Multifactorial Analysis of Differences Between Sporadic Breast Cancers and Cancers Involving BRCA1 and BRCA2 Mutations

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Background: **We have previously demonstrated that breast cancers associated with inherited BRCA1 and BRCA2 gene mutations differ from each other in their histopathologic appearances and that each of these types differs from breast cancers in patients unselected for family history (i.e., sporadic cancers). We have now conducted a more detailed examination of cytologic and architectural features of these tumors.** ***Methods:*** **Specimens of tumor tissue (5-µm-thick sections) were examined independently by two pathologists, who were unaware of the case or control subject status, for the presence of cell mitosis, lymphocytic infiltration, continuous pushing margins, and solid sheets of cancer cells; cell nuclei, cell nucleoli, cell necrosis, and cell borders were also evaluated. The resulting data were combined with previously available information on tumor type and tumor grade and further evaluated by multifactorial analysis. All statistical tests are two-sided.** ***Results:*** **Cancers associated with BRCA1 mutations exhibited higher mitotic counts (*P* = .001), a greater proportion of the tumor with a continuous pushing margin (*P*<.0001), and more lymphocytic infiltration (*P* = .002) than sporadic (i.e., control) cancers. Cancers associated with BRCA2 mutations exhibited a higher score for tubule formation (fewer tubules) (*P* = .0002), a higher proportion of the tumor perimeter with a continuous pushing margin (*P*<.0001), and a lower mitotic count (*P* = .003) than control cancers.** ***Conclusions:*** **Our study has identified key features of the histologic phenotypes of breast cancers in carriers of mutant BRCA1 and BRCA2 genes. This information may improve the classification of breast cancers in individuals with a family history of the disease and may ultimately aid in the clinical management of patients. [J Natl Cancer Inst 1998;90:1138–45]**

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See ''Notes'' following ''References.''

Two highly penetrant breast cancer predisposition genes, BRCA1 and BRCA2, have recently been identified *(1,2)*. BRCA1 is located on chromosome 17q21 *(3)* and encodes a protein of 1863 amino acids. BRCA2 is located on chromosome 13q12–q13 *(4)* and encodes a protein of 3418 amino acids.

We recently compared the pathology of breast cancers in patients carrying mutations in BRCA1 and BRCA2 with that of breast cancers from a series of control subjects of similar age distribution unselected for family history *(5)*. In this first review, we compared breast cancer subtype and grade and the presence of carcinoma *in situ*. Overall, the results showed that breast cancers in BRCA1 and BRCA2 mutation carriers differed from those in control subjects as well as from each other. Breast cancers due to mutations in the BRCA1 gene were characterized by higher scores for mitosis (i.e., higher numbers of cells undergoing mitosis per tumor), pleomorphism (i.e., a greater degree of pleomorphism), and tubule formation (i.e., less tubule formation) than breast cancers from control subjects. Breast cancers due to BRCA2 mutations showed a higher score for tubule formation and included a smaller proportion of tubular carcinomas than the breast cancers in control subjects. The findings with respect to cancers involving BRCA1 mutations are broadly in agreement with previously published series *(6,7)*. Only a small number of breast cancers involving BRCA2 mutations have previously been evaluated for their histology *(7)*.

Medullary and atypical medullary carcinomas were reported more frequently in carriers of BRCA1 mutations (13%) than in control breast cancer patients without a family history (2%; $P<.0001$). Medullary carcinoma is a form of invasive breast cancer characterized by the presence of solid sheets of large, pleomorphic cells with vesicular nuclei, prominent nucleoli, and indistinct cell borders that lead to a ''syncitial'' appearance *(8,9)*. The border of the tumor is well defined with a pushing edge. The stroma may be sparse but always contains a dense lymphocytic infiltrate. The whole tumor should exhibit these features. Carcinoma *in situ* is usually not seen. Despite the apparent high grade of the tumor, it has been associated with a relatively favorable prognosis *(9)*. Atypical medullary carcinoma is diagnosed either when up to 25% of the tumor is invasive ductal carcinoma of no special type and the remainder is classic medullary or when the complete tumor shows less lymphoid infiltration and less circumscription or areas of dense fibrosis while still having the other features of medullary carcinoma *(8)*.

In the first review *(5)*, the agreement between pathologists was low (κ score of .41) for medullary and atypical medullary carcinomas. This result was consistent with previous reports indicating poor reproducibility for this diagnostic category *(10,11)*.

To clarify the observation of an excess of medullary and atypical medullary carcinomas reported in BRCA1 mutation carriers, we carried out a second review in which the morphologic features that are associated with medullary and atypical medullary carcinomas were specifically evaluated. Subsequently, we combined the data from both evaluations and performed a multifactorial analysis to identify the features that are independently associated with cancers in carriers of mutations in the BRCA1 and BRCA2 genes.

Patients and Methods

Breast Cancer Specimens

As described previously in our first review *(5)*, we obtained specimens from case subjects with familial breast cancer (at least one relative affected with the disease) in the form of 5-μm sections either unstained or stained with hematoxylin–eosin (H&E). These specimens came from the United Kingdom, United States, Ireland, France, Germany, Iceland, Switzerland, and The Netherlands. The vast majority of familial cases were from the last two decades, since it was predominantly from that period that blocks were available.

