Pathological Features and *BRCA1* Mutation Screening in Premenopausal Breast Cancer Patients[1]

Jenny Chang,[2] Susan G. Hilsenbeck, Jen Hwei Sng, John Wong, and G. C. Ragu

Breast Center, Baylor College of Medicine, Houston Texas 77030, and Departments of Medical Oncology and Pathology, National University of Singapore, Singapore 0511

ABSTRACT

Purpose: **Risk calculations for carrying *BRCA1/BRCA2* mutations are based on family history and the age of onset of cancers. However, women may carry these deleterious mutations without a strong family history. Additional criteria for risk estimation would be of value. It has been recently established that *BRCA1*-associated breast cancers are associated with poor tumor differentiation (TD3) and estrogen receptor (ER) negativity. The aim of this study is to determine whether morphological features of breast cancers in premenopausal patients (age < 45 years) could determine additional women who may benefit from *BRCA1* screening.**

Experimental Design: **In a prospective, systematic study of 76 consecutive breast cancer patients (age < 45 years), genomic DNA was obtained from peripheral blood, and eight mutations in *BRCA1* (10.5%) were found. Archival paraffin-embedded breast cancer specimens were then analyzed for tumor differentiation and ER status.**

Results: **In patients < 45 years of age, 25% (6 of 24) of ER-negative and TD3 breast cancers were found to harbor mutations in *BRCA1*. Only 5.6% (2 of 36) of *BRCA1*-associated breast cancers did not have this morphological profile, compared with 94.4% (34 of 36) patients without *BRCA1* mutations, giving an odds ratio of 5.67 (95% confidence interval, 1.04–32; $P = 0.05$). Finally, only one patient with *BRCA1* mutations had a significant family history.**

Conclusions: **In patients with early-onset breast cancer, the use of morphological criteria provides an additional strategy to determine those patients who might benefit from genetic testing.**

INTRODUCTION

Hereditary breast cancers with genomic mutations in *BRCA1* and *BRCA2* account for less than 10% of all breast cancers (1, 2). Asymptomatic carriers of these mutations are at increased risk of bilateral breast cancer, ovarian cancer, and other cancers, and specific interventions with respect to lifestyle changes, multimodality screening, prophylactic surgery, and chemoprevention with pharmacological agents are proposed (3). In addition, the locoregional and systemic management of breast cancer patients with these mutations may differ from that of patients with sporadic cancers, with some women choosing bilateral mastectomy and reconstruction instead of breast conservation, together with an increased vigilance or prophylactic oophorectomy for her increased ovarian cancer risk (3).

At present, the indications for performing genomic mutational analysis are based on a significant family history of breast and/or ovarian cancer, together with the age of onset of these cancers (4, 5). Because of the low prevalence of these mutations even in early-onset breast cancer patients, general population-based screening for these mutations has not been recommended (6). The estimated population-based risk of alterations in *BRCA1* is less than 10% in patients < 40 years old (7).

Because the knowledge of *BRCA1* status may impact management, the effectiveness of mutational screening could be improved by the selection of appropriate patients based on additional criteria other than family history to detect more women with genomic mutations. It has recently been published that *BRCA1*-associated breast cancers have distinct morphological features. Most studies report lower hormone receptor status, poor TD[3] (TD3), more frequent aneuploidy, higher proliferation rate, and higher frequency of p53 mutations for *BRCA1*-associated breast cancers (8–10). In particular, ER negativity and poor TD (TD3) were found on multivariate analysis to have high predictive value for ascertaining *BRCA1* status (11). In one report, approximately 30% of patients < 35 years old who had ER-negative and poorly differentiated breast cancers were found to have alterations in *BRCA1* (12). The aim of this study is to extend this strategy to determine whether morphological features of breast cancers in premenopausal breast cancer patients < 45 years of age could determine those who would benefit from mutational analysis of *BRCA1*. In this context, we analyzed 76 consecutive patients with breast cancer onset at <45 years of age for *BRCA1* mutations, and we used morphological criteria (ER status and TD) to determine whether these parameters had predictive value in ascertaining *BRCA1* mutational status.

