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Reproductive Factors and Risk of Breast Cancer by Tumor Subtypes among Ghanaian Women: A Population-based Case-control Study

Reproductive Factors and Risk of Breast Cancer by Tumor Subtypes among Ghanaian Women: A Population-based Case-control Study

Short title: Breast cancer risk by tumor subtypes in Ghana

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Novelty and Impact:

Black-African women have a higher incidence of aggressive hormone negative breast cancer than white women. In the first population-based study of breast cancer in sub-Saharan Africa--parity and breastfeeding were the two major identified factors among Ghanaian women, which exhibited risk differences by age at diagnosis and hormone receptor status, consistent with racial disparities. Promotion of extended breastfeeding could help reduce incidence for early-onset hormone negative and all later onset breast cancers.

List of abbreviations:

ER: estrogen receptor

OR: odds ratios

CI: confidence interval

PR: progesterone receptor

HER2: human epidermal growth factor receptor 2

AMBER: African American Breast Cancer Epidemiology and Risk

IHC: immunohistochemical

NCI: National Cancer Institute

ABCS: African Breast Cancer Study

Article category: Research Articles

Abstract

Higher proportions of early-onset and estrogen receptor (ER) negative cancers are observed in women of African ancestry than in women of European ancestry. Differences in risk factor distributions and associations by age at diagnosis and ER status may explain this disparity. We analyzed data from 1,126 cases (aged 18–74 years) with invasive breast cancer and 2,106 controls recruited from a population-based case-control study in Ghana. Odds ratios (OR) and 95% confidence intervals (CI) were estimated for menstrual and reproductive factors using polytomous logistic regression models adjusted for potential confounders. Among controls, medians for age at menarche, parity, age at first birth, and breastfeeding/pregnancy were 15 years, 4 births, 20 years, and 18 months, respectively. For women $\geq$ 50 years, parity and extended breastfeeding were associated with decreased risks: >5 births vs. nulliparous, OR 0.40 (95% CI 0.20–0.83) and 0.71 (95% CI 0.51–0.98) for $\geq$19 vs. <13 breastfeeding months/pregnancy, which did not differ by ER. In contrast, for earlier onset cases (<50 years) parity was associated with increased risk for ER-negative tumors (*P*-heterogeneity by ER = 0.02), which was offset by extended breastfeeding. Similar associations were observed by intrinsic-like subtypes. Less consistent relationships were observed with ages at menarche and first birth. Reproductive risk factor distributions are different from European populations but exhibited etiologic heterogeneity by age at diagnosis and ER status similar to other populations. Differences in reproductive patterns and subtype heterogeneity are consistent with racial disparities in subtype distributions.

Keywords: Reproductive risk factors, subtype heterogeneity, racial disparities, breast cancer

Introduction

Reproductive factors have been well documented as key breast cancer risk factors with direct associations observed with early ages at menarche, nulliparity, late ages at first birth and limited breastfeeding. Breast cancer is a heterogeneous disease, with differential etiologic associations for tumor subtypes defined by estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) status.[1] Most of these results derive from studies on European ancestry populations. Similar investigations among African ancestry populations are crucial given the differences in demographic and risk factor distributions and their disproportionately high incidence of early-onset breast cancer and ER-negative aggressive subtypes.[1-4]

Analyses of risk factors by the African American Breast Cancer Epidemiology and Risk (AMBER) consortium have revealed differential risk factor associations by tumor subtypes defined by ER, PR, and HER2 status.[5,6] Parity was associated with a decreased risk for ER-positive cancers but an increased risk for triple-negative breast tumors; furthermore, ever breastfeeding in parous women was strongly inversely related to the risk of triple-negative tumors.[6] Accumulating data support similar observations in other studies on women of African American and European ancestry, although distributions of risk factors differ.[1,7-11]

With substantially increasing rates of breast cancer in sub-Saharan Africa, identifying risk factors and strategies for reducing incidence are essential.[12,13] A population-based case-control study of breast cancer in Ghana aimed to overcome challenges of previous African studies that were unable to select population-based controls and properly classify hormone receptor-negative cases.[3,12,14,15] Using a census-based sampling of controls [16] and standardized protocols for collecting tumor biopsy samples for immunohistochemical

(IHC) staining from cases prior to treatment (17), we sought to determine the associations between menstrual and reproductive risk factors and breast cancer subtypes.

Materials and Methods

Study population

In brief, cases were women presenting with lumps suspected to be breast cancer at three hospitals [Korle Bu Teaching Hospital (Accra), Komfo Anokye Teaching Hospital (Kumasi), and Peace and Love Hospital (Kumasi)] were recruited from 2013–2015 and controls frequency matched to cases by age and districts of residence. Recent data cleaning efforts identified some duplicate subjects, leading to a few changes in eligibility status. Supplemental Fig. 1 details the 1,126 invasive breast cancer cases and 2,106 controls included in the present analysis. Details of the multi-disciplinary population-based case-control study in Ghana have been previously described.(17-18) Our primary analyses focused on ER status because this was the key marker of etiological heterogeneity demonstrated in previous studies.(19-21)

The study was approved by the Special Studies Institutional Review Board of the National Cancer Institute (Rockville, MD, USA), the Ghana Heath Service Ethical Review Committee and institutional review boards at the Noguchi Memorial Institute for Medical Research (Accra, Ghana), the Kwame Nkrumah University of Science and Technology (Kumasi, Ghana), the School of Medical Sciences at Komfo Anokye Teaching Hospital (Kumasi, Ghana) and Westat (Rockville, MD, USA). All participants provided written informed consent.

Risk factor information

Subjects were asked about their pregnancies and outcomes, the month and year that each pregnancy was completed, whether the baby (or babies) was breastfed, and for how many months they breastfed. Women were also asked questions on age at first menstruation, whether they were still menstruating, and if no longer menstruating, the age at which menstrual periods stopped and the reason for stopping.

Tumor characteristics

Prior to treatment, 4–8 core-needle biopsies (14-gage) were fixed in 10% neutral buffered formalin for 24–72 h and then processed into formalin-fixed paraffin embedded blocks for diagnosis using standardized protocols.(17) Blocks that were not required for diagnosis were sent to the National Cancer Institute (NCI) for additional pathological review (80% of the 1,126 invasive cases). Because organized mammography screening is not routine in Ghana, 96% of tumors presented as lumps > 2 cm based on clinical examination.(18) We obtained information on key IHC ER, PR, and HER2 markers from pathology departments in Ghana for 776 cases (69%). ER and PR status were considered positive if ≥10% of tumor cells stained positive. The proportion of cases that were classified as 1%–9% ER positive cells was minimal (1.8%). For HER2, tumors were considered positive if they demonstrated a homogeneous, dark pattern of staining in ≥10% of tumor cells. Indeterminate and negative cases were combined and considered HER2 negative.

We assessed the agreement of IHC assays performed in pathology departments in Ghana with those performed at an NCI laboratory in 87 cases, using two tumor tissue samples from the same patient. We observed good agreement for ER and HER2 (79% for ER, n = 87, $P < 0.0001$ and 78% for HER2, n = 76, $P < 0.0001$). PR showed a 65% agreement (n = 86, $P = 0.002$). To determine if associations differed by proxies for intrinsic subtypes based on IHC data, we further classified tumors as luminal A-like (ER+ or PR+ and HER2−), luminal B-

like (ER+ or PR+ and HER2+), HER2-enriched-like (ER−, PR−, and HER2+), or triple-negative/basal-like (ER−, PR−, and HER2−).