Given the diverse origin of the familial cases, it was logistically impossible to obtain locally matched control subjects in all instances. However, almost all the familial case and control subjects were Caucasian. A higher proportion of mutation carriers than control subjects would be Ashkenazi Jewish, but this proportion would still be a very small minority of the cases. Moreover, ethnic origin is unlikely to be strongly related to grade or other histopathologic features of the tumor. We therefore chose control specimens from the Department of Histopathology, Royal Marsden Hospital National Health Service Trust, Sutton, Surrey, U.K., to give an age distribution similar to that of familial case subjects. These control subjects constitute a series of breast cancer patients unselected for family history. Some of these control subjects may have carried mutations in BRCA1 and BRCA2. However, the proportion would have been very small, and precise estimates were not available.

We selected one, or occasionally two, representative H&E-stained sections from each primary breast cancer and coded each section with a random number. We arranged the slide sets from familial cancer patients and control subjects with sporadic cancers in sequential order according to their random number for the review. If slides from two or more tumors from the same woman were available, results obtained from the earliest tumor only were included in the analysis, unless the second tumor was clearly recorded as a second primary cancer (nine cases: two involving BRCA1, five involving BRCA2, and two familial cases that were not classifiable as involving either gene).

The studies were carried out with the informed consent of the patients and after approval from the local institutional review board.

Conduct of the Second Histologic Review

The second review was carried out by seven pathologists (J. P. Sloane, T. J. Anderson, J. Jacquemier, M. J. van de Vijver, B. A. Gusterson, L. M. Farid, and D. Venter); each pathologist scored the slides independently. The slides were randomly sorted into boxes containing 100 slides each. The pathologists were assigned to review a certain number of boxes, and it was arranged so that no pair of pathologists reviewed more than one box. Each slide was read independently by two pathologists. The study was conducted blindly, so that the pathologists were not aware if the slide being read was from a case subject or a control subject. The pathologists were asked to complete a form that included an assessment of the percentage of tumor present as solid sheets of cells (<25%, 25%–75%, and >75%) determined by low-power scrutiny of the section, the total mitotic count per 10 high-power fields (hpf) by use of ×40 magnification lens, the presence of continuous pushing margins (i.e., a smooth, noninfiltrative edge to the tumor—subdivided into absent and present [<25%, 25%–75%, and >75% of tumor perimeter]) determined by low-power scrutiny of the section, the presence of confluent necrosis, the presence of lymphocytic infiltrate (if present, whether mild or prominent), the presence of discernible cell borders, the presence of vesicular nuclei (defined as nuclei with cleared chromatin, often divided by septae into sac-like compartments), and the presence of prominent, eosinophilic nucleoli. Because the same slide set had been used in the first review, each pathologist reviewed a subset of slides that he/she had not examined previously. No attempt was made to reconcile differences between the pathologists because it was difficult to design such a process that would not introduce other biases. Although there were clear differences in frequency of diagnoses between the pathologists, each pathologist reviewed tumors from case individuals carrying BRCA1 and BRCA2 mutations as well as control tumors from individuals unselected for a family history. Moreover, all variables examined were adjusted for the pathologist. To assess the degree of agreement between the pathologists and, hence, the reproducibility of evaluation of each feature, κ scores were calculated.

Classification of Families

As previously described *(5)*, familial cases were attributed to BRCA1 or BRCA2 on the basis of either strong linkage evidence or a clearly disease-associated mutation generating a higher than 90% posterior probability of being

due to either gene. We classified the residual cases as ''unknown.'' The posterior probability of linkage to BRCA1 was determined by the following formula:

$$(1 - \mu_1)\ \alpha_1\ 10^{LOD1}/[(1 - \mu_1)\ \alpha_1\ 10^{LOD1} + \alpha_2\ 10^{LOD2} + (1 - \alpha_1 - \alpha_2)].$$

α_1 and α_2 are the prior probabilities of linkage to BRCA1 and BRCA2. These probabilities were estimated from the numbers of individuals with breast cancer (both female and male) and ovarian cancer in the family, as reported in recent studies by the Breast Cancer Linkage Consortium *(12).* Although α_1 and α_2 theoretically depend on ages of cancer occurrence in a family, precise prior probabilities by exact ages are not known. We have therefore based the prior probabilities on number of cases. μ_1 is the estimated sensitivity of the BRCA1 mutation testing used on the family [for details, *see (12)*]. Methods of mutation testing included DNA sequencing, single-strand conformation polymorphism analysis, the protein truncation test, and heteroduplex analysis *(12).* We ignored the test sensitivity to BRCA2 because only a small proportion of families had been examined for BRCA2 mutations at the time of analysis. For the purposes of this study, there are essentially no effects of this assumption. Only 10 cases were classified as involving BRCA2 on the basis of linkage rather than mutations status. On the basis of mutation studies now completed, a more realistic estimate of the mutation sensitivity of BRCA2 would still have included all these cases as being due to BRCA2. LOD1 and LOD2 (logarithm of odds ratios) are the LOD scores for linkages to BRCA1 and BRCA2, respectively, employing markers close to the gene. In most cases, we calculated LOD1 from a multipoint analysis by use of the markers D17S579 and either D17S250 or Thra1. LOD2 was based on markers D13S260 and D13S267. We calculated posterior probabilities for BRCA2 in a similar way. We made the assumption that case subjects in mutation-positive families were mutation carriers unless information from mutation or linkage analyses indicated that they were not carriers. (These noncarriers were excluded from all analyses.)