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[2] To whom requests for reprints should be addressed, at Breast Center, Baylor College of Medicine, 6550 Fannin, Suite 701, Houston, TX 77030. Phone: (713) 798-1655; Fax: (713) 798-8884; E-mail: jcchang@breastcenter.tmc.edu.

[3] The abbreviations used are: TD, tumor differentiation; ER, estrogen receptor; nt, nucleotide(s).

Table 1 The clinical characteristics, family histories, and mutational analysis of the *BRCA1* gene in 76 patients with breast cancer diagnosis before age 45 years

Patient	Age (yrs)	ER status	TD status	Exon/codon	Nucleotide change	Family history of	
						Breast (yr)	Ovarian
1	35	ER−	TD2	11A/464	1510del C, ter 474	None	None
2	37	ER−	TD3	11A/468	1523delG, ter 475	None	None
3	34	ER+	TD1	11B/770	2430insC, ter 776	None	None
4	39	ER−	TD3	11C/1088	3378/81delG, ter 1108	None	None
5	39	ER−	TD3	11B/871	2732insT, ter 902	MA (39)[a]	None
6	33	ER−	TD3	13/1443	4446C →T, ter 1443	M (60) MGM (50) MA (46)	None
7	39	ER−	TD3	11C/1183	3667A →G,LK1183AR 3240T →A,SS1040T	None	None
8	38	ER−	TD3	9/119	690G →A, Va191I	None	None

[a] MA, maternal aunt; M, mother; MGM, maternal grandmother.

PATIENTS AND METHODS

Patient Selection. A prospective study of the prevalence of *BRCA1* mutations was conducted in Singaporean Chinese patients presenting to a single institution (National University Hospital, Singapore, Singapore). Consecutive, unrelated patients were eligible for *BRCA1* testing if they had premenopausal breast cancer and/or at least one affected first-degree relative with either breast or ovarian cancer. This study was approved by the institutional ethical committee approval, signed written informed consent was obtained from all patients, and the results have been published previously (13). In this systematic study of BRCA1 mutations, 76 consecutive patients with breast cancer diagnosed before the age of 45 years were tested for *BRCA1* mutations. Genomic mutations in *BRCA1* were determined in 10.5% of patients (8 of 76 patients). In addition, 12 polymorphisms of unlikely significance were also detected. A detailed family pedigree was obtained by direct interviews at the time of consent.

Laboratory Methods. The archival tumor specimens from these patients were analyzed for grade of TD and ER immunohistochemistry. Standard methods for immunohistochemistry have been described in detail elsewhere. Briefly, for ER staining, the slides were incubated with ER antibody (Abbott ER-ICA monoclonal antibody; 1:40 dilution), and then secondary antibody (biotinylated antirat IgG) was applied. After rinsing, the slides were incubated with streptavidin horseradish peroxidase (1:100) for 30 min, rinsed with PBS, exposed to diaminobenzidine tetrahydrochloride chromogen for 10 min, rinsed with autobuffer and PBS, counterstained with 1% methyl green, rinsed with deionized water, and then mounted.

Genomic DNA was obtained from whole blood for *BRCA1* mutational analysis. Briefly, single-strand conformational polymorphism and DNA sequence analysis was performed using primer pairs that span the BRCA1 coding region and intron-exon boundaries for all coding exons except for exon 11. PCR amplification was carried out with 50 ng of genomic DNA, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 200 μM deoxynucleotide triphosphates (Promega), 0.8 μM each primer, and 0.75 unit of Taq polymerase (Promega). Amplification was performed for 35 cycles in a Perkin-Elmer 480 DNA thermal cycler. The PCR product was then subjected to electrophoresis and sequenced. If mutations were detected, a second single-strand conformational polymorphism sequencing analysis was performed for confirmation. For exon 11, the protein transcription translation assay analysis was used to detect truncating mutations (14, 15). Exon 11 was amplified in three overlapping fragments (16), and PCR was performed (50-μl volumes containing 1× PCR reaction buffer, 0.2 μM deoxynucleotide triphosphate, 0.8 μM primer, 0.75 unit of Taq polymerase, and 50 ng of template DNA). The reactions were amplified for 35 cycles. The PCR products were then purified, and the mRNA was translated into radiolabeled peptides using the TnT T7 Coupled Reticulocyte Lysate System or Wheat Germ System (Promega). If truncations were detected, DNA sequencing was performed as described above.

