Prediction of *BRCA1* Status in Patients with Breast Cancer Using Estrogen Receptor and Basal Phenotype

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Abstract

Purpose: To investigate the proportion of breast cancers arising in patients with germ line *BRCA1* and *BRCA2* mutations expressing basal markers and developing predictive tests for identification of high-risk patients.

Experimental Design: Histopathologic material from 182 tumors in *BRCA1* mutation carriers, 63 *BRCA2* carriers, and 109 controls, collected as part of the international Breast Cancer Linkage Consortium were immunohistochemically stained for CK14, CK5/6, CK17, epidermal growth factor receptor (EGFR), and osteonectin.

Results: All five basal markers were commoner in *BRCA1* tumors than in control tumors (CK14: 61% versus 12%; CK5/6: 58% versus 7%; CK17: 53% versus 10%; osteonectin: 43% versus 19%; EGFR: 67% versus 21%; $P < 0.0001$ in each case). In a multivariate analysis, CK14, CK5/6, and estrogen receptor (ER) remained significant predictors of *BRCA1* carrier status. In contrast, the frequency of basal markers in *BRCA2* tumors did not differ significant from controls.

Conclusion: The use of cytokeratin staining in combination with ER and morphology provides a more accurate predictor of *BRCA1* mutation status than previously available, that may be useful in selecting patients for *BRCA1* mutation testing. The high percentage of *BRCA1* cases positive for EGFR suggests that specific anti-tyrosine kinase therapy may be of potential benefit in these patients.

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In a large collaborative study carried out on behalf of the Breast Cancer Linkage Consortium, we have characterized the histopathologic and immunohistochemical features of breast cancers arising in patients harboring germ line mutations in the *BRCA1* and *BRCA2* genes (1–3). Breast cancers in patients with *BRCA1* mutations were of higher grade, had higher mitotic counts, a greater degree of nuclear pleomorphism, and less tubule formation. They were more likely to be steroid receptor and HER-2 negative and harbor mutations in *TP53* gene than age-matched sporadic breast cancers unselected for family history. Breast cancers due to *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, pleomorphism, steroid receptor expression, or mutation in *TP53*.

The vast majority of breast carcinomas are of invasive ductal, no special type. Breast cancers, as determined morphologically, are thought to arise exclusively from the inner, luminal epithelial cell compartment of the terminal-duct lobular unit of the breast. Irrespective of the true histogenesis (cell of origin) of breast carcinoma, it has been apparent for some time, that a proportion (2-18%) of cancers have a basal/myoepithelial phenotype as defined by immunohistochemical positivity for intermediate filaments such as CK5 and CK14 (4–7). Recent microarray studies have also identified a basal-like group of breast tumors based on their patterns of gene expression (8, 9).

Although a definition or comprehensive characterization of basal carcinomas is lacking, there are a number of features reported to be associated with this type of tumor. Morphologically, they are usually high grade (7), and some of them have been reported to contain large central acellular zones comprising necrosis, tissue infarction, collagen, and hyaline material (10, 11). Immunohistochemically, as well as expression of a number of myoepithelial markers, these tumors are often estrogen receptor (ER), progesterone receptor, and HER-2 negative (7, 12), an immunophenotype resembling *BRCA1* tumors (3). Microarray analysis has also shown a similarity between sporadic, basal-like tumors and those familial tumors harboring a *BRCA1* mutation, based upon their patterns of gene expression (13, 14).

We have investigated the Breast Cancer Linkage Consortium pathology data set for the expression of basal markers to clarify what proportion of cancers exhibit this phenotype and to investigate whether this could help refine the predictive value of pathology in identifying carriers of *BRCA1* or *BRCA2* mutations.

