BRCA1 and *BRCA2* Mutation Carriers, Oral Contraceptive Use, and Breast Cancer Before Age 50

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Abstract

Background: **Understanding the effect of oral contraceptives on risk of breast cancer in *BRCA1* or *BRCA2* mutation carriers is important because oral contraceptive use is a common, modifiable practice.**

Methods: **We studied 497 *BRCA1* and 307 *BRCA2* mutation carriers, of whom 195 and 128, respectively, had been diagnosed with breast cancer. Case-control analyses were conducted using unconditional logistic regression with adjustments for family history and familial relationships and were restricted to subjects with a reference age under 50 years.**

Results: **For *BRCA1* mutation carriers, there was no significant association between risk of breast cancer and use of oral contraceptives for at least 1 year [odds ratio (OR), 0.77; 95% confidence interval (95% CI), 0.53-1.12] or duration of oral contraceptive use (P_{trend} = 0.62). For *BRCA2* mutation carriers, there was no association with use of oral contraceptives for at least 1 year (OR, 1.62; 95% CI, 0.90-2.92); however, there was an association of elevated risk with oral contraceptive use for at least 5 years (OR, 2.06; 95% CI, 1.08-3.94) and with duration of use (OR_{trend} per year of use, 1.08; P = 0.008). Similar results were obtained when we considered only use of oral contraceptives that first started in 1975 or later.**

Conclusions: **We found no evidence overall that use of oral contraceptives for at least 1 year is associated with breast cancer risk for *BRCA1* and *BRCA2* mutation carriers before age 50. For *BRCA2* mutation carriers, use of oral contraceptives may be associated with an increased risk of breast cancer among women who use them for at least 5 years. Further studies reporting results separately for *BRCA1* and *BRCA2* mutation carriers are needed to resolve this important issue.** (Cancer Epidemiol Biomarkers Prev 2006;15(10):1863–70)

Introduction

Reproductive factors have been consistently associated with breast cancer risk for women unselected for predisposing genetic mutations (1, 2). A first birth is associated with an increased risk of breast cancer in the first 10 years after the birth, but after this time (i.e., after 10 years), if the birth occurs before age 32 years, the birth is associated with a decreased risk and the magnitude of this decrease increases with increasing time since the birth. If the first birth occurs after about age 32, the decreased risk does not occur, and such a woman has a lifelong increased risk compared with a nulliparous woman. Further births have similar effects, so that, for example, a woman with an early first birth and high parity has a much reduced risk if the further births take place at a young age. Women with an early age at menarche and/or a late age at menopause and a greater number of menstrual cycles are all at an increased risk of breast cancer. Taken together, these associations suggest that endogenous steroid hormones play an important role in the etiology of breast cancer. This raises the question of whether use of exogenous steroid hormones, such as oral contraceptives, might also be associated with risk. In a comprehensive meta-analysis of 54 studies, including about 90% of the relevant data that were available worldwide at the time, current oral contraceptive use was associated with a 24% increase in risk, and risk was doubled for young women who used oral contraceptives within the past 5 years and who were under 20 years of age at first use. These risks did not seem to be modified by family history of breast cancer (3).

Reproductive factors have not been well studied for *BRCA1* or *BRCA2* mutation carriers (4). Ursin et al. (5) suggested that

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oral contraceptive use might increase risk more for *BRCA1* and *BRCA2* mutation carriers than for noncarriers. Narod et al. (6) reported that oral contraceptive use was not associated with risk of breast cancer for *BRCA2* mutation carriers, but ever use of oral contraceptives by *BRCA1* mutation carriers was associated with a 20% increase in risk, and this increase was greater for those who had either used oral contraceptives for at least 5 years, used oral contraceptives before age 30 years, were diagnosed with breast cancer before age 40 years, or who first used oral contraceptives before 1975. Milne et al. (7), using data obtained from the Breast Cancer Family Registry (Breast CFR), compared cases (stratified by *BRCA1* and *BRCA2* mutational status) with unrelated, population-based controls not tested for *BRCA1* and *BRCA2* mutations and reported a possible protective effect of oral contraceptive use for cases carrying a *BRCA1* mutation, particularly for use of oral contraceptives after 1975, and essentially no effect of oral contraceptive use for cases carrying a *BRCA2* mutation, regardless of use before or after 1975. The possibility that oral contraceptive use might influence risk for *BRCA1* and *BRCA2* mutation carriers is particularly important to resolve because oral contraceptive use is relatively common, and there is some evidence that oral contraceptive use might protect against ovarian cancer for *BRCA1* and *BRCA2* mutation carriers (8-10), although a protective effect was not observed in one study (11).

We investigated associations between breast cancer risk and oral contraceptive use, separately for *BRCA1* and *BRCA2* mutation carriers, based on data from 804 *BRCA1* and BRCA2 mutation carriers ages under 50 years who were ascertained from a number of large breast cancer family registries. Information on oral contraceptive use was obtained using a single structured questionnaire, and mutations in *BRCA1* and *BRCA2* were detected using techniques that were validated against each other (12).

Materials and Methods

Participating Sites. Eligible subjects were living, White non-Hispanic women with a reference age under 50 years who carried a deleterious mutation in either *BRCA1* or *BRCA2*, completed an epidemiologic questionnaire, and had no personal history of ovarian cancer who were ascertained from eight sources of breast cancer families, six of which were from the Breast CFR, a consortium of research groups in the United States, Australia, and Canada (13). The other two were the Kathleen Cuningham Foundation Consortium for Research into Familial Breast Cancer (kConFab; http://www.kconfab.org) in Australia and the Ontario Cancer Genetics Network in Canada. Subjects affected with a first primary invasive breast cancer were defined as cases (this includes 37 *BRCA1* and 30 *BRCA2* cases that were included in the study of Milne; ref. 7), and unaffected subjects were defined as controls. The unaffected subjects included sisters, mothers, nieces, and cousins of the probands.

