# Estrogen Receptor Status in *BRCA1*- and *BRCA2*-Related Breast Cancer: The Influence of Age, Grade, and Histological Type

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## ABSTRACT

***Purpose:*** ***BRCA1*-related breast cancers are more frequently estrogen receptor (ER) negative than are either *BRCA2*-related or nonhereditary breast cancers. The relationship between ER status and other clinical features of hereditary breast cancers has not been well studied.**

***Experimental Design:*** **ER status, grade, and histological tumor type were evaluated in 1131 women with invasive breast cancer, ascertained at 10 centers in North America. There were 208 *BRCA1* mutation carriers, 88 *BRCA2* carriers, and 804 women without a known mutation. We stratified the patients by mutation status, grade, age, and histological type and calculated the percentage of ER-positive tumors within each stratum.**

***Results:*** ***BRCA1*** **mutation carriers were more likely to have ER- negative breast cancers than were women in other groups, after adjustment for age, grade, and histological subtype ($P < 0.001$). Only 3.9% of *BRCA1*-related breast cancers were ER-positive cancers occurring in women in their postmenopausal years. The direction and magnitude of the change in ER status with increasing age at diagnosis in *BRCA1* carriers was significantly different from in *BRCA2* carriers ($P_{intercept} = 0.0002$, $P_{slope} = 0.04$). Notably, changes in ER status with age at diagnosis for *BRCA1* carriers and noncarriers were almost identical ($P_{slope} = 0.98$).**

***Conclusions:*** **The strong relationship between the presence of a *BRCA1* mutation and the ER-negative status of the breast cancers is neither a consequence of the young age at onset nor the high grade but is an intrinsic property of *BRCA1*-related cancers. The ER-negative status of these cancers may reflect the cell of origin of *BRCA1*-related cancers.**

## INTRODUCTION

It has been recognized for several years that *BRCA1*-related breast cancers are more likely to be estrogen receptor (ER) negative than are non-*BRCA1*-related breast cancer and *BRCA2*-related breast cancers (1, 2). Tamoxifen appears to be effective in reducing both local recurrence and contralateral breast cancer among women with *BRCA1* mutations (3). These two observations appear to stand in contradiction to each other because in general, hormone-based treatments such as tamoxifen are not effective in preventing or treating ER-negative breast cancer (4, 5). The widely voiced opinion that tamoxifen is likely to be ineffective in preventing *BRCA1*-related breast cancers is mainly based on an analysis of the NSABP-P1 (prevention) trial of tamoxifen *versus* placebo. Among 8 *BRCA1* mutation carriers (hereafter *BRCA1* carriers) who developed breast cancer, 5 had been allocated to tamoxifen and 3 had been allocated to placebo (6). The estimated risk ratio was 1.67 [95% confidence interval (CI) 0.32–10.70], which favors no protective effect of tamoxifen. The study design is ideal (prospective and randomized), but the number of *BRCA1* carriers was small ($n = 8$). In contrast, a large case-control study of *BRCA1* carriers with and without contralateral breast cancer suggested that tamoxifen is a highly effective method of preventing second primary breast cancers in *BRCA1* carriers (7). The adjusted odds ratio for contralateral breast cancer in association with tamoxifen use among *BRCA1* carriers was 0.38 (95% CI 0.19–0.74). By inference, the authors concluded that tamoxifen would likely also be effective in preventing primary breast cancer in *BRCA1* carriers. This interpretation fits with the results of a study of prophylactic oophorectomy in *BRCA1* carriers, where removal of the ovaries, and the resulting estrogen deprivation, resulted in highly significant 47% reduction in the risk of breast cancer (8). Given all of the data, the most parsimonious explanation of

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these conflicting data are that the NSABP-P1 study was underpowered to detect a protective effect of tamoxifen in *BRCA1/2* carriers. However, an alternative hypothesis is that the ER status of the two tumors is highly correlated. In this scenario, if the first cancer is ER positive, then the contralateral tumor is also likely to be ER positive. Thus, tamoxifen treatment of the ER-positive first cancer would be effective in preventing the second cancer because of its predicted ER-positive status. Finally, it is possible that menopause results in a significant increase in the proportion of *BRCA1*-related cancers that are ER positive, favoring successful prevention of the second breast cancer by tamoxifen in postmenopausal women. The case-control study (7) did not contain sufficient information regarding the ER status of either the first or the second cancer to eliminate the possibility that the observed effect was due to prevention of second cancers that were destined to be ER-positive (9).

