

Reproductive factors and ovarian cancer risk in Jewish *BRCA1* and *BRCA2* mutation carriers (United States)

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Received 7 August 2002; accepted in revised form 13 February 2003

Key words: *BRCA1*, oral contraceptives, ovarian cancer, parity.

Abstract

Objective: To determine whether oral contraceptive (OC) use, childbearing, breastfeeding and tubal ligation differ between ovarian cancer cases with and without a *BRCA1/2* mutation.

Methods: A case-only study of 242 Jewish women with invasive epithelial ovarian cancer. Women were genotyped for three Ashkenazi founder mutations (185delAG and 5382insC in *BRCA1* and 6174delT in *BRCA2*). We obtained data on OC use, childbearing, breastfeeding, gynecologic surgeries and other reproductive factors from each woman. We compared the frequencies of these risk factors in carriers and non-carriers using unconditional logistic-regression, controlling for other covariates.

Results: Among the 242 cases, 64 (26.4%) carried one of the *BRCA1* founder mutations, and 31 (12.8%) carried the *BRCA2* mutation. Although there were no differences in the percent of nulliparous women between carriers and non-carriers, parous *BRCA1* carriers reported fewer live births than non-carriers (average of 2.1 versus 2.5 live births, OR = 0.61, 95%CI = 0.39–0.95, adjusted for age at diagnosis, tubal ligation and duration of OC use). Carriers and non-carriers did not differ in their history of breastfeeding, or in their lifetime use of OCs. *BRCA1* carriers were more likely than non-carriers to have had a tubal ligation (25.0 versus 10.2%, OR = 3.67, 95%CI = 1.55–8.70, adjusted for age at diagnosis, number of live births and OC duration).

Conclusions: In general, OC use, childbearing and breastfeeding do not differ between *BRCA1/2* carriers and non-carriers with ovarian cancer. However, the effects of tubal ligation may differ between *BRCA1* carriers and non-carriers.

Introduction

Mortality from invasive ovarian cancer is very high, with a five year survival rate of approximately 40% [1].

Survival is better with early stage disease, but the majority of patients present with metastatic disease [1]. To date, no effective early detection techniques have been identified and primary prevention represents an important opportunity for reducing ovarian cancer morbidity and mortality. Women with mutations in the cancer predisposing *BRCA1* and *BRCA2* genes have a lifetime ovarian cancer risk of 16–36% [2–5]. Using oral contraceptives (OCs), bearing children and

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breast-feeding have consistently been shown to reduce ovarian cancer risk among women in general [6, 7]. Tubal ligation has also been shown to reduce ovarian cancer risk [6, 8]. However, little is known about the impact of these factors on ovarian cancer risk in *BRCA1/2* mutation carriers. In a case-control study comparing 207 women with hereditary ovarian cancer to 161 of their unaffected sisters without the disease, OC use was less common among women with the disease [9]. This suggests that OC use may reduce the risk of ovarian cancer in women with a mutation in the *BRCA1* or *BRCA2* genes. However, the results of that study have been questioned, because the *BRCA1/2* carrier status of some of the sisters was unknown. This unknown data can potentially invalidate the findings. More recently, a case-control study of Israeli Jewish women found that the risk of ovarian cancer among carriers of a *BRCA1* or *BRCA2* mutation decreases with each birth but not with increased duration of use of oral contraceptives [10]. These conflicting data suggest the need to further investigate the potential of OCs as a chemopreventive agent among women with a *BRCA1/2* mutation.

In this study, we aimed to evaluate the potential benefit associated with OC use among women at high risk for ovarian cancer because they carry a mutated *BRCA1* or *BRCA2* gene. We also sought to determine the benefit or risk associated with other reproductive factors, including childbearing, breastfeeding, and tubal ligation in these women.

