reduced risk of basal-like, but not luminal A breast cancer (16). These inconsistencies could be due to some important differences between the North Carolina study and ours, such as the participants' age distribution (ranges: 20–74 vs. 35–64 years), the breast cancer case patients' stage (*in situ* and invasive vs. only invasive), control subjects' participant rates (57% vs. 74%), analysis approach (each subtype separately compared with control group using unconditional logistic regression models vs. all subtypes simultaneously compared to control group using unconditional polychotomous logistic regression models), and different reference groups and potential confounders considered when testing the effects of age at first full-term pregnancy and breastfeeding (reference group including nulliparous women vs. analysis restricted to parous women; unadjusted number of full-term pregnancy vs. adjusted).

To our knowledge, this study is the first to examine whether p53 status modifies the association of OC use and reproductive factors with risk of invasive triple negative or luminal A breast cancer. p53 gene mutations may cause loss of tumor suppressor function and gain of oncogenic activity (21). Approximately 69% of p53 IHC positive cancers have p53 gene mutations (41). While 92% of missense p53 gene mutations are associated with p53 IHC-positivity, only 45% of p53 non-sense or other gene mutations are associated with IHC-positivity (41). Since the vast majority of p53 gene mutations are missense mutations, p53 protein overexpression and p53 gene mutation are highly correlated. We speculated that this would result in a stronger association between hormone-related risk factors and the risk of breast cancer with p53 protein overexpression since hormones drive cell proliferation and therefore, increase the probability for the accumulation of random genetic errors (50). However, our data did not show any

differences in associations of OC use or reproductive factors when triple negative tumors and luminal A tumors were further classified by p53 overexpression status. We did observe that number of full-term pregnancies was statistically significantly associated with luminal A tumors without p53 overexpression and that duration of breastfeeding was statistically significantly associated with both triple negative and luminal A tumors without p53 overexpression. These findings may have resulted from the larger sample sizes for tumor subtypes lacking p53 overexpression than subtypes that overexpress p53.

Where p53 has previously been examined alone, reproductive factor associations did not differ by p53 status (22-24), which is consistent with our results. One previous study showed that longer duration of OC use was more strongly associated with an increase in the risk of p53+ than p53- breast cancer among women at ages 20-54 (24). Another study found this difference in the association with OC use between p53- and p53+ tumors existed among younger (age < 45 years), but not older women (22). In a third study, OC use was not associated with either p53+ or p53- tumors (23).

This study has several limitations. We obtained paraffin-embedded tissue for 80% of the samples requested; however, we did not request tissue for all eligible case patients because of funding constraints. We compared demographic characteristics, family history of breast cancer, aspects of OC use, reproductive factors, tumor size, and tumor stage between our eligible case patients with and without known ER, PR, HER2, and p53 status from the centralized laboratory (Results not shown). Case patients with information on these four biomarkers were more likely to have been diagnosed in LA than in Detroit, to have been better educated, and to have had larger tumors than those without this information. Parous case patients with known ER, PR, HER2, and p53 status had, on average, breast fed 1.4 months longer, but no statistically significant differences were detected for other factors examined. The difference in duration of breastfeeding could lead us to underestimate any protective effect of breastfeeding on overall breast cancer risk in the patients with biomarker results, but it is unlikely that this bias would differ across tumor subtypes. Further, although previous research shows that p53 protein expression and p53 mutation status determined by fluorescent in situ hybridization analysis are strongly correlated, assessment of p53 protein expression by IHC may misclassify some tumors.

