Genotypic and phenotypic analysis of familial male breast cancer shows under representation of the HER2 and basal subtypes in BRCA-associated carcinomas

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Abstract

Background: Male breast cancer (MBC) is an uncommon and relatively uncharacterised disease accounting for <1% of all breast cancers. A significant proportion occurs in families with a history of breast cancer and in particular those carrying BRCA2 mutations. Here we describe clinicopathological features and genomic BRCA1 and BRCA2 mutation status in a large cohort of familial MBCs.

Methods: Cases (n=60) included 3 BRCA1 and 25 BRCA2 mutation carriers, and 32 non-BRCA1/2 (BRCAX) carriers with strong family histories of breast cancer. The cohort was examined with respect to mutation status, clinicopathological parameters including TNM staging, grade, histological subtype and intrinsic phenotype.

Results: Compared to the general population, MBC incidence was higher in all subgroups. In contrast to female breast cancer (FBC) there was greater representation of BRCA2 tumours (41.7% vs 8.3%, p=0.0008) and underrepresentation of BRCA1 tumours (5.0% vs 14.4%, p=0.0001). There was no correlation between mutation status and age of onset, disease specific survival (DSS) or other clinicopathological factors. Comparison with sporadic MBC studies showed similar clinicopathological features. Prognostic variables affecting DSS included primary tumour size (p=0.003, HR:4.26 95%CI 1.63-11.11), age (p=0.002, HR:4.09 95%CI 1.65-10.12), lymphovascular (p=0.019, HR:3.25 95%CI 1.21-8.74) and perineural invasion (p=0.027, HR:2.82 95%CI 1.13-7.06). Unlike familial FBC, the histological subtypes seen in familial MBC were more similar to those seen in sporadic MBC with 46 (76.7%) pure invasive ductal carcinoma of no special type (IDC-NST), 2 (3.3%) invasive lobular carcinomas and 4 (6.7%) invasive papillary carcinoma. A further 8 (13.3%) IDC-NST had foci of micropapillary differentiation, with a strong trend for co-occurrence in BRCA2 carriers (p=0.058). Most tumours were of the luminal phenotype (89.7%), with infrequent HER2 (8.6%) and basal (1.7%) phenotype tumours seen.

Conclusion: MBC in BRCA1/2 carriers and BRCAX families is different to females. Unlike FBC, a clear BRCA1 phenotype is not seen but a possible BRCA2 phenotype of micropapillary histological subtype is suggested. Comparison with sporadic MBCs shows this to be a high-risk population making further recruitment and investigation of this cohort of value in further understanding these uncommon tumours.

Keywords: Male breast cancer, BRCA1, BRCA2, BRCAX, Micropapillary, Familial
Background
Male breast cancer (MBC) is an infrequent and poorly characterised disease. Limited data to date suggests it is epidemiologically and biologically different from female breast cancer (FBC) but it is unknown whether current paradigms and treatment of female disease can be extrapolated to the pathobiology and management of MBC and vice versa. Although some recent large MBC studies have been undertaken, these are population-based and this current report is the largest to describe the genotype, tumour phenotype, complete clinicopathological parameters and survival in MBC from high-risk families.

Accounting for less than 1% of all male cancers, and 0.65% of all breast tumours [1-3], the incidence of MBC has increased steadily from approximately 0.86 to 1.06 per 100,000 males over a 26 year period [4,5] There is controversy surrounding mortality with some suggestion that MBC disproportionately accounts for a higher number of deaths than breast cancer in women [4-7] while other studies suggest parity when comparing age and stage matched cases [8].

Inherited risk factors for MBC appears to be a more significant contributor than in women with estimates of 10% of all MBC cases arising with a family pedigree suggestive of a genetic predisposition [2,9-11]. Unlike women, BRCA2 germline mutation in men confers a significantly higher lifetime risk of developing breast cancer than BRCA1 [2,9-11]. Other genes also implicated in the development of MBC including PTEN [12], P53 [13] and CHEK2 1100delC [14]. Kleinfelter’s syndrome (XXY) [15], environmental and hormonal states that alter the ratio of androgens to estrogens are also thought to contribute to MBC [16]. Recent meta-analysis has also shown an association between previous breast disease, in particular gynaecomastia, and occurrence of MBC [17]. It is still unclear, however, whether this is a precursor lesion, a risk factor for MBC or whether the aetiology and pathogenesis is the same for both conditions.

Despite extensive knowledge about female BRCA1, BRCA2 and other inherited familial breast tumours at present, little is know of male tumours from high-risk families. Comparison of sporadic tumours in both sexes shows; a steady linear increase in incidence in men with age in contrast to the bimodal distribution seen in FBC [2,3,18], an older median age of diagnosis in men [6,8,18], more advanced stage-related tumour characteristics (tumour size >2cm, positive axillary nodes) [2,18] but with more favourable histopathological characteristics (lower tumour grade) and biology (hormone receptor positive tumours) [2,18]. Most MBC studies have been performed with cohorts predominantly composed of “sporadic” population based patients whereas this study is focused on one of the largest groups of MBCs arising in high-risk families evaluating both clinicopathological and genetic associations.