Methods

Subjects

Because of the high prevalence of three *BRCA1/2* founder mutations among Ashkenazi Jewish women with invasive epithelial ovarian cancer [11], we limited our study to Jewish women with epithelial ovarian cancer and with no prior history of breast cancer. Data on subjects were pooled from four sources: two population-based case-control studies of epithelial ovarian cancer in the United States (100 cases) [12, 13], a hospital-based study of Jewish women with epithelial ovarian cancer among 11 centers in North America and Israel (208 cases) [11], and a genetic counseling center in Chicago (14 cases). The Chicago clinic had been one of the sites for the hospital-based study, but the 14 incident, invasive cases included in this analysis were in addition to those participating in the original study. There was some overlap between cases included in the current study and those in the previous report of OCs

and ovarian cancer in *BRCA1/2* carriers [9] but this was less than 10%. Unfortunately, because subject links from the original studies to this study were not maintained, we were unable to identify which cases included in this study were also included in the previous report of OCs and ovarian cancer [9].

Moslehi *et al.* [11] (the hospital-based study) classified a woman as Jewish if three out of four grandparents were Jewish. Questions about place of birth of parents and grandparents further identified Ashkenazi women in that study. In Lu *et al.* [13] (one of the population-based studies), a woman was considered to be Jewish if she indicated that her childhood religious upbringing was Jewish. For the other two sources of subjects, a woman was considered to be Jewish if she classified herself as Jewish on medical records.

Specific descriptions of each study methodology are provided in the original publications [11–13] and are summarized in Table 1. Briefly, Moslehi *et al.* [11] used medical records to identify 465 Jewish women with ovarian cancer. Of these, 80 women were dead, 33 women were found not to have invasive disease on pathology review, 98 women were unreachable, and 49 women refused to participate. The remaining 208 (44.7%) women completed an in-person interview and provided a blood sample. Ness *et al.* [12] identified all women age 20–69 diagnosed with ovarian cancer in the Delaware Valley between 1994 and 1998. Of the 957 eligible women, 69 were too ill to participate, 15 were untraceable, and 92 refused to participate. Fourteen physicians did not consent to their patients' participating, for a total of 767 (80.1%) eligible women who completed an in-person interview. For the study presented here, we used medical records to identify successfully the religious affiliation of 437 of the 767 women, 46 of whom were Jewish, and we used banked pathology specimens (normal tissue blocks) to determine *BRCA1/2* carrier status of 36 of these women. Lu *et al.* [13] used tumor registries to identify 1080 women with ovarian cancer in eastern Massachusetts and New Hampshire between May 1992 and March 1997. Of the 1080 women, 203 had died or were unreachable, 126 were not contacted because their physician denied permission, 136 women declined participation, and 52 had non-epithelial ovarian cancer. The remaining 563 (52%) women were interviewed, during which time they provided a blood sample and answered questions about their childhood religious upbringing. Of the 563 women, 54 identified Jewish as the religion of their upbringing.

Each study obtained written informed consent from participants and was approved by the appropriate institutional review boards.

Table 1. Summary recruitment and eligibility characteristics of four pooled studies

Study	Moslehi <i>et al.</i> [11]	Ness <i>et al.</i> [12]	Lue <i>et al.</i> [13]	Chicago	Present study
Year of diagnosis	1980–1999 ^a	1993–1998	1992–1997	1990–1999	1990–1999
Place	11 centers in North America and Israel	Delaware Valley, USA	Massachusetts and New Hampshire	Chicago	na
Total eligible	465	957	1080	14	na
Total participants	208	767 (437 with known religion)	563	14	na
Method of determining Jewish descent	3 of 4 grandparents Jewish	Self disclosure of current religion	Childhood upbringing	Self disclosure of current religion	na
Total eligible Jewish women	208	46	54	14	322
Total with complete exposure data	191	46	54	14	305
Total with <i>BRCA1/2</i> status known	191	36 ^b	54	14	295
Total confirmed invasive	162	31	35	14	242
Total with <i>BRCA1/2</i> founder mutation	65	15	13	2	95

^a 26/162 final participants included in this analysis were from 1980–1989; 1 final participant was from 1972.

^b Ten tissue blocks were unobtained.

