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Factors influencing ovulation and the risk of ovarian cancer in *BRCA1* and *BRCA2* mutation carriers

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The role of the lifetime number of ovulatory cycles has not been evaluated in the context of *BRCA*-associated ovarian cancer. Thus, we conducted a matched case–control study to evaluate the relationship between the cumulative number of ovulatory cycles (and contributing components) and risk of developing ovarian cancer in *BRCA* mutation carriers (1,329 cases and 5,267 controls). Information regarding reproductive and hormonal factors was collected from a routinely administered questionnaire. Conditional logistic regression was used to evaluate all associations. We observed a 45% reduction in the risk of developing ovarian cancer among women in the lowest *vs*. highest quartile of ovulatory cycles (OR = 0.55; 95% CI 0.41–0.75, $p = 0.0001$). Breastfeeding for more than 12 months was associated with a 38% (95% CI 0.48–0.79) and 50% (95% CI 0.29–0.84) reduction in risk among *BRCA1* and *BRCA2* mutation carriers, respectively. For oral contraceptive use, maximum benefit was seen with five or more years of use among *BRCA1* mutation carriers (OR = 0.50; 95% CI 0.40–0.63) and three or more years for *BRCA2* mutation carriers (OR = 0.42; 95% CI 0.22–0.83). Increasing parity was associated with a significant inverse trend among *BRCA1* (OR = 0.87; 95% CI 0.79–0.96; *p*-trend = 0.005) but not *BRCA2* mutation carriers (OR 0.98; 95% CI 0.81–1.19; *p*-trend = 0.85). A later age at menopause was associated with an increased risk in women with a *BRCA1* mutation (OR trend = 1.18; 95% CI 1.03–1.35; $p = 0.02$). These findings support an important role of breastfeeding and oral contraceptive use for the primary prevention of ovarian cancer among women carrying *BRCA* mutations.

Epidemiology

Key words: BRCA1, BRCA2, ovulation, ovarian cancer

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What's new?

The number of ovulatory cycles a woman has in her lifetime may influence her risk for ovarian cancer, though how cancer-associated *BRCA* mutations factor into that relationship remains uncertain. Here, ovarian cancer risk was found to be significantly reduced among *BRCA* mutation carriers in the lowest quartile of ovulatory cycles. Likewise, risk was reduced in women with *BRCA1* or *BRCA2* mutations who used oral contraceptives for five or three years, respectively, or who breastfed for more than 12 months. The findings lend support to the idea that factors that suppress or interrupt ovulation protect against *BRCA*-associated ovarian cancer.

Inherited mutations in the breast and ovarian cancer susceptibility genes *BRCA1* and *BRCA2* confer high lifetime risks of developing ovarian cancer, estimated at 40 and 20%, respectively, compared to <2% for women in the general population.[1–3] Women with *BRCA* mutations tend to develop high-grade serous ovarian cancers.[4] Given the high mortality rate associated with ovarian cancer, surgical bilateral salpingo-oophorectomy is currently recommended to women carrying *BRCA* mutations at age 35 to decrease the risks of both breast and ovarian cancer.[5]

Oral contraceptive use is the most effective, nonsurgical prevention option for this high-risk population.[6] We and others have reported an approximately 50% reduction in ovarian cancer risk with a history of oral contraceptive use, with 3–5 years of use offering the maximum level of protection.[7] A recent meta-analysis, which included our earlier study, reported a highly significant 42% reduction in *BRCA*-associated ovarian cancer risk with oral contraceptives use (95% CI 0.46–0.73).[6] This level of risk reduction is comparable to estimates reported among women in the general population.[8] There is also evidence to suggest protective roles of parity and breastfeeding for *BRCA1* mutation carriers but not for *BRCA2* mutation carriers.[7,9,10] In a recent meta-analysis, Friebel *et al.* concluded that the protective effects of breastfeeding and tubal ligation were limited to women with *BRCA1* mutations.[11]

Among women in the general population, several hypotheses regarding the pathogenesis of ovarian cancer have been proposed, including "incessant ovulation" whereby factors that suppress or interrupt ovulation (*i.e.*, pregnancy, breastfeeding and oral contraceptives) protect against ovarian cancer.[12–14] The "incessant ovulation" hypothesis, originally proposed by Fathalla in 1971, is supported by limited epidemiologic evidence of an inverse association between lifetime ovulatory cycles and ovarian cancer risk in the general population. Other suggested mechanisms include stimulation by hormonal exposures (including gonadotropins, estrogens, androgens, insulin and IGF-1), inflammation and retrograde transport of endogenous and/or exogenous carcinogens through the fallopian tubes (14-18). To our knowledge, the role of the lifetime number of ovulatory cycles has not been evaluated specifically in the context of *BRCA*-associated ovarian cancer. Thus, the goal of our study was to evaluate the relationship between the cumulative number of ovulatory cycles and the risk of developing ovarian cancer in *BRCA1* and *BRCA2* mutation carriers. We also update our analyses on the relationship between individual menstrual and reproductive factors that influence ovulation and may impact ovarian cancer risk.

Material and Methods

Study population

This study population, as well as the data and sample collection methodology, has previously been described in detail (see Refs. 7 and 15). Briefly, eligible study subjects were identified from 72 participating centers in 20 countries. These women were participants in research studies or sought testing for *BRCA1* and *BRCA2* mutations because of a personal or family history of breast and/or ovarian cancer. The institutional review boards of the host institutions approved the study. All subjects 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. Mutation detection was performed using a range of techniques, but all nucleotide sequences were confirmed by direct sequencing of DNA. A woman was eligible if she was a carrier of a deleterious mutation in the *BRCA1* or *BRCA2* gene.

