***Original Article***

**Title: Genetic testing in a cohort of young patients with *HER2* amplified breast cancer**

Authors: D.M. Eccles1, N. Li2, 2b, R. Handwerker1, T. Maishman1, E.R. Copson1, L.T. Durcan1, S.M.Gerty1, L. Jones3, D.G. Evans4, L. Haywood3, I. Campbell2,2a.

1. Faculty of Medicine and Cancer Sciences, University of Southampton, Southampton, UK

2. Cancer Genetics Laboratory, Peter MacCallum Cancer Centre, East Melbourne, Australia

2a. Sir Peter MacCallum Department of Oncology, University of Melbourne, Melbourne, Australia

2b. Cancer Biology Research Center Tongji Hospital, Huazhong University of Science and Technology, Wuhan, China

3. Barts Cancer Institute (CRUK) and Centre for Tumour Biology, Queen Mary University of London, London, UK

4. Genomic Medicine Institute of Human Development, University of Manchester and St Mary's Hospital, Manchester, UK

Corresponding author: Professor Diana M Eccles, Cancer Sciences, University of Southampton, Somers Cancer Research Building, Southampton General Hospital, Tremona Road, Southampton SO16 6YA, UK

Phone: +44(0)2381206593

Email: d.m.eccles@soton.ac.uk

Na Li

Ronja Handwerker

Tom Maishman

Ellen Copson

Lorraine Durcan

Sue Gerty

Louise Jones

Gareth Evans

Linda Haywood

Ian Campbell

# Background

### Introduction: Young age at diagnosis for breast cancer raises the question of genetic susceptibility. We explored breast cancer susceptibility genes testing amongst patients with *HER*2 amplified invasive breast cancer aged 40 years or younger.

### Patients and methods: Patients were selected from a large UK cohort study and were aged ≤40 at diagnosis with confirmed *HER*2 amplified breast cancer. The probability of finding a BRCA gene mutation was calculated based on family history. Genetic testing was either clinical testing for *BRCA*1 and *BRCA*2 with a subset also tested for *TP53* mutations, or research based testing using a typical panel comprising 17 breast cancer susceptibility genes (CSGs) including *BRCA*1, *BRCA*2 and *TP53*.

### Results: There were 591 eligible patients. Clinical testing results were available for 133 cases; an additional 263 cases had panel testing. BRCA testing across 396 cases found 8 *BRCA*2 (2%) and 6 *BRCA*1 (2%) pathogenic mutations. Of 304 tested for *TP53* mutations overall 9 (3%) had deleterious *TP53* mutations.

### Of 396 patients, 101 (26%) met clinical criteria for BRCA testing (≥10% probability), amongst whom BRCA testing yielded 11% with pathogenic *BRCA* mutations (6 *BRCA*2, 5 *BRCA*1). Where the probability was calculated to be <10%, only 4/295 (1%) had BRCA mutations. Amongst the 59 patients meeting the 10% threshold who had *TP53* testing, there were 7 mutations (12%). Likely functionally deleterious mutations in 14 lower penetrance CSGs were present in 12/263(5%) panel tested patients.

### Conclusion: Patients under 41 at diagnosis with *HER*2+ breast cancer and no family history of breast cancer can be reassured that they have a low chance of being a high risk gene carrier. If there is a strong family history, not only BRCA but also *TP*53 gene testing should be considered. The clinical utility of testing lower penetrance CSGs remains unclear.

# Keywords

*HER2* positive, breast cancer, young onset, genetic testing, gene panel

# Key message

### Young breast cancer patients diagnosed with *HER*2+ breast cancer and no family history of breast cancer can be reassured that they have a low chance of being a high risk gene carrier. If there is a strong family history, not only BRCA but also *TP*53 gene testing should be considered. The clinical utility of testing lower penetrance CSGs remains unclear.

# Introduction

Amplification of the EGFR2 tyrosine kinase receptor in breast cancers (*HER2*+ BC) is reported in about 15-25% of all breast cancers[1-3]. *HER2*+ BCs are more frequently diagnosed in younger patients. Triple negative breast cancers (TNBC, malignant cells do not express oestrogen, progesterone receptor or have *HER2* amplification) are also reported more frequently in younger onset patients [4] and account for a high proportion (>70%) of the breast cancers diagnosed in *BRCA1* mutation carriers[5]. In contrast to 15-25% of patients with breast cancer in general, *HER2* amplification is less frequent in *BRCA1* (10%) and *BRCA2* (13%) carrier breast cancers [5]. However, patients with a germline mutation in the *TP53* gene, which confers a higher risk of breast cancer with a younger average age at onset than *BRCA*1 or *BRCA*2, predominantly develop *HER2*+ BC[6-8].