We have recently shown that tamoxifen is effective at reducing both local recurrence and contralateral breast cancer among women with *BRCA1* mutations (3). However, in that study, much of the ER data were missing. One possible way to circumvent the missing ER data are to identify other features of the breast cancer that predict ER status and then use these tumor characteristics as surrogate markers of ER status. In a small series of cases from Montreal, Quebec, Canada, we observed that 16 of 18 (89%) grade 3, infiltrating ductal breast cancers, which occurred among *BRCA1* carriers, were ER negative. Here, we attempted to confirm this initial observation in an expanded series of cases. We also wished to establish whether *BRCA1*-related breast cancer is more likely to be ER negative than non-*BRCA1*-related breast cancer, even after adjusting for the effects of age, grade, and histological type.

*Table 1* Percentage of estrogen receptor (ER)-positive breast cancers by grade

| Class | N (%) | ER+% | $P^a$ |
|---|---|---|---|
| Noncarriers[b] | | | |
| Grade 1 | 71 (29.7) | 90.1 | $P_{1/2} = 0.0024$ |
| Grade 2 | 98 (41.0) | 69.4 | $P_{1/3} < 0.0001$ |
| Grade 3 | 70 (29.3) | 45.7 | $P_{2/3} = 0.0006$ |
| Total | 239 (100) | 68.6 | $P_{trend} < 0.0001$ |
| *BRCA1* carriers[b] | | | |
| Grade 1 | 7 (3.3) | 71.4 | $P_{1/2} = 0.070$ |
| Grade 2 | 48 (23.1) | 41.7 | $P_{1/3} = 0.0004$ |
| Grade 3 | 153 (73.6) | 15.7 | $P_{2/3} = 0.0001$ |
| Total | 208 (100) | 23.6 | $P_{trend} < 0.0001$ |
| *BRCA2* carriers[b] | | | |
| Grade 1 | 5 (5.7) | 80.0 | $P_{1/2} = 0.403$ |
| Grade 2 | 42 (47.7) | 95.3 | $P_{1/3} = 0.297$ |
| Grade 3 | 41 (46.6) | 61.0 | $P_{2/3} = 0.0001$ |
| Total | 88 (100) | 78.4 | $P_{trend} = 0.0017$ |
| Untested[b] | | | |
| Grade 1 | 86 (14.4) | 88.4 | $P_{1/2} = 0.798$ |
| Grade 2 | 239 (40.1) | 87.0 | $P_{1/3} < 0.0001$ |
| Grade 3 | 271 (45.5) | 55.7 | $P_{2/3} < 0.0001$ |
| Total | 596 (100) | 72.9 | $P_{trend} < 0.0001$ |

[a] $P_{a/b}$ (*i.e.*, $P_{1/2}$, $P_{1/3}$, and $P_{2/3}$, column 4) represents the *P* for the *t* test between ER+% in classes a and b, therefore $P_{1/2}$ is the *P* value for the *t* test comparing the ER+% in grade 1 and grade 2 tumors. $P_{trend}$ was calculated using the two-sided Cochran-Armitage trend test.

[b] Including all histologies ($n = 1131$): 992 ductal and 209 nonductal cancers. The 209 nonductal cancers are distributed as follows: noncarrier, $n = 54$ (22.6% of all noncarriers); *BRCA1* carrier, $n = 20$ (9.6%); *BRCA2* carrier, $n = 12$ (13.6%); untested, $n = 123$, (20.6%).