In conclusion, our results support the contention that some hormone-related risk factor profiles differ between triple negative and other breast cancer subtypes defined by ER, PR, and HER2 status, but no further consistent differences are noted when p53 is also considered.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Multivariable adjusted* odds ratio (OR) and 95% confidence interval (CI) for invasive breast cancer associated with oral contraceptive use by expression status of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2)

		No. controls		No. cases			OR (95% CI)			
		Triple negative	ER-/PR-/HER2+	Luminal A	Luminal B	Triple negative	ER-/PR-/HER2+	Luminal A	Luminal B	
Oral contraceptive use										
Never use	410	59	19	155	21	Reference	Reference	Reference	Reference	
Ever use	1605	276	78	490	99	1.00 (0.72–1.39)	1.21 (0.69–2.11)	0.93 (0.74–1.17)	1.23 (0.73–2.10)	
Test for homogeneity <i>P</i> -value						0.65				
Duration of use, years										
<1	358	57	18	116	21	0.94 (0.63–1.42)	1.22 (0.61–2.43)	0.98 (0.73–1.32)	1.17 (0.62–2.24)	
1–4	540	86	25	179	29	0.93 (0.63–1.36)	1.15 (0.59–2.23)	1.04 (0.79–1.37)	1.12 (0.60–2.07)	
5–9	396	75	13	101	29	1.12 (0.75–1.66)	0.86 (0.40–1.85)	0.78 (0.57–1.06)	1.50 (0.80–2.78)	
≥10	311	58	22	94	20	1.06 (0.70–1.61)	1.59 (0.81–3.10)	0.87 (0.63–1.19)	1.20 (0.62–2.32)	
Trend <i>p</i> -value						0.48	0.39	0.14	0.39	
Test for homogeneity of trends <i>P</i> -value						0.18				
Age at first use, years										
≥25	399	54	21	136	22	0.99 (0.66–1.48)	1.26 (0.66–2.42)	0.91 (0.69–1.20)	1.07 (0.57–2.00)	
20–24	569	89	28	194	34	0.96 (0.65–1.42)	1.31 (0.68–2.55)	1.03 (0.78–1.36)	1.26 (0.68–2.34)	
18–19	332	62	17	80	26	1.00 (0.65–1.55)	1.13 (0.53–2.42)	0.78 (0.55–1.11)	1.66 (0.84–3.27)	
<18	305	71	12	80	17	1.12 (0.72–1.74)	0.85 (0.36–2.00)	0.89 (0.62–1.28)	1.23 (0.57–2.64)	
Trend <i>p</i> -value						0.61	0.80	0.36	0.33	
Test for homogeneity of trends <i>P</i> -value						0.49				
Duration between menarche and first use, years										
<5	264	58	12	62	13	1.01 (0.63–1.60)	0.88 (0.37–2.11)	0.78 (0.53–1.16)	1.11 (0.49–2.53)	
5–9	662	119	41	188	53	0.96 (0.66–1.40)	1.61 (0.84–3.10)	0.87 (0.65–1.16)	1.72 (0.94–3.14)	
10–14	437	64	13	152	21	0.99 (0.66–1.47)	0.85 (0.40–1.79)	1.01 (0.77–1.34)	1.02 (0.53–1.94)	
≥15	242	35	12	88	12	1.09 (0.69–1.72)	1.31 (0.61–2.78)	0.94 (0.68–1.29)	1.00 (0.47–2.09)	
Trend <i>p</i> -value						0.89	0.55	0.99	0.90	
Test for homogeneity of trends <i>P</i> -value						0.96				

	No. controls	No. cases				OR (95% CI)			
		Triple negative	ER-/PR-/HER2+	Luminal A	Luminal B	Triple negative	ER-/PR-/HER2+	Luminal A	Luminal B
Time since last use, years									
<5	165	29	9	44	10	0.84 (0.50–1.44)	1.57 (0.62–4.01)	0.78 (0.51–1.20)	1.13 (0.48–2.69)
5–9	116	27	7	39	8	1.13 (0.66–1.95)	1.63 (0.61–4.35)	1.10 (0.70–1.72)	1.40 (0.56–3.49)
10–14	219	45	13	68	14	1.04 (0.65–1.65)	1.44 (0.64–3.23)	0.99 (0.69–1.43)	1.27 (0.59–2.70)
15–19	327	51	11	86	25	0.87 (0.56–1.34)	0.92 (0.41–2.07)	0.83 (0.60–1.15)	1.62 (0.85–3.11)
≥20	778	124	38	253	42	1.05 (0.74–1.48)	1.18 (0.65–2.14)	0.95 (0.74–1.21)	1.12 (0.64–1.97)
Trend <i>p</i> -value						0.80	0.95	0.71	0.55
Test for homogeneity of trends <i>P</i> -value									
0.89									