New guidelines for germline genetic testing emphasise the significant pick up of germline *BRCA1* and 2 mutation carriers amongst patients presenting with triple negative breast cancer particularly at younger ages[9]. Increasingly patients are being referred to consider genetic testing purely based on young age at onset. Oncologists and geneticists are more frequently facing the option of requesting a panel of cancer susceptibility genes, most with much lower penetrance than *BRCA1* and *BRCA2*. This has emerged as a consequence of technology advances that make sequencing larger amounts of DNA for less money possible. However many of the genes on these panels are associated with rather limited knowledge about clinical consequences of carrying a variant even where that variant is clearly deleterious from a molecular perspective[10].

In order to better inform young patients with breast cancer who may wish to discuss genetic testing, we undertook to determine the prevalence of variants in a typical panel of breast cancer susceptibility genes in a large series of young onset *HER2*+ BC cases with and without family history based criteria that qualified them for *BRCA1*/2 mutation analysis based on most current national guidelines.

# Patients and methods

**Study Design**

Patients included in this study were recruited to the parent UK population based study Prospective study of Outcomes in sporadic versus Hereditary breast cancer (POSH). The protocol and study cohort description for POSH are published [3, 11].

*HER2*+ sub-study: The cases included in the present study all presented with *HER2* amplified primary breast cancer. For inclusion in the study patients had to have provided a blood sample with DNA successfully extracted and available for analysis. In addition the diagnosed breast cancer had to have clear evidence of *HER2* positive status (supplementary figure 1). Lines of evidence for *HER2*+ tumour were (1) Immunohistochemistry - strong membrane staining of tumour either on whole section or tissue core (score 3+) from the clinical diagnostic report; (2) Immunohistochemistry on research tissue micro-arrays (TMAs) - cases were represented in 0.6mm cores in triplicate on TMAs and scored by experienced breast cancer pathologist (LJ); (3) Fluorescent (or other) In Situ Hybridisation (ISH) method showing clear amplification (Clinical report); (4) Clinical data - no pathology report was available but the patient had received a *HER2* receptor antagonist as part of their documented treatment. All negative, unconfirmed and borderline cases were excluded including those where tumours scored IHC 2+ (borderline) from pathology report or on TMA or both if no supplementary FISH report was available.

**Genetic data**

*Family history data*: Family history was recorded from a family history questionnaire completed by patients at recruitment. The occurrence of any subsequent primary breast or ovarian cancer was taken from the annual clinical research forms. Family history was used to estimate the probability of being a *BRCA1*/2 carrier using the freely available and validated online software BOADICEA without adjustment for tumour pathology (<https://pluto.srl.cam.ac.uk/cgi-bin/bd3/v3/bd.cgi>)[12]. The BOADICEA score was recalculated if a patient had developed a contralateral new primary breast cancer. Based on a threshold probability of 10% or greater of being a *BRCA1*/2 gene carrier as specified in the UK National guidelines for genetic testing (<https://www.nice.org.uk/guidance/cg164>, we assigned cases to those who did or did not meet clinical criteria.

Family histories were also scrutinised manually against the CHOMPRET Criteria for evidence in favour of possible *TP53* mutation[13] and the Chompret 2015 criteria[14] which incorporates age at onset of breast cancer <31 years.

*Genetic testing*: Patients were either tested as part of their clinical care in an NHS genetic testing laboratory and clinical testing report available (Group C, supplementary figure 2) or had a panel of breast cancer susceptibility genes tested (Group B1), or remained untested (Group B2). Group B1 cases were selected consecutively from the cases not previously tested for *BRCA1/2* up to the total number of assays available. Panel testing was effected using HaloPlex™ Target Enrichment System followed by next generation sequencing for a panel of 17 known or likely breast cancer susceptibility genes commonly tested on commercial gene panels (*BRCA1*, *BRCA2* and *TP53* then less well characterised susceptibility genes *ATM, ATR, BARD1, BLM, BRIP1, CDH1, CHEK2, MRE11A, NBN, NF1, PALB2, PTEN, RAD50* and *STK11*). Those undergoing panel testing were matched to those that remained untested so were representative of the untested group, the selection scheme is summarised in supplementary figure 2. Group D is all patients tested for BRCA gene mutations (B1+C). A further sub-group of patients (Group E) had been tested for *TP53* either clinically, through the research panel test or as part of a previous study specifically examining the frequency of *TP53* mutations in patients in the POSH cohort aged 30 or younger at diagnosis[6]

**Statistical analysis**

This was a complete case analysis. Patient characteristics including FH data and tumour characteristics were summarised and compared between groups. Summary data for *BRCA1*/2 and *TP53* clearly pathogenic mutations were summarised. Data on variants across all tested CSGs for the panel testing were also summarised.