Statistical analysis

We observed a high correlation between total breastfeeding years and number of births (rho = 0.87 among controls) and a lower correlation with median breastfeeding months per pregnancy (rho = 0.15 among controls), the latter of which was used to avoid collinearity in the models. Polytomous logistic regression estimated the OR and 95% CI for each breast cancer subtype (comparing case IHC-defined subtypes with controls). Heterogeneity between menstrual and reproductive risk factors was assessed using polytomous logistic regression analyses restricted to cases (case-only analyses) with tumor characteristics and IHC as the outcome variable. To test differences in ORs by age, a likelihood-ratio test was performed by fitting the logistic regression models with and without interaction terms. Further, stratified analyses were performed to determine risk associations according to older and younger women.

Odds ratios (ORs) and 95% confidence intervals (95% CIs) were estimated to determine menstrual and reproductive factors using polytomous logistic models adjusted for study site and age (as a categorical variable) as well as key risk factors, including education, a family history of breast cancer, self-reported body size based on pictograms (17) and menopausal status or age at menopause. Trend tests were based on ordinal categories of variables and a missing category was used in models to retain all women in the models. All the statistical tests were two-sided. Analyses were performed using STATA/MP 14.2 (StataCorp, College Station, TX). Plots on the means and standard deviations of a 3-point running average for reproductive factors stratified by case/control status were presented to illustrate how these reproductive exposures have changed over time and were performed using R version 3.4.4.

Results

Descriptive characteristics of cases and controls

Cases were slightly older than controls reflecting that the controls were initially frequency matched to all women with a suspicion of breast cancer prior to diagnosis confirmation. Approximately half of the cases had non-malignant breast diseases and tended to be younger than those with malignant breast disease.(17) The cases more often than the controls reported late ages at menarche, few births, late ages at first birth and low median breastfeeding months (Table 1).

A total of 50%, 52% and 23% of cases were ER positive, PR positive, and HER2 positive, respectively (Table 1). Luminal A-like breast cancer was the most common subtype (49%) followed by triple-negative/basal-like (28%), HER2-enriched (15%) and luminal B-like breast cancers (8%) (Supplemental Figure 1). There were no significant differences in cases missing ER, PR and HER2 status by risk factor data (data not shown). We did not find significant differences in distributions of molecular subtypes between women <50 years compared to ≥50 years of age (Supplemental Figure 1, χ^2 *P*=0.24).

We assessed descriptively if reproductive factors varied by age which is highly correlated with birth cohort (1945–1975, Figure 1). Number of births was lower in older cases (correlated with older birth cohorts) compared to younger ages born in more recent birth cohorts, with cases having fewer births on average compared with the controls (among

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women 70 years of age, mean 4.1 for cases and 6.4 for controls; among women 40 years of age, mean 2.5 for cases and 3.3 for controls). Age at first birth increased by approximately 1 year in the later compared with earlier birth cohorts for both cases and controls (21.7 at ages 70 and 22.3 at ages 40 among controls). Age at menarche showed no apparent trends, hovering around 15 years across the birth cohorts. Breastfeeding months per pregnancy among controls declined until the 1960s and steadily increased until 1975. Among the cases, breastfeeding months per pregnancy increased over time by 1 month per pregnancy from 17 to 18 months.

Associations with reproductive factors overall and stratified by age

Associations for age at menarche, number of births, age at first birth, and median breastfeeding months per pregnancy overall and stratified by age are shown in Table 2. Analyses of all cases combined showed number of births as the only risk factor with a statistically significant risk association (P-trend=0.005). Among women aged <50 years, we observed an inverse association with parity (≥5 vs 0 births: OR 0.70, 95% CI 0.42–1.18, P-trend = 0.06) and an increased risk with older ages at first birth (≥26 vs <19 years: OR 1.40, 95% CI 0.97–2.01, P-trend = 0.05). In more discrete categories of age, we observed a significant trend ($P = 0.01$) with advancing age at first birth among women aged <40 years (Supplementary Table 1). Age at menarche and median breastfeeding months were not significantly associated with breast cancer risk among younger women. Among women aged ≥50 years, a strong inverse association was observed with parity (≥5 vs 0 births: OR 0.40, 95% CI 0.20–0.83); a test for interaction with age was significant ($P = 0.02$). Similarly, median breastfeeding months among older women were inversely associated with risk (≥19 vs <13 months: OR 0.71, 95% CI 0.51–0.98) and demonstrated a significant interaction with age ($P = 0.01$). Age at menarche was unrelated to risk among the older women (Table 2). Evaluation of these associations with more detailed categories of age revealed a significant

interaction by age for parity and median breastfeeding months per pregnancy, with the strongest inverse associations of parity and extended breastfeeding among women aged ≥60 years (Supplementary Table 1).

Associations with reproductive factors by ER and stratified by age

Analyses for all cases combined did not show statistically significant differences for ER-negative compared with ER-positive cases (Supplementary Table 2). When we evaluated the associations by ER status among women aged <50 years (Table 3), we observed a strong inverse association with parity for ER-positive tumors and a positive association for ER-negative tumors, with the test for heterogeneity being statistically significant (*P*-het = 0.02). Among women <50 years, older ages at first birth showed a slightly stronger direct association for ER-positive than ER-negative breast tumors, but the test for heterogeneity was not statistically significant. Extended breastfeeding only showed an inverse association among ER-negative tumors, with evidence of significant heterogeneity compared with ER-positive tumors (≥19 vs <13 months: ER-positive tumors OR 1.39, 95% CI 0.82–2.34; ER-negative tumors 0.71, 0.45–1.12; *P*-het = 0.04). There was no additional relationship for ≥19 breastfeeding months; when we compared women with ≥13 breastfeeding months per pregnancy to <13 months, the resultant OR for ER-negative tumors was OR 0.69 (95% CI 0.45–1.03). There was a suggestion of a positive association with older ages at menarche for ER-positive breast tumors that was not apparent for ER-negative breast tumors.

Among the women aged ≥50 years (Table 4), parity was inversely associated with risk for both ER-negative and ER-positive tumors (although there were few nulliparous women, p-het = 0.33). Although extended breastfeeding showed an inverse association regardless of ER status, a stronger association was observed among ER-positive tumors (*P*-het = 0.07). Age at first birth did not demonstrate any consistent associations with risk.

We further assessed the joint effects of parity and breastfeeding per pregnancy (Figure 2 and Supplemental Table 3). Among women aged ≥50 years, increasing parity, and breastfeeding were associated with reduced risks for both ER-negative and ER-positive tumors, with the lowest risks observed among women with ≥3 births who breastfed for ≥13 months/pregnancy compared with nulliparous women (ER-negative cases: OR 0.45, 95% CI 0.21–0.95; ER-positive cases: OR 0.31, 95% CI 0.13–0.75). This trend was less apparent among women aged <50 years with ER-positive tumors [≥3 births who breastfed for ≥13 months/pregnancy compared with nulliparous women (OR 0.69, 95% CI 0.36–1.30)]. In contrast, among women aged <50 years with ER-negative tumors, compared with nulliparous women, the highest risk was for those with ≥3 births who breastfed <13 months/pregnancy (OR 1.91, 95% CI 0.89–4.10). Women with ≥3 births who breastfed, on average, ≥13 months per pregnancy were not at increased risk (OR 1.09, 95% CI 0.56–2.10), due to the multiplicative joint association of two factors associated with risk in opposite directions.