Exposure information, *BRCA1/2* mutation status and data quality

From each study source, data were requested on the use of OCs, including age at first and last use, and duration of use. Data were also obtained on number of live births, age at first and last live birth, and total duration of breastfeeding. We further requested information on other factors including age at menarche, body mass index, history of hysterectomy and history of tubal ligation. Because data on age at menopause and hormone replacement therapy were inconsistent among the studies, we were not able to include them in our analyses. We obtained details of tumor histology on all subjects, and we restricted our analyses to invasive ovarian cancers of the epithelial type. All data were checked for internal consistency and corrections or clarifications were requested from the original investigators when necessary.

All subjects were screened for the three Ashkenazi founder mutations (185delAG and 5382insC in *BRCA1* and 6174delT in *BRCA2*). Mutation analysis was performed by the original study investigators using several established detection techniques, including heteroduplex analysis, single-strand conformation analysis and allele-specific oligonucleotide hybridization. In addition, Moslehi *et al.* [11] tested all subjects for mutations in exon 11 of *BRCA1* and exons 10 and 11 of *BRCA2* using the protein-truncation test [14]. Truncating mutations in these exons represent about 70% of the *BRCA1/2* mutations found to date [11]. No women from that study included in the analysis reported here were found to have any *BRCA1/2* mutations other than one

of the three *BRCA1/2* founder mutations. Regardless of the technique employed, all mutations were confirmed by direct sequencing of DNA. Non-carriers were defined as women with none of the three mutations (for the studies employing only the Ashkenazi panel) and no other detected mutations (for subjects from Moslehi *et al.* [11]). *BRCA1* carriers were defined as women with either the 185delAG or the 5382insC in *BRCA1*. Women with the 6174delT in *BRCA2* were defined as *BRCA2* mutation carriers.

All subject data submitted for the pooled analysis were anonymous. Approval for the pooled analysis was obtained from the University of Pittsburgh Institutional Review Board.

Study design and statistical analyses

To determine whether carriers and non-carriers differed in OC use, parity, breast-feeding, and tubal ligation, we employed a case-only study design [15]. In a case-only study, cases with the genotype (carriers) form the ‘pseudo-cases’ and cases without the susceptibility genotype (non-carriers) form the ‘pseudo-control’ group. The two groups are compared with respect to the prevalence of each exposure. The odds ratio (OR) reflects the association between the exposure and the genotype (assuming independence of genotype and exposure). If this ratio is different from one, then the relative risk associated with the exposure differs for carriers and non-carriers. For a protective factor such as OC use, childbearing and breastfeeding in ovarian cancer, an OR greater than one indicates that the factor was more prevalent among the carriers (‘pseudo-cases’);

thus, the factor provides less protection to carriers than to non-carriers. Conversely, an OR of less than one indicates that the factor was less prevalent among the carriers, and suggests that the factor provides greater protection for carriers than for non-carriers.

To control for potentially confounding effects of other factors, we used unconditional logistic-regression analyses and included as covariates age at diagnosis and year of birth as continuous terms. Age at diagnosis was included in all models because univariate analyses showed a significant difference between carriers and non-carriers. Because the studies differed in the time period in which they were performed, year of birth was included in order to control for secular trends in OC use, parity and breastfeeding. However, there were no differences in results between analyses including year of birth and those excluding the variable. We therefore present the most parsimonious model in this paper. To check the reasonableness of pooling data from diverse sources, we calculated a Mantel–Haenszel test for heterogeneity for all major results. In none of the associations between BRCA status and reproductive factors did we find statistically significant heterogeneity among subject source. In addition, models that included a variable for study site did not differ in results from models excluding the variable; thus, the final models presented in this paper do not include a variable for study site. All analyses were performed with the STATA statistical software package (STATA Corporation, Release 5.0) and all *p* values given are from two-sided tests.

We analyzed all cases with complete exposure data. Because one of the parent studies [11] noted a difference in age at onset between *BRCA1* and *BRCA2* carriers, and because univariate analyses showed other differences in risk factors between *BRCA1* and *BRCA2* carriers for the entire study population, we analyzed the data for *BRCA1* and *BRCA2* carriers both jointly and separately.

