Published in final edited form as:

Cancer. 2011 April 1; 117(7): 1350–1359. doi:10.1002/cncr.25642.

CK8/18 expression, the basal phenotype and family history in identifying BRCA1-associated breast cancer in the Ontario site of the Breast Cancer Family Registry

Anna Marie Mulligan^{1,2}, Dushanthi Pinnaduwa³, Anita L. Bane^{4,5}, Shelley B. Bull^{3,6}, Frances P. O'Malley^{*,1,2}, and Irene L. Andrulis^{*,2,3,7,8}

¹Keenan Research Centre in the Li Ka Shing Knowledge Institute of St. Michael's Hospital and the Department of Laboratory Medicine, St. Michael's Hospital, Toronto, Ontario, Canada

²Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada

³Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada

⁴Juravinski Cancer Centre, Hamilton, Ontario, Canada

⁵Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada

⁶Dalla Lana School of Public Health, University of Toronto, Toronto, Ontario, Canada

⁷Department of Medical Genetics and Microbiology, University of Toronto, Toronto, Ontario, Canada

⁸Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Ontario, Canada

Abstract

Background—BRCA1-associated breast cancer has been shown to be morphologically and genetically distinct from sporadic cancers. The aim of our study was to determine the association of CK8/18 with BRCA1-associated tumors and if, by using CK8/18 and basal biomarkers in conjunction with morphologic features and family history characteristics, we could improve the specificity of the BRCA1-associated tumor profile in a pathologically well-characterised cohort.

Methods—Fifty-eight patients with known BRCA1 germline mutations and 221 control (familial non-BRCA) patients were selected from the Ontario Familial Breast Cancer Registry. From this database, information on family history and morphologic features was abstracted. TMAs were constructed and immunohistochemistry to determine expression of several biomarkers was performed. After a logistic regression (LR) fit, a best-subsets variable-selection procedure using model performance and predictive ability measures was applied to find a best predictor to distinguish BRCA1-associated tumors from non-BRCA associated tumors.

Results—BRCA1-associated tumors differed significantly from control tumors in terms of morphology, family history and biomarker profile. CK8/18 was highly significantly associated with BRCA1 tumors. Consistently, BRCA1 cancers showed low levels of CK8/18 compared to non-BRCA tumors whether they were basal-like or not. A combination of seven factors, including CK8/18 and family history, best predicted the BRCA1-associated cancers.

Corresponding Author: Anna Marie Mulligan, Department of Laboratory Medicine, St. Michael's Hospital, 30 Bond St., Toronto, Ontario, M5B 1W8, Canada, mulligana@smh.toronto.on.ca, Tel: + 1 416 8646060, ext. 2367, Fax: +1 416 8645648.

*Co-senior authors

The authors have no financial disclosures to declare.

Conclusions—CK8/18 expression is independently associated with BRCA1-associated breast cancers. Reduced CK8/18 expression in conjunction with the basal-like phenotype and family history may improve our ability to identify which tumors are likely to be associated with a BRCA1 germline mutation and thereby help streamline genetic testing.

Keywords

CK8/18; BRCA1; breast cancer; basal subtype

Introduction

BRCA1, located on chromosome 17q12–21 was cloned in 1994¹ and is involved in many transcriptional processes.² As a tumor suppressor, it maintains genomic stability, playing a role in DNA recognition and repair.^{3, 4} Germline mutations in BRCA1 as well as in BRCA2 confer susceptibility to breast and ovarian cancer. Mutations in these genes account for 2–3% of all breast cancers and around 30–40% of all familial breast cancers.⁵

A substantial body of work indicates that tumors arising in patients with germline mutations in the BRCA1 gene are morphologically and genetically distinct from those arising in carriers of BRCA2 mutations and from tumors in patients lacking mutations. In gene expression studies, BRCA1-associated tumors are classified as basal subtype tumors.^{6, 7} This is reflected in their morphology, being of higher grade, and the morphologic features of medullary carcinoma, which include a lymphocytic infiltrate, pushing margins and syncytial growth are also more frequently present. Being basal-like they express several markers that are normally expressed in the basal/myoepithelial cells of the breast, including stratified epithelial cytokeratins 5/6, 14 and 17. BRCA1-associated tumors are more likely to be hormone receptor (HR) and HER2 negative and harbor mutations in the TP53 gene than age-matched sporadic breast cancers^{8–10}. CK17, osteonectin and EGFR have also been found to be more common in BRCA1-associated tumors, although, these lost significance in multiple regression analysis once CK5/6, CK14 and ER status was taken into account.¹⁰

Identifying patients with breast cancer who are likely to harbor a BRCA germline mutation has important clinical consequences. Current selection processes for mutation testing are based on personal history at a young age or family history for breast and/or ovarian cancer.^{11, 12} Typically, a risk of 10% or greater is the accepted level at which genetic testing is recommended.^{13, 14} This approach is both insensitive and non-specific;^{15, 16} thus, refinement of the selection criteria is highly desirable. Incorporating tumor morphologic features into the selection process has been shown to facilitate the identification of which patients are likely to harbor a germline mutation.^{17–23}

It has been suggested that, in the breast, BRCA1 may play a role in the differentiation of undifferentiated stem cells or progenitor cells into a glandular epithelial phenotype,²⁴ the latter characterized by the expression of luminal cytokeratins, CK8, CK18 and CK19. We, therefore, hypothesized that CK8/18 expression may be reduced in tumors arising in patients with a BRCA1 germline mutation. Thus, within a large cohort of patients with BRCA1-associated breast cancer and controls, we examine the association between BRCA1 status and CK8/18 expression, in addition to specific family history characteristics, morphologic features and the expression of several other biomarkers including HR, HER2 and markers of basal-like tumors (CK5, CK14 and epidermal growth factor receptor (EGFR)). We further attempt to identify combinations of parameters that may aid in distinguishing BRCA1 from non-BRCA tumors. These parameters could subsequently be used in the clinical setting to complement current methods used in mutation testing decision-making.

