Triple-negative breast cancer and PTEN (phosphatase and tensin homologue) loss are predictors of BRCA1 germline mutations in women with early-onset and familial breast cancer, but not in women with isolated late-onset breast cancer

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Abstract

Introduction: Given that breast cancers in germline BRCA1 carriers are predominantly estrogen-negative and triple-negative, it has been suggested that women diagnosed with triple-negative breast cancer (TNBC) younger than 50 years should be offered BRCA1 testing, regardless of family cancer characteristics. However, the predictive value of triple-negative breast cancer, when taken in the context of personal and family cancer characteristics, is unknown. The aim of this study was to determine whether TNBC is a predictor of germline BRCA1 mutations, in the context of multiple predictive factors.

Methods: Germline mutations in BRCA1 and BRCA2 were analyzed by Sanger sequencing and multiple ligation-dependent probe amplification (MLPA) analysis in 431 women from the Malaysian Breast Cancer Genetic Study, including 110 women with TNBC. Logistic regression was used to identify and to estimate the predictive strength of major determinants. Estrogen receptor (ER) and phosphatase and tensin homologue (PTEN) status were assessed and included in a modified Manchester scoring method.

Results: Our study in an Asian series of TNBC patients demonstrated that 27 (24.5%) of 110 patients have germline mutations in BRCA1 (23 of 110) and BRCA2 (four of 110). We found that among women diagnosed with breast cancer aged 36 to 50 years but with no family history of breast or ovarian cancer, the prevalence of BRCA1 and BRCA2 mutations was similar in TNBC (8.5%) and non-TNBC patients (6.7%). By contrast, in women diagnosed with breast cancer, younger than 35 years, with no family history of these cancers, and in women with a family history of breast cancer, the prevalence of mutations was higher in TNBC compared with non-TNBC (28.0% and 9.9%; P = 0.045; and 42.1% and 14.2%; P < 0.0001, respectively). Finally, we found that incorporation of estrogen-receptor and TNBC status improves the sensitivity of the Manchester Scoring method (42.9% to 64.3%), and furthermore, incorporation of PTEN status further improves sensitivity (42.9% to 85.7%).

Conclusions: We found that TNBC is an important criterion for highlighting women who may benefit from genetic testing, but that this may be most useful for women with early-onset breast cancer (35 years or younger) or with a family history of cancers. Furthermore, addition of TNBC and PTEN status improves the sensitivity of the Manchester scoring method and may be particularly important in the Asian context, where risk-assessment models underestimate the number of mutation carriers.

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Introduction
Discovery of the breast cancer-predisposition genes BRCA1 and BRCA2 has enabled us to identify carriers accurately, to target the reduction of risk of breast and ovarian cancers in carriers, and to develop a new generation of targeted therapies (PARP inhibitors) [1]. However, given that deleterious mutations in these genes account for only 1% to 4% of all breast cancer cases across different populations [2] and that genetic testing and genetic counseling have hitherto been relatively expensive, genetic testing for BRCA1 and BRCA2 has typically been offered only in clinical genetics settings to women who have early-onset breast cancer, and/or to individuals with significant family history of breast and ovarian, or other BRCA-related cancers.

Recently, it was suggested that screening women with early-onset triple-negative breast cancer (TNBC) may be a cost-effective method with which to identify BRCA1 mutation carriers in Caucasian women [3-5]. This is because, in the majority of BRCA1 carriers, breast tumors have distinctive morphologic features and immunohistochemical phenotypes characteristic of basal-like breast cancers, including negative expression of the estrogen receptor, high expression of basal markers, such as basal cytokeratins CK5/6 and CK14, and loss of tumor-suppressor PTEN [6-8]. Moreover, molecular gene-expression profiling of BRCA1 tumors showed that the tumors have significant similarities with the basal-like subtype of breast cancer [9]. Up to 50% of women diagnosed with breast cancer, younger than 50 years, and women who have a family cancer history may have mutations in BRCA1 or BRCA2 [10]. However, it is notable that although more than 10% women in whom an isolated TNBC develops at younger than 40 years old may have a mutation in BRCA1 [3-5], insufficient evidence exists for those aged 41 to 50 years, with no family history of breast or ovarian cancer [11].

