Clinical and pathological features of BRCA1/2 tumors in a sample of high-risk Moroccan breast cancer patients

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Abstract

Background: BRCA1 and BRCA2 genes explain a large part of hereditary breast cancer. Several studies have shown that BRCA1 and BRCA2 tumors exhibit some specific morphological and immunohistochemical characteristics. The aim of our study is to compare the clinicopathological characteristics between Moroccan breast cancers associated or not with BRCA1 and BRCA2 mutations. Previously, we had identified 11 BRCA carriers in a series of 40 selected breast cancer patients at increased risk for carrying a mutation in the BRCA1 and BRCA2 genes. The clinical and pathological features of patients carrying BRCA1 or BRCA2 mutation (n = 11) were evaluated and compared to those of non-mutated patients (n = 29).

Results: In comparison with non carriers, women with BRCA1/2 mutation present younger mean age at diagnosis (37.90 vs. 44.48 years, p = 0.05), younger mean age of 1st menarche (13.08 vs. 14.24 years, p = 0.05) and shorter duration of breastfeeding (8.71 vs. 19.35 months, p = 0.05). Moreover, 63.6 and 62.5 % of BRCA1/2 carriers present SBR grade III and triple negative tumors respectively (p = 0.02).

Conclusions: In this first Moroccan study comparing clinical and pathological characteristics of women carrying or not BRCA1/2 mutation, patients with BRCA mutation tend to develop early breast cancer with high-grade and triple negative tumors. However, further large scale research including more data is needed to better characterize BRCA1/2 cases and to evaluate the survival rate associated with these mutations in our population tumors. Moreover, it would be more interesting to study women with BRCA1 and BRCA2 mutations separately in order to determine if they exhibit distinct characteristics.

Keywords: Breast cancer, BRCA1, BRCA2, Clinicopathological, Characteristics, Morocco

Background

Breast cancer can occur in sporadic or hereditary forms. In the case of hereditary forms a germline mutation in a specific gene predisposes to cancer. Two major genes involved in the pathogenesis of breast and ovarian cancer have been identified. BRCA1 gene located on chromosome 17q21 [1, 2] and BRCA2 gene located on chromosome 13q12 [3] are tumor suppressor genes involved in maintaining of genome integrity by engaging in many processes such as repair of DNA double strand breaks, cell cycle control and transcription [4]. Both genes explain a large part of families with a predisposition to breast and ovarian cancer [5]. Indeed, the risk of developing breast cancer in carriers of BRCA1 or BRCA2 mutation is about 45–80 % [6, 7].

Several studies have focused on clinical and pathological features of breast and ovarian cancer associated with BRCA1 and BRCA2 mutations [8–11]. These studies finding have shown that BRCA1 and BRCA2 tumors exhibit some specific morphological and immunohistochemical characteristics.

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This study aims to compare the clinicopathological features between Moroccan breast cancers associated or not with BRCA1 and BRCA2 mutations in the order to find some clinical and pathological characteristics specific to this population especially that a recent study identified a specific founder BRCA1 mutation in the Moroccan population [12].

Methods
In our previous study [13], a total of 40 clinically high-risk breast and/or ovarian cancer patients, treated in Mohammed VI Cancer Treatment Center of Ibn Rochd University of Casablanca, were selected and referred for BRCA genetic testing to the Genetics and Molecular Pathology Laboratory of the Medical school of Casablanca between 2009 and 2010.

Breast cancer patients were selected according to specific criteria:

- Three or more first or second degree relatives with breast cancer diagnosed at any age in the same familial branch;
- Two first degree relatives with breast cancer, with at least one early onset breast cancer case (≤40 years) or male breast cancer case or ovarian cancer case.
- Single cases diagnosed with breast cancer before age 40.