Statistical Methods

As in the previous analysis, the effects of each morphologic feature on cancer status was summarized in terms of odds ratios, as in standard case–control analyses *(13),* for patients carrying mutations in the BRCA1 gene versus control patients and, separately, for patients carrying mutations in the BRCA2 gene versus control patients. Analyses comparing tumors associated with BRCA1 and BRCA2 mutations directly were also performed. To analyze the joint effect of several features simultaneously, we performed multiple logistic regression analysis, using the program S-Plus (version 3.4; MathSoft Inc., Seattle, WA). All analyses were adjusted for age in groups (i.e., <30 years old, 30–39 years old, 40–49 years old, 50–59 years old, 60–69 years old, and ⩾70 years old) and by pathologist, by including these as covariates in the regression. This procedure adjusts for systematic differences in scoring between pathologists. The main complication in the analysis is that the observations by different pathologists on the same slide cannot be considered independent. Using standard logistic regression, therefore, involves maximizing a quasi-likelihood rather than a true likelihood; this maximization of a quasi-likelihood leads to unbiased odds ratio estimates but underestimates the standard errors and confidence intervals (CIs). To correct for this situation, we computed confidence limits by using Huber's sandwich estimator for the variance–covariance matrix of maximum quasi-likelihood estimates *(14),* by use of specially written S-Plus macros. This quasi-likelihood approach allows for the variation in scoring individual samples between the pathologists without explicitly modeling the error distribution. The CIs were also estimated by bootstrapping *(15),* in which 1000 bootstrap samples were created by resampling (unit of resampling was the case with observations from the two pathologists) the cases (with replacement) within each age group. This method, which allows confidence limits to be derived without assuming an asymptotic normal distribution, gave results very similar to those of the sandwich estimator. For simplicity and consistency with previous analyses, the confidence limits with the use of the sandwich estimator are quoted. Significance levels for each factor in multiple regression analysis were derived from the parameter estimates and the covariance matrix (adjusted by use of the sandwich estimator). Since all the factors scored on more than one level (e.g., mitotic count) are naturally considered as ordered categories, we constructed 1-degree-of-freedom significance tests based on testing for linear trends in log(odds ratio) with increasing category *(13).* (Estimated odds ratios were, however, derived separately for each level.) Significance levels less than .10 are quoted in the tables. Heterogeneity chi-squared statistics (based on $k - 1$ degree of freedom for factors with k levels) have also been presented for those factors with the best fitting models. All P values are two-sided.

In the multiple regression analysis, all factors that were significant at the 5% level for presence of mutations in either BRCA1 or BRCA2 genes, together with the patient's age and the pathologist who reviewed the slides, were initially included. Factors (other than patient's age and pathologist) were then removed from the model on a stepwise basis until no further factors could be removed at the 5% level.

On the basis of the estimated odds ratios in the final genetic model, we computed BRCA1 carrier probabilities for breast cancer cases with given combinations of histologic features such that the overall carrier probability agreed with the predicted mutation prevalence in each age group *(12).*

Results

Classification of Familial Cases

This study included 440 female patients with familial breast cancer. The age distribution of the control and familial groups was similar. The results reported here are based on 360 observations from familial case subjects, 219 observations from 114 subjects with mutations in the BRCA1 gene, 141 observations from 73 subjects with mutations in the BRCA2 gene, and 1046 observations from 528 control subjects with cancer unselected for family history. Details of actual mutations are given in *(5).* The numbers of case and control subjects differed slightly from the previously published first study *(5)* because the present analysis was restricted to invasive cancers and excluded slides where only *in situ* cancer was reported. Because two pathologists reviewed slides from each case and control subject, the number of observations should be double the number of cancers. However, in a few cancers, one or both pathologists were unable to review the section because of poor quality. Overall, the slides from familial case subjects were of poorer quality than those from control subjects; however, sections from case subjects with BRCA1 and BRCA2 mutations were of similar quality; therefore, quality is unlikely to account for the observed differences between the two genes. The characteristics of the BRCA1 and BRCA2 mutations in the set have been reported previously *(5).* Of the case subjects classified as BRCA1 mutation carriers, 111 were classified as such on the basis of a clear disease-causing mutation and three on the basis of genetic linkage analysis. Of the case individuals classified as BRCA2 mutation carriers, 63 were classified as such on the basis of a clear disease-causing mutation and 10 on the basis of genetic linkage analysis. Results from the familial set as a whole and from ''unknown'' cases (of which there were 253) are not presented in this article.

Analysis of Morphologic Features

The agreement between the pathologists on the features evaluated, expressed as κ scores, is shown in Table 1. A κ score of 1 indicates complete agreement, whereas a κ score of 0 indicates no agreement. Best agreement was achieved for necrosis (κ score of .68 [standard error = .03]), and least agreement was achieved for the definition of cell borders (κ score of .21 [standard error = .03]).

