

# The Pathology of Familial Breast Cancer: Predictive Value of Immunohistochemical Markers Estrogen Receptor, Progesterone Receptor, HER-2, and p53 in Patients With Mutations in *BRCA1* and *BRCA2*

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**Purpose:** The morphologic and molecular phenotype of breast cancers may help identify patients who are likely to carry germline mutations in *BRCA1* and *BRCA2*. This study evaluates the immunohistochemical profiles of tumors arising in patients with mutations in these genes.

**Materials and Methods:** Samples of breast cancers obtained from the International Breast Cancer Linkage Consortium were characterized morphologically and immunohistochemically using antibodies to estrogen receptor, progesterone receptor, HER-2 (*c-erbB-2* oncogene), and p53 protein.

**Results:** Breast cancers in patients with *BRCA1* germline mutations are more often negative for estrogen re-

ceptor, progesterone receptor, and HER-2, and are more likely to be positive for p53 protein compared with controls. In contrast, *BRCA2* tumors do not show a significant difference in the expression of any of these proteins compared with controls.

**Conclusion:** *BRCA1* has a distinctive morphology and immunohistochemical phenotype. The combined morphologic and immunohistochemical data can be used to predict the risk of a young patient harboring a germline mutation in *BRCA1*. The *BRCA2* phenotype is currently not well defined.

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IN A LARGE COLLABORATIVE study carried out on behalf of the Breast Cancer Linkage Consortium, we have characterized the histopathologic features of breast cancers arising in patients harboring germline mutations in the *BRCA1* and *BRCA2* genes.<sup>1,2</sup> Breast cancers in patients with *BRCA1* mutations were of higher grade and had higher mitotic counts, a greater degree of nuclear pleomorphism, and less tubule formation than age-matched sporadic breast cancers unselected for family history. *BRCA1* carriers also had an excess of medullary and atypical medullary cancers.

However, multifactorial analysis demonstrated that many of these factors were associated with each other. A high mitotic count, presence of a lymphocytic infiltrate, and the presence of a smooth noninfiltrative pushing border were independently associated with *BRCA1*, but all other features became nonsignificant.

Breast cancers caused by *BRCA2* mutations were also of higher overall grade as a result of exhibiting less tubule formation, but were not significantly different from controls with respect to mitoses and pleomorphism. In the multifactorial analysis, both the reduction in tubule formation and the presence of continuous pushing margins were significantly associated with *BRCA2*. The findings with respect to *BRCA1* are broadly in agreement with other series, but only a limited number of breast cancers caused by *BRCA2* have been evaluated in detail by other groups.<sup>3-11</sup>

The use of immunohistochemical and molecular analysis of cancer-associated genes and the encoded proteins has been important in understanding tumor biology. Immunohistochemical studies in sporadic cancers have led to identification of novel targets with roles in diagnosis, prognostication, and therapeutics. Examples include hormones (eg, estrogen receptor) and growth factor receptors (eg, epidermal growth factor receptor), tumor-specific oncogene products (eg, HER-2), and cell cycle proteins (eg, cyclin D1). The development of Herceptin (Genentech, Inc, San Francisco, CA), a humanized monoclonal antibody against HER-2, is a triumph in translation of molecular and cell biology to the clinic.<sup>12</sup>

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In this report, we present the immunohistochemical profile (estrogen receptor [ER], progesterone receptor [PR], HER-2, and p53) of tumors associated with mutations in *BRCA1* and *BRCA2* genes. Clearly, this question is of more than just scientific curiosity, because the phenotype of the tumor may provide vital diagnostic and prognostic information for the patient. Although genetic testing for *BRCA1* and *BRCA2* mutations within high-risk families is available, it is expensive and is associated with psychological morbidity.<sup>13</sup> The morphologic and molecular phenotype of breast cancers may help identify patients who are likely to carry mutations in these genes.

