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Breastfeeding and the risk of epithelial ovarian cancer among women with a *BRCA1* or *BRCA2* mutation

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HIGHLIGHTS

- Whether a history of breastfeeding is associated with the risk of ovarian cancer among *BRCA* mutation carriers is not known.
- In this matched analysis, ever-breastfeeding was associated with a significant 23% reduction in risk of ovarian cancer.
- We observed an additive effect of both oral contraceptive use and breastfeeding which was strongly protective.
- Delineating the underlying mechanism(s) conferring the protective effect of breastfeeding is necessary.

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ABSTRACT

Objective. *BRCA* mutation carriers face a high lifetime risk of developing ovarian cancer. The strong inverse association between breastfeeding and the risk of ovarian cancer is established in the general population but is less well studied among women with a germline *BRCA1* or *BRCA2* mutation.

Method. Thus, we conducted a matched case-control analysis to evaluate the association between breastfeeding history and the risk of developing ovarian cancer. After matching for year of birth, country of residence, *BRCA* gene and personal history of breast cancer, a total of 1650 cases and 2702 controls were included in the analysis. Conditional logistic regression was used to estimate the odds ratio (OR) and 95% confidence intervals (CI) associated with various breastfeeding exposures.

Results. A history of ever-breastfeeding was associated with a 23% reduction in risk (OR = 0.77; 95%CI 0.66–0.90; $P = 0.001$). The protective effect increased with breastfeeding from one month to seven months

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after which the association was relatively stable. Compared to women who never breastfed, breastfeeding for seven or more months was associated with a 32% reduction in risk (OR = 0.68; 95%CI 0.57–0.81; $P < 0.0001$) and did not vary by *BRCA* gene or age at diagnosis. The combination of breastfeeding and oral contraceptive use was strongly protective (0.47; 95%CI 0.37–0.58; $P < 0.0001$).

Conclusions. These findings support a protective effect of breastfeeding for at least seven months among women with a *BRCA1* or *BRCA2* mutation, that is independent of oral contraceptive use.

1. Introduction

There are few options available to prevent ovarian cancer. The gold standard is prophylactic bilateral salpingo-oophorectomy, which is recommended for women at high-risk of developing ovarian cancer due to a hereditary predisposition [1]. Ovarian cancer screening with ultrasound and CA125 fails to detect most cases at a curable stage [2]. Factors which reduce the risk of ovarian cancer include oral contraceptive use, childbirth, tubal ligation and breastfeeding, but it is not clear how these reproductive and hormonal exposures can be incorporated into a public health strategy to reduce disease incidence [3].

Breastfeeding has been associated with a reduction in the risk of ovarian cancer risk [4]. In a recent pooled analysis of 13 case-control studies, which included 9973 cases of ovarian cancer and 13,843 controls, ever vs. never breastfeeding was associated with a 24% reduction in the risk of developing ovarian cancer [5]. The benefit increased with increasing duration of breastfeeding. The association was strongest for high-grade serous and endometrioid subtypes and the benefit persisted for more than 30 years after breastfeeding ended. Breastfeeding and parity are naturally correlated, and thus, it is important to dissociate the protective effect of breastfeeding from parity as well as other reproductive factors. Improving our understanding of the underlying biology behind this protective effect is critical to advance knowledge of the etiology of this disease and to develop effective prevention strategies. Preventive hormonal therapies which mimic the effect of breastfeeding may have the potential to disrupt ovarian carcinogenesis.

In an earlier matched case-control study of *BRCA1* and *BRCA2* carriers, we showed that breastfeeding for more than 12 months was associated with a 38% (95% CI 0.48–0.79) reduction in risk among *BRCA1* and a 50% (95% CI 0.29–0.84) reduction in risk among *BRCA2* mutation carriers [6]. The overall goal of the current analysis was to further evaluate the association between breastfeeding and ovarian cancer in a larger study population of *BRCA* mutation carriers, accounting for timing and duration of breastfeeding as well as other reproductive and hormonal exposures.

