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## No evidence of excess breast cancer risk among mutation-negative women from *BRCA* mutation-positive families

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### Abstract

This analysis addresses risk of breast cancer among women in *BRCA*-positive families who test negative for the family mutation. We compared the number of prospectively diagnosed breast cancers in 395 mutation-negative women from 28 *BRCA1/2*-positive families to an age-, race-, and calendar time-specific expected number of breast cancers derived from the SEER 9 Cancer Registry. Study participants contributed a total of 7008.1 person-years of follow-up. The mean age at study entry was 31.3 years; mean follow-up was 17.7 years. Ten women developed breast cancer yielding an observed-to-expected ratio of 0.82 (95% CI 0.39–1.51). Adjustment for possible reduction in breast cancer risk due to oophorectomy by two different methods resulted in O/E ratios in the range of 0.80–0.99. Stratification by degree of relatedness to the nearest mutation carrier did not substantially alter these results, however, women with at least one first degree relative with breast cancer appeared to have a slightly increased, though not statistically significant, risk of breast cancer (O/E ratio = 1.33, 95% CI 0.41–2.91). Our data suggest that breast cancer risk among mutation-negative women from *BRCA1/2* mutation-positive families is similar to that observed in the general population, with a possible slight increase in risk among mutation-negative women with a family history of breast cancer in a first degree relative.

Although this is the largest prospective cohort yet assembled to address this important question, the number of breast cancer events is still relatively small.

## Keywords

*BRCA* mutation; Breast cancer risk; Mutation negative; Cohort

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## Introduction

Women with deleterious mutations in *BRCA1* and *BRCA2* have an estimated 40–85% lifetime risk of breast cancer [1], and are more likely to develop early onset and bilateral breast cancer. Differences in cancer penetrance and median age at diagnosis between families suggest that other modifier genes may influence cancer risk in these families; however, little data exist regarding the risk of breast cancer among female members of *BRCA1/2* mutation-positive families who do not carry the family mutation. These women have traditionally been counseled that, in the absence of the family's *BRCA* mutation, their risk is similar to that of the general population, which is on the order of a 7–12% lifetime risk [2], with risk estimates based on known personal and environmental risk factors.

The data regarding risk of breast cancer among mutation-negative members of *BRCA*-positive families is inconsistent. Recently, Smith and colleagues reported a 5-fold increase in breast cancer risk among mutation-negative women from 277 *BRCA1/2*-positive families [3]. In a smaller, prospectively collected subset of this cohort, there was a non-significant increase in risk of about 2-fold. Other authors have noted similar, small increases in risk [4, 5], but a recent study in 375 mutation-negative women observed no increase in risk of breast cancer [6]. Additional data are required to draw firm conclusions regarding the magnitude of risk among women in *BRCA1/2*-positive families who test negative for the known family mutation, since screening recommendations might be altered if this risk is sufficiently large.

In order to further investigate this issue, we compared the number of prospectively diagnosed breast cancers among mutation-negative women in an IRB-approved National Cancer Institute (NCI) cohort study of families with known deleterious *BRCA1/2* mutations with an age-, race-, and calendar time-specific expected number of breast cancer cases derived from the Surveillance, Epidemiology and End Results (SEER)-9 Cancer Registry.

## Patients and methods

### Study participants

The familial breast cancer cohort for this analysis includes 415 consented mutation-negative women from 28 *BRCA1/2*-positive families ascertained between 1969 and 1997. The family population at risk was defined to include all bloodline individuals within three degrees of relatedness to a known mutation carrier. Of the 415 eligible women, 14 were excluded due to missing date of birth and six were excluded because we had not had contact with the individual or a family member within at least three degrees of relatedness, leaving 395 women.