Table 2 compares morphologic characteristics evaluated in the second review with odds ratios adjusted for age of the patient and for the pathologist. Cancers associated with BRCA1 mutations showed higher mitotic counts than cancers associated with BRCA2 mutations or control breast cancers, with an elevated relative risk for BRCA1 at all mitotic counts above 5 per 10 hpf and a maximum odds ratio of 6.79 (95% CI = 3.94–11.67) at 20–39 mitoses per 10 hpf. Cancers associated with BRCA1 mutations also showed more lymphocytic infiltrate (P for trend <.0001), both mild (odds ratio = 3.43; 95% CI = 2.26–5.21)

Table 1. κ scores indicating level of agreement between the observers evaluating histopathologic features of breast cancers

Characteristic	κ score (SE)*
Mitotic count	.39 (.02)
Lymphocytic infiltrate	.34 (.03)
Continuous pushing margins	.39 (.02)
Solid sheets of cells	.51 (.02)
Nucleoli	.45 (.03)
Nuclei	.23 (.03)
Necrosis	.68 (.03)
Cell borders	.21 (.03)

*SE = standard error.

and prominent (odds ratio = 7.01; 95% CI = 3.38–15.05), were more likely to have continuous pushing margins occupying a higher proportion of the tumor perimeter (odds ratio = 6.47; 95% CI = 3.68–11.36; for >75% pushing margins, *P* for trend <.0001), were more likely to include solid sheets of cells occupying more than 75% of the tumor area (odds ratio = 4.90; 95% CI = 2.93–8.17; *P* for trend <.0001), and were more likely to include areas of necrosis (odds ratio = 3.95; 95% CI = 2.63–5.93; *P*<.0001). However, the discernibility of cell borders, the presence of vesicular nuclei, or the prominence of nucleoli did not differ significantly from those of control patients.

Cancers associated with BRCA2 mutations also tended to have continuous pushing margins occupying a greater proportion of the tumor perimeter (odds ratio = 2.82; 95% CI = 1.31–6.08; for >75% continuous pushing margins, *P* for trend =

Table 2. Unifactorial analysis of features of breast cancers associated with BRCA1 and BRCA2 mutations compared with control breast cancers unselected for family history*

Characteristic	Control breast cancers, No. of observations (%)	Breast cancers associated with BRCA1 mutations: No. of observations (%)	Breast cancers associated with BRCA1 mutations: Odds ratio (95% CI)	Breast cancers associated with BRCA2 mutations: No. of observations (%)	Breast cancers associated with BRCA2 mutations: Odds ratio (95% CI)
Mitotic count					
0–4	586 (56)	51 (23)	1.0 (referent)	70 (50)	1.0 (referent)
5–9	141 (13)	27 (12)	2.12 (1.22–3.69)	26 (18)	1.56 (0.91–2.68)
10–19	153 (15)	52 (24)	3.89 (2.29–6.59)	27 (19)	1.49 (0.83–2.67)
20–39	96 (9)	61 (28)	6.79 (3.94–11.67)	16 (11)	1.43 (0.69–2.97)
⩾40	70 (7)	28 (13)	4.13 (2.09–8.14)	2 (1)	0.24 (0.06–1.01)
		χ^2_1 = 37.79 (*P*<.0001)		χ^2_1 = 0.83	
Lymphocytic infiltrate					
Absent	639 (61)	74 (34)	1.0 (referent)	75 (53)	1.0 (referent)
Mild	372 (36)	117 (53)	3.43 (2.26–5.21)	61 (43)	1.77 (1.06–2.96)
Prominent	35 (3)	28 (13)	7.01 (3.38–15.05)	5 (4)	1.33 (0.40–4.47)
		χ^2_1 = 35.02 (*P*<.0001)		χ^2_1 = 0.75	
Continuous pushing margins					
Absent	842 (80)	109 (49)	1.0 (referent)	90 (64)	1.0 (referent)
Present					
<25%	67 (6)	25 (11)	3.46 (1.90–6.29)	16 (11)	2.42 (1.23–4.78)
25%–75%	81 (8)	40 (18)	3.84 (2.33–6.33)	20 (14)	2.63 (1.39–4.69)
>75%	56 (5)	47 (21)	6.47 (3.68–11.36)	15 (11)	2.82 (1.31–6.08)
		χ^2_1 = 53.36 (*P*<.0001)		χ^2_1 = 13.05 (*P* = .0003)	
Solid sheets of cells					
<25%	745 (72)	100 (46)	1.0 (referent)	89 (64)	1.0 (referent)
25%–75%	175 (17)	46 (21)	2.42 (1.49–3.92)	34 (24)	1.95 (1.19–3.21)
>75%	120 (12)	73 (33)	4.90 (2.93–8.17)	17 (12)	1.49 (0.72–3.09)
		χ^2_1 = 37.31 (*P*<.0001)		χ^2_1 = 2.84 (*P* = .09)	
Nucleoli					
Absent	597 (57)	99 (45)	1.0 (referent)	74 (52)	1.0 (referent)
Present	449 (43)	120 (55)	1.63 (1.11–2.42)	67 (48)	1.29 (0.81–2.05)
		χ^2_1 = 6.16 (*P* = .013)		χ^2_1 = 1.15	
Nuclei					
Nonvesicular	388 (37)	76 (35)	1.0 (referent)	57 (40)	1.0 (referent)
Vesicular	658 (63)	143 (65)	1.19 (0.78–1.82)	84 (60)	0.81 (0.47–1.41)
		χ^2_1 = 0.64		χ^2_1 = 0.54	
Necrosis					
Absent	857 (82)	113 (52)	1.0 (referent)	111 (79)	1.0 (referent)
Present	189 (18)	106 (48)	3.95 (2.63–5.93)	30 (21)	1.27 (0.72–2.23)
		χ^2_1 = 43.69 (*P*<.0001)		χ^2_1 = 0.68	
Cell borders					
Absent	380 (36)	77 (35)	1.0 (referent)	47 (33)	1.0 (referent)
Present	666 (64)	142 (65)	1.11 (0.76–1.62)	94 (67)	1.29 (0.83–1.99)
		χ^2_1 = 0.30		χ^2_1 = 1.31	