2. Materials and methods

2.1. Study population

The current study included women enrolled in an on-going, longitudinal study of *BRCA1* and *BRCA2* mutation carriers housed at the Women's College Research Institute (Toronto, Canada) and has previously been described in detail [6,7]. Briefly, eligible study participants were women carrying a deleterious germline *BRCA1* or *BRCA2* mutation from one of 61 participating centers in 15 countries. These women had sought genetic testing because of a personal or family history of breast and/or ovarian cancer or were part of a research study. Mutation detection was performed using a range of techniques, but all nucleotide sequences were confirmed by direct sequencing of DNA. The study protocol was approved by the institutional review boards of the respective host institutions and all study participants provided written informed consent. All study subjects (with the exception of some of those from the University of Utah and the University of California Irvine) received genetic counseling prior to genetic testing.

2.2. Data collection

Participants completed a baseline questionnaire at the individual center at the time of a clinic appointment, at their home at a later date, or at the time of recruitment into the research study. These questionnaires were either mailed to each participant to complete and return or administered over the phone by a genetic counselor or research assistant. The questionnaire requested detailed information on family or personal history of cancer, medical and reproductive history, as well as, exogenous hormone and medication use. A follow-up questionnaire was administered every two years thereafter to obtain updated exposure information and collect information on incident disease as well as any treatments received.

For the current analysis, we included information collected regarding parity and breastfeeding history. Specifically, the baseline questionnaire asked if the woman had ever been pregnant (yes/no) and if 'yes', was asked to consider all pregnancies in order from first to last and provide detailed information for each pregnancy including: year of pregnancy, length of pregnancy (weeks), pregnancy outcome (life, still, miscarriage, twins, etc.), live birth or caesarean section, date of childbirth, weight of child, and months of breastfeeding ('0' if she did not breastfeed). Furthermore, she was asked if she ever had difficulty breastfeeding (yes/no), and if 'yes' to select a reason and indicate for which pregnancy. We also queried about use of medication to stop milk production (yes/no), including name and method of medication and for which pregnancy. In the follow-up questionnaire, women were asked if they had any pregnancies since completion of the last questionnaire (yes/no), and if 'yes', were asked to complete the same questions regarding pregnancy outcome and breastfeeding. Using this information, we created several breastfeeding variables including ever vs. never breastfed and total months of breastfeeding.

Detailed information regarding oral contraceptive use was also collected. Women were asked if they ever used birth control pills to prevent pregnancy or for any other reason. If 'yes', they were asked the start and end dates (year) and duration of use (in months and years). We also asked information on current oral contraceptive use. Women were asked if they ever had a tubal ligation (i.e., fallopian tubes tied), and if 'yes', the year of surgery.

Pathology reports were requested for all women who reported incident cancers on the follow-up questionnaires. Information regarding histology (serous, endometrioid, mucinous, other), stage (I–IV), primary site of origin (ovary, fallopian tube, uterus, peritoneum, other), and whether the tumor had spread locally (if yes, to which sites: fallopian tubes, peritoneum, uterus, other) was abstracted from pathology report and/or medical record review if the latter was not available. We did not have pathology reports for those cases diagnosed prior to the baseline questionnaire.

2.3. Case and control subjects

Women were potentially eligible for inclusion in the current analysis if they carried a deleterious germline *BRCA1* or *BRCA2* mutation, were between the ages of 18 and 80 at the time of baseline questionnaire

and had ovarian cancer status available. There were 17,341 eligible women. Case subjects were women with a diagnosis of invasive epithelial ovarian cancer diagnosed between the ages of 30 and 80 years. Cases could have the diagnosis prior to the baseline questionnaire or during the follow-up period. We excluded 19 potential cases who had a diagnosis of a primary cancer other than breast cancer prior to their ovarian cancer. Women with a primary peritoneal or fallopian tube cancer were not eligible for inclusion. Control subjects were women who never had ovarian cancer and had two ovaries intact prior to the diagnosis of the case. Potential subjects were excluded if they had been diagnosed with a primary cancer other than breast, ovarian, thyroid or skin cancer ($n = 364$) or if information on their personal history of breast or ovarian cancer was missing ($n = 272$). Women were excluded if pertinent information was missing on breastfeeding ($n = 1838$), oophorectomy status ($n = 422$), pregnancy history ($n = 859$) or other pertinent information ($n = 58$). After exclusions, there were 13,528 eligible women, including 1771 women with ovarian cancer (potential case subjects) and 11,757 women without ovarian cancer (potential controls).