Materials and Methods

Study Population

Fifty-eight patients with known BRCA1 germline mutations were selected from the Ontario Familial Breast Cancer Registry (OFBCR).²⁵ In addition, 221 age at diagnosis (± 2 years) and ethnicity matched controls, known to be negative for BRCA mutations, were selected from the registry at a ratio of 1:m where m=1 to 6. Additional analyses were performed using a cohort of patients with sporadic breast cancer and lymph node negative disease (LNN) as controls (n=510). This cohort has been previously described.²⁶ Approval of the study protocol was obtained from the research ethics boards of Mount Sinai Hospital and the University Health Network, Toronto and written informed consent was received from all study participants.

Tumor Morphology and Family History

As part of the OFBCR a centralized pathology review of tumors was carried out by an expert in breast pathology. Included in this comprehensive review was assessment of established pathologic prognostic factors including tumor type, stage, grade, as well as morphologic features specifically designed to identify those tumors with medullary-like features, including margin circumscription, syncytial growth and lymphocytic infiltration. This information was recorded in the registry database along with detailed information on the patient's family history. From this database information on morphologic parameters and proband family history (Table 1) was abstracted.

Mutational Analysis of BRCA1

Testing for germline mutations in BRCA1 and BRCA2 was performed using an RNA/DNA-based protein truncation test with complementary 5' sequencing, as previously described,²⁷ or by complete gene sequencing by Myriad Genetics. All mutations were confirmed by DNA sequencing. Mutations were classified as deleterious if they were protein-truncating, missense mutations, or splice-site mutations as defined by the Breast Informatics Consortium (<http://research.nhgri.nih.gov/bic/>).

Tissue Microarray Construction and Immunohistochemical Staining

Areas of invasive carcinoma were selected from an H&E stained section of each tumor and duplicate 0.6mm cores of tissue were taken from the corresponding areas of the paraffin block for use in the construction of tissue microarrays (Beecher Instruments, Sun Prairie, WI). Microwave antigen retrieval was carried out in a Micromed T/T Mega Microwave Processing Lab Station (ESBE Scientific, Markham, Ontario, Canada). Four-micron TMA sections were cut and used for immunohistochemical staining using methods as listed in Table 2. Staining for HR, HER2, CK5, CK14, CK8/18 and EGFR was performed. Sections were developed with diaminobenzidine tetrahydrochloride and counterstained in Mayer's hematoxylin.

Interpretation and Scoring of Immunohistochemistry

Each of the immunohistochemically stained sections was scored using Allred's scoring method,²⁸ which adds scores for the intensity of staining (absent: 0, weak: 1, moderate: 2, and strong: 3) to the percentage of cells staining (none: 0, <1%: 1, 1–10%: 2, 11–33%: 3, 34–66%: 4 and 67–100%: 5) to yield a 'raw' score of 0 or 2–8. Previously validated cut-offs for ER and PgR were used (0,2=negative, 3–8=positive;).^{29, 30} Moderate to strong complete membranous staining was assessed for HER2 and the validated cut-off of ≥ 5 was used to indicate positivity.³¹ For each of the remaining antibodies, a score of ≥ 4 was considered positive. The raw score data were reformatted using a TMA deconvoluter

software program³² into a format suitable for statistical analysis. The highest score from each tumor pair was entered into the statistical analysis. Interpretable scores were obtained on 89 to 92% of tumors.

Guided by previous reports, tumors from each group were further defined as ‘basal-like’ if they showed the following profile: ER negative, HER2 negative and positivity for one or more of the following: CK5, CK14 or EGFR.^{33, 34}

Statistical Analysis

The family history characteristics, morphologic parameters and biomarker status between carriers and controls were compared using a logistic regression (LR) model controlling for matching variables. Ideally, a conditional LR model would have been applied if there was not sparse data for some strata. Odds ratios (ORs), 95% confidence intervals (CI) and P values were calculated for each of the variables. A best subsets LR approach was used to study the prognostic value of CK8/18 and to identify a best model for distinguishing BRCA1 cancers from control cancers. Spearman's rank correlation test was used to identify correlations among the variables. Variables that were significant and not highly correlated were used as the important predictors in the best subsets analysis. For each possible subset of important predictors, an LR model was fitted controlling for matching variables. As tumor grade, ER and HER2 have been shown to significantly differ between BRCA1 and non-BRCA tumors,^{6, 8–10, 20–23} these features were incorporated into all models. The idea here is to reduce the level of overfitting by decreasing the number of possible subsets. Models were ranked by Bayesian Information Criteria (BIC) or Schwarz Information Criteria (SIC)³⁵ according to the model's performance (goodness of fit). Adjusted R^2 was also calculated as another model performance measure.³⁶ Predictive ability measures, such as sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated. To calculate predictive measures a cut-off probability of 0.5 (default cut-off) was used; tumors with an estimated probability above 0.5 were predicted to be carriers of a BRCA1 germline mutation. Finally, the predictive value of CK818 and robustness of the best model was verified by using the cohort of sporadic LNNs as controls and fitting an LR model for the best subset. All P values were 2 sided. Statistical analyses were performed using SAS 9.1 software (SAS Inc., Cary, NC, USA).