Methods

Study group
Males with breast cancer were obtained from the kConFab repository (http://www.kconfab.org). Criteria for admission to the kConFab study has been previously published [19] (Additional file 1: Table S1) and patients were attained from within Australia and New Zealand between 1998 and 2009. The cases used in the analysis

Table 1 Mutation carrier status and male breast cancer with the kConFab cohort

| Gene | Mutation | Effect |
|------|----------|--------|
| BRCA1 | del exons 21_24 | LGR |
| BRCA1 | del GAAA (STOP 998) | P |
| BRCA1 | 5382_5383 ins C (STOP 1829) | P |
| BRCA2 | del exons 1_2 | LGR |
| BRCA2 | del exons 14_16 | LGR |
| BRCA2 | del del 6024_6025 del TA (STOP 2098) | P |
| BRCA2 | del 6714_6717 del ACAA (STOP 2166) | P |
| BRCA2 | del 6854_6855 del TA (STOP 2223) | P |
| BRCA2 | del 6971_6983 del ATGCCACACATT (STOP 2275) | P |
| BRCA2 | del del 698_702 del AGTCA (STOP 180) | P |
| BRCA2 | del del 7708 C>T (R2494X) | P |
| BRCA2 | del del 8168_8169 ins C (STOP 2661) | P |
| BRCA2 | del del 9132 del C (STOP 2975) | P |
| BRCA2 | del 9161 C>A (S2978X) | P |
| BRCA2 | del del 9610 C>T (R3128X) | P |
| BRCA2 | del del 9610 C>T (R3128X) | P |
| BRCA2 | del del 9610 C>T (R3128X) | P |
| BRCA2 | del del 9610 C>T (R3128X) | P |
| BRCA2 | del del 9708 C>T (R3128X) | P |
| BRCA2 | del del 983_986 del ACAG (STOP 275) | P |
| BRCA2 | del del 995 del C (STOP 276) | P |
| BRCA2 | del del 995 del C (STOP 276) | P |
| BRCA2 | del del 995 del C (STOP 276) | P |
| BRCA2 | del del 995 del C (STOP 276) | P |

Table 2 Characterisation of BRCA1 and 2 mutations of males included within this study

| Gene | Mutation | Effect |
|------|----------|--------|
| BRCA1 | BRCA1 del exons 21_24 | LGR |
| BRCA1 | del GAAA (STOP 998) | P |
| BRCA1 | 5382_5383 ins C (STOP 1829) | P |
| BRCA2 | del exons 1_2 | LGR |
| BRCA2 | del exons 14_16 | LGR |
| BRCA2 | del del 6024_6025 del TA (STOP 2098) | P |
| BRCA2 | del del 6714_6717 del ACAA (STOP 2166) | P |
| BRCA2 | del del 6854_6855 del TA (STOP 2223) | P |
| BRCA2 | del del 6971_6983 del ATGCCACACATT (STOP 2275) | P |
| BRCA2 | del del 698_702 del AGTCA (STOP 180) | P |
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| BRCA2 | del del 9610 C>T (R3128X) | P |
| BRCA2 | del del 9708 C>T (R3128X) | P |
| BRCA2 | del del 983_986 del ACAG (STOP 275) | P |
| BRCA2 | del del 995 del C (STOP 276) | P |
| BRCA2 | del del 995 del C (STOP 276) | P |

Classification of Variants: P = Pathogenic, LGR = Large Genomic Rearrangement, UV = Unclassified Variant.
### Table 3 Clinicopathological features

| Feature                        | All patients (n=60) | BRCA1 (n=3) | BRCA2 (n=25) | BRCAx (n=32) | P-value |
|-------------------------------|---------------------|-------------|--------------|--------------|---------|
| **AGE AT DIAGNOSIS**          |                     |             |              |              |         |
| Median                        | 62.5 (30.1 - 85.6)  | 65.6 (49.5-80.1) | 61 (31.0 - 85.7) | 63.2 (30.1 - 81.8) |         |
| <60 yoa                       | 26 (43.3%)          | 1 (33.3%)   | 11 (44.0%)   | 14 (43.8%)   | NS      |
| >60 yoa                       | 34 (56.7%)          | 2 (66.6%)   | 14 (56.0%)   | 18 (56.3%)   | NS      |
| **DISEASE SPECIFIC MORTALITY**|                     |             |              |              |         |
|                              | 35.0%               | 33.3%       | 40.0%        | 31.3%        |         |
| **SIDE**                      |                     |             |              |              |         |
| Right                        | 36 (60.0%)          | 1 (33.3%)   | 17 (68.0%)   | 18 (56.2%)   | NS      |
| Left                         | 24 (40.0%)          | 2 (66.7%)   | 8 (32.0%)    | 14 (43.8%)   | NS      |
| Unifocal                     | 56 (93.3%)          | 3 (100%)    | 22 (88.0%)   | 31 (96.9%)   |         |
| Multifocal                   | 2 (3.3%)            | 0           | 2 (8.0%)     | 0            |         |
| Bilateral                    | 2 (3.3%)            | 0           | 1 (4.0%)     | 1 (3.1%)     | NS      |
| **HISTOLOGICAL SUBTYPE**      |                     |             |              |              |         |
| Invasive Ductal Carcinoma - No special type | 46 (76.7%)      | 2 (66.7%)   | 18 (72%)     | 28 (87.5%)   |         |
| IDC with Micropapillary component | 8 (13.3%)    | 0           | 6 (24%)      | 2 (6.3%)     |         |
| Invasive Papillary Carcinoma | 4 (6.7%)            | 1 (33.3%)   | 1 (4%)       | 2 (6.3%)     |         |
| Invasive Lobular Carcinoma   | 2 (3.3%)            | 0           | 0            | 2 (6.3%)     | NS      |
| **BRE GRADE**                |                     |             |              |              |         |
| 1                             | 2 (3.3%)            | 0           | 1 (4%)       | 1 (3.1%)     |         |
| 2                             | 31 (51.7%)          | 0           | 12 (48%)     | 19 (59.4%)   |         |
| 3                             | 27 (45.0%)          | 3 (100%)    | 12 (48%)     | 12 (37.5%)   | NS      |
| **ER STATUS (ALLRED 0-8)**   |                     |             |              |              |         |
| 0                             | 1 (1.7%)            | 0           | 0            | 1 (3.3%)     |         |
| 1-5                           | 5 (8.6%)            | 1 (33.3%)   | 2 (8.0%)     | 2 (6.7%)     |         |
| 6-8                           | 52 (89.7%)          | 2 (66.7%)   | 23 (92.0%)   | 27 (90.0%)   |         |
| NA                            | 2                   | 0           | 0            | 2            | NS      |
| **PR STATUS (ALLRED 0-8)**   |                     |             |              |              |         |
| 0                             | 5 (8.8%)            | 0           | 1 (4%)       | 4 (13.8%)    |         |
| 1-5                           | 8 (14.0%)           | 0           | 5 (20%)      | 3 (10.3%)    |         |
| 6-8                           | 44 (77.2%)          | 3 (100%)    | 19 (76%)     | 22 (75.9%)   |         |
| NA                            | 3                   | 0           | 0            | 3            | NS      |
| **HER2**                      |                     |             |              |              |         |
| Amplification                 | 5 (9.1%)            | 0           | 2 (8.3%)     | 3 (10.7%)    |         |
| Non-amplified                 | 50 (90.9%)          | 3 (100%)    | 22 (91.7%)   | 25 (89.3%)   |         |
| NA                            | 5                   | 0           | 1            | 4            | NS      |
| **PHENOTYPE**                 |                     |             |              |              |         |
| Basal                         | 1 (1.7%)            | 0           | 0            | 1 (3.3%)     |         |
| Luminal                       | 52 (89.7%)          | 3 (100%)    | 23 (92.0%)   | 26 (86.7%)   |         |
| HER2                          | 5 (8.6%)            | 0           | 2 (8.0%)     | 3 (10.0%)    |         |
| NA                            | 2                   | 0           | 0            | 2            | NS      |
| **TUMOUR SIZE**               |                     |             |              |              |         |
| Median                        | 17mm (2-50mm)       | 15mm (9-25mm) | 17mm (6-40mm) | 16 (2-50mm)  |         |
| **TUMOUR STAGE**              |                     |             |              |              |         |
| T1a                           | 1 (1.7%)            | 0           | 0            | 1 (3.1%)     |         |
| T1b                           | 8 (13.3%)           | 1 (33.3%)   | 4 (16.0%)    | 3 (9.4%)     |         |
| T1c                           | 31 (51.7%)          | 1 (33.3%)   | 10 (40.0%)   | 19 (59.4%)   |         |
Table 3 Clinicopathological features (Continued)