## MATERIALS AND METHODS

**Subjects Included in Study.** Three different sets of breast cancer patients were included in this study. The first set consists of a historical cohort of 276 Ashkenazi Jewish women who were diagnosed with a first primary invasive breast cancer, at <65 years of age, at a single Montreal institution between January 1980 and November 1995. They were all tested for the three founder mutations in *BRCA1/2* that are common in the Ashkenazi Jewish population. The histological type, grade and ER status were determined by one pathologist (L. R. B.). ER status was determined by immunohistochemistry, and tumors were deemed positive if ≥10% of the total number of tumor cells examined showed clear and intense staining with the anti-ER antibody used, with no background cytoplasmic staining, as described previously (1). The ascertainment criteria and mutation analysis performed for these women have been described elsewhere (10, 11). Tumors ≥ 51 mm were excluded, so as to match the second series (see below). Among the 246 eligible women, 30 were *BRCA1* carriers, 7 were *BRCA2* carriers, and 239 were noncarriers.

The second series of women consists of a historical cohort of *BRCA1* and *BRCA2* carriers affected with breast cancer. Four hundred ninety-two *BRCA1/2* carriers (328 *BRCA1*, 152 *BRCA2*, and 12 *BRCA1*+*BRCA2*) women were ascertained as part of a North American study of the effects of various treatments of hereditary breast cancer. There were 10 participating centers. The inclusion criteria were as follows: presence of a germ-line *BRCA1* or *BRCA2* mutation; invasive breast cancer; diagnosed in 1975 or thereafter; at age ≤ 65 years; and residents of North America at the time of diagnosis. Exclusion criteria were as follows: women with a history of breast cancer or any other cancer; before 1975; carcinoma *in situ* with no invasive component; tumor size > 50 mm; and evidence for locoregional or distant metastases at time of diagnosis and those with fixed mass of axillary nodes. Histological type, histological grade, and ER status were extracted from the patient charts by local collaborators. ER status was measured both biochemically and by using immunohistochemistry, but we do not have details of individuals scores because the entry sheet used allowed for three responses: positive; negative; and unknown. There were 178 *BRCA1* carriers and 81 *BRCA2* carriers with complete information, and these were included in the present study. In total, there were 208 *BRCA1* carriers and 88 *BRCA2* carriers (both study sets combined).

The third study group consisted of consecutive cases of women diagnosed with invasive breast cancer at <65 years of age at Women's College Hospital, Toronto, Ontario, Canada, from 1987 to 1997, for whom histological subtype, ER status, and histological grade were available. In all cases, ER was measured biochemically in all cases at the time of diagnosis, and a level of >10 fmol/mg protein was recorded as positive for ER. The range of values observed was 0–776 fmol/mg protein. No immunohistochemical results were used in the analyses reported here. Patients with primary tumors 51 mm and larger in size

*Table 2* Influence of mutation status on estrogen receptor (ER) status of grade 3, infiltrating ductal breast cancers

| Class | Class description | N | ER+% | $P^a$ |
|---|---|---|---|---|
| 1 | Noncarriers | 65 | 46.2 | $P_{1/2} < 0.0001$ |
| 2 | *BRCA1* | 138 | 15.2 | $P_{1/3} = 0.065$ |
| 3 | *BRCA2* | 36 | 63.9 | $P_{1/4} = 0.183$ |
| 4 | Untested | 217 | 54.8 | $P_{2/3} < 0.0001$ |
| | Total | 456 | 42.3 | $P_{2/4} < 0.0001$ |
| | | | | $P_{3/4} = 0.276$ |

[a] $P_{a/b}$ represents the *P* for the *t* test between ER+% in classes a and b, therefore, $P_{1/2}$ is the *P* value for the *t* test comparing ER+% in noncarriers and *BRCA1* carriers.

*Table 3* Percentage of estrogen receptor (ER)-positive breast cancers by age at diagnosis

