Manuscript title:

Multifocal breast cancers are more prevalent in BRCA2 versus BRCA1 mutation carriers

Running title:

Multifocality in BRCA-associated breast cancer

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Abstract

Multifocal breast cancer is generally considered to be where two or more breast tumours are present within the same breast, but are clearly separated with no intervening in situ or invasive disease. It is seen in ~10% of breast cancer cases. This study investigates multifocality prevalence in BRCA1/2 mutant patients via cross-sectional analysis. Data from 211 women with BRCA1/2 mutations (BRCA1 - 91), (BRCA2 - 120), with breast cancer were collected including age, tumour focality, size, type, grade, and receptor profile. The prevalence of multifocality within this group was 25%, but within subgroups, prevalence amongst BRCA2 carriers was more than double that of BRCA1 carriers (p=0.001). Women affected by multifocal tumours had proportionately higher oestrogen receptor positivity (p=0.001), lower triple negativity (p=0.004), and were more likely to be younger at diagnosis compared with those with unifocal tumours (p=0.039). Odds of a BRCA2 carrier developing multifocal cancer were almost four-fold higher than a BRCA1 carrier (OR: 3.71, CI: 1.77-7.78, p=0.001). BRCA2 carriers show much greater multifocality than those carrying BRCA1 – multifocal tumours are strongly associated with being both BRCA2 mutant and oestrogen receptor positive.

Key words: Multifocal; Breast cancer; BRCA; Mutation; Pathology; Prevalence; Epidemiology
Introduction

A large meta-analysis of 22 studies, including over 67,000 women, estimated prevalence of multifocal breast cancer to be 9.5%[1]. Evidence suggests that multifocal cancers are associated with reduced disease-free survival compared with unifocal tumours[2]. Moreover, treatments offered for multifocal breast cancer vary widely, with some women undergoing multiple breast conserving procedures, and others mastectomy, with no clear treatment guidelines[3].

Definitions of multifocal and multicentric disease have varied historically. Multifocal cancers have been defined as two or more distinct invasive breast carcinomas within the same breast quadrant, whereas multicentric disease has been defined as separate tumours in different breast quadrants. However, several studies have demonstrated that in both cases, tumours are predominantly of independent clonal origin i.e. are independent tumours arising in the same breast[4,5]. Therefore, for the purposes of this study we have considered multifocal and multicentric tumours together, defining them as clearly separated tumours without any intervening in situ or invasive disease.

BRCA1 and BRCA2 are tumour suppressor genes located on chromosomes 17 and 13 respectively. They encode proteins involved in the cellular DNA damage response pathway, particularly DNA double strand break repair[6]. Germline mutations in these genes predispose female carriers to a significantly increased risk of breast and ovarian cancer, with up to 80% lifetime risk of breast cancer. Given this elevated breast cancer risk, we hypothesised that these women may be more likely than non-mutation carriers to develop multifocal disease. Surprisingly, despite biological plausibility for the existence of an association between BRCA1/2 mutations and multifocal tumours, at time of writing, there were no studies investigating this. Therefore, this study aimed to investigate the prevalence of multifocal breast cancer in a group of BRCA1/2 mutation carriers, with exploration of the clinicopathological characteristics of all tumours occurring in this group.

Materials and methods
Data from 252 women with an known pathogenic germline \( BRCA1/2 \) mutation diagnosed with breast cancer (1994-2017) were retrospectively extracted from a database containing all known female \( BRCA1/2 \) mutation carriers in Northern Ireland (Figure 1). Information about histological tumour type and focality (unifocal or multifocal) was extracted from pathology records for 211 women, with 41 patients excluded due to missing focality information (n=30), or because of a diagnosis of DCIS without invasion (n=11). Additional clinicopathological data was collected, including age at initial cancer diagnosis, tumour grade and size, hormone receptor status, HER2 status, nodal involvement, presence/absence of other primary cancer. Data was entered into Microsoft Excel® for stratification and calculation of prevalence. 23 randomly selected cases (10%) underwent review of the original diagnostic slides by an independent pathologist for validation of multifocality reporting.