Two control subjects were selected for each case subject and matched according to mutation in the same gene (*BRCA1* or *BRCA2*), year of birth (within three years), country of residence and personal history of breast cancer (yes, no). A control was eligible to be matched to a given case if the date of interview or date of prophylactic bilateral salpingo-oophorectomy in the matched control occurred at or after the year of ovarian cancer diagnosis of the case. For each matched set, up to two controls were selected. If there were more than two eligible controls, the two with the closest date of birth to the matched case were selected. In total, 1650 matched sets were identified and included 1650 ovarian cancer cases and 2702 controls, with each case matched to at least one control.

2.4. Statistical analyses

A matched case-control analysis was performed to evaluate the association between breastfeeding and the risk of ovarian cancer. The distributions of continuous and categorical variables between cases and controls were compared using the Student's *t*-test and χ^2 test, respectively. Conditional logistic regression was used to estimate the univariate and multivariate odds ratios (OR) and 95% confidence intervals (CI) for ovarian cancer associated with breastfeeding. The following covariates were included in the multivariate model: parity, oral contraceptive use, and tubal ligation. For the controls, we only considered exposures that took place prior to the date of diagnosis of the matched case. We performed subgroup analyses to evaluate the relationship between breastfeeding and risk by *BRCA* gene, age at diagnosis, oral contraceptive and tubal ligation.

All analyses were performed using the SAS statistical package, version 9.1.3 (SAS Institute, Cary, NC). *P*-values were based on two-sided tests and were considered statistically significant if $P < 0.05$.

3. Results

Table 1 summarizes the 1650 cases and 2702 controls included in the analysis. Cases and controls did not differ with respect to date of birth, age at menarche, *BRCA* gene, personal history of breast cancer or country of residence. *BRCA1* mutation carriers were diagnosed with ovarian cancer at a younger age, on average, than *BRCA2* mutation carriers (49.7 vs. 54.8 years). Cases were less likely to have a history of oral contraceptive use than controls (46% vs. 57.6%; $P < 0.0001$) and a similar proportion of cases and controls had a tubal ligation (15.3% vs. 17.4%; $P = 0.08$). Mean parity was slightly lower in the cases than in controls (2.0 vs. 2.2; $P < 0.0001$).

Fewer cases reported a history of breastfeeding than controls (58.9% vs. 64.9%; $P < 0.0001$) (Table 1). The mean cumulative duration of breastfeeding was significantly lower in the cases vs. controls ($P = 0.01$). Among those who breastfed, cases breastfed for a total of 13.0 months on average (range 1.0–137) compared to 14.5 months (range 1.0–148) among the controls (Table 2).

Table 1
Baseline characteristics of ovarian cancer cases and controls with a *BRCA1* or *BRCA2* mutation.