*CI = confidence interval. All χ^2 tests are tests for trend and are two-sided. The subscript numbers to χ^2 are equal to the degrees of freedom. Different numbers of observations were recorded for different features because of the variable quality of the histopathologic material; hence, there is variation in the evaluability of certain features on some sections. Analyses were adjusted for age of the patients and for the pathologist.

.0003). None of the other features examined in the second review differed significantly from those in control cancers.

In a direct comparison of BRCA1 and BRCA2, tumors involving BRCA1 mutations had significantly greater scores for mitotic count ($P<.0001$), lymphocytic infiltrate ($P = .002$), continuous pushing margins ($P = .03$), solid sheets of cells ($P = .004$) and necrosis ($P = .001$).

Multifactorial Analysis

All factors significant at the 5% level from both reviews (analyses adjusted for age of the patients and for the pathologist) were included in a multiple regression analysis (Table 3). For BRCA1, all factors had weaker effects than in the unifactorial analysis. In particular, the odds ratios (95% CI) for medullary and atypical medullary carcinomas were reduced from 5.16 (2.42–11.03) *(5)* in the unifactorial analysis to 1.46 (0.85–2.52) in the multifactorial analysis. Odds ratios for mitotic count were also reduced, although less markedly, whereas the odds ratios for other components of grade (i.e., pleomorphism and tubule score) were close to 1. Table 4 shows the final model after stepwise removal of nonsignificant factors for breast cancers arising in BRCA1 mutation carriers. The only factors significant in the multifactorial model were mitotic count (P for trend = .001), continuous pushing margins (P for trend <.0001), lymphocytic infiltrate (P for trend = .002), ductal carcinoma *in situ* (DCIS) ($P = .022$), and lobular carcinoma *in situ* (LCIS) ($P = .042$). The features of solid sheets of cells, necrosis, and nucleoli, all of which were statistically significant in the unifactorial analysis, did not appear to be so in the final model, since they were correlated with mitotic count, continuous pushing margins, and lymphocytic infiltrate.

Similar analyses were performed for BRCA2 (*see* Table 3). After stepwise removal of nonsignificant factors, the final model for BRCA2 included the following factors: tubule score (P for trend = .0002) and continuous pushing margins (P for trend <.0001). In contrast to the unifactorial analysis, tumors in BRCA2 mutation carriers also had a lower mitotic count (P for trend = .003) than those in control subjects after adjustment for tubule formation and continuous pushing margins (Table 5). DCIS was more common in tumors associated with BRCA2 mutations after adjustment for certain factors (i.e., mitotic count, lymphocytic infiltrate, and tubule formation) (odds ratio = 1.39; 95% CI = 0.98–1.99) but did not quite reach statistical significance ($P = .053$)

In the corresponding analysis in which tumors with BRCA1 and BRCA2 mutations were compared directly with each other, the significant factors in the final model were a higher score for mitotic count ($P<.0001$) and lymphocytic infiltrate ($P = .001$) in tumors associated with BRCA1 mutations and a higher score for tubule formation (i.e., fewer tubules) ($P<.0001$) in tumors associated with BRCA2 mutations.

Discussion

In this study, by evaluating several features that are used in the diagnosis of medullary and atypical medullary carcinomas, we have extended our comparison of the histopathology of cancers arising in BRCA1 mutation carriers, BRCA2 mutation carriers, and control patients unselected for family history. In addition, the data have been combined with previously published

Table 3. Multifactorial analysis of features of breast cancers in BRCA1 and BRCA2 mutation carriers compared with breast cancers in control patients unselected for family history*