Note: Triple negative = ER-/PR-/HER2-, Luminal A = ER+ or PR+ plus HER2-, Luminal B = ER+ or PR+ plus HER2+.

* Adjusted for study site, race, education, age, family history of breast cancer, age at menarche, menopausal status, body mass index, parity.

Table 2

Multivariable adjusted* odds ratio (OR) and 95% confidence interval (CI) for invasive breast cancer associated with reproductive history by expression status of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2)

No. controls		No. cases				OR (95% CI)			
		Triple negative	ER-/PR-/HER2+	Luminal A	Luminal B	Triple negative	ER-/PR-/HER2+	Luminal A	Luminal B
Number of full-term pregnancies									
Never pregnant	176	28	9	83	14	Reference	Reference	Reference	Reference
1	314	55	22	109	29	0.98 (0.60–1.62)	1.07 (0.47–2.41)	0.80 (0.56–1.14)	1.17 (0.59–2.31)
2	574	106	27	187	34	1.12 (0.71–1.78)	0.75 (0.34–1.64)	0.73 (0.53–1.00)	0.79 (0.41–1.52)
3	391	56	18	115	18	0.91 (0.55–1.50)	0.69 (0.30–1.60)	0.65 (0.46–0.92)	0.61 (0.29–1.29)
≥4	402	63	15	104	18	1.00 (0.60–1.67)	0.47 (0.19–1.15)	0.55 (0.38–0.79)	0.55 (0.26–1.20)
Trend <i>p</i> -value						0.84	0.02	0.0006	0.01
Test for homogeneity of trends <i>P</i> -value									
						0.06			
Only non-full-term pregnancy	158	27	6	47	7	0.91 (0.51–1.63)	0.67 (0.23–1.94)	0.66 (0.43–1.00)	0.55 (0.22–1.42)
Age at first full-term pregnancy [†] , years									
≤19	583	103	26	139	28	Reference	Reference	Reference	Reference
20–24	614	91	34	195	42	0.92 (0.66–1.27)	1.34 (0.76–2.34)	1.20 (0.92–1.56)	1.42 (0.84–2.41)
25–29	279	45	14	124	18	1.01 (0.66–1.55)	1.23 (0.59–2.58)	1.71 (1.23–2.36)	1.15 (0.58–2.28)
≥30	205	41	8	57	11	1.32 (0.80–2.17)	0.91 (0.35–2.36)	1.03 (0.67–1.57)	0.78 (0.33–1.85)
Trend <i>p</i> -value						0.40	0.92	0.15	0.71
Test for homogeneity of trends <i>P</i> -value									
						0.74			
Duration of breastfeeding [†] , months									
Never	712	131	43	240	45	Reference	Reference	Reference	Reference
<1	202	38	5	65	18	1.03 (0.69–1.54)	0.45 (0.17–1.17)	0.91 (0.66–1.26)	1.34 (0.74–2.41)
1–6	334	54	14	92	15	0.80 (0.56–1.15)	0.79 (0.41–1.51)	0.70 (0.53–0.94)	0.67 (0.36–1.25)
7–23	302	36	13	91	14	0.58 (0.38–0.89)	0.86 (0.44–1.68)	0.78 (0.58–1.05)	0.72 (0.37–1.38)
≥24	131	21	7	27	7	0.80 (0.47–1.36)	1.31 (0.53–3.21)	0.55 (0.34–0.88)	0.91 (0.38–2.20)
Trend <i>p</i> -value						0.03	0.96	0.004	0.27

