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Breastfeeding: a reproductive factor able to reduce the risk of luminal B breast cancer in premenopausal White women

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In the medical literature, the role of breastfeeding and reproductive factors in the risk of breast carcinoma is still an open debate in premenopausal women. We highlight the role of breastfeeding and reproductive factors in luminal A and luminal B, the most frequent breast cancers. This case–control study analyzes a White premenopausal population of 286 breast cancer patients, divided into molecular subtypes, and 578 controls matched by age. Multivariate logistic regression models were used to assess the relationships of breastfeeding and other reproductive factors (age at menarche, parity, age at first pregnancy, number of children) with the risk of breast cancers. Among the variables examined, reproductive factors did not alter the risk of cancer, whereas breastfeeding up to 12 months was a significant protective factor against luminal B breast cancer (multivariate odds ratio: 0.22, 95% confidence interval: 0.09–0.59, P = 0.002). In contrast, luminal A cases did not significantly correlate with breastfeeding or other reproductive factors. Breastfeeding up to 12 months is strongly protective against the more aggressive luminal B, but not against the less aggressive luminal A breast cancer in premenopausal White women. *European Journal of Cancer Prevention* 26:217–224 Copyright © 2017 The Author(s). Published by Wolters Kluwer Health, Inc.

Introduction

Breast cancer is the leading cause of cancer death among women worldwide (Siegel *et al.*, 2015). Several studies and meta-analyses have shown that reproductive factors are associated with the risk of breast cancer (Kelsey *et al.*, 1993; Yang and Jacobsen, 2008; Butt *et al.*, 2014). The underlying biological mechanisms are not yet clearly understood, but epidemiological evidence indicates that early menarche, older age at first full-term pregnancy, low parity, and lack of breastfeeding increase the risk of breast cancer (Kelsey *et al.*, 1993; Collaborative Group on Hormonal Factors in Breast Cancer, 2002).

A protective effect of breastfeeding has been indicated (Collaborative Group on Hormonal Factors in Breast Cancer, 2002; Ursin *et al.*, 2005; Ma *et al.*, 2006; Horn *et al.*, 2014), but the majority of published findings often present results for a mix of both premenopausal and postmenopausal women (Ursin *et al.*, 2005; Butt *et al.*, 2014) or only for postmenopausal women (Phipps *et al.*, 2008; Horn *et al.*, 2014). Recently, several studies have assessed the role of breastfeeding and other reproductive factors in different breast cancer subtypes, suggesting that the association of reproductive factors with the risk of breast cancer may vary according to breast cancer molecular profiles (Ursin *et al.*, 2005; Phipps *et al.*, 2008; Horn *et al.*, 2014). The identification of which factors are related to the risk of breast cancer in populations of women identified by menopausal status, age, race, and molecular cancer subtypes still deserves further investigation (Warner *et al.*, 2013; Horn *et al.*, 2014). In particular, it remains to be clarified whether breastfeeding could exert a protective effect on breast cancers in premenopausal women (Negri *et al.*, 1996; Ferlay *et al.*, 2013; Butt *et al.*, 2014).

This study investigates in a case–control population of White premenopausal women living in a country with a high breast cancer incidence the role of breastfeeding and reproductive factors on the risk of breast cancer stratified into molecular cancer subtypes. In particular, we analyzed the luminal breast cancers, accounting for about 75% of all breast cancers (Azim and Partridge, 2014; Rotunno *et al.*, 2014).

Patients and methods
Study cohort

We selected a homogeneous population of White premenopausal women living for at least 10 years in Trieste (Friuli-Venezia Giulia), an Italian region with the highest incidence of breast cancer: estimated age-standardized

rates (ASRs) for Europe 94.2, ASRs for Italy 118.0, and ASRs for Friuli-Venezia Giulia 129.1 (European standard) (Ferlay et al., 2013; Zucchetto et al., 2013).

Premenopausal women were enrolled by Azienda Ospedaliera 'Ospedali Riuniti' of Trieste (Italy) between January 2006 and January 2014. The case patients had received a histopathologically confirmed first diagnosis of primary breast cancer between 2006 and 2013. The control participants were women who spontaneously joined breast cancer-prevention campaigns between 2009 and 2014; they had no breast diseases, which was confirmed by medical records. The cohort of women between 21 and 49 years of age were premenopausal (regular menstrual flow in the last year at the interview).