The purpose of this study was to determine whether TNBC is an independent criterion for stratifying women with an increased risk of having a BRCA1 mutation and to determine whether the addition of immunohistologic features of basal-like breast cancers helps to define a subset of women who are likely to have germline mutations in BRCA1.

Materials and methods
MyBrCa Breast cancer cohort
The recruitment of breast cancer patients into the Malaysian Breast Cancer Genetic Study (MyBrCa) started in January 2003 at the University Malaya Medical Centre in Kuala Lumpur. All were histopathology-proven breast carcinomas. Histomorphologic and biomarker parameters were retrieved from the histopathology reports. Blood, demographic, and family-history data were collected from breast cancer patients who consented to participate in this study. This study was approved by the ethics committee of University Malaya Medical Centre.

Analysis of BRCA1 and BRCA2
From January 2003 to February 2012, 1,454 breast cancer patients were recruited into the MyBrCa study. Germline DNA samples were screened for BRCA1 and BRCA2 mutations for all women with (a) early-onset breast cancer (≤35 years of age) (35 with and 96 without family history of breast and ovarian cancer); (b) family history of breast or ovarian cancer in first- and second-degree relatives (193 women); or (c) isolated triple-negative breast cancer diagnosed at between 36 and 50 years old in the absence of family history (47 women). In addition, of the 432 women who were diagnosed aged 36 to 50 years with non-TNBC, 60 women with the highest risk were analyzed (bilateral breast cancer, breast and ovarian cancer in the index patient, family history of breast and ovarian cancer in third-degree or isolated breast cancer (≤45 years of age)). Mutation detection for germline BRCA1 and BRCA2 mutations was conducted by using direct DNA sequencing and multiple ligation-dependent probe amplification (MLPA), as previously described [12,13].

Pathologic analysis
For this cohort, patients were classified as having TNBC when we found <10% ER, <10% PR, and 0 or ≤2+ HER2 staining with immunohistochemistry. HER2 was not routinely tested for in all patients prior to 2006, and therefore, in 259 patients, HER2 status was unavailable. Of the 110 women for whom germline BRCA1 and BRCA2 mutations were analyzed, adequate archived paraffinized invasive tumor tissue for evaluation was available from 32 index patients and two relatives. All cases were tested with immunohistochemistry for cytokeratin 5/6 (D5/16B4; Dako Ltd.), cytokeratin 14 (LL02; Novocastra Labs), and PTEN (6H2.1; Dako). Cytokeratin 5/6 or cytokeratin 14 was defined as positive if >10% of invasive tumor cells showed cytoplasmic staining [14], and PTEN was defined as negative if PTEN staining was undetectable in tumor cells in contrast to adjacent normal stromal cells [6-8]. Description of other pathologic features was conducted as previously described [14].

Statistical methods
Unconditional multiple linear logistic regression was used to model the probability that the proband was a mutation carrier as a function of her personal and family history and age at diagnosis, as described in [15].

Manchester score analysis
Manchester score was calculated as previously described [16]. In brief, this system assigns scores depending on the type of cancer and age at diagnosis and developed such
that a score of 15 was equivalent to a 10% chance of identifying a BRCA1 or BRCA2 mutation. The modified method [17-19] includes upward adjustments for TNBC (+4), estrogen-negative (+1), and high-grade invasive cancers (+2), and downward adjustments for human epidermal growth factor receptor 2 (HER2)-positive (-4) or estrogen-receptor positive (-1), lobular (-2), and low-grade noninvasive cancers (-2). For individuals in whom tumor tissue was available for PTEN staining, upward (+1) and downward (-1) adjustments were made for the absence or presence of PTEN staining, respectively.

Results
Prevalence of BRCA1 and BRCA2 germline mutations is higher among subsets of women with TNBC compared with non-TNBC
Of the 1,454 breast cancer patients in the MyBrCa Study, 177 (12.2%) had TNBC based on pathology reports. Of these 177 women, 50 older than 50 years developed breast cancer and did not have any family history of breast or ovarian cancer in first- or second-degree relatives and were therefore excluded from the study. Of the remaining 127 women, 63 had already been analyzed because of family history of either breast and/or ovarian cancer (38 individuals, 13 BRCA1 and three BRCA2 carriers, mutation prevalence, 42.1%) or early-onset breast cancer (25 individuals, seven BRCA1 and no BRCA2 carriers; mutation prevalence, 28.0%). Of the remaining 64 women diagnosed at ages 36 through 50 years, but with no family history of breast or ovarian cancer, 47 women were screened for germline mutations in BRCA1 and BRCA2 genes, and three BRCA1 and one BRCA2 carriers were identified (mutation prevalence, 8.5%). Overall, of the 110 women who developed TNBC and were analyzed (63 with and 47 without family history of breast and ovarian cancers), 23 BRCA1 and 4 BRCA2 carriers were identified, giving a mutation prevalence of 24.5% (Table 1).