Characteristic	Controls ($n = 2702$)	Cases ($n = 1650$)	*P*
Year of birth, mean (range)	1950.8 (1917–1981)	1951.0 (1917–1981)	0.46
Age at diagnosis, mean (range)	n/a	50.7 (30–78)	
BRCA1	n/a	49.7 (30–78)	
BRCA2	n/a	54.8 (31–75)	
Age at study enrollment, mean (range)	55.2 (31–80)	54.5 (32–79)	0.04
Mutation type, n (%)			
BRCA1	2162 (80.0)	1333 (80.8)	Matched
BRCA2	540 (20.0)	317 (19.2)	
Personal history of breast cancer, n (%)			
No	1909 (70.7)	1235 (74.8)	Matched
Yes	793 (29.4)	415 (25.2)	
Country of residence, n (%)			
USA	905 (33.5)	551 (33.4)	
Poland	846 (31.3)	509 (30.8)	
Canada	782 (28.9)	477 (28.9)	
Israel	69 (2.5)	44 (2.7)	
Italy	25 (0.9)	16 (1.0)	
Austria	22 (0.8)	13 (0.8)	
United Kingdom	10 (0.4)	9 (0.5)	
Norway	10 (0.4)	6 (0.4)	
Other	33 (1.2)	25 (1.5)	
Age at menarche, mean (range)	13.1 (9–20)	13.1 (9–28)	0.64
Parity[a], n (%)			
Never	372 (13.8)	265 (16.1)	0.04
Ever	2330 (86.2)	1385 (83.9)	<0.0001
Mean parity	2.2 (0−10)	2.0 (0−11)	
Oral contraceptive use[a], n (%)			
Never	1139 (42.4)	883 (54.0)	<0.0001
Ever	1549 (57.6)	753 (46.0)	
Missing	14	14	
Tubal ligation[a], n (%)			
Never	2174 (82.6)	1342 (84.6)	0.08
Ever	459 (17.4)	244 (15.3)	
Missing	69	64	
Breastfeeding, n (%)			
Never	949 (35.1)	679 (41.2)	<0.0001
Ever	1753 (64.9)	971 (58.9)	0.01
Months of breastfeeding, mean (range)	14.5 (1.0–148)	13.0 (1.0–137)	

[a] Variables were censored one year prior to the date of diagnosis of the matched case.

In the univariate model, ever vs. never breastfeeding was associated with a 25% reduction in the risk of developing ovarian cancer (OR = 0.75; 95%CI 0.65–0.86; $P < 0.0001$). The relationship was similar after adjusting for oral contraceptive use, parity and tubal ligation (OR = 0.77; 95%CI 0.0.66–0.90; $P = 0.001$). The protective effect increased with breastfeeding from one month to seven months after which the association was relatively stable (Fig. 1). The odds ratio associated with seven or more months of breastfeeding was 0.71 for women with a *BRCA1* mutation (95%CI 0.58–0.86) and 0.62 for women with a *BRCA2* mutation (95%CI 0.42–0.89; $P = 0.01$).

There were 654 women diagnosed with ovarian cancer prior to age 50 and 568 diagnosed after age 50 years. Breastfeeding for seven or more months was associated with a 37% reduction in the risk of developing disease among those diagnosed with ovarian cancer prior to age 50 (OR = 0.63; 95%CI 0.50–0.81; $P = 0.0003$) and a 27% reduction in risk among those diagnosed with ovarian cancer after age 50 (OR = 0.73; 95%CI 0.62–0.97; $P = 0.02$).

The protective effect of breastfeeding for seven or more months was stronger for those with a history of oral contraceptive use (OR = 0.55;

Table 2
Association between reproductive and hormonal factors and the risk of ovarian cancer among *BRCA* mutation carriers.

Variable	# of Cases/total[a]	Univariate OR (95%CI)	*P*	Multivariate OR (95%CI)[b]	*P*
Breastfeeding					
Never	679/1628	1		1	0.001
Ever	971/2724	0.75 (0.65–0.86)	<0.0001	0.77 (0.66–0.90)	
Oral contraceptive use					
Never	883/2022	1.00 (reference)		1.00 (reference)	<0.0001
Ever	753/2302	0.58 (0.50–0.67)	<0.0001	0.59 (0.51–0.68)	
Parity					
Never	265/637	1.00 (reference)		1.00 (reference)	0.90
Ever	1385/3715	0.76 (0.63–0.91)	0.003	0.99 (0.79–1.23)	
Tubal ligation					
Never	1342/3516	1.00 (reference)		1.00 (reference)	0.26
Ever	244/703	0.83 (0.70–1.00)	0.05	0.90 (0.75–1.08)	

[a] Total = case and control subjects.
[b] Mutually adjusted for all characteristics in column 1.