Cases were age adjusted and matched with two controls with an age interval of 5 years. The Mann–Whitney test confirmed the pairing.

Data collection questionnaire

Women were invited to fill out a questionnaire on demographics, reproductive history, breast pathology, BMI, and lifestyle. Reproductive history included questions on age an menarche, parity (defined by the number of full-term pregnancies), age at first full-term pregnancy, overall duration of breastfeeding (≤ 12 months, > 12 months), and menopausal status.

Questions on familial breast cancer history were included according to NICE guidelines (National Institute for Health and Care Excellence Pathway, created: October 2011, last updated: December 2013). Consent to the processing of data in an anonymous form for clinical research, epidemiology, and training (and with the aim of improving knowledge, treatment and prevention), in agreement with the Italian law art.81 D.lgs 196/2003, sez. D, was obtained at the diagnosis of cases by the hospital. For each case and control, we obtained a signed informant consent during the interview to use the data in an anonymous form, in agreement with the Italian law art.81 D.lgs 196/2003, sez. D.

Physicians always conducted the structured questionnaire and the interviews lasted about 20 min.

Data collection on breast cancer and molecular profiles

Histopathological features and data on estrogen receptor (ER), progesterone receptor (PR), and HER2 expression status were obtained from records. All invasive tumors were re-evaluated by pathologists to confirm aggressiveness and tumor type according to the WHO classification. Classification of molecular subtypes takes into account the expression of ER, PR, HER2, and Ki-67 according to St. Gallen 2013 (Goldhirsch et al., 2013).

All tests of ER, PR, and HER2 expression were based on immunohistochemistry testing. In equivocal HER2 cases, data from fluorescent in-situ hybridization assay records were used for molecular stratification. Patients with incomplete data on tumor markers were excluded from the study population. Breast cancer subtypes were grouped into the following categories: in-situ carcinomas, luminal A cases (ER + /PR + /HER2 −, Ki-67 < 20%), luminal B cases (luminal B HER2 −: HER + /HER2 − and at least one of Ki-67 ≥ 20% or PR −, luminal B HER2 +: ER + /HER2 + /any Ki-67/any PR), HER2 + (ER − /PR − /HER2 +), and triple-negative breast cancers (ER − /PR − /HER2 −).

The Ki-67 protein was evaluated on formalin-fixed tissue samples by the Joint Commission Accredited Hospital Laboratory using an automatic stainer (Lab Vision; Thermo Scientific, Runcorn, UK). The reaction with monoclonal antibody clone MIB-1 (Bio-Optica MILANO S.p.A, Milan, Italy; 1 : 200 dilution) was followed by HRP–DAB staining [UltraVision LP (Thermo Scientific); and DAB Quanto (Thermo Scientific)].

The HER2 monoclonal antibody was clone CB-11 (1 : 300 dilution; Aczon Biotech S.p.A, Bologna, Italy); the reactivity was evaluated using the detection kit HRP–DAB (Thermo Scientific). Every slide was evaluated by a pathologist on an optical microscope with a low magnification (×10) and classified as positive, negative, or equivocal, in agreement with the guidelines of the American Society of Clinical Oncology and College of American Pathologists (ASCO-CAP, 2006; Wolff et al., 2007).

Statistical analysis

Continuous variables (age, age at menarche, and age at first full pregnancy) were analyzed using the Mann–Whitney test. In the univariate analysis, associations between reproductive factors and breast cancer were evaluated using the χ^2-test. Multiple logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) as measures of the relative risk of cancers adjusted for age, age at menarche, BMI, oral contraceptive use, benign breast disease, and family history of breast cancer.

We fited different multivariate models: all cancer cases (independent of molecular subtype) versus controls, and luminal A and luminal B cases versus controls (selected at random within 5-year age strata among 578 controls of the study).

Statistical analyses were carried out using R (the R Foundation for Statistical Computing; Version 3.0.3) and P-values less than 0.05 were considered statistically significant.

Results

Study population

The study population included a total of 864 premenopausal White women: 286 breast cancer patients diagnosed between 2006 and 2013 and 578 control

participants selected between 2009 and 2014. The age of the patients ranged from 21 to 49 years (median age: 44.5 years, interquartile range: 41–49), and that of control participants ranged from 23 to 49 years (median age: 44 years, interquartile range: 41–49).