Results

Table 1 presents a summary of the subjects participating in this study from the four parent studies. A total of 322 cases of epithelial ovarian cancer in Jewish women were identified. Of these, complete exposure data were obtained on 305 cases and *BRCA1/2* status was confirmed on 295 cases. Of the 295 cases, invasive histology was confirmed on all but 53 cases, for a total of 242 cases included in this analysis.

The characteristics of the 64 subjects with a *BRCA1* mutation, the 31 subjects with a *BRCA2* mutation, and the 147 non-carriers are presented in Table 2. As

expected, *BRCA1* carriers with invasive tumors were diagnosed at a significantly earlier average age than non-carriers (51.2 versus 57.5 years, *p* = 0.001). In contrast, *BRCA2* carriers were diagnosed at later ages than non-carriers (60.8 versus 57.5 years), although this difference was not significant. The difference in age at diagnosis between *BRCA1* carriers and *BRCA2* carriers, however, was significant (*p* < 0.001).

Only 11.7% of non-carriers reported a family history of ovarian cancer, compared to 16.1% of *BRCA1* carriers (*p* = 0.39) and 29.0% of *BRCA2* carriers (*p* = 0.017 for comparison to non-carriers). Similarly, non-carriers were less likely to report a family history of breast cancer (15.2% for non-carriers versus 22.6% for *BRCA1* carriers and 35.5% for *BRCA2* carriers). The difference between *BRCA2* carriers and the non-carriers was significant (*p* = 0.011).

Table 3 compares reproductive factors among carriers and non-carriers. After adjusting for possible confounders, there were no significant differences between the groups for age at menarche, ages at first and last live birth, or breastfeeding. There was also no difference in the percent of nulliparous women between carriers and non-carriers. However, parous *BRCA1* carriers reported fewer live births than parous non-carriers. The average number of live births among parous women was 2.5 among non-carriers, but only 2.1 among *BRCA1* carriers (OR = 0.61, 95%CI = 0.39–0.95, adjusted for age at diagnosis, tubal ligation and duration of OC use). Although parous *BRCA2* carriers also reported fewer live births than non-carriers, the difference between parous *BRCA2* carriers and non-carriers was not significant.

Interestingly, compared to non-carriers, *BRCA1* carriers were more likely to report having had a tubal ligation (25.0 versus 10.2%, OR = 3.67, 95%CI = 1.55–8.70 adjusted for age at diagnosis, number of live births and OC use). *BRCA2* carriers were less likely to report a history of tubal ligation compared to non-carriers, but the difference was not significant. However, the difference between *BRCA1* and *BRCA2* carriers was significant (*p* < 0.05). No differences in hysterectomy were found between carriers and non-carriers.

We compared additional characteristics of oral contraceptive use between carriers and non-carriers (Table 4). No significant differences were found in ever use of OCs or in duration of OC use. However, *BRCA1* carriers were likely to have begun using OCs at a later mean age than non-carriers (24.0 versus 23.2 years of age, OR = 1.15, 95%CI = 1.01–1.30 adjusted for age at diagnosis, number of live births, tubal ligation and OC duration). *BRCA1* carriers were also more likely to report recent use of OCs. The mean interval from last