| Class | Class description | N (%) | ER+% | $P^a$ |
|---|---|---|---|---|
| 1 | Noncarriers | | | |
| | Age <45 yrs | 59 (24.7) | 61.2 | $P_{1/2} = 0.872$ |
| | Age 45–55 yrs | 72 (30.1) | 59.7 | $P_{1/3} = 0.018$ |
| | Age 55–65 yrs | 108 (45.2) | 78.7 | $P_{2/3} = 0.007$ |
| | | | | $P_{trend} = 0.008$ |
| 2 | *BRCA1* | | | |
| | Age <45 yrs | 142 (68.3) | 19.0 | $P_{1/2} = 0.095$ |
| | Age 45–55 yrs | 45 (21.6) | 31.1 | $P_{1/3} = 0.054$ |
| | Age 55–65 yrs | 21 (10.1) | 38.0 | $P_{2/3} = 0.562$ |
| | | | | $P_{trend} = 0.020$ |
| 3 | *BRCA2* | | | |
| | Age <45 yrs | 43 (48.8) | 83.7 | $P_{1/2} = 0.196$ |
| | Age 45–55 yrs | 35 (39.8) | 71.4 | $P_{1/3} = 0.799$ |
| | Age 55–65 yrs | 10 (11.4) | 80.0 | $P_{2/3} = 0.566$ |
| | | | | $P_{trend} = 0.418$ |
| 4 | Untested | | | |
| | Age <45 yrs | 177 (29.7) | 65.5 | $P_{1/2} = 0.061$ |
| | Age 45–55 yrs | 240 (40.3) | 73.6 | $P_{1/3} = 0.003$ |
| | Age 55–65 yrs | 179 (30.0) | 79.3 | $P_{2/3} = 0.202$ |
| | | | | $P_{trend} = 0.003$ |

[a] $P_{a/b}$ represents the *P* for the *t* test between ER+% in classes a and b, therefore $P_{1/2}$ for noncarriers is the *P* value for the t test comparing ER+% in those diagnosed <45 years (class 1) and those diagnosed 45–55 years (class 2). $P_{trend}$ represents the *P* calculated from the two-sided Cochran-Armitage trend test.

were excluded, so as to match set 2 (*BRCA1/2* carriers). The 596 women in this data set were not tested for *BRCA1* or *BRCA2* mutations. This set is referred to as the untested set in the tables.

**Statistical Analysis.** The percentages of ER-positive tumors were calculated by histological grade (Table 1), by mutation status (Table 2), and by age at diagnosis (Table 3). The significance of differences was tested using the *t* test. The trend in percent ER positivity as histological grade increased was tested by the Cochran-Armitage test. A multiple regression model (12) was created:

$$Y = \alpha_1 + \alpha_2 I_2 + \alpha_3 I_3 + \alpha_4 I_4 + \beta_1 \text{ageca} + I_2\beta_2 \text{ageca} + I_3\beta_3 \text{ageca} + I_4\beta_4 \text{ageca ca.} \quad (1)$$

This model was used to analyze how ER positivity changes with the age at diagnosis and to compare the parameters of the regression equations in each of the different mutation groups: Y represents the ER status, $I_j = 1$ if sample from mutation group j, else $I_j = 0$; j = 1, 2, 3, 4 means subgroups of noncarriers, *BRCA1* carriers, *BRCA2* carriers, and untested individuals, respectively (Fig. 1). As a result of regression of model (1), the four regression equations of the four groups were as follows: $Y = \alpha_1 + \beta_1 \text{ageca}$ (for group 1), and $Y = \alpha_1 + \alpha_j + (\beta_1 + \beta_j)\text{ageca}$, for j = 2, 3, 4 (for groups 2, 3, 4), respectively.

The model (1) was altered to analyze the same relationship when samples were divided by histological grade (Fig. 2).

Pearson correlation coefficients were examined between the variables percent ER positive, histological grade, and age of diagnosis. Stepwise regression was conducted to compare the two variables (histological grade and age of diagnosis) in the prediction of ER positivity. In this case, the model was as follows: $ER = \alpha + \beta_1 BR + \beta_2$ ageca, where BR is Bloom and Richardson histological grade. All statistical analyses were done using SAS, version 8.2.