Data collection

All study subjects completed baseline questionnaires at the individual centers at the time of clinic appointments or at their homes at a later date. The questionnaire requested information on family and personal histories of cancer, reproductive and medical histories, including preventive oophorectomy and mastectomy. Detailed information regarding ages at menarche and menopause, pregnancy, breastfeeding history and hormone use was also obtained. We also gathered information on menstrual cycle regularity. The majority of the participating centers (~95%) utilize the same questionnaire, while the remaining centers query information in a manner that easily allows for extraction of the exposure of interest. For each live birth, women were asked to indicate the number of months of breastfeeding for each child. We estimated the total duration of breastfeeding for each woman by summing the months of breastfeeding for each live birth. For oral contraceptive use, women were asked if they ever used birth control pills to prevent pregnancy or for any other reason. If they answered "yes," they were asked the start and end date (year) and duration of use (in months and years).

Table 1. Baseline characteristics of ovarian cancer cases and controls with *BRCA1* and *BRCA2* mutations

Characteristic	Controls ($n = 5,267$)	Cases ($n = 1,329$)	p
Year of birth, mean (range)	1949.4 (1916–1980)[1]	1949.6 (1913–1982)	0.57
Age of diagnosis, mean (range)	n/a	49.99 (20–70)	n/a
Age of interview, mean (range)	54.83 (28–85)[2]	53.85 (26–83)	0.22
Mutation, *n* (%)			
BRCA1	4,291 (82%)	1,095 (82%)	Matched
BRCA2	947 (18.5%)	233 (17.5%)	
BRCA1 and *2*	2 (0.04%)	1 (0.08%)	
Prior diagnosis of breast cancer, *n* (%)			
Yes	2,033 (38%)	334 (25%)	Matched
No	3,244 (62%)	995 (75%)	
Country of residence, *n* (%)			
United States	1,839 (35%)	407 (31%)	Matched
Poland	1,801 (34%)	406 (31%)	
Other Canada	997 (19%)	305 (23%)	
Norway	192 (4%)	64 (5%)	
Israel	123 (2%)	36 (3%)	
French Canadian (in Canada)[2]	121 (2%)	39 (3%)	
Netherlands	81 (2%)	18 (1%)	
Italy	43 (1%)	25 (2%)	
Austria	28 (1%)	8 (0.6%)	
France	16 (0.3%)	6 (0.5%)	
Sweden	14 (0.3%)	6 (0.5%)	
United Kingdom	10 (0.2%)	7 (0.5%)	
China	2 (0.04%)	2 (0.2%)	
Ethnicity, *n* (%)			
Other White	4,054 (77%)	991 (75%)	0.007
Jewish	933 (18%)	241 (18%)	
French-Canadian	145 (3%)	40 (3%)	
Other	135 (2%)	57 (4%)	

[1]For the control subjects, the mean represents the mean of all the controls for the corresponding matched set.
[2]Twenty-five French-Canadians lived in the United States.

Information on current use was also obtained. We limited this analysis to the use of birth control pills. Women were asked if they ever had a tubal ligation (*i.e.*, fallopian tubes tied) and the year of surgery. Age at menopause was based on the age at which a woman reported that her periods had completely ceased. The cumulative number of ovulatory cycles for each woman was estimated by using the following equations: (*i*) if premenopausal: ovulatory cycles = 12* [(current age for controls or age at diagnosis for cases) − age at menarche − years of oral contraceptive use − parity * 0.77 − years of breastfeeding)]; (*ii*) if postmenopausal, current age was replaced with age at menopause. Controls who underwent a hysterectomy (with or without a unilateral oophorectomy), salpingectomy or partial hysterectomy before the diagnosis of their matched case, and cases who underwent any of the aforementioned surgeries before their diagnosis were excluded from the analysis of ovulatory cycles.

Case and control subjects

Information on cancer status was available for a total of 14,536 women who carried a *BRCA1* or *BRCA2* mutation. Case subjects were women with a diagnosis of invasive epithelial ovarian cancer. Control subjects were women who never had ovarian cancer. Potential subjects were excluded if they had been diagnosed with a cancer other than breast or ovarian cancer ($n = 1,638$) or if information on their personal history of breast or ovarian cancer was missing ($n = 218$). Women were also excluded if pertinent information on oral contraceptive use or pregnancy history was missing ($n = 857$). Controls were excluded if they had missing

Table 2. Relationship between factors affecting ovulation and risk of ovarian cancer risk among *BRCA1* mutation carriers