As described previously [13], DNA was extracted from whole blood samples using the salting out method and all exons and exon–intron boundaries of BRCA1 and BRCA2 genes were amplified in a final volume of 25 μl containing: 1× reaction buffer, 1.5 mM MgCl2, 0.2 mM dNTPs, 5 μM primers (sequences available on request), 1.25 U Taq polymerase and 50 ng genomic DNA. Amplification cycles were: 94 °C for 7 min followed by 4 cycles of 94 °C for 0.1 min, 64 °C for 0.1 min, and 72 °C for 1 min, 4 cycles of 94 °C for 0.1 min, 64 °C for 0.1 min, and 72 °C for 1 min, 35 cycles of 94 °C for 0.1 min, 58 °C for 0.1 min, and 72 °C for 1 min and 1 cycle at 72 °C for 7 min, except for exon 15 of BRCA2 for which the amplification conditions were: 94 °C for 7 min followed by 40 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 2 min and ended with a 7 min incubation at 72 °C. Amplicons were purified and sequenced in both forward and reverse strands using BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems) then runned on a ABIPRISM 3130 XL Genetic analyzer (Applied Biosystems) after purification and denaturation. Sequence analyses were performed using SeqScape v2.6 (Applied Biosystems) software. All mutations and variants are cited according to Human Genome Variation Sequence systematic nomenclature (HGVS; http://www.hgvs.org/mutnomen/) using GenBank entries: U14680 for BRCA1 and U43746 for BRCA2.

Sequencing results of the entire and exon/intron sequences of both genes have showed that 11 patients were mutated in BRCA1/2 genes, and 29 women were not associated to BRCA1/2 mutations.

A detailed semi-structured face to face interview including information on family history and risk factors for breast cancer (interview guide available upon request) such as age at diagnosis of breast cancer, age at menarche and menopause, parity (parous and nulliparous), breast-feeding (presence and absence), oral contraceptive use (presence and absence) and tumor location (unilateral or bilateral involvement) was conducted by HJ (a male professor of Radiation Oncology and PhD candidate with experience in conducting qualitative research) and AT (a female PhD researcher) at Mohammed VI Cancer Treatment Center of Ibn Rochd University Hospital of Casablanca after explaining the aim and the objectives of the study and obtaining written consent from all eligible women. All interviews were conducted in Moroccan Arabic language and lasted approximately 30 min. There were no third parties present for any interview. Collected data were recorded on transcripts which were not returned to interviewees then coded by both authors. None of the participants refused to participate and no repeat interviews were carried out. The histological analysis of the tumor, tumor size and lymph node involvement according to the TNM classification [14], SBR grade according to Nottingham modification of Scarff–Bloom–Richardson system [15], hormone receptor status and HER2 status based on CAP guidelines [16, 17] were collected by review of medical records.

The clinical and pathological features of patients carrying BRCA1 or BRCA2 mutation were evaluated and compared to those of non-mutated patients. Statistical analysis was performed using Epi Info Version 3.5.4. Quantitative variables with normal distribution were analyzed by Student's t test. Comparison of qualitative data was performed using Fisher’s Exact test. The correlation is statistically significant between two variables if the P value is less than or equal to 0.05.

Results
In this study, we tried to find a correlation between the clinicopathological characteristics of breast cancer and BRCA1/2 mutation status. Indeed, our previous study [13] have revealed among 40 breast cancer patients, at increased risk of carrying a mutation, 29 women with negative BRCA1/2 testing and 11 patients with a positive BRCA1/2 status (Table 1) including six patients with BRCA1 mutation and five patients carrying BRCA2 mutation.
The main characteristics of breast cancer patients at diagnosis are shown in Table 2. In the present study, 90.9 % of BRCA1/2 carriers versus 82.8 % of non-carriers reported a family history (p = 0.66). The mean age at diagnosis of breast cancer and the mean age of first menarche was younger in BRCA1/2 mutation carriers than in non-carriers (p = 0.05). Similarly, the average duration of breastfeeding was shorter among BRCA1/2 carriers than non-carriers (p = 0.05). Conversely, no difference was observed between both groups regarding the use of oral contraceptives, age at first full-term pregnancy, parity, breastfeeding, age of menopause and tumor localization.