Results

Patient, Family and Tumor Characteristics

Age at diagnosis ranged from 27–71 years in both BRCA1-carrier and control groups with a median of 42.5 and 43 years respectively. Age at diagnosis of the LNN cohort ranged from 22–76 years with a median of 55 years. Table 3 shows the morphologic features of tumors from both groups in univariate LR analysis. BRCA1-associated tumors were significantly more likely to be high grade with 91% of carriers having grade III tumors compared with 41% of controls ($P<0.0001$). Specific morphologic features associated with the medullary phenotype were also significantly more commonly associated with tumors from BRCA1 mutation carriers, including a greater lymphocytic infiltrate ($P=0.0007$), the presence of a pushing margin ($P=0.0151$) and a syncytial growth pattern ($P<0.0001$). As shown in Table 4, three family history characteristics were associated with BRCA1 mutation carrier status in univariate LR analysis. BRCA1-carriers were significantly more likely to have at least one first degree relative with breast or ovarian cancer ($P<0.0001$) (Characteristic 1) and were significantly more likely to have three first degree relatives with cancer (breast, ovarian, colon, prostate, pancreatic carcinoma or sarcoma with at least one diagnosis ≤ 50) than controls ($P=0.0018$) (Characteristic 7). They were also more likely to have a proband diagnosed with both breast and ovarian cancer or multiple breast primaries ($P=0.0451$)

(Characteristic 5). Other family characteristics were not significantly associated with BRCA1-status. Odds ratios of the comparisons are given in Table 3 and Table 4.

Using the sporadic LNN cohort as controls, BRCA1-associated tumors were significantly more likely to be high grade (grade III) compared with controls (93% vs. 36%; $P<0.0001$),

Biomarker Profiling

Expression of several immunohistochemical biomarkers, ER, PR, CK5, CK14, CK8/18 and EGFR, was strongly associated with BRCA1-carrier status whereas HER2 showed borderline significance (Table 5). ER, PR and HER2 expression in BRCA1-tumors was low compared to controls (12% vs. 58%, 5% vs. 50%, and 2% vs. 12% respectively). In addition, expression of CK8/18 was also lower in tumors from BRCA1 mutation carriers compared to control tumors (43% vs. 94%). Conversely, tumors from BRCA1 mutation carriers were more likely to express basal cytokeratins and EGFR. When grade 3 cancers were considered only, CK8/18 expression was again significantly lower in tumors from BRCA1 mutation carriers compared with controls (40% vs. 88% $P<0.0001$; data not shown). Furthermore, BRCA1-tumors were more likely to have a basal-phenotype, as defined previously, than control tumors (69% vs. 19%, $P<0.0001$, data not shown) and, in this subset, CK8/18 expression was seen in 42% of tumors from BRCA1 mutation carriers compared with 83% of controls ($P=0.0005$, data not shown).

Using the sporadic LNN cohort as controls, ER and CK8/18 expressions were significantly lower in tumors from BRCA1 mutation carriers compared with controls (13% vs. 70% and 42% vs. 99% respectively; $P<0.0001$ for both comparisons; data not shown). HER2 expression in BRCA1-tumors was low compared to sporadic controls (2% vs. 8%; not significant, data not shown). Conversely, tumors from BRCA1 mutation carriers were more likely to express CK5 than sporadic controls (64% vs. 19%; $P<0.0001$; data not shown) and to be triple negative (86% vs. 20%, $P<0.0001$, data not shown). When grade 3 cancers were considered only, CK8/18 expression was again significantly lower in tumors from BRCA1 mutation carriers compared with sporadic controls (40% vs. 96%; $P<0.0001$; data not shown). In triple negative tumors, CK8/18 expression was seen in 39% of tumors from BRCA1 mutation carriers compared with 93% of controls ($P<0.0001$; data not shown).

Best-subsets Selection

Strong correlations were found between mitotic score and grade, ER and PR, and CK5 and CK14 (data not shown). To reduce colinearity problems, mitotic score, PR and CK14 were dropped from further analyses. From the remaining variables (excluding grade, ER and HER2), those that were significant in the univariate model were used to form possible subsets. These included margin circumscription, syncytial growth pattern, lymphocytic infiltration, CK5, CK8/18, EGFR and the two most significant family characteristics 1 and 7. Later, the subsets were fitted by LR models along with matching variables, grade, ER and HER2 as explained in the 'Statistical Analysis' section. To further reduce the number of possible subsets, family history characteristic 5 ($P=0.0451$) was not included in the subset analysis. In the first screening, the first 50 models ranked according to BIC were selected. The sensitivity values for the models varied from 51.1 to 66.7. In the second screening, the models with sensitivities > 60 were kept, leaving a total of 22 models (Table 6). These models had very similar values for R^2 and predictive ability measures. Thus, without trying to find a best predictive model based on these measures, we looked at the most frequently occurring variables. While CK5 was selected in half of the models, CK8/18 and family characteristic 7 were contained in all of the models. Family characteristic 1 was included in 59% of the models, whereas syncytial growth pattern and lymphocytic infiltration were present in 32% of the models. Margin circumscription and EGFR were each included in

approximately 25% of the models. Thus, the model containing CK8/18, CK5 and family history was selected as the best model, along with the standard variables: grade, ER and HER2 (Table 7). This model predicted for an underlying BRCA1 mutation with a sensitivity and specificity of 62 and 93% respectively. The models containing only family history or family history together with grade, ER and HER2 had low sensitivities (7% and 47% respectively) compared to the selected best model, although, they had similar specificities. In all the models in Table 6, CK8/18 showed an independent association with BRCA1 mutation carriers after adjusting for other variables (data not shown). When the best model was validated, with the sporadic LNN cohort as controls, the significance of each parameter remained unchanged except for a larger OR for CK8/18 (Table 8). CK8/18 was again shown as an independent predictor of BRCA1 status. This model predicted for an underlying BRCA1 mutation with a sensitivity and specificity of 55.6 and 98.5% respectively. Family history variables were omitted from the model as there was insufficient family history data available for the sporadic cohort. When family history variables were omitted from the model in Table 7, an underlying BRCA1 mutation was predicted with a sensitivity and specificity of 53.3 and 92.0% respectively. Thus, exclusion of family history criteria may explain the lower sensitivity of the model in Table 8.