Pairing showed no differences with respect to each 5-year age group ($P = 0.90$).

Reproductive factors and cancer risk in premenopausal women

As shown in Table 1, reproductive factors did not significantly relate to the risk of breast cancer in univariate analysis. The mean age at menarche was 12.33 ± 1.49 years in the case group and 12.51 ± 1.58 years in the control group, with no differences on statistical analysis ($P = 0.11$); the number of births was similar in the two groups: 69.2% of parous women in the case group and 67.5% of parous women in the control group ($P = 0.60$). The mean age at first full-term pregnancy was 28.88 ± 5.51 years among the patients and 28.78 ± 5.25 years among the control participants ($P = 0.69$). Therefore, the above-mentioned reproductive factors were not associated with the risk of breast cancer in premenopausal women.

Breastfeeding and cancer risk in premenopausal women

The distribution of risk factors among premenopausal women with breast cancers compared with the controls is shown in Table 1. In the multivariate analysis, parous women who breastfed for up to 12 months were at a lower risk of developing breast cancer (estimated OR: 0.41, 95% CI: 0.24–0.70, $P = 0.0009$). Women who had breastfed for more than 12 months showed an increase in the risk of breast cancer (OR: 2.32, 95% CI: 1.26–4.34, $P = 0.007$). The other reproductive factors were not significantly related to the risk of breast cancers (Table 2).

Reproductive factors and molecular subtypes of breast cancer

To determine whether reproductive factors were associated with different molecular subtypes of breast cancer, 280 out of 286 breast cancer archive tissue samples were analyzed: 21 (8%) in-situ carcinoma, 117 (42%) luminal A tumors, 94 (33%) luminal B tumors, 15 (5%) HER2 + tumors, and 33 (12%) triple-negative cancers. We excluded six cases: two because of missing data and four because they did not fulfill the St. Gallen classification criteria. For Ki-67 status, we chose the threshold greater than or equal to 20%, which is used widely by pathologists (Goldhirsch *et al.*, 2013). We compared this classification of our cohort with that suggested by Maisonneuve *et al.* (2014): only one of our cases of luminal A was classifiable as luminal B and one of our cases of luminal B was classifiable as luminal A. Thus, our classification of the cohort did not differ significantly from that suggested by Maisonneuve *et al.* (2014). Moreover, in the analysis, patients with in-situ carcinoma

Table 1 Distribution of risk factors among women with breast cancer (cases) and unaffected women (controls)[a]

Characteristics	Women with breast cancer (286 cases)	Unaffected women (578 controls)	P_{trend}
Age			
Mean ± SD	43.3 ± 4.8	43.5 ± 4.23	0.90
Median (interquartile range)	44.5 (41–49)	44.0 (41–49)	
Range	21–49	23–49	
Parity [n (%)]			0.60
Nulliparous	88 (30.8)	188 (32.5)	
Parous	198 (69.2)	390 (67.5)	
Age at menarche [n (%)]			
Mean ± SD	12.3 ± 1.5	12.5 ± 1.6	0.11
< 12	80 (28.5)	134 (23.4)	0.33
12	80 (28.5)	158 (27.6)	
13	63 (22.4)	149 (26.0)	
≥ 14	58 (20.6)	132 (23.0)	
BMI (kg/m²) [n (%)]			0.34
< 18.5	14 (5.0)	36 (6.3)	
18.5–24.9	199 (71.3)	407 (70.7)	
25–29.9	38 (13.6)	92 (16.0)	
≥ 30	28 (10.0)	41 (7.0)	
Oral contraceptive use [n (%)]			0.60
Never	169 (61.2)	324 (58.0)	
Current (at least for 5 years)	34 (12.3)	69 (12.3)	
Former (at least for 5 years)	73 (26.5)	166 (29.7)	
Parity [n (%)]			
Number of births (mean ± SD)	1.7 ± 0.7	1.7 ± 0.8	0.60
0	88 (30.8)	188 (32.5)	0.59
1	88 (30.8)	174 (30.1)	
2	87 (30.4)	183 (31.7)	
≥ 3	23 (8.0)	33 (5.7)	
Benign breast disease [n (%)]			0.02*
Yes	17 (5.9)	16 (2.8)	
No	269 (94.1)	562 (97.2)	
Family history of breast cancer			0.14
None	160 (58.2)	350 (65.1)	
First-degree familial (< 50 years)	24 (8.7)	39 (7.3)	
Second-degree familial	91 (33.1)	148 (27.6)	