Table 2. Characteristics of *BRCA1* and *BRCA2* carriers and non-carriers

	<i>BRCA</i> - (n = 147)	<i>BRCA1</i> + (n = 64)		<i>BRCA2</i> + (n = 31)	
Demographic characteristics					
Mean year of birth	1936 ± 12.8	1941 ± 9.9	<i>p</i> = 0.005	1932 ± 12.1	<i>p</i> = 0.14
Mean age at diagnosis (years)	57.5 ± 12.5	51.2 ± 9.9	<i>p</i> = 0.001	60.8 ± 11.3	<i>p</i> = 0.20
Mean body mass index (kg/m ²)	24.8 ± 5.6	25.1 ± 5.5	<i>p</i> = 0.753	25.8 ± 6.8	<i>p</i> = 0.40
Family history of ovarian cancer, n (%)	17 (11.7)	10 (16.1)	<i>p</i> = 0.391	9 (29.0)	<i>p</i> = 0.017
Family history of breast cancer, n (%)	22 (15.2)	14 (22.6)	<i>p</i> = 0.200	11 (35.5)	<i>p</i> = 0.011
Family history of ovarian or breast cancer, n (%)	26 (17.7)	16 (25.0)	<i>p</i> = 0.223	11 (35.5)	<i>p</i> = 0.030
Reproductive characteristics					
Mean age at menarche (years)	12.7 ± 1.4	12.6 ± 1.6	<i>p</i> = 0.746	12.3 ± 1.6	<i>p</i> = 0.139
Parous, n (%)	124 (84.4)	49 (76.6)	<i>p</i> = 0.178	29 (93.6)	<i>p</i> = 0.196
Mean number of livebirths ^a	2.5 ± 1.2	2.1 ± 0.8	<i>p</i> = 0.027	2.3 ± 0.9	<i>p</i> = 0.508
Mean age at first birth ^a	26.0 ± 4.7	26.6 ± 4.2	<i>p</i> = 0.453	27.1 ± 5.1	<i>p</i> = 0.261
Mean age at last birth ^a	31.3 ± 4.8	30.1 ± 4.3	<i>p</i> = 0.159	32.1 ± 5.4	<i>p</i> = 0.388
Mean time since first birth (years) ^a	32.7 ± 13.8	26.9 ± 11.6	<i>p</i> = 0.012	33.6 ± 11.8	<i>p</i> = 0.747
Mean time since last birth (years) ^a	27.4 ± 12.9	23.3 ± 11.7	<i>p</i> = 0.061	28.6 ± 11.0	<i>p</i> = 0.646
Breastfeeding, n (%)	47 (32.0)	22 (34.4)	<i>p</i> = 0.732	8 (25.8)	<i>p</i> = 0.501
Mean duration of breastfeeding (months) ^a	5.6 ± 16.2	6.5 ± 16.4	<i>p</i> = 0.743	6.8 ± 12.3	<i>p</i> = 0.721
Tubal Ligation, n (%)	15 (10.2)	16 (25.0)	<i>p</i> = 0.007	2 (6.5)	<i>p</i> = 0.522
Hysterectomy, n (%)	19 (12.9)	7 (10.9)	<i>p</i> = 0.687	6 (19.4)	<i>p</i> = 0.353
OC characteristics					
OC use, n (%)	58 (39.2)	36 (56.3)	<i>p</i> = 0.025	11 (35.5)	<i>p</i> = 0.680
Mean duration of use (years) ^b	5.1 ± 4.9	3.7 ± 3.6	<i>p</i> = 0.163	3.4 ± 5.2	<i>p</i> = 0.294
Mean age at first use ^b	23.2 ± 4.9	24.0 ± 5.3	<i>p</i> = 0.425	23.6 ± 6.2	<i>p</i> = 0.801
Mean time since last use (years) ^b	21.4 ± 9.1	19.6 ± 8.0	<i>p</i> = 0.360	23.8 ± 9.9	<i>p</i> = 0.429

Plus-minus values are means ± SD. *p*-values are for comparison of carriers to non-carriers.

Bolded entries are significant at *p* < 0.05.

Missing data are as follows: four subjects (2 *BRCA*-, 2 *BRCA1* +): family history of breast or ovarian cancers; 1 *BRCA2* + subject: BMI; 1 *BRCA*-subject: age at first and last birth.

^a Among women who had a live birth; ^b Among ever users.

Table 3. Adjusted ORs and 95% CIs for reproductive characteristics according to *BRCA1/2* carrier status