Characteristic	Odds ratio (95% CI) BRCA1	Odds ratio (95% CI) BRCA2
Mitotic count		
0–4	1.0 (referent)	1.0 (referent)
5–9	1.42 (0.81–2.48)	1.22 (0.69–2.64)
10–19	2.13 (1.28–3.53)	0.91 (0.52–1.61)
20–39	2.56 (1.39–4.70)	0.69 (0.31–1.50)
⩾40	1.22 (0.60–2.47)	0.11 (0.02–0.47)
	$\chi^2_1 = 4.09$ ($P = .043$)	$\chi^2_1 = 6.40$ ($P = .011$)
Tubule score		
1	1.0 (referent)	1.0 (referent)
2	0.89 (0.42–1.88)	4.87 (1.47–16.14)
3	1.01 (0.49–2.07)	13.11 (3.74–45.98)
	$\chi^2_1 = 0.01$	$\chi^2_1 = 13.02$ ($P = .0003$)
Pleomorphism score		
1	1.0 (referent)	1.0 (referent)
2	0.96 (0.57–1.60)	1.35 (0.71–2.57)
3	1.03 (0.57–1.87)	1.00 (0.49–2.04)
	$\chi^2_1 = 0.00$	$\chi^2_1 = 0.09$
Medullary/atypical medullary		
Absent	1.0 (referent)	1.0 (referent)
Present	1.46 (0.85–2.52)	0.61 (0.27–1.37)
	$\chi^2_1 = 1.84$	$\chi^2_1 = 1.45$
Solid sheets of cells		
<25%	1.0 (referent)	1.0 (referent)
25%–75%	0.94 (0.60–1.49)	1.47 (0.88–2.44)
>75%	0.93 (0.51–1.70)	0.77 (0.33–1.81)
	$\chi^2_1 = 0.073$	$\chi^2_1 = 0.016$
Continuous pushing margins		
Absent	1.0 (referent)	1.0 (referent)
Present		
<25%	1.86 (1.11–3.11)	2.41 (1.30–4.45)
25%–75%	1.77 (1.05–3.00)	2.73 (1.46–5.12)
>75%	2.72 (1.48–5.00)	4.02 (1.60–10.08)
	$\chi^2_1 = 11.62$ ($P = .0007$)	$\chi^2_1 = 13.46$ ($P = .0002$)
Lymphocytic infiltrate		
Absent	1.0 (referent)	1.0 (referent)
Mild	1.76 (1.19–2.60)	1.28 (0.80–2.06)
Prominent	1.83 (0.89–3.79)	0.54 (0.18–1.61)
	$\chi^2_1 = 4.34$ ($P = .037$)	$\chi^2_1 = 0.69$
DCIS		
Absent	1.0 (referent)	1.0 (referent)
Present	0.77 (0.57–1.04)	0.56 (0.22–2.07)
	$\chi^2_1 = 2.95$ ($P = .086$)	$\chi^2_1 = 1.41$
LCIS		
Absent	1.0 (referent)	1.0 (referent)
Present	0.27 (0.11–0.68)	0.56 (0.22–2.07)
	$\chi^2_1 = 7.71$ ($P = .005$)	$\chi^2_1 = 1.41$
Nucleoli		
Absent	1.0 (referent)	1.0 (referent)
Present	0.78 (0.55–1.11)	1.06 (0.69–1.62)
	$\chi^2_1 = 1.96$	$\chi^2_1 = 0.07$
Necrosis		
Absent	1.0 (referent)	1.0 (referent)
Present	1.54 (1.00–2.35)	0.74 (0.40–1.35)
	$\chi^2_1 = 3.91$ ($P = .048$)	$\chi^2_1 = 0.97$

*CI = confidence interval; DCIS = ductal carcinoma *in situ;* LCIS = lobular carcinoma *in situ.* All χ^2 tests are tests for trend and are two-sided. The subscript numbers to χ^2 are equal to the degrees of freedom. Analyses were adjusted for age of the patients and for the pathologist.

Table 4. Final model for breast cancers arising in BRCA1 mutation carriers after stepwise regression*

Characteristic	Odds ratio (95% CI)
Mitotic count	
0–4	1.0 (referent)
5–9	1.53 (0.90–2.59)
10–19	2.35 (1.50–3.67)
20–39	2.98 (1.79–4.96)
⩾40	1.58 (1.58–2.98)
	χ^2_1 = 10.59 (P = .001)† χ^2_4 = 23.09 (P = .0001)‡
Continuous pushing margins	
Absent	1.0 (referent)
Present	
<25%	1.82 (1.11–2.98)
25%–75%	1.89 (1.16–3.09)
>75%	2.87 (1.73–4.76)
	χ^2_1 = 17.64 (P<.0001)† χ^2_3 = 18.62 (P = .0003)‡
Lymphocytic infiltrate	
Absent	1.0 (referent)
Mild	1.90 (1.31–2.76)
Prominent	2.46 (1.26–4.79)
	χ^2_1 = 9.69 (P = .002)† χ^2_2 = 12.99 (P = .002)‡
DCIS	
Absent	1.0 (referent)
Present	0.71 (0.53–0.95)
	χ^2_1 = 5.23 (P = .022)
LCIS	
Absent	1.0 (referent)
Present	0.38 (0.15–0.97)
	χ^2_1 = 4.14 (P = .042)

*CI = confidence interval; DCIS = ductal carcinoma *in situ;* LCIS = lobular carcinoma *in situ*. Analyses were adjusted for age of the patients and for the pathologist.

†All χ^2 tests are tests for trend and are two-sided. The subscript numbers to χ^2 are equal to the degrees of freedom.

‡Heterogeneity tests.