Only parous women	Women with breast cancer (198 cases)	Unaffected women (390 controls)	P_{trend}
Age at first pregnancy [n (%)]			
Mean ± SD	28.9 ± 5.5	28.7 ± 5.3	0.51
< 25	46 (23.2)	92 (23.8)	0.85
25–29	58 (29.3)	120 (31.1)	
≥ 30	94 (47.5)	174 (45.1)	
Breastfeeding [n (%)]			0.13
Yes	160 (80.8)	334 (85.6)	
No	38 (19.2)	56 (14.4)	
Breastfeeding duration [n (%)]			< 0.001*
≤ 12 months		91 (45.0)	
		292 (74.9)	
> 12 months	69 (34.8)	42 (10.7)	
No	38 (19.2)	56 (14.4)	

[a]Numbers do not add up to the total because of missing values.
*Statistically significant.

and nonluminal breast cancer were not included because very few patients had these cancers.

We compared luminal A cases and luminal B cases separately with a group of controls selected among 578

Table 2 Multivariable-adjusted odds ratios for breast cancer (only parous women)

	Women with breast cancer (198 cases) Unaffected women (390 controls)		
Characteristics	OR[a]	95% CI	P-value
Age at menarche			
< 12	1.01	0.60–1.69	0.97
12	1	Reference	–
13	0.87	0.51–1.51	–
≥ 14	0.81	0.45–1.43	–
BMI (kg/m^2)			
< 18.5	0.5	0.17–1.29	0.18
18.5–24.9	1	Reference	–
25–29.9	0.68	0.37–1.19	0.18
≥ 30	1.25	0.58–2.62	0.56
Oral contraceptive use			
Never	1.16	0.75–1.83	0.51
Current (at least for 5 years)	1	Reference	–
Former (at least for 5 years)	1.15	0.59–2.22	0.68
Benign breast disease			
No	1	Reference	–
Yes	1.96	0.77–4.88	0.22
Family history of breast cancer			
None	1	Reference	–
First-degree familial (< 50 years)	1.37	0.64–2.83	0.40
Second-degree familial	1.31	0.84–2.02	0.23
Breastfeeding duration			
No	1	Reference	–
≤ 12 months	0.41	0.24–0.70	< 0.001*
> 12 months	2.32	1.26–4.34	0.007*

CI, confidence interval; OR, odds ratio.
[a]Odds ratios are adjusted for age, age at menarche, BMI, oral contraceptive use, benign breast disease, and family history of breast cancer.
*Statistically significant.

Table 3 Distribution of risk factors among women with luminal A breast cancer and unaffected women[a]

Characteristics	Women with luminal A breast cancer (N = 117)	Unaffected women (controls) (N = 234)	P$_{trend}$
Age			
Mean ± SD	44.4 ± 4.4	44.0 ± 4.2	
Median (interquartile range)	45 (43–48)	45 (42–47)	0.37
Range	25–49	23–49	
Parity [n (%)]			0.29
Nulliparous	32 (27.4)	77 (32.9)	
Parous	85 (72.6)	157 (67.1)	
Age at menarche [n (%)]			
Mean ± SD	12.4 ± 1.5	12.3 ± 1.5	0.73
< 12	35 (30.2)	63 (27.0)	0.20
12	27 (23.4)	64 (27.5)	
13	27 (23.4)	59 (25.3)	
≥ 14	27 (23.4)	47 (20.2)	
BMI (kg/m^2) [n (%)]			0.75
< 18.5	7 (6.1)	13 (5.6)	
18.5–24.9	82 (71.3)	160 (68.4)	
25–29.9	15 (13.0)	41 (17.5)	
≥ 30	11 (9.6)	20 (8.5)	
Oral contraceptive use [n (%)]			0.19
Never	64 (57.1)	137 (60.9)	
Current (at least for 5 years)	18 (16.1)	21 (9.3)	
Former (at least for 5 years)	30 (26.8)	67 (29.8)	
Parity [n (%)]			
Number of births (mean ± SD)	1.7 ± 0.7	1.6 ± 0.6	0.65
0	32 (27.4)	77 (32.9)	0.52
1	39 (33.3)	35 (32.5)	
2	36 (30.8)	31 (29.5)	
≥ 3	10 (8.5)	9 (5.2)	
Benign breast disease [n (%)]			0.19
Yes	7 (6.0)	7 (3.0)	
No	110 (94.0)	227 (97.0)	
Family history of breast cancer [n (%)]			0.22
First-degree familial (< 50 years)	7 (6.3)	19 (9.5)	
Second-degree familial	43 (38.4)	59 (29.5)	
None	62 (55.4)	122 (61.0)	