Materials and Methods

Case selection. The selection of cases and controls is essentially identical to that for the previous analyses of immunohistochemical markers from the Breast Cancer Linkage Consortium (3). Three hundred and seventy-five tumors were studied; these included 182 tumors in *BRCA1* carriers, 63 in *BRCA2* carriers, and 109 control tumors unselected for mutation status. Specimens were from case subjects with familial breast cancer from collaborating centres in the Breast Cancer Linkage Consortium from the United Kingdom, United States, Ireland, France, Germany, Iceland, Switzerland, Hungary, and The Netherlands. Cases were ascertained through clinical genetics centers or research studies based on the family history of breast and ovarian cancer and were eligible for this study if they were shown to carry a pathogenic *BRCA1* or *BRCA2* mutation, as classified by the Breast Cancer Information Core (http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/). Both prevalent and incident cases were included. Specimens were obtained in the form of blocks or unstained 3-μm-thick sections. The control set comprised a series of cancers obtained sequentially from the archives of University College Hospital (London, United Kingdom), whose age distribution was frequency matched to the familial set (15).

For consistency with previous analyses, 46 tumors were excluded where the tumor was a lymph node metastasis or was suspected to be a primary from another site, or where one of the pathologists in the previous review scored the tumor as nonmalignant/noninvasive. Four cases of male breast cancer were also excluded. There were two tumors from breast cancer families in which a *BRCA1* or *BRCA2* mutation was detected but who did not themselves carry the mutation (excluded from all analyses). The final analyses were therefore based on 158 *BRCA1* and 48 *BRCA2* tumors and 100 controls. The work was carried out with local ethical approval.

Immunohistochemistry. Formalin-fixed, paraffin-embedded tissue sections were immunohistochemically stained for CK14 (1:40, clone LL002, Novocastra, Newcastle upon Tyne, United Kingdom), CK5/6 (1:600, clone D5/16B4 Chemicon, Temecula, CA), CK17 (1:25, clone E3, DAKO, Glostrup, Denmark), epidermal growth factor receptor (EGFR; 1:50, clone 31G7, Zymed, South San Francisco, CA), and osteonectin (1:2, clone 15G12 Novocastra). CK14, CK5/6, CK17, and EGFR were chosen as they are established markers of basal/myoepithelial cells (6, 16, 17). Osteonectin was chosen as this molecule was differentially expressed in myoepithelial cells in studies of expression profiling of normal human luminal and myoepithelial cells (18). Furthermore, in that study, osteonectin was reported to be a poor prognostic indicator for patients with sporadic breast cancer. All sections were dewaxed in xylene, taken to ethanol (99.7-100%, v/v), and blocked for endogenous peroxidase H2O2 in methanol for 10 minutes or Envision kit blocking reagent for 5 minutes. All sections were subjected to a high-temperature unmasking technique [CK17 and osteonectin; 3 minutes pressure cooking in citrate buffer (pH 6.0); CK5/6 and CK14; microwave in DAKO (pH 6.0) antigen retrieval solution for 18 minutes; EGFR; chymotrypsin for 10 minutes in 37°C water bath].

The sections for CK14, CK5/6, and EGFR were blocked in normal horse serum 2.5% (Vector Labs, Peterborough, United Kingdom) for 20 minutes and the primary antibodies were applied for 1 hour at 25°C. All antibodies were diluted in TBS (pH 7.4). The primary antibody was rinsed off in TBS/0.05% Tween 20 and developed using Vectastain Universal avidin-biotin complex method kit (Vector Labs). All antibodies were visualized with diaminobenzidine (DAKO). The primary antibodies CK17 and osteonectin 1:2 were applied for 30 minutes at room temperature and visualized using the ENVISION system (DAKO). All sections were counterstained with Harris' hematoxylin on the LEICA XL staining machine.

Slide review. As with previous reviews (2, 3), the slides were organized according to an assigned random number so that the pathologists were unaware of the BRCA status of the tumors. Each slide was read independently by two pathologists (CK14 and CK5/6 were scored by F.P.L. and M.V.V. and CK17, osteonectin, and EGFR by J.R.F. and L.F.).

Statistical analysis. As in previous studies, formal analysis of the associations between cytokeratin staining and carrier status was evaluated by logistic regression. All analyses were carried out using Stata (version 7.0).