Table 5. Final model for breast cancers arising in BRCA2 mutation carriers after stepwise regression*

Characteristic	Odds ratio (95% CI)
Mitotic count	
0–4	1.0 (referent)
5–9	1.21 (0.71–2.07)
10–19	0.87 (0.51–1.48)
20–39	0.63 (0.30–1.31)
⩾40	0.09 (0.02–0.39)
	χ^2_1 = 9.06 (P = .003)† χ^2_4 = 12.95 (P = .002)‡
Tubule score	
1	1.0 (referent)
2	5.13 (1.57–16.75)
3	13.37 (3.95–45.32)
	χ^2_1 = 14.03 (P = .0002)† χ^2_2 = 27.77 (P = .0001)‡
Continuous pushing margins	
Absent	1.0 (referent)
Present	
<25%	2.57 (1.41–4.68)
25%–75%	2.85 (1.55–5.25)
>75%	3.19 (1.55–6.54)
	χ^2_1 = 17.11 (P<.0001)† χ^2_3 = 18.82 (P = .0003)‡

*CI = confidence interval. Analyses were adjusted for age of the patients and for the pathologist.

†All χ^2 tests are tests for trend and are two-sided. The subscript numbers to χ^2 are equal to the degrees of freedom.

‡Heterogeneity tests.

results and have been subjected to a multifactorial analysis to determine the morphologic features that are associated with cancers in BRCA1 and BRCA2 mutation carriers independently of other factors.

For BRCA1, the multifactorial analysis showed that high mitotic count, the presence of continuous pushing margins, and lymphocytic infiltrate (both mild and prominent) remained statistically highly significantly different from those in control cases, independent of other factors. The odds ratios associated with these factors (twofold to threefold) were markedly lower when considered in the multifactorial analysis than when considered in the unifactorial analysis (more than sixfold in each case). This finding reflects the fact that these factors are positively correlated with one another. The multifactorial analysis indicates that the differences previously observed with respect to pleomorphism, tubule formation, and the frequency of medullary and atypical medullary carcinomas are explicable in terms of these three significant factors and are not independent of them. Tumors involving BRCA1 mutations were also associated in the multifactorial analysis with a lower rate of associated DCIS and LCIS, although the evidence was of only marginal significance. The evidence for the association with LCIS in particular may be exaggerated in this analysis, in that one of the BRCA1 mutation carriers with LCIS in the first review was not scored for factors in the second review and hence was excluded from the multifactorial analysis. It is interesting, however, that both DCIS and LCIS were also less common in tumors associated with BRCA2 mutations to a similar extent, although the differences were not significant in this case.

As with all such multiple regression analyses in which a large number of factors are considered, the results must be interpreted cautiously. First, there is a multiple testing problem: With such a large number of factors being considered, some associations may have occurred by chance. The three important factors in the BRCA1 analysis were, however, all highly significant so that chance association is unlikely.

Another potential difficulty is colinearity, whereby factors are so strongly correlated that more than any one combination of factors can explain the data equally well. Qualitatively, this does not appear to be a problem here, in that the three important factors retained large and highly significant effects in the multifactorial analysis, whereas the other factors associated with them had nonsignificant effects with odds ratios close to 1.

However, there remains some quantitative uncertainty as to the relative importance of the three factors mitotic count, continuous pushing margins, and lymphocytic infiltrate, since they are positively associated with one another. Some other factors may, of course, differ between hereditary (familial) cancer case subjects and control subjects unselected for family history to a more moderate extent, but a much larger study would be required to detect such differences.

Of the three features that are independently associated with cancers involving BRCA1 mutations, two (continuous pushing margins and lymphoid infiltrate) constitute a subset of the characteristics that define medullary carcinoma. A high mitotic count, which is the third feature associated with these tumors, is also often present in medullary carcinoma, but it is not regarded as a defining feature. However, vesicular nuclei, indistinct cell borders leading to a ''syncitial'' appearance, and the presence of

prominent nucleoli, all of which are listed among the criteria for medullary carcinoma *(8,9)*, are not independently associated with cancers involving BRCA1 mutations. Moreover, in many cancers involving BRCA1 mutations, the lymphoid infiltrate was not as prominent as would usually be required for diagnosis of classic medullary carcinoma. To further address the issue of the role of medullary and atypical medullary carcinomas in breast cancers associated with BRCA1 mutations, we performed an additional multifactorial analysis in which cancers registered as medullary and atypical medullary cancers were excluded. In that analysis, mitotic count, continuous pushing margins, and lymphoid infiltrate remained statistically highly significantly different between patients carrying BRCA1 mutations and patients with sporadic cancers, and odds ratios were similar to those shown in Table 4. Therefore, although an increased frequency of classic and atypical medullary carcinomas may contribute to the observed BRCA1 phenotype, these cancers are likely to account for only a small proportion of the differences observed between BRCA1 mutation-associated cancers and the control group of sporadic cancers.

We have previously described the potential clinical implications of the histopathologic features of breast cancers developing in individuals carrying BRCA1 mutations with respect to prognosis and detectability by mammographic screening. These conclusions are essentially unchanged by the results included in this analysis. As we also outlined in the previous report, it may be possible to use the data to predict the likelihood of a breast cancer patient carrying a BRCA1 mutation, particularly when a clear family history of the disease is not reported. The results of our multifactorial analysis should increase the effectiveness of this approach. For example, based on previous estimates, 7.5% of breast cancer patients in Britain who were diagnosed between the ages of 20 and 29 years carry a BRCA1 mutation *(16)*. Assuming that the odds ratios from our analysis are independent of age, only about 2% of case subjects in this age group in whom the mitotic count is below 5 per 10 hpf, without continuous pushing margins, and in whom there is no lymphocytic infiltrate would be expected to carry a BRCA1 mutation. By contrast, about 45% of case subjects in the 20- to 29-year-old group with 20–39 mitoses per 10 hpf, continuous pushing margins occupying more than 75% of the tumor perimeter, and a prominent lymphocytic infiltrate would be expected to be BRCA1 carriers. The corresponding proportions based on mitotic count would be 4% and 16%. Thus, the combination of these three factors provides a much clearer discrimination than the use of mitotic count alone. Although the level of agreement between pathologists for these features, as reflected in the kappa scores, may restrict the general application of this approach, it may be possible in the future to define these features more reliably and hence to improve their utility.