	No. controls	No. cases				OR (95% CI)			
		Triple negative	ER-/PR-/HER2+	Luminal A	Luminal B	Triple negative	ER-/PR-/HER2+	Luminal A	Luminal B
Test for homogeneity of trends <i>P</i> -value									
0.68									

Note: Triple negative = ER-/PR-/HER2-, Luminal A = ER+ or PR+ plus HER2-, Luminal B = ER+ or PR+ plus HER2+.

* Adjusted for study site, race, education, age, family history of breast cancer, age at menarche, menopausal status, body mass index.

† Also adjusted for parity and mutually adjusted for age at first full-term pregnancy and duration of breastfeeding among parous women.

Table 3

Multivariable adjusted* odds ratio (OR) and 95% confidence interval (CI) for triple negative and luminal A breast cancer associated with oral contraceptive use by age

		Younger (age 35–44 years)					Older (age 45–64 years)				
	No. controls	No. triple negative	No. luminal A	OR (95% CI) of triple negative	OR (95% CI) of luminal A	No. controls	No. triple negative	No. luminal A	OR (95% CI) of triple negative	OR (95% CI) of luminal A	
Oral contraceptive use											
Never use	74	21	22	Reference	Reference	336	38	133	Reference	Reference	
Ever use	647	133	175	0.72 (0.42–1.24)	0.97 (0.57–1.64)	958	143	315	1.25 (0.83–1.88)	0.92 (0.71–1.20)	
Test for homogeneity <i>P</i> -value				0.38					0.18		
Duration of use, years											
<1	135	18	33	0.44 (0.22–0.90)	0.91 (0.48–1.72)	223	39	83	1.49 (0.91–2.45)	1.03 (0.73–1.45)	
1–4	222	42	72	0.66 (0.36–1.22)	1.20 (0.68–2.11)	318	44	107	1.15 (0.70–1.89)	0.98 (0.70–1.35)	
5–9	158	39	35	0.87 (0.46–1.62)	0.78 (0.42–1.45)	238	36	66	1.26 (0.75–2.12)	0.78 (0.54–1.12)	
≥10	132	34	35	0.92 (0.49–1.73)	0.90 (0.49–1.68)	179	24	59	1.06 (0.60–1.88)	0.85 (0.58–1.24)	
Trend <i>p</i> -value				0.25	0.54				0.95	0.18	
Test for homogeneity of trends <i>P</i> -value				0.16					0.36		
Age at first use, years											
≥25	52	8	17	0.55 (0.22–1.38)	1.26 (0.60–2.66)	347	46	119	1.23 (0.77–1.95)	0.88 (0.65–1.18)	
20–24	164	35	50	0.74 (0.39–1.40)	1.11 (0.61–2.01)	405	54	144	1.11 (0.68–1.83)	1.03 (0.74–1.42)	
18–19	183	40	49	0.78 (0.42–1.44)	0.94 (0.52–1.70)	149	22	31	1.17 (0.62–2.20)	0.65 (0.40–1.06)	
<18	248	50	59	0.70 (0.38–1.27)	0.82 (0.46–1.47)	57	21	21	2.87 (1.44–5.74)	1.36 (0.75–2.48)	
Trend <i>p</i> -value				0.46	0.23				0.04	0.85	
Test for homogeneity of trends <i>P</i> -value				0.11					0.05		
Duration between menarche and first use, years											
<5	200	44	46	0.75 (0.40–1.40)	0.82 (0.44–1.50)	64	14	16	1.59 (0.75–3.39)	0.79 (0.42–1.51)	
5–9	329	64	95	0.69 (0.38–1.23)	1.02 (0.59–1.77)	333	55	93	1.28 (0.77–2.14)	0.80 (0.56–1.15)	
10–14	93	20	25	0.76 (0.38–1.56)	0.98 (0.50–1.92)	344	44	127	1.16 (0.71–1.88)	1.02 (0.75–1.40)	
≥15	25	5	9	0.71 (0.24–2.16)	1.41 (0.56–3.54)	217	30	79	1.28 (0.76–2.14)	0.90 (0.64–1.26)	
Trend <i>p</i> -value				0.42	0.45				0.48	0.81	
Test for homogeneity of trends <i>P</i> -value				0.22					0.43		