Associations with reproductive factors by ER, PR, HER2 status and stratified by age

We evaluated if associations with parity and breastfeeding differed using the IHC proxy for intrinsic subtypes. We focused our analyses on triple-negative compared with luminal A-like cases because previous studies have shown differences between these two groups (6-9,20,21) and these were also the two most common tumor subtypes (Supplementary Tables 4–5). Parity was inversely related to the risk of luminal A-like tumors regardless of age, as well as with risk of triple-negative tumors among women aged ≥50 years (Supplementary Tables 4–5). In contrast, a positive association was observed for triple-negative tumors among women aged <50 years (*P*-het = 0.03). Among younger women with triple-negative tumors, extended breastfeeding was inversely associated with risk, a relationship not observed for luminal A-like tumors. In contrast, among older women, we observed a strong inverse association of breastfeeding with luminal A-like tumors (OR 0.52,

95% CI 0.33–0.82) that was not observed for triple-negative tumors (*P* -het = 0.04) (Supplementary Table 5).

Discussion

Among Ghanaian women, we observed substantial heterogeneity of the parity association with breast cancer risk by age at diagnosis and ER status, with strong inverse associations for all tumor subtypes in older (≥50 years) women and for younger-onset ER-positive tumors, but an opposite association for younger-onset ER-negative tumors (i.e., increased risk with increasing birth numbers). Higher median breastfeeding months per pregnancy were strongly inversely associated with later-onset breast tumor risk (particularly ER-positive or luminal A-like tumors); among younger women, it was an apparent protective factor for ER-negative tumors. Similar to previous reports,(6-9,20,21) our study population allowed an evaluation of associations for a wide range of number of births and breastfeeding months per pregnancy.

Few studies have addressed the relation of reproductive risk factors in women of African ancestry. The largest dataset derives from the African Breast Cancer Study (ABCS),(22) a hospital-based case-control study in Nigeria, Cameroon and Uganda, comprising 1,995 cases and 2,631 controls (with 81% of the cases from Nigeria). Analyses from this study showed changing reproductive patterns over time (particularly number of births) and an inverse association of risk with parity; however, it did not show statistically significant heterogeneity of risk associations by menopausal status or age at diagnosis.(22,23) Notably, in contrast to our study, ABCS was not population-based and lacked information on hormone receptor status of the tumors, thereby limiting the comparability of the findings. Data from the AMBER consortium, a pooled analysis of four studies of African-American women with available tumor IHC data found that among 1,252 ER-negative breast tumors

parous women were at elevated risk compared with nulliparous women, increasing to 1.60 among those aged <40 years.(6) Our data are consistent with AMBER and other recent studies,(9,11) supporting a cross-over association between parity on breast cancer risk that is dependent on age at onset and ER status.

In our Ghanaian population, number of births and breastfeeding years were highly correlated. Our data showed a significant inverse risk relationship with median breastfeeding months per pregnancy, with a 15% reduced risk for those with 13–18 vs <13 months/pregnancy. In pooled analyses of populations of European ancestry, breastfeeding has been shown to have a weak inverse association with breast cancer risk. However, recent data that includes molecular subtyping information provides evidence of a possible stronger inverse association for hormone-negative breast tumors.(6-9,20,21) In the AMBER study, the inverse association of breastfeeding was most pronounced for younger-onset ER-negative and triple-negative breast tumors. In fact, for such tumors, analyses demonstrated that extended breastfeeding could reduce the adverse risks associated with parity, which has also been seen in other studies that included African-American women.(9,11) Our results revealed similar associations given that extended breastfeeding appeared to largely counteract the adverse relationship with multiparity among younger women with ER-negative tumors.

Recent studies assessing associations by molecular subtypes using IHC and mRNA expression profiling have shown increased risk with parity that may predominate for triple-negative or basal-like breast tumors.(20,24) In our study, the modifications in risk associations between parity and breastfeeding by age reflected different temporal trend patterns by birth cohorts in cases and controls: the rate of decrease in number of births was faster for controls than cases in early birth cohorts (i.e., older women); a decreasing trend of breastfeeding months per pregnancy in early birth cohorts was seen in controls but not in cases. Given that multiparity and increased breastfeeding are inversely associated with later-

onset breast cancers (with somewhat stronger associations with ER-positive tumors), if the observed temporal trends of decreasing parity and breastfeeding continue, they are likely to result in an increased incidence of later-onset breast cancer.(13) This indicates the importance of public health measures to maintain high rates of breastfeeding,(25) which could potentially attenuate the projected increase in risk due to changes in reproductive patterns and demographics.(13,26)

Older age at first birth has been associated with increases in breast cancer risk in numerous studies, particularly for ER-positive tumors.(8,10,20,27,28) The AMBER consortium also found increased risks for older ages at first birth for ER-positive but not for ER-negative tumors. Our data were consistent with these findings, suggesting that this association may be stronger or limited to early-onset ER-positive breast cancer cases.(6) However, in African populations, this is a difficult exposure to assess given that few women actually delay their first births until truly late ages. With increasing adoption of westernized lifestyles and access to birth control, continued monitoring of maternity data are needed to determine if ages at first birth continue to increase.

Despite the observed trends in reproductive patterns toward westernization, our study population still maintained higher parity and breastfeeding frequencies compared with other populations. The reproductive patterns in our study are consistent with recent nationally representative surveys.(29,30) For example, the decline in fertility rate from 6.4 in 1988 to 3.9 in 2017 reported in surveys by the Ghana Maternal Health Survey ages 15–49 years is similar to the decline in average number of live births in our control population from 6.4 to 3.3 for women born in 1945 (i.e., 43 years old in 1988) and 1975 (i.e., 42 years old in 2017).(29) Median breastfeeding months per pregnancy were 17 to 18 months in our study controls and in a 2011 survey median months breastfeeding were 17.4 and 17.9 months for Greater Accra and Ashanti regions, respectively.(30) The strong inverse associations of these

factors with late onset, mostly ER-positive tumors, together with a lack of population-based screening, are likely important factors contributing to historically low incidence of late onset ER-positive breast cancers. In contrast, for early-onset cancers, higher parity was directly associated with ER-negative disease in our study. It is doubtful, however, that high parity explains the higher incidence of ER-negative early-onset cancers in our population given the high prevalence of breastfeeding, which appeared to offset the higher risk from multiparity. Instead, the younger demographics in Ghana and other sub-Saharan African countries probably explains the higher proportion of these early-onset cancers compared with populations of European ancestry.(3) It may be that rather than a population with an "excess" of early-onset ER-negative cancers that there could be fewer diagnoses of late onset ER-positive breast cancer compared with other populations, as suggested in other studies.(31) To specifically address this, further studies comparing age- incidence rates of breast cancer subtypes in Africa are needed, similar to U.S. studies that have addressed racial differences by age.(32)

Age at menarche has been inversely associated with risk in European ancestry populations.(33) In the studies of African-American women, later ages at menarche were inversely associated with breast cancer regardless of hormone receptor status.(5,9) In contrast, we observed no such relationship. The median age of menarche of 15 years in Ghanaian women is quite different from the reported age of 12 years among African-American women, with our study having limited variation in ages at menarche. Increased nutrition has been suggested to lower the age at menarche; this variable could reflect early exposures that may differ between populations (e.g., early adolescent weight).(34) In addition, a substantial number of women in our study could not recall their ages at menarche, suggesting that measurement error could have impacted our ability to assess relationships reliably.

Strengths of this study are the population-based design, detailed risk factor assessment, and tissue collection for quality assessment of IHC markers to examine etiologic heterogeneity in sub-Saharan Africa. A limitation is that although IHC data can be used as a proxy for molecular subtypes, mRNA expression assays are required to classify previously described intrinsic molecular subtypes, especially HER2-enriched and luminal B subtypes. Further, although our study is one of the largest breast cancer epidemiological studies conducted in sub-Saharan Africa, analyses by age and subtypes resulted in small numbers within strata of these critical factors.