In total, 321 women with non-TNBC were analyzed. This included all women with a family history of breast and/or ovarian cancer (190 individuals, 10 BRCA1 and 17 BRCA2 carriers, mutation prevalence 14.2%) and women diagnosed at 35 years or younger with no family history of breast and ovarian cancer (71 individuals, three BRCA1 and four BRCA2 carriers; mutation prevalence, 9.9% (Table 2)). In addition, of the 432 women who were diagnosed aged 36 through 50 years with non-TNBC, 60 women with the highest risk were analyzed (bilateral breast cancer, breast and ovarian cancer in the index patient, family history of breast and ovarian cancer in third degree or isolated breast cancer (aged 45 years or younger)). Of these 60 women, one BRCA1 and three BRCA2 carriers were found [mutation prevalence, 6.7%]. Overall, of the 321 non-TNBC women analyzed, 14 BRCA1 and 24 BRCA2 carriers were identified, giving a mutation prevalence of 11.8%.

We compared the prevalence of BRCA1 and BRCA2 mutations in the women who developed TNBC and non-TNBC. Of the women with low familial risk of breast and ovarian cancer (diagnosed 36 to 50 with no family history of breast or ovarian cancer), 6.4% and 1.7% of women with TNBC and non-TNBC were found to be BRCA1 carriers, respectively, whereas 2.1% and 5.0% were BRCA2 carriers. Overall, 8.5% of women with TNBC and 6.7% of women with non-TNBC were found to have germline BRCA1 or BRCA2 mutations. This suggests that, regardless of the TNBC status, in the absence of family history of breast or ovarian cancer in this age group, a low (<10%) probability exists of having a BRCA1 or BRCA2 mutation.

By contrast, in two other groups of women, a significantly higher prevalence of BRCA1 mutations was found in women who developed TNBC versus non-TNBC. First, of the women in whom breast cancer developed at 35 years or younger, 28.0% of women with TNBC were BRCA1 or BRCA2 carriers compared with only 9.9% among those who developed non-TNBC (P = 0.045). Notably, all 28.0% of women diagnosed with TNBC at younger than 35 years were BRCA1 carriers, compared with 4.2% BRCA1 and 5.6% BRCA2 among women diagnosed with non-TNBC.

Second, of women who had a family history of breast and/or ovarian cancer, 42.1% of women with TNBC were BRCA1 or BRCA2 carriers compared with 14.2% of those with non-TNBC (P = < 0.0001). This is largely because of a difference in prevalence of BRCA1 mutations in TNBC compared with non-TNBC (34.2% compared with 5.3%; P = < 0.0001), as no significant difference occurred in the prevalence of BRCA2 in both subsets of women (7.9% compared with 8.9%; P = 1.00). Notably, no significant difference was noted in average age at onset of breast cancer in the index patient, or the mean number of first-degree relatives of women in whom TNBC developed compared with the non-TNBC (seven relatives), but the mean number of affected relatives in the non-TNBC group was higher than that in the TNBC group (0.8 compared with 0.6; P = 0.003). This suggests that the higher prevalence of BRCA1 mutations in the women in whom TNBC developed compared with women in whom non-TNBC developed is not due to a difference in the age at onset or strength of family history. Taken together, the results suggest that early onset and familial breast cancer patients in whom TNBC develops are more likely to have mutations in the BRCA1 genes compared with those in whom non-TNBC develops (24.5% versus 11.8%; P = 0.001).