Data were analysed using SPSS®. Heterogeneity of clinicopathological characteristics between those diagnosed with unifocal disease and those diagnosed with multifocal disease were compared using \( \chi^2 \). Mean age and tumour size between groups was compared using t-test. Binary logistic regression was performed to calculate the unadjusted odds ratio (OR) of developing multifocal disease in patients with \( BRCA2 \)-associated breast cancer versus those with \( BRCA1 \)-associated breast cancer. Thereafter, adjusted OR was calculated using a manually controlled backward stepwise elimination approach[7]. Potentially confounding variables with a biological association to breast cancer were entered into the regression model and sequentially removed until only those with statistical significance remained. A p value of <0.05 indicates significance at the 95% confidence interval throughout. Institutional approval was granted by the Belfast Health and Social Care Trust (Ref: 5805).

Results

90 (42.7%) women had a \( BRCA1 \) mutation and 121 (57.3%) a \( BRCA2 \) mutation. Mean age at diagnosis was 45 years (range: 25-77 years) with a lower mean age at diagnosis for multifocal tumours compared with unifocal tumours (43 vs. 46 years) (p=0.109). Mean tumour size was 24mm (range: 2-150mm) with no significant difference in mean size between the largest multifocal tumour foci and unifocal
tumours (24.8mm vs. 23.2mm) (p=0.587). There were 52 diagnoses of multifocal disease and 159 diagnoses of unifocal disease. Prevalence of multifocal disease was 13.3% in BRCA1 mutation carriers and 33.1% in BRCA2 mutation carriers. Therefore, prevalence of multifocal disease in BRCA2 carriers was 2.5-fold greater than BRCA1 carriers (p=0.001). Clinicopathological findings are documented in Table 1. The majority of multifocal and unifocal tumours were invasive ductal carcinomas (86.5% and 96.2% respectively), grade III (73.6% and 63.5% respectively), and HER2-negative (75.0% and 73.6% respectively). Additionally, BRCA1/2 carriers with multifocal disease were more likely to be oestrogen receptor positive than negative (75.0% vs. 45.9%) (p=0.001).

Of the 52 women diagnosed with multifocal disease, 23.1% (n=12) had a BRCA1 mutation and 76.9% (n=40) a BRCA2 mutation. 50% (n=6) women with a BRCA1 mutation were oestrogen receptor positive whilst 82.5% (n=33) women with a BRCA2 mutation were oestrogen receptor positive (p=0.039). See Supplementary Table. Unadjusted odds of breast cancer being multifocal in BRCA2 mutation carriers were 3.2 times greater than in BRCA1 mutation carriers (CI:1.57–6.57, p=0.001). Age was found to be a significant confounding factor in logistic regression (CI:0.22–0.85, p=0.015). Therefore, after adjusting for age, odds of a BRCA2 mutation carrier developing breast cancer were 3.7-fold greater than in BRCA1 mutation carriers (CI:1.77–7.78, p=0.001). Oestrogen receptor and HER2 status had no significant effect on the association between BRCA subtype and multifocality (Table 2).

Discussion

A systematic review of multifocal breast cancer conducted by Vera-Badillo et al included twenty-two studies encompassing 67,557 women. This study calculated a prevalence of 9.5% amongst women with sporadic breast cancer (BRCA status unknown)[1]. In contrast, prevalence of multifocality this cohort of 211 BRCA1/2 mutation carriers was 24.6%, more than double that reported by Vera-Badillo et al.

Our study found that prevalence of multifocality in BRCA2 mutation carriers was double that of BRCA1 mutation carriers – a finding mirrored in a small-scale study by Bergthorsson et al[8]. The odds of a woman who has developed breast cancer,
exhibiting multifocal disease, are over three times greater if she has a \textit{BRCA2} mutation compared to a \textit{BRCA1} mutation. This rises to an almost four-fold increase in odds of a \textit{BRCA2} carrier developing multifocal disease once the effect of age at diagnosis is taken into account.