Our study indicates that while reproductive factors showed important temporal trends and distinct distributions compared with African-American or European ancestry populations, their associations with breast cancer risk were generally consistent with those observed in these populations. Our data support the importance of breastfeeding to prevent early-onset ER-negative breast cancer associated with multiparty and the longer-term protection of parity and breastfeeding for later-onset breast tumors, irrespective of their ER status. Further studies including more detailed molecular characterization of tumors and additional risk factors may provide additional insights into breast cancer etiology in sub-Saharan Africa.

Conflict of Interest: None declared.

Author contributions

Conception & Design of the study: JDF, BDL, LE, NT, EA, JNCL, JY, BW, BA, MD, SW, KN, FA, DA, SH, TA, MGC, LAB; Data collection: JDF, BDL, LE, NT, EA, JNCL, JY,

BW, BA, MD, SW, KN, FA, DA, SH, TA, MGC, LAB**;** Interpretation of data JDF, BDL, LE, NT, EA, JNCL, JY, BW, BA, MD, SW, KN, FA, DA, SH, TA, MGC, LAB**;** Drafting of the manuscript: JDF, BDL, LAB, MGC; Revised work and provided important intellectual content: JDF, BDL, LE, NT, EA, JNCL, JY, BW, BA, MD, SW, KN, FA, DA, SH, TA, MGC, LAB.

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References

1.   Anderson KN, Schwab RB, Martinez ME. Reproductive risk factors and breast cancer subtypes: a review of the literature. Breast Cancer Res Treat 2014; 144: 1–10

2.   Ademuyiwa FO, Tao Y, Luo J, Weilbaecher K, Ma CX. Differences in the mutational landscape of triple-negative breast cancer in African Americans and Caucasians. Breast Cancer Res Treat 2017; 161: 491–499

3.   Brewster AM, Chavez-MacGregor M, Brown P. Epidemiology, biology, and treatment of triple-negative breast cancer in women of African ancestry. Lancet Oncol 2014; 15: e625–634

4.   Newman LA, Kaljee LM. Health disparities and triple-negative breast cancer in African American women: A review. JAMA Surg 2017; 152: 485–493

5.   Ambrosone CB, Zirpoli G, Hong CC, Yao S, Troester MA, Bandera EV, Schedin P, Bethea TN, Borges V, Park SY, Chandra D, Rosenberg L, Kolonel LN, Olshan AF, Palmer JR. Important role of menarche in development of estrogen receptor-negative breast cancer in African American women. J Natl Cancer Inst 2015; 107

6.   Palmer JR, Viscidi E, Troester MA, Hong CC, Schedin P, Bethea TN, Bandera EV, Borges V, McKinnon C, Haiman CA, Lunetta K, Kolonel LN, Rosenberg L, Olshan AF, Ambrosone CB. Parity, lactation, and breast cancer subtypes in African American women: results from the AMBER Consortium. J Natl Cancer Inst 2014; 106

7.   Fortner RT, Sisti J, Chai B, Collins LC, Rosner B, Hankinson SE, Tamimi RM, Eliassen AH. Parity, breastfeeding, and breast cancer risk by hormone receptor status and molecular phenotype: results from the Nurses' Health Studies. Breast Cancer Res 2019; 21: 40

8.   Gaudet MM, Gierach GL, Carter BD, Luo J, Milne RL, Weiderpass E, Giles GG, Tamimi RM, Eliassen AH, Rosner B, Wolk A, Adami HO, Margolis KL, Gapstur SM,

Garcia-Closas M, Brinton LA. Pooled analysis of nine cohorts reveals breast cancer risk factors by tumor molecular subtype. Cancer Res 2018; 78: 6011–6021

9.   Ma H, Ursin G, Xu X, Lee E, Togawa K, Duan L, Lu Y, Malone KE, Marchbanks PA, McDonald JA, Simon MS, Folger SG, Sullivan-Halley J, Deapen DM, Press MF, Bernstein L. Reproductive factors and the risk of triple-negative breast cancer in white women and African-American women: A pooled analysis. Breast Cancer Res 2017; 19: 6

10.   Lambertini M, Santoro L, Del Mastro L, Nguyen B, Livraghi L, Ugolini D, Peccatori FA, Azim HA Jr. Reproductive behaviors and risk of developing breast cancer according to tumor subtype: A systematic review and meta-analysis of epidemiological studies. Cancer Treat Rev 2016; 49: 65–76.

11.   John EM, Hines LM, Phipps AI, Koo J, Longacre TA, Ingles SA, Baumgartner KB, Slattery ML, Wu AH. Reproductive history, breast-feeding and risk of triple negative breast cancer: The Breast Cancer Etiology in Minorities (BEM) study. Int J Cancer 2018; 142: 2273–2285

12.   Akarolo-Anthony SN, Ogundiran TO, Adebamowo CA. Emerging breast cancer epidemic: evidence from Africa. Breast Cancer Res 2010; 12: S8

13.   Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87–108

14.   Bird PA, Hill AG, Houssami N. Poor hormone receptor expression in East African breast cancer: evidence of a biologically different disease? Ann Surg Oncol 2008; 15: 1983–1988

15.   Brinton LA, Figueroa JD, Awuah B, Yarney J, Wiafe S, Wood SN, Ansong D, Nyarko K, Wiafe-Addai B, Clegg-Lamptey JN. Breast cancer in Sub-Saharan Africa: opportunities for prevention. Breast Cancer Res Treat 2014; 144: 467–478

16.   Nyante SJ, Biritwum R, Figueroa J, Graubard B, Awuah B, Addai BW, Yarney J, Clegg-Lamptey JN, Ansong D, Nyarko K, Wiafe S, Oppong J, Boakye I, Brotzman M, Adjei R, Afriyie LT, Garcia-Closas M, Brinton LA. Recruiting population controls for case-control studies in sub-Saharan Africa: The Ghana Breast Health Study. PLoS One 2019; 14: e0215347

17.   Brinton LA, Awuah B, Nat Clegg-Lamptey J, Wiafe-Addai B, Ansong D, Nyarko KM, Wiafe S, Yarney J, Biritwum R, Brotzman M, Adjei AA, Adjei E, Aitpillah F, Edusei L, Dedey F, Nyante SJ, Oppong J, Osei-Bonsu E, Titiloye N, Vanderpuye V, Brew Abaidoo E, Arhin B, Boakye I, Frempong M, Ohene Oti N, Okyne V, Figueroa JD. Design considerations for identifying breast cancer risk factors in a population-based study in Africa. Int J Cancer 2017; 140: 2667–2677

18.   Brinton L, Figueroa J, Adjei E, Ansong D, Biritwum R, Edusei L, Nyarko KM, Wiafe S, Yarney J, Addai BW, Awuah B, Clegg-Lamptey JN. Factors contributing to delays in diagnosis of breast cancers in Ghana, West Africa. Breast Cancer Res Treat 2017; 162: 105–114

19.   Allred DC, Brown P, Medina D. The origins of estrogen receptor alpha-positive and estrogen receptor alpha-negative human breast cancer. Breast Cancer Res 2004; 6: 240–245

20.   Holm J, Eriksson L, Ploner A, Eriksson M, Rantalainen M, Li J, Hall P, Czene K. Assessment of breast cancer risk factors reveals subtype heterogeneity. Cancer Res 2017; 77: 3708–3717