In addition, in this cohort, a marked difference appears in prevalence of BRCA1 and BRCA2 in the
TNBC and non-TNBC patients. TNBC patients are more likely to have \textit{BRCA1} than \textit{BRCA2} mutations (20.9\% and 4.4\%; \(p < 0.0001\)), whereas no statistically significant difference is present in \textit{BRCA1} and \textit{BRCA2} mutations in non-TNBC patients (3.6\% and 7.5\%; \(P = 0.158\)).

Logistic regression analyses

Given the possible confounding between the age of diagnosis, subtype of breast cancer, and family history of cancer, it has been demonstrated that the predictive effects of factors should be based on an analysis that takes into account all factors simultaneously [15]. By using binary logistic regression analysis, we found that age at diagnosis of breast cancer, having any family history of breast or ovarian cancer, and having triple-negative breast cancer were associated with a 2.6-fold (confidence interval (CI), 1.4 to 4.8; \(P < 0.003\)), 3.5-fold (CI, 1.9 to 6.8; \(P < 0.0001\)), and 3.5-fold (CI, 1.91 to 6.3; \(P < 0.0001\)) increase in risk of being a \textit{BRCA1} or \textit{BRCA2} carrier, respectively. No evidence of interaction effects between these factors was seen. With multiple linear logistic regression analysis, the strongest predictors of mutation status were breast cancer in the proband if age at diagnosis was younger than 35 years (\(P = 0.043\)), with bilateral breast cancer (\(P = 0.025\)), with triple-negative breast cancer (\(P < 0.0001\)), with breast cancer in a first-degree relative if age at diagnosis was younger than 60 years (\(P < 0.01\)), or ovarian cancer was present in a first- or second-degree relative (\(P < 0.025\)) (Table 3).

The probability that a proband with a given set of personal and family characteristics is a mutation carrier can be estimated from the model fit shown in Table 4. Starting with the log odds score of \(\theta\) (the baseline coefficient), add the respective regression coefficient (\(\beta\)) for each personal characteristic and the respective regression for each family characteristic multiplied by the number of affected relatives. In this model, an overall score of -1.5 is equivalent to a 15\% probability of being a \textit{BRCA1} or \textit{BRCA2} carrier. Notably, the overall scores

| Table 1 Characteristics of women tested for germline \textit{BRCA1} and \textit{BRCA2} mutations |
|-----------------|-----|-----|-----|-----|
| Characteristics                           | Total | \textit{BRCA1} | \textit{BRCA2} | \textit{BRCA1} and -2 |
|                                              | \(n\) | (%)  | \(n\) | (%)  | \(n\) | (%)  | \(n\) | (%)  |
| Female breast, age at index diagnosis, years |      |      |      |      |      |      |      |      |
| \(\leq 30\)                                    | 50   | 11.6 | 8    | 16.0 | 2    | 4.0  | 10   | 20.0 |
| \(31-40\)                                     | 164  | 38.1 | 17   | 10.4 | 13   | 7.9  | 30   | 18.3 |
| \(41-50\)                                     | 144  | 33.4 | 8    | 5.6  | 8    | 5.6  | 16   | 11.1 |
| \(>50\)                                       | 73   | 16.9 | 4    | 5.5  | 5    | 6.8  | 9    | 12.3 |
| Breast or ovarian cancers in family (first and second degree only) |      |      |      |      |      |      |      |      |
| Female breast                                | 217  | 50.3 | 21   | 9.7  | 20   | 9.2  | 41   | 18.9 |
| Female ovary                                 | 19   | 4.4  | 6    | 31.6 | 3    | 15.8 | 9    | 47.4 |
| Manchester score                             |      |      |      |      |      |      |      |      |
| \(\leq 10\)                                   | 205  | 47.6 | 5    | 2.4  | 6    | 2.9  | 11   | 5.4  |
| \(11-17\)                                     | 146  | 33.9 | 16   | 11.0 | 10   | 6.8  | 26   | 17.8 |
| \(>18\)                                      | 80   | 18.6 | 16   | 20.0 | 12   | 15.0 | 28   | 35.0 |
| Ancestry                                     |      |      |      |      |      |      |      |      |
| Malay                                        | 115  | 26.7 | 8    | 7.0  | 9    | 7.8  | 17   | 14.8 |
| Chinese                                      | 248  | 57.5 | 15   | 6.0  | 16   | 6.5  | 31   | 12.5 |
| Indian                                       | 59   | 13.7 | 12   | 20.3 | 3    | 5.1  | 15   | 25.4 |
| Others                                       | 9    | 2.1  | 2    | 22.2 | 0    | 0.0  | 2    | 22.2 |
| Referral characteristic                      |      |      |      |      |      |      |      |      |
| Early onset \(\leq 35\) years, regardless of family history | 131  | 30.4 | 17   | 13.0 | 8    | 6.1  | 25   | 19.1 |
| Two cases of breast cancer, one <50 years    | 126  | 29.2 | 10   | 7.9  | 11   | 8.7  | 21   | 16.7 |
| Three cases of breast or ovarian cancer      | 76   | 17.6 | 13   | 17.1 | 12   | 15.8 | 25   | 32.9 |
| One case of bilateral breast cancer <50 years, in index or first- and second-degree relative | 39   | 9.0  | 10   | 25.6 | 3    | 7.7  | 13   | 33.3 |
| One case of breast and ovarian cancer in same individual in index or first- and second-degree relative | 8    | 1.9  | 3    | 37.5 | 1    | 12.5 | 4    | 50.0 |
| Triple-negative breast cancer, <50 years     | 98   | 22.7 | 20   | 20.4 | 3    | 3.1  | 23   | 23.5 |