Characteristic	Controls ($n = 4,291$)	Cases ($n = 1,095$)	Univariate OR (95% CI)	p	Multivariate OR (95% CI)[1]	p
Age at menarche (years)						
≤10	130 (3%)	40 (4%)	1.00 (reference)	0.40	1.00 (reference)	
11	367 (9%)	87 (9%)	0.82 (0.51–1.31)	0.42	0.82 (0.51–1.32)	0.42
12	830 (21%)	201 (20%)	0.84 (0.55–1.29)	0.32	0.82 (0.53–1.27)	0.37
13	1,094 (27%)	255 (26%)	0.81 (0.53–1.23)	0.45	0.80 (0.52–1.22)	0.30
≥14	1,583 (40%)	414 (42%)	0.85 (0.56–1.29)	0.98	0.84 (0.55–1.28)	0.42
Trend	3,874 (7%)	957 (96%)	1.00 (0.93–1.08)	0.36	1.00 (0.93–1.08)	0.98
≥11			0.83 (0.56–1.24)		0.82 (0.55–1.23)	0.34
Parity, *n* (%)						
Never	690 (16%)	152 (14%)	1.00 (reference)		1.00 (reference)	
Ever	3,601 (84%)	943 (86%)	0.68 (0.54–0.85)	0.0007	0.91 (0.68–1.21)	0.52
Parity (per birth)						
1	688 (16%)	172 (16%)	0.92 (0.70–1.21)	0.55	1.11 (0.82–1.52)	0.54
2	1,678 (39%)	437 (40%)	0.69 (0.54–0.87)	0.002	0.86 (0.64–1.17)	0.34
≥3	1,234 (29%)	334 (31%)	0.57 (0.44–0.73)	<0.0001	0.74 (0.53–1.02)	0.07
Trend			0.82 (0.76–0.88)	<0.0001	0.87 (0.79–0.96)	0.005
Age at first birth, *n* (%)[2]						
≤21	779 (28%)	276 (31%)	1.00 (reference)		1.00 (reference)	
22–24	826 (29%)	242 (28%)	0.88 (0.71–1.09)	0.25	0.84 (0.68–1.05)	0.13
25–27	565 (20%)	181 (21%)	0.96 (0.76–1.21)	0.71	0.90 (0.70–1.14)	0.37
>27	660 (23%)	180 (20%)	0.88 (0.69–1.14)	0.33	0.81 (0.62–1.05)	0.11
Trend			0.97 (0.89–1.05)	0.43	0.94 (0.86–1.02)	0.15
Age at last birth, *n* (%)[2]						
≤26	859 (30%)	301 (34%)	1.00 (reference)		1.00 (reference)	
27–30	842 (30%)	265 (30%)	0.91 (0.74–1.12)	0.36	0.95 (0.77–1.17)	0.63
31–33	555 (20%)	140 (16%)	0.72 (0.56–0.93)	0.01	0.78 (0.60–1.02)	0.07
>33	574 (20%)	173 (20%)	0.71 (0.55–0.90)	0.006	0.80 (0.61–1.04)	0.09
Trend			0.88 (0.82–0.95)	0.002	0.92 (0.84–1.00)	0.04
Breastfeeding, *n* (%)						
Never	1,285 (30%)	366 (33%)	1.00 (reference)		1.00 (reference)	
Ever	2,586 (60%)	604 (55%)	0.71 (0.60–0.84)	<0.0001	0.75 (0.61–0.93)	0.007
Missing	424 (10%)	125 (11%)				
Duration (months)						
≤12	1,597 (37%)	413 (38%)	0.79 (0.66–0.95)	0.01	0.83 (0.67–1.04)	0.10
>12	985 (23%)	191 (17%)	0.59 (0.48–0.73)	<0.0001	0.62 (0.48–0.79)	0.0002
OC use, *n* (%)						
Never	1,975 (46%)	636 (58%)	1.00 (reference)		1.00 (reference)	
Ever	2,361 (54%)	459 (42%)	0.57 (0.69–1.10)	<0.0001	0.60 (0.50–0.71)	<0.0001
Duration (years)						
<1	518 (12%)	145 (13%)	0.77 (0.60–0.98)	0.03	0.82 (0.64–1.05)	0.12
1–<3	434 (10%)	84 (8%)	0.53 (0.39–0.71)	<0.0001	0.56 (0.41–0.75)	<0.0001
3–<5	348 (8%)	67 (6%)	0.51 (0.37–0.70)	<0.0001	0.54 (0.39–0.75)	0.0002
≥5	995 (23%)	157 (14%)	0.49 (0.39–0.62)	<0.0001	0.50 (0.40–0.63)	<0.0001

Table 2. Relationship between factors affecting ovulation and risk of ovarian cancer risk among *BRCA1* mutation carriers (Continued)

Characteristic	Controls ($n = 4,291$)	Cases ($n = 1,095$)	Univariate OR (95% CI)	p	Multivariate OR (95% CI)[1]	p
Missing	21 (<1%)	6 (<1%)				
Trend			0.83 (0.79–0.88)	<0.0001	0.83 (0.78–0.88)	<0.0001
Tubal ligation, *n* (%)						
No	3,478 (81%)	856 (78%)	1.00 (reference)		1.00 (reference)	
Yes	456 (11%)	143 (13%)	0.87(0.69–1.10)	0.23	0.89 (0.69–1.13)	0.34
Missing	357 (8%)	96 (9%)				
Age at menopause, *n* (%)[3]						
≤42	239 (29%)	96 (23%)	1.00 (reference)		1.00 (reference)	
43–46	185 (22%)	93 (22%)	1.48 (1.00–2.18)	0.05	1.51 (1.02–2.25)	0.04
47–50	245 (30%)	117 (28%)	1.49 (1.00–2.22)	0.05	1.49 (1.00–2.24)	0.05
>50	156 (19%)	110 (26%)	1.71 (1.13–2.60)	0.01	1.75 (1.14–2.68)	0.01
Trend			1.18 (1.03–1.35)	0.02	1.18 (1.03–1.35)	0.02