21.   Work ME, John EM, Andrulis IL, Knight JA, Liao Y, Mulligan AM, Southey MC, Giles GG, Dite GS, Apicella C, Hibshoosh H, Hopper JL, Terry MB. Reproductive risk factors and oestrogen/progesterone receptor-negative breast cancer in the Breast Cancer Family Registry. Br J Cancer 2014; 110: 1367–1377

22.   Sighoko D, Ogundiran T, Ademola A, Adebamowo C, Chen L, Odedina S, Anetor I, Ndom P, Gakwaya A, Ojengbede O, Huo D, Olopade OI. Breast cancer risk after full-term pregnancies among African women from Nigeria, Cameroon, and Uganda. Cancer 2015; 121: 2237–2243

23.   Huo D, Adebamowo CA, Ogundiran TO, Akang EE, Campbell O, Adenipekun A, Cummings S, Fackenthal J, Ademuyiwa F, Ahsan H, Olopade OI. Parity and breastfeeding are protective against breast cancer in Nigerian women. Br J Cancer 2008; 98: 992–996

24.   Sun X, Nichols HB, Tse CK, Bell MB, Robinson WR, Sherman ME, Olshan AF, Troester MA. Association of parity and time since last birth with breast cancer prognosis by intrinsic subtype. Cancer Epidemiol Biomarkers Prev 2016; 25: 60–67

25.   Victora CG, Bahl R, Barros AJ, França GV, Horton S, Krasevec J, Murch S, Sankar MJ, Walker N, Rollins NC. Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. Lancet 2016; 387: 475–490

26.   Tamimi RM, Spiegelman D, Smith-Warner SA, Wang M, Pazaris M, Willett WC, Eliassen AH, Hunter DJ. Population attributable risk of modifiable and nonmodifiable breast cancer risk factors in postmenopausal breast cancer. Am J Epidemiol 2016; 84: 884–893

27.   Ma H, Wang Y, Sullivan-Halley J, Weiss L, Marchbanks PA, Spirtas R, Ursin G, Burkman RT, Simon MS, Malone KE, Strom BL, McDonald JA, Press MF, Bernstein L. Use of four biomarkers to evaluate the risk of breast cancer subtypes in the women's contraceptive and reproductive experiences study. Cancer Res 2010; 70: 575–587

28.   Tamimi RM, Colditz GA, Hazra A, Baer HJ, Hankinson SE, Rosner B, Marotti J, Connolly JL, Schnitt SJ, Collins LC. Traditional breast cancer risk factors in relation to molecular subtypes of breast cancer. Breast Cancer Res Treat 2012; 131: 159–167

29.   Ghana Statistical Service (GSS) GHSG, and ICF. Ghana Maternal Health Survey 2017. In: Accra GG, GHS, and ICF, editor. Rockville, MD: Department of Health Services ICF; 2018

30.   Service GS. Ghana multiple indicator cluster survey with an enhanced malaria module and biomarker, 2011, Final Report. Accra, Ghana; 2011

31.   Dickens C, Duarte R, Zietsman A, Cubasch H, Kellett P, Schüz J, Kielkowski D, McCormack V. Racial comparison of receptor-defined breast cancer in Southern African women: subtype prevalence and age-incidence analysis of nationwide cancer registry data. Cancer Epidemiol Biomarkers Prev 2014; 23: 2311–2321

32.   Clarke CA, Keegan TH, Yang J, Press DJ, Kurian AW, Patel AH, Lacey JV Jr. Age-specific incidence of breast cancer subtypes: understanding the black-white crossover. J Natl Cancer Inst 2012; 104: 1094–1101

33.   Collaborative Group on Hormonal Factors in Breast C. Menarche, menopause, and breast cancer risk: individual participant meta-analysis, including 118 964 women with breast cancer from 117 epidemiological studies. Lancet Oncol 2012; 13: 1141–1151

34.   Cheng G, Buyken AE, Shi L, Karaolis-Danckert N, Kroke A, Wudy SA, Degen GH, Remer T. Beyond overweight: nutrition as an important lifestyle factor influencing timing of puberty. Nutr Rev 2012; 70: 133–152

Figure legends

Figure 1: Temporal trends of menstrual and reproductive risk factors for cases and controls in the Ghana Breast Health Study by birth cohorts from 1945 to 1975

(A) Age at menarche, (B) parity, (C) age at first birth and (D) median breastfeeding months per pregnancy. The means and standard deviations plotted are the results of a 3-point running average. Gray indicates standard deviation.

Figure 2: ORs and 95% CIs for joint effects of parity and breastfeeding (vs nulliparous) by ER status and age of onset

Polytomous logistic regression models were used to calculate ORs and 95% CI, adjusted for age, education, study site, body size, family history of breast cancer, age at menarche, age at first birth, menopausal status, and age at menopause. bars indicate standard deviations = breastfeeding. ER = Estrogen receptor. Details of sample sizes, effect estimates and pvalues are presented in Supplemental Table 3.

Table legends

Table 1. Demographic and reproductive characteristics of 1,126 diagnosed invasive breast cancer cases and 2,106 controls from the Ghana Breast Health Study

IQR, interquartile range; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2

Table 2. ORs and 95% CIs for select reproductive risk factors and overall breast cancer risk in women younger and older than 50 years in 1,122 cases and 2,096 controls

Logistic regression models were adjusted for age, education, study site, body size, family history of breast cancer, menopausal status, age at menopause, and all reproductive factors listed above. OR, odds ratio; CI, confidence interval; LRT, Likelihood ratio test for interaction term

Table 3. Association between reproductive risk factors and breast cancer risk in women <50 years of age in 378 cases and 1,294 controls stratified by ER status

Polytomous logistic regression models were adjusted for age, education, study site, body size, family history of breast cancer, menopausal status, and all reproductive factors listed above. ER, estrogen receptor; OR, odds ratio; CI, confidence interval; *P*-het, *P*-heterogeneity test

Table 4. Reproductive risk factors in women ≥50 years of age in 398 cases and 802 controls stratified by ER status

Polytomous logistic regression models were adjusted for age, education, study site, body size, family history of breast cancer, menopausal status, age at menopause, and all reproductive factors listed above. ER, estrogen receptor; OR, odds ratio; CI, confidence index; *P*-het, *P*-heterogeneity test

Table 1. Demographic and reproductive characteristics of 1,126 diagnosed invasive breast cancer cases and 2,106 controls from the Ghana Breast Health Study

Study Population Characteristics	Controls N = 2,106		Cases N = 1,126	
	N	%	N	%
Age (years)				
<35	435	21	114	10
35–44	561	27	277	25
45–55	554	26	330	29
≥55	546	26	401	36
Unknown	10		4	
Study site				
Accra	736	35	384	34
Kumasi	1370	65	742	66
Education				
No formal education	498	24	254	24
Primary school	369	18	153	15
Junior secondary school	654	32	260	25
> Senior secondary school	512	25	387	37
Unknown	73		72	
Family history of breast cancer				
No	2036	98	1034	93
Yes	46	2	78	7
Unknown	24		14	1
Bodysize				
Slight	585	29	253	24
Average	827	40	434	41
Slightly heavy	470	23	261	25
Heavy	163	8	104	10
Unknown	61		74	
Age at menarche (years)				
Median age at menarche (IQR)	15 (15–15)		15 (15–15)	
<15	568	30	266	27
15	548	29	255	26
16	383	20	223	23
≥17	395	21	228	23
Unknown	212		154	
Parity				
Median parity (IQR)	4 (2–5)		3 (2–5)	
Nulliparous	228	11	107	10
1–2	533	25	319	28
3–4	685	33	365	33
≥5	652	31	331	30
Unknown	8		4	
Age at first birth (years)				
Median age (IQR)	20 (18–24)		21 (19–25)	