Younger (age 35–44 years)					Older (age 45–64 years)					
	No. controls	No. triple negative	No. luminal A	OR (95% CI) of triple negative	OR (95% CI) of luminal A	No. controls	No. triple negative	No. luminal A	OR (95% CI) of triple negative	OR (95% CI) of luminal A
Time since last use, years										
<5	130	26	38	0.68 (0.35–1.32)	0.97 (0.52–1.81)	35	3	6	0.69 (0.19–2.45)	0.39 (0.15–0.98)
5–9	89	23	34	0.88 (0.44–1.76)	1.35 (0.71–2.55)	27	4	5	1.13 (0.36–3.58)	0.50 (0.18–1.38)
10–14	153	33	35	0.76 (0.40–1.45)	0.82 (0.44–1.52)	66	12	33	1.42 (0.67–2.98)	1.35 (0.82–2.22)
15–19	155	31	33	0.70 (0.37–1.34)	0.78 (0.41–1.46)	172	20	53	0.96 (0.52–1.76)	0.87 (0.58–1.29)
≥20	120	20	35	0.59 (0.29–1.21)	1.10 (0.58–2.08)	658	104	218	1.31 (0.87–2.00)	0.93 (0.70–1.22)
Trend <i>p</i> -value				0.26	0.64				0.17	0.90
Test for homogeneity of trends <i>P</i> -value										
				0.56					0.19	

Note: Triple negative = ER-/PR-/HER2-, Luminal A = ER+ or PR+ plus HER2-.

* Adjusted for study site, race, education, age, family history of breast cancer, age at menarche, menopausal status, body mass index, parity.

Table 4

Multivariable adjusted* odds ratio (OR) and 95% confidence interval (CI) for triple negative and luminal A breast cancer associated with reproductive history by age