[1]Adjusted for age at menarche (≤10/≥11 years old), parity (ever/never), breastfeeding (never, ≤12, >12 months), OC use (never, <1, 1–<3, 3–<5, ≥5 years), tubal ligation (yes/no) and ethnicity (other white, Jewish, French-Canadian, other).
[2]Among 891 matched case–control sets with at least one birth.
[3]Among 416 matched case–control sets that were menopausal and in instances where the controls underwent menopause before the age of diagnosis of the matched case.

information on oophorectomy status ($n = 202$) or if their year of surgery was missing ($n = 16$). After exclusions, there was a total of 11,605 eligible women, including 1,509 women with ovarian cancer (potential case subjects) and 10,096 women without ovarian cancer (potential controls). Each case was matched to a variable number of controls (on average, 1:4 matching) according to mutation in the same gene (*BRCA1* or *BRCA2*), year of birth (within 3 years), country of residence (Canada was divided into French Canadian and other) and a previous diagnosis of breast cancer (date of diagnosis was matched within 5 years). 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. In total, 1,329 matched sets were identified with a mean of 3.96 controls for each case.

Statistical analysis

A matched case–control analysis was performed to evaluate associations between various factors affecting ovulation 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 chi-square test, respectively. Conditional logistic regression for a variable number of cases to controls was used to estimate the univariate and multivariate odds ratios (ORs) and 95% confidence intervals (CIs) for ovarian cancer associated with various exposures. Only exposures in the control that took place before the date of diagnosis in the matched cases were considered in the analysis. In the analysis of age at first birth and age at last birth, we limited the analysis to parous women, while the analysis of age at menopause was limited to menopausal women. All analyses were performed using the SAS statistical package, version 9.1.3 (SAS Institute, Cary, NC). All p values were based on two-sided tests and were considered statistically significant if $p \leq 0.05$.

Results

Table 1 displays the baseline characteristics of the 1,329 cases and 5,267 controls included in our study. There was no significant difference with respect to year of birth and age at interview between the cases and controls in the matched sets. There was a significant difference in the ethnic distribution of the cases and controls, although the absolute difference was small ($p = 0.007$).

The relationship between individual components affecting ovulation and the risk of ovarian cancer in *BRCA1* and *BRCA2* mutation carriers is presented in Tables 2 and 3, respectively. Among women with a *BRCA1* mutation, there was no significant relationship between parity *per se* (*i.e.*, nulliparous *vs.* parous) and the risk of developing ovarian cancer (OR = 0.91; 95% CI 0.68–1.21); however, there was a significant inverse trend with increasing number of live births (OR per birth = 0.87; 95% CI 0.79–0.96; $p = 0.005$). Among parous women, age at last birth was significantly and inversely related to risk (OR for trend = 0.92; 95% CI 0.84–1.00; $p = 0.04$). There was a significant inverse relationship between ever having breastfed (OR = 0.75; 95% CI 0.61–0.93) and the duration of breastfeeding among *BRCA1* mutation carriers although the latter was limited to breastfeeding for more than 12 months (OR = 0.62; 95% CI 0.48–0.79). A history of oral contraceptive use was associated with a 40%

Table 3. Relationship between factors affecting ovulation and risk of ovarian cancer risk among *BRCA2* mutation carriers