Scores for each pathologist were used; that is, no attempt was made to provide a consensus score. The numbers in Table 1 refer therefore to numbers of scores rather than tumors. The odds ratios (OR) estimate the associations based on an individual observer and therefore reflect the effects likely be found in practical application. Analyses based on a consensus score would be expected to provide higher ORs because some random error would be removed but would exaggerate the effects obtained by a single observer. Although the logistic regression analysis leads to consistent estimates of the ORs, the nonindependence of the

Table 1. Summary of cytokeratin staining by mutation status, with ORs for staining positivity for *BRCA1* and *BRCA2* carriers compared with controls

Marker	Level	Control	BRCA1			BRCA2		
		n (%)	*n* (%)	OR (95% CI)	*P*	n (%)	OR (95% CI)	*P*
CK14	Neg	150 (88.2)	95 (39.4)	1.0		66 (75.9)	1.0	
	Pos	20 (11.8)	146 (60.6)	12.47 (5.52-28.21)	<0.0001	21 (24.1)	2.25 (0.89-5.67)	0.085
CK14, % staining	<1%	150 (88.8)	96 (39.8)	1.0		66 (75.9)		
	1-10%	14 (8.3)	68 (28.2)	8.08 (3.25-20.06)		12 (13.8)		
	11-50%	3 (1.8)	41 (17.0)	47.8 (6.15-371.0)		4 (4.6)		
	51-100%	2 (1.2)	36 (14.9)	23.5 (3.00-185.0)	0.0001	5 (5.7)		
CK14, intensity	None	150 (88.2)	95 (39.4)	1.0		66 (75.9)		
	+	17 (10.0)	96 (39.8)	9.32 (3.89-22.31)		14 (16.1)		
	++	1 (0.6)	10 (4.1)	32.58 (7.43-143)	<0.0001	2 (2.3)		
	+++	2 (1.2)	40 (16.6)			5 (5.7)		
CK5/6	Neg	155 (93.4)	86 (42.4)	1.0		44 (84.6)		
	Pos	11 (6.6)	117 (57.6)	22.27 (7.78-63.75)	<0.0001	8 (15.4)	2.73 (0.77-9.75)	
CK5/6, % staining	<1%	155 (93.4)	87 (42.9)	1.0		44 (84.6)		
	1-10%	8 (4.8)	51 (25.1)	11.07 (3.65-33.53)	<0.0001	5 (9.6)		
	11-50%	3 (1.8)	37 (18.2)	85.7 (12.66-580)	<0.0001	0 (0.0)		
	51-100%	0 (0.0)	28 (13.8)			3 (5.8)		
CK5/6, intensity	None	155 (93.4)	86 (42.4)	1.0		44 (84.6)		
	+	9 (5.4)	83 (40.9)	22.24 (8.11-61.0)		7 (13.5)		
	++	2 (1.2)	26 (12.8)	22.36 (4.77-104.7)	<0.0001	0 (0.0)		
	+++	0 (0.0)	8 (3.9)			1 (1.9)		
CK17	Neg	179 (90.4)	99 (46.7)	1.0		39 (92.9)	1.0	
	Pos	19 (9.6)	113 (53.3)	7.1 (3.29-15.29)	<0.0001	3 (7.1)	0.56 (0.1-3.16)	
Osteo	Neg	161 (81.3)	121 (56.8)	1.0		32 (76.2)	1.0	
	Pos	37 (18.7)	92 (43.2)	4.09 (2.0-8.39)	0.0001	10 (23.8)	1.61 (0.44-5.91)	
EGFR	Neg	85 (78.7)	56 (32.7)	1.0		33 (91.7)	1.0	
	Pos	23 (21.3)	115 (67.3)	6.88 (2.88-16.45)	<0.0001	3 (8.3)	0.27 (0.04-1.62)	

Abbreviations: CK, cytokeratin; Osteo, osteonectin; Neg, negative; Pos, positive.

scores for the same tumor inflates the SEs and confidence intervals (CI). Confidence limits were therefore computed using a robust variance approach, using the robust option in Stata. For CK14 and CK5/6, analyses were conducted based on positivity, the proportion of cells stained (<1%, 1-10%, 10-50%, or >50%) and staining intensity (on a three-point scale). CK17, osteonectin, and EGFR were scored negative (<1%), focally positive (1-10%), and positive (>10%); for the purposes of the analysis, focally positive has been treated as positive. ORs have been computed, separately for *BRCA1* and *BRCA2*, adjusted for age (categorized as <30, 30-39, 40-49, 50-59, 60-69, >70) and pathologist. Because all slides were read by two pathologists, concordance between pathologists for each stain (classified as positive or negative) was assessed using kappa statistics, using the kap command in Stata. Receiver operator characteristic (ROC) analyses were done using the roctab command in Stata.