Discussion

This study includes a large cohort of BRCA1-carriers in which pathologic parameters have been used in conjunction with family history to identify patient and tumor characteristics that are most likely to be associated with a BRCA1 germline mutation. We found that CK8/18 negativity was independently associated with BRCA1 mutation carriers in multivariate analyses. Furthermore, our data (Table 6) are very promising in terms of developing a predictor for BRCA1 germline mutation and, therefore, streamlining genetic testing. CK8/18 together with the basal-like phenotype (high grade, ER/HER negativity, CK5 positivity) and family history were found to be the best combination for predicting BRCA1 carrier status as they featured fairly consistently in all models.

Identifying which patients are at higher risk of harboring a BRCA1 mutation is important in terms of subsequent personal and family risk of developing breast and/or ovarian cancer. Furthermore, it is becoming clear that specific therapeutic options must be considered for patients with BRCA1-associated cancers. Being mostly HR and HER2 negative, these tumors will typically not respond to targeted hormonal or trastuzumab treatment. However, recent studies show that BRCA1/2 deficient cells are extremely sensitive to inhibitors of the base excision DNA repair protein Poly(ADP-ribose) polymerase or PARP.³⁷⁻³⁸ Currently, age and family history are used to predict the likelihood of carrying a BRCA1 mutation. Obvious family cancer syndromes are likely to be present in a minority of patients with an inherited predisposition and population-based series of early-onset breast cancer suggest that a high proportion of patients with BRCA-associated cancers, in fact, present as sporadic cancers.³⁹ This has led many investigators to examine whether specific morphologic features and immunophenotype of these tumors can contribute to the predictive accuracy of genetic testing.¹⁷⁻²³

Using morphology to detect possible carriers of germline mutations is in widespread use in the setting of colonic adenocarcinoma.⁴⁰⁻⁴¹ Loss of immunohistochemical staining for a number of mismatch repair genes, including MSH2 and MLH1 can be used to identify tumors that are likely to be MSI-High and therefore target individuals at higher risk of carrying a germline mutation and, thereby those that may benefit from genetic testing. In breast cancer, van't Veer et al used the fact that BRCA1-associated tumors are typically ER negative as a starting point to define the expression profile in these tumors.⁶ Lakhani et al found ER negativity to be the single best predictor of an underlying BRCA1 mutation and

that combining this with basal cytokeratin positivity provided a more accurate predictor of carrier status.¹⁰ James et al²¹ found the sensitivity of BRCA-PRO, a traditional model for predicting mutations in the BRCA genes using family history, was increased by including the ER and PR receptor status and pathologic grade of the tumor. In a study that included 11 BRCA1-associated tumors, Farshid et al²⁰ showed that using morphologic criteria, pathologists could identify tumors that possibly or probably had a BRCA1 germline mutation with high sensitivity and specificity.

Our data support previous studies that identified features that distinguish BRCA1 from non-BRCA tumors as largely a consequence of the prominence of the basal-like phenotype in the former group. On the other hand, we found that BRCA1 associated tumors were significantly more likely to show reduced CK8/18 expression compared to control tumors (43% vs. 94%; $p < 0.0001$). When we derived a predictive model, CK8/18 negative status was important in all 50 models (data not shown) showing that CK8/18 associates independently with BRCA1 mutation and gives an additional gain to the prediction models. This property of CK8/18 negativity was seen in the overall group, as well as in each tumor subgroup and, therefore, was not specific to any particular subgroup including those considered basal-like (descriptive analysis - data not shown). To test the predictive value of CK8/18 and robustness of the best model, an independent sporadic cohort was used as controls. CK8/18 was again found to be independently associated with BRCA1 status. To our knowledge, this is the first study to show CK8/18 to be independently associated with BRCA1 carrier status.

Previous studies have examined CK8/18 expression in unselected basal-like tumors and it has been reported that most co-express CK8/18 as well as basal cytokeratins.³⁴⁻⁴² However, Laakso et al⁴³ found that while all basal cancers in a cohort of sporadic carcinomas co-expressed CK8/18, 5 of 20 BRCA1-related tumors showed complete absence of CK8/18 staining and 5 more showed reduced staining. In another study,⁴⁴ keratin 8 was found to be one of 176 genes that showed different expression in tumors with BRCA1 mutations compared with those with BRCA2 mutations. Our findings are consistent with these studies and suggest that lack of or reduced CK8/18 expression is associated with BRCA1-associated breast cancers, but not non-BRCA tumors.

The prominence of the basal phenotype in BRCA1-associated tumors has raised the possibility that BRCA1 may play a role in the differentiation of mammary epithelial cells.²⁴⁻⁴⁵ Breast cancers are generally thought to arise from luminally differentiated epithelial cells as evidenced by strong expression of CK8, CK18 and CK19.⁴⁶⁻⁴⁸ However, CK5, expressed in the basal group of cancers, is normally found in the basal layer of the mammary duct.⁴⁶⁻⁴⁹ Foulkes²⁴ has proposed that BRCA1 may act as a stem cell regulator where it plays a role in promoting the transition from the basal to the glandular epithelial phenotype and it is the failure of this mechanism that leads to the persistence of the 'primitive' basal phenotype. Depletion of BRCA1 mRNA has been shown to block differentiation and stimulate proliferation of these 'basal' cells in a 3D cell culture system.⁴⁵ Furthermore, BRCA1 deficient epithelial cells lose the ability to form differentiated mammary structures in mouse models, producing instead structures expressing ALDH1, a putative stem cell marker.⁵⁰ Lim et al⁵¹ recently found that benign breast tissue from prophylactic mastectomy specimens from unaffected BRCA1 mutation carriers harbored an expanded luminal progenitor cell population which showed ligand-independent clonogenic activity. Furthermore, using gene expression profiling, upregulated luminal progenitor signature genes were more highly expressed in basal tumors than in other tumor subtypes and in breast tissue heterozygous for a BRCA1 mutation compared to normal breast tissue. The findings in this study suggest that luminal progenitor cells represent a probable cancer-initiating population in BRCA1 mutation carriers, and that the perturbed growth properties of these luminal progenitor cells are likely to aberrantly influence luminal cell differentiation. We

have found tumor expression of CK8/18 – a marker of luminal differentiation - to be significantly reduced in BRCA1-tumors suggesting disruption of the pathway that drives differentiation of breast epithelial cells to a glandular/luminal phenotype.