Characteristic	Controls ($n = 974$)	Cases ($n = 233$)	Univariate OR (95% CI)	p	Multivariate OR (95% CI)[1]	p
Age at menarche (years)						
≤10	49 (5%)	18 (8%)	1.00 (reference)		1.00 (reference)	
11	134 (15%)	24 (11%)	0.43 (0.20–0.95)	0.04	0.55 (0.24–1.24)	0.15
12	286 (31%)	50 (23%)	0.49 (0.24–0.97)	0.04	0.61 (0.30–1.26)	0.18
13	240 (26%)	68 (32%)	0.66 (0.33–1.32)	0.24	0.77 (0.37–1.62)	0.50
≥14	204 (22%)	54 (25%)	0.64 (0.31–1.32)	0.22	0.73 (0.34–1.56)	0.41
Trend			1.05 (0.91–1.22)	0.51	1.05 (0.90–1.22)	0.58
≥11	846 (95%)	196 (92%)	0.54 (0.28–1.04)	0.07	0.66 (0.33–1.31)	0.23
Parity, *n* (%)						
Never	234 (24%)	40 (17%)	1.00 (reference)		1.00 (reference)	
Ever	740 (76%)	193 (83%)	0.74 (0.49–1.14)	0.17	1.15 (0.67–1.96)	0.61
Parity (per birth)						
1	102 (11%)	21 (9%)	0.90 (0.48–1.70)	0.76	1.33 (0.65–2.74)	0.43
2	343 (35%)	83 (36%)	0.75 (0.47–1.18)	0.21	1.14 (0.65–2.01)	0.64
≥3	295 (30%)	89 (38%)	0.69 (0.43–1.16)	0.13	1.03 (0.56–1.90)	0.92
Trend			0.88 (0.76–1.03)	0.11	0.98 (0.81–1.19)	0.85
Age at first birth, *n* (%)[2]						
≤21	118 (21%)	41 (23%)	1.00 (reference)		1.00 (reference)	
22–24	124 (22%)	46 (25%)	1.01 (0.60–1.70)	0.97	0.87 (0.50–1.51)	0.62
25–27	114 (20%)	45 (25%)	1.43 (0.83–2.46)	0.20	1.36 (0.76–2.44)	0.30
>27	203 (36%)	49 (27%)	1.08 (0.64–1.82)	0.76	0.98 (0.54–1.77)	0.93
Trend			1.05 (0.99–1.24)	0.57	1.03 (0.85–1.24)	0.80
Age at last birth, *n* (%)[2]						
≤26	109 (20%)	50 (28%)	1.00 (reference)		1.00 (reference)	
27–30	176 (31%)	54 (30%)	0.64 (0.38–1.05)	0.09	0.66 (0.38–1.26)	0.13
31–33	133 (24%)	33 (18%)	0.54 (0.31–0.95)	0.03	0.54 (0.30–0.98)	0.04
>33	141 (25%)	44 (24%)	0.77 (0.45–1.32)	0.35	0.80 (0.44–1.47)	0.48
Trend			0.92 (0.77–1.09)	0.32	0.92 (0.76–1.12)	0.41
Breastfeeding, *n* (%)						
Never	412 (42%)	110 (47%)	1.00 (reference)		1.00 (reference)	
Ever	491 (50%)	99 (43%)	0.68 (0.49–0.93)	0.02	0.67 (0.45–1.00)	0.05
Missing	71 (7%)	24 (10%)				
Duration (months)						
≤12	261 (27%)	67 (29%)	0.80 (0.55–1.17)	0.25	0.79 (0.51–1.23)	0.30
>12	230 (24%)	32 (14%)	0.51 (0.32–0.81)	0.005	0.50 (0.29–0.84)	0.009
OC use, *n* (%)						
Never	225 (23%)	96 (41.2)	1.00 (reference)		1.00 (reference)	
Ever	749 (77%)	137 (58.8)	0.56 (0.39–0.80)	0.0002	0.63 (0.44–0.92)	0.01
Duration (years)						
<1	154 (15%)	52 (22%)	1.00 (0.63–1.60)	0.99	1.09 (0.68–1.79)	0.70
1–<3	137 (14%)	28 (12%)	0.55 (0.32–0.93)	0.03	0.62 (0.36–1.08)	0.09
3–<5	111 (11%)	14 (6%)	0.39 (0.20–0.75)	0.005	0.42 (0.22–0.83)	0.01
≥5	347 (36%)	43 (19%)	0.47 (0.30–0.73)	0.0008	0.51 (0.32–0.81)	0.005

Table 3. Relationship between factors affecting ovulation and risk of ovarian cancer risk among *BRCA2* mutation carriers (Continued)

Characteristic	Controls ($n = 974$)	Cases ($n = 233$)	Univariate OR (95% CI)	p	Multivariate OR (95% CI)[1]	p
Missing	9 (<1%)	0 (0%)				
Trend			0.81 (0.73–0.90)	<0.001	0.81 (0.72–0.90)	<0.001
Tubal ligation, *n* (%)						
No	703 (72%)	166 (71%)	1.00 (reference)		1.00 (reference)	
Yes	206 (21%)	47 (20%)	0.77 (0.52–1.14)	0.18	0.76 (0.50–1.16)	0.20
Missing	65 (7%)	20 (9%)				
Age at menopause, *n* (%)[3]						
≤42	68 (29%)	33 (26%)	1.00 (reference)		1.00 (reference)	
43–46	50 (22%)	19 (15%)	0.90 (0.43–1.86)	0.77	0.89 (0.40–1.98)	0.78
47–50	59 (26%)	36 (28%)	1.18 (0.61–2.27)	0.63	1.17 (0.57–2.38)	0.67
>50	53 (23%)	40 (31%)	1.37 (0.71–2.67)	0.35	1.38 (0.67–2.83)	0.38
Trend			1.13 (0.91–1.39)	0.28	1.13 (0.89–1.42)	0.31

[1]Adjusted for age at menarche (≤10/≥11 years old), parity (ever/never), breastfeeding (never, ≤12, >12 months), OC use (never, <1, 1–<3, 3–<5, ≥5 years), tubal ligation (yes/no) and ethnicity (other white, Jewish, French-Canadian, other).
[2]Among 183 matched case–control sets with at least one birth.
[3]Among 128 matched case–control sets that were menopausal and in instances where the controls underwent menopause before the age of diagnosis of the matched case.

reduction in ovarian cancer (OR = 0.60; 95% CI 0.50–0.71) and increasing duration of use conferred a greater reduction in risk (p-trend < 0.0001); the maximum benefit was seen among women who used oral contraceptives for five or more years (OR = 0.50; 95% CI 0.40–0.63). Increasing age at menopause was associated with an increased risk of ovarian cancer (p-trend = 0.02). Women who achieved menopause at age 50 had a 75% greater risk of ovarian cancer compared to those at or before age 42 (95% CI 1.14–2.68). There was no significant relationship between increasing age at menarche (p-trend = 0.98), a tubal ligation (OR = 0.89; 95% CI 0.69–1.13) or age at first birth (p-trend = 0.15) and the risk of ovarian cancer in women with *BRCA1* mutations.