	<i>BRCA</i> - (n = 147)	<i>BRCA1/2</i> + (all carriers combined) (n = 95)		<i>BRCA1</i> + (n = 64)		<i>BRCA2</i> + (n = 31)	
		Adj ^a OR	95% CI	Adj ^a OR	95% CI	Adj ^a OR	95% CI
Age at menarche	Referent	0.93	0.78–1.11	1.01	0.81–1.26	0.80	0.60–1.06
Parous ^b	Referent	0.89	0.42–1.87	0.67	0.29–1.52	2.50	0.54–11.68
Number of livebirths ^{b,d}	Referent	0.70	0.49–0.99	0.61	0.39–0.95	0.85	0.55–1.31
Age at first birth ^d	Referent	1.02	0.95–1.09	0.98	0.90–1.07	1.05	0.96–1.15
Age at last birth ^d	Referent	1.01	0.94–1.08	0.96	0.88–1.04	1.06	0.97–1.16
Time since first birth ^d	Referent	0.98	0.92–1.06	1.02	0.94–1.11	0.95	0.87–1.04
Time since last birth ^d	Referent	1.00	0.93–1.06	1.04	0.96–1.13	0.95	0.86–1.03
Breastfeed	Referent	1.09	0.61–1.97	1.36	0.68–2.73	0.70	0.28–1.72
Duration of breastfeeding ^d	Referent	1.02	0.99–1.04	1.01	0.98–1.04	1.02	0.99–1.05
Tubal ligation ^c	Referent	2.32	1.06–5.11	3.67	1.55–8.70	0.65	0.14–3.16
Hysterectomy	Referent	1.56	0.69–3.54	1.79	0.63–5.07	1.37	0.48–3.91

^a Each row represents a separate model. All models were adjusted for age at diagnosis, number of live births (continuous variables) and OC use and history of tubal ligation (yes/no), except for those noted by (^b), which were not adjusted for number of live births, and those noted by (^c), which were not adjusted for tubal ligation. ORs in bold are significant at the *p* < 0.05 level.

^d Among women who had a live birth.

use to diagnosis was 19.6 years for *BRCA1* carriers and 21.4 years for non-carriers (OR = 0.89, 95%CI = 0.79–0.99 adjusted for age at diagnosis, number of live births,

tubal ligation and OC duration). The differences in age at first OC use or recent OC use between *BRCA2* carriers and non-carriers were not significant.

Table 4. Adjusted ORs and 95% CIs for OC use according to *BRCA1* and *BRCA2* carrier status

	BRCA- (n = 147)	<i>BRCA1/2+</i> (all carriers combined) (n = 95)		<i>BRCA1+</i> (n = 64)		<i>BRCA2+</i> (n = 31)	
		Adj ^a OR	95% CI	Adj ^a OR	95% CI	Adj ^a OR	95% CI
OC use	Referent	1.21	0.67–2.17	1.29	0.66–2.52	1.11	0.44–2.76
Duration of use (years) ^{b,c}	Referent	0.93	0.83–1.03	0.92	0.80–1.04	0.92	0.78–1.09
Age at first use ^c	Referent	1.11	0.99–1.24	1.15	1.01–1.30	0.96	0.80–1.16
Time since last use ^c	Referent	0.91	0.82–1.01	0.89	0.79–0.99	1.01	0.86–1.19

^a Each row represents a separate model. Each model is adjusted for age at diagnosis, year of birth, number of live births, OC duration (continuous variables) and history of tubal ligation, except for that noted by (^b), which was adjusted for OC duration. ORs in bold are significant at the $p < 0.05$ level.

^c Among ever users.

Discussion

We pooled data on Jewish women with invasive ovarian cancer from four sources in order to determine whether there were differences in OC use, childbearing, breastfeeding and tubal ligation between *BRCA1/2* mutation carriers with invasive ovarian cancer and non-carriers with the disease.

We found no difference in the percent of nulliparous women between carriers and non-carriers, although parous *BRCA1* carriers had experienced fewer live births than non-carriers (2.1 versus 2.5). This suggests that the effect of bearing children is similar for both *BRCA1* carriers and non-carriers. It is possible that the earlier age at diagnosis among *BRCA1* carriers may partially explain fewer live births in that group. However, in our population-based case-control study [16], healthy controls with a mean age of 49.5 years had on average 2.8 live births. This suggests that the earlier age of diagnosis cannot fully explain the observed reduced parity. Moreover, our analyses showed a similar finding (fewer live births compared to non-carriers) for parous *BRCA2* carriers, despite that fact that compared with non-carriers and *BRCA1* carriers, *BRCA2* carriers had a later age at diagnosis. We are careful to note, however, that this result failed to reach statistical significance, possibly due to the small number of *BRCA2* carriers in our study.