## MATERIALS AND METHODS

### Breast Cancer Specimens

We reviewed 182 tumors from 119 families in *BRCA1* mutation carriers, 63 tumors from 34 families in *BRCA2* carriers, and 109 control tumors. Twenty-one familial cases in which no *BRCA1* or *BRCA2* mutation could be found were also reviewed but are not included in these analyses. After exclusion of tumors where there was no evidence of invasion, or where the primary site appeared to be other than the breast, the final analyses were derived from tumors from 165 individuals from *BRCA1* families, 52 individuals from *BRCA2* families, and 103 controls. Two cases from mutation-positive families who were known not to carry the mutation were excluded. Samples were analyzed for morphologic features using the same format as in the previous reviews.<sup>2,14</sup> We obtained specimens from case subjects with familial breast cancer in the form of blocks or unstained 3- $\mu$ m-thick sections from the United Kingdom, United States, Ireland, France, Germany, Iceland, Switzerland, Italy, Hungary, and the Netherlands. The control set of breast cancers was drawn from the archives of University College Hospital, London. Cases were selected at random such that the frequency of controls in each decade of age was similar to that in the familial cases.<sup>14</sup> The cases were identified only by an allocated number and not by personal details. A computer-generated random number was allocated to each case familial and control sample.

### Immunohistochemistry

Sections 3  $\mu$ m thick were cut onto DAKO Capillary Gap slides (S2024) (DAKO Corp, Carpinteria, CA) and dried at 60°C overnight. Slides were dewaxed in xylene, taken to absolute alcohol, and incubated in 3% hydrogen peroxide in methanol for 10 minutes to block endogenous peroxidase. The slides were then transferred to running tap water before pressure cooking.

Slides were transferred to 3 L of boiling citrate buffer pH 6.0 in a 15-lb pressure cooker. Once full pressure was achieved, the slides were cooked for 2 minutes and the pressure cooker flushed with tap water.

After antigen retrieval, slides were rinsed in Tris-buffered saline (TBS) pH 7.4 and incubated in normal goat serum (1:10) for 10 minutes. The serum was tipped off and the sections incubated in primary antibody for 60 minutes at the appropriate dilution (Table 1). The slides were rinsed in TBS and incubated in DAKO Duet (K0492) biotinylated goat antimouse/rabbit secondary reagent (1:100) for 35 minutes. After rinsing in TBS, the slides were then incubated in DAKO Duet (K0492) streptavidin-biotin-horseradish peroxidase complex for 35 minutes, rinsed in TBS, and treated with DAB chromogen (896102,

**Table 1. Details of Antibodies Used in the Study**

Antibody	Dilution	Supplier
ER	1:30	DAKO M7047
PR	1:40	Novacastra NCL-
p53 (DO-7)	1:50	DAKO M7001
HER-2	1:100/n	Novacastra NCL-

Abbreviation: o/n, incubated in primary antibody overnight at room temperature.

Kem-En-Tec, Copenhagen, Denmark) for 10 minutes. The slides were then rinsed in tap water, counterstained in Mayer's hematoxylin, and mounted in synthetic mountant.

### Conduct of the Histologic Review

In the review, each slide was read independently by two pathologists (two of M.J.V., P.P.O., T.J.A., and J.J.). Because the slides were arranged in order according to a random number, the pathologists were not aware of whether the slide being read was from a case subject or a control subject. No attempt was made to reconcile differences between pathologists, as it was difficult to design such a process that would not introduce other biases. For ER, PR, and p53, the intensity of staining was recorded as negative, low, moderate, or strong. The pathologists were provided with identical color charts to aid consistency in scoring the intensity of the staining (ranging from white [negative] to dark brown [strong]). The proportion of positive cells was divided into six categories; 0 to less than 1%, 1% to 5%, 6% to 25%, 26% to 50%, 51% to 75%, and more than 75%. For HER-2, tumors in which the majority (> 75%) of cells showed a strong complete membrane staining (equivalent to score 3, DAKO scoring system) were classed as positive. All other cases were recorded as negative.