The Breast CFR has collected pedigree and epidemiologic risk factor data and biospecimens from >12,000 families that span a wide range of breast cancer risk. kConFab has collected the same data for >900 multiple-case breast cancer families ascertained by 14 family cancer clinics throughout Australia and New Zealand. The Ontario Cancer Genetics Network has collected similar data from ~2,500 multiple-case breast cancer families identified through cancer clinics in the province of Ontario, Canada.

Family Ascertainment. Three approaches were used to ascertain the families that are the source of *BRCA1* and *BRCA2* carriers. Breast CFR sites in San Francisco, Ontario, and Australia ascertained families through probands with incident breast cancer who were sampled from a population-based cancer registry. In San Francisco and Ontario, breast cancer cases at increased genetic risk were over-sampled based on family history of cancer and age at diagnosis, whereas in Australia, all incident cases were included in the family registry, as described elsewhere (13). The Breast CFR sites in New York, Philadelphia, and Utah, as well as kConFab and Ontario Cancer Genetics Network ascertained families through cancer clinics and community outreach. Probands included those whose first- and second-degree relatives included a minimum number of persons affected with breast or ovarian cancer (13, 14). In addition, Ashkenazi Jewish breast and/or ovarian cancer families were ascertained by groups in New York, Philadelphia, Ontario, and Australia by community outreach efforts.

All probands completed the same questionnaires regarding family history of cancer and demographic attributes and epidemiologic exposures and were asked to donate a blood sample. Probands were asked to permit researchers to contact their first-degree relatives from whom demographic and epidemiologic data and blood samples were also obtained. The study protocol was approved by all relevant institutional review boards, and written informed consent was obtained from all participants.

Personal Characteristics. Data on reproductive factors and oral contraceptive use were obtained from all subjects using the same structured questionnaire. Except for subjects from the Utah, New York, and Ontario study sites, data were obtained by personal interview whenever possible and by telephone interview when required because of a subject's distance or other needs. Data from subjects in Utah, New York, and Ontario were obtained by mailed questionnaires with follow-up telephone calls in the event of missing, inconsistent, or ambiguous information. All information obtained on reproductive factors preceded the date of breast cancer diagnosis for cases or the date of interview for controls. Each subject was assigned a reference age: for cases, this was age at diagnosis of invasive breast cancer; for controls, this was age at the earliest of the following events: interview, bilateral mastectomy, bilateral oophorectomy, or diagnosis of *in situ* breast cancer. We restricted analysis to subjects with a reference age <50 years. To minimize survival bias and recall bias, we also restricted analysis to subjects interviewed no more than 5 years after their reference age, with ~82% of subjects being interviewed within 3 years of their reference age. We duplicated the main regression analyses for this subset of subjects and found that the odds ratio (OR) estimates for the two groups were similar. The main exposures of interest in this analysis were (*a*) use of oral contraceptives for at least 1 year because use of oral contraceptives of a shorter duration may not be a meaningful exposure in terms of cancer risk and such short use may be recalled more accurately by cases than controls and (*b*) duration of oral contraceptive use, including age at first and last use.

Identification of *BRCA1* and *BRCA2* Mutation Carriers. Varying criteria were used to select individuals for *BRCA1* and *BRCA2* mutation testing. The three population-based sites tested the case proband from each family. The five clinic-based sites initially chose one family member for testing, generally, the youngest in the family affected with breast cancer. When a subject was identified as a mutation carrier, that mutation was subsequently evaluated in the subject's relatives. All participants of Ashkenazi Jewish ancestry were tested for the three founder mutations. Thus, the carriers included in this analysis came from families with a wide range of risk, including families with a single or multiple breast cancer cases.

Testing was done either by complete gene sequencing at Myriad Genetics or by one of several other methods: enzymatic mutation detection (15), two-dimensional gene scanning (16, 17), chemical cleavage of mismatch (18), the protein truncation test (19), single-strand conformation polymorphism

analysis (20), and denaturing high-performance liquid chromatography (21). All mutations were confirmed by sequencing. Description of these methods and of their performance relative to that of full gene sequencing can be found in Andrulis et al. (12).

Statistical Analyses. We used unconditional logistic regression implemented in SAS (SAS 9.2) to compare oral contraceptive use in cases and controls. ORs and 95% confidence intervals (95% CI) were estimated by modeling the probability distribution of attributes of ascertained carriers, conditional on reported pedigree structure and family disease occurrences. Logistic regression provides consistent OR estimates under a marginal covariate model, with intra-family attribute correlations accommodated by a robust variance estimator (22). This approach accommodates the possibility that carriers may have been ascertained because of their breast cancer status. Valid inferences for association variables can be obtained by ignoring other ascertainment issues provided that the exposure-disease OR among ascertained carriers equals the corresponding OR among unascertained carriers having the same family history (22). This condition would be violated only if ascertainment modified the relation between exposure and breast cancer.