Only parous women	Women with luminal A breast cancer (N = 85)	Unaffected women (controls) (N = 157)	P$_{trend}$
Age at first pregnancy [n (%)]			
Mean ± SD	29.7 ± 5.7	28.7 ± 5.2	0.59
< 25	16 (18.8)	36 (23.4)	0.30
25–29	22 (25.9)	49 (31.8)	
≥ 30	47 (55.3)	69 (44.8)	
Breastfeeding [n (%)]			0.90
Yes	72 (84.7)	132 (84.0)	
No	13 (15.3)	25 (16.0)	
Breastfeeding duration [n (%)]			< 0.001*
≤ 12 months	46 (54.1)	115 (73.2)	
> 12 months	26 (30.6)	17 (10.8)	
No	13 (15.3)	25 (16.0)	

[a]Numbers do not add up to the total because of missing values.
*Statistically significant.

negative women of the study, respectively, always matched by age. Tables 3 and 4 show the distribution of risk factors among luminal A and luminal B cases compared with the controls. In the multivariate analysis, reproductive factors were not correlated significantly with luminal A cancers (Table 5). In contrast, breastfeeding for up to 12 months did show a significant protective role for luminal B breast cancers (OR: 0.22, 95% CI: 0.09–0.59, $P = 0.002$), and, more interestingly, it was the only reproductive factor that was statistically significant in the multivariate analysis (Table 6). However, breastfeeding for more than 12 months was not a protective factor in both luminal A and luminal B molecular subtypes (NS).

Discussion

Our study analyzed the role of breastfeeding and reproductive factors in the risk of breast cancer in a homogenous selected population of White premenopausal women living in a country with a high risk of breast cancer, focusing on luminal molecular phenotypes, the most frequent cancers occurring in the population sample (75%).

Parity was shown to be a protective factor for breast cancer in postmenopausal women, but not in premenopausal women (Clavel-Chapelon, 2002). Accordingly, our results confirm that in premenopausal women, the parity did not reduce the risk of breast cancer. In contrast to the results reported by other authors, we cannot conclude that the lack of a protective effect of multiparity is because of the low

Table 4 Distribution of risk factors among women with luminal B breast cancer and unaffected women[a]

Characteristics	Women with luminal B breast cancer (N = 94)	Unaffected women (controls) (N = 188)	P_trend
Age			
Mean ± SD	42.8 ± 4.9	42.8 ± 5.4	
Median (interquartile range)	44 (40–46)	44 (41–47)	0.72
Range	21–49	21–49	
Parity [n (%)]			0.21
Nulliparous	27 (28.7)	68 (36.2)	
Parous	67 (71.3)	120 (63.8)	
Age at menarche [n (%)]			
Mean ± SD	12.4 ± 1.5	12.5 ± 1.5	0.28
< 12	24 (26.1)	41 (21.8)	0.51
12	29 (31.5)	57 (30.3)	
13	18 (19.6)	52 (27.7)	
≥ 14	21 (22.8)	38 (20.2)	
BMI (kg/m^2)			0.58
< 18.5	4 (4.3)	15 (7.9)	
18.5–24.9	66 (71.7)	134 (71.3)	
25–29.9	14 (15.2)	28 (14.9)	
≥ 30	8 (8.8)	11 (5.9)	
Oral contraceptive use			0.06
Never	60 (65.9)	93 (51.1)	
Current (at least for 5 years)	9 (9.9)	31 (17.0)	
Former (at least for 5 years)	22 (24.2)	58 (31.9)	
Parity			
Number of births (mean ± SD)	1.7 ± 0.7	1.7 ± 0.6	0.80
0	27 (28.7)	68 (36.2)	0.25
1	31 (33.0)	44 (23.4)	
2	28 (29.8)	64 (34.0)	
≥ 3	8 (8.5)	12 (6.4)	
Benign breast disease [n (%)]			0.38
Yes	3 (3.2)	3 (1.6)	
No	91 (96.8)	185 (98.4)	
Family history of breast cancer [n (%)]			0.26
First-degree familial (< 50 years)	7 (7.7)	15 (8.0)	
Second-degree familial	27 (29.7)	42 (22.3)	
None	57 (62.6)	131 (69.7)	

