Inherited breast cancer predisposition in Asians: multigene panel testing outcomes from Singapore

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Genetic testing for germline mutations in breast cancer predisposition genes can potentially identify individuals at a high risk of developing breast and/or ovarian cancer. There is a paucity of such mutational information for Asians. Panel testing of 25 cancer susceptibility genes and BRCA1/2 deletion/duplication analysis was performed for 220 Asian breast cancer patients or their family members referred for genetics risk assessment. All 220 participants had at least one high-risk feature: having a family history of breast and/or ovarian cancer in first- and/or second-degree relatives; having breast and ovarian cancer in the same individual or bilateral breast cancer; having early-onset breast cancer or ovarian cancer (<40 years of age). We identified 67 pathogenic variants in 66 (30.0%) patients. Of these, 19 (28.3%) occurred in BRCA1, 16 (23.9%) in BRCA2, 7 (10.4%) in PALB2, 6 (9.0%) in TP53, 2 (3.0%) in CDH1 and 15 (22.4%) in other predisposition genes. Notably, 47.8% of pathogenic variants were in non-BRCA1/2 genes. Of the 66 patients with pathogenic mutations, 63.6% (42/66) were under the age of 40 years. Family history of breast and/or ovarian cancer is enriched in patients with BRCA1/2 pathogenic variants but less predictive for non-BRCA1/2 related pathogenic variations. We detected a median of three variants of unknown significance (VUS) per gene (range 0–21). Custom gene panel testing is feasible and useful for the detection of pathogenic mutations and should be done in the setting of a formal clinical cancer genetics service given the rate of VUS.

Results

Study population

Patients suspected of hereditary breast cancer in this study were referred from Singapore and the region for genetic risk assessment at the National Cancer Centre Singapore. Of the patients with established ethnicity, 181 (82.3%) were Chinese, 17 (7.7%) Malay, and 6 (2.7%) of South Indian descent (Table 1). The remaining 16 (7.3%) were of Burmese, Eurasian, Japanese, Filipino, Vietnamese and other races, respectively. Age at diagnosis of patients with breast and/or ovarian cancer ranged from 19 to 72 years, with an average age of 39 years. Of the 120 patients with

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Table 1. Characteristics of the study participants

| Characteristics                          | Study participants | n = 220 |
|------------------------------------------|--------------------|---------|
| Race/ethnicity                           |                    |         |
| Chinese                                  | 181                | 82      |
| Malay                                    | 17                 | 8       |
| Indonesians                              | 7                  | 3       |
| Indians                                  | 5                  | 2.5     |
| Sri Lankan                               | 1                  | 0.5     |
| Vietnamese                               | 3                  | 1       |
| Burmese                                  | 1                  | 0.5     |
| Filipino                                 | 1                  | 0.5     |
| Japanese                                 | 1                  | 0.5     |
| Eurasian                                 | 1                  | 0.5     |
| Other races                              | 2                  | 1       |
| Personal history of breast cancer        |                    |         |
| Unilateral                               | 177                | 80      |
| Bilateral                                | 18                 | 8       |
| Age at first breast cancer diagnosis, years |                    |         |
| Mean                                     | 46                 |         |
| Median                                   | 50.5               |         |
| Range (unknown age for 3 patients)       | 15–65              |         |
| Age at ovarian cancer diagnosis, years   |                    |         |
| Mean                                     | 46                 |         |
| Median                                   | 50.5               |         |
| Range (unknown age for 3 patients)       | 15–65              |         |
| Family history of breast cancer          | 104                | 47      |
| Family history of ovarian cancer         | 16                 | 7       |

Available family history information, 104 (86.7%) had at least one first- or second-degree relative with breast cancer, and 16 (13.3%) had a relative with ovarian cancer.