Conclusions

In conclusion, BRCA1-associated cancers differ significantly in terms of morphology and immunophenotype from non-BRCA cancers, largely a result of the prominent representation of the basal subgroup in the former; however, this study shows differential expression of CK8/18 to be independent of tumor subgroup. Thus, CK8/18 in conjunction with basal phenotype and family history may improve our ability to identify which tumors are likely to be associated with a BRCA1 germline mutation and thereby help streamline genetic testing. It is possible that the predictor developed may overfit the data. Therefore, these findings require validation in an independent data set before they can be employed to complement or replace current screening methods. Furthermore, the finding of diminished CK8/18 expression in these tumors may provide greater insight into the biology of these tumors.

Acknowledgments

The authors would like to thank Nayana Weerasooriya for her assistance with data preparation.

Research support:

This work was supported by the National Cancer Institute, National Institutes of Health under RFA # CA- 06-503 and through cooperative agreements with members of the Breast Cancer Family Registry and Principal Investigators, including Cancer Care Ontario (U01 CA69467). The content of this manuscript does not necessarily reflect the views or policies of the National Cancer Institute or any of the collaborating centers in the BCFR, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government or the BCFR.

References

1. Miki Y, Swensen J, Shattuck-Eidens D, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science*. 1994 Oct 7; 266(5182):66–71. [PubMed: 7545954]
2. Cable PL, Wilson CA, Calzone FJ, et al. Novel consensus DNA-binding sequence for BRCA1 protein complexes. *Mol Carcinog*. 2003 Oct; 38(2):85–96. [PubMed: 14502648]
3. Jhanwar-Uniyal M. BRCA1 in cancer, cell cycle and genomic stability. *Front Biosci*. 2003 Sep 18:s1107–s1117. [PubMed: 12957814]
4. Venkitaraman AR. Cancer susceptibility and the functions of BRCA1 and BRCA2. *Cell*. 2002 Jan 25; 108(2):171–182. [PubMed: 11832208]
5. Wooster R, Weber BL. Breast and ovarian cancer. *N Engl J Med*. 2003 Jun 5; 348(23):2339–2347. [PubMed: 12788999]
6. van 't Veer LJ, Dai H, van de Vijver MJ, et al. Gene expression profiling predicts clinical outcome of breast cancer. *Nature*. 2002 Jan 31; 415(6871):530–536. [PubMed: 11823860]
7. Sorlie T, Tibshirani R, Parker J, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. *Proc Natl Acad Sci U S A*. 2003 Jul 8; 100(14):8418–8423. [PubMed: 12829800]
8. Lakhani SR, Van De Vijver MJ, Jacquemier J, et al. 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. *J Clin Oncol*. 2002 May 1; 20(9):2310–2318. [PubMed: 11981002]
9. Lakhani SR, Jacquemier J, Sloane JP, et al. Multifactorial analysis of differences between sporadic breast cancers and cancers involving BRCA1 and BRCA2 mutations. *J Natl Cancer Inst*. 1998 Aug 5; 90(15):1138–1145. [PubMed: 9701363]