Among women with *BRCA2* mutations, ever having breastfed was associated with a borderline significant reduction in ovarian cancer risk (OR = 0.67; 95% CI 0.45–1.00) (Table 3). The protective effect was limited to women who breastfed for more than 12 months, which conferred a 50% reduction in risk (95% CI 0.29–0.84). Ever use of oral contraceptives significantly reduced the risk of ovarian cancer in *BRCA2* mutation carriers (OR = 0.63; 95% CI 0.44–0.92; p-trend < 0.0001); 3 years of use was required to achieve maximal benefit (OR = 0.42; 95% CI 0.22–0.83; $p = 0.01$). There was no significant relationship between parity (OR trend = 0.98; 95% CI 0.81–1.19; p-trend = 0.85), increasing age at menarche (p-trend = 0.58), age at first birth (p-trend = 0.80), age at last birth (p-trend = 0.41), a tubal ligation (OR = 0.76; 95% CI 0.50–1.16) or age at menopause (p-trend = 0.31) and the risk of ovarian cancer in *BRCA2* mutation carriers.

The cumulative number of ovulatory cycles was inversely associated with a significant 45% reduction in the risk of ovarian cancer overall ($p = 0.0001$), as well as in an analysis stratified by *BRCA* mutation status (Table 4). The OR comparing the lowest *vs*. highest quartile of the number of ovulatory cycles was 0.58 (95% CI 0.41–0.82; $p = 0.0002$) and 0.40 (95% CI 0.18–0.86; $p = 0.02$) for *BRCA1* and *BRCA2* mutation carriers, respectively. This protective association with ovulatory cycles was primarily driven by the effects of parity, oral contraceptive use and age at menopause in *BRCA1* mutation carriers and by breastfeeding and oral contraceptive use in *BRCA2* mutation carriers (Table 5). The proportion of women who experienced one or more irregular menstrual cycle was not different between the cases (11%) and controls (11%) ($p = 0.44$) (data not shown).

In a secondary analysis designed to dissociate the effects of parity and breastfeeding from each other, we matched cases and controls based on their history of breastfeeding (ever/never), as well as other factors, and re-evaluated the effect of parity among women who never breastfed. In this analysis, we observed a protective effect of increasing parity among *BRCA1,* but not *BRCA2* mutation carriers, independent of breastfeeding. The risk estimates for one unit increase in parity were 0.84 (95% CI 0.76–0.94; p-trend = 0.001) and 0.87 (95% CI 0.75–1.02; p-trend = 0.08) for women with a *BRCA1* mutation who ever breastfed and never breastfed, respectively. The corresponding estimates for *BRCA2* mutation carries were 1.03 (95% CI 0.84–1.26; p-trend = 0.82) and 0.93 (95% CI 0.72–1.21; p-trend = 0.60).

Discussion

We observed an inverse relationship between the number of lifetime ovulatory cycles and the risk of developing ovarian cancer among *BRCA* mutation carriers. In women with a

Table 4. Relationship between cumulative number of ovulatory cycles and risk of ovarian cancer among *BRCA1* and *BRCA2* mutation carriers

Ovulatory cycles	Controls ($n = 5,267$)	Cases ($n = 1,329$)	p	Univariate RR (95% CI)	p	Multivariate[1] RR (95% CI)	p
Mean (range)	322.3 (58.6–525.5)	338.6 (0–552)	<0.0001				
All subjects							
>398.8	2,317 (54%)	253 (25%)		1.00 (reference)		1.00 (reference)	
>348.0–≤398.8	852 (20%)	255 (25%)		0.89 (0.68–1.16)	0.30	0.91 (0.69–1.18)	0.46
>293.5–≤348.0	684 (16%)	256 (25%)		0.96 (0.73–1.26)	0.77	0.97 (0.73–1.28)	0.82
≤293.5	461 (11%)	253 (25%)	<0.0001	0.55 (0.40–0.74)	<0.0001	0.55 (0.41–0.75)	0.0001
Missing/hysterectomy[2]	675/278	217/95					
Trend				0.83 (0.76–0.92)	0.0002	0.84 (0.76–0.92)	0.0003
BRCA1 mutation carriers							
>398.8	1,900 (54%)	225 (26%)		1.00 (reference)		1.00 (reference)	
>348.0–≤398.8	714 (20%)	216 (25%)		0.87 (0.64–1.16)	0.34	0.88 (0.65–1.18)	0.38
>293.5–≤348.0	574 (16%)	218 (25%)		0.91 (0.67–1.12)	0.54	0.91 (0.66–1.24)	0.54
≤293.5	342 (10%)	198 (23%)	<0.0001	0.58 (0.41–0.73)	0.0002	0.58 (0.41–0.82)	0.0002
Missing/hysterectomy[2]	558/203	175/63					
Trend				0.85 (0.76–0.91)	0.003	0.85 (0.76–0.94)	<0.003
BRCA2 mutation carriers							
>398.8	415 (53%)	28 (18%)		1.00 (reference)		1.00 (reference)	
>348.0–≤398.8	138 (18%)	38 (24%)		1.00 (0.54–1.83)	0.99	1.08 (0.57–2.05)	0.81
>293.5–≤348.0	110 (14%)	38 (24%)		1.27 (0.30–2.33)	0.46	1.46 (0.76–2.79)	0.26
≤293.5	119 (15%)	55 (35%)	<0.0001	0.35 (0.17–0.74)	0.006	0.40 (0.18–0.86)	0.02
Missing/hysterectomy [2]	117/75	42/32					
Trend				0.79 (0.64–0.98)	0.03	0.81 (0.65–1.02)	0.07

[1]Estimate adjusted for ethnicity (other white, Jewish, French-Canadian, other) and tubal ligation (yes/no).
[2]Controls who underwent a hysterectomy (with or without a unilateral oophorectomy), salpingectomy or partial hysterectomy before the diagnosis of their matched case, and cases who underwent any of the aforementioned surgeries before their diagnosis, were excluded from the analysis of ovulatory cycles.