Data were obtained via mailed questionnaire or in-person follow-up. Cancer status was obtained from the subjects directly (33%), their first-degree relatives (34%), second-degree relatives (16%), and third-degree relatives (13%). All cancer diagnoses were confirmed by review of pathology reports. Mutation status was not assigned probabilistically, but based on either direct testing or direct inference (participants were inferred to be mutation-negative if they were descendants of an individual who tested negative). Prospective individual follow-

up began at date of family ascertainment or at age 25 for those younger than 25 years of age at family ascertainment. Study follow-up was completed at the date of the most recent family or personal contact, date of death, date of breast cancer diagnosis, or date of bilateral mastectomy, whichever was first.

### Statistical methods

The number of breast cancers diagnosed in the familial population was compared with the age-, race-, and calendar time-specific expected number of breast cancer cases derived from the Surveillance, Epidemiology, and End Results (SEER)-9 Cancer Registry.

In order to account for the possibility that women in this familial breast/ovarian cancer cohort may have been counseled to undergo oophorectomy at a higher rate than the general population, which might bias results, we utilized two adjustment strategies. For the first, we assumed that oophorectomy reduces the risk of breast cancer by approximately 29%, based on data derived from women at average risk of breast cancer [7], and multiplied the expected number of cancers derived from the SEER registry by a factor of 0.71 for those women who underwent oophorectomy. For the second scenario, we utilized estimates of risk reduction due to oophorectomy derived from *BRCA* mutation carriers and taking into account age at oophorectomy. We calculated adjusted O/E ratios by adjusting the expected number of cancers by the appropriate reduction factor for those women that underwent oophorectomy in the given age range (see Table 1) [8].

We assessed the effect of relatedness to a known mutation carrier by conducting analyses stratified by closest relationship to a mutation-positive family member. In order to assess whether having a closely related, mutation-positive family member with breast cancer had an effect on risk among mutation-negative women, we also stratified the analysis by whether or not the subject had a first-degree relative with breast cancer.

### Results

Three hundred and ninety-five study participants from 28 families with a known *BRCA1* or *BRCA2* mutation contributed a total of 7,008.1 person-years of follow-up. The mean age at study entry was 31.3 years, and mean length of follow-up was 17.7 years. Overall, 10 study participants developed breast cancer compared with an expected 12 invasive plus in situ breast cancer cases, yielding an observed-to-expected ratio (O/E ratio) of 0.82 (95% CI 0.39–1.51). Since all cases of breast cancer among study participants were invasive, we also estimated the O/E ratio for invasive disease only, which was 0.95 (95% CI 0.45–1.74). Stratification of these analyses by degree of relatedness to the closest mutation carrier yielded similar results in all three strata (see Table 2). When stratifying for family history, women with a first degree relative with breast cancer had a slightly, though not significantly increased risk (O/E = 1.33, 95% CI 0.49–2.91).

The majority of study participants came from families with *BRCA1* mutations (322 women from 21 families). When limiting the analysis to women from *BRCA1*-positive families, the O/E was not substantially different from that seen in the entire study cohort (O/E = 0.66, 95% CI 0.27–1.37). We did not look separately at *BRCA2*-positive families due to small numbers (73 participants from 7 families).

Many women in our study cohort were ascertained prior to the identification of the *BRCA* genes, and were therefore counseled regarding an increased risk of breast and ovarian cancer based on their family history alone. Thus, we considered the possibility that our results may have been biased due to a greater likelihood of oophorectomy than that seen in the general population. We adjusted the expected number of breast cancers in our analysis for the

known decrease in breast cancer risk from oophorectomy using two scenarios, as described above. The analysis adjusted for the risk reduction seen with oophorectomy in the general population [7] yielded an adjusted O/E ratio for invasive plus in situ breast cancer of 0.89, 95% CI 0.42–1.63. When adjusting for the expected reduction in breast cancer risk seen in *BRCA* mutation carriers [8], the adjusted O/E was 0.94, 95% CI 0.45–1.74.