10. Lakhani SR, Reis-Filho JS, Fulford L, et al. Prediction of BRCA1 status in patients with breast cancer using estrogen receptor and basal phenotype. *Clin Cancer Res*. 2005 Jul 15; 11(14):5175–5180. [PubMed: 16033833]
11. Genetic risk assessment and BRCA mutation testing for breast and ovarian cancer susceptibility: recommendation statement. *Ann Intern Med*. 2005 Sep 6; 143(5):355–361. [PubMed: 16144894]
12. Eisinger F, Alby N, Bremond A, et al. Recommendations for medical management of hereditary breast and ovarian cancer: the French National Ad Hoc Committee. *Ann Oncol*. 1998 Sep; 9(9): 939–950. [PubMed: 9818066]
13. Lancaster JM, Powell CB, Kauff ND, et al. Society of Gynecologic Oncologists Education Committee statement on risk assessment for inherited gynecologic cancer predispositions. *Gynecol Oncol*. 2007 Nov; 107(2):159–162. [PubMed: 17950381]
14. Euhus DM, Smith KC, Robinson L, et al. Pretest prediction of BRCA1 or BRCA2 mutation by risk counselors and the computer model BRCAPRO. *J Natl Cancer Inst*. 2002 Jun 5; 94(11):844–851. [PubMed: 12048272]
15. King MC, Marks JH, Mandell JB. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. *Science*. 2003 Oct 24; 302(5645):643–646. [PubMed: 14576434]
16. Moller P, Hagen AI, Apold J, et al. Genetic epidemiology of BRCA mutations--family history detects less than 50% of the mutation carriers. *Eur J Cancer*. 2007 Jul; 43(11):1713–1717. [PubMed: 17574839]
17. Laloo F, Evans DG. The pathology of familial breast cancer: Clinical and genetic counselling implications of breast cancer pathology. *Breast Cancer Res*. 1999; 1(1):48–51. [PubMed: 11250683]
18. Lidereau R, Eisinger F, Champeme MH, et al. Major improvement in the efficacy of BRCA1 mutation screening using morphoclinical features of breast cancer. *Cancer Res*. 2000 Mar 1; 60(5): 1206–1210. [PubMed: 10728676]
19. Chang J, Hilsenbeck SG, Sng JH, Wong J, Ragu GC. Pathological features and BRCA1 mutation screening in premenopausal breast cancer patients. *Clin Cancer Res*. 2001 Jun; 7(6):1739–1742. [PubMed: 11410514]
20. Farshid G, Balleine RL, Cummings M, Waring P. Morphology of breast cancer as a means of triage of patients for BRCA1 genetic testing. *Am J Surg Pathol*. 2006 Nov; 30(11):1357–1366. [PubMed: 17063074]
21. James PA, Doherty R, Harris M, et al. Optimal selection of individuals for BRCA mutation testing: a comparison of available methods. *J Clin Oncol*. 2006 Feb 1; 24(4):707–715. [PubMed: 16446345]
22. Gadzicki D, Schubert A, Fischer C, et al. Histopathological criteria and selection algorithms for BRCA1 genetic testing. *Cancer Genet Cytogenet*. 2009 Mar; 189(2):105–111. [PubMed: 19215791]
23. Atchley DP, Albarracin CT, Lopez A, et al. Clinical and pathologic characteristics of patients with BRCA-positive and BRCA-negative breast cancer. *J Clin Oncol*. 2008 Sep 10; 26(26):4282–4288. [PubMed: 18779615]
24. Foulkes WD. BRCA1 functions as a breast stem cell regulator. *J Med Genet*. 2004 Jan; 41(1):1–5. [PubMed: 14729816]
25. Ozcelik H, Knight JA, Glendon G, et al. Individual and family characteristics associated with protein truncating BRCA1 and BRCA2 mutations in an Ontario population based series from the Cooperative Family Registry for Breast Cancer Studies. *J Med Genet*. 2003 Aug; 40(8):e91. [PubMed: 12920083]
26. Mulligan AM, Pinnaduwa D, Bull SB, O'Malley FP, Andrulis IL. Prognostic effect of basal-like breast cancers is time dependent: evidence from tissue microarray studies on a lymph node-negative cohort. *Clin Cancer Res*. 2008 Jul 1; 14(13):4168–4174. [PubMed: 18593996]
27. Andrulis IL, Anton-Culver H, Beck J, et al. Comparison of DNA- and RNA-based methods for detection of truncating BRCA1 mutations. *Hum Mutat*. 2002 Jul; 20(1):65–73. [PubMed: 12112659]
28. Allred DC, Harvey JM, Berardo M, Clark GM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. *Mod Pathol*. 1998 Feb; 11(2):155–168. [PubMed: 9504686]

29. Harvey JM, Clark GM, Osborne CK, Allred DC. Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. *J Clin Oncol*. 1999 May; 17(5):1474–1481. [PubMed: 10334533]
30. Mohsin SK, Weiss H, Havighurst T, et al. Progesterone receptor by immunohistochemistry and clinical outcome in breast cancer: a validation study. *Mod Pathol*. 2004 Dec; 17(12):1545–1554. [PubMed: 15272277]
31. O'Malley FP, Parkes R, Latta E, et al. Comparison of HER2/neu status assessed by quantitative polymerase chain reaction and immunohistochemistry. *Am J Clin Pathol*. 2001 Apr; 115(4):504–511. [PubMed: 11293897]
32. Liu CL, Prapong W, Natkunam Y, et al. Software tools for high-throughput analysis and archiving of immunohistochemistry staining data obtained with tissue microarrays. *Am J Pathol*. 2002 Nov; 161(5):1557–1565. [PubMed: 12414504]
33. Nielsen TO, Hsu FD, Jensen K, et al. Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. *Clin Cancer Res*. 2004 Aug 15; 10(16):5367–5374. [PubMed: 15328174]
34. Livasy CA, Karaca G, Nanda R, et al. Phenotypic evaluation of the basal-like subtype of invasive breast carcinoma. *Mod Pathol*. 2006 Feb; 19(2):264–271. [PubMed: 16341146]
35. Schwarz G. Estimating the Dimension of a Model. *The Annals of Statistics*. 1978; 6(2):461–464.
36. Nagelkerke NJD. A Note on a General Definition of the Coefficient of Determination. *Biometrika*. 1991; 78(3):691–692.
37. Bryant HE, Schultz N, Thomas HD, et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature*. 2005 Apr 14; 434(7035):913–917. [PubMed: 15829966]
38. Farmer H, McCabe N, Lord CJ, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature*. 2005 Apr 14; 434(7035):917–921. [PubMed: 15829967]
39. Cui J, Hopper JL. Why are the majority of hereditary cases of early-onset breast cancer sporadic? A simulation study. *Cancer Epidemiol Biomarkers Prev*. 2000 Aug; 9(8):805–812. [PubMed: 10952097]
40. Aaltonen LA, Peltomäki P, Mecklin JP, et al. Replication errors in benign and malignant tumors from hereditary nonpolyposis colorectal cancer patients. *Cancer Res*. 1994 Apr 1; 54(7):1645–1648. [PubMed: 8137274]
41. Løthe RA, Peltomäki P, Meling GI, et al. Genomic instability in colorectal cancer: relationship to clinicopathological variables and family history. *Cancer Res*. 1993 Dec 15; 53(24):5849–5852. [PubMed: 8261392]
42. Abd El-Rehim DM, Pinder SE, Paish CE, et al. Expression of luminal and basal cytokeratins in human breast carcinoma. *J Pathol*. 2004 Jun; 203(2):661–671. [PubMed: 15141381]
43. Laakso M, Loman N, Borg A, Isola J. Cytokeratin 5/14-positive breast cancer: true basal phenotype confined to BRCA1 tumors. *Mod Pathol*. 2005 Oct; 18(10):1321–1328. [PubMed: 15990899]
44. Hedenfalk I, Duggan D, Chen Y, et al. Gene-expression profiles in hereditary breast cancer. *N Engl J Med*. 2001 Feb 22; 344(8):539–548. [PubMed: 11207349]
45. Furuta S, Jiang X, Gu B, Cheng E, Chen PL, Lee WH. Depletion of BRCA1 impairs differentiation but enhances proliferation of mammary epithelial cells. *Proc Natl Acad Sci U S A*. 2005 Jun 28; 102(26):9176–9181. [PubMed: 15967981]
46. Moll R, Franke WW, Schiller DL, Geiger B, Krepler R. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. *Cell*. 1982 Nov; 31(1):11–24. [PubMed: 6186379]
47. Bocker W, Moll R, Poremba C, et al. Common adult stem cells in the human breast give rise to glandular and myoepithelial cell lineages: a new cell biological concept. *Lab Invest*. 2002 Jun; 82(6):737–746. [PubMed: 12065684]
48. Chu PG, Weiss LM. Keratin expression in human tissues and neoplasms. *Histopathology*. 2002 May; 40(5):403–439. [PubMed: 12010363]