95%CI 0.45–0.68; $P < 0.0001$) than for those who never used oral contraceptives (OR = 0.87; 95%CI 0.71–1.07; $P = 0.20$) (Table 3). Breastfeeding was associated with a significant reduction in risk among women who did not have a tubal ligation (OR = 0.62; 95%CI 0.50–0.77; $P < 0.0001$). Among women who had a tubal ligation, the level of risk reduction with breastfeeding was similar, although not statistically significant (OR = 0.61; 95%CI 0.30–1.25; $P = 0.18$). The magnitude of the association did not vary substantially by a personal history of breast cancer or country of residence.

For women who completed childbearing prior to age 35 years, breastfeeding was associated with a 19% reduction in risk (OR = 0.81; 95%CI 0.69–0.96; $P = 0.01$). For women who had a baby at age 35 or older, breastfeeding was associated with a 40% reduction in risk (OR = 0.60; 95%CI 0.48–0.76; $P < 0.0001$) (Table 4). Among women with a livebirth after age 35, the protective effect of breastfeeding on risk was greater for women diagnosed with ovarian cancer after age 60 (OR = 0.43; 95%CI 0.22–0.84; $P = 0.01$) than before age 60 (OR = 0.63; 95%CI 0.49–0.81; $P = 0.0003$).

Table 5 summarizes the joint effects of breastfeeding and oral contraceptive use on the risk of ovarian cancer, overall and by *BRCA* gene. The risk reduction was greatest among women with a history of both breastfeeding and oral contraceptive use (OR = 0.47; 95%CI 0.37–0.58; $P < 0.0001$). Among women with at least seven months of breastfeeding, the odds ratio for ovarian cancer with oral contraceptive use (ever vs. never) was 0.60 (95% CI 0.43–0.85).

4. Discussion

In this case-control study, a history of breastfeeding was associated with a 23% reduction in the risk of ovarian cancer among *BRCA* mutation carriers. The protective effect was optimal with at least seven months of breastfeeding, after which additional breastfeeding did not appear to further diminish the risk. Other reproductive and hormonal factors, including oral contraceptives, parity and tubal ligation, are correlated with breastfeeding and also confer protection against ovarian cancer [8,9]. In a multivariate model incorporating all these factors, we found that parity per se (OR = 0.99; 95%CI 0.79–1.23) and tubal ligation (OR = 0.90; 95%CI 0.75–1.08) were not significant protective factors in this study population; however, oral contraceptive use was a significant independent protective factor. Further, the impact of breastfeeding was present in women with a history of oral contraceptive use. We observed an additive effect when we examined the impact of both exposures on risk, compared to women who neither breastfed nor used oral contraceptives. Among women who breastfed for seven or more months, additional months of breastfeeding did not further decrease the risk (Fig. 1) but with the addition of an oral contraceptive, the odd ratio declined from 0.66 (95%CI 0.53–0.82) to 0.47 (95%CI 0.37–0.58).

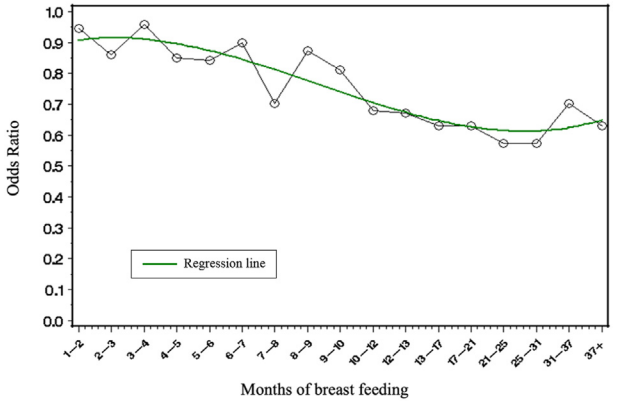

Fig. 1. Odds ratio for ovarian cancer among BRCA mutation carriers by cumulative duration of breastfeeding in months.

Breastfeeding was inversely associated with risk irrespective of age at last birth, and the association was particularly strong among women with a recent birth. Our findings are in line with reports conducted among women in the general population, suggesting a strong inverse association between breastfeeding and the risk of ovarian cancer [4,5,10,11]. In a recent collaborative analysis by Babic et al., which included data from 13 case-control studies with 9973 ovarian cancer cases and 13,843 controls [5], the authors also reported a stronger protective effect of breastfeeding among women who breastfed at an older age than among those who breastfed farther in the past.