Outcomes were then summarised for all *BRCA1* and *BRCA2* testing (Group B1+C) and grouped according to their eligibility for clinical testing based on a BOADICEA probability of 10% or greater. *TP53* tested cases were separately summarised as not all those seen clinically had *TP53* testing and criteria for suspecting Li Fraumeni Syndrome are not incorporated into the BOADICEA model.

# Results

*Cohort characteristics*

A total of 591 patients with a *HER2*+ BC diagnosed aged 40 years or younger were included in this study. The amplified *HER2* status was based on immunohistochemistry (IHC) in 461 (78%) cases, on FISH in 72 (12%) of cases and based on treatment given in 59 (10%) cases The characteristics of patients in whom there was no record of clinical testing (group B1), or in whom clinical testing for *BRCA1*/2 had been completed (group C) were compared with the whole cohort characteristics (group A), as set out in table 1a. As might be expected, more of the patients meeting family history based testing criteria had already been clinically tested for BRCA mutations.

Clinical classification: of the whole group (group A), 117/591 (20%) met the current threshold for BRCA testing in the UK (i.e. >=10% probability of being a BRCA carrier). Amongst those who had clinical testing (group C), nearly half 61/133 (46%), met current guidelines for BRCA testing. Amongst the remaining cases, more of those selected for panel testing (group B1) met the 10% threshold, compared with the untested group B2 (40/263 (15%) compared with 16/195, (8%) respectively, table 1a). For 6 patients who did not provide a family history or were adopted, BOADICEA score was estimated based on age at onset and family history unknown, these cases all technically fell below the threshold for testing (2 in group C and 4 in group B1).  For the combined Group D receiving BRCA testing 101/396 (26%) met the clinical testing threshold (table 1b, figure 1a).

Bilateral breast cancer: In total 32/591 (5%) patients had developed a contralateral primary breast cancer of whom 30 had genetic testing. Of those tested 20 (67%) presented with a family history which already took them over the 10% threshold. In the remaining 10 (33%) cases where at initial presentation the threshold was not met, all had scores over 10% after entering the second primary breast cancer.

*Genetic testing results*

The overall outcome of genetic testing according to clinical testing criteria is summarised in supplementary figure 2a and b. Table 2a provides a summary of all deleterious *BRCA1, BRCA2* and *TP53* mutations detected in Group D, Table 2b lists the likely deleterious mutations in other genes.

*Clinical interpretation and implications for genetic testing in young patients with HER2 amplified breast cancer*

*BRCA1*/2 mutation testing (Figure 1a, table 2a)

Amongst the whole cohort of patients presenting with *HER2* amplified breast cancer aged 40 years or younger at diagnosis, 20% could have been selected for BRCA mutation testing based on current guidelines. In practice just below half of those eligible for testing were offered and accepted clinical testing for BRCA mutations. Amongst 396 patients tested for *BRCA*1/2 mutation, 8 (2%) *BRCA*2 and 6 (2%) *BRCA*1 mutations were detected (total 4%). However by using a testing threshold based on the family history data or the development of a second primary cancer, without adjustment for pathology sub-type, the rate of detection of pathogenic BRCA mutations reaches 11% (figure 3a). This is similar to the detection rate amongst young patients with no family history but who present with a triple negative breast cancer (TNBC) phenotype. It is worth noting that young TNBC cases with a strong FH yield closer to 30% of cases with pathogenic mutations [15]. Amongst those who were BRCA tested who were aged 30 or younger at diagnosis, 1/62 only had a BRCA1 mutation. This patient had a BOADICEA score above the 10% threshold. Testing patients with even very early onset *HER2*+ breast cancer who do not meet clinical testing criteria based on family history or second primary, will yield less than 1% of cases with a positive result.

*TP53* mutation testing criteria (figure 1b, table 2b)

Clinical testing for *TP53* mutations was infrequent amongst this patient group reflecting typical clinical practice. In total we had results in 304 (51%) cases for *TP53* mutations. Overall 9/304 (3%) had pathogenic *TP53* mutations. Amongst the 304 cases, 59 (19%) met the 10% BOADICEA threshold, all were BRCA negative but 7/59 (12%) had deleterious *TP53* mutations compared to 2/245 (2%) of those not meeting the 10% threshold (Figure 1b).

There were 71 patients aged 30 or younger at onset in the group tested for *TP53* mutations and therefore met the new criterion suggested as an addition to the Chompret criteria in 2015 (onset of breast cancer below 31 years). Of the 71 patients meeting this criterion, 5 (8.5%) had pathogenic *TP53* mutations. However 4 of these 5 cases also met the clinical threshold for BOADICEA testing based on a family history of breast cancer and none had a history of Li Fraumeni spectrum cancers in addition to young onset breast cancer.