In total, 431 breast cancer patients were analyzed for germline mutations in \textit{BRCA1} and \textit{BRCA2} by DNA sequencing and multiple ligation-dependent probe amplification (MLPA) analysis. Table 1 shows the distribution of women according to their age at diagnosis, family history of breast and ovarian cancer in first- and second-degree relatives, Manchester score and self-declared ethnicity, and the prevalence of \textit{BRCA1} and \textit{BRCA2} mutations in each category.
for women with isolated TNBC who were diagnosed aged ≤35 years old, 36 to 39 years old, or 40 to 49 years old, are -1.1, -1.6, and -2.1, respectively, which correlates with a 29%, 14%, and 9% probability of being a BRCA1 or BRCA2 carrier. The overall scores for women with isolated non-TNBC who were diagnosed at younger than 35 years, 36 to 39 years old, and 40 to 49 years old, are -2.4, -2.9, and -3.4, respectively, which correlates with a 9%, 4%, and 4% probability of being a BRCA1 or BRCA2 carrier. These results show that women with isolated TNBC have a higher probability of being carriers compared with women with isolated non-TNBC and that, of the women with isolated breast cancers, only women with isolated TNBC diagnosed at younger than 40 years have a greater than 10% probability of having germline mutations in BRCA1 or BRCA2.

| Table 2 Characteristics of women tested for germline BRCA1 and BRCA2 mutations |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Characteristics                              | Triple negative                               | Not triple negative                           |
|                                               | n = 110                                       | n = 321                                       | P value |
|                                               | n | BRCA1 | BRCA2 | Total (%) | n | BRCA1 | BRCA2 | Total (%) |
| With family history                          |   |       |       |           |   |       |       |           |
| Early onset, ≤35 years old                   | 6 | 4     | 0     | 4 (66.7)  | 29 | 3     | 4     | 7 (24.1)  | 0.063a   |
| >35 years old                                | 32| 9     | 3     | 12 (37.5)| 161| 7     | 13    | 20 (12.4) | 0.0005b  |
| Overall                                      | 38| 13    | 3     | 16 (42.1)| 190| 10    | 17    | 27 (14.2) | <0.0001b |
| Without family history                       |   |       |       |           |   |       |       |           |
| Early onset, ≤35 years old                   | 25| 7     | 0     | 7 (28.0)  | 71 | 3     | 4     | 7 (9.9)   | 0.045a   |
| 36 to 50 years old                           | 47| 3     | 1     | 4 (8.5)   | 60 | 1     | 3     | 4 (6.7)   | 1.000a   |
| Overall                                      | 72| 10    | 1     | 11 (15.3)| 131| 4     | 7     | 11 (8.4)  | 0.131b   |
| All, regardless of family history or age     | 110| 23   | 4     | 27 (24.5)| 321| 14    | 24    | 38 (11.8) | 0.001b   |
| Mean age at diagnosis (years)                | 40.6| 42.0 |       |           |   |       |       |           |
| Mean number of first-degree relatives        | 7.2| 7.1   |       |           |   |       |       |           |
| Mean number of affected (breast or ovarian) relatives, first or second degree | 0.6| 0.8 |       |           |   |       |       |           |
| Prevalence of BRCA1 mutations                | 20.9%|       |       |           |   |       |       |           |
| Prevalence of BRCA2 mutations                | 4.4%|       |       |           |   |       |       |           |
|                                               | <0.0001b |       |       |           |   |       |       |           |