Only parous women	Women with luminal B breast cancer (N = 67)	Unaffected women (controls) (N = 120)	P_trend
Age at first pregnancy [n (%)]			
Mean ± SD	28.2 ± 5.3	28.4 ± 4.9	0.65
< 25	16 (22.9)	33 (27.5)	0.75
25–29	24 (35.8)	37 (30.8)	
≥ 30	27 (40.3)	50 (41.7)	
Breastfeeding [n (%)]			0.01*
Yes	51 (76.1)	108 (90.0)	
No	16 (23.9)	12 (10.0)	
Breastfeeding duration [n (%)]			< 0.001*
≤ 12 months	25 (37.3)	97 (80.8)	
> 12 months	26 (38.8)	11 (9.2)	
No	16 (22.9)	12 (10.0)	

[a]Numbers do not add up to the total because of missing values.
*Statistically significant.

Table 5 Multivariable-adjusted odds ratios for breast cancer (only parous women)

Characteristics	Women with luminal A breast cancer (N = 85) Unaffected women (controls) (N = 157)		
	OR[a]	95% CI	P-value
Age at menarche			
< 12	1.27	0.57–2.85	0.56
12	1	Reference	–
13	0.95	0.39–2.3	0.90
≥ 14	1.1	0.48–2.73	0.84
BMI (kg/m^2)			
< 18.5	0.73	0.14–3.0	0.67
18.5–24.9	1	Reference	–
25–29.9	0.46	0.16–1.15	0.11
≥ 30	1.05	0.32–3.15	0.93
Oral contraceptive use			
Never	1.13	0.57–2.25	0.73
Current (at least for 5 years)	1	Reference	–
Former (at least for 5 years)	2.09	0.74–6.0	0.16
Benign breast disease			
No	1	Reference	–
Yes	3.64	0.92–5.0	0.06
Family history of breast cancer			
None	1	Reference	–
First-degree familial (< 50 years)	0.82	0.23–2.60	0.74
Second-degree familial	1.2	0.61–2.35	0.58
Breastfeeding duration			
No	1	Reference	–
≤ 12 months	0.55	0.18–1.65	0.28
> 12 months	2.43	0.87–7.01	0.09

CI, confidence interval; OR, odds ratio.
[a]Odds ratios are adjusted for age, age at menarche, BMI, oral contraceptive use, benign breast disease, and family history of breast cancer.

cancer was found both for multiparity and for age at menarche. Interestingly, familial history of breast cancer of both first and second grade in our cohort of premenopausal women was not significantly related to the risk of breast cancer. This can be attributed to the lack of a significant number of cases with first-grade familial history of breast or ovarian cancer at a young age, which is highly related to an increased risk of breast cancer (six cases).

Literature data show that reproductive factors are related to the molecular subtypes of breast cancer (Althuis *et al.*, 2004; Ursin *et al.*, 2005; Tamimi *et al.*, 2012); hormonal factors have a greater impact on luminal than nonluminal types of breast cancer, the risk being predominantly influenced by hormonal mechanisms (Anderson, 2002; Dickson and Stancel, 2000; Anderson *et al.*, 2014). Hormonal risk factors such as parity and early age at first birth seem to relate positively to luminal subtypes compared with nonluminal tumors (Ma *et al.*, 2006). Previous studies have suggested a protective effect of multiparity and young age at first full-term pregnancy on breast cancer, but this protective effect appears to be different among the various breast cancer receptor statuses: multiparity seems to exert a protective effect on ER + /PR + tumors, but not on receptor-negative ones (Yoo *et al.*, 1997; Cotterchio *et al.*, 2003; Ursin *et al.*, 2005; Kwan *et al.*, 2009). Recently, a large population-based prospective

number of multiparous young women: the births were not correlated to the risk of breast cancer in a statistically significant sample of multiparous women. Moreover, we found that younger age at first full-term pregnancy did not confer protection; no significant increase in the risk of