## RESULTS

We studied 1131 women with breast cancer from the three study sets to determine the relationship between ER status and histological type, histological grade, age at diagnosis, and *BRCA1/2* mutation status. As expected, among women with either ductal or nonductal cancers, the proportion of cancers that

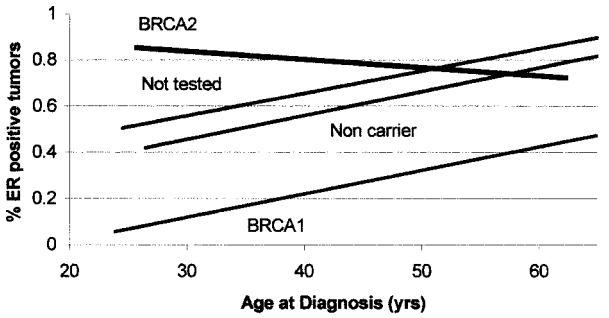


*Fig. 1* Estrogen receptor (ER) status and age of diagnosis of breast cancer by mutation status. We regressed ER percent positive (*y* axis) against age at diagnosis (*x* axis), dividing the data by mutation status. Sample size: 239 noncarriers (group 1); 208 *BRCA1* carriers (group 2); 88 *BRCA2* carriers (group 3); 596 untested (group 4); total 1131.

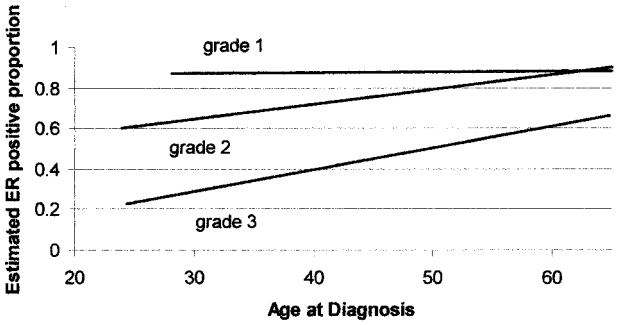


*Fig. 2* Estrogen receptor (ER) status and age at diagnosis of breast cancer by histological grade. We regressed ER percent positive (*y* axis) against age at diagnosis (*x* axis), dividing the data by histological grade. Sample size: 169 grade 1; 427 grade 2; 535 grade 3, total 1131.

*Table 4* Parameters estimated for model (1), as shown in Figure 1

| Class | Class description | $\alpha_i$ | *P* | $\beta_i$ | *P* |
|---|---|---|---|---|---|
| 1 | Noncarriers | 0.147 | 0.370 | 0.01 | 0.0009 |
| 2 | *BRCA1* | −0.336 | 0.130 | −0.001 | 0.984 |
| 3 | *BRCA2* | 0.799 | 0.010 | −0.014 | 0.034 |
| 4 | Untested | −0.224 | 0.251 | −0.003 | 0.403 |

Regression results: $R^2 = 0.180$. Additional tests: $P(\alpha_2 = \alpha_3) = 0.0002$; $P(\beta_2 = \beta_3) = 0.041$; $P(\alpha_2 = \alpha_4) = 0.002$; $P(\beta_2 = \beta_4) = 0.460$; $P(\alpha_3 = \alpha_4) = 0.043$; $P(\beta_3 = \beta_4) = 0.079$.

are ER-positive decreases as the histological grade increases (Table 1). This is observed in patients with and without mutations in *BRCA1/2* (Table 1). However, for tumors of grade 2 or 3, *BRCA1*-related breast cancers are significantly more likely to be ER negative than are *BRCA2*-related noncarriers or untested individuals (all $P < 0.001$). There are too few grade 1 *BRCA1*-related cancers to observe an effect, but the trend is in the same direction. The proportion of cancers that are ER positive decreases dramatically from grade 1 to grade 3 in noncarriers: (grade 1, 90.1% ER positive and grade 3, 45.7% ER positive; $P < 0.0001$) and in *BRCA1* carriers (71.4 and 15.7%, respectively; $P = 0.0024$). In contrast to these groups, the difference in ER positivity between grade 1 and grade 3 tumors is not significant for *BRCA2*-related tumors (80.0 *versus* 61.0%, $P = 0.64$), although the sample size is small (Table 1). There were 209 nonductal cancers (18.5% of the total). The same relationship between grade, age, and ER status is observed in nonductal cancers as in ductal cancers, but because of small numbers, statistical significance is not reached in all comparisons for nonductal cancers (data not shown).