## Discussion

Our data suggest that breast cancer risk among mutation-negative women from *BRCA1/2* mutation-positive families is similar to that observed in the general population. These results are similar to those reported by Domchek et al., who saw no increased risk of breast cancer among a similar cohort of 375 mutative negative women [6]. While Domchek et al. did not report an aggregate SIR for invasive and non-invasive cancer, based on the total of four observed and 4.9 expected invasive plus in situ diagnosis, the risk does not appear to be elevated in this study. Conversely, Smith et al. reported a 5-fold increase in risk, although prospectively obtained data in a subset of their cohort suggested about a doubling of risk [3]. Several other studies [4, 5, 9] report risk estimates on the order of a 2-fold increase in risk as well. A comparison of pertinent study characteristics and findings is presented in Table 3.

There are several potential reasons for the wide variability in results from different studies. The Smith study had several fundamental limitations. One major criticism was the possibility of ascertainment bias—i.e., families that meet criteria for genetic testing will necessarily have multiple breast cancer cases, and thus families containing phenocopies are more likely to be tested than those without. In order to overcome this bias, Gronwald et al. looked at a Polish population of 261 sisters of 188 *BRCA1* mutation carriers from an unselected population [4]. They assumed that 50% of these women (130.5 individuals) would be mutation-negative. One breast cancer was reported among 72 non-carriers; based on these numbers they estimated that 2.5 cases would occur in the estimated 130.5 mutation-negative individuals—a number roughly double the expected number of cases in Poland. While this study design does account for selection bias, several other methodologic issues arise. First, the data are retrospective in nature, and the estimated risk ratio is based on a calculated probability of carrier or non-carrier status. Second, and more importantly, the estimate of risk, presented without confidence intervals, is extrapolated from only one case of breast cancer, and thus comes with a wide degree of uncertainty.

The Rowan series [5] presents a prospective study of 104 mutation-negative women and includes three incident cases of breast cancer. Again the study is small, and the confidence limits surrounding the point estimate for risk is wide, although the lower boundary does not cross unity, suggesting at least a modest increase in risk. Interestingly, of the three women diagnosed with breast cancer, one had a maternal aunt with bilateral breast cancer who tested positive for a mutation in *BRCA1*, and a mother who tested negative but who also developed bilateral breast cancer. This example illustrates the point that at least in some families, there is likely some component of risk that is familial, but that is not explained solely by mutations in *BRCA1* and *BRCA2*.

Our study is the largest prospective study to date, with the longest follow-up, and mutation status in our participants was known rather than being assigned probabilistically. Similar methodology was used by Domchek et al., who also noted no increase in risk of invasive breast cancer among mutation-negative women [6]. One strength of the current study was our ability to perform stratified analyses. We saw no difference by degree of relatedness to a known *BRCA*-positive individual, but, interestingly, there was a slight though not statistically significant increase in risk among women with a first degree relative with breast cancer. This finding suggests the possibility that there exist genetic and/or environmental

modifiers that may influence the risk of breast cancer among mutation carriers as well as non-carriers, and highlight the importance of individual assessment of family history when performing risk assessment for women who test negative for mutations in *BRCA1/2*.

Our study takes into account the protective effect of oophorectomy, which no other analysis to date has done. We calculated adjusted O/E ratios using two methods—one which accounted for the breast cancer risk reduction seen in the general population after oophorectomy, and the second using estimates of risk reduction from a population of *BRCA* mutation carriers. Both methods have potential strengths and weaknesses. The general population estimate of the reduction in breast cancer risk from oophorectomy is probably most accurate for this population of mutation-negative women, but the study from which we derived this estimate did not take into account age at oophorectomy, which we know to be important. Thus, we also present the second model, which taken into account age at oophorectomy, but which may overestimate the effect of surgical menopause because it is derived from a population of *BRCA* mutation carriers. The adjusted O/E ratios using these two methods were 0.89 and 0.94, respectively. Thus, with optimal adjustment for oophorectomy, our point estimate would likely have been somewhere between these two figures.