Table 6 Multivariable-adjusted odds ratios for breast cancer (only parous women)

| Characteristics | Women with luminal B breast cancer (N = 67) Unaffected women (controls) (N = 120) | | |
	OR[a]	95% CI	P-value
Age at menarche			
<12	0.99	0.38–2.60	0.99
12	1	Reference	–
13	0.81	0.28–2.29	0.70
≥14	1.17	0.42–3.24	0.76
BMI (kg/m^2)			
<18.5	0.33	0.04–1.84	0.25
18.5–24.9	1	Reference	–
25–29.9	0.91	0.32–2.39	0.84
≥30	1.20	0.32–4.49	0.78
Oral contraceptive use			
Never	1.42	0.61–3.28	0.42
Current (at least for 5 years)	1	Reference	–
Former (at least for 5 years)	0.62	0.17–2.05	0.44
Benign breast disease			
No	1	Reference	–
Yes	1.52	0.15–11.60	0.69
Family history of breast cancer			
None	1	Reference	–
First-degree familial (<50 years)	1.28	0.33–4.59	0.70
Second-degree familial	1.29	0.55–2.96	0.54
Breastfeeding duration			
No	1	Reference	–
≤12 months	0.22	0.09–0.59	0.002*
>12 months	2.15	0.70–6.78	0.18

CI, confidence interval; OR, odds ratio.
[a]Odds ratios are adjusted for age, age at menarche, BMI, oral contraceptive use, benign breast disease, and family history of breast cancer.

study with long-term follow-up (Horn *et al.*, 2014) confirmed that the associations of reproductive factors with the risk of breast cancer may vary according to different molecular subtypes. They found that early menarche, older age at first birth, and low parity were associated with an increased risk for luminal breast cancer, whereas for the nonluminal type, there were no associations.

We found that age at menarche, parity, and age at first full-term pregnancy did not affect luminal A and luminal B breast cancers in our cohort of premenopausal women.

Reports of large series observed a protective effect in the cohorts of both premenopausal and postmenopausal women (Ursin *et al.*, 2005; Kobayashi *et al.*, 2012; Anderson *et al.*, 2014). In the population of premenopausal women, breastfeeding was found to be protective in some studies (Collaborative Group on Hormonal Factors in Breast Cancer, 2002; Shema *et al.*, 2007; Nelson *et al.*, 2012; Warner *et al.*, 2013; Trentham-Dietz *et al.*, 2014), but not in others (Tessaro *et al.*, 2003; Shantakumar *et al.*, 2007; Butt *et al.*, 2014). Several studies (Phipps *et al.*, 2008; Butt *et al.*, 2014; Horn *et al.*, 2014) and meta-analyses (Collaborative Group on Hormonal Factors in Breast Cancer, 2002; Althuis *et al.*, 2004) were published to better clarify the effect of breastfeeding and reproductive factors on the incidence

of different breast cancer subtypes, and the majority of published data were from postmenopausal women (Phipps *et al.*, 2008; Horn *et al.*, 2014) or a large study population of both premenopausal and postmenopausal breast cancer patients (Kelsey *et al.*, 1993; Ursin *et al.*, 2005).

On multivariate analysis, we found that breastfeeding up to 12 months reduced the risk of luminal B but not luminal A breast cancer in this selected population. Breastfeeding was found to protect against both ER +/ PR + and ER −/PR − tumors (Huang *et al.*, 2000a; Ursin *et al.*, 2005; Ma *et al.*, 2006; Kwan *et al.*, 2009). Other studies showed a reduced risk of basal-like triple negative breast cancer among women who breastfed for more than 4 months in African-American and White women, or in African-American women only (Millikan *et al.*, 2008; Palmer *et al.*, 2014). It is noteworthy that, in our study, among luminal cancers, only luminal B cancer was significantly related to breastfeeding. It might be conceivable, as suggested by several studies (Ma *et al.*, 2006; Phipps *et al.*, 2008; Kobayashi *et al.*, 2012), that breastfeeding acts through different hormonal mechanisms than parity, thus determining the risk for different subtypes of breast cancer. In this respect, it cannot be excluded that the same factors might also play a role in determining the tumor aggressiveness in those established cancers. However, we cannot support any conclusion on the incidence of HER2 + or triple-negative cancers and breastfeeding because of the low number of cases.