Statistical Methods. Statistical analysis was conducted using Fisher's exact two-tailed test for comparisons. The odds ratio was determined by the Mantel-Haenszel inference test.

RESULTS

From the 76 cases of premenopausal breast cancer with known *BRCA1* status, archival tissue was available for 70 women, whereas both tumor grade and ER status were available for 60 patients. Of these, 55.3% (36 of 65 cases) were TD3, and 56.2% (36 of 64 cases) were ER negative. In these patients, eight mutations (10.5%) were detected. In the 22 patients diagnosed with cancer at <35 years of age, three mutations (13.6%) were found. Of these eight *BRCA1* carriers, only one patient had a significant family history. This patient, who had an affected mother and grandmother, had a nonsense mutation with a C to T substitution at nt 4446, resulting in a termination codon. Other mutations included an insertion of T at nt 2732, resulting in chain termination at codon 902 (Table 1). This particular mutation has been described previously in 14 different families including 1 family with 11 breast and ovarian cancers (17). Missense mutations or unclassified variants were detected in two patients (K1183R/S1040T and V191I, respectively). An A→G mutation at nt 3667 (K1183R) was detected in two patients. One of these patients also had a disease-causing mutation (3378/3381delG). The other patient had an additional missense mutation at nt 3240 (T→A, S1040T). The V191I missense mutation has now been described in three Chinese

Table 2 Distribution of morphology, *BRCA1* mutation status in breast cancer patients <45 years of age

Genetic status	ER− & TD3 (%)	Other pathology (%)	Total	*P*	OR (95% CI)[a]
BRCA1 mutations	6 (25)	2 (5.6)	8		
No *BRCA1* mutation	18 (75)	34 (94.4)	52		
Total	24	36	60	0.05	5.7 (1.0–32.0)

[a] OR, odds ratio; CI, confidence interval.

women in the literature (13). The issue of whether or not missense mutations cause disease remains problematic. Substitutions that occur in highly conserved regions like the ring finger domain show segregation with disease in high-risk families, and those that are not commonly observed in controls are typically classified as pathogenic. The missense mutation V191I is located close to the ring finger domain and is highly conserved as compared with murine *BRCA1* (18).

In this population-based, prospective, systematic study of premenopausal breast cancer patients < 45 years of age, 25% (6 of 24) of tumors that were ER negative and had poor TD (TD3) were found to harbor mutations in *BRCA1*. This is in comparison to 10.5% (8 of 76) of women < 45 years of age, independent of pathological morphology. Only two patients with *BRCA1* mutation (5.6%) did not have ER-negative and TD3 cancers, compared with 34 non-*BRCA1*-associated cancers (34 of 36; 94.4%), giving an odds ratio of 5.7 (95% confidence interval, 1.0–32.0; $P = 0.05$; Table 2).

In this series of 76 consecutive breast cancer patients, a full family history was determined prospectively at the time of consent. Of all the patients screened for BRCA1 mutations, 11 had a family history with at least 1 affected first-degree relative with breast or ovarian cancer (13). Two of the eight *BRCA1* mutation carriers had affected relatives, and only one of these had first-degree relatives with breast cancer. The other patient had only one second-degree relative (a maternal aunt) with breast cancer diagnosed at age 39 years. Despite direct questioning, there were no cases of bilateral breast cancers or family history of ovarian cancers (Table 2).