*Fisher Exact test. **χ² test. The prevalence of germline mutations in BRCA1 and BRCA2 (combined) in the 431 breast cancer patients analyzed, in whom 110 developed triple-negative breast cancer, and 321 did not. Family history includes presence of breast or ovarian cancer in first- and second-degree relatives, bilateral breast cancer in the index patient or relative, or breast and ovarian cancer in the same individual in the index patient or relative. P values were calculated by using Fisher Exact or χ² test, and mean values were calculated by using independent t test.

| Table 3 Logistic regression coefficients (β), standard errors, and nominal significance levels of potentially predictive factors for BRCA1 or BRCA2 carriers |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Characteristic                               | β                | Standard error | P     |
| Baseline                                     | -5.169           | 1.388           | 0.000 |
| Proband breast cancer diagnosis ≤35 years    | 2.791            | 1.379           | 0.043 |
| Proband breast cancer diagnosis 36-39 years  | 2.248            | 1.365           | 0.100 |
| Proband breast cancer diagnosis 40-49 years  | 1.763            | 1.355           | 0.193 |
| Proband breast cancer diagnosis 50-59 years  | 1.065            | 1.354           | 0.431 |
| Proband bilateral breast cancer at any age    | 1.036            | 0.462           | 0.025 |
| Proband ovarian cancer at any age             | 0.238            | 1.469           | 0.871 |
| Proband triple-negative breast cancer at any age | 1.323           | 0.331           | 0.000 |
| For each relative with cancer:               |                  |                  |       |
| First degree, breast cancer diagnosis ≤39 years | 0.950            | 0.369           | 0.010 |
| First degree, breast cancer diagnosis 40-49 years | 1.450            | 0.386           | 0.000 |
| First degree, breast cancer diagnosis 50-59 years | 1.445            | 0.450           | 0.001 |
| First degree, breast cancer diagnosis 60+ years | 0.207            | 0.646           | 0.749 |
| First degree, ovarian cancer at any age       | 2.556            | 0.769           | 0.001 |
| Second degree, breast cancer diagnosis at any age | 0.359            | 0.272           | 0.187 |
| Second degree, ovarian cancer diagnosis at any age | 1.861            | 0.832           | 0.025 |

The multiple linear logistic regression analysis of the probability of breast cancer patients having a germline mutation in the BRCA1 or BRCA2 gene on the basis of age at diagnosis and family history of breast or ovarian cancers.
Table 4 Overall number of carriers for each predicted range of probability of being a BRCA1 or a BRCA2 carrier

| Overall score | Number of women | Number of carriers | Percentage of carriers |
|---------------|-----------------|--------------------|------------------------|
| ≤3.5          | 25              | 0                  | 0                      |
| -3.0 to -3.5  | 49              | 2                  | 4                      |
| -2.5 to -3.0  | 47              | 2                  | 4                      |
| -2.0 to -2.5  | 144             | 13                 | 9                      |
| -1.5 to -2.0  | 58              | 8                  | 14                     |
| -1.0 to -1.5  | 59              | 17                 | 29                     |
| 0 to -1.0     | 33              | 11                 | 33                     |
| 0 to 1        | 16              | 12                 | 75                     |

The number of carriers for each predicted range of probability calculated through incorporation of logistic regression coefficients generated in Table 3.

Pathologic features of BRCA1 and non-BRCA1 triple-negative breast cancer

We analyzed the pathology reports of BRCA1 carriers and compared them with those in BRCA2 and non-BRCA carriers. Of the 31 BRCA1 index carriers and affected relatives where pathology reports were available, 26 (83.9%) developed TNBC. By contrast, of the 270 BRCA2 carriers and non-BRCA carriers, 88 (32.6%) developed TNBC.