Younger (age 35–44 years)				Older (age 45–64 years)						
	No. controls	No. triple negative	No. luminal A	OR (95% CI) of triple negative	OR (95% CI) of luminal A	No. controls	No. triple negative	No. luminal A	OR (95% CI) of triple negative	OR (95% CI) of luminal A
Number of full-term pregnancies										
Never pregnant	70	19	26	Reference	Reference	106	9	57	Reference	Reference
1	140	24	40	0.62 (0.31–1.23)	0.81 (0.45–1.45)	174	31	69	1.77 (0.80–3.91)	0.80 (0.52–1.25)
2	219	50	64	0.89 (0.48–1.64)	0.84 (0.49–1.45)	355	56	123	1.70 (0.81–3.58)	0.68 (0.46–1.01)
3	127	29	30	0.89 (0.45–1.74)	0.67 (0.36–1.25)	264	27	85	1.15 (0.51–2.55)	0.66 (0.43–1.00)
≥4	76	16	15	0.76 (0.35–1.67)	0.56 (0.26–1.17)	326	47	89	1.48 (0.68–3.21)	0.56 (0.36–0.86)
Trend <i>p</i> -value				0.87	0.09				0.77	0.006
Test for homogeneity of trends <i>P</i> -value										
Only non-full-term pregnancy	89	16	22	0.62 (0.29–1.30)	0.66 (0.34–1.27)	69	11	25	1.61 (0.63–4.13)	0.67 (0.38–1.19)
Age at first full-term pregnancy [†] , years										
≤19	163	31	33	Reference	Reference	420	72	106	Reference	Reference
20–24	154	39	51	1.40 (0.80–2.43)	1.61 (0.96–2.69)	460	52	144	0.73 (0.48–1.11)	1.10 (0.81–1.51)
25–29	129	25	41	1.14 (0.59–2.20)	1.50 (0.84–2.68)	150	20	83	0.91 (0.50–1.65)	1.95 (1.31–2.90)
≥30	116	24	24	1.43 (0.66–3.09)	0.92 (0.45–1.90)	89	17	33	1.41 (0.71–2.80)	1.22 (0.71–2.09)
Trend <i>p</i> -value				0.48	0.95				0.66	0.04
Test for homogeneity of trends <i>P</i> -value										
				0.58					0.35	
Duration of breastfeeding [†] , months										
Never	180	46	60	Reference	Reference	532	85	180	Reference	Reference
<1	61	14	14	0.83 (0.41–1.67)	0.66 (0.34–1.31)	141	24	51	1.11 (0.67–1.84)	1.04 (0.71–1.52)
1–6	144	28	36	0.63 (0.36–1.11)	0.61 (0.37–1.02)	190	26	56	0.86 (0.53–1.41)	0.73 (0.51–1.04)
7–23	123	20	31	0.45 (0.24–0.87)	0.60 (0.34–1.05)	179	16	60	0.60 (0.34–1.07)	0.86 (0.60–1.23)
≥24	54	11	8	0.55 (0.24–1.28)	0.35 (0.15–0.86)	77	10	19	0.82 (0.39–1.72)	0.69 (0.40–1.21)
Trend <i>p</i> -value				0.02	0.01				0.14	0.09

Younger (age 35–44 years)				Older (age 45–64 years)			
No. controls	No. triple negative	No. luminal A	OR (95% CI) of triple negative	OR (95% CI) of luminal A	No. controls	No. triple negative	No. luminal A
Test for homogeneity of trends <i>P</i> -value				0.97			0.80

Note: Triple negative = ER-/PR-/HER2-, Luminal A = ER+ or PR+ plus HER2-.

* Adjusted for study site, race, education, age, family history of breast cancer, age at menarche, menopausal status, body mass index.

† Also adjusted for parity and mutually adjusted for age at first full-term pregnancy and duration of breastfeeding among parous women.

	No. controls	No. cases			OR (95% CI)				
		Triple negative/p53+	Triple negative/p53−	Luminal A/p53+	Luminal A/p53−	Triple negative/p53+	Triple negative/p53−	Luminal A/p53+	Luminal A/p53−
Time since last use, years									
<5	165	15	14	10	34	1.06 (0.51–2.23)	0.71 (0.35–1.45)	1.17 (0.49–2.78)	0.72 (0.45–1.15)
5–9	116	14	13	7	32	1.39 (0.66–2.96)	0.97 (0.47–2.01)	1.23 (0.48–3.19)	1.09 (0.67–1.76)
10–14	219	23	22	9	59	1.21 (0.63–2.30)	0.92 (0.50–1.69)	0.80 (0.34–1.89)	1.02 (0.69–1.51)
15–19	327	21	30	19	67	0.81 (0.43–1.54)	0.96 (0.55–1.67)	1.20 (0.60–2.40)	0.77 (0.54–1.10)
≥20	778	52	72	46	207	0.93 (0.56–1.56)	1.18 (0.75–1.85)	1.24 (0.71–2.18)	0.91 (0.70–1.19)
Trend <i>p</i> -value						0.53	0.31	0.48	0.53
Test for homogeneity of trends <i>P</i> -value									
0.46									

Note: Triple negative = ER-/PR-/HER2-, Luminal A = ER+ or PR+ plus HER2-.

* Adjusted for study site, race, education, age, family history of breast cancer, age at menarche, menopausal status, body mass index, parity.