<19	555	31	235	25
19–21	510	28	265	28
22–25	412	23	260	27
≥26	322	18	197	21
Unknown	79		62	
Median breastfeeding per pregnancy (months)				
Median months(IQR)	18 (15–24)		18 (12–24)	
<13	352	20	239	26
13–18	692	39	341	37
≥19	747	42	347	37
Unknown	87		92	
Menopausal status				
Premenopausal	1276	61	495	44
Postmenopausal	816	39	629	56
Unknown	14		2	
Age at menopause				
median years (IQR)	49 (45-51)		49 (45-51)	
<45	119	18	86	17
45-49	222	33	162	33
50-54	267	39	192	39
≥55	68	10	54	11
Unknown	147		137	
ER status				
Positive			393	50
Negative			387	50
Unknown			346	
PR status				
Positive			402	52
Negative			374	48
Unknown			350	
HER2 status				
Positive			181	23
Negative			544	70
Inconclusive			54	7
Unknown			347	

Table 2. ORs and 95% CIs for select reproductive risk factors and overall breast cancer risk in women younger and older than 50 years in 1,122 cases and 2,096 controls

	All women					Women <50 years old (N = 564)							Women ≥50 years old (N = 558)							
	OR	95% CI		P	P-trend	Controls	Cases	OR	95% CI		P	P-trend	Controls	Cases	OR	95% CI		P	P-trend	P-int (LRT)
Age at menarche (years)																				
<15	1.00					391	148	1.00					174	118	1.00					
15	0.88	0.70	1.10	0.25		323	121	0.88	0.65	1.20	0.42		225	134	0.81	0.57	1.14	0.22		
16	1.13	0.89	1.44	0.31		238	114	1.17	0.86	1.61	0.31		143	108	0.94	0.64	1.38	0.75		
≥17	1.08	0.85	1.37	0.53	0.30	249	117	1.08	0.79	1.48	0.63	0.46	146	111	0.97	0.66	1.42	0.87	0.82	0.34
Parity																				
Nulliparous	1.00					209	73	1.00					19	34	1.00					
1–2	1.04	0.72	1.51	0.83		406	204	0.93	0.58	1.50	0.78		122	115	0.59	0.28	1.24	0.16		
3–4	0.80	0.55	1.15	0.23		429	187	0.79	0.49	1.27	0.32		254	176	0.41	0.20	0.84	0.01		
≥5	0.73	0.50	1.07	0.10	0.005	246	99	0.70	0.42	1.18	0.18	0.06	403	230	0.40	0.20	0.83	0.01	0.01	0.02
Age at first birth (years)																				
<19	1.00					310	103	1.00					242	132	1.00					
19–21	1.14	0.90	1.43	0.28		290	121	1.18	0.85	1.64	0.32		219	143	1.15	0.82	1.60	0.42		
22–25	1.27	1.00	1.62	0.05		247	127	1.42	1.01	2.00	0.04		164	133	1.15	0.81	1.65	0.43		
≥26	1.18	0.91	1.54	0.22	0.135	204	121	1.40	0.97	2.01	0.07	0.05	118	76	1.03	0.68	1.56	0.88	0.74	0.28
Median months breastfeeding per pregnancy (among parous women)																				
<13	1.00					189	89	1.00					163	150	1.00					
13–18	0.85	0.68	1.06	0.16		416	182	0.98	0.71	1.36	0.92		272	158	0.74	0.54	1.03	0.07		
≥19	0.84	0.67	1.05	0.12	0.159	434	184	1.04	0.75	1.44	0.82	0.77	308	160	0.71	0.51	0.98	0.04	0.05	0.01

Table 3. Association between reproductive risk factors and breast cancer risk in women <50 years of age in 378 cases and 1,294 controls stratified by ER status

	Controls	ER-positive	ER-negative	ER-positive N = 185 OR	95% CI		P-trend	ER-negative N = 193 OR	95% CI		P-trend	ER-negative/ER-positive P-het
Age at menarche (years)												
<15	391	40	54	1.00				1.00				
15	323	44	37	1.21	0.76	1.93		0.69	0.43	1.09		0.08
16	238	37	43	1.38	0.85	2.25		1.21	0.77	1.90		0.79
≥17	249	47	38	1.61	1.00	2.58	0.05	0.97	0.61	1.55	0.81	0.09
Parity												
Nulliparous	209	23	19	1.00				1.00				
1–2	406	62	64	0.57	0.26	1.22		1.70	0.82	3.51		0.06
3–4	429	66	67	0.51	0.24	1.09		1.62	0.78	3.36		0.04
≥5	246	34	43	0.46	0.20	1.06	0.19	1.80	0.82	3.95	0.32	0.02
Age at first birth (years)												
<19	310	31	40	1.00				1.00				
19–21	290	45	40	1.43	0.86	2.36		1.08	0.66	1.76		0.54
22–25	247	37	52	1.34	0.78	2.30		1.64	1.01	2.65		0.49
≥26	204	45	32	1.72	0.99	2.97	0.08	1.15	0.66	1.99	0.30	0.29
Median months breastfeeding per pregnancy (among parous women)												
<13	189	24	39	1.00				1.00				
13–18	416	65	57	1.37	0.82	2.29		0.67	0.42	1.05		0.02
≥19	434	64	61	1.39	0.83	2.34	0.29	0.71	0.45	1.12	0.25	0.04

Table 4. Reproductive risk factors in women ≥50 years of age in 398 cases and 802 controls stratified by ER status

				ER-positive N = 205				ER-negative N = 193				ER-negative/ER-positive
	Controls	ER-positive	ER-negative	OR	95% CI		P-trend	OR	95% CI		P-trend	P-het
Age at menarche (years)												
<15	177	51	39	1.00				1.00				
15	225	50	46	0.69	0.43	0.85		0.85	0.51	1.39		0.60
16	145	37	27	0.73	0.43	0.69		0.69	0.39	1.22		0.84
≥17	146	42	43	0.84	0.50	1.13	0.74	1.13	0.67	1.92	0.77	0.36
Parity												
Nulliparous	19	12	14	1.00				1.00				
1–2	127	48	36	0.82	0.31	2.16		0.34	0.13	0.88		0.13
3–4	256	72	53	0.58	0.23	1.49		0.23	0.09	0.57		0.83
≥5	406	76	91	0.49	0.19	1.26	0.24	0.28	0.11	0.70	0.004	0.33
Age at first birth (years)												
<19	245	46	50	1.00				1.00				
19–21	220	49	56	1.10	0.67	1.78		1.26	0.80	2.00		0.55
22–25	165	48	42	1.10	0.66	1.83		1.06	0.64	1.75		1.00
≥26	118	32	24	1.09	0.61	1.93	0.72	1.02	0.57	1.84	0.95	0.84
Median months breastfeeding per pregnancy (among parous women)												
<13	163	68	42	1.00				1.00				
13–18	276	54	53	0.61	0.39	0.95		0.81	0.50	1.30		0.35
≥19	313	51	66	0.54	0.34	0.85	0.01	0.89	0.56	1.42	0.82	0.07

Reproductive Factors and Risk of Breast Cancer by Tumor Subtypes among Ghanaian Women: A Population-based Case-control Study