| T2     | 19 (31.7%) | 1 (33.3%) | 11 (44.0%) | 7 (21.9%) |
|--------|------------|-----------|------------|-----------|
| T3     | 1 (1.7%)   | 0         | 0          | 1         |
|        |            |           |            | NS        |

LYMPHOVASCULAR INVASION

| Absent | 32 (57.1%) | 2 (66.7%) | 14 (60.9%) | 16 (53.3%) |
|--------|------------|-----------|------------|------------|
| Present| 24 (42.9%) | 1 (33.3%) | 9 (39.1%)  | 14 (46.7%) |
| NA     | 4          | 0         | 2          | 2          |
|        |            |           |            | NS         |

PERINEURAL INVASION

| Absent | 31 (56.4%) | 3 (100%)  | 12 (50.0%) | 16 (57.1%) |
|--------|------------|-----------|------------|------------|
| Present| 24 (43.6%) | 0         | 12 (50.0%) | 12 (42.9%) |
| NA     | 5          | 0         | 1          | 4          |
|        |            |           |            | NS         |

PAGET’S DISEASE OF NIPPLE

| Absent | 44 (84.6%) | 2 (100%)  | 19 (86.4%) | 23 (82.1%) |
|--------|------------|-----------|------------|------------|
| Present| 8 (15.4%)  | 0         | 3 (13.6%)  | 5 (17.9%)  |
| NA     | 8          | 1         | 3          | 4          |
|        |            |           |            | NS         |

NODAL STATUS

| Cases with nodes examined | 46 (76.7%) | 3 (100%)  | 20 (80.0%) | 23 (71.9%) |
|---------------------------|------------|-----------|------------|------------|
| Cases with positive nodes | 20 (43.4%) | 2 (66.7%) | 9 (45.0%)  | 9 (39.1%)  |
| Average numbers of nodes examined per case | 12.9 (1-30) | 16.3 (13-24) | 15.9 (1-30) | 10.1 (1-29) |

NODAL STAGE

| N0     | 26 (56.5%) | 1 (33.3%) | 11 (55.0%) | 14 (60.1%) |
|--------|------------|-----------|------------|------------|
| N1     | 18 (39.1%) | 2 (66.7%) | 8 (40.0%)  | 8 (34.8%)  |
| N2     | 2 (4.3%)   | 0         | 1 (5.0%)   | 1 (4.3%)   |
| Cases with extranodal extension | 8 (17.4%) | 0         | 5 (25.0%)  | 3 (13.0%)  |
|        |            |           |            | NS         |

MARGINS

| Clear | 29 (48.3%) | 1 (33.3%) | 12 (48.0%) | 16 (50.0%) |
|-------|------------|-----------|------------|------------|
| Involved | 15 (25.0%) | 0 | 6 (24.0%) | 9 (28.1%) |
| Not assessable | 16 (26.7%) | 2 (66.7%) | 7 (28.0%) | 7 (21.9%) |
|        |            |           |            | NS         |

DCIS

| Absent | 14 (25.0%) | 0 | 7 (29.2%) | 7 (24.1%) |
|--------|------------|---|-----------|-----------|
| NA     | 4          | 0 | 1         | 3         |
| Present| 42 (75.0%) | 3 (100%)  | 17 (70.8%) | 22 (75.9%) |
| Nuclear Grade | Low | 2 (4.8%) | 0 | 2 (11.8%) | 0 |
| Intermediate | 26 (61.9%) | 1 (33.3%) | 10 (58.8%) | 15 (68.0%) |
| High   | 14 (33.3%) | 2 (66.7%) | 5 (29.4%) | 7 (31.8%) |
|        |            |           |            | NS         |

NS – Not significant.

had a diagnosis of breast cancer between 1980 – 2009. Clinical parameters, including TNM staging, tumour recurrence, occurrence of non-breast primary tumours and death were obtained from referring clinical centres, kConFab questionnaires and state death registries. Information on pedigree, mutational status and testing were available from the kConFab central registry. All available slides from all cases were reviewed by a pathologist for relevant histopathological parameters. Histological classification was based on criteria set by the World Health Organisation. This work was carried out with approval from the Peter MacCallum Cancer Centre Ethics Committee (Project No: 11/61).