### Classification of Families

Familial cases were attributed to *BRCA1* or *BRCA2* on the basis of either a mutation clearly associated with disease or strong linkage evidence generating a more than 90% posterior probability of being caused by one or the other gene, as described previously.<sup>1,2</sup> We made the assumption that cases in mutation-positive families were mutation carriers unless information from mutation or linkage analyses indicated that they were noncarriers (these noncarriers were excluded from all analyses). In practice, only one family was attributed to *BRCA1* and one family to *BRCA2* on the basis of linkage alone.

### Statistical Methods

We performed separate analyses comparing tumors in *BRCA1* carriers and *BRCA2* carriers with control tumors. As in the previous analyses, the effects of each morphologic feature on cancer status were summarized in terms of odds ratios. All analyses were adjusted for age, in groups less than 30, 30 to 39, 40 to 49, 50 to 59, and 60 to 69 years, by the pathologist conducting the immunohistochemical and by the pathologist conducting the morphologic review. These adjusted analyses were carried out using multiple logistic regression analysis, using the program S-Plus (Version 3.4, MathSoft, Inc, Seattle, WA).

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 leads to unbiased odds ratio estimates but underestimates their standard errors and confidence intervals. To correct for this, we computed confidence limits

**Table 2. Kappa Coefficients for Interobserver Variation**

	Positivity		Intensity		Percentage of Cells Stained	
	Kappa	95% CI	Kappa	95% CI	Kappa	95% CI
ER	0.95	0.92-0.98	0.87	0.83-0.89	0.87	0.84-0.89
PR	0.70	0.63-0.76	0.67	0.62-0.72	0.70	0.65-0.75
HER-2	0.83	0.72-0.92				
p53	0.63	0.59-0.72	0.75	0.71-0.78	0.76	0.72-0.81

Abbreviation: CI, confidence interval.

using the robust Huber's sandwich estimator for the variance-covariance matrix of maximum quasi-likelihood estimates (18) using 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. Significance levels for each factor were derived from the parameter estimates and the covariance matrix (adjusted using the sandwich estimator). Significance tests for the effects of intensity of staining and percentage of cells stained on carrier probability were constructed as 1 degree of freedom tests on the basis of testing for linear trends in log (odds ratio) with increasing category.<sup>15</sup> Estimated odds ratios were, however, derived separately for each level. Significance levels less than .10 are quoted in the tables. Heterogeneity  $\chi^2$  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.

To determine which factors were independently predictive of genetic status, we also performed multiple regression analyses. In these analyses, all factors that were significant at the 5% level in either the *BRCA1* or *BRCA2* analysis, together with age of the patient and pathologist who reviewed the slides, were initially included. Factors (other than age and pathologist) were then removed from the model on a stepwise basis until no further factors could be removed at the 5% level.

Concordance between pathologists was assessed using kappa statistics. For characteristics on an ordinal scale (ie, intensity of staining and percentage of cells stained), weighted kappa statistics were used. Confidence limits were constructed by bootstrapping, using 1,000 bootstrap replicates.

To derive the predicted *BRCA1* carrier probabilities on the basis of pathologic status, we used the predicted carrier probabilities for breast cancer cases at different ages derived from a previous population-based segregation analysis of breast cancer.<sup>16</sup> The predicted carrier probabilities were then derived from the frequency of each pathologic subgroup in each age group and the estimated odds ratios from the model, so as to agree with the overall *BRCA1* carrier probability.

## RESULTS

The kappa coefficients for interobserver variation for the different characteristics are listed in Table 2. Coefficients are highest for ER and lowest for PR and p53, but all coefficients are reasonably high.