Unexpectedly, overall, breastfeeding for more than 12 months was not linked to breast cancer risk reduction in our population of premenopausal women compared with those who never breastfed, for both luminal A and B cancers (Tables 5 and 6). These findings seem to be in contrast to the results of a large meta-analysis (Collaborative Group on Hormonal Factors in Breast Cancer, 2002) and other reports (Shema *et al.*, 2007; Nelson *et al.*, 2012; Trentham-Dietz *et al.*, 2014) that indicate that the longer women breastfeed, the more they are protected against breast cancer. However, we would like to report observations of two other studies (Huang *et al.*, 2000b; Yang and Jacobsen, 2008) that did not show an association between increased duration of lactation and reduction of the risk of breast cancer. Moreover, Gustbee *et al.* (2013) reported a high risk for early events of breast cancer for a total duration of breastfeeding of more than 12 months, perhaps because of the proproliferation effect of prolactin. Furthermore, three case–control studies showed that longer breastfeeding duration was a risk factor for breast cancers: a multicenter case–control study carried out in Italy (Negri *et al.*, 1996), a Turkish study (Ulusoy *et al.*, 2010), and a recent Serbian study (Ilic *et al.*, 2015). A large Italian study (Negri *et al.*, 1996) included premenopausal and postmenopausal women and excluded any appreciable protective role for

lactation independent of the duration of breastfeeding. Ulusoy *et al.* (2010) investigated a population of both premenopausal and postmenopausal Turkish women, showing that a 10-month increase in breastfeeding increased the risk of breast cancer by 8% (OR: 1.008, 95% CI: 1.003–1.012, $P = 0.0001$). Nevertheless, this study does not indicate whether the OR has been adjusted for the other factors analyzed in a multivariate manner. Finally, Ilic *et al.* (2015) reported that in women living in Serbia, the risk of breast cancer increases with the duration of breastfeeding (OR: 3.44, 95% CI: 1.15–10.24, $P = 0.023$; for women who breastfed >13 months). Also, in this case, a sample of both premenopausal and postmenopausal women was analyzed. Our analysis indicates that breastfeeding for more than 12 months increases the risk of breast cancer, considering all breast cancer cases, but we cannot conclude on a general negative relation because of the fact that significance was not achieved on stratifying luminal A and luminal B cases.

In contrast to the results reported by these authors (Negri *et al.*, 1996; Ulusoy *et al.*, 2010; Ilic *et al.*, 2015), we found lactation up to 12 months to be a protective factor for luminal B cancer and our study showed some differences: (a) our cohort was homogeneous and included premenopausal White women; (b) our results were obtained by matching one case with two controls (1 : 2 case–control study); and (c) our study considered the molecular phenotype of breast cancers. It is noteworthy that we did not observe a reduction in the risk of breast cancer in relation to breastfeeding, but a less aggressive cancer in breastfeeding women that depends on the duration of lactation. In women with more than 12 months of lactation, the lack of breastfeeding protection was not expected. As in the majority of the literature, we considered the total duration of breastfeeding derived from the sum of the breastfeeding months for all children (if more than one) for each woman. To clarify this point, we analyzed whether there is a difference related to more than 12 months of lactation in women with one child (88 cases/ 174 controls). The results confirmed that breastfeeding for more than 12 months was not protective in multivariate analysis. The same results were found for the case group of women with two children. In this respect, we underline that, in our cohort of cases, the majority of women had one or two children (175 cases/357 controls). Thus, the results obtained on the total duration of breastfeeding appear to be consistent. We cannot exclude that breastfeeding for more than 12 months might be a protective factor or might contribute toward increasing the risk of breast cancer depending on the type of population analyzed (i.e. race). Although overall observations showed that breastfeeding for more than 12 months was not protective, further investigations on a higher number of cases are necessary.

In conclusion, this study of a homogeneous cohort of White premenopausal women showed no statistical associations between age at menarche, parity, age at first birth, and number of births and the risk of breast cancer. In contrast, the study supports evidence that, among luminal breast cancers, breastfeeding for up to 12 months is strongly protective against the more aggressive luminal B rather than the less aggressive luminal A type.

Conflicts of interest
There are no conflicts of interest.

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