49. Boecker W, Buerger H. Evidence of progenitor cells of glandular and myoepithelial cell lineages in the human adult female breast epithelium: a new progenitor (adult stem) cell concept. *Cell Prolif.* 2003 Oct.36 Suppl 1:73–84. [PubMed: 14521517]
50. Liu S, Ginestier C, Charafe-Jauffret E, et al. BRCA1 regulates human mammary stem/progenitor cell fate. *Proc Natl Acad Sci U S A.* 2008 Feb 5; 105(5):1680–1685. [PubMed: 18230721]
51. Lim E, Vaillant F, Wu D, et al. Aberrant luminal progenitors as the candidate target population for basal tumor development in BRCA1 mutation carriers. *Nat Med.* 2009 Aug; 15(8):907–913. [PubMed: 19648928]

Table 1

Family History Characteristics Criteria Description

Characteristic	Description
1	At least 1 first degree relative with breast or ovarian cancer
2	At least 2 second degree relatives with breast or ovarian cancer
3	Proband diagnosed with breast cancer at age ≤ 35
4	At least 1 second or 1 third degree relative with breast cancer at age ≤ 35 or ovarian cancer at age ≤ 60
5	Proband diagnosed with both breast and ovarian cancer or multiple breast primaries
6	At least 1 second or 1 third degree relative with multiple breast primaries
7	Family has at least three relatives in a first degree relationship with any combination of breast, ovarian, colon, prostate, or pancreatic cancer or sarcoma with at least 1 diagnosis ≤ 50

Table 2

Summary of antibodies and conditions of use

Antibody	Clone	Dilution	Source	Pretreatment
ER	6F11	1:75	Vector	Tris (pH 9.0)
PgR	PgR1294	1:1,000	DAKO	Tris (pH 9.0)
CK5	XM26	1:400	Vector	Tris (pH 9.0)
HER2	CB11, TAB250 (cocktail)	1:300	Novocastra, Zymed	Proteinase K
CK14	LL002	1:40	Novocastra	Citrate buffer
EGFR	31G7	1:25	Zymed	Pepsin
CK8/18	5D3	1:6400	Novocastra	Tris (pH 9.0)

Table 3
Results of Logistic Regression Analysis* of Carrier Status on Morphology Features

	BRCA1 Mutation Carriers No. (%)	Non-carriers No. (%)	OR (95% CL)	P
Number of tumors	58	221		
Grade				
I/II	5 (8.6)	131 (59.3)	1.0 (reference)	
III	53 (91.4)	90 (40.7)	16.16 (6.11, 42.71)	< 0.0001
Lymphocytic infiltration				
Mild/Absent	31 (53.5)	171 (77.4)	1.0 (reference)	
Moderate/Marked	27 (46.5)	48 (21.7)	2.92 (1.57, 5.44)	0.0007
Missing	0 (0.0)	2 (0.9)		
Margin circumscriptio				
Negative (0)	40 (69.0)	181 (81.9)	1.0 (reference)	
Positive (1)	18 (31.0)	38 (17.2)	2.36 (1.18, 4.70)	0.0151
Missing	0 (0.0)	2 (0.9)		
Syncytial growth pattern				
Negative (0)	44 (75.9)	208 (94.1)	1.0 (reference)	
Positive (1)	14 (24.1)	11 (5.0)	6.08 (2.51, 14.71)	<0.0001
Missing	0 (0.0)	2 (0.9)		
Mitotic score				
1/2	11 (19.0)	146 (66.1)	1.0 (reference)	
3	47 (81.0)	75 (33.9)	8.01 (3.88, 16.52)	< 0.0001

* Analysis was controlled for matching variables: age at diagnosis and ethnicity. Missing category was not included in the analysis.

Table 4

Results of Logistic Regression Analysis* of Carrier Status on Family History Characteristics described in Table 1

	BRCA1 Mutation Carriers No. (%)	Non-carriers No. (%)	OR (95% CL)	P
No. tumors	58	221		
Characteristic 1				
Yes	34 (58.6)	73 (33.0)	3.55 (1.88, 6.70)	<0.0001
No	24 (41.4)	148 (70.0)		
Characteristic 2				
Yes	6 (10.3)	22 (9.9)	1.04 (0.40, 2.72)	0.9418
No	52 (89.7)	199 (90.1)		
Characteristic 3				
Yes	17 (29.3)	50 (22.6)	1.56 (0.65, 3.77)	0.3216
No	41 (70.7)	171 (77.4)		
Characteristic 4				
Yes	13 (22.4)	35 (15.8)	1.65 (0.80, 3.41)	0.1787
No	45 (77.6)	186 (84.2)		
Characteristic 5				
Yes	17 (29.3)	40 (18.1)	1.99 (1.02, 3.90)	0.0451
No	41 (70.7)	181 (81.9)		
Characteristic 6				
Yes	4 (6.9)	7 (3.2)	2.31 (0.64, 8.38)	0.2036
No	54 (93.1)	214 (96.8)		
Characteristic 7				
Yes	23 (39.7)	47 (21.3)	2.81 (1.47, 5.36)	0.0018
No	35 (60.3)	174 (78.7)		