Table 3 lists the distributions of immunohistochemical features in *BRCA1* and *BRCA2* tumors and controls. The corresponding odds ratios for each factor, adjusted for age and pathologist, are listed in Table 4. There was a highly significant difference in the distribution of ER status between *BRCA1* tumors and controls ( $P < .0001$ ). Only 10%

of *BRCA1* tumors showed any positive staining for ER, compared with 65% of controls. In contrast, *BRCA2* tumors showed a distribution of ER staining similar to controls (in terms of both intensity and percentage of cells stained), with 66% of tumors being positive. *BRCA1* carriers were also significantly less likely to be PR-positive, although the effect was slightly weaker than for ER (21% v 59%,  $P < .0001$ ). Again, the distribution of PR status in *BRCA2* carriers (55% positive) was similar to that in controls.

*BRCA1* tumors were significantly less likely to show positive staining of HER-2 (3% v 15% in controls;  $P = .018$ ). The frequency of HER-2 positivity in *BRCA2* tumors (3%) was similar to that in *BRCA1* tumors, although this was not significantly different from the frequency in controls.

p53 staining showed a more complex relationship with carrier status. The distribution of p53 staining was significantly different between *BRCA1* tumors and controls ( $P = .006$ ). However, the *BRCA1* group contained a lower frequency of tumors with low-intensity staining than with no staining (odds ratio, 0.32), but a higher frequency of tumors with high-intensity staining (odds ratio, 2.28). A similar pattern was seen with percentage of cells stained, with a reduced frequency of tumors with 1% to 10% cells stained (odds ratio, 0.31), but an increasing trend thereafter, reaching an odds ratio of 3.19 for tumors with more than 75% cells stained. There was some suggestion of a similar effect in *BRCA2* tumors, although this was not significant.

The factors that were individually significant predictors of *BRCA1* status (ER, PR, and HER-2) were included in a multiple logistic regression analysis together with those significant predictors from the morphologic analysis (mitotic count, lymphocytic infiltration, and continuous pushing margins) (Table 5). ER status was the most significant risk factor in the regression analysis. No other factor was individually significant once ER status was taken into account. The odds ratio associated with HER-2 positivity (0.16) was identical to that in the single-factor analysis, but this was not quite statistically significant in the multiple regression ( $P = .067$ ).

Table 3. Distribution of Immunohistochemical Features in Familial and Unselected Breast Cancers

	Controls		BRCA1		BRCA2	
	No.	%	No.	%	No.	%
<b>ER</b>						
Intensity						
Negative	72	35	293	90	35	34
Low	66	32	16	5	20	19
Medium	40	20	7	2	28	27
High	26	13	9	3	21	20
Percentage of cells stained						
< 1%	74	36	293	90	35	34
1-10%	3	1	2	1	4	4
11-25%	7	3	5	2	4	4
26-50%	14	7	6	2	5	5
51-75%	30	15	9	3	21	20
76%+	76	37	9	3	35	34
<b>PR</b>						
Intensity						
Negative	75	41	255	79	48	45
Low	39	21	42	13	20	19
Medium	33	18	14	4	26	25
High	58	31	10	4	12	11
Percentage of cells stained						
< 1%	79	39	264	82	49	46
1-10%	34	17	19	6	13	12
11-25%	14	7	14	4	6	6
26-50%	12	6	11	3	11	10
51-75%	20	10	7	3	13	12
76%+	45	22	6	2	14	13
<b>HER-2</b>						
Negative	175	85	317	97	107	97
Positive	31	15	9	3	3	3
<b>p53</b>						
Intensity						
Negative	80	39	137	42	48	45
Low	53	26	25	8	22	21
Medium	39	19	52	16	22	21
High	34	17	111	34	15	14
Percentage of cells stained						
< 1%	106	51	148	45	59	55
1-10%	34	17	13	4	7	7
11-25%	11	5	13	4	4	4
26-50%	10	5	9	3	6	6
51-75%	17	8	33	10	12	11
76%+	28	14	110	34	19	18

We also conducted an analysis including grade and ER status, because grade is more likely to be routinely available than the more detailed morphologic characteristics. There was some suggestion of higher grade in *BRCA1* tumors after adjusting for ER status. This was apparent in both the ER-negative and ER-positive groups, but again the effect was not statistically significant (Table 6).