Short title: Breast cancer risk by tumor subtypes in Ghana

Jonine D Figueroa, Brittny C Davis Lynn, Lawrence Edusei, Nicholas Titiloye , Ernest Adjei , Joe-Nat Clegg-Lamptey , Joel Yarney , Beatrice Wiafe-Addai , Baffour Awuah , Maire A. Duggan , Seth Wiafe , Kofi Nyarko , Francis Aitpillah , Daniel Ansong , Stephen M Hewitt , Thomas Ahearn , Montserrat Garcia-Closas and Louise A Brinton on behalf of the Ghana Breast Health Study team

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Supplemental Figure 1: Details of cases and controls for analysis of reproductive factors and breast cancer risk by tumor characteristics in the Ghana Breast Health Study
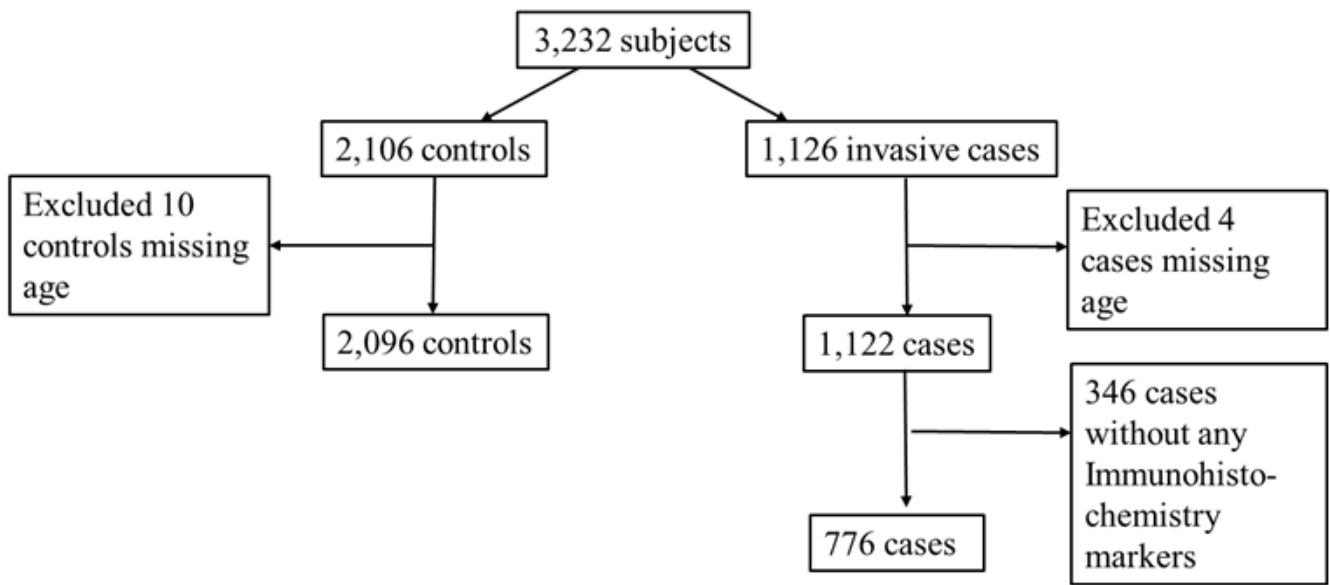
	Cases age <50 (N=375) %	Cases age ≥50 (N=401) %
Luminal A-like	46.4	51.1
Triple-negative	27.2	28.7
Luminal B-like	9.3	7.0
HER2-enriched	17.1	13.2
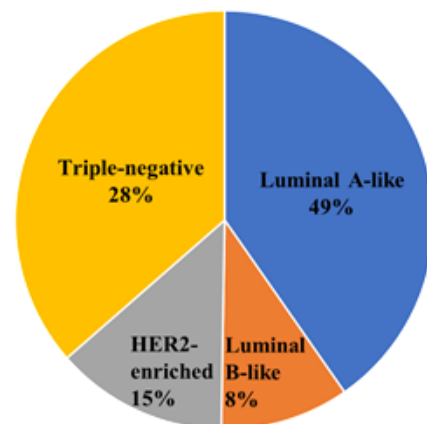
Intrinsic-like subtype distribution for cases with immunohistochemical data on ER, PR, and HER2 defined as luminal A-like (ER+ or PR+ and HER2−), luminal B-like (ER+ or PR+ and HER2+), HER2-enriched-like (ER− or PR− and HER2+), or triple-negative/basal-like (ER−, PR− and HER2−). We did not find significant differences in distributions of molecular subtypes between women <50 years compared to ≥50 years of age, χ^2 P=0.24.

Supplementary Table 1. Association between reproductive factors and breast cancer risk among 1,122 cases and 2,096 controls stratified by age groups in the Ghana Breast Health Study

	Co	Ca				Co	Ca				Co	Ca				Co	Ca					
			<40 years old					**40–49 years old**					**50–59 years old**					**≥60 years old**					
	N = 654	N = 235	OR	95% CI	p-trend	N = 640	N = 329	OR	95% CI	p-trend	N = 468	N = 297	OR	95% CI	p-trend	N = 334	N = 261	OR	95% CI	p-trend	LRT	age interaction (continuous age)
Age at menarche (years)																						
<15	246	71	1·00			145	77	1·00			113	67	1·00			61	51	1·00				
15	157	43	0·93	0·58 1·50		166	78	0·86	0·57 1·31		129	75	0·95	0·61 1·50		96	59	0·59	0·33 1·05			
16	101	39	1·41	0·86 2·33		137	75	1·07	0·70 1·62		85	58	1·04	0·63 1·72		58	50	0·75	0·40 1·38			
≥17	114	52	1·76	1·10 2·81	0·031	135	65	0·82	0·53 1·27	0·509	90	62	1·02	0·62 1·67	0·793	56	49	0·91	0·48 1·71	0·968	0·0795	0·162
Parity																						
Nulliparous	169	42	1·00			40	31	1·00			13	21	1·00			6	13	1·00				
1–2	243	106	1·03	0·49 2·16		163	98	0·65	0·32 1·31		87	77	0·48	0·19 1·22		35	38	0·56	0·14 2·27			
3–4	191	67	1·01	0·49 2·06		238	120	0·63	0·31 1·26		172	93	0·28	0·11 0·70		82	83	0·46	0·12 1·74			
≥5	48	19	1·07	0·43 2·68	0·928	198	80	0·56	0·27 1·14	0·183	194	105	0·34	0·14 0·85	0·029	209	125	0·34	0·09 1·27	0·023	0·002	<0·001
Age at first birth (years)																						
<19	135	32	1·00			175	71	1·00			140	67	1·00			102	65	1·00				
19–21	125	41	1·17	0·66 2·08		165	80	1·22	0·81 1·84		129	84	1·40	0·89 2·19		90	59	0·86	0·51 1·44			
22–25	124	60	1·73	0·98 3·06		123	67	1·27	0·82 1·99		103	74	1·39	0·86 2·23		61	59	0·94	0·53 1·64			
≥26	89	51	1·91	1·03 3·54	0·01	115	70	1·34	0·84 2·13	0·221	70	39	1·19	0·68 2·08	0·438	48	37	0·87	0·45 1·69	0·783	0·0689	0·58
Median months breastfeeding per pregnancy (among parous women)																						
<13	104	36	1·00			85	53	1·00			94	68	1·00			69	82	1·00				
13–18	178	64	1·07	0·64 1·79		238	118	0·96	0·62 1·49		167	103	0·99	0·64 1·52		105	55	0·53	0·32 0·90			
≥19	182	76	1·38	0·84 2·26	0·171	252	108	0·90	0·58 1·41	0·667	172	80	0·79	0·50 1·23	0·259	136	80	0·66	0·40 1·08	0·161	0·0002	0·04