BRCA1 mutation, this association appears to be driven by the significant protective effects of increasing parity, oral contraceptive use and age at menopause, whereas in women with a *BRCA2* mutation, we observed stronger roles for breastfeeding and oral contraceptive use. This represents, to our knowledge, the first evaluation of ovulatory cycles and risk of ovarian cancer specifically in *BRCA* mutation carriers. Our study represents an extension of our earlier analysis and includes an additional 530 cases and 2,843 controls.[7]

Oral contraceptive use was associated with a 40% reduction in the risk of developing ovarian cancer overall, with maximum benefit observed after 5 years of use for *BRCA1* (OR = 0.50) and 3 years of use for *BRCA2* (OR = 0.42) mutation carriers. We reported a similar protective effect with oral contraceptive use in our earlier analysis, which was based on a subset of the women included in our study ($n = 799$ matched pairs).[7] This protective finding has been observed in prior studies of *BRCA* mutation carriers.[6,9,16,17]

Breastfeeding for more than 12 months was associated with a significant reduction in ovarian cancer risk in carriers of either mutation (OR = 0.62; 95% CI 0.48–0.79 for *BRCA1* mutation carriers and 0.50; 95% CI 0.29–0.84 for *BRCA2* mutation carriers). This finding is different from the published literature to date that has generally reported no relationship between breastfeeding and risk,[9,18] including one prospective study from the International *BRCA1/2* Carrier Cohort Study with 2,281 *BRCA1* and 1,038 *BRCA2* mutation carriers (HR = 0.88; 95% CI 0.62–1.26 for ever *vs.* never breastfeeding)[9]; however, the number of ovarian cancer cases in that study was small (range 150–253 cases *vs.* 1,329 in our study).

We found some evidence for a modest protective effective of increasing parity (but not parity *vs.* nulliparity *per se*) for *BRCA1*, but no relationship with *BRCA2*-associated ovarian cancer. This finding differs from our earlier report of a higher risk for carriers of *BRCA2* mutations.[7] The effect of parity was independent of breastfeeding. Other groups have generally reported a reduced risk of ovarian cancer with increasing parity for *BRCA1* but not *BRCA2* mutation carriers (although mostly inverse),[9,10] while Gronwald *et al.* reported no relationship between parity and risk among women with a *BRCA1* mutation.[18] In the report of Antoniou

Table 5. Relationship between individual components of ovulatory cycles and risk of ovarian cancer among *BRCA1* and *BRCA2* mutation carriers

Characteristic	Univariate RR (95% CI)	*p*	Multivariate RR (95% CI)[1]	*p*
All subjects				
Age at menarche, per year	1.01 (0.95–1.08)	0.79	1.00 (0.94–1.08)	0.91
Parity*0.77	0.85 (0.79–0.91)	<0.0001	0.86 (0.79–0.93)	0.0003
Breastfeed, per year	0.86 (0.80–0.94)	0.0003	0.92 (0.84–1.00)	0.05
OC use, per year	0.95 (0.93–0.96)	<0.0001	0.94 (0.92–0.96)	<0.0001
Age at menopause, per year[2]	1.03 (1.01–1.05)	0.009	1.03 (1.00–1.05)	0.02
BRCA1 mutation carriers				
Age at menarche, per year	1.00 (0.93–1.08)	0.98	1.00 (0.93–1.08)	0.99
Parity*0.77	0.84 (0.78–0.91)	<0.0001	0.84 (0.76–0.93)	0.0005
Breastfeed, per year	0.88 (0.81–0.96)	0.004	0.95 (0.86–1.04)	0.27
OC use, per year	0.95 (0.93–0.97)	<0.0001	0.95 (0.92–0.97)	<0.0001
Age at menopause, per year[1]	1.04 (1.01–1.06)	0.003	1.03 (1.00–1.06)	0.01
BRCA2 mutation carriers				
Age at menarche, per year	1.05 (0.91–1.22)	0.51	1.04 (0.88–1.22)	0.64
Parity*0.77	0.88 (0.76–1.02)	0.10	0.91 (0.76–1.08)	0.28
Breastfeed, per year	0.76 (0.61–0.95)	0.01	0.77 (0.60–0.98)	0.03
OC use, per year	0.94 (0.91–0.97)	<0.0001	0.93 (0.90–0.97)	0.0005
Age at menopause, per year[1]	1.00 (0.97–1.04)	0.85	1.01 (0.96–1.05)	0.85

[1]In the multivariate analysis, age at menopause was coded as premenopausal *vs*. postmenopausal when included as a covariate.
[2]Among postmenopausal women only; censored at the age of diagnosis of the matched case for the control subjects.

et al., the authors reported a significant reduction in risk among nulliparous *BRCA1* mutation carriers compared to those with one full-term birth.[9] In their meta-analysis of the published literature, Friebel *et al*. reported a significant reduction in risk only among *BRCA1* mutation carriers with four or more live births and no relationship with increasing parity for *BRCA2* mutation carriers.[11] The inconsistency in the earlier findings is likely attributed to the small sample number of *BRCA* mutation carriers in the earlier analyses (range 150–253) or may be a reflection of a more complex role of BRCA1 (and possibly BRCA2) expression during pregnancy on the ovarian surface epithelium. Despite this, a possible relationship between parity and risk requires further exploration.