#### DISCUSSION

To date, most if not all studies investigating the molecular profile of *BRCA*-associated tumors have been performed on

relatively small numbers of cases, making statistical analysis of individual studies difficult. However, the combined data in the literature is beginning to provide a glimpse of the unique immunohistochemical and molecular profile of *BRCA1*- and *BRCA2*-associated tumors.<sup>6,17,18</sup>

In previous reports,<sup>1,2</sup> we have demonstrated that the pathology of breast cancers arising in *BRCA1* mutation carriers differs from that observed in cancers from *BRCA2* mutation carriers and that both differ from age-matched breast cancers unselected for family history. We have now compared the immunophenotype of tumors from patients

Table 4. Odds Ratios for Individual Factors, Adjusting for Age and Pathologist

Factor/Level	BRCA1		BRCA2	
	Odds Ratio	95% CI	Odds Ratio	95% CI
ER intensity				
Negative	1.0		1.0	
Low	0.062	0.017-0.23	0.56	0.26-1.21
Medium	0.037	0.008-0.17	1.29	0.54-3.05
High	0.13	0.024-0.65	1.70	0.64-4.53
	$\chi^2_3 = 27.81 (P < .0001)$		$\chi^2_3 = 6.00 (P = .11)$	
	$\chi^2_1 = 18.72 (P < .0001)$		$\chi^2_1 = 0.48$	
ER percentage of cells stained				
< 1%	1.0		1.0	
1-10%	0.14	0.023-0.91	1.35	0.12-15.42
11-25%	0.15	0.040-0.58	0.88	0.27-2.88
26-50%	0.12	0.031-0.49	0.76	0.25-2.29
51-75%	0.082	0.020-0.34	1.49	0.64-3.49
76%+	0.028	0.003-0.27	0.88	0.42-1.85
	$\chi^2_5 = 20.11 (P = .001)$		$\chi^2_5 = 2.43$	
	$\chi^2_1 = 18.42 (P < .0001)$		$\chi^2_1 = 0.00$	
PR intensity				
Negative	1.0		1.0	
Low	0.26	0.11-0.62	0.65	0.31-1.38
Medium	0.11	0.029-0.39	1.11	0.47-2.64
High	0.083	0.019-0.37	0.43	0.16-1.14
	$\chi^2_3 = 16.61 (P = .0008)$		$\chi^2_3 = 4.71$	
	$\chi^2_1 = 16.05 (P < .0001)$		$\chi^2_3 = 1.58$	
HER-2				
Negative	1.0		1.0	
Positive	0.16	0.029-0.72	0.13	0.008-2.0
	$\chi^2_1 = 5.55 (P = .018)$		$\chi^2_1 = 2.15$	
p53 intensity				
Negative	1.0		1.0	
Low	0.32	0.14-0.72	0.69	0.32-1.52
Med	0.93	0.39-2.21	1.15	0.51-2.61
High	2.28	0.81-6.42	0.84	0.33-2.13
	$\chi^2_3 = 12.89 (P = .005)$		$\chi^2_3 = 1.56$	
	$\chi^2_1 = 0.28$		$\chi^2_1 = 0.08$	
p53 percentage of cells stained				
< 1%	1.0		1.0	
1-10%	0.31	0.13-0.71	0.36	0.12-1.06
11-25%	1.79	0.31-10.26	1.25	0.30-5.21
26-50%	1.43	0.28-7.20	2.05	0.42-9.90
51-75%	2.29	0.47-11.16	2.12	0.61-7.32
76-100%	3.19	1.09-9.30	1.34	0.54-3.34
	$\chi^2_5 = 16.36 (P = .006)$		$\chi^2_5 = 6.36$	
	$\chi^2_1 = 2.37 (P = .12)$		$\chi^2_1 = 1.03$	
51%+	3.25	1.30-8.13	1.66	0.77-3.55
	$\chi^2_1 = 6.36 (P = .01)$		$\chi^2_1 = 1.69$	

with *BRCA1* or *BRCA2* mutations with breast cancers unselected for family history.