Mutation detection

Mutation test results were generated through two avenues. If a clinic had performed mutation screening, the clinic report was passed onto the kConFab central registry. If no clinic mutation testing had been performed, the kConFab core research laboratory performed mutation testing. Testing for BRCA1 and BRCA2 mutations was performed on DNA extracted from 18 ml sample of anticoagulated
blood or mouthwash kit [20]. The blood processing protocol [21] generated a nucleated cell product for DNA extraction. DNA was extracted as required (QIAamp DNA blood kit, Qiagen GmbH, Hilden, Germany). Testing of index cases in kConFab families was carried out by denaturing high performance liquid chromatography or multiplex ligation-dependent probe amplification [22]. BRCA1 and BRCA2 variants were classified into the following categories with criteria as posted on kConFab’s website [23]: pathogenic, splice-site variant, variant of unknown significance and polymorphism. Once the family mutation had been identified, all pathogenic (including splice site) variants of BRCA1 and BRCA2 were genotyped by kConFab in all available family members’ DNA.

Tissue microarrays (TMAs) and expression analysis by immunohistochemistry (IHC)
TMAs were created from archival paraffin material. Two 1mm cores were taken for each tumour. TMA sections were cut at 4 μm thick intervals, de-waxed and hydrated. Antigen retrieval was performed according to manufacturers’ instructions and endogenous peroxidase activity blocked before incubating sections with desired antibodies. Tumours were separated into molecular phenotypes as per Nielsen et al [24]. Expression of estrogen receptor-α (ER) (Ventana, clone SP1), progesterone receptor (PgR) (Ventana, clone 1E2), epidermal growth factor receptor (EGFR) (Zymed, clone 31G7) and cytokeratin (CK) 5 (Cell Marque, clone EP1601Y) was performed. HER2 amplification was assessed by silver in situ hybridisation (SISH) using the INFORM HER2 DNA probe (Ventana). Nuclear expression of ER and PgR was scored as per the Allred scoring system [25] (intensity + percentage of tumour cells staining, 0–8) and separated into absent (score 0/8), low (1-5/8) and high (6-8/8). HER2 gene status was reported as the average number of copies of the HER2 gene per cell in 30 tumour cells. Gene status was assessed as per the guidelines recommended by Wolff et al [26]. EGFR was scored positive for any membranous staining of tumour cells. Expression of CK5 was defined as positive when cytoplasmic and/or membranous staining was observed in tumour cells. Tumours were assigned to the following subtypes; Luminal (ER positive, HER2 negative), HER2 (HER2 positive), Basal (ER PgR and HER2 negative, CK5 and/or EGFR positive), and Null/negative (ER, PgR, HER2, CK5/6 and EGFR negative).

Statistical analysis
Comparison of groups was made with using Mann–Whitney U for non-parametric continuous distributions and chi-square test for threshold data. Kaplan-Meier survival curves were plotted using breast cancer related death as the endpoint and compared using a log rank test. Regression analyses as time to fail curves were plotted for age of diagnosis and occurrence of second non breast primary tumours. Cox proportional hazard regression model was used to identify independent prognostic factors for disease specific survival (DSS). Analysis was performed with GraphPad Prism 5 software (GraphPad Prism version 5.04 for Windows, GraphPad Software, La Jolla California USA). A two-tailed P-value test was used in all analyses and a P-value or less than 0.05 was considered statistically significant.

Results
Mutation analysis
The prevalence of MBCs in the kConFab registry with known gene mutations is summarised in Table 1 and 2. There were 5 (1.2%) of 429 known BRCA1 mutation carriers and 35 (10.3%) of 339 BRCA2 carriers who developed breast cancer. Of these, 3 and 25 cases respectively had reports, slides and tissues available for examination and were included in the study. Of the 3 BRCA1 cases, 2 had a pathogenic mutation with 1 large genomic rearrangement. Of the 25 BRCA2 cases, 22 had a pathogenic mutation, 2 large genomic rearrangements and 1 an unclassified variant. Within non-BRCA1/2 families, of a total of 19,137 males, 78 (0.4%) developed breast cancer with 32 cases available for use in the study.

Clinicopathological features
The clinicopathological features are summarised in Table 3. The overall median age of diagnosis was 62.5 years (range 30.1-85.6 years), and mean age of diagnosis 60.0 years. There was no significant difference in clinicopathological factors between BRCA1, BRCA2 carriers and BRCAX males including age of onset (Figure 1). Surgical treatment was by wide local excision (33.3%, 20/60) and mastectomy (66.6%, 40/60). All tumours
were present within 30mm of the subareolar region and the nipple. Four cases (6.6%) had multifocal disease with 2 cases of bilateral breast cancer, of which one was a metachronous BRCAx tumour with a 10 year interval and the other a BRCA2 carrier with contralateral tumour occurring 12 years after the primary lesion.

Tumour size ranged from 2 mm to 50 mm (median 17 mm). The most common histological subtype was infiltrating ductal carcinoma of no special type (IDC-NST) (90%, 54/60) (Figure 2a) with 2 cases of invasive lobular carcinoma (3.3%) (Figure 2b and c) and 4 cases of invasive papillary carcinoma (6.7%) (Figure 2d and e). Of the IDC-NST tumours, 8 had areas between 15 to 40% of invasive micropapillary carcinoma (Figure 2f).