Histologically (Table 3), the infiltrating ductal carcinoma was the most common histological type in both groups (90.9 and 93.3 %). The medullary carcinoma accounted for 9.1 % in BRCA1/2 carriers and only 3.4 % in non-carriers (not significant). T1 and T2 tumor sizes were observed in mutated patients with a frequency of 72.7 %. Moreover, SBR grade III was found in 63.6 % of women with BRCA1/2 mutation against a frequency of 20.7 % among non-carriers, this difference appears to be statistically significant (p = 0.02). On the other hand, lymph node involvement, hormone receptors expression and Her2/neu status showed no statistically significant difference between both studied groups. However, BRCA1/2 carriers were more likely to be triple-negative breast cancer compared with non-carriers (62.5 vs. 16.7 %, p = 0.02). Nevertheless, it should be emphasized that ER and PR status was not available in four patients (10 %) while Her2/neu data was missing in 8 (20 %). This may be due to the fact that some patients prefer to perform the tests in outside laboratories.

**Discussion**

Although the family history is widely established as a risk factor for breast cancer, there is a disagreement about its impact on prognosis with reported conflicting series results [18–25]. The discovery of BRCA1 and 2 genes predisposing to breast cancer has improved identification of cases linked to genetic susceptibility.

The probability of an individual to carry a BRCA1 or BRCA2 germline mutation is based primarily on clinical data such as family history, age at diagnosis of breast cancer and ethnicity. Indeed, family history with a

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**Table 1** BRCA1 and BRCA2 mutations [13]

| Gene | Mutation | Manifestation, age at diagnosis | Family history |
|------|----------|---------------------------------|----------------|
| BRCA1 | c.181T > G | BC, 34 years | Mother, BC 45 years |
| BRCA1 | c.798-799delTT | BC, 42 years | M Cousin, BC 47 years |
| BRCA1 | c.2805delA | BC, 41 years | M aunt, BC 42 years |
| BRCA1 | c.3279delC | BC, 49 years | Daughter, BC 32 years |
| BRCA1 | c.5062-5064delGTT | BC, 25 years | M aunt, CS 40 years |
| BRCA2 | c.745-1G > A | BC, 33 years | No family history |
| BRCA2 | c.3381delT | BC, 38 years | M aunt, BC 43 years |
| BRCA2 | c.7110delA | BC, 40 years | M aunt, BC 40 years |
| BRCA2 | c.7235insG | BC, 40 years | M aunt, BC 38 years |
| BRCA2 | c.7235insG | BBC, 45 years | Sister, BC 33 years |

**Table 2** Personal and clinical characteristics of patients carrying or not BRCA1/2 mutations

| Variable | BRCA1/2+ | BRCA1/2− | p value |
|----------|----------|----------|---------|
| Family history | No | 1 (9.1 %) | 5 (17.2 %) | 0.66 |
| Breastfeeding | None | 10 (90.9 %) | 24 (82.8 %) |
| Mean age at diagnosis (years) | 37.90 (SD = 6.67) | 44.48 (SD = 9.74) | 0.05 |
| Mean age at menarche (years) | 13.08 (SD = 1.60) | 14.24 (SD = 1.62) | 0.05 |
| Mean age at first delivery (years) | 25.15 (SD = 5.76) | 25.57 (SD = 6.60) | 0.88 |
| Mean age at menopause (years) | 47.50 (SD = 0.70) | 48.66 (SD = 5.60) | 0.79 |
| Average duration of breastfeeding (months) | 8.71 (SD = 5.40) | 19.35 (SD = 12.92) | 0.05 |
| Oral contraceptive use | Yes | 6 (54.5 %) | 11 (37.9 %) | 0.48 |
| Parity | Nulliparous | 4 (36.4 %) | 10 (34.5 %) | 1.00 |
| Parous | 7 (63.6 %) | 17 (58.6 %) |
| Tumor localization | Unilateral | 10 (90.9 %) | 28 (96.6 %) | 1.00 |
| Bilateral | 1 (9.1 %) | 1 (3.4 %) |

Values in Italic are statistically significant (p value ≤ 0.05)
concentration of breast and ovarian cancers is the most important risk factor in developing the disease. However, this criterion presents some problems as it is based on the collection of the cancer events in the family without pathological confirmation. In addition, a number of population studies have revealed that a large proportion of breast cancer patients with a BRCA1 or BRCA2 germline mutation have no history of the disease in the family [26, 27].