These findings suggest that the mechanisms of protection may not be the same for breastfeeding and oral contraceptives, and in particular, cannot be solely explained by the diminishment of ovulatory cycles. The ovulatory cycle theory proposes the repeated trauma and repair of the ovarian epithelium with each ovulatory cycle increases the possibility of early neoplastic transformation through mutation or inflammation [12,13]. Under this paradigm, both breastfeeding and oral contraceptives diminish cancer risk by a similar mechanism through the diminishment of ovulatory cycles. However, after seven months of breastfeeding, further breastfeeding did not diminish the risk (despite preventing more ovulatory cycles) whereas adding an oral contraceptive reduced the risk beyond that of maximal breastfeeding.

An alternate (although not entirely independent) hypothesis includes changes in circulating hormones that occur during breastfeeding, specifically a reduction in pituitary gonadotropin secretion and a concomitant decrease in estrogen levels, inhibiting epithelial proliferation and the potential for malignant transformation [14]. A decrease in gonadotropins further inhibits ovulation and subsequently circulating estrogen and progesterone [15].

It may be that the risk of ovarian cancer is determined in large part by the cumulative number of ovulatory cycles, but the protective effects

Table 3
Association between breastfeeding for seven or more months compared to never breastfeeding and risk ovarian cancer in *BRCA* mutation carriers, overall and stratified by various factors[a].

Strata	# of Cases/total[a]	Univariate OR (95%CI)	*P*	Multivariate OR (95%CI)[b]	*P*
All women					
Never	679/1628	1.00 (reference)		1.00 (reference)	<0.0001
Yes ≥7 months	543/1646	0.66 (0.57–0.77)	<0.0001	0.68 (0.57–0.81)	
Age at diagnosis					
<50 years					
Never breastfed	377/927	1.00 (reference)		1.00	0.0003
Breastfed ≥7 months	277/938	0.58 (0.47–0.71)	<0.0001	(reference)(0.50–0.81)	
>50 years					
Never breastfed	302/701	1.00 (reference)	0.02	1.00 (reference)	0.02
Breastfed ≥7 months	266/708	0.77 (0.62–0.97)		0.73 (0.62–0.97)	
Oral contraceptive use					
Never breastfed	672/1613	1.00 (reference)	0.02	1.00 (reference)	0.20
Never user and breastfed ≥7 months	309/809	0.86 (0.71–1.03)		0.87 (0.71–1.07)	
Ever user and breastfed ≥7 months	232/831	0.54 (0.44–0.65)	<0.0001	0.55 (0.45–0.68)	<0.0001
BRCA1 mutation carrier					
Never breastfed	529/1237	1.00 (reference)		1.00 (reference)	0.0005
Breastfed ≥7 months	457/1358	0.67 (0.57–0.80	<0.0001	0.71 (0.58–0.86)	
BRCA2 mutation carrier					
Never breastfed	159/391	1.00 (reference)		1.00 (reference)	0.01
Breastfed ≥7 months	86/288	0.62 (0.44–0.86)	0.005	0.62 (0.42–0.89)	
Tubal ligation					
Never					
Never breastfed	523/1241	1.00 (reference)		1.00 (reference)	<0.0001
Breastfed ≥7 months	448/1372	0.61 (0.51–0.74)	<0.0001	0.62 (0.50–0.77	
Ever					
Never breastfed	109/293	1.00 (reference)		1.00 (reference)	0.18
Breastfed ≥7 months	83/246	0.68 (0.34–1.34)	0.26	0.61 (0.30–1.25)	
Personal history of breast cancer					
Yes					
Never breastfed	192/509	1.00 (reference)		1.00 (reference)	0.01
Breastfed ≥7 months	118/386	0.71(0.53–0.96)	0.02	0.65(0.47–0.91)	
No					
Never breastfed	487/1119	1.00 (reference)		1.00 (reference)	0.002
Breastfed ≥7 months	425/1260	0.65 (0.55–0.78)	<0.0001	0.72 (0.58–0.88)	
Parity					
Never breastfed[c]	679/1628	1.00 (reference)		1.00 (reference)	0.96
Primiparous and breastfed ≥7 months	53/132	0.99 (0.68–1.43)	0.85	0.99 (0.68–1.44)	<0.0001
Multiparous and breastfed ≥7 months	490/1514	0.64 (0.55–0.75	<0.0001	0.66 (0.56–0.77)	