Tumours were of mainly grade 2 (51.7%) and grade 3 (45.0%). Lymphovascular and perineural invasion (PNI) was identified in 42.9% (24/56) and 43.6% (24/55) of cases respectively when able to be assessed. Paget's involvement of the nipple was seen in 15.4% of cases (8/52) when assessable. Most tumours had a component of DCIS present (75%, 42/56). Normal breast tissue and gynaecomastia was observed in 65.1% (28/43) and 11.6% (5/43) of cases respectively. Forty six cases had lymph node sampling with 7 sentinel node biopsy only (15.2%) and

![Figure 2](http://www.biomedcentral.com/1471-2407/12/510)
the remainder axillary dissection (84.7%). On average 1.6 sentinel nodes (median 1, range 1–3) were examined and an average of 15 nodes from axillary dissections (median 13, range 4–30). Of these, 1 (14.3%) sentinel node had metastatic disease and 19 axillary dissections had positive nodal disease (48.7%) with extranodal extension in 8 cases.

Most tumours were ER and PgR positive (Additional file 2: Figures S1 and Additional file 3: Figure S2), with 89.7% (52/58) and 77.2% (44/57) of cases respectively scored as high (Allred score 6-8/8) (Figure 3). HER2 amplification was seen in 9.1% (5/55) of cases (Figure 4). The range of HER2 amplification was 6.1-10.5 signals per nuclei in amplified cases. Two tumours were unable to be immunophenotyped completely. Based on analysis of the remainder, the most common intrinsic subtype was Luminal (89.7%, 52/58) followed by HER2 (8.6%, 5/58) and Basal (1.7%, 1/58). The Basal subtype (Figure 5) was a BRCAX tumour with prominent CK5 and EGFR staining but also low ER nuclear positivity. Morphology of this tumour was more consistent with a basal subtype rather than a luminal type tumour.

There was a trend towards BRCA2 tumours having an invasive micropapillary component (24% 6/25, p=0.0574) and high Bloom Richardson Ellis (BRE) grade for BRCA1 tumours (100% grade 3 3/3, p=0.0855), however these observations did not reach statistical significance. Overall, clinicopathological factors and intrinsic subtypes were not associated with BRCA1 or 2 mutation carrier status and unlike in female breast cancer [27], there was no association between BRCA1 mutational status and basal cell phenotype.

Characteristics are compared with other recent large MBC studies containing >50 patients and completed within the last 4 years [6-8,28-40] (Additional file 4: Table S2) and with the previous study of female breast cancers within the kConFab cohort [41].
Disease specific survival

The overall 5 and 10 year disease specific survival rates were 84.6% and 40.6% for all cases, 100% and 0% for BRCA1 case, 80.6% and 42.2% for BRCA2 cases and 86.7% and 41.2% for BRCAX cases (Figure 6). Clinico-pathological variables (Figure 7) that were of prognostic significance for DSS included a primary tumour size >2.0 cm (HR:4.26 95%CI 1.63-11.11, p=0.003), age at diagnosis > 65 years (HR:4.09 95%CI 1.65 -10.12, p=0.002), lymphovascular invasion (HR:3.25 95%CI 1.21-8.74, p=0.019) and PNI (HR:2.82 95% CI 1.13-7.06, p=0.027) (Table 4). A strong adverse trend for loss or low progesterone receptor expression was also seen (HR:2.59 95% CI 0.86-7.80, p=0.091) but fell short of being statistically significant.

Comparisons of mutation carrier status, tumour grade, presence of nodal disease, involvement of surgical margins and multifocality were not prognostically significant (all p>0.05).

Second cancers

Ten patients had a second major malignancy (5/25 BRCA2 mutation carriers, 5/31 BRCAX cases) (Table 5). No BRCA1 patients developed a second malignancy. In eight (80%) cases, the diagnosis of the primary breast tumour was the sentinel event while in two cases (20%) another malignancy was diagnosed preceding the breast cancer. The median time to diagnosis was 3.8 years after the diagnosis of the breast cancer (range 3 years previous to 15.5 years after). The most common second malignancy was prostatic acinar adenocarcinoma (50%, 5/10). Of note, one patient had an adenocarcinoma of the abdominal wall of unknown primary origin with exclusion of a breast metastasis. Mutation carrier status was not prognostic of development of a second malignancy when comparing BRCA2 and BRCAX cohorts (Figure 8).

Discussion

To the best of our knowledge this is the largest high-risk population based study to date describing the genotypic, conventional clinicopathological and intrinsic phenotypic characteristics of MBCs arising within breast cancer families. Previous studies have either not contained large numbers of patients with a significant family history [30,34,35,37,43,47], not commented or examined family history [6-8,28,29,36,39,40], or have contained large numbers of such cases with strong family pedigree but not described clinicopathological features [32] (Table 4). As a large proportion of MBCs are purported to arise in families with breast cancer and in particular BRCA2 mutation carriers, further description of this cohort is of significance in understanding and characterising the disease.

The incidence of MBC in BRCA2, BRCA1 and BRCAX males is significantly higher than the lifetime cumulative incidence of 0.1% in the general population [17,48] confirming this group as a high risk for MBC. However, the representation of carriers is different to that of familial FBC with direct comparison within the kConFab registry [41] showing an increased proportion of BRCA2 male carriers and underrepresentation of BRCA1 male tumours. This suggests that significant gender associated modifiers such as high estrogen levels may affect BRCA1 penetrance over BRCA2. Comparing studies of sporadic MBC [6-8, 28-32,35,37-40,44], the median and mean age of onset in our patients is also younger, and this together with the observation of frequent multifocality or bilateral disease reflects the pattern of cancer often seen with underlying genetic predisposition as seen in familial FBC. A recent large population based study by Ottini et al. [45] containing 46 BRCA2 mutation carriers also observed a high rate (15.2%) of contralateral breast cancer in these carriers, thus supporting this observed pattern.