ER has become one of the most important prognostic and predictive markers for breast cancer.<sup>19</sup> The expression of ER is inversely correlated with tumor grade.<sup>20</sup> Because *BRCA1*- and *BRCA2*-associated tumors are overall higher grade than sporadic cancers, they would be predicted to be more often ER-negative. Indeed, a number of studies have

shown a low level of ER expression in familial breast cancers,<sup>6,11,21-23</sup> particularly those associated with *BRCA1*. Our study has confirmed that the large majority of tumors in *BRCA1* carriers are ER-negative, but that, in contrast, the expression of ER in *BRCA2* tumors appears to be similar to that in sporadic breast cancers.<sup>21,22</sup> (Although the *BRCA2* series was smaller than the *BRCA1* series, the study still had adequate power to detect an effect of ER status—a differ-

**Table 5. Multiple Regression Analysis of Factors Related to *BRCA1***

Factor	Odds Ratio	95% CI
ER		
Positive	0.082	0.022-0.30
	$\chi^2_1 = 13.87 (P = .0002)$	
HER-2		
Positive	0.12	0.006-2.16
	$\chi^2_1 = 2.08 (P = .15)$	
Mitotic count		
0-4	1.0	
5-9	0.56	0.16-2.01
10-19	0.84	0.22-3.30
20-39	1.77	0.37-8.39
40+	1.27	0.15-10.88
	$\chi^2_4 = 2.47$	
	$\chi^2_1 = 0.06$	
Pushing margins		
None	1.0	
< 25%	0.92	0.092-9.14
25-50%	1.33	0.069-25.75
> 50%	1.20	0.10-14.33
	$\chi^2_3 = 0.06$	
	$\chi^2_1 = 0.04$	
Lymphocytic infiltration		
None	1.0	
Mild	1.68	0.51-5.57
Severe	2.36	0.15-37.67
	$\chi^2_2 = 1.02$	
	$\chi^2_1 = 0.58$	

ence comparable to that seen in *BRCA1* would have been highly significant.) This difference in behavior is mirrored by the different age distribution of tumors in *BRCA1* and *BRCA2* carriers: tumors in *BRCA1* carriers occur at a younger age, with the incidence rate relative to population rates decreasing rapidly with increasing age, whereas *BRCA2* tumors have an age distribution much closer to that in the general population.<sup>16</sup>

The multiple regression analysis indicated that ER status was the strongest predictor of *BRCA1* status. The effect of factors including grade and lymphocytic infiltration were

**Table 6. Odds Ratios for *BRCA1* Positivity Related to ER and Grade, by Multiple Regression Analysis**

Factor	Odds Ratio	95% CI
ER		
Positive	0.076	0.026-0.22
	$\chi^2_1 = 23.09 (P < .0001)$	
Grade		
1	1.0	
2	1.46	0.29-7.28
3	2.24	0.43-11.68
	$\chi^2_2 = 1.20$	
	$\chi^2_1 = 0.68$	

much weaker after ER status had been accounted for. The effect of ER status was seen clearly even in low-grade tumors, suggesting that the tumors arose in receptor-negative cells. This is consistent with the previous observations that both the invasive and in situ component in *BRCA1* tumors has a similar status with respect to steroid hormone receptor expression. Taken together, these observations suggest a model in which *BRCA1* tumors are arising as a result of a quite distinct, hormone-independent mechanism than *BRCA2* tumors and tumors in noncarriers, possibly in a different subpopulation of cells. However, the alternative hypothesis that *BRCA1* tumors develop aggressively and lose hormone dependence in progression cannot be ruled out definitively. Whatever the mechanism, these data predict that cancers arising in *BRCA1* carriers will be relatively resistant to hormonal therapy. They also cast doubt (particularly if the *BRCA1* tumors are receptor-negative ab initio) on the likely efficacy of hormonal chemoprevention, such as tamoxifen, in *BRCA1* carriers, although this clearly requires empiric evaluation. Because most of the ER-positive cells in the normal breast are not the same as the proliferative compartment, this argument may not be entirely valid.<sup>24</sup>