To study the effect of mutation status on ER status independent of grade and histological type, we stratified all ductal grade 3 cancers by mutation status (Table 2). *BRCA1*-related breast cancers are significantly more likely to be ER negative than tumors in *BRCA2* carriers, noncarriers, or untested individuals (all $P < 0.0001$). Among grade 3 ductal cancers, *BRCA1* tumors are 4.8 times (95% CI 2.4–9.36) more likely to be ER negative than were tumors in noncarriers. Among *BRCA1* carriers who were <45 years when they were diagnosed with a grade 3 ductal breast cancer, 87.1% (88 of 101) were ER negative. This fell to 70% (7 of 10) in women 55–65 years old at diagnosis ($P = 0.44$). Interestingly, *BRCA2* carriers with grade 2 or 3 cancers are more likely to have an ER-positive cancer than are noncarriers (80.8 *versus* 60.9%, $P = 0.0015$; Tables 1 and 2).

For each age group (<45, 45–54, and 55–64 years), *BRCA1*-related breast cancers are more likely to be ER negative than cancers occurring in any other group (Table 3). For all groups other than *BRCA2* carriers, a similar increase in the percentage of tumors that were ER positive with increasing age was observed. For *BRCA2*-related breast cancers, there is a much smaller change in ER status with increasing age at diagnosis (3.7%, $P = 0.78$; Table 3) or grade (19%, $P = 0.64$; Table 1) compared with the other subtypes studied.

The relationship between age at diagnosis and percentage of ER-positive cancers was studied further by a regression analysis (model 1). The percentage of ER-positive cancers (*y* axis) was plotted against age at diagnosis (*x* axis; Fig. 1), with the parameters. Notwithstanding the different starting points of the slopes (noncarrier *versus* *BRCA1*, $P = 0.0.13$; noncarriers *versus* untested, $P = 0.25$), *BRCA1* carriers, noncarriers, and untested women have slopes that are almost parallel from ages 24 to 64.9 years (noncarrier *versus* *BRCA1*, $P = 0.98$; noncarriers *versus* untested, $P = 0.40$; Table 4). From Fig. 1, it can be deduced that a 60-year-old *BRCA1* carrier has the same probability of an ER-negative breast cancer as a 25-year-old noncarrier. At the youngest ages at diagnosis, the proportion of cancers that were ER positive was 0.42 (noncarriers, youngest age at diagnosis: 26.5 years), 0.06 (*BRCA1* carriers, 23.9 years), 0.85 (*BRCA2* carriers, 25.7 years), and 0.54 (untested individuals, 24.6 years). Despite these differences, the difference in the proportion of cancers that are ER positive between the youngest and oldest ages is almost identical in *BRCA1* carriers (0.40) and noncarriers (0.41, $P = 0.98$; Fig. 1). The relationship between age at diagnosis and ER status in *BRCA2* carriers is significantly different from that of the noncarrier group in both intercept ($P = 0.01$) and slope ($P = 0.034$) and that from *BRCA1* carriers ($P = 0.0002$ for intercept and $P = 0.041$ for slope; Table 4). For the untested group ($n = 596$), we also plotted absolute level of ER in fmol/mg of protein against the age at diagnosis. This resulted in a slope with an $R^2$ of 0.06 (data not shown).

The proportion of cancers that are ER positive increases with age for all tumor grades, but the increase is most marked for grade 3 tumors (Fig. 2) in keeping with the results shown in Tables 1 and 3. The intercept and slope for grade 3 cancers are statistically significantly different from that seen in grade 1 cancers. When comparing the intercepts and slopes between grade 2 and grade 3 cancers, the intercepts are very different, but the slopes (*i.e.*, the change in ER status with increasing age at diagnosis) are similar (*P* for differences between intercepts = 0.002, *P* for slope = 0.34; Fig. 2). Table 5 shows in more detail the parameters estimated using model (1). The intercept ($\alpha$) and slope ($\beta$) for grade 3 cancers are both significantly different from the grade 1 values, whereas the parameters for grade 2 cancers are not different from grade 1 cancers. This indicates that the relationship between the ER status of a breast cancer and the age at diagnosis is dependent on the grade of the cancer, insofar as the relationship is not uniform across all grades. As the age at breast cancer diagnosis increases, the difference between the three groups becomes less noticeable. This is in contrast to the results shown in Figure 1. Grade 1 cancers are likely to be ER positive, no matter the age at diagnosis; this is not the case for grade 3 cancers. Notably, a woman who develops a grade 3 breast cancer at 40 years of age is five times more likely to have an ER-negative cancer than a woman diagnosed