Logistic regression models were adjusted for education, study site, body size, family history of breast cancer, menopausal status, and all reproductive factors listed above. P-values < 0·05 were considered statistically significant. Co, controls; Ca, cases; OR, odds ratio; CI, confidence interval; LRT, likelihood ratio test for the interaction term

Supplemental Table 2. Association between reproductive risk factors and breast cancer risk among 776 cases and 2,106 controls stratified by ER status in the Ghana Breast Health Study

				ER-positive N = 390				ER-negative N = 386				ER-negative/ER-positive
	Controls	ER-positive	ER-negative	OR	95% CI		*P*-trend	OR	95% CI		*P*-trend	*P*-het
Age at menarche (years)												
<15	565	91	93	1·00				1·00				
15	548	94	83	0·99	0·72	0·83		0·83	0·60	1·16		0·42
16	381	74	69	1·12	0·79	1·04		1·04	0·74	1·48		0·77
≥17	395	89	81	1·24	0·89	1·15	0·22	1·15	0·82	1·61	0·42	0·71
Parity												
Nulliparous	228	35	33	1·00				1·00				
1–2	528	110	100	0·96	0·56	1·10		1·10	0·63	1·90		0·71
3–4	683	136	120	0·80	0·47	0·88		0·88	0·51	1·53		0·78
≥5	649	109	133	0·67	0·38	1·17	0·05	0·95	0·54	1·67	0·59	0·34
Age at first birth (years)												
<19	552	77	90	1·00				1·00				
19–21	509	94	95	1·18	0·84	1·13		1·13	0·82	1·57		0·85
22–25	411	85	94	1·18	0·83	1·31		1·31	0·93	1·84		0·65
≥26	322	77	56	1·31	0·89	1·01	0·17	1·01	0·68	1·50	0·59	0·31
Median months breastfeeding per pregnancy (among parous women)												
<13	352	92	81	1·00				1·00				
13–18	688	119	109	0·84	0·61	1.15		0·78	0·56	1·08		0·73
≥19	742	112	127	0·79	0·57	1.08	0·14	0·84	0·61	1·16	0·42	0·74

Polytomous logistic regression models were adjusted for age, education, study site, body size, family history of breast cancer, menopausal status, age at menopause, and all reproductive factors listed above. *P*-values <0·05 were considered statistically significant. ER, estrogen receptor; OR, odds ratio; CI, confidence interval; *P*–het, *P*-heterogeneity test

Supplemental Table 3. Joint effects of parity and breastfeeding among 776 cases and 2,106 controls stratified by ER status and age group

Subtype	Age group	Controls (N)	Cases (N)	Parity/breastfeeding category	OR	95%CI	
ER-positive	**<50**	209	19	Nulliparous	**Referent**		
		100	17	1-2 births & <13 months breastfeeding	0.62	0.27	1.44
		286	41	1-2 births & >13 months breastfeeding	0.77	0.39	1.53
		89	22	3+ births & <13 months breastfeeding	0.44	0.18	1.07
		564	77	3+ births & >13 months breastfeeding	0.69	0.36	1.30
ER-negative	**<50**	209	23	Nulliparous	**Referent**		
		100	14	1-2 births & <13 months breastfeeding	1.46	0.65	3.27
		286	44	1-2 births & >13 months breastfeeding	1.27	0.63	2.54
		89	10	3+ births & <13 months breastfeeding	1.91	0.89	4.10
		564	85	3+ births & >13 months breastfeeding	1.09	0.56	2.10
ER-positive	**>50**	19	14	Nulliparous	**Referent**		
		34	7	1-2 births & <13 months breastfeeding	0.79	0.28	2.23
		73	21	1-2 births & >13 months breastfeeding	0.48	0.18	1.28
		129	35	3+ births & <13 months breastfeeding	0.55	0.22	1.36
		507	97	3+ births & >13 months breastfeeding	0.31	0.13	0.75
ER-negative	**>50**	19	19	Nulliparous	**Referent**		
		34	34	1-2 births & <13 months breastfeeding	0.91	0.37	2.24
		73	73	1-2 births & >13 months breastfeeding	0.63	0.27	1.47
		129	129	3+ births & <13 months breastfeeding	0.51	0.23	1.13
		507	507	3+ births & >13 months breastfeeding	0.45	0.21	0.95

Supplemental Table 4. Association between reproductive factors and breast cancer risk in women <50 years of age in 375 cases and 1,294 controls stratified by molecular subtypes defined by ER, PR, and HER2 tumor tissue expression

| | Luminal A-like | Triple-negative | HER2-enriched-like | Luminal B-like | Luminal A-like (N = 174) | | | Triple negative (N = 102) | | | |
					OR	95% CI		*P*-trend	OR	95% CI		*P*-trend	*P*-het
Parity													
Nulliparous	24	10	2	8	1·00				1·00				
1–2	60	33	13	21	0·60	0·28	1·31		1·64	0·62	4·35		0·10
3–4	59	33	14	26	0·52	0·24	1·14		1·52	0·57	4·06		0·08
≥5	33	27	7	10	0·48	0·21	1·11	0·12	2·01	0·71	5·68	0·31	0·03
Median months breastfeeding per pregnancy (among parous women)													
<13	23	20	8	10	1·00				1·00				
13–18	59	30	11	23	1·30	0·76	2·22		0·65	0·35	1·22		0·08
≥19	62	34	9	21	1·45	0·84	2·48	0·19	0·79	0·43	1·47	0·57	0·13

Polytomous logistic regression models were adjusted for age, education, study site, body size, family history of breast cancer, menopausal status, and all reproductive factors listed above. Reference for p-het was luminal A-like cases (ER+/PR+HER2−). P-values <0·05 were considered statistically significant. ER = estrogen receptor. PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; OR, odds ratio; CI, confidence interval; *P*-het, *P*-heterogeneity test

Supplemental Table 5. Association between reproductive factors and breast cancer risk in women ≥50 years of age in 401 cases and 802 controls stratified by molecular subtypes defined by ER, PR, and HER2 tumor tissue expression

| | Luminal A-like | Triple-negative | HER2-enriched-like | Luminal B-like | Luminal A-like (N = 205) | | | Triple negative (N = 115) | | | |
					OR	95% CI	*P*-trend	OR	95% CI	*P*-trend	*P*-het
Parity											
Nulliparous	13	9	1	3	1·00			1·00			
1–2	47	20	4	13	0·74	0·27 2·01		0·23	0·07 0·72		0·07
3–4	71	34	4	16	0·49	0·19 1·28		0·18	0·06 0·56		0·12
≥5	73	52	20	23	0·42	0·16 1·11	0·02	0·22	0·07 0·65	0·16	0·28
Median months breastfeeding per pregnancy (among parous women)											
<13	66	22	7	15	1·00			1·00			
13–18	49	36	7	15	0·54	0·34 0·85		1·06	0·59 1·93		0·05
≥19	51	40	10	17	0·52	0·33 0·82	0·004	1·06	0·59 1·91	0·85	0·04

Polytomous logistic regression models were adjusted for age, education, study site, body size, family history of breast cancer, menopausal status, and all reproductive factors listed above. Reference for *P*-het was luminal A-like cases (ER+/PR+HER2−). *P*-values <0·05 were considered statistically significant.

ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; OR, odds ratio; CI, confidence interval; *P*-het, *P*-heterogeneity test