Of the 110 TNBC patients included in this study and for whom germline status of BRCA1 and BRCA2 has been characterized (see earlier), 34 had adverse invasive tumor tissue for evaluation. Slides were cut and immunostained for several markers that have been reported to be useful in defining the basal-like phenotype, including basal cytokeratins CK5/6, CK14, and PTEN. Although some higher grade were present, higher basal cytokeratin, loss of PTEN, high grade of pleomorphism, presence of pushing margins, solid sheets, necrosis, and mitosis in BRCA1 carriers compared with non-BRCA1 carriers, these differences were not statistically significant (see Additional file 1).

Inclusion of pathologic features in Manchester scores

To determine whether the addition of pathologic features can define a subset of women who are likely to have germline mutations in BRCA1 or BRCA2, we calculated the Manchester score for each individual based on the original Manchester score [16] and on the updated Manchester score where pathology was included [18]. Upward adjustments in BRCA1 mutation-prediction scores were made for grade 3 ductal cancers, estrogen receptor (ER), and triple-negative tumors, and downward adjustments in the score were made for grade 1 tumors, lobular cancer, ductal or lobular carcinoma in situ, noninvasive breast cancer, and ER/HER2 positivity, as described previously [18].

Of the 431 women in this study, 86 were excluded because pathology reports were incomplete. Without adjustment, 72 of 345 women had a Manchester score of ≥15, and this included only 12 of the 28 BRCA1 carriers (sensitivity, 42.9%; specificity, 81.1%; PPV, 16.7%). With the adjustment, 82 of the 345 women had a Manchester score of ≥15, and this included 18 of the 28 BRCA1 carriers (sensitivity, 64.3%; specificity, 79.8%; PPV, 22.0%) (Table 5). These results show that adjustment in this cohort resulted in 14% increase in the number of tests (72 to 82) and 21% increase in sensitivity (43% to 64%).

In addition, given that PTEN loss was associated with BRCA1 germline mutations [8], we made a further upward adjustment for PTEN loss (+1 point) for all patients for whom PTEN status was available. Without adjustment, five of 26 women had a >10% probability of BRCA1 and BRCA2 mutations, and this included only three of the seven BRCA1 carriers (sensitivity, 42.9%; specificity, 89.5%; PPV, 60.0%). With the adjustment for PTEN loss, 10 of the 26 women had a Manchester score ≥15, and this included six of the seven BRCA1 carriers (sensitivity, 85.7%; specificity, 78.9%; PPV, 60.0%; Table 5). Although PTEN results were available for only a small subset of patients, these results suggest that upward adjustment for PTEN may aid the identification of BRCA1 carriers.

Discussion

Our study in an Asian series of triple-negative breast cancer patients demonstrated that up to 24.5% (27 of 110) women have germline mutations in BRCA1 (23 of 110) and BRCA2 (four of 110), and that the addition of negative estrogen-receptor status and PTEN loss improves the sensitivity of the Manchester Scoring method in our Asian cohort.

The results in this study are consistent with that in other cohorts of triple-negative breast cancer patients, in whom 11% to 39% have germline mutations in BRCA1 and BRCA2 [3,6,20-23], and cohorts of estrogen-receptor-negative breast cancer patients, of whom 24% to 29% have germline mutations in BRCA1 and BRCA2 [4,6,24,25]. A recent single-institution study showed that 50% of high-risk patients with TNBC had mutations in BRCA1/2, but notably, 76% of this cohort had a family history of breast cancer [10]. For all of these series and for our study, BRCA1 mutations are more common than BRCA2 mutations.

Cost-effectiveness analyses have suggested that mutation testing for all TNBC patients younger than 50 years old may be a cost-effective approach, assuming that 10% to 25% of these patients have BRCA1 or BRCA2 mutations [5]. However, we find that although TNBC is associated with an increased prevalence of BRCA1 and BRCA2 mutations among those younger than 35 years old (28.0% in TNBC versus 9.9% in non-TNBC; P = 0.045), TNBC is not associated with an increased prevalence of mutations among those aged 36 to 50 years without a family history of breast or ovarian cancer (BRCA prevalence of 8.5% and
Table 5 Performance of Manchester Scoring method before and after adjustment with ER status or ER and PTEN status

| Manchester Scoring method       | Cohort, n | Total BRCA1 carriers, n | No pathology adjustment | With pathology adjustment |
|--------------------------------|-----------|-------------------------|-------------------------|--------------------------|
|                                |           |                         | Individuals tested, n   | BRCA1 carriers tested, n  |
| Adjustment + estrogen-receptor  | 345       | 28                      | 72                      | 12                       |
| status                         |           |                         | 82                      | 18                       |
| Adjustment + estrogen-receptor  | 26        | 7                       | 5                       | 3                        |
| + PTEN status                  |           |                         | 10                      | 6                        |