*Table 5* Parameters estimated for model (1), as shown in Figure 2

| Class | Class description | $\alpha_i$ | *P* | $\beta_i$ | *P* |
|---|---|---|---|---|---|
| 1 | Grade 1 | 0.865 | 0.0002 | 0.0003 | 0.944 |
| 2 | Grade 2 | −0.442 | 0.092 | 0.007 | 0.157 |
| 3 | Grade 3 | −0.901 | 0.0004 | 0.009 | 0.039 |

Regression results: $R^2 = 0.186$. Additional tests: $P(\alpha_2 = \alpha_3) = 0.002$; $P(\beta_2 = \beta_3) = 0.337$.

with a grade 1 cancer at the same age (ER percent negative = 60 *versus* 12%).

To compare the relative importance of age and grade in the predicting of the ER status of any given tumor, stepwise regression was used. This analysis showed that both age and grade are highly predictive ($\alpha = 0.74$, $P < 0.0001$; $\beta_1 = -2.7$, $P < 0.0001$ for grade; and $\beta_2 = 0.009$, $P < 0.0001$ for age), but histological grade is the most important variable in predicting ER status.

## DISCUSSION

To some extent, many of the clinical and pathological features of hereditary breast cancer are a reflection of the early age at diagnosis observed in most *BRCA1/2* carriers. In general, young women tend to develop high-grade, ER-negative breast cancers. For example, it has been difficult to identify statistically significant differences in immunophenotypes between *BRCA1* carriers and age-matched noncarriers (13). However, detailed histological analysis, combined with immunohistochemical and expression array studies, has been successful in distinguishing *BRCA1*-related breast cancers from non-*BRCA1*-related breast cancers (14–17). In the general population, as women age, the likelihood of developing an ER-positive cancer increases (18, 19). In one small study, breast cancers occurring in *BRCA1* carriers older than 50 years at diagnosis were equally as likely to be ER-positive as noncarriers. This has been taken as evidence that sporadic breast cancer can develop in *BRCA1* carriers (20). Here, in a much larger study, we show that in every age interval, breast cancers that develop in *BRCA1* carriers are significantly more likely to be ER negative than are breast cancers that occur in noncarriers. In particular, breast cancers occurring in older *BRCA1* carriers are much more likely to be ER negative than are breast cancer developing in older noncarriers (62.0 *versus* 21.3%, $P = 0.0004$; Table 3). Our findings suggest that ER-negative status is an intrinsic feature of *BRCA1*-related breast cancer. As suggested previously, it is possible that most, if not all ER-positive breast cancers occurring in *BRCA1* carriers are, in fact, sporadic cancers. We could not address this question in this study, but *BRCA1* sequencing or loss of heterozygosity analysis to compare the frequency of complete inactivation of *BRCA1* in ER-negative and ER-positive breast cancers could help to resolve this issue. Even if this hypothesis were supported, the magnitude of the reduction in risk for contralateral breast cancer in *BRCA1* carriers who received tamoxifen treatment appears to be too large to be attributable to the successful treatment of sporadic, *BRCA1*-unrelated breast cancers (3).

Although this hypothesis remains a possibility, it is notable that the slope of the change in ER positivity with increasing age is almost identical for *BRCA1* carriers, noncarriers, and untested individuals (Fig. 1). This similarity in the slopes suggests that *BRCA1* carriers are likely to be susceptible to the same hormonal and/or environmental factors that result in the change in ER positivity with age in the general population. The hormonal milieu of the woman at risk of breast cancer due to a *BRCA1* mutation probably influences the ER status of the cancer, but the presence of the mutation sets the limits of the response in terms of the likely ER status of the breast cancer. *BRCA1* carriers in the oldest age group were 2.6 times as likely as those in the youngest age group to have an ER-positive cancer ($P = 0.08$), but this phenotype was less than half as likely to occur in older *BRCA1* carriers, when compared with older noncarriers (38.0 *versus* 78.7%, $P = 0.0004$; Table 3). The lack of change with age in the slope for *BRCA2* carriers (Fig. 1) suggests that the ER status of breast cancer occurring in *BRCA2* carriers is not under the same hormonal and environmental control as is nonhereditary breast cancer.