With regards to breastfeeding, we found no differences between *BRCA1/2* carriers and non-carriers. Thus, the effect of breastfeeding on ovarian cancer risk appears to be similar for both carriers and non-carriers. Similarly, no difference between *BRCA1/2* carriers and non-carriers were found for ever having a hysterectomy, suggesting that the effect of hysterectomy on ovarian cancer risk does not differ between carriers and non-carriers.

We found that OC use also appeared to be similar for both carriers and non-carriers, confirming a previous report [9]. Although we did find a statistically significant

difference in age at first use and recency of use between *BRCA1* carriers and non-carriers, these differences were small and may be due to chance. We failed to demonstrate a similar association between early OC use or recency of OC use for *BRCA2* carriers. Again, this may be due to differences in the effects of OC use in *BRCA2* carriers, or it may be due to the small number of *BRCA2* carriers in our study.

We further found that the protection associated with early OC use differed between *BRCA1* and *BRCA2*, although this difference may be due to the small number of *BRCA2* carriers. Notably, the direction of the ORs for the age and timing data among *BRCA2* carriers was opposite to that of the ORs for the *BRCA1* carriers, suggesting that the difference between the two groups may be real and not an artifact of sample size.

These results are in contrast to those of Modan *et al.* [10] who reported that the use of OC provided no protection to Israeli Jewish *BRCA1/2* carriers. While we cannot exclude the possibility that our finding is due to chance, we believe that there are differences between the two studies that may explain these disparate findings. In particular, the duration and frequency of use of OCs were far less in the Israeli population than in the population studied here. Moreover, there may be differences in OC formulations between the two populations. In addition, as discussed below, the differences between the study designs (case-control versus case-only) and our small sample size may account for the different findings.

Interestingly, *BRCA1* carriers were more likely to report having had a tubal ligation than non-carriers. Several studies have shown an association between tubal ligation and a reduction in ovarian cancer risk [8, 17–19], although the exact mechanism remains unknown. Our results suggest that if the procedure does protect against ovarian cancer, it may not provide the same degree of protection to *BRCA1* carriers. This finding is in contrast to those of Narod *et al.* [20] who report a

reduction in risk from tubal ligation among *BRCA1* carriers (OR = 0.39, 95%CI = 0.21–0.63, adjusted for OC use, parity, history of breast cancer and ethnic group). Data from that study were obtained from a database containing information on women from high-risk families in Canada, the United States and the United Kingdom. The differences between that study and the results presented here may be due to differences in study populations (high-risk women with any *BRCA1/2* mutation versus Ashkenazi Jewish women with one of three mutations), study design (matched case–control versus case-only), or chance. In particular, the *BRCA1* gene has over 850 known mutations, and it is unknown whether risk factors for ovarian cancer vary by mutation type. Confounding with other factors, such as family history of breast or ovarian cancer, may also explain our findings.

Care must be taken in interpreting our results. First, subjects were drawn from several sources. It is possible that the different study designs and data collection methods could have resulted in differences among the data sets that would affect our results. We note that while tests for heterogeneity between BRCA status and reproductive factors revealed no significant heterogeneity among subject source, it is possible that the tests may be underpowered in this instance because of the small sample size and the amount of stratification needed to perform the analyses. Therefore, such a test may not be very meaningful.

Second, we tested for a subset of mutations associated with ovarian cancer within a well-defined ethnic population. This raises the question of the generalizability of our results to the non-Jewish population or to women with other mutations.

Third, three of the four sources, which provided 80 subjects (33% of the data) for this study, tested for only the three mutations found in the Ashkenazim. Recently, Frank *et al.* [21] reported that among 322 Ashkenazi individuals who underwent full sequence analysis only after negative results from a three-mutation test, six (1.9%) carried a non-founder deleterious mutation. Therefore, we may have missed some mutations and classified some carriers as non-carriers, although in light of the Frank data, we would expect that number to be less than three. Moreover, the study providing the majority of cases [11] tested for most of the truncating mutations in *BRCA1* and *BRCA2* reported to date in addition to the three founder mutations analyzed here. No additional mutations were found. That is, no mutations (other than the three founder mutations) were identified among the subjects reported here. Therefore, the occurrence of carrier misclassification would likely be small. Assuming that this misclassification is non-differ-

ential with respect to the exposures we examined, it would bias our results towards the null value.