Women diagnosed with multifocal breast cancer were proportionately more likely to be oestrogen receptor positive, had a lower prevalence of triple receptor negativity, and were more likely to be aged less than 40 years at initial diagnosis. These findings are in keeping with numerous studies documenting significantly higher rates of oestrogen receptor positivity amongst \textit{BRCA2} carriers compared with \textit{BRCA1} mutation carriers. Therefore, it is unlikely that oestrogen signalling itself drives multifocal disease\cite{9,10}. Indeed, the large-scale meta-analysis described earlier found no association between ER status and sporadic multifocal breast cancer, suggesting that ER does not play a role in the specific development of multifocal disease\cite{1}. Precisely why \textit{BRCA2} carriers are more likely to develop multifocal disease than \textit{BRCA1} carriers is unclear. Recent evidence suggests that \textit{BRCA1}-related breast cancer is driven by aberrant RANK/RANKL signalling in \textit{BRCA1} heterozygous luminal progenitor cells, coupled with increased DNA damage/defective DNA repair in these cells, resulting in development of basal breast cancers\cite{11}. In contrast, this has not been reported in \textit{BRCA2} carriers, who predominately develop luminal breast cancer. Additionally, \textit{BRCA2}'s predominant reported function is its direct role in homologous recombination-mediated double strand break repair\cite{12}. Clearly, a better understanding of molecular and genetic processes resulting in the development of basal and luminal breast cancers at the single cell level is required. Moreover, given the apparent predominant development of synchronous but distinct cancers in \textit{BRCA2} mutation carriers, the contribution of genomic instability at a single cell level also needs to be investigated. Finally, given recent data demonstrating activation of cell intrinsic innate immune responses to the loss of \textit{BRCA1/2}, the role of early immunoediting in control of tumours in \textit{BRCA1} versus \textit{BRCA2} carriers needs to be investigated\cite{13}.

The association between younger age at diagnosis and increased likelihood of a tumour exhibiting multifocality may be partly explained by enhanced breast screening uptake amongst women with \textit{BRCA} mutations. This leads to a greater proportion of
tumours, including those exhibiting multifocality, being diagnosed earlier, possibly confounding the association between age of diagnosis and multifocality[14,15].

The primary strength of this study is data completeness, and independent pathological validation of histopathology reports. Data for all 211 women is complete, with regard to tumour focality, BRCA mutation type, age at diagnosis, and tumour type. Furthermore, information about tumour grade, oestrogen receptor status, lymph node involvement, and presence or absence of other primary tumours are all in excess of 96% complete. The considerable quantity of missing HER2 status data reflects the fact HER-2 testing was not routinely carried out at time of diagnosis for many of these women[16].

In conclusion, we report a higher than anticipated prevalence of multifocality amongst a group of 211 female BRCA1/2 mutation carriers diagnosed with breast cancer. Findings suggest multifocality is more common in BRCA2-associated breast cancer. Those with multifocal disease were more likely to be younger at diagnosis, and to be oestrogen receptor positive than those with unifocal disease. Further prospective studies are required for confirmatory purposes, and to establish the underlying mechanistic basis for these findings.

**Additional information**

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**Author contributions statement**: study idea was conceived by SMcl and KS. Data was collected by ADM and SA. Statistical analysis was performed by ADM, SA, and CM. Verification was performed by PM and CB. Manuscript was drafted and critically revised by all authors; all authors have approved the final version of the manuscript.
Table and Figure Legends

**Table 1:** Clinical and pathological characteristics of BRCA1/2 mutation carrier patients diagnosed with breast cancers between 1994-2017. *Pearson’s $\chi^2$ where p<0.05 indicates significance.