Compared with other MBC groups, our study appeared to have a higher proportion of high grade tumours with only 3.3% of tumours of BRE grade I, the lowest within any MBC cohort reported to date. We also reported the highest proportion of invasive papillary carcinomas with 6.7% of cases, the next highest in the literature being 5.5% by Ottini et al. [45]. The histopathological tumour characteristics of our group otherwise is comparable to that seen in previous studies of sporadic MBC with the majority of cancers being invasive ductal carcinoma. This is higher than that seen in FBCs from kConFab [41]. Unlike FBC, we also observed proportionately less lobular carcinoma which is thought to reflect paucity of lobular and acinar units in males [49].

We also report a relatively higher proportion of tumours with invasive micropapillary areas particularly within BRCA2-associated tumours, an association not previously reported. Recent studies suggest that these
Figure 7 (See legend on next page.)
lesions are a distinct entity with more aggressive behaviour than IDC-NST [50]. The distinct histological features of these tumours correlate with distinct molecular genetic profiles [42], however, in female cancer a correlation with BRCA2 mutation has not been described or suggested [10]. Ottini et al [45], also describe a BRCA2 MBC phenotype with a high proportion of BRE grade 3 tumours (54.8%), loss of PgR expression (67.9%) and HER2 amplification (63.2%). Similar to them, our BRCA2 carriers contained a large proportion of BRE grade 3 but was not significantly different to the BRCA1 and BRCAX population. The expression of ER and PgR in our familial MBCs is similar to that seen in sporadic MBC, with proportionately higher levels than seen in FBC, and absence of PgR expression did not discriminate a BRCA2 phenotype. Subsequently, the majority of our cases were also of the luminal subtype. Reported HER2 amplification in MBC has been more variable than ER and PgR with studies demonstrating between 3.3% [40] to 28.4% [45] of cases showing HER2 amplification. While our study and Ottini are the only to date to examine the association with BRCA status, using routine diagnostic testing for HER2 we see lower frequency of HER2 amplification both overall (9.1%) and within our BRCA2 carriers (8.3%) as a subgroup. Our results are consistent with most MBC studies that suggest HER2 amplification is seen half as frequently as that in FBC [41].

The few numbers of BRCA1 MBCs in our cohort precludes extensive clinicopathological analysis, however, in contrast and unlike tumours seen in BRCA1 female carriers [27,51], cancers of medullary/basal cell phenotype in BRCA1 males has not been reported in the literature and was also not observed in our cohort of BRCA1 males. The paucity of tumours of basal phenotype in our cohort overall also reflected observations of other MBC studies.

Several prognostic markers in our study are also reported in both FBC and sporadic MBC. In our study, we confirmed many but also identified PNI as being of prognostic significance, which has not been reported previously in MBC. Its presence, being double most rates reported in FBC [52,53], may be due to frequent subareolar tumour location which is less frequently seen in women, and comparable to frequent perineural involvement seen in other epithelial tumours such as pancreatic [54] and prostatic [55] adenocarcinoma where the organs have closer proximity to nerve bundles. While mixed prognostic significance of PNI has been seen in FBC studies [53], PNI positive tumours have been shown to be more often associated with positive nodal status and hormonal positivity [53], both of which are more commonly seen in MBC in general, and in our study cohort when compared with FBC.

While our numbers are not large, a considerable proportion (16.6%) of the BRCA2 and BRCAX patients developed a second non-breast primary malignancy. The onset or histological type of these tumours did not correlate with mutation carrier status. These findings are consistent with those previously reported in MBC cohorts where the relative risk for developing prostate cancer of 19.08 (p<0.0001, 95%CI 4.50-80.91) and 20.56 (p<0.0001, 95%CI 6.30-67.12) times the normal population for BRCA2 and BRCAX male patients with breast cancer respectively. These data show that patients with MBC may be a high-risk group for developing second malignancies, even when comparing with BRCA2 carriers.

### Table 4 Clinicopathological variables of prognostic significance

| Variable                                | P-value | Hazard ratio | 95% confidence interval |
|-----------------------------------------|---------|--------------|-------------------------|
| Lymphovascular Invasion                 | 0.0194  | 3.25         | 1.21 - 8.74             |
| Perineural Invasion                     | 0.0266  | 2.82         | 1.13 - 7.06             |
| Tumour Size > 20mm                      | 0.0030  | 4.26         | 1.63 - 11.11            |
| Age of Diagnosis > 65 years             | 0.0024  | 4.09         | 1.65 - 10.12            |
| Low Progesterone Receptor Expression    | 0.0909  | 2.59         | 0.86 - 7.80             |

(See figure on previous page.)

**Figure 7** Clinicopathological variables and disease specific survival: (a) BRE grade, (b) lymphovascular invasion, (c) perineural invasion, (d) primary tumour size, (e) Paget’s disease of the nipple, (f) nodal status, (g) age at diagnosis, (h) histological subtype, (i) Intrinsic phenotype, (j) PgR immunohistochemical expression, (k) ER immunohistochemical expression, (l) HER2 amplification, (m) involvement of margins, (n) diagnosis of second non-breast primary malignancy, (o) multifocal disease.
without MBC. Whether this is due to hormonal influence driving both tumour types or underlying genetic factors requires further study in a larger data set.

Conclusions

This is the largest clinicopathological study of male breast cancers arising in breast cancer families. It identifies three high-risk population groups (BRCA1/2, BRCAX) which may be important for screening for male breast cancer. The clinical and pathological characteristics are different to familial female breast cancer but similar to previously described male breast cancer studies which have contained but not separately analysed sporadic and familial breast cancers. Notably, our study in comparison contains proportionately more multifocal disease, a younger age of onset and a significant proportion with a second major malignancy, features often seen in tumours that arise with a genetic predisposition. BRCA2 mutation status did not appear to correlate with a distinct clinicopathological phenotype or disease behaviour, and a strong trend was seen within BRCA2 carrier tumours containing areas of micropapillary carcinoma possible suggesting a possible BRCA2 male breast cancer phenotype. Further subgroup analysis, in particular of BRCA1 tumours, was limited by the number of cases available. Further recruitment of well characterised tumours in breast cancer families, in particular a focused collection of BRCA1 cases, is warranted to validate and characterise familial MBC further.