Germline mutations

All coding exons and consensus splice sites of 25 known cancer predisposition genes were screened for mutations in the 220 patients. Overall, 67 pathogenic mutations were identified in 66 patients (30.0% (66/220); Table 2). Eight mutations were detected in more than 1 patient, and 10 patients were carriers for more than one mutation (Table 2). Of these, 19 (28.4%) occurred in patients. Overall, 67 pathogenic mutations were identified in 66 patients (30.0% (66/220); Table 2). Eight mutations were detected in 10.5% (23/220) of patients, including

| BRCA1 | BRCA2 | PALB2 | PMS2 | PTEN | RAD51C | RAD51D | TP53 |
|-------|-------|-------|------|------|--------|--------|------|
| 19.4  | 19.4  | 19.4  | 19.4 | 19.4 | 19.4   | 19.4   | 19.4 |

Manchester scores were available for 56 of 66 individuals with deleterious mutations, and 124 of 154 individuals with no mutations.

Family history

We also evaluated whether patients with mutations in the 25 predisposition genes were associated with a greater family history of breast and/or ovarian cancers than non-mutated patient cases (Table 2). Patients with BRCA1 mutations were enriched for a family history of breast (5/23 (21.7%)) and ovarian cancers (2/23 (8.7%)), whereas patient cases with BRCA2 mutations were enriched for a family history of breast (7/17 (41.2%)) but none of the family members had ovarian cancers. (Table 2). This is reflected in the differences in Manchester and Boadicea scores seen between the two groups of patients (Table 3). However, patient cases with mutations in the non-BRCA1/2 genes were not significantly associated with an enriched family history for either breast or ovarian cancer (Table 2). In particular, only 8 (24.2% (8/33)) non-BRCA1/2 gene mutation carriers had a family history of breast or ovarian cancer.

Variants of unknown significance

A total of 94 VUS were identified in 23 genes in 96 of 220 participants. Per participant, the average number of VUS across all genes was 0.67 (s.d., 0.9) (Figure 3a). Of the 220 participants, 103 (46.8%) had at least one VUS among the 25 genes sequenced. Per gene, the median number of VUS detected across all 220 participants was 3, ranging from zero (PTEN and NBN) to 21 (ATM; Figure 3b). Among the 7 high-risk genes, 10 VUS were found in BRCA1, 15 in BRCA2, 10 in PALB2, 2 in CDH1, 2 in STK11, 1 in TP53 and none in PTEN. In the remaining 18 genes, a median of 3.5 VUS per gene (range 0–21) were detected. All VUS were missense mutations and within exonic regions. Of the 94 VUS, 41 (43.6%) were novel, not previously reported in the databases or dbSNP. No statistically significant difference was detected in VUS frequency between ethnicities.

DISCUSSION

We present here a comprehensive mutation analysis of Asian patients suspected of having hereditary breast cancer. To our knowledge, this is the largest Asian series to date for the NGS screening of germline mutations using a panel of known breast cancer predisposition genes. We found 67 germline deleterious mutations in 17 of 25 predisposition genes tested. BRCA1 and BRCA2 mutations were found in 17.7% (39/220) of patients, consistent with other studies using panel testing, whereas mutations in 15 other genes were found in 32 (14.5%) patients. The frequency of these mutations, especially in PALB2, which has recently been associated with a high lifetime risk of breast cancer, was similar to the frequency in high- and moderate-risk breast cancer families. This is a significant higher yield of potentially actionable results, compared with the 5 to 10% probability threshold endorsed by guidelines for testing for HBOC and Lynch syndrome testing.

In Asia and many parts of the world, while there is a growing appreciation for the testing of patients identified as being at high risk of hereditary cancer, it is still not as yet ‘mainstream’ practice, as such patients are often referred after the development of multiple cancers in a patient. This may account for the relatively high number of TP53 (9.0%) and PTEN (3.0%) germline mutations seen in our cohort. Notably, only 63.6% (42/66) of patients with pathogenic variants were under the age of 40 years at the age of first cancer diagnosis, suggesting that age alone as a cut-off may miss significant numbers of patients (Table 2). Currently, there is no data as yet on the risk-benefit ratio of increased breast surveillance among patients with pathogenic

