Pathological Features and \textit{BRCA1} Mutation Screening in Premenopausal Breast Cancer Patients\textsuperscript{1}

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\textbf{ABSTRACT}

\textit{Purpose:} Risk calculations for carrying \textit{BRCA1}/\textit{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 \textit{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 \textit{BRCA1} screening.

\textit{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 \textit{BRCA1} (10.5\%) were found. Archival paraffin-embedded breast cancer specimens were then analyzed for tumor differentiation and ER status.

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

\textit{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.

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1 Supported in part by National Medical Research Council Grants RP6600070 and RP3902352, Singapore Cancer Society Grant GR6672, and the Academic Research Fund.

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\textsuperscript{3}The abbreviations used are: TD, tumor differentiation; ER, estrogen receptor; nt, nucleotide(s).
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 autobody 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 MgCl2, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 200 μM deoxyribonucleotide 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 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 breast 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>
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<tr>
<th>Table 1</th>
<th>The clinical characteristics, family histories, and mutational analysis of the BRCA1 gene in 76 patients with breast cancer diagnosis before age 45 years</th>
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</thead>
<tbody>
<tr>
<td>Patient</td>
<td>Age (yrs)</td>
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<td>---------</td>
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<tr>
<td>1</td>
<td>35</td>
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<td>2</td>
<td>37</td>
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<td>3</td>
<td>34</td>
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<td>6</td>
<td>33</td>
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<td>7</td>
<td>39</td>
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<td>8</td>
<td>38</td>
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*MA, maternal aunt; M, mother; MGM, maternal grandmother.*
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, systemic 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. 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 small number of BRCA1 mutation status in breast cancer patients <45 years of age

<table>
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<tr>
<th>Genetic status</th>
<th>ER− &amp; TD3 (%)</th>
<th>Other pathology (%)</th>
<th>Total</th>
<th>P</th>
<th>OR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1 mutations</td>
<td>6 (25)</td>
<td>2 (5.6)</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No BRCA1 mutation</td>
<td>18 (75)</td>
<td>34 (94.4)</td>
<td>52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>36</td>
<td>60</td>
<td>0.05</td>
<td>5.7 (1.0–32.0)</td>
</tr>
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* OR, odds ratio; CI, confidence interval.

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.

REFERENCES


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