The predicted and observed numbers of BRCA1 and BRCA2 (combined) carriers in women with low (1 to 14) and high Manchester score, either without adjustment for pathologic features, or with adjustment for ER status and other features [17–19], or further adjustment including +1 for loss of PTEN.

6.7%, respectively). This may be because BRCA1 is a high-penetrance gene and is associated with both early-onset disease and multiple affected family members, and therefore, in the absence of these features, a low prevalence of BRCA1 mutations is found, even in TNBC. Further studies with larger population-based datasets are needed to determine the prevalence of BRCA1 and BRCA2 mutations and the cost-effectiveness of testing women diagnosed with isolated breast cancer, aged 40 to 49 years old.

We find that the prevalence of BRCA1 mutations is higher in TNBC compared with non-TNBC in women in whom early-onset breast cancer develops and in women with a family history of breast and ovarian cancer. This effect is largely due to a difference in prevalence of BRCA1 mutations and is consistent with the observation that the majority of BRCA1 carriers develop early-onset triple-negative basal-like breast tumors that have distinctive morphologic and immunohistochemical characteristics [6–8]. Intriguingly, the significant proportion of TNBC patients who have BRCA1 germline mutations in these two subgroups of patients (28.0% and 34.2%) suggests that mutation in BRCA1 is a key driver of the development of TNBC. This could be due to the roles of BRCA1 in determining cell fate of luminal progenitor cells [26], its effect on transcriptional regulation of ER-gene expression [27], its effect on regulation of mi155 [27], or a combination of these.

Taken together, we suggest that TNBC status may be helpful in stratifying women with a moderate risk of having BRCA1 mutations (for example, a weak family history or isolated case of early-onset breast cancer), but may have limited utility in the absence of such features (for example, women with a single case of TNBC aged 40 to 50 years old).

Finally, we find that addition of negative estrogen receptor and TNBC status improves the sensitivity and specificity of the Manchester Scoring method in our cohort and that addition of PTEN loss further improves the sensitivity of the method. PTEN loss is highly associated with BRCA1 breast cancers (28 (82.4%) of 34 of tumor samples from BRCA1 carriers showed the loss of PTEN by immunohistochemistry) and can result from gene rearrangements involving DNA double-strand breaks, intragenic inversions on insertions, homozygous deletions, and focalized CNIs [8]. However, given that the data on PTEN loss were available on only a small subset of patients, this result requires further validation in a larger cohort of patients. We believe that methods for stratifying the likelihood of carrying a BRCA1 and BRCA2 mutation that is independent of family history are important, particularly in the Asian context, because the familial and social stigma associated with cancer makes accurate family-history reporting challenging [28].

Conclusions

In previous studies and in our cohort of TNBC, a significant proportion of women have germline BRCA1 mutations. Our study shows that among women with early-onset breast cancer (≤35 years old) and with a family history of breast cancer, a higher prevalence of BRCA1 mutations is present in women with TNBC compared with women with non-TNBC. However, no difference in prevalence of BRCA1 and BRCA2 mutations is noted among women who develop isolated breast cancer aged 36 to 50 (7% to 8% prevalence in both TNBC and non-TNBC). Our study suggests that the current clinical recommendations of offering BRCA1 and BRCA2 genetic testing, even to women with isolated TNBC younger than 60 years, warrant further analysis.

Additional material

Additional file 1: CK, cytokeratin Characteristics of BRCA1 carriers Additional File 1 shows the pathologic features of breast cancers in eight BRCA1 carriers compared with 26 non-BRCA1 carriers.
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Authors' contributions

PSY carried out the genetic analyses; LLM, AR, and SD conducted the pathological analyses; and NH conducted the statistical analyses. NAMT and YCH recruited patients and collected clinical data. PSY and TSH conceived the study and drafted the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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