Additional files

Additional file 1: Table S1. Eligibility criteria for recruitment of families into kConFab.
Additional file 2: Figure S1. Distribution of ER Allred histoscores.
Additional file 3: Figure S2. Distribution of PgR Allred histoscores.
Additional file 4: Table 2. Comparison of previous MBC studies.

Abbreviations
MBC: Male Breast Cancer; FBC: Female Breast Cancer; DSS: Disease Specific Survival; ER: Estrogen Receptor; PgR: Progesterone Receptor; CK: Cytokeratin; EGFR: Epidermal Growth Factor Receptor; IDC-NST: Invasive Ductal Carcinoma of No Special Type; PNI: Perineural Invasion; BRE: Bloom Richardson Ellis.

Competing interests
The authors declare they have no competing interests.

Authors’ contributions
SD – Pathology review of all cases, conception and design of study, analysis and interpretation of data, statistical analysis, manuscript preparation, NJ – Immunohistochemistry and ISH, kConFab Investigators – BRCA1/2 testing, acquisition of all data, manuscript preparation, SBF – Conception and design of study, analysis and interpretation of data, manuscript preparation. All authors read and approved the final manuscript.

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References

1. Weiss JR, Moyisch KB, Swede H: Epidemiology of male breast cancer. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 2005, 14(13):26.

2. Korde LA, Zuzewski JA, Kamin L, Giordano S, Domchek S, Anderson WF, Bartlett JM, Melkonian L, Nahleh Z, Bergh J, et al: Multidisciplinary meeting on male breast cancer: summary and research recommendations. J Clin Oncol 2010, 28(12):2114–223.

3. SEER Database. http://seer.cancer.gov.

4. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ: Cancer statistics, 2004. CA: a cancer journal for clinicians 2004, 54(1):8–29.

5. Nilsson C, Holmqvist M, Bergkvist L, Hedenfalk I, Lambre M, Fialkovic ML: Similarities and differences in the characteristics and primary treatment of breast cancer in men and women - a population based study (Sweden). Acta oncologica (Stockholm, Sweden) 2011, 50(7):1088–1098.

6. Anderson WF, Jatoi I, Tse J, Rosenberg PS: Male breast cancer: a population-based comparison with female breast cancer. J Clin Oncol 2010, 28(22):239.

7. Foerster R, Foerster FG, Woff V, Schubotz B, Baaske D, Wolfarten M, Kuhn WC, Rudolphi C: Matched-pair analysis of patients with female and male breast cancer: a comparative analysis. BMC cancer 2011, 11:335.

8. Basham VM, Lipscombe JM, Ward JM, Gayther SA, Ponder BA, Easton DF, Pharaoh PD: BRCA1 and BRCA2 mutations in a population-based study of male breast cancer. Breast cancer research; 2002, 4(1):R2.

9. Evans DG, Bulman M, Young K, Gohlale D, Lalor F: High detection rate for BRCA2 mutations in male breast cancer families from North West England. Familial cancer 2001, 1(3):131–133.

10. Thompson D, Easton D: Variation in cancer risks, by mutation position, in BRCA2 mutation carriers. Am J Hum Genet 2001, 68(2):410–419.

11. Fackenthal JD, Marsh DJ, Richardson AL, Cummins SA, Eng C, Robinson BG, Olopade OI: Male breast cancer in Cowden syndrome patients with germline PTEN mutations. J Med Genet 2001, 38(3):159–164.

12. Anevi A, Anvei TF, Youngson B, Rosen PF, Borgen PI: Mutations of the p53 gene in male breast cancer. Cancer 1995, 75(9):2233–2238.

13. Wasilewski M, den Bakker MA, van den Ouweland A, Meijer-van Gelder ME, Portegies H, Klijn JG, Meijers-Heijboer H, Foeckens J, Schutte M, CHEK2 11000cDei and male breast cancer in the Netherlands. Breast Cancer Res Treat 2009, 116(3):297–401.

14. Evans DB, Critchlow RW: Carcinoma of the male breast and Kleneifeister’s syndrome: is there an association? CA: cancer journal for clinicians 1987, 37(4):246–251.

15. Brinton LA, Richarson DS, Gierach GL, Lacey Jv Jr, Park Y, Hollenbeck AR, Schatzkin A: Prospective evaluation of risk factors for male breast cancer. J Nat Cancer Inst 2008, 100(20):1477–1484.

16. Sasco AJ, Lowenberg MB, Pakie – de Jong P: Review article: epidemiology of male breast cancer. A meta-analysis of published case–control studies and discussion of selected aetiological factors. Int J Cancer J Int du cancer 1993, 53(4):583–549.

17. Anderson WF, Althuis MD, Brinton LA, Devesa SS: Is male breast cancer similar or different than female breast cancer? Breast Cancer Res Treat 2004, 83(1):57–66.

18. Mann GJ, Thome H, Balleine RL, Butow PN, Clarke CL, Edkins E, Evans GM, Fereday S, Haen E, Gattas M, et al: Analysis of cancer risk and BRCA1 and BRCA2 mutation prevalence in the kConFab familial breast cancer resource. Breast Cancer Res 2006, 8(1):R12.

19. Lumm A, Le Marchand L: A simple mouthwash method for obtaining genomic DNA in molecular epidemiological studies. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 1998, 7(8):719–724.

20. kConFab Biospecimen Protocol: http://www.konfab.org/epidemiology/biospecimen_protocol.html.