Results

Kappa scores for agreement on staining positivity between pathologists were 0.74 (SE, 0.06) for CK14, 0.82 (SE, 0.07) for CK5/6, 0.90 for CK17 (SE, 0.06), 0.79 for osteonectin (SE, 0.06), and 0.84 for EFGR (SE, 0.08).

All five markers were strongly associated with *BRCA1* carrier status (Table 1). Thus, 60.6% of *BRCA1* carriers stained positive for CK14 compared with 11.8% of control tumors. For CK5/6, the proportions were 57.6% in *BRCA1* carriers and 6.6% for controls; for CK17, 53.3% of *BRCA1* carriers and 9.6% of controls; for osteonectin, 43.2% of *BRCA1* carriers and 18.7% of controls; and for EGFR, 67.3% of *BRCA1* carriers and 21.3% of controls. The corresponding estimated ORs for each marker individually, adjusted for age and pathologist were 22.3 (95% CI, 7.78-63.8) for CK5/6, 12.5 (95% CI, 5.5-22.2) for CK14, 7.1 (95% CI, 3.3-15.3) for CK17, 4.1 (95% CI, 2.0-8.4) for osteonectin, and 6.9 (95% CI, 2.9-16.5) for EGFR (all $P < 0.0001$).

There was some suggestion that both intensity and extent of staining for CK14 and extent of staining for CK5/6 were also associated with carrier status, but this trend was only significant for CK5/6 ($P = 0.044$ for percentage cells stained).

There was no significant evidence for trend in marker positivity with age, in either *BRCA1* carriers or controls, except for CK17, which seemed to decline with age ($P = 0.001$ overall, $P = 0.02$ in controls). However, the OR associated with CK5/6 increased with age ($P = 0.018$ for interaction between age and CK5/6 positivity).

None of the five markers were significantly associated with *BRCA2* status. Although the point estimates of the OR were >1 for both CK14 (OR, 2.25) and for CK5/6 (OR, 2.73), the 95% CIs included 1 in each case. Moreover, the prevalence was

significantly lower than that in *BRCA1* carriers for all five markers.

Multiple regression analyses. CK14, CK5/6, CK17, and EGFR positivity were all strongly correlated (Table 2; r = 0.42-0.61, all $P < 0.0001$), whereas the correlations between these markers and osteonectin was somewhat weaker (r = 0.16-0.26). All cytokeratin staining was highly correlated with ER negativity; in controls, 23% of ER-negative tumors were CK14 positive and 16% were CK5/6 positive compared with 6% and 2%, respectively, of ER-positive tumors. Staining was also strongly associated with tumor grade. The prevalence of CK14 positivity in controls was 6% in grade 1 tumors, 7% in grade 2 tumors, and 16% in grade 3 tumors. The effect was even more marked for CK5/6, which was present in 12% of grade 3 tumors but 0% of grade 1 and 2 tumors.

When the five markers typed in this study were all included in a multiple regression model together with ER and grade, neither CK17, osteonectin, EGFR, nor grade remained significant. After stepwise removal of markers not significant at the 5% level, CK5/6 and ER remained highly significant and CK14 also remains significant ($P = 0.02$; Table 3).

There was some evidence of an interaction between ER positivity and CK5/6 staining ($P = 0.034$), with a stronger (relative) effect in ER-negative tumors. In fact, there was no significant effect of either CK5/6 or CK14 in ER-positive tumors; however, these types were very rare. Among ER-negative tumors, both CK5/6 and CK14 were independently associated with *BRCA1* positivity (OR, 7.83; $P = 0.001$ and OR, 3.57; $P = 0.036$, respectively).