The detection of ER immunohistochemically does not necessarily reflect functional competence, and a proportion of breast cancers expressing ER are known to be resistant to antiestrogen therapy. The function of ER is dependent on the ability to transactivate so-called ER-dependent genes. Expression of PR and PS2 protein is indirect evidence of retained transcriptional activation activity of ER, and it has been shown that PR and PS2 expression have stronger correlation with prognosis in breast cancer than ER expression alone.<sup>25</sup> Osin et al<sup>21</sup> have shown that although nine of 40 familial breast cancer cases were ER-positive, only two of these were also PR-positive. This suggests that even in cases where ER receptors could be identified immunohistochemically, their functional ability may be compromised.

A large number of studies have been performed on the functional role of HER-2 oncogene in breast cancer. HER-2 product is a tyrosine kinase receptor belonging to the same family as epidermal growth factor receptor. It is overexpressed in approximately 20% to 30% of high-grade invasive breast cancers and has been shown to be a valuable prognostic indicator. HER-2 status also predicts response to antiestrogen and cytotoxic chemotherapy. Antibodies directed against the HER-2 protein have attracted a lot of attention recently because of the availability of the monoclonal antibody Herceptin for treatment of breast cancer.<sup>12</sup> Clearly, the role of HER-2 in familial breast cancer would therefore be of interest.

Data on HER-2 in familial breast cancer are limited and conflicting. Robson et al<sup>11</sup> and Armes et al<sup>23</sup> have not

shown a difference in HER-2 expression between sporadic and familial cancers. However, the study by Johannsson et al<sup>6</sup> demonstrated that HER-2 expression in *BRCA1*-associated cancers is lower than would be predicted on the basis of their histologic grade. This is indeed the conclusion of this study. Only 3% of *BRCA1* and 3% of *BRCA2* cases were positive for HER-2, compared with 15% of sporadic cases. The proportion of sporadic cancers positive for HER-2 is on the lower side of data within the literature. This may be a reflection of the choice of antibody, the stringent criteria used to score positive results, or a real population difference. In any event, the difference to familial breast cancer is striking, especially in view of the overall higher grade of *BRCA1* and *BRCA2* tumors. Taken at face value, Herceptin is unlikely to play a large role in the management of breast cancer in patients harboring mutations in *BRCA1* and *BRCA2*.

p53 protein is an important guardian of stability and integrity of the genome, acting to prevent cell proliferation after DNA damage and activating apoptosis in case of unreparable damage.<sup>26</sup> Mutations in the *TP53* gene are the most common genetic alterations in human cancers and are encountered in 20% to 40% of sporadic breast cancers. The frequency of these mutations correlates with tumor grade. It has been demonstrated that p53 missense mutations results in p53 protein with an increased half-life. It is also clear, however, that not all truncating mutations lead to protein changes that can be detected using immunohistochemistry. Despite this, detection of p53 protein by immunohistochemistry has become a routine method in pathology practice, and the presence of detectable p53 protein is an important prognostic marker that correlates with higher histopathologic grade, increased mitotic activity, aggressive behavior, and therefore a worse prognosis.<sup>27-30</sup>