Table 6

Multivariable adjusted* odds ratio (OR) and 95% confidence interval (CI) for triple negative and luminal A breast cancer associated with reproductive history by p53 status

	No. controls		No. cases				OR (95% CI)			
	Triple negative/p53+	Triple negative/p53−	Luminal A/p53+	Luminal A/p53−	Triple negative/p53+	Triple negative/p53−	Luminal A/p53+	Luminal A/p53−		
Number of full-term pregnancies										
Never pregnant	176	10	18	14	69	Reference	Reference	Reference	Reference	
1	314	27	28	17	92	1.33 (0.62–2.83)	0.81 (0.43–1.53)	0.67 (0.32–1.42)	0.84 (0.58–1.22)	
2	574	48	58	35	152	1.41 (0.69–2.86)	0.97 (0.55–1.71)	0.75 (0.39–1.45)	0.73 (0.52–1.03)	
3	391	24	32	20	95	1.10 (0.51–2.39)	0.81 (0.44–1.51)	0.67 (0.32–1.39)	0.66 (0.46–0.96)	
≥4	402	31	32	16	88	1.36 (0.63–2.95)	0.80 (0.42–1.52)	0.50 (0.23–1.11)	0.57 (0.38–0.84)	
Trend <i>p</i> -value						0.79	0.55	0.15	0.002	
Test for homogeneity of trends <i>P</i> -value										
						0.22				
Only non-full-term pregnancy	158	11	16	10	37	0.99 (0.40–2.40)	0.87 (0.42–1.78)	0.74 (0.31–1.72)	0.64 (0.40–1.02)	
Age at first full-term pregnancy [†] , years										
≤19	583	54	49	29	110	Reference	Reference	Reference	Reference	
20–24	614	34	57	32	163	0.68 (0.42–1.09)	1.14 (0.74–1.74)	0.99 (0.57–1.71)	1.24 (0.93–1.66)	
25–29	279	26	19	18	106	1.17 (0.67–2.05)	0.84 (0.46–1.54)	1.29 (0.65–2.55)	1.81 (1.27–2.57)	
≥30	205	16	25	9	48	0.94 (0.46–1.94)	1.70 (0.90–3.24)	0.86 (0.35–2.15)	1.07 (0.68–1.68)	
Trend <i>p</i> -value						0.92	0.31	0.91	0.12	
Test for homogeneity of trends <i>P</i> -value										
						0.83				
Duration of breastfeeding [†] , months										
Never	712	57	74	39	201	Reference	Reference	Reference	Reference	
<1	202	18	20	14	51	1.08 (0.61–1.90)	0.98 (0.57–1.66)	1.25 (0.65–2.40)	0.85 (0.60–1.22)	
1–6	334	27	27	19	73	0.95 (0.57–1.57)	0.68 (0.42–1.11)	0.92 (0.51–1.66)	0.66 (0.48–0.91)	
7–23	302	20	16	10	81	0.75 (0.43–1.32)	0.44 (0.24–0.79)	0.50 (0.24–1.05)	0.82 (0.60–1.13)	
≥24	131	8	13	6	21	0.68 (0.30–1.53)	0.91 (0.46–1.78)	0.78 (0.30–2.02)	0.52 (0.31–0.87)	
Trend <i>p</i> -value						0.23	0.03	0.13	0.008	

		No. controls		No. cases				OR (95% CI)			
				Triple negative/p53+	Triple negative/p53−	Luminal A/p53+	Luminal A/p53−	Triple negative/p53+	Triple negative/p53−	Luminal A/p53+	Luminal A/p53−
Test for homogeneity of trends <i>P</i> -value											
		0.93									

Note: Triple negative = ER-/PR-/HER2-, Luminal A = ER+ or PR+ plus HER2-.

* Adjusted for study site, race, education, age, family history of breast cancer, age at menarche, menopausal status, body mass index.

† Also adjusted for parity and mutually adjusted for age at first full-term pregnancy and duration of breastfeeding among parous women.