Bilateral breast cancer: In total 32 patients had developed a contralateral primary breast cancer of whom 30 were tested (24 in group B1 and 6 in group C including *TP53* testing). 7/30 (23%) of these patients had pathogenic mutations (*TP53* = 4, BRCA1=2, BRCA2 = 1).

Outcomes for testing a broader panel of BC susceptibility genes (supplementary table 1)

Likely deleterious mutations in other putative breast cancer susceptibility genes were detected in 13/263 (4.9%) patients in this cohort within *ATM* (5 patients), *BLM* (1 patient), *BRIP1* (2 patients), *CHEK2* (3 patients), *NBN* (1 patient) and *PALB2* (1 patient). In the cohort presented here, most of the cases with mutations in these other genes had no significant family history (11/13) commensurate with low or at best moderate penetrance for this type of mutation. The frequency and spectrum of mutations in patients with HER2+ breast cancer is not dissimilar to that in many other cohorts of breast cancer patients tested for mutations in a similar panel of susceptibility genes[9, 16, 17]. Detecting mutations in genes where the published data allows only a very broad assertion of a possible association with increased breast cancer risk is of limited value in the clinical setting and may lead to inappropriate decisions about health care options[10].

# Discussion

Overall germline *TP53* mutations are a rare explanation for breast cancer but finding a mutation does have implications for treatment including the late effects of cytotoxic radio and chemotherapy treatment, specifically second malignancies. The finding of an underlying *TP53* mutation is difficult for families because of the implications for potential childhood malignancy, particularly since in this age group many patients will already have young children. Again we note that the mutation spectrum in the patients ascertained largely through a young onset breast cancer phenotype includes a higher proportion of truncating mutations rather than the more severe dominant negative missense mutations. This is important to consider in counselling patients about risk[14]. The potential hazard of repeated exposure to imaging using even low doses of X-irradiation including mammograms, CT and PET scanning present a challenge for long term surveillance of at risk family members[18]. Families where a *TP53* gene mutation has been identified may want to know about options for prenatal and preimplantation diagnosis if they are planning to have further children. Within the context of increasing BRCA1/2 testing being facilitated by oncologists to inform treatment decisions, it is important that oncologists are educated to appreciate the importance of referring families with a strong family history but a negative outcome from BRCA1/2 testing for more specialist genetics service advice.

The strength of this study is the large number of young patients with confirmed HER2 amplified breast cancer available for the study of inherited genetic mutations. However clearly there is some potential for a slight change to the overall estimates of proportions of germline gene mutation carriers if the whole cohort could have been tested across all genes. However since the groups who were tested are very similar to each other we believe that these observations are representative of the whole cohort and therefore merit consideration in managing young breast cancer patients. Although HER2 status was not routinely established in breast cancer cases in the UK prior to 2005, we have tested tumours on tissue microarrays and filled HER2 testing gaps to allow representative selection of cases across the time course of the study. The estimation of probability of each individual being a gene carrier was based on a family history questionnaire completed at the time of recruitment soon after diagnosis. Family histories are dynamic and some may have evolved to be more significant after the study participant was recruited. We did not attempt to update family histories over the follow up period but we did update the estimate based on the development of a second primary breast cancer. This situation is very representative of an oncology clinic – family histories are not reviewed over time but genetic testing is likely to be reconsidered when a patient presents with a second primary. The general perception that HER2 amplified breast cancer is less likely to be due to an underlying BRCA gene mutation than many other tumour types in young breast cancer patients is reasonable but in the face of a strong family history BRCA testing is valuable. The most striking finding in this study though is the large number of *TP53* germline mutations in those with only breast cancer family histories. Furthermore the mutations detected as previously noted are more likely to be truncating than missense mutations where the family history is not Li Fraumeni-like.

**Conclusion**

The diagnosis of breast cancer in a young person (under 41 years at diagnosis) may raise the question of whether they could have an underlying high risk due to an inherited gene mutation in a breast cancer susceptibility gene. For patients with HER2 positive breast cancer where the family history provides no support to the likelihood of an inherited risk then the probability of finding a clinically useful inherited gene mutation (in *BRCA1*,2 or *TP53*) is low. If the family history evolves or a second new primary breast cancer is diagnosed then the patient should have genetic testing options reviewed. If the family history meets the guideline threshold of 10% or greater for the probability of finding an underlying *BRCA1*/2 gene mutation, then close to 10% will have pathogenic mutations in BRCA genes. However, in almost all scenarios, the detection rate of deleterious *TP53* mutations in patients with young onset *HER2+* breast cancer, is higher than the detection rate of BRCA1 or BRCA2 and slightly higher than both BRCA genes combined. Clinicians should therefore consider this when discussing options for genetic testing in a young woman with HER2+ breast cancer where age at diagnosis is less than 31 or where a second primary breast cancer has occurred and in whom BRCA testing is negative. In contrast the clinical utility of testing a large range of lower penetrance genes has yet to be established.