In breast cancers arising in carriers of BRCA1 mutations, it is believed that function(s) of the BRCA1 protein are absent or are severely inhibited. The increased proliferative activity in cancers from BRCA1 mutation carriers, reflected in the high mitotic count, is therefore consistent with recently published observations suggesting that overexpression of normal BRCA1 reduces the rate of cell proliferation *(17)*, that reduction of normal BRCA1 expression increases the rate of cell proliferation *(18)*, and that BRCA1 activity is regulated through the cell cycle *(19)*. However, some cancers in BRCA1 mutation carriers do show a low mitotic count (23% have 0–4 mitoses per 10 hpf) and, overall, are of low or intermediate grade. It is, therefore, possible that the phenotype of cancers in BRCA1 mutation carriers is related to relatively quick passage of clones of breast cancer cells through a low-grade phase into a higher grade state. This rapid progression may be related to a role for BRCA1 in preserving genomic stability, similar to the role proposed for p53. This hypothesis would be consistent with the observation that BRCA1 forms a complex with Rad 51, the human homologue of a yeast protein that protects against radiation-induced damage and with which p53 also complexes *(20)*.

Perhaps more surprising, however, is the finding of an increase in lymphoid infiltrate that is associated with breast cancers in BRCA1 mutation carriers. It is possible that this is a reflection of a particularly aggressive response by the immune system. If so, this may account for some preliminary reports that cancers in BRCA1 mutation carriers are associated with a better prognosis *(7,21)*. Alternatively, it can be argued that normal breast epithelium and many breast cancers also contain a scattering of lymphocytes and that the excess lymphoid infiltrate in breast cancers involving BRCA1 mutations is simply an exaggeration of a phenotypic feature that has no bearing on disease progression. Either way, further investigation of the mechanisms responsible for the accumulation of lymphoid cells near and within breast cancers involving BRCA1 mutations is indicated.

Of particular interest is the excess of continuous pushing margins, i.e., the presence of a smooth, noninfiltrative edge to the tumor, which is the only morphologic feature strongly associated with cancers involving both BRCA1 and BRCA2 mutations. Although the biologic basis of this feature is not immediately obvious, it suggests that cancers involving BRCA1 and BRCA2 mutations may be associated with a reduced potential for stromal infiltration by individual or small groups of cells. Studies of the expression of adhesion molecules (e.g., E-cadherin) or matrix metalloproteinases that mediate stromal invasion may elucidate this issue.

For BRCA2, the multifactorial analysis indicates that, in addition to continuous pushing margins, reduced tubule formation is an independent factor that differs significantly from controls. As discussed previously, the reduction in tubule formation suggests a defect in the formation of tissue architecture, particularly with respect to cell–cell and cell–stroma interactions. The multifactorial analysis also indicated a significant inverse relationship with mitotic count. This significant inverse relationship was seen despite the fact that there was no difference from controls in the unifactorial analysis and reflects the fact that tumors associated with BRCA2 mutations, unlike those associated with BRCA1 mutations, do not have the high mitotic counts that one would expect, given their high scores for tubule formation and continuous pushing margins.

Although there is no strong sequence homology between BRCA1 and BRCA2, there are many other similarities between the two genes. Abnormalities in both genes predispose to breast and ovarian cancers; both genes encode large proteins and have a large exon 11 and an untranslated first exon *(1,2,22)*; both are poorly conserved during evolution *(23,24)*; they have a remarkably similar tissue pattern of expression *(23)*; both act as transcriptional activators in reporter systems *(25,26)*; homozygous

knockouts of both genes in mice can be lethal at early stages of development, and heterozygotes show no abnormality *(27,28);* both form a complex (directly or indirectly) with Rad 51 *(20,27);* both have a similar pattern of regulation through the cell cycle *(29).*

Although analysis of the histopathologic appearances of the cancers confirms some similarities between tumors associated with BRCA1 and tumors associated with BRCA2 mutations (e.g., with respect to continuous pushing margins), it also re-emphasizes differences between the two genes (with respect to mitotic count, tubule formation, and lymphocytic infiltrate). Taken together with differences in the clinical phenotype associated with mutations in the two genes, including a lower risk of ovarian cancer conferred by BRCA2 mutations [*(4,30);* Breast Cancer Linkage Consortium: unpublished data], a lower risk of male breast cancer conferred by BRCA1 mutations [*(4,30);* Breast Cancer Linkage Consortium: unpublished data], and differing risks of other cancers such as pancreatic cancer [*(31);* Breast Cancer Linkage Consortium: unpublished data] and ocular melanoma *(30),* our results indicate that there are likely to be substantial differences in the biologic activities of the proteins encoded by the BRCA1 and BRCA2 genes.

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Notes

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