We did not see a clear relationship between p53 staining and *BRCA1* status. The proportion of tumors showing strong p53 staining was higher than in controls, as was the proportion with staining in more than 50% of cells (44% v 22%). However, the proportion with weak staining was actually lower in *BRCA1* carriers. These differences are less marked than those reported by Crook et al,<sup>31,32</sup> who reported that BRCA-associated tumors were more often p53-positive compared with grade-matched sporadic breast cancers (77% *BRCA1*, 45% *BRCA2*, and 35% sporadic). Sobol et al<sup>33</sup> reported 41% positivity in *BRCA1* cases compared with 17.5% in sporadic cancers using the DO1 antibody. Further evidence for an important role for p53 in familial breast cancer comes from the detection of mutations at a higher frequency compared with sporadic cancers. The mutations in BRCA-associated cancers were often multiple and their locations unusual, which is in marked

contrast to sporadic cancer.<sup>34</sup> Studies of p53 gene function in BRCA tumors have been performed using in vitro models. These show that the identified mutants are unique not only in their number and location but also in their function. The mutants retain some of their wild p53-dependent activities, such as transactivation, suppression of proliferation, and apoptosis induction (in particular, through PIG3 transactivation). At the same time, these mutants fail to suppress transformation and exhibit gain of function.<sup>35</sup> The retained ability of some of these novel mutants to transactivate MDM2 may explain the absence of immunodetectable p53 in some BRCA tumors with p53 mutations. This can occur because of the degradation of the p53 protein by the MDM2-regulated ubiquitin-dependent pathway.

*BRCA1* and *BRCA2* proteins are thought to have a role in DNA repair.<sup>36</sup> Inactivation of the gene(s) will lead to cell cycle arrest because of activation of p53. This may explain why breast cancers in patients with inactivation of *BRCA1* and *BRCA2* show mutations in p53. This view is consistent with data from *BRCA1* knockout mice where the embryonic lethality can be partially rescued by knocking out p53.<sup>37,38</sup>

The immunohistochemical analysis provides a new and powerful predictor of *BRCA1* mutation status that could augment risk assessment on the basis of family history of breast/ovarian cancer, particularly because ER status is routinely available in many centers. The estimated carrier probabilities in women with breast cancer, on the basis of age at diagnosis, ER status, grade, and age, are listed in Table 7. The observation that a tumor is ER-negative will roughly double the probability that the individual is a *BRCA1* carrier, whereas finding that the tumor is ER-positive will reduce the probability by approximately fivefold. Thus, for example, the probability that women diagnosed with breast cancer at age 30 to 34 harbor a *BRCA1* mutation is approximately 5%. On the basis of our results, this would rise to approximately 27% for ER-negative grade 3 tumors, and would be at least 10% for ER-negative tumors regardless of grade. At this level of risk, predictive testing might well be considered justified, even in the absence of information on family history, particularly in view of the high risk of a subsequent breast cancer or ovarian cancer.<sup>39</sup> Thus, inclusion of pathologic features into risk assessment would help identify patients most likely to harbor mutations, hence reducing the costs and psychological morbidity associated with genetic testing. There is emerging data from smaller studies that the use of morphology and immunohistochemical profile is likely to be useful in clinical practice.<sup>40-42</sup> Hence, although this is not the first report to consider this element, it is the largest series of cases analyzed to date that provided risk estimates that can be used in clinical practice. Further prospective studies will be

**Table 7. Predicted Probabilities of Carrying a BRCA1 Mutation, by Age, ER Status, and Grade**

	All Histologies (%)	ER-Positive			ER-Negative		
		Grade 1 (%)	Grade 2 (%)	Grade 3 (%)	Grade 1 (%)	Grade 2 (%)	Grade 3 (%)
Age group							
< 30 years	8	1.1	1.6	2.7	14.4	21.0	35.0
30-34 years	5	0.8	1.2	2.0	10.9	15.9	26.5
35-39 years	2	0.2	0.3	0.5	2.7	4.0	6.6
40-44 years	1.5	0.1	0.2	0.3	1.5	2.2	3.7
45-49 years	1	0.1	0.1	0.2	1.0	1.5	2.5
50-59 years	0.3	0.03	0.04	0.07	0.4	0.6	0.9

needed to evaluate its utility in the clinic. The inclusion of HER-2 positivity would further add to the predictive value. Unfortunately, prediction of *BRCA2* status remains problematic.

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#### APPENDIX

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