# Acknowledgements:

The ethical approval for the POSH study is MREC /00/6/69. We thank Cancer Research UK for funding (C1275/A11699). THE POSH study steering committee are Professor D Eccles, Dr P. Simmonds, Professor DG Altman, Professor P. Pharoah, Professor L. Jones, Professor R Eeles, Professor DG Evans, Professor A Hanby, Professor A. Thompson, Professor S. Hodgson, Mr H. Hammad, Dr R. Warren, Professor F. Gilbert, Professor S. Lakhani. Thanks to Dr David Bunyan, Wessex Regional Genetics Laboratory for confirming mutations detected using NGS panel, Professor Nazneen Rahman and Dr Helen Hanson, Institute of Cancer Research UK for previous TP53 sequencing. For management of pedigree data we thank Will Tapper (University of Southampton), Alex Cunningham (University of Cambridge, BOADICEA software), Harvey Johnson (University of Bristol) and Kim Fitzgerald (University of Oxford), for pedigree and data checking, Nikki Graham and Sylvia Diaper, University of Southampton DNA Banking facility.

# Funding

Funding for the POSH study was provided by the Wessex Cancer Trust, Cancer Research UK grants A7572, A11699, C22524).

# Conflict of Interest

The authors have no conflicts of interest to declare

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Table 1a and 1b. Demographic dataTable 1a – Comparison of Group A (whole cohort) with Group B1 and Group C

|  |  |  |  |
| --- | --- | --- | --- |
| **Study** | **Group A** | **Group B1** | **Group C** |
| **(n=591)** | **(n=263)** | **(n=133)** |
| Age at diagnosis, in years – median (IQR, range) | 36 (33-38, 20 to 40) | 36 (33-39, 20 to 40) | 35 (32-38,22 to 40) |
| Body mass index – median (IQR, range) | 24.5 (22.1-28.3,17.5 to 59.5) | 24.6 (22.3-29.0,17.9 to 59.5) | 24.3 (22.1, 27.9,18.2 to 37.4) |
| Missing/unknown | 26 (4.4%) | 12 (4.6%) | 5 (3.8%) |
| Ethnicity |  |  |  |
| Caucasian/white | 546 (93%) | 238 (92%) | 128 (97%) |
| Black | 16 (3%) | 9 (3%) | 1 (1%) |
| Asian | 19 (3%) | 9 (3%) | 2 (2%) |
| Other | 4 (1%) | 3 (1%) | 1 (1%) |
| Missing/unknown | 6 (1%) | 4 (2%) | 1 (1%) |
| Number with children | 405 (69%) | 177 (69%) | 94 (71%) |
| Number of children – median (IQR, range) | 2 (1-2, 1 to 7) | 2 (1-2, 1 to 7) | 2 (1-3, 1 to 5) |
| Number without children | 178 (31%) | 79 (31%) | 39 (29%) |
| Missing/unknown | 8 (1%) | 7 (3%) | 0 |
| Presentation |  |  |  |
| Symptomatic | 585 (99.2%) | 263 (100%) | 129 (97.0%) |
| Screen detected | 4 (0.7%) | 0 | 4 (3.0%) |
| Other | 1 (0.2%) | 0 | 0 |
| Missing/unknown | 1 (0.2%) | 0 | 0 |
| Family History score estimated by BOADICEA – median (IQR, range) | 0.03 (0.02-0.07,0.01 to 0.99) | 0.03 (0.02-0.06,0.01 to 0.83) | 0.09 (0.03-0.23,0.01 to 99) |
| BOADICEA<10% | 474 (80%) | 223 (85%) | 72 (54%) |
| BOADICEA≥10% | 117 (20%) | 40 (15%) | 61 (46%) |
| Histological grade |  |  |  |
| 1 | 10 (2%) | 7 (3%) | 1 (1%) |
| 2 | 177 (31%) | 74 (29%) | 37 (28%) |
| 3 | 386 (67%) | 172 (68%) | 92 (71%) |
| Missing/unknown | 18 (3%) | 10 (4%) | 3 (2%) |
| Localisation of the cancer |  |  |  |
| Multifocal | 335 (65%) | 144 (63%) | 78 (67%) |
| Localised | 181 (35%) | 86 (37%) | 39 (33%) |
| Missing/unknown | 75 (13%) | 33 (13%) | 16 (12%) |
| Oestrogen Receptor (ER) status |  |  |  |
| Negative | 211 (36%) | 98 (37%) | 51 (38%) |
| Positive | 380 (64%) | 165 (63%) | 82 (62%) |
| Progesterone Receptor (ER) status |  |  |  |
| Negative | 223 (45%) | 98 (43%) | 51 (46%) |
| Positive | 271 (55%) | 130 (57%) | 59 (54%) |
| Missing/unknown | 97 (16%) | 35 (13%) | 23 (17%) |