All regression models included reference age (<40 and 40-49 years) plus reference age as a continuous variable, dummy variables for study sites (Canada, Australia, Utah, other U.S. sites), one of three measures of family history of breast or ovarian cancer (see below), and number of full-term pregnancies (livebirths and stillbirths) as a categorical (0, 1-2, ≥3) variable. To reduce the effect of outliers in trend tests for continuous variables, these tests were done by first categorizing a variable and then replacing the reported values in each category by the median for that category. In addition, for the main exposure variables of interest (use of oral contraceptives for at least 1 year and duration of use), we conducted analyses that further adjusted for age at first full-term pregnancy, time since first full-term pregnancy, time since last full-term pregnancy, smoking, alcohol, and clinic- versus population-based ascertainment. To address the question of possible confounding by birth year, we conducted conditional logistic regression stratifying on 5-year birth year group, study center, and reference age.

We were concerned about potential confounding of the oral contraceptive use and breast cancer association by family history of breast cancer because this attribute is a risk factor for breast cancer, and it may also be associated with oral contraceptive use. Therefore, we analyzed Breast CFR data to assess whether breast cancer occurrence in a woman's mother or sister was associated with age at starting oral contraceptive use or with duration of oral contraceptive use among users. There was no effect of family history on the age at which oral contraceptive use started: the hazard ratio of having a mother with breast cancer before oral contraceptive use started was 1.04 (95% CI, 0.98-1.1); that is, women with a mother with breast cancer started use slightly earlier. The hazard ratio of having a sister with breast cancer was 0.91 (95% CI, 0.68-1.21). Duration of oral contraceptive use once oral contraceptive use had started was slightly shorter in the subjects with either a mother or a sister with breast cancer, with the hazard ratio for stopping equal to 1.08 (95% CI, 1.03-1.15) for a mother and a hazard ratio equal to 1.08 (95% CI, 0.95-1.24) for a sister.

To address potential confounding of any association of breast cancer with oral contraceptive use due to family history, we included in the regressions one or more of the following covariates: (*a*) any first-degree relative with breast or ovarian cancer; (*b*) any family history of breast cancer by the case or control's 20th birthday; and (*c*) for estimating duration effects only, any new diagnosis of breast cancer in the family after the start of oral contraceptive use. Because the ORs for oral contraceptive use were similar regardless of which of these covariates were included, we report only the ORs obtained from models that included any first-degree relative with breast or ovarian cancer.

The analysis includes 497 *BRCA1* mutation carriers, 195 of whom had breast cancer, and 307 *BRCA2* mutation carriers, 128 of whom had breast cancer.

Results

Table 1 shows that, on average, cases were older than controls. The mean ages of *BRCA1* cases and controls and *BRCA2* cases and controls were 38.0, 35.6, 38.7, and 38.4, respectively. There was also a case-control difference in the distribution of study site, with more cases than controls from Canada and more controls than cases from Utah. More controls than cases were ascertained from clinic- versus population-based sources. A lower percentage of cases than controls reported a history of breast or ovarian cancer in first-degree relatives. This observation is simply the result of the ascertainment and *BRCA1* and *BRCA2* mutation testing protocols described earlier because the majority of controls (i.e., unaffected women) were ascertained through a family member with breast or ovarian cancer and only tested for mutations if a mutation was detected in at least one case (usually the proband) in the family; thus, by definition, they had a positive family history. This was not necessarily true for cases that were tested. Among *BRCA1* mutation carriers, cases were more often married and parous than controls, but we did not observe similar differences for *BRCA2* carriers.

Breast cancers from *BRCA1* mutation carriers were less likely than those from *BRCA2* carriers to be estrogen receptor positive and progesterone receptor positive. Finally, we note that oral contraceptive use differed substantially between study sites. For *BRCA1* and *BRCA2* controls, respectively, the prevalence of oral contraceptive use was 0.86 and 0.87 in Australia, 1.00 and 0.77 in Canada, 0.51 and 0.29 in Utah, and 0.65 and 0.79 in other U.S. sites (data not shown in table).

All of the differences noted above were also observed when we evaluated these characteristics in subjects who were <40 years of age, with the exception of parity, where the frequency of parous subjects was higher in cases than controls in both *BRCA1* and *BRCA2* mutation carriers.

In Table 2, we present results for various categories of oral contraceptive use separately for *BRCA1* and *BRCA2* mutation carriers. For *BRCA1* mutation carriers, there was no significant association between oral contraceptive use for at least 1 year and risk of breast cancer (OR, 0.77; 95% CI, 0.53-1.12). For subgroups, most point estimates were less than unity. There was decreased risk for use within the last 10 years compared with nonusers (OR, 0.63; 95% CI, 0.42-0.95), and 1 to 3 years of use before age 30 compared with nonusers (OR, 0.42; 95% CI, 0.20-0.85); however, only the trend estimate for time since last use was significant ($P = 0.002$).

For *BRCA2* mutation carriers, there was no significant association between oral contraceptive use for at least 1 year and risk of breast cancer (OR, 1.62; 95% CI, 0.90-2.92). An increased risk was associated with ≥5 years of oral contraceptive use (OR, 2.06; 95% CI, 1.08-3.94), and the trend for increasing duration of use was significant, with an OR per year of use of 1.08 ($P = 0.008$). For subgroups, increased risk was associated with ≥4 years of oral contraceptive use before the first full-term pregnancy (OR, 3.46; 95% CI, 2.10-5.70) and for ≥4 years of oral contraceptive use before age 30 (OR, 2.20; 95% CI, 1.26-3.85). The *P*s for trend were significant for both subanalyses ($P < 0.0001$ and $P = 0.008$, respectively). Due to colinearity, it was not possible to discriminate between use before the first full-term pregnancy, use before age 30, or use for ≥5 years as independent predictors of risk. We observed no

Table 1. Characteristics of non-Hispanic White *BRCA* mutation carriers with and without invasive breast cancer, by mutation status

	BRCA1 mutation carriers		*BRCA2* mutation carriers	
	Cases* (n = 195), n (%)	Controls* (n = 302), n (%)	Cases* (n = 128), n (%)	Controls* (n = 179), n (%)
Reference age (y)†				
<30	23 (12)	80 (27)	10 (8)	34 (19)
30-39	88 (45)	107 (35)	61 (48)	60 (34)
40-49	84 (43)	115 (38)	57 (44)	85 (47)
Year of birth				
1940-1949	16 (8)	30 (10)	16 (13)	34 (19)
1950-1959	103 (53)	87 (29)	61 (48)	53 (30)
1960-1969	65 (33)	98 (32)	49 (38)	56 (31)
1970+	11 (6)	87 (29)	2 (1)	36 (20)
Study site				
Australia	91 (47)	121 (40)	65 (51)	91 (51)
Canada	47 (24)	15 (5)	35 (27)	13 (7)
United States	42 (21)	81 (27)	22 (17)	43 (24)
(except Utah)	15 (8)	85 (28)	6 (5)	32 (18)
Utah				
Ascertainment				
Clinic based	120 (62)	284 (94)	76 (59)	158 (88)
Population based	75 (38)	18 (6)	52 (41)	21 (12)
College graduate	66 (34)	119 (39)	38 (30)	65 (36)
Ever married	146 (89)	186 (79)	86 (88)	112 (90)
Family history‡	114 (58)	229 (76)	67 (52)	127 (71)
Age at menarche (y)				
<13	95 (50)	114 (38)	46 (37)	71 (40)
13	57 (30)	78 (26)	37 (30)	65 (36)
≥14	39 (20)	106 (36)	42 (33)	42 (24)
Parous§	145 (75)	199 (66)	101 (79)	142 (79)
Estrogen receptor status, cases only				
Positive	33 (17)		70 (55)	
Negative	111 (57)		19 (15)	
Unknown	51 (26)		39 (30)	
Progesterone receptor status, cases only				
Positive	39 (20)		65 (51)	
Negative	98 (50)		22 (17)	
Unknown	58 (30)		41 (32)	

*The totals for the different variables may not equal the total number of subjects in a category because of missing data.
†For cases, age at breast cancer diagnosis and for controls, age at the earliest of these events: bilateral mastectomy, bilateral oopherectomy, diagnosis of *in situ* breast cancer, and interview.
‡Having first-degree relatives with breast cancer or ovarian cancer.
§Livebirths and stillbirths.

substantial differences in risk estimates for estrogen receptor–positive compared with estrogen receptor–negative cases and progesterone receptor–positive compared with progesterone receptor–negative cases for *BRCA1* or *BRCA2* mutation carriers, although the power to detect significant heterogeneity between these groups was low (data not shown).

No heterogeneity test for differences in the estimates between *BRCA1* and *BRCA2* carriers described above was significant, although for one comparison (the effect of oral contraceptive use for at least 4 years before the first full-term pregnancy), the heterogeneity test approached statistical significance (P = 0.07).

We then restricted our analyses to *BRCA1* and *BRCA2* mutation carriers whose reference ages were under 40 years of age, to facilitate comparison with reports by others who similarly restricted their analyses (5-7). Results are presented in Table 3.

For *BRCA1* mutation carriers under age 40, ORs associated with oral contraceptive use averaged around 0.60. For subgroups, a decreased risk was associated with first use after age 20 (OR, 0.45; 95% CI, 0.22-0.92), for <10 years since last use (OR, 0.55; 95% CI, 0.30-1.01), and 1 to 3 years of use before the age of 30 (OR, 0.20; 95% CI, 0.06-0.68). The trend estimate was significant only for time since last use (P = 0.01).

For *BRCA2* mutation carriers under age 40, in a subgroup analysis, risk was associated with ≥4 years of oral contraceptive use before the first full-term pregnancy (OR, 2.08; 95% CI, 1.02-4.25; P_{trend} estimate = 0.01). The pattern of change in the estimates comparing those aged under 50 (Table 2) with those aged under 40 (Table 3) was not as strong or consistent as that observed for *BRCA1* carriers.

We repeated all analyses presented in Tables 2 and 3 after including factors for family history of breast cancer in any first-degree relative before the subject reached 20 years of age (because most women who used oral contraceptives had started by this age), and for the duration-related exposures, any first-degree relative who was diagnosed with breast cancer after the subjects started oral contraceptive use (hypothesizing that a new occurrence of breast cancer in a close relative while a subject was taking oral contraceptives may have affected duration of oral contraceptive use). Adjusting for these factors resulted in only trivial changes to point and interval estimates (data not shown).

Adjusting for birth year (in 5-year intervals), smoking (pack-years), and alcohol (using drink-years as a combined variable for years of use and amount of alcohol consumed) did not make any appreciable difference to the point and interval estimates presented above (data not shown). Similarly, adjustment for age at first full-term pregnancy, time since first full-term pregnancy, or time since last full-term pregnancy made no difference in results (data not shown). We also examined the distribution of Ashkenazi Jewish subjects by case-control status and oral contraceptive use. There was a greater proportion of Ashkenazi Jewish subjects among *BRCA1* than *BRCA2* mutation carriers (0.2 and 0.11, respectively). However, within either *BRCA1* or *BRCA2* mutation

carriers, the case-control difference in oral contraceptive use was similar for Ashkenazi Jewish and non-Ashkenazi Jewish subjects; thus, Ashkenazi Jewish descent is not responsible for the results we observed.

Because the case-control distributions were different for clinic- versus population-based ascertainment, we conducted additional analyses of the main exposures of interest (oral contraceptive use for at least 1 year and duration of use) adjusting for this variable. Results for *BRCA2* mutation carriers did not change appreciably. For *BRCA1* mutation carriers, the suggestion of a possible protective effect presented in Table 2 is no longer observed (for use of oral contraceptives for at least 1 year: OR, 1.15; 95% CI, 0.75-1.74 and for durations of use of 1-4 years and 5+ years: OR, 0.97; 95% CI, 0.59-1.61 and OR, 1.20; 95% CI, 0.78-1.85, respectively; OR_{trend}, 1.02; $P = 0.24$). We are unable to explore this possible confounding effect among *BRCA1* mutation carriers in more detail because of small numbers when we adjust simultaneously for study site and clinic- versus population-based ascertainment. We also ran the main models separately for clinic- versus population-based families (data not shown). The results for population-based families were not significant due to low numbers; for clinic-based families, the ORs with oral contraceptive use were elevated for *BRCA2* mutation carriers. There was no statistically significant evidence of heterogeneity between clinic- and population-based families.

Finally, a question particularly relevant to current clinical and public health practice is what are the associations with risk of breast cancer among *BRCA1* and *BRCA2* mutation carriers who started using oral contraceptives in 1975 or later, when the dose of estrogen in oral contraceptives was reduced substantially (6, 7). Therefore, we analyzed the key oral contraceptive use variables (use of oral contraceptives for at least 1 year and duration of use) among subjects who first started using oral contraceptives in or after 1975, excluding all subjects who used oral contraceptives before 1975. Results are presented in Table 4. For *BRCA1* mutation carriers (who started use in 1975 or later), we continued to observe a nonsignificant reduction in risk associated with use of oral contraceptives for at least 1 year (OR, 0.76; 95% CI, 0.48-1.20) and no consistent association with duration of use (OR_{trend}, 0.98; $P = 0.50$). For *BRCA2* mutation carriers, there was a significant association with use of oral contraceptives for at least 1 year (OR, 2.11; 95% CI, 1.05-4.23); increased risk was also associated with use of oral contraceptives for at least 5 years (OR, 2.72; 95% CI, 1.26-5.85). For subjects under 40 years of age (Table 4B), there were no significant associations with oral contraceptive use of at least 1 year or with increasing duration of use.

In all analyses presented above, we included six subjects with ductal carcinoma *in situ* as controls; excluding them from the analyses or including them as cases made no material difference in our results. We also repeated the main analysis (of oral contraceptive use for at least 1 year) separately for each country of residence (data not shown) and observed no significant association between risk of breast cancer and oral contraceptive use for *BRCA1* or *BRCA2* carriers in any country and no significant evidence of heterogeneity between countries, although power to detect significant associations within countries and significant heterogeneity between countries was low.

Discussion

In the present study, which was restricted to carriers with a reference age under 50, oral contraceptive use of at least 1 year and duration of oral contraceptive use were not associated

Table 2. Risk of invasive breast cancer among *BRCA* mutation carriers according to oral contraceptive use, by *BRCA* mutation status for all subjects (under 50 years of age)

	BRCA1 mutation carriers			*BRCA2* mutation carriers		
	Cases* (n = 195), n (%)	Controls* (n = 302), n (%)	OR (95% CI)	Cases* (n = 128), n (%)	Controls* (n = 179), n (%)	OR (95% CI)
Oral contraceptive use for ≥1 y †						
No	49 (25)	86 (29)	1.00	19 (15)	45 (26)	1.00
Yes	146 (75)	214 (71)	0.77 (0.53-1.12)	109 (85)	132 (74)	1.62 (0.90-2.92)
Duration of use (y) †						
<1	49 (25)	86 (29)	1.00	19 (15)	45 (26)	1.00
1-4	40 (21)	84 (28)	0.68 (0.43-1.08)	24 (19)	45 (26)	1.16 (0.58-2.34)
≥5	102 (54)	128 (43)	0.80 (0.54-1.18)	84 (66)	83 (48)	2.06 (1.08-3.94)
Trend per year			0.99 (P = 0.62)			1.08 (P = 0.008)
Age at first use (y) †						
Nonusers ‡	49 (26)	86 (29)	1.00	19 (15)	45 (26)	1.00
<20	87 (45)	129 (44)	0.79 (0.53-1.17)	73 (57)	76 (45)	1.82 (0.96-3.44)
≥20	58 (29)	80 (27)	0.81 (0.51-1.26)	36 (28)	49 (29)	1.65 (0.87-3.16)
Trend per year			1.00 (P = 0.94)			0.93 (P = 0.41)
Time since last use (y) †						
Nonusers ‡	49 (26)	86 (29)	1.00	19 (15)	45 (26)	1.00
≥10	68 (35)	70 (24)	1.00 (0.64-1.57)	48 (38)	50 (30)	1.92 (0.97-3.82)
<10	75 (39)	138 (47)	0.63 (0.42-0.95)	60 (47)	75 (44)	1.62 (0.91-2.87)
Trend per year			1.04 (P = 0.002)			1.02 (P = 0.34)
Years of use before first full-term§ pregnancy †						
<1	73 (38)	121 (41)	1.00	30 (24)	83 (47)	1.00
1-3	38 (20)	73 (25)	0.82 (0.54-1.24)	25 (20)	36 (21)	1.53 (0.86-2.74)
≥4	79 (42)	102 (34)	0.91 (0.62-1.32)	72 (56)	56 (32)	3.46 (2.10-5.70)
Trend per year			0.99 (P = 0.73)			1.16 (P < 0.0001)
Years of use before age 30 †						
<1	51 (27)	89 (30)	1.00	21 (16)	53 (30)	1.00
1-3	6 (3)	25 (8)	0.42 (0.20-0.85)	5 (4)	13 (7)	1.49 (0.51-4.34)
≥4	134 (70)	183 (62)	0.93 (0.64-1.35)	101 (80)	109 (63)	2.20 (1.26-3.85)
Trend per year			1.00 (P = 0.93)			1.08 (P = 0.008)

*The totals for the different variables may not equal the total number of subjects in a category because of missing data.
†Adjusted for age, study site, family history, and number of full-term pregnancies.
‡Never or <1 year of use.
§Livebirths and stillbirths.

Table 3. Risk of invasive breast cancer according to oral contraceptive use for women <40 years of age, by *BRCA* mutation carrier status

	BRCA1 mutation carriers			*BRCA2* mutation carriers		
	Cases* (n = 111), n (%)	Controls* (n = 185), n (%)	OR (95% CI)	Cases* (n = 71), n (%)	Controls* (n = 94), n (%)	OR (95% CI)
Oral contraceptive use for ≥1 y †						
No ‡	26 (24)	49 (26)	1.00	10 (14)	26 (28)	1.00
Yes	85 (76)	136 (74)	0.64 (0.35-1.16)	61 (86)	68 (72)	1.29 (0.61-2.76)
Duration of use (y) †						
<1	26 (24)	49 (26)	1.00	10 (14)	26 (28)	1.00
1-4	24 (22)	56 (31)	0.61 (0.31-1.17)	10 (14)	20 (21)	0.79 (0.26-2.37)
≥5	58 (54)	79 (43)	0.61 (0.32-1.16)	50 (72)	48 (51)	1.45 (0.64-3.27)
Trend per year			0.97 (P = 0.27)			1.06 (P = 0.24)
Age at first use (y) †						
Nonusers ‡	26 (24)	49 (26)	1.00	10 (14)	26 (28)	1.00
<20	64 (58)	84 (47)	0.84 (0.45-1.55)	47 (66)	46 (50)	1.64 (0.77-3.46)
≥20	20 (18)	48 (27)	0.45 (0.22-0.92)	14 (20)	20 (22)	0.89 (0.33-2.40)
Trend per year			0.89 (P = 0.08)			0.89 (P = 0.11)
Time since last use (y) †						
Nonusers ‡	26 (24)	49 (26)	1.00	10 (14)	26 (28)	1.00
>10	24 (22)	17 (9)	1.10 (0.48-2.53)	13 (19)	11 (12)	0.83 (0.30-2.33)
<10	59 (54)	115 (65)	0.55 (0.30-1.01)	47 (67)	55 (60)	1.48 (0.70-3.14)
Trend per year			1.06 (P = 0.01)			0.95 (P = 0.17)
Years of use before first full-term § pregnancy †						
<1	37 (35)	66 (36)	1.00	13 (19)	35 (37)	1.00
1-3	21 (20)	45 (25)	0.71 (0.40-1.28)	12 (17)	19 (20)	0.81 (0.36-1.81)
≥4	49 (45)	72 (39)	0.69 (0.41-1.16)	45 (64)	40 (43)	2.08 (1.02-4.25)
Trend per year			0.95 (P = 0.21)			1.11 (P = 0.01)
Years of use before age 30 †						
<1	28 (26)	49 (27)	1.00	11 (16)	26 (28)	1.00
1-3	2 (2)	19 (10)	0.20 (0.06-0.68)	2 (3)	4 (4)	1.30 (0.15-11.0)
≥4	78 (72)	115 (63)	0.89 (0.50-1.57)	57 (81)	64 (68)	1.48 (0.68-3.20)
Trend per year			1.00 (P = 0.90)			1.03 (P = 0.36)

*The totals for the different variables may not equal the total number of subjects in a category because of missing data.
†Adjusted for age, study site, family history, and number of full-term pregnancies.
‡Never or <1 year of use.
§Livebirths or stillbirths.

overall with an increased risk of breast cancer among *BRCA1* mutation carriers. For *BRCA2* mutation carriers, oral contraceptive use of at least 1 year was not associated with an increased risk of breast cancer; however, there was an elevated risk of breast cancer associated with use of oral contraceptives for ≥5 years, and there was a significant trend of increasing risk with increasing duration of use. Regarding the important question of oral contraceptive use starting in 1975 or later, we observed results similar to our overall results (i.e., no significant associations among *BRCA1* mutation carriers but an elevated risk among *BRCA2* mutation carriers who used oral contraceptives for at least 5 years).

We first consider the validity of our results. The scientists who conducted the *BRCA1* and *BRCA2* mutation testing were unaware of the subjects' reported use of oral contraceptives or their breast cancer status at the time of testing, and all deleterious mutations were confirmed by direct sequencing (except for the three founder mutations from Ashkenazi cases in New York). Regarding the lifestyle/environmental variables, there is some error inherent in questionnaire-based data; however, a questionnaire was used that was based on carefully developed and validated instruments, and standardized questions were used across centers. The errors should be similar for *BRCA1* and *BRCA2* mutation carriers; thus, the likely effects of misclassification would be to bias the *BRCA1/BRCA2* stratum–specific results and the heterogeneity between them towards the null. We restricted this study to cases who were interviewed within 5 years of diagnosis to reduce the potential for recall bias. In addition, we observed results very similar to the ones reported here when we conducted an analysis restricted to cases interviewed within 3 years of diagnosis.

It is not possible to calculate meaningful response rates for participation in this study because many of the subjects in this study were ascertained through high risk clinics; however, for selection bias to have affected the ORs, selection into this study would not only had to have been dependent on disease status or oral contraceptive use but interactively with respect to both factors to have caused a bias in the ORs. To explain the possible suggestion of heterogeneity of the oral contraceptive effect between *BRCA1* and *BRCA2* subjects, the selection of subjects would have had to depend jointly on disease and exposure and be differential between *BRCA1* and *BRCA2* mutation carriers, which seems unlikely.

Confounding is an important issue to address. All ORs were adjusted for age, study site (including separate strata for Utah versus the other sites in the United States), parity, and family history of breast and ovarian cancer. We were aware that *BRCA1* and *BRCA2* mutation status is associated with a family history of breast and/or ovarian cancer and the possibility that this family history could affect the use of oral contraceptives in family members who served as controls. Therefore, we conducted an analysis of three different family history variables. None of the oral contraceptive results seemed to be confounded in a substantial manner by any of the family history variables we included, which is similar to results reported by Milne et al. (7). In fact, controlling for study site, including separate strata for Utah and other U.S. sites combined, had a stronger effect on the risk estimates than family history. Age and parity are generally reported with a high degree of accuracy; thus, the potential for residual confounding by these variables should be small.

It is of interest to compare the current results with the published literature. The two most relevant publications in this regard are the study by Narod et al. (6), because their design is similar to the one used here, and the study by Milne et al. (7), because their case-control study is based on the Breast CFR

data. The study by Milne et al. (7) differs from the design of the present study in that they used population-based cases stratified by *BRCA* mutation status (i.e., *BRCA1* mutation carriers, *BRCA2* mutation carriers, and noncarriers) and compared them with population-based, unrelated controls who were not tested for *BRCA1* or *BRCA2* mutations and restricted the study to women under the age of 40 years. They reported a possible protective effect of oral contraceptives among cases with a *BRCA1* mutation and essentially no effect associated with oral contraceptives among cases with a *BRCA2* mutation. We believe the question in this area of research that is most relevant to clinical and public health practice is whether use of oral contraceptives is associated with risk of breast cancer in women who carry a mutation in *BRCA1* or *BRCA2*. The case-control design that most appropriately addresses this question is the one employed in this study (10), comparing oral contraceptive use between *BRCA1* and *BRCA2* mutation carriers with a history of breast cancer (cases) and mutation carriers with no history of breast cancer (controls), as opposed to comparing oral contraceptive use between affected carriers and unaffected noncarriers, which is essentially the design used by Milne et al. (7), because the majority of untested, population-based controls would not carry a mutation in either *BRCA1* or *BRCA2*. Thus, the case series are similar between studies (although the present study included clinic-based subjects as well), but the control groups are fundamentally different. The prevalence of oral contraceptive use for at least 1 year is 0.71 to 0.74 among controls in the present study and 0.86 among controls in the study by Milne et al. (7), which seems to be the major reason for the differences in results. To further address this point, we stratified controls by 10-year birth strata and observed that the clinic-based controls, in particular, in the current study reported less oral contraceptive use in every 10-year birth stratum compared with the population-based controls in the Milne study (7).

The study by Narod et al. (6) employed a design very similar to the current study. They reported a small increase in risk associated with use of oral contraceptives among *BRCA1* mutation carriers, but no effect of oral contraceptive use among *BRCA2* mutation carriers. A few features of their study warrant comment. They did not control for family history of breast or ovarian cancer. Although family history of breast or ovarian cancer is probably not a strong confounder of the oral contraceptive effects in the present family-based study, the control of family history is a requirement to address the issue of ascertainment bias in this design. In addition, their study involved data from 52 centers in 11 countries. They controlled for country by matching cases and controls on country and taking this into account in their analyses. Results from the current study suggest the possibility of minor residual confounding if one does not also adjust for specific study sites within a country (e.g., Utah versus the other U.S. sites in our study). A list of within-country study sites is not provided in the article by Narod et al. (6). The 52 centers did not all use the same, standardized set of questions, raising the possibility of inconsistent data quality on oral contraceptive use across centers. In addition, they conducted their interviews on average 8 years after the breast cancer diagnosis. In the current study, all subjects were interviewed within 5 years of their reference age, and 82% were interviewed within 3 years. When the time between date of diagnosis and date of interview is long, potential for inaccurate recall and possibly biased recall increases. Cases who have experienced breast cancer may be more motivated to recall (accurately) their use of oral contraceptives than controls, and the potential for this differential recall would grow as the interval between diagnosis and interview grows. This rationale would provide a possible explanation for their observation of a slightly increased risk associated with use of oral contraceptives among *BRCA1* carriers but would not explain their observation of no oral contraceptive effect among *BRCA2* carriers. The subjects with

Table 4.

A. Risk of invasive breast cancer among *BRCA* mutation carriers who first used oral contraceptives in 1975 or later according to oral contraceptive use, by *BRCA* mutation status

	BRCA1 mutation carriers			*BRCA2* mutation carriers		
	Cases* (n = 140), n (%)	Controls* (n = 242), n (%)	OR (95% CI)	Cases* (n = 87), n (%)	Controls* (n = 123), n (%)	OR (95% CI)
Oral contraceptive use for ≥1 y †						
No	49 (35)	86 (36)	1.00	19 (22)	45 (37)	1.00
Yes	91 (65)	156 (64)	0.76 (0.48-1.20)	68 (78)	78 (63)	2.11 (1.05-4.23)
Duration of use (y) †						
<1	49 (35)	86 (36)	1.00	19 (22)	45 (37)	1.00
1-4	30 (22)	63 (26)	0.71 (0.41-1.25)	11 (13)	25 (20)	1.02 (0.44-2.40)
≥5	60 (43)	91 (38)	0.78 (0.49-1.26)	57 (66)	53 (43)	2.72 (1.26-5.85)
Trend per year			0.98 (P = 0.50)			1.06 (P = 0.004)

B. Risk of invasive breast cancer according to oral contraceptive use for women less than 40 years of age, by *BRCA* mutation carrier status, among BRCA mutation carriers who first used oral contraceptives in 1975 or later

	BRCA1 mutation carriers			*BRCA2* mutation carriers		
	Cases* (n = 100), n (%)	Controls* (n = 179), n (%)	OR (95% CI)	Cases* (n = 64), n (%)	Controls* (n = 91), n (%)	OR (95% CI)
Oral contraceptive use for ≥1 y †						
No	26 (26)	49 (27)	1.00	10 (16)	26 (29)	1.00
Yes	74 (74)	130 (73)	0.65 (0.36-1.19)	54 (84)	65 (71)	1.21 (0.56-2.58)
Duration of use (y) †						
<1	26 (26)	49 (28)	1.00	10 (16)	26 (29)	1.00
1-4	24 (24)	55 (31)	0.63 (0.32-1.24)	7 (11)	20 (22)	0.43 (0.13-1.42)
≥5	49 (50)	74 (42)	0.62 (0.33-1.17)	47 (73)	45 (49)	1.56 (0.68-3.58)
Trend per year			0.97 (P = 0.24)			1.09 (P = 0.08)

*The totals for the different variables may not equal the total number of subjects in a category because of missing data.
†Adjusted for age, study site, family history, and number of full-term pregnancies.

BRCA2 mutations, however, were only one third the sample size of the subjects with *BRCA1* mutations; thus, CI estimates were quite wide.

When we consider the studies published to date and results reported here, the weight of evidence suggests that the use of oral contraceptives produced after 1975 among carriers of *BRCA1* mutations is most likely not associated with an increased risk of breast cancer. We have less data on *BRCA2* mutation carriers, and results are mixed. In the current study, there was no association overall with use of oral contraceptives for at least 1 year; however, there was a modest and significant increase in risk among subjects who took oral contraceptives for ≥5 years, and this elevated risk persisted among *BRCA2* mutation carriers who first started taking oral contraceptives in 1975 or later, raising a possible concern with current formulations of oral contraceptives. We also observed an increased risk for *BRCA2* mutation carriers who used oral contraceptives for at least 4 years before the first full-term pregnancy or before age 30 years. As we explored plausible explanations for the possibility of heterogeneity in the oral contraceptive effects between *BRCA1* and *BRCA2* mutation carriers, we observed that *BRCA2* cases reported using oral contraceptives for a longer duration than *BRCA1* cases (e.g., the proportions of *BRCA1* cases who reported using oral contraceptives for <1, 1-4, and ≥5 years were 0.26, 0.21, and 0.53, respectively, whereas the corresponding proportions for *BRCA2* cases were 0.15, 0.19, and 0.66, respectively). We observed no differences among controls in either *BRCA1* or *BRCA2* mutation carriers; thus, the apparent difference in effect of oral contraceptives results from differences in the case groups, not the control groups.

The current findings from both basic and population-based sciences suggest some important differences between *BRCA1* and *BRCA2* in terms of breast cancer risk, functions, and downstream genes affected by *BRCA1* or *BRCA2* mutations. For example, the age-specific cumulative risks for breast cancer are strikingly different for *BRCA1* mutation carriers compared with *BRCA2* mutation carriers (23). In addition, *BRCA1*- and *BRCA2*-associated breast cancers express markedly different hormone receptor profiles. *BRCA1* tumors are predominantly estrogen receptor and progesterone receptor negative, whereas *BRCA2* tumors are typically estrogen receptor and progesterone receptor positive (24, 25). These opposing receptor profiles may in part explain the increased risk observed in this study with prolonged use of oral contraceptives in *BRCA2* carriers only. A similar argument has been employed to explain the seemingly greater chemoprotective effect of antiestrogens, such as tamoxifen, in breast cancer prevention in *BRCA2* carriers compared with *BRCA1* carriers (26). Until the possibility of heterogeneity in effects of selected exposures (e.g., use of oral contraceptives in this study) is elucidated with further studies, it would seem prudent not to lump *BRCA1* and *BRCA2* mutation carriers together in epidemiologic studies as if they were one and the same gene. Because oral contraceptives have been reported to protect against ovarian cancer among *BRCA1* mutation carriers in some studies, generating informative results on the oral contraceptive-related risk of breast cancer among *BRCA1* and *BRCA2* mutation carriers remains an important clinical and public health objective. Given that any case-control approach to this issue may be subject to biases that cannot be fully addressed, it would seem that a cohort study of *BRCA1* and *BRCA2* mutation carriers designed to minimize losses to follow-up and maximize ascertainment of cancer outcomes is warranted.

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BRCA1 and *BRCA2* Mutation Carriers, Oral Contraceptive Use, and Breast Cancer Before Age 50

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