**Table 2:** Odds of cancer being multifocal in patients with BRCA2 vs. BRCA1 mutation where $a =$ unadjusted odds ratio, $b =$ odds ratio adjusted for age (>40 years vs. <40 years), and $c =$ effect of being >40 years adjusted for effect of being BRCA2 mutation carrier

**Supplementary table:** Oestrogen receptor status of a cohort of female BRCA1/2 mutation carriers diagnosed with multifocal breast cancer between 1994-2017. **Pearson’s $\chi^2$ where p<0.05 indicates significance

**Figure 1:** participant flow diagram showing inclusions/exclusions
Table 1: Clinical and pathological characteristics of BRCA1/2 mutation carriers

| Clinical and pathological features of breast cancers | Multifocality N (%) | Unifocality N (%) | p-value (*) |
|-----------------------------------------------------|---------------------|------------------|-------------|
| **BRCA mutation** |                     |                  |             |
| BRCA1 | 12 (13.3) | 78 (86.7) | 0.001 |
| BRCA2 | 40 (33.1) | 81 (66.9) |             |
| **Age at first diagnosis** | | | |
| <40 years | 23 (32.9) | 47 (67.1) | 0.039 |
| ≥40 years | 29 (20.6) | 112 (79.4) |             |
| **Tumour subtype** | | | |
| Invasive ductal | 45 (22.7) | 153 (77.3) |             |
| Invasive lobular | 6 (66.7) | 3 (33.3) | 0.011 |
| Other | 1 (25.0) | 3 (75.0) |             |
| **Tumour grade** | | | |
| I | 3 (42.9) | 4 (57.1) |             |
| II | 15 (30.0) | 35 (70.0) |             |
| III | 33 (22.0) | 117 (78.0) |             |
| Unknown | 1 (25.0) | 3 (75.0) | 0.460 |
| **Oestrogen receptor** | | | |
| Positive | 39 (34.8) | 73 (65.2) |             |
| Negative | 12 (12.9) | 81 (87.1) | 0.001 |
| Unknown | 1 (16.7) | 5 (83.3) |             |
| **HER2 status** | | | |
| Positive | 3 (27.3) | 8 (72.7) |             |
| Negative | 39 (25.0) | 117 (75.0) | 0.933 |
| Unknown | 10 (22.7) | 34 (77.3) |             |
| **Triple negativity** | | | |
| Yes | 7 (12.5) | 49 (87.5) | 0.004 |
| No | 30 (36.1) | 53 (63.9) |             |
| Unknown | 15 (20.8) | 57 (79.2) |             |
| **Lymph node involvement** | | | |
| Yes | 26 (33.3) | 52 (66.7) | 0.072 |
| No | 25 (20.0) | 100 (80.0) |             |
| Unknown | 1 (12.5) | 7 (87.5) |             |
| **Presence of other primary cancer** | | | |
| Yes | 9 (23.7) | 29 (76.3) | 0.957 |
| No | 42 (25.0) | 126 (75.0) |             |
| Unknown | 1 (20.0) | 4 (80.0) |             |
**Table 2:** Odds of cancer being multifocal in patients with *BRCA2* vs. *BRCA1* mutation

| Variable       | Odds ratio (95% CI) | p-value |
|----------------|---------------------|---------|
| BRCA status\(^a\) | 3.21 (1.57-6.57)    | 0.001   |
| BRCA status\(^b\) | 3.71 (1.77-7.78)    | 0.001   |
| Age\(^c\)       | 0.43 (0.22-0.85)    | 0.015   |

\(^a\) Refer to [original reference](https://doi.org/10.1101/19006478) (which was not peer-reviewed).
Supplementary table: Oestrogen receptor status of a cohort of female BRCA1/2 mutation carriers

| Women with pathologically confirmed multifocal breast cancer | BRCA1 mutation N (%) | BRCA2 mutation N (%) | p-value (*) |
|-----------------|----------------------|----------------------|-------------|
| Oestrogen receptor status | Positive | 6 (15.4) | 33 (84.6) | 0.039 |
|                 | Negative | 6 (50.0) | 6 (50.0) |
|                 | Missing  | 0 (0.0)  | 1 (100.0) |
Figure 1: details of patient ascertainment

BRCA register used to identify patients with a diagnosis of breast cancer between 1994 and 2017

Pathology reports (electronic and paper) used to gather diagnostic data (N=252)

Excluded
- Missing focality data (N=30)
- Non-invasive DCIS (N=11)

Additional clinical and pathological data collected for remaining BRCA patients (N=211)

Patients divided into BRCA1 and BRCA2 subtypes for statistical analysis

BRCA1 mutation patients (N=90)

BRCA2 mutation patients (N=121)
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