[a] Total = case and control subjects.
[b] Mutually adjusted for all characteristics in column 1.
[c] Nulliparous included in the never breastfed group.

of reproductive risk factors act through other mechanisms. As a woman ages, premalignant cells accumulate in the ovary as a result of inflammation and genetic mutations associated with cyclical ovulation and subsequent tissue repair. There is a small chance that, at any age, the premalignant lesions progress to invasive cancers. In 1994, Adami and colleagues proposed that pregnancy helps clear existing premalignant lesions from the ovary [16]. Risch extended the theory and suggested that elevated levels of progesterone during pregnancy were responsible for the apoptosis and subsequent clearance of premalignant cancer cells [14]. Experimental data supports a pro-apoptotic effect of progesterone on the ovarian epithelium [17–20]. This theory may likewise explain the protective effect observed with oral contraceptive use which contain

Table 4
Association between history of breastfeeding and risk of ovarian cancer according to age at last birth, overall and by age at diagnosis.

Variable	# of Cases/total[a]	Univariate OR (95%CI)	*P*	Multivariate OR (95%CI)[b]	*P*
All subjects					
Never breastfed	679/1628	1.00 (reference)		1.00 (reference)	
Ever breastfed	971/2724	0.75 (0.65–0.86)	<0.0001	0.77 (0.66–0.90)	0.001
Ever breastfed and age at last birth ≤34	806/2172	0.80 (0.67–0.92)	0.001	0.81 (0.69–0.96)	0.01
Ever breastfed and age at last birth >34	165/552	0.57 (0.46–0.70)	<0.0001	0.60 (0.48–0.76)	<0.0001
Age at diagnosis, ≤60 years					
Never breastfed	579/1430	1.00 (reference)		1.00 (reference)	
Ever breastfed	860/2434	0.77 (0.67–0.89)	0.0004	0.80 (0.67–0.95)	0.01
Ever breastfed *and* age at last birth ≤34	714/1941	0.82 (0.71–0.96)	0.01	0.84 (0.70–1.00)	0.06
Ever breastfed *and* age at last birth >34	146/493	0.59 (0.47–0.74	<0.0001	0.63 (0.49–0.81)	0.0003
Age at diagnosis, >60 years					
Never breastfed	100/198	1.00 (reference)		1.00 (reference)	
Ever breastfed	114/290	0.60 (0.41–0.88)	0.009	0.58 (0.38–0.88)	0.01
Ever breastfed *and* age at last birth ≤34	92/231	0.66 (0.44–0.97)	0.04	0.62 (0.40–0.97)	0.04
Ever breastfed *and* age at last birth >34	19/59	0.42 (0.22–0.84)	0.01	0.43 (0.22–0.84)	0.01

[a] Total = case and control subjects.
[b] Mutually adjusted for all characteristics in column 1.

Table 5
Association between independent and combined effects of history breastfeeding and oral contraceptive use and risk ovarian cancer in *BRCA* mutation carriers, overall and stratified by *BRCA* mutation type.