* Analysis was controlled for matching variables: age at diagnosis and ethnicity

Table 5

Results of Logistic Regression Analysis* of Carrier Status on Biomarkers

No. tumors	BRCA1 Mutation Carriers No. (%)	Non-carriers No. (%)	OR (95% CL)	P
ER				
Negative	44 (75.8)	73 (33.0)	13.03 (5.36, 31.71)	< 0.0001
Positive	7 (12.1)	129 (58.4)	1.0(reference)	
Missing	7 (12.1)	19 (8.6)		
PR				
Negative	46 (79.3)	93 (42.1)	19.96 (5.93, 67.22)	< 0.0001
Positive	3 (5.2)	111 (50.2)	1.0(reference)	
Missing	9 (15.5)	17 (7.7)		
HER2				
Negative	48 (82.8)	179(81.0)	7.55 (0.99, 57.38)	0.0507
Positive	1 (1.7)	27 (12.2)	1.0(reference)	
Missing	9 (15.5)	15 (6.8)		
CK5				
Negative	22 (37.9)	155 (70.0)	1.0(reference)	
Positive	32 (55.2)	44 (20.0)	5.43 (2.80, 10.55)	< 0.0001
Missing	4 (6.9)	22 (10.0)		
CK8/18				
Negative	29 (50.0)	12 (5.4)	23.27 (9.98, 54.21)	< 0.0001
Positive	22 (37.9)	189 (85.5)	1.0(reference)	
Missing	7 (12.1)	20 (9.1)		
CK14				
Negative	38 (65.5)	176 (79.6)	1.0(reference)	
Positive	13 (22.4)	22 (10.0)	2.81 (1.27, 6.22)	0.0108
Missing	7 (12.1)	23 (10.4)		
EGFR				
Negative	41 (70.7)	181 (81.8)	1.0(reference)	
Positive	15 (25.9)	20 (9.1)	3.65 (1.69, 7.90)	0.001

	BRCA1 Mutation Carriers No. (%)	Non-carriers No. (%)	OR (95% CL)	<i>P</i>
Missing	2 (3.4)	20 (9.1)		

* Analysis was controlled for matching variables: age at diagnosis and ethnicity. Missing category was not included in the analysis.

Table 6

Selected models

			Model*	Sensitivity	Specificity	PPV	NPV	RSQ	BIC rank
CK8/18	FH1	FH7	syngnth	66.7	92.0	68.2	91.4	0.61	9
CK8/18	FH1	FH7	push marg	66.7	91.4	66.7	91.4	0.61	43
CK8/18	FH1	FH7		64.4	91.5	65.9	91.0	0.60	1
CK8/18	FH1	FH7	push marg	64.4	91.4	65.9	90.9	0.60	12
CK8/18	FH7	CK5	syngnth	64.4	93.1	70.7	91.0	0.60	18
CK8/18	FH7	syngnth	LI	64.4	93.1	70.7	91.0	0.58	37
CK8/18	FH7	push marg	LI	64.4	92.5	69.0	91.0	0.58	40
CK8/18	FH7	CK5		62.2	92.0	66.7	90.5	0.60	4
CK8/18	FH1	FH7	CK5	62.2	93.2	70.0	90.6	0.62	5
CK8/18	FH1	FH7	LI	62.2	93.1	70.0	90.5	0.61	6
CK8/18	FH1	FH7	EGFR	62.2	92.6	68.3	90.6	0.61	10
CK8/18	FH7	LI		62.2	93.1	70.0	90.5	0.58	11
CK8/18	FH1	FH7	CK5	62.2	93.1	70.0	90.5	0.62	20
CK8/18	FH7	CK5	LI	62.2	92.5	68.3	90.4	0.60	21
CK8/18	FH1	FH7	CK5	62.2	92.5	68.3	90.4	0.62	22
CK8/18	FH7	CK5	EGFR	62.2	92.6	68.3	90.6	0.60	23
CK8/18	FH7	CK5	push marg	62.2	92.5	68.3	90.4	0.60	24
CK8/18	FH1	FH7	CK5	62.2	93.2	70.0	90.6	0.62	25
CK8/18	FH1	FH7	CK5	62.2	92.5	68.3	90.4	0.62	26
CK8/18	FH1	FH7	syngnth	62.2	92.0	66.7	90.4	0.61	39
CK8/18	FH1	FH7	push marg	62.2	92.5	68.3	90.4	0.61	41
CK8/18	FH7	CK5	syngnth	62.2	92.5	68.3	90.4	0.60	46

* Each model contains Grade, ER and HER2 in addition to matching variables; syngnth=syncytial growth; push marg= pushing margins; LI=lymphocytic infiltrate ; FH=family history characteristic

Table 7

Best model with extended biomarkers, pathology and family history

Parameter	OR (95% CI)	P
Grade	6.66 (1.12, 39.53)	0.0368
ER	1.91(0.40, 9.02)	0.4159
HER2	2.69 (0.26, 28.28)	0.4091
CK5	2.89 (0.89, 9.36)	0.0770
CK818	16.57 (5.21, 52.71)	<0.0001
FH1	3.39 (1.15,10.02)	0.0269
FH7	5.16 (1.58, 16.80)	0.0065

FH=family history characteristic

Table 8

Best model with extended biomarkers and pathology using sporadic controls

Parameter	OR (95% CI)	<i>P</i>
Grade	6.53 (1.62, 26.30)	0.0083
ER	2.13 (0.60, 7.59)	0.2439
HER2	1.67 (0.20, 14.02)	0.6367
CK5	2.53 (0.88, 7.28)	0.0849
CK818	30.34 (9.79, 94.04)	<0.0001