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| ID  | Race | Ca site                        | Subtype                       | Age at diagnosis (years) | Affected gene | Nucleotide change                  | Type of mutation | Amino-acid change | Family Ca history                      | MC score | Bo BRCA1 | Bo BRCA2 | Ref |
|-----|------|--------------------------------|-------------------------------|--------------------------|---------------|------------------------------------|------------------|------------------|----------------------------------------|----------|----------|----------|-----|
| 119 | C    | Bil Br Ca Histology Unk/Ov Ca | Unk                           | 35                       | Br Ca (50/50)  | c.3381T > A                         | N                | p.Y1127fs*          | Sis Br Ca (37)                      | 46       | 64.6     | 22.1     |     |
| 153 | C    | Bil Br Ca, Serous type        | Ov Ca, Serous type             | 35                       | Br Ca (50/50)  | c.3381T > A                         | N                | p.Y1127fs*          | Sis Br Ca (40); Sis Ov Ca (60); Fa Thy Ca (54); Co Pat Br Ca (40) | 46       | 33.2     | 10.2     | 6   |
| 121 | C    | Br IDC                        | ER−/PR−/Her2−                 | 35                       | BrCA1         | c.67_68delinsAG                      | Fr_ins           | p.E23Rfs*18          | Mo Br Ca (43); GM Mat Br Ca (45); Au Mat Br Ca (45); Ov Br Ca (50) | 75       | 22.6     | 4.2      | 6,16-19 |
| 152 | C    | Ov Ca                          | ER−/PR−/Her2− Na              | 35                       | BrCA1         | c.67_68delinsAG                      | Fr_ins           | p.E23Rfs*18          | Sis Ov Ca (47)                      | 46       | 27.2     | 1        | 16-19 |
| 163 | C    | Bill Br IDC                    | ER−/PR−/Her2− Endometrioid    | 38, 46                    | BRCAl         | c.3333delA                          | Fr_del           | p.E1112Nfs*5         | GM Mat Ov Ca (40)                      | 42       | 32.2     | 6.1      | 20  |
| 166 | C    | Bill Br IDC/Atypical medullary type | ER−/PR−/Her2− No personal Ca history, Predictive testing | 39, 46                    | BRCAl         | c.5072C > A                          | Mis              | p.T1691K             | Unk FH                              | 51       | 88.8     | 9.7      | 21  |
| 125225960 | C | FH83 Br IDC                   | ER−/PR−/Her2− Unk             | 32                       | BRCAl         | c.5072C > A                          | Mis              | p.T1691K             | Twin Sis Br Bil Ca (30s, 40s); Sis Br Ca (40s) | 30       | 10.1     | 3.3      | 6,23 |
| 104b | C    | Br IDC                         | ER+ PR+/Her2−                 | 33                       | BRCAl         | c.5068A > C                          | Mis              | p.K1690Q             | Unk FH                              | 1        | 0.5      | 2.1      | 5,22 |
| 172  | M    | Br IDC                         | ER−/PR−/Her2−                 | 37                       | BRCAl         | c.4327C > T                          | N                | p.R1443*              | Unk FH                              | 2        | 4.5      | 20.7     | 24  |
| 105b | C    | Br IDC                         | ER−/PR−/Her2−                 | 39                       | BRCAl         | c.4327C > T                          | N                | p.R1443*              | Unk FH                              | 2        | 4.5      | 20.7     | 24  |
| 159  | I    | Br IDC                         | ER−/PR−/Her2−                 | 22                       | BRCAl         | c.2766delA                          | Fr_del           | p.V923Lfs*77          | Unk FH                              | 34       | 18.6     | 6.8      | 6,26 |
| 65   | C    | Br IDC                         | ER−/PR−/Her2− + ER−/PR−/Her2− | 44, 51, 53                | BRCAl         | c.2635G > T                          | N                | p.R679*               | Unk FH                              | 22       | 85.6     | 1        | 6,27 |