The combined effects of the cytokeratin markers and ER are summarized in Table 4. ER was strongly associated with *BRCA1* status, with over 90% of BRCA1 tumors being receptor negative. However, whereas it is a highly sensitive predictor (i.e., ER-positive cases are rarely mutation carriers), it is not very specific because ~33% of control tumors with similar age distribution are ER negative. The use of cytokeratins in combination provides a potentially much more specific test; ER-negative cytokeratin-positive (CK14 and/or CK5/6) patients constitute an estimated 70% of *BRCA1* patients but only 9% of controls. The combined effect of CK14 and CK5/6 was also apparent; 44% of carriers but only 2% of controls were ER negative and positive for both markers (estimated OR, 148 compared with ER positive, cytokeratin negative). The last effect was however particularly imprecise because only four controls were scored in this category.

Cytokeratin-positive tumors were also more likely to be HER-2 negative (2.9% of CK5/6-positive patients were HER-2 positive compared with 10.8% of CK5/6-negative patients). We and others have previously reported that *BRCA1* tumors were less likely to be HER-2 positive (3). However, the association between *BRCA1* and HER-2 positivity seemed of similar magnitude after adjustment for cytokeratin and ER (OR, 0.06 after adjustment; OR, 0.11 before adjustment), although it was no longer significant ($P = 0.08$). Therefore, HER-2 positivity is probably a (negative) predictor of *BRCA1* status that is independent of cytokeratin and ER.

Predicted carrier probabilities. Assuming an overall prevalence of *BRCA1* mutations among breast cancer cases diagnosed below age 60 is ~2%, the prevalence of *BRCA1* mutations among various risk categories based on pathology can be computed from the estimated ORs given in Table 4 and the frequencies of these categories in the control series. The estimated prevalences are ER positive, 0.4%; ER negative and cytokeratin negative, 1.6%; ER negative and CK14 positive, 5.2%; ER negative and CK5/6 positive, 10.1%. The estimated prevalence in the ER-negative, CK14- and CK5/6-positive group is 55% but is particularly uncertain given the low population frequency.

Table 2. Associations between immunohistochemical markers

	Correlations between basal markers				
	CK14	**CK5/6**	**CK17**	**Osteo**	**EGFR**
CK5/6	0.60*				
CK17	0.51*	0.61*			
Osteo	0.16‡	0.26*	0.24*		
EGFR	0.42*	0.47*	0.52*	0.26†	
Proportion of tumors positive for each marker by tumor characteristic (no. of observations in brackets)					
ER positive	6% (12)	2% (4)	1% (2)	11% (30)	5% (6)
ER negative	22% (28)	16% (18)	28% (36)	33% (42)	47% (40)
Grade					
1	6% (3)	0% (0)	0% (0)	9% (7)	0% (0)
2	7% (7)	0% (0)	2% (2)	13% (15)	11% (6)
3	16% (30)	12% (22)	17% (36)	24% (52)	34% (40)

Abbreviations: CK, cytokeratin; Osteo, osteonectin.
*$P < 0.0001$.
†$P < 0.001$.
‡$P < 0.01$.

Table 3. Multiple logistic regression analysis of factors associated with BRCA1 positivity

		OR (95% CI)
Initial model		
CK14	+	3.45 (0.83-14.22)
CK5/6	+	27.33 (4.36-171.5)
CK17	+	0.62 (0.13-3.06)
Osteo	+	1.03 (0.26-4.02)
EGFR	+	2.13 (0.61-7.37)
ER	–	3.39 (0.89-12.9)
Grade		
1		1
2		0.65 (0.18-2.28)
3		0.97 (0.21-4.45)
Final model		
CK14	+	3.22 (1.2-8.66)
CK5/6	+	6.41 (2.11-19.43)
ER	–	12.34 (4.08-37.31)
Final model (ER-negative tumors)		
CK14	+	3.57 (1.09-11.75)
CK5/6	+	7.83 (2.23-27.37)

Abbreviations: CK, cytokeratin; Osteo, osteonectin.