Table 1b – Comparison of Group D (all tested cases) versus Group A (whole cohort)

|  |  |  |
| --- | --- | --- |
| **Study** | **Group A** | **Group D** |
| **(n=591)** | **(n=396)** |
| Age at diagnosis, in years – median (IQR, range) | 36 (33-38, 20 to 40) | 36 (33-38, 20 to 40) |
| Body mass index – median (IQR, range) | 24.5 (22.1-28.3,17.5 to 59.5) | 24.5 (22.1-28.4,17.9 to 59.5) |
| Missing/unknown | 26 (4.4%) | 17 (4.3%) |
| Ethnicity |  |  |
| Caucasian/white | 546 (93%) | 366 (94%) |
| Black | 16 (3%) | 10 (3%) |
| Asian | 19 (3%) | 11 (3%) |
| Other | 4 (1%) | 4 (1%) |
| Missing/unknown | 6 (1%) | 5 (1%) |
| Number with children | 405 (69%) | 271 (70%) |
| Number of children – median (IQR, range) | 2 (1-2, 1 to 7) | 2 (1-2, 1 to 7) |
| Number without children | 178 (31%) | 118 (30%) |
| Missing/unknown | 8 (1%) | 7 (2%) |
| Presentation |  |  |
| Symptomatic | 585 (99.2%) | 392 (99.0%) |
| Screen detected | 4 (0.7%) | 4 (1.0%) |
| Other | 1 (0.2%) | 0 |
| Missing/unknown | 1 (0.2%) | 0 |
| Family History score estimated by BOADICEA – median (IQR, range) | 0.03 (0.02-0.07,0.01 to 0.99) | 0.03 (0.02-0.11,0.01 to 0.99) |
| BOADICEA<10% | 474 (80%) | 295 (74%) |
| BOADICEA≥10% | 117 (20%) | 101 (26%) |
| Histological grade |  |  |
| 1 | 10 (2%) | 8 (2%) |
| 2 | 177 (31%) | 111 (29%) |
| 3 | 386 (67%) | 264 (69%) |
| Missing/unknown | 18 (3%) | 13 (3%) |
| Localisation of the cancer |  |  |
| Multifocal | 335 (65%) | 222 (64%) |
| Localised | 181 (35%) | 125 (36%) |
| Missing/unknown | 75 (13%) | 49 (12%) |
| Oestrogen Receptor (ER) status |  |  |
| Negative | 211 (36%) | 149 (38%) |
| Positive | 380 (64%) | 247 (62%) |
| Progesterone Receptor (ER) status |  |  |
| Negative | 223 (45%) | 149 (44%) |
| Positive | 271 (55%) | 189 (56%) |
| Missing/unknown | 97 (16%) | 58 (15%) |

 |

# Table 2: summary of all pathogenic/likely pathogenic mutations detected

Table 2a): summary of all high risk susceptibility gene mutations and BOADICEA score

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| ***Gene*** | **Variants Detected** | **Type of mutation**  | **Clinically useful?** | **BOADICEA** |
| ***BRCA1*** | c.5266dupC, p.Q1756fs | Frameshift | Yes | 0.43 |
| c.2681\_2682delAA, p.Lys894fs | Frameshift | Yes | 0.31 |
| Deletion exons 1-17 | Large deletion | Yes | 0.22 |
| c.1556\_1556delA, p.Lys519Argfs | Frameshift | Yes | 0.17 |
| c.2199\_2199delG, p.Glu733fsX3 | Frameshift | Yes | 0.07 |
| c.594-2A>C | Splice Mutation | No\* | 0.04 |
| ***BRCA2*** | c.7985G>A, p.W2586X | Nonsense | Yes | 0.81 |
| c.2034\_2038delTAATA, p.N678fs | Frameshift | Yes | 0.41 |
| Deletion of exons 14 - 16  | Large deletion | Yes | 0.26 |
| c.9196C>T, p.Q3066X | Nonsense | Yes | 0.20 |
| c.517-2A>G | Splice Mutation | Yes | 0.13 |
| c.2836\_2837delAG, p.Asp946PhefsTer12 | Frameshift | Yes | 0.07 |
| c.4415\_4418delAAGA, p.Lys1472ThrfsTer6 | Frameshift | Yes | 0.02 |
| c.9382C>T, p.Arg3128Ter | Nonsense | Yes | 0.06 |
| ***TP53*** | c.733G>A, p.Gly245Ser | Missense | Yes | 0.27 |
| c.733G>A, p.Gly245Ser  | Missense | Yes | 0.02 |
| c.672+1G>T | Splice Mutation | Yes | 0.14 |
| c.625A>T, p.R209X | Nonsense | Yes | 0.11 |
| c.919+1G>A | Splice Mutation | Yes | 0.03 |
| c.437G>A, p.W146X | Nonsense | Yes | 0.77 |
| c.586C>T, p.R196X | Nonsense | Yes | 0.76 |
|  c.659A>G, p.Y220C | Missense | Yes | 0.60 |
| c.633\_640delCAGCTTTT, p.Phe212Ter | Frameshift  | Yes | 0.05 |
|  |  |  |  |
| \*See Rosenthal et al 2014\*\* Mutations reported in Wilson et al 2010 |