| 61   | M    | Bil Br Ca Unk type             | ER+/PR+/Her2− Unk             | 34                       | BRCAl         | c.2145A > T                          | Fr_del           | p.R7625               | No FH Ca                            | 1        | 2        | 1.8      |     |
| 103  | M    | Bil Br IDC                     | ER+/PR+/Her2−/ILC             | 24                       | BRCAl         | c.981_982del                          | Fr_del           | p.C328*              | No FH Ca                            | 22       | 18.7     | 33.2     | 28  |
| 150b | B    | Br IDC                         | ER+/PR+/Her2−                 | 28                       | BRCAl         | c.172C > G                           | Mis              | p.S98A                | No FH Ca                            | 10       | 4.4      | 0.9      | 6   |
| 59   | C    | Br mixed IDC ILC               | ER+/PR+/Her2−                 | 43                       | BRCAl         | Del                                  | Deletion of Exon 13-16-19* | Fr_del | Deletion of Exon 13-16-19* | Unk FH                              | 31       | 3        | 3.6      | 6   |
| MR0017 | C | Br IDC                         | ER−/PR−/Her2−                 | 41                       | BRCAl         | Dup                                  | Duplication of Exon 13* | Fr_del | Duplication of Exon 13* | Unk FH                              | 22       | 85.6     | 1        | 6,27 |
| 79   | C    | Br DCIS/Ov Ca                  | ER−/PR−/Her2−                 | 38                       | BRCAl         | c.442_15del10*                      | SE               | p.L709FS*13F         | Sis Br Ca (37), Fa Br Ca (72)         | 38       | 68.1     | 1.7      | 5.6  |
| MR0027 | C | Br IDC                         | ER+/PR+/Her2−                 | 36                       | BRCAl         | c.483T > G                           | Fr_del           | p.C161W               | Unk FH                              | 10       | 3.3      | 0.7      | 5   |
| FH87 | C    | Br Ca                          | ER+/PR+/Her2−                 | 32                       | BRCAl         | c.483T > G                           | Fr_del           | p.C161W               | Unk FH                              | 22       | 9.3      | 8.7      | 5   |
| FH60  | C    | Br IDC                         | ER+/PR+/Her2−                 | 56                       | BRCAl         | c.275delC                           | Fr_del           | p.L709FS*13F         | Sis Br Ca (37), Fa Br Ca (72)         | 26       | 1.6      | 44.8     | 6   |
| YP33 | C    | Br IDC                         | ER−/PR−/Her2−                 | 40                       | BRCAl         | c.3847_3848delIGT                    | Fr_del           | p.L709FS*13F         | Sis Br Ca (37), Fa Br Ca (72)         | 7        | 4.3      | 1.1      | 6,29|
| 168  | C    | No Ca                          | ER−/PR−/Her2−                 | 40                       | BRCAl         | c.4151delT                          | Fr_del           | p.L709FS*13F         | Sis Br Ca (37), Fa Br Ca (72)         | 1        | 4.3      | 1.1      | 6,29|

Table 2. Pathogenic variants with their Manchester and Boadicea scores

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| ID   | Race | Ca site              | Subtype                     | Age at diagnosis (years) | Affected gene | Nucleotide change | Type of mutation | Amino-acid change | Family Ca history | MC score | Bo BRCA1 | Bo BRCA2 | Ref |
|------|------|----------------------|-----------------------------|--------------------------|---------------|-------------------|------------------|-------------------|------------------|----------|----------|----------|-----|
| HR0029 | C    | Br IDC               | ER+/PR+/Her2-               | 51                       | BRCA2         | c.5576_5579delITTAA  | Fr_del           | p.I1859Kfs*3     | Sis Br Ca (53), Sis Br Ca (60), Sis Br Ca (51), Au Mat Br Ca (60), Mo Br Ca (58), Au Mat Br Ca (60), Un Mat Ga Ca (50) | 18 | 1.4 | 2.2 | 6,30 |
| 151  | C    | Clear Cell Ov Ca    |                             | 51                       | BRCA2         | c.5799_5802delCCCA  | Fr_del           | p.N1933Kfs