21. Hugenvorst FB, Nederlif PM, Gilles J, McEneaney C, Giepplandt M, Prunel R, Regenius R, van Welken T, van Speaendonk R, Menko, FH, et al: Large genomic deletions and duplications in the kConFab1 gene identified by a novel quantitative method. Cancer Res 2003, 63(7):1449–1453.

22. kConFab Classification of BRCA1 and BRCA2 Mutations. http://www.kconfab.org/progress/mutations.asp.

23. Nielsen TO, Hsu FD, Jensen K, Cheung M, Karaca G, Hu Z, Hernandez-Boussard T, Livasy C, Cowan D, Dresler L, et al: Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. Clinical Cancer Res: an official journal of the American Association for Cancer Research 2004, 10(16):5367–5374.

24. Lakhani SR, Reis-Filho JS, Penault-Llorca F, van der Vijver M, Parry S, Bishop T, Benitez J, Rivais C, Bignon YJ, et al: Prediction of BRCA1 status in patients with breast cancer using estrogen receptor and basal phenotype. Clinical Cancer Res: an official journal of the American Association for Cancer Research 2005, 11(14):5175–5180.

25. Arslan UY, Oksuzoglu B, Ozdemir N, Aksoy S, Alkis N, Gok A, Kaplan MA, Gunus M, Berk V, Uncu D, et al: Outcome of non-metastatic male breast cancer: 118 patients. Med Oncol (Northwood, London, England) 2011, 29(2):554–60.

26. Bourhafour M, Belbaraka R, Souadka A, M'Rabti H, Tijami F, Errihani H: Male breast cancer: a report of 127 cases at a Moroccan institution. BMC Res notes 2011, 4:219.

27. Cutuli LB, Le-Nir CC, Senin D, Kirova Y, Gaci Z, Lernamski C, De Lafontan B, Zoubir M, Maingon P, Mignotite H, et al: Male breast cancer. Evolution of treatment and prognostic factors. Analysis of 489 cases. Critical reviews in oncology/hematology 2010, 73(3):246–254.

28. Ding YC, Steele L, Kuan CJ, Greilac S, Neuhausen SL: Mutations in BRCA2 and PALB2 in male breast cancer cases from the United States. Breast Cancer Res Treat 2011, 126(2):771–778.

29. Evans DG, Bulman M, Young K, Howard E, Baylis S, Wallace A, Laloo F: BRCA1/2 mutation analysis in male breast cancer families from North West England. Familial cancer 2008, 7(2):113–117.

30. Johannson I, Nilsson C, Berglund P, Strand C, Jonsson G, Staaf J, Ringner M, Nevanlinna H, Bardkottardt RB, Borg A, et al: High-resolution genomic profiling of male breast cancer reveals differences hidden behind the similarities with female breast cancer. Breast cancer research and treatment 2011, 129(5):747–760.

31. Kluk Jv, Lee MC, Park CK, Meade T, Minton S, Harris E, Kim J, Laronga C: Male breast cancer: management and follow-up recommendations. The Breast 2011, 175(5):503–509.

32. Liukkonen S, Saarto T, Maenpaa H, Sjostrom-Mattson J: Male breast cancer: a survey at the Helsinki University Central Hospital during 1981–2006. Acta Oncol(Stockholm, Sweden) 2010, 49(3):322–327.

33. Mans H, Verkooijen HM, Chia KS, Bouchardy C, Pukall E, Laronningen S, Mellemkjaer L, Czene K, Hartman M: Incidence and outcome of male breast cancer: an international population-based study. J Clin Oncol 2011, 29(33):4381–4386.

34. Nahleh ZA, Srikantiah R, Safa M, Jazieh AR, Muhleman A, Komrokji R: Male breast cancer in the veterans affairs population: a comparative analysis. Cancer 2007, 109(1):1471–1477.

35. Otni L, Rizzolo P, Zanna I, Falchetti M, Ceccarelli K, Vezzosi V, Gulino A, Giannini G, Bianchi S, et al: BRCA1/BRCA2 mutation status and clinical-pathologic features of 108 male breast cancer cases from Tuscany: a population-based study in central Italy. Breast Cancer Res Treat 2009, 116(3):577–586.
46. Tavassoli FA, Devilee P, Organization WH, Cancer IAR: Ouriel K, Lotze MT, Hinshaw JR: Analysis of prognostic factors in male breast cancer: a report of 72 cases from a single institution. Chinese J Cancer 2010, 29(2):184-188.

41. Loughrey M, Provan PJ, Byth K, Balleine RL: Perineural invasion has no prognostic significance of lymph node metastasis and intrapancreatic perineural tumor characteristics in male breast cancer: a population-based study in Italy. Cancer Res 2003, 63(2):342-347.

39. Shaaban AM, Ball GR, Brannan RA, Cserni G, Benedetto AD, Dent J, Fulford L, Honarpisheh H, Jordan L, Jones J, et al: A comparative biomarker study of 514 matched cases of male and female breast cancer reveals gender-specific biological differences. Breast Cancer Res Treat 2011, 128(3):949-958.

40. Zhou FF, Xia LP, Wang X, Guo GF, Rong YM, Qiu HJ, Zhang B: Genomic and immunophenotypical characterization of pure micropapillary carcinomas of the breast. J Pathol 2008, 215(4):398-410.

48. Fentiman IS, Fourquet A, Prognostic factors of carcinoma of the breast and female genital organs. Lyon, France: IARC Press; 2003.

58. Willems AJ, Dawson SJ, Samarutunga H, De Luca A, Antill YC, Hopper JL, Thorne HJ: Loss of heterozygosity at the BRCA2 locus detected by multiplex ligation-dependent probe amplification is common in prostate cancers from men with a germline BRCA2 mutation. Clinical Cancer Res: an official journal of the American Association for Cancer Research 2008, 14(10):2953-2961.

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