Limited studies of BRCA gene mutations have been carried out in Morocco but none have described the clinicopathological characteristics in detail. Thus, the study of tumor phenotypes associated with BRCA1/2 mutations may be useful to predict the probability to carry a germline mutation. In this study, we analyzed the clinicopathological characteristics of breast cancer patients based on their BRCA status.

Based on our results, the frequency of BRCA1 and BRCA2 mutations among Moroccan women with hereditary breast and/or ovarian cancer is 25.64 % [13]. Consistent with this result, a recent study [28] examining the prevalence of BRCA1/2 germline mutations in 21,401 families with breast and ovarian cancer history has reported a prevalence of 24.0 % (95 % CI 23.4–24.6 %) [28].

Several studies have reported that breast cancer related to BRCA1/2 mutations is often associated with an early age of diagnosis [10, 29–33]. Consistent with these findings, the comparison of the average age of diagnosis between our groups of breast cancer with or without BRCA1/2 mutation showed a statistically significant difference. Semple et al. [34] recently reported that the annual breast cancer risks for BRCA1 mutation is not affected by age of breast cancer diagnosis in the first-degree relative, which is not the case for BRCA2 mutation carriers where women with a first-degree relative diagnosed before the age of 30 years have an annual breast cancer risk of 4.5 %.

On the other hand, the authors report that cumulative exposure to sex hormones, especially estrogen, is probably associated to breast cancer risk in BRCA1 mutation carriers. Thus, women who had menarche at a later age or who have breastfed seem to be protected against breast cancer development, while the role of gender is ambiguous [35]. In this study, the age of the first menstruation seems to be statistically similar between BRCA mutation carriers and non-carriers. This observation was consistent with some previous studies [10, 30].

Our results showed that patients with or without BRCA1/2 mutation were similar with regard to oral contraceptives use, age at first full-term pregnancy, parity, lactation and average age of menopause. These data are consistent with those reported in other investigations [8, 30]. However, the average duration of breastfeeding was statistically shorter in women carrying mutations. Jernström et al. [36] has observed a significantly shorter period of breastfeeding in BRCA1 mutation carriers who breastfed for at least one year (OR 0.68; 95 % CI 0.52–0.91; p = 0.008) and for two or more years (OR 0.51; 95 % CI 0.35–0.74; p = 0.0003), respectively. However, no significant association was observed between breastfeeding and breast cancer risk among BRCA2 mutation carriers [35].

It is well known that breast cancer women with BRCA1/2 mutation have a high risk of developing contralateral breast cancer. Indeed, a recent publication has reported a higher frequency of bilateral breast cancer in the BRCA-positive group [37]. However, Kwong et al. [30] had concluded that the bilateral nature of breast cancer was not significantly associated with BRCA1 and BRCA2 mutation which is in line with the results of the present study.

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### Table 3 Pathological characteristics of patients carrying or not BRCA1/2 mutations

| Variables                        | BRCA1/2+ | BRCA1/2− | p value |
|----------------------------------|----------|----------|---------|
| Histological type                |          |          |         |
| Invasive ductal carcinoma        | 10 (90.9 %) | 27 (93.1 %) | 0.63    |
| Invasive lobular carcinoma       | 0 (0 %)   | 1 (3.4 %)   |         |
| Medullary carcinoma              | 1 (9.1 %)  | 1 (3.4 %)   |         |
| Tumor size                       |          |          |         |
| T1–T2                            | 8 (72.7 %) | 19 (65.5 %) | 1.00    |
| T3                               | 2 (18.2 %) | 4 (13.8 %)   |         |
| T4                               | 1 (9.1 %)  | 4 (13.8 %)   |         |
| SBR grade                        |          |          |         |
| I–II                             | 4 (36.4 %) | 23 (79.3 %) | 0.02    |
| III                              | 7 (63.6 %) | 6 (20.7 %)   |         |
| Node involvement                 |          |          |         |
| N−                               | 5 (45.5 %) | 13 (44.8 %) | 1.00    |
| N+                               | 6 (54.5 %) | 16 (55.2 %) |         |
| Estrogen receptors status        |          |          |         |
| ER−                              | 7 (63.6 %) | 10 (40.0 %) | 0.28    |
| ER+                              | 4 (36.4 %) | 15 (60.0 %) |         |
| Progesterone receptors status    |          |          |         |
| PR−                              | 7 (63.6 %) | 11 (44.0 %) | 0.47    |
| PR+                              | 4 (36.4 %) | 14 (56.0 %) |         |
| Her2/neu                         |          |          |         |
| Her2−                            | 5 (62.5 %) | 16 (66.7 %) | 1.00    |
| Her2+                            | 3 (37.5 %) | 8 (33.3 %)   |         |
| Triple negative (ER−, PR− and HER2/neu−) | 5 (62.5 %) | 4 (16.7 %) | 0.02 |