About 40% of the cases included in this study were interviewed more than one year after their diagnoses. Women with a *BRCA1* or *BRCA2* mutation may have improved survival compared to women with non-hereditary ovarian cancer [22]. Therefore, it is possible that mutation carriers would be over represented among those interviewed more than a year after diagnosis. Indeed, among those women interviewed more than one year after diagnosis, 43% were *BRCA1/2* mutation carriers; among women interviewed within one year of diagnosis, only 36% carried a mutation. However, this methodological issue would only impact our findings if OC use, parity, breastfeeding and/or tubal ligation affect prognosis.

An additional limitation of this study is the sample size, which limits the detectable differences in OC use, parity and other factors between carriers and non-carriers, and may explain some of our negative findings.

Finally, our choice of a case-only approach has limitations that may have affected our findings. In particular, the case-only design assumes independence between the genetic marker and the environmental exposure [15]. However, it is often difficult to make this assessment, even in a large-scale study [10]. Hence, in the absence of such evidence, as is the case here, point estimates and confidence intervals must be interpreted cautiously. In particular, if there is uncertainty about the assumption that OC use and parity are independent of carrier status among Jewish women, then it is possible that the estimates reported here are less precise than the data suggest [23]. The estimates may also be biased. Specifically, if there were a positive association between genotype and exposure in the underlying population, then the interaction OR above one would be biased towards one when compared to the ratio of relative risks that we are attempting to estimate. A case–control analysis would address these limitations. Unfortunately, because our data came from four sources with separate study designs, we lacked a valid control group to which we could compare the distribution of risk factors found among the different case groups. Moreover, because of the low prevalence of *BRCA1/2* mutations in the general population, it is unlikely that we would have had enough carriers in any control population to employ a standard interaction analysis.

In conclusion, our data suggest that in women with ovarian cancer, using oral contraceptives, bearing children and breastfeeding do not differ between women with and without a *BRCA1/2* mutation. While the data presented here confirm previous findings [9], they stand in contrast to those reported recently by Modan *et al.* [10] which suggested that OCs may not be protective in

women with a *BRCA1* or *BRCA2* mutation. Moreover, our results contradict the recent report that tubal ligation provides protection against ovarian cancer in *BRCA1* mutation carriers [20]. The disagreement between our study and these other studies on the protectiveness of OCs and tubal ligation indicate a substantial lack of clarity on how to counsel women at high risk for ovarian cancer.

Acknowledgements

We thank Drs J.M. Friedman, J.L. Weissfeld, and J.L. Eppinger for their support of this research. We further thank the members of the various study groups who facilitated this research: Dr Thomas Rocereto, Cooper Hospital; Dr Thomas Sedlacek, Graduate Hospital; Dr Joel Noumoff, Crozer-Chester Medical Center; Dr Matthew Boente, Hospital of the Fox-Chase Cancer Center; Drs Charles Mangan and Mary Cunnane, Pennsylvania Hospital; Drs Nathan Freed and Paul Krueger, Kennedy Memorial Hospital-University Medical Center; Dr Michael Hogan, Lankenau Hospital; Dr William Helm, Temple University Hospital; Drs Jack Carlson and Pamela Edmonds, Thomas Jefferson University Hospital; and Ron Vergona, University of Pittsburgh.

We also thank the anonymous reviewers, whose comments greatly improved this manuscript.

Supported by Department of Defense Ovarian Cancer Research Program grants DAMD 17-94-J-4249 and DAMD17-001-0569; National Cancer Institute grants K07-CA80668-01A1, R25-CA57703-06A1, R01-CA-61095, R01-CA63678-05, R01-CA54419 and NIH/NCI Early Detection Research Program U01CA85133-01.

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