Supplementary table 1) Mutations detected in genes other than *BRCA1*/2 or *TP53* using a panel testing of 263 cases of young onset *HER2*+ breast cancer

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| ***Gene*** | **Variants Detected** | **Type of mutation**  | **BOADICEA** | **Clinically useful?\*** |
| ***ATM*** | c.8149A>T, p.Lys2717Terc.6100C>T, p.Arg2034Terc.3801delG, p.Val1268Terc.7271T>G, p.Val2424Glyc.7271T>G, p.Val2424Gly | NonsenseNonsenseFrameshiftMissenseMissense | 0.0770.1370.0190.0990.08 | NKNKNKPossiblyPossibly |
| ***BLM*** | p.Gln975LysfsTer24 | frameshift | 0.019 | NK |
| ***BRIP1*** | c.2392N>T, p.Arg798Terc.2255\_2256delTT, p.Lys752ArgfsTer12 | Nonsense Frameshift | 0.0370.015 | NKNK |
| ***CHEK2*** | c.784delC, p.Glu262AsnfsTer16c.534delT, p.Lys178AsnfsTer26p.Asp246Ter | FrameshiftFrameshiftNonsense | 0.1080.0230.032 | NKNKNK |
| ***NBN*** | c.643C>T, P.Arg215Trp | Missense | 0.027 | NK  |
| ***PALB2*** | p.Ser172GlyfsTer4 | frameshift | 0.073 | Probably |
| ***PTEN, ATR, BARD1, CDH1, MRE11A, RAD50, STK11*** had no detectable mutations |
| \*Could this variant be used to accurately predict future cancer risks to the individual or their close relatives at the present time? [10]\*\*NB. the p.Val2424Gly variant is associated with higher risk than truncating variants |

Supplementary figure 1: CONSORT diagram for case selection

POSH cohort

n=2956

HER2 negative/borderline=2009

HER2 missing/unknown=351

POSH HER2 positive

n=593/2956 (20%)

No DNA Sample

n=5

**GROUP A**

POSH HER2 positive (DNA available)

n=591/596 (99%)

Supplementary figure 2: Sampling for panel testing cohort

**GROUP A**

POSH HER2 positive (DNA available)

n=591

**GROUP C**

BRCA Tested

n=133/591 (23%)

**GROUP B**

Untested

n=458/591 (77%)

**GROUP B2**

No Testing

n=195/458 (43%)

**GROUP B1**

Panel Testing

n=263/458 (57%)

# Figure 1: Outcome of genetic testing according to clinical selection criteria

Figure 1a: Outcome of BRCA testing

**GROUP A**

POSH HER2 positive (DNA available)

n=591

**GROUP D (=B1+C)**

Panel + BRCA clinical

n=396/591 (67%)

**GROUP D2**

Do not meet clinical criteria for BRCA testing (included FH unknown n=14)

n=295/396 (74%)

**GROUP D1**

Meet clinical criteria for BRCA testing

n=101/396 (26%)

BRCA1 pathogenic– 5/101 (5%)

BRCA2 pathogenic – 6/101 (6%)

BRCA1 pathogenic – 1/295 (0.3%)

BRCA2 pathogenic – 3/295 (1%)

Figure 1b: Outcome of *TP53* testing

**GROUP A**

POSH HER2 positive (DNA available)

n=591

**GROUP E (TP53 testing)**

n=304/591 (51%)

**Chrompet +**

n=30/59 (51%)

**Chompret –**

n=29/59(49%)

**Pathogenic mutation on TP53 found**

n=6/30 (20%)

**Pathogenic mutation on TP53 found**

n=1/29 (3 %)

**Meet criteria for BRCA testing**

n=59/304 (19%)

**Do not meet criteria for BRCA testing**

n=245/304 (81%)

**Chrompet +**

n=48/245 (20%)

**Chrompet -**

n=197/245 (80%)

**Pathogenic mutation in TP53**

n=1/39 (3%)

**Pathogenic mutation in TP53**

n=1/195 (0.5%)

|  |
| --- |
|  |

1. Rakha EA, Pinder SE, Bartlett JM et al. Updated UK Recommendations for HER2 assessment in breast cancer. J Clin Pathol 2015; 68: 93-99.