Among *BRCA1* carriers, grade 3, ductal breast cancers are highly likely to be ER negative: only 21 of 138 of this type were ER positive (15.2%; Table 2). This suggests that in any study of breast cancer outcomes after adjuvant hormonal treatment, it is reasonable to assume that the great majority of *BRCA1*-related, grade 3 invasive ductal cancers are ER negative. This finding is relevant to the debate as to the potential benefit of tamoxifen for the primary prevention of *BRCA1*-related breast cancer. The data presented here do not suggest that ER-positive cancers predominate in any subgroup of *BRCA1*-related breast cancers, whether defined by age at diagnosis, grade, or histological type. Recent case-control (7) and retrospective (3) studies of contralateral breast cancer in *BRCA1* and *BRCA2* carriers have shown that tamoxifen is capable of reducing the incidence of second primary breast cancers by ~50%. The reduction was equally large in women whose first cancer was a high-grade ductal cancer as it was for other groups. Moreover, as stated above, our results suggest that the percentage of all *BRCA1* carriers who develop an ER-positive breast cancer in the postmenopausal years is too small (8 of 208, or 3.9%) to account for the observed risk reductions, even if ER-positive breast cancers in *BRCA1* carriers are actually sporadic breast cancers.

There are two main weaknesses in our study that may limit the interpretation of our results. Because of the diverse nature of the three study groups and the partly retrospective collection of data, we were unable to arrange central pathology review. Similarly, we were unable to determine ER status using one standardized method. Both these weaknesses mean that some of our analyses may be subject to error. However, should grade or ER measurement errors exist, they are likely to bias the results toward the null. It would be more troubling if our lack of standardization were to lead to differential misclassification. However, in group 1, all tumors were analyzed by a single pathologist without knowledge of the *BRCA1/2* status, and given the era, very few of the pathologists could have been aware of the *BRCA1/2*-status in group 2. It is therefore implausible that there the lack of standardization has introduced a systematic bias in the measuring or reporting of either grade or ER status. It has been suggested that tumor-associated infiltrates, which are common in *BRCA1*-related breast cancers, may express ER (21), and ligand-binding assays could therefore overestimate tumor ER levels. If this observation were to contribute significantly to our findings, it would mean that tumor cells in *BRCA1*-related cancers actually contain less ER than reported here. In this case, *BRCA1*-related breast cancers are even more likely to be ER-negative than we observed. Finally, we do not know the *BRCA1/2* status of the untested cases (group 3). It is difficult to be sure of the expected numbers of *BRCA1/2* carriers, but it is likely to be substantially <5% in most outbred populations (22),

and therefore, these hidden carriers are likely to contribute very little to the overall results obtained from the untested group.

The observation here and elsewhere (23) that *BRCA1*-related breast cancer is likely to be ER negative, regardless of the grade or age at diagnosis, suggests that *BRCA1*-related breast cancers may arise from an ER-negative breast cell. This conjecture is supported by the observation that ductal carcinoma *in situ* occurring in *BRCA1* carriers is usually ER negative (24). Some studies suggest that the breast stem cell is an ER-negative cell but is surrounded by ER-positive cells (25, 26). If the regulation of ER-negative breast stem cells is partly controlled by paracrine factors released by the adjacent ER-positive cells (26), then inhibiting estrogenic stimulation of these supporting cells either directly by estrogen deprivation, or indirectly by use of tamoxifen, could play a role in reducing the incidence of breast cancers in *BRCA1* carriers.

Our observations led us to conclude that the beneficial effect of tamoxifen on both local recurrence and contralateral cancer rates in *BRCA1* carriers is due to the prevention of breast cancers that arose from breast cells that either were always ER negative or those that became ER negative very soon after an initiating event. Research focused on the mechanisms by which tamoxifen reduces risk of breast cancer in *BRCA1* carriers is to be encouraged.

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