DISCUSSION

Initial studies of germ-line mutations in *BRCA1* and *BRCA2* were based on analyses of pedigrees with multiple cases of cancers within each family (19). From these pedigrees, most calculated risk estimate models for carrying *BRCA* mutations are based on the number of affected relatives and the age of onset of cancer (20). Most population-based studies report a family history in carriers of *BRCA1* mutations (7, 21–23). In one study involving 388 patients < 40 years old, 27.7% (5 of 18) of *BRCA1* mutation cancers had one affected first-degree relative (7). In another study of 80 women with breast cancer diagnosed before the age of 35 years, 1 of 6 *BRCA1* mutation carriers had a first-degree relative with breast cancer (21). These results are consistent with our study of 76 patients, in which one patient with *BRCA1* mutation-associated breast cancer had a significant family history (13).

However, some carriers of genomic mutations may not report a significant number of affected relatives because of a lack of knowledge of family history or because the number of family members is small. Singapore practiced a two-child policy from the 1960s through the 1980s, which has limited the number of large family pedigrees. Hence, although a significant family history remains the gold standard in risk calculations, additional criteria for ascertaining at-risk women who might benefit for mutation analysis are indicated, especially in populations where the family pedigrees are small.

The management of *BRCA1*-associated breast cancer is becoming increasingly complex. The local failure rate with breast conservation and radiation may be higher in patients with *BRCA1/BRCA2* mutations (24), although this finding is still controversial. (25) The possible higher risk of ipsilateral recurrence, together with the increased risk of contralateral breast cancer, has led to the practice of bilateral mastectomy with reconstruction in breast cancer patients with *BRCA1/BRCA2* mutations (3). Moreover, the role of bilateral oophorectomy in decreasing both breast cancer and ovarian cancer risk is another feasible alternative for patients at risk of these malignancies (26). There is evidence that chemoprevention with agents like tamoxifen may decrease the risk of contralateral breast cancer by 50% in high-risk women (27, 28).

We have noted previously that breast cancers in this population tend to occur in younger women, with a higher proportion of ER-negative tumors.[4] Because mutation screening based on age alone is not feasible, additional criteria for selecting at-risk women is indicated in this population with small family pedigrees. If the criterion for screening was based on a family history (defined as one affected first-degree relative), we would have detected 1 woman of 11 patients tested and missed 7 potential *BRCA1* carriers. Using age as the sole criterion, 65 additional women would be screened to detect genomic alterations in 7 patients. Using age and morphological features, we would have screened an additional 23 women to detect 5 extra genomic alterations while missing 2 *BRCA1* carriers.

This study has shown that the risk of carrying *BRCA1* mutations in premenopausal patients < 45 years of age with ER-negative, poorly differentiated cancers is about 25%. These results are consistent with an earlier study that showed that 29% of patients < 35 years old with these morphological criteria carried mutations in *BRCA1* (12). We have extended this previous study to evaluate the predictive value of morphological features in a different population of patients with a different ethnic background. Because of the difficulty and high costs of doing large-scale population-based studies, the major flaw of this study is the small number of *BRCA1* mutation carriers and

[4] S. E. Lim, J. Wong, J. Chang, A-B. Ong, C. Chua, K-C. Lun, and W. Tan. Breast cancer in Singapore: a clinicopathologic analysis of 848 Asian women with invasive breast cancer from a single institution, submitted for publication.

the marginal statistically significant results of this study. However, the observation that morphology of the primary cancers may aid in determining additional women who might benefit from testing needs further investigation in larger studies.

In summary, this preliminary study indicates that pathological features in patients with early-onset breast cancer may be useful in determining a different subset of women who might benefit from mutation screening. If these observations are confirmed in other population-based studies, then the use of morphological criterion in premenopausal women with breast cancer could serve as a useful adjunct to family history in selecting additional women who could benefit from mutational analysis. As recommendations regarding the medical management of patients with these hereditary cancers change over the next few years, additional models for predicting germ-line mutation status will become increasingly important.

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