Variable	# of Cases/total[a]	Univariate OR (95%CI)	*P*	Multivariate OR (95%CI)[b]	*P*
All subjects					
Never breastfed/never OC[c] user	326/650	1.00 (reference)		1.00 (reference)	
Ever breastfed *only*	541/1391	0.65 (0.53–0.79)	<0.0001	0.66 (0.53–0.82)	0.0001
Ever OC use *only*	335/935	0.56 (0.45–0.69)	<0.0001	0.56 (0.45–0.70)	<0.0001
Ever breastfed and used OCs	416/1324	0.45 (0.37–0.55)	<0.0001	0.47 (0.37–0.58)	<0.0001
BRCA1 mutation carriers					
Never breastfed/never OC user	263/532	1.00 (reference)		1.00 (reference)	
Ever breastfed *only*	485/1254	0.66 (0.53–0.82)	0.0001	0.69 (0.55–0.88)	0.0002
Ever OC use *only*	242/662	0.60 (0.47–0.76	<0.0001	0.60 (0.47–0.77)	<0.0001
Ever breastfed and used OCs	322/1010	0.48 (0.38–0.59)	<0.0001	0.50 (0.39–0.65)	<0.0001
BRCA2 mutation carriers					
Never breastfed/never OC user	63/118	1.00 (reference)		1.00 (reference)	
Ever breastfed *only*	56/137	0.63 (0.39–1.02)	0.06	0.58 (0.34–0.96)	0.03
Ever OC use *only*	93/273	0.44 (0.28–0.71)	0.0007	0.44 (0.27–0.70)	0.0005
Ever breastfed and used OCs	94/314	0.37 (0.23–0.58)	<0.0001	0.34 (0.21–0.56)	<0.0001

[a] Total = case and control subjects.
[b] Mutually adjusted for all characteristics in column 1.
[c] OC = oral contraceptive use.

high levels of synthetic progestins that are more potent than natural progesterone [14].

There are many hormonal changes that take place during pregnancy and breastfeeding. Less is known about other hormones closely related to milk production and secretion, particularly prolactin and oxytocin and cancer risk [15,21]. To our knowledge there have been no large-scale analyses of circulating levels of these hormones and ovarian cancer risk.

There is a great deal of support for the hypothesis that the fallopian tube is the cell of origin for a large proportion of high-grade serous cancers [22–24] and that pre-malignant ovarian cancer cells reside predominantly in the fallopian tube. In the current study, we excluded cases of fallopian tube cancer because these were too few in number to consider them separately and for most women diagnosed with ovarian cancer early in the study, pathological examinant of the fallopian tube was not done. In a previous study, we reported that breastfeeding was not a risk factor for fallopian tube cancer although this was based on a small number of cases [25]. In contrast, a recent report of women undergoing preventive bilateral salpingo-oophorectomy found that the presence of a fallopian tube lesion (including STIC, STIL, or invasive carcinoma) correlated with breastfeeding duration (7.4 vs. 14.5 months for those with a lesion versus without; $P = 0.07$) [26]. This is a topic of further interest.

To our knowledge, this represents the largest evaluation of breastfeeding and risk of ovarian cancer among women with a *BRCA1* or *BRCA2* mutation. Women from our earlier report also were included in the current study; however, we have expanded the number of cases from 1329 to 1650 and have conducted a more detailed analysis [6]. This sample size allowed us to perform relevant subgroup analyses. Our statistical approach was robust given the similarity in the univariate and multivariate risk estimates. One other study also reported an inverse, albeit not significant association, that was based on a small number of cases ($n = 253$) [27].

There are several limitations to the study. Although based on self-report, recollection of breastfeeding and other reproductive factors has previously been demonstrated to be highly accurate [28–30]. Furthermore, recall bias is unlikely given that women with a germline *BRCA* mutation are unlikely to attribute their heightened risk of developing ovarian cancer to reproductive choices such as parity and breastfeeding. We did not have information on histologic subtype for a large proportion of cases; however *BRCA*-associated ovarian cancers tend to be high-grade serous subtypes [31]. Finally, infertility per se was not considered; however, we previously reported low prevalence of treatment for infertility in our cohort of *BRCA* mutation carriers [32].

The results of this study suggest a strong, protective role of breastfeeding in women with a germline *BRCA1* or *BRCA2* mutation that appears to be independent of oral contraceptive use. It is important to uncover the underlying mechanism for this effect for the development of targeted prevention options.

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Author contribution

JK and SN conceived the study. PS was responsible for the statistical analysis. SN and JK drafted the manuscript. All authors were involved in participant enrollment and data collection. All authors reviewed the final manuscript.

Declaration of Competing Interest

All the authors declare that they have no conflict of interest.

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