Table 4. Prevalence of combined immunotypes defined by ER, CK14, and CK5/6 and adjusted ORs

ER	CK14	CK5/6	BRCA1 (%)	Control (%)	Adj OR (95% CI)
Pos			9.6	67.2	1
Neg	Neg	Neg	20.9	24.0	4.15 (1.76-9.82)
Neg	Neg	Pos	13.4	2.4	26.9 (7.18-101.1)
Neg	Pos	Neg	12.4	4.8	14.01 (3.41-57.5)
Neg	Pos	Pos	43.8	1.6	147.9 (35.2-621.3)

Abbreviations: CK, cytokeratin; adj OR, adjusted OR; Neg, negative; Pos, positive.

Discussion

It has become increasingly clear that a proportion of breast cancers exhibit a basal/myoepithelial phenotype; meaning, they express molecules normally seen in the basal/myoepithelial compartment of the normal breast (4–7). This differentiation towards the basal cells is interesting as it has generally been assumed that most breast cancers arise from the luminal cell compartment, whereas the myoepithelial cell functions in a contractile fashion to extrude milk during lactation. It is not clear at this stage whether all breast cancers do indeed arise from luminal cells and show differentiation towards various lineages or whether the tumors exhibiting a basal/myoepithelial phenotype arise from myoepithelial cells. It has also been postulated that such tumors may be a result of transformation of progenitor/stem cells (19). Such basal tumors have been shown to have a distinct morphology (10, 11), immunophenotype and more recently, a distinct genotype using comparative genomic hybridization (7) and an expression profile on microarray analysis (8, 9). There are also data to suggest that basal breast cancers have a distinct metastatic propensity and prognosis compared with the nonbasal, usual type of ductal carcinoma of the breast (11).

It has been noted by us as well as others that *BRCA1* tumors share a similar immunohistochemical profile to sporadic basal carcinomas (ER negative, progesterone receptor negative, HER-2 negative; ref. 3), and recently, this has been confirmed on expression analysis (9, 13).

The current study, which comprises the largest set of *BRCA1/2* tumors analyzed for basal keratins, confirmed that expression of basal markers is frequent in *BRCA1*-related cancers. It is consistent with and expands on a recent small study reported by Foulkes et al. in which they reported that 15 of 17 (88%) *BRCA1* tumors expressed CK5/6 (20). The CK5/6/14-positive tumor category was strongly associated with *BRCA1*. In this study, we estimate that if the ER or grade is ignored, CK5/6- and CK14-positive tumors represents 1% to 2% of all breast cancer cases but ~44% of all *BRCA1* carriers. Staining for CK17, osteonectin, and EGFR were also more common in *BRCA1* carriers. Multiple regression analysis, however, indicated these markers were not significant once CK5/6, CK14, and ER status had been taken into account. Given the number of stains considered, however, the precise ORs associated with each marker should be interpreted cautiously. In contrast to *BRCA1*, there were no significant differences between *BRCA2* tumors and controls in the frequency of any of the basal makers.

In this study, all stains were scored by two pathologists. Both CK5/6 and CK14 scoring was highly reproducible, with kappa statistics of 0.74 and 0.82, respectively. These correspond to concordance rates of 88% and 92%, respectively. This suggests that these stains should give reliable results in routine histopathologic practice.

One potential bias in this study is that carrier cases were ascertained through high-risk families selected for genetic testing. Thus, there may be some selection towards better prognosis cases. Expression of basal keratins has been shown to be associated with a poorer prognosis, which would imply that the association between *BRCA1* positivity and cytokeratin staining may have been, if anything, underestimated.