2. Fasching P, Weihbrecht S, Haeberle L et al. HER2 and TOP2A amplification in a hospital-based cohort of breast cancer patients: associations with patient and tumor characteristics. Breast Cancer Research and Treatment 2014; 145: 193-203.

3. Copson E, Eccles B, Maishman T et al. Prospective observational study of breast cancer treatment outcomes for UK women aged 18-40 years at diagnosis: The POSH study. Journal of the National Cancer Institute 2013; 105: 978-988.

4. Parise CA, Bauer KR, Brown MM, Caggiano V. Breast Cancer Subtypes as Defined by the Estrogen Receptor (ER), Progesterone Receptor (PR), and the Human Epidermal Growth Factor Receptor 2 (HER2) among Women with Invasive Breast Cancer in California, 1999–2004. The Breast Journal 2009; 15: 593-602.

5. Mavaddat N, Barrowdale D, Andrulis IL et al. Pathology of Breast and Ovarian Cancers among BRCA1 and BRCA2 Mutation Carriers: Results from the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA). Cancer Epidemiology Biomarkers & Prevention 2012; 21: 134-147.

6. Wilson JR, Bateman AC, Hanson H et al. A novel HER2-positive breast cancer phenotype arising from germline TP53 mutations. J Med Genet 2010; 47: 771-774.

7. Melhem-Bertrandt A, Bojadzieva J, Ready KJ et al. Early onset HER2-positive breast cancer is associated with germline TP53 mutations. Cancer 2012; 118: 908-913.

8. Masciari S, Dillon DA, Rath M et al. Breast cancer phenotype in women with TP53 germline mutations: a Li-Fraumeni syndrome consortium effort. Breast Cancer Res Treat 2012; 133: 1125-1130.

9. Couch FJ, Hart SN, Sharma P et al. Inherited mutations in 17 breast cancer susceptibility genes among a large triple-negative breast cancer cohort unselected for family history of breast cancer. Journal of Clinical Oncology 2015; 33: 304-311.

10. Easton DF, Pharoah PDP, Antoniou AC et al. Gene-Panel Sequencing and the Prediction of Breast-Cancer Risk. New England Journal of Medicine 2015; 372: 2243-2257.

11. Eccles D, Gerty S, Simmonds P et al. Prospective study of outcomes in sporadic versus hereditary breast cancer (POSH): Study protocol. BMC Cancer 2007; 7.

12. Antoniou AC, Hardy R, Walker L et al. Predicting the likelihood of carrying a BRCA1 or BRCA2 mutation: Validation of BOADICEA, BRCAPRO, IBIS, Myriad and the Manchester scoring system using data from UK genetics clinics. Journal of Medical Genetics 2008; 45: 425-431.

13. Tinat J, Bougeard G, Baert-Desurmont S et al. 2009 version of the Chompret criteria for Li Fraumeni syndrome. J Clin Oncol 2009; 27: e108-109; author reply e110.

14. Bougeard G, Renaux-Petel M, Flaman JM et al. Revisiting Li-Fraumeni Syndrome From TP53 Mutation Carriers. J Clin Oncol 2015; 33: 2345-2352.

15. Couch FJ, Hart SN, Sharma P et al. Inherited mutations in 17 breast cancer susceptibility genes among a large triple-negative breast cancer cohort unselected for family history of breast cancer. J Clin Oncol 2015; 33: 304-311.

16. Maxwell KN, Wubbenhorst B, D'Andrea K et al. Prevalence of mutations in a panel of breast cancer susceptibility genes in BRCA1/2-negative patients with early-onset breast cancer. Genet Med 2014.

17. Tung N, Battelli C, Allen B et al. Frequency of mutations in individuals with breast cancer referred for BRCA1 and BRCA2 testing using next-generation sequencing with a 25-gene panel. Cancer 2015; 121: 25-33.

18. Evans DG, Birch JM, Ramsden RT et al. Malignant transformation and new primary tumours after therapeutic radiation for benign disease: substantial risks in certain tumour prone syndromes. J Med Genet 2006; 43: 289-294.