Values in italic are statistically significant (p value ≤ 0.05)
In general, a number of studies have raised some important biological and pathological differences between BRCA1/2 mutations carriers and non-carriers. In our study, we observed a significant predominance of SBR grade III tumors (p = 0.02). Consistent with these findings, various reports have found that tumors related to BRCA1/2 mutations seem to be of higher grade compared to non carriers [8, 30–32].

Additionally, tumor size and axillary dissection showed no statistically significant difference between both studied groups. In accordance with our results, Kwong et al. [30] had observed no difference in axillary lymph node involvement between breast cancers associated with BRCA mutations to those not related to mutations but they have found that BRCA carriers developed significantly smaller tumors [OR (T1 vs. T2–4) 0.41; 95 % CI 0.17–0.98; p = 0.05]. Contrariwise, another study had reported that BRCA positive patients tended to have positive lymphnodes [8].

Furthermore, our results showed no significant difference between both groups with regard to the expression of hormone and HER-2/neu receptors which is similar to the findings related to an Italian study [8]. Contrary to these results, some reports have found a significant difference between both groups regarding hormone and HER-2/neu receptors with a predominance of negative status in carriers of mutations [30, 38]. In this study, BRCA1 and BRCA2 carriers were more likely to have triple negative tumors (ER−, PR− and HER2/neu−) which is in line with literature [30–32, 39].

In considering the results of the present report, we should note that the sample size is very small which means that the differences or similarities observed between BRCA1/2 carriers and non carriers regarding the clinical and pathological characteristics studied maybe due to random variability. A limitation which may also be due to the fact that we restricted the study population to Moroccan women. Also, this study includes selected breast cancer patients with a high probability of carrying a pathogenic BRCA1/2 germline mutation so it is not reasonable to generalize these results to the entire population. Therefore, these findings should be interpreted cautiously and need to be confirmed by larger trials.

Conclusions
In this first Moroccan study comparing clinical and pathological characteristics of women carrying or not BRCA mutation, patients with BRCA1/2 mutation tend to develop early breast cancer with high-grade tumors. On the other hand, early menarche and short duration of breastfeeding appear to characterize patients with BRCA mutation. Nevertheless, this study has a number of limitations; the main limitation is the reduced statistical power due to small sample size. Finally, further large scale research including more data is needed to better characterize the BRCA1/2 cases and to evaluate the survival rate associated with these mutations in our population tumors. Moreover, it would be more interesting to study women with BRCA1 and BRCA2 mutations separately due to their differences in tumor characteristics.

Abbreviations
BRCA1: breast cancer susceptibility gene 1; BRCA2: breast cancer susceptibility gene 2; SBR: Scarff–Bloom–Richardson; ER: estrogen receptor; PR: progesterone receptor; HER-2: human epidermal growth factor receptor 2.

Authors’ contributions
HJ and AT recruited the patients, performed the analysis and drafted the manuscript. AN participated in patient recruitment and acquisition of data. HA, SN and AB participated in the conception, the design and the coordination of the study. All authors read and approved the final manuscript.

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Availability of data and material
Any request of data and material may be sent to the corresponding author.

Competing interests
The authors declare that they have no competing interests.

Ethics approval and consent to participate
Written informed consent was obtained from all subjects and the study was approved by the Ethics Committee of the Faculty of Medicine of Casablanca.

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