Although further validation in a large prospective series of *BRCA1* cases is required, there are a number of significant implications of these data. First, the information can be used to predict more accurately the probability of carrying a *BRCA1* mutation. To illustrate this, consider a screening procedure based on selecting women who are ER negative and CK5/6 positive. This test alone would have a sensitivity in *BRCA1* carriers of ~56% and a specificity of 97%, with a positive predictive value (i.e., the proportion of CK5/6 positive's which are *BRCA1* positive) of 28% and a negative predictive value of 99%. For this simple test, the area under the ROC curve is 0.77. If the five categories in Table 4, based on ER, CK5/6, and CK14 status are used, with their respective frequencies and ORs, the area under the ROC curve rises to 0.87. Patients are routinely selected for mutation testing on the basis of a family history, but this may be poorly predictive because only a minority of breast cancer cases report a positive first degree family history and that <10% of cases with a positive family history harbor a *BRCA1* mutation (21). This is exacerbated by the fact that some aspects of family history are often poorly recorded. For example, the study of Peto et al. (21), based on 617 cases of breast cancer diagnosed below age 45, identified 16 *BRCA1* mutation carriers. Five of the carriers had a first-degree relative with breast cancer diagnosed below age 60, or of ovarian cancer, compared with 50 of 601 noncarriers. A test based on this definition of family history would therefore have a sensitivity of 31%, a positive predictive value of 9%, and an area under the ROC curve of 0.61. Thus, immunostaining provides better sensitivity and positive predictive value than family history. The effect of combining both family history and immunostaining can also be evaluated, providing one assumes that the immunostatus is not related to family history except through the presence of a *BRCA1* mutation. Using a classification of cases by family history (as classified above) and immunostaining status (in five categories as above), the area under the ROC curve would be 0.89, only slightly greater than that for immunostaining alone. In reality, this analysis underestimates the true predictive value of family history, because finer classifications that incorporate the number of ovarian cancers and bilaterality would provide further information. Nevertheless, it illustrates that the immunohistochemical-based assays may substantially improve the power to predict carrier status. This may be particularly true for early-onset cases, where the proportion of women with a positive family history is low.

To illustrate how this would work in practice, consider a breast cancer case diagnosed at age 45. The threshold for

genetic testing in the United Kingdom is a mutation prevalence (for *BRCA1* and *BRCA2* combined) of ≥20%, and this would only be reached for cases with a reasonably strong family history (e.g., at least two cases of breast cancer diagnosed below age 50 years). Based on our data, this PPV threshold would also be reached by CK5/6-positive, ER-negative tumors. In contrast, for ER-positive tumors, the prevalence of *BRCA1* mutations is approximately one fifth that of all tumors. They represent ~70% of cases but only 14% of carriers. To achieve a carrier probability in this group of 20%, the prior probability of carrying a mutation based on family history would need to be ~98%. Thus, logically, only a very strong family history should lead to predictive testing for *BRCA1* mutations in such cases. (It is important to emphasize, however, that testing for *BRCA2* mutations is not affected by these considerations.) The addition of basal markers to age of diagnosis, family history, and ER status can therefore considerably enhance our ability to identify patients with *BRCA1* mutations by defining more precisely those subgroups of patients with a high probability of harboring a mutation. This is important given that mutation testing is very expensive and carries implications both for the individual and their family. Unlike expression profiling, the use of cytokeratin markers and ER assessment could be easily incorporated in the vast majority of pathology laboratories, providing a widely available and relatively inexpensive tool with which to identify patients for referral for genetic testing.

The second major implication relates to prognosis. The data regarding prognosis of *BRCA1/2* associated breast cancers are confusing with different groups suggesting better, worse, or same prognosis as sporadic cancers (22–25). The association of basal cancers with poorer prognosis (9) would seem to lend support to a worse prognosis for *BRCA1* patients. However, there are data to indicate that even basal cancers are heterogeneous at a genomic level and that there may be subsets with very poor as well as very good outcomes (26, 27). The difference in data in the literature regarding the differing prognosis has been assigned to methodologic issues or population differences. Whereas these are likely to have played a part, it is forgotten that *BRCA1*-associated tumors, although sharing germ line mutations in the same gene, are also likely to exhibit some heterogeneity at the molecular level and in their behavior. Lumping them together as one group may have contributed to the confusion in their behavior.

Finally, the expression of the basal marker, EGFR, in *BRCA1*-associated cancers raises the possibility of specific anti-EGFR therapies (such as gefitinib; ref. 28) in this particular group of patients.

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