Similar to findings of two earlier case–control studies, we found no relationship between age at first birth and risk among both *BRCA1* and *BRCA2* mutation carriers.[9,10] Interestingly, there was a significant inverse relationship between age at last birth and risk that was limited to women with a *BRCA1* mutation. To our knowledge, there are no prior reports of this relationship specifically in this high risk population; however, studies conducted among women in the general population have shown similar findings.[19–21] It has been suggested that the high levels of progesterone during pregnancy result in apoptosis and allow for the clearance of precancerous cells from the ovary.[22,23] In contrast, nulliparity or an earlier age at childbirth may result in an accumulation of more transformed cells thereby increasing cancer risk.

We also observed a significant positive association between age at menopause and risk of ovarian cancer that was limited to *BRCA1* mutation carriers. This relationship is consistent with prior reports among women at baseline population risk, further supporting an important role of incessant ovulation in the etiology of this disease.

Consistent with our earlier report, we found no association between a tubal ligation and ovarian cancer risk. Antoniou *et al*. reported a protective effect of tubal ligation in *BRCA1* (HR = 0.42; 95% CI 0.22–0.80; $p = 0.008$) and *BRCA2* mutation carriers (OR = 0.47; 95% CI 0.18–1.21; $p = 0.12$), although the latter association did not achieve statistical significance.[9] Similar to previously published studies, we found no relationship between age at menarche and risk.[9,18]

In studies conducted among women at general population risk of developing ovarian cancer, factors that interrupt ovulation, specifically reproductive factors such as breastfeeding and parity along with oral contraceptive use, have all been associated with a decreased risk of ovarian cancer.[24] The repeated damage to the ovarian surface epithelium with recurrent ovulation is thought to predispose to malignant transformation.[25] On the other hand, tubal ligation has been hypothesized, in part, to lower risk by affecting ovulation, reducing access of inflammatory, hormonal or carcinogenic agents (*e.g*., talc) to the ovary surface[13,26] or increasing immunity against the surface glycoprotein human mucin 1 (MUC1).[27,28] For *BRCA* mutation carriers

specifically, our findings only support an important role of oral contraceptive use and breastfeeding, implicating not only the interruption of ovulation but also other (potentially not mutually exclusive) pathways including the modulation of steroid hormone levels.[22,26] Oral contraceptive use reduces androgens but increases progesterone levels, while both breastfeeding and oral contraceptives suppress ovulation[29] and decrease levels of gonadotropins (*i.e.*, LH and FSH), all of which have been associated with a reduction in ovarian cancer risk.[22,26]

Given that the majority of *BRCA*-associated ovarian cancers are serous invasive tumors, our findings generally reflect what has been reported in studies evaluating risk factor relationships by histologic subtype among women at baseline population risk.[4,30,31] In the general population, factors including tubal ligation, oral contraceptive use, parity and breastfeeding confer protection while increasing age at natural menopause and the number of ovulatory cycles may increase risk, although there is emerging evidence that some of these associations may differ by histologic subtype. For example, Gates *et al.* have shown that the association with breastfeeding is stronger for mucinous tumors while the number of ovulatory cycles was a stronger predictor of serous and endometrioid *versus* mucinous tumors.[30]

Evidence from pathology studies suggests that as many as half of high-grade serous tumors may originate in the distal fallopian tube.[32–36] A step-wise progression to the development of invasive cancer in the fallopian tube has been supported from studies carefully examining the distal fallopian tubes of *BRCA* mutation carriers (as well as noncarriers) undergoing risk-reducing bilateral salpingo-oophorectomy.[37–40] Given the fallopian tube origin of many invasive epithelial cancers, the hypothesis that disruption and healing of the ovarian surface epithelium influences risk may be less relevant in this group.

Strengths of our study include the large number of women with known *BRCA1* or *BRCA2* mutations, and the ability to match for important factors and adjust for potential confounders. Importantly, this represents the first analysis of many important reproductive characteristics including age at menopause, age at last birth and ovulatory cycles in this high-risk population. Although questionnaires were used to collect information on important exposures, studies have shown that women are able to accurately recall reproductive and hormonal events[41–43] and there is no reason to suspect that a cancer diagnosis or knowledge of mutation status would have influenced recall of such events. The cases included in our study were interviewed, on average, 4 years following their diagnosis, thus introducing survivorship bias in our analysis. A superior survival experience has previously been shown in women who undergo genetic testing.[44] We did not address other less common factors that might impact ovulation and lead to periods of amenorrhea; however, the proportion of women in our study indicting at least one episode of irregularity was low and similar in the cases and controls (11%).

In summary, it appears that parity, breastfeeding and oral contraceptive use are associated with a reduction in the risk of developing *BRCA*-associated ovarian cancer. Breastfeeding for more than 12 months should be recommended to carriers of either mutation. Increasing parity protects against ovarian cancer among *BRCA1*, but not *BRCA2*, mutation carriers. This effect is independent of breastfeeding. With respect to oral contraceptive use for the primary prevention of ovarian cancer, *BRCA2* mutation carriers should be advised to use for at least 3 years; however, given the increased risk of breast cancer associated with oral contraceptive use before age 25 among *BRCA1* mutation carriers,[45] women with a *BRCA1* mutation should be advised to initiate use after the age of 25 and continue for 5 years.

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