An additional potential source of bias in this study is the phenomenon of “informative censoring,” which occurs when a given co-variate affects both the outcome of interest and the probability of being censored. In this case, if subjects who were most likely to develop breast cancer preferentially underwent prophylactic mastectomy, and thus did not develop breast cancer, the number of observed cancers would be lower than expected. In our study, only three participants underwent bilateral mastectomy during the study period (at ages 60, 46, and 44, contributing 16, 2, and 7 person-years of follow-up, respectively); if all three of these women were “destined” to develop breast cancer, which would have resulted in a total of 13 incident cases, the adjusted O/E ratio in this study may have been slightly greater than one.

It is also possible that there are women in our mutation-positive families on whom we did not have information who did develop breast cancer. The NCI Clinical Genetics Branch Hereditary Breast and Ovarian Cancer Registry sends periodic follow-up questionnaires to all study family members with known addresses; follow-up for the current analysis was truncated at the date of return of the most recent questionnaire. The questionnaires request updates on personal history as well as family cancer diagnoses, and we attempt to verify all reported cancer diagnoses in the family with pathology reports or physician records. In this analysis, we included any woman with known or inferable *BRCA* mutation status on whom we had a recent cancer status update from either the individual or a first-, second- or third-degree relative. It is possible that the study participants who completed the questionnaires would not know about breast cancer diagnoses in more distant relatives. However, in our study, cancer status information came from third-degree relatives for only 13% of participants, and data suggest that family reporting of breast cancer diagnoses in the context of familial syndromes is quite accurate [10].

Lastly, although the study had a mean of 17.7 years of follow-up, this cohort is still relatively young, and thus an excess risk of later-onset breast cancer may still emerge as the cohort ages.

In summary, we were unable to detect an excess risk of breast cancer among mutation-negative women in families with known *BRCA* mutations, with the possible exception of those with a family history of breast cancer in a first-degree relative. Even in that context, the O/E ratios and confidence intervals noted were in keeping with risk imparted by a first-

degree family history in the general population. We saw no increase in risk after adjustment for the higher rate of prophylactic oophorectomy in our study population. Taken in concert with other prospective studies, these data suggest that current information is insufficient to mandate changes in screening practices for mutation-negative women from *BRCA1/2* mutation-positive families. Additional research regarding both genetic modifiers and environmental factors that influence breast cancer risk may help to clarify individual breast cancer risk among women in this population.

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**Table 1**

Proportional adjustment of expected rate of breast cancer by oophorectomy status and age at oophorectomy, derived from [8]

	No. of subjects	Relative risk adjustment
Intact ovaries	345	1.0
Oophorectomy at age $\leq 40$	24	0.41
Oophorectomy at age 41–50	18	0.47
Oophorectomy at age $>50$	8	0.70



**Table 2**

Observed-to-expected ratio for invasive plus in situ breast cancer, stratified by degree of relatedness to closest relative with known *BRCA* mutation

	No. of Subjects	Mean age at cohort entry (years)	Mean follow-up (years)	O/E (95% CI)
1st degree	102	36.8	19.9	0.66 (0.13–1.94)
2nd degree	182	31.8	17.8	0.97 (0.35–2.11)
3rd degree	111	25.4	15.4	0.69 (0.01–3.83)



**Table 3**

Comparison of studies evaluating risk of breast cancer among mutation-negative women in *BRCA* mutation-positive families

Study	No. of subjects	Person-years	No. of breast cancers	Risk ratio (95% CI)
Smith (full cohort) [3]	258	n/a	28	5.3 (3.5–7.7)
Smith (prospective subset) [3]	153	818	3	2.1 (0.4–6.2)
Rowan [5]	101	720	3	2.9 (1.0–8.6)
Gronwald [4]	130.5	n/a	2.5	2 (not given)
Domchek [6]	375	1,962	2 (invasive) 2 (DCIS)	0.52 (0.13–2.09) 2.30 (0.57–9.19)
Korde (current study)	395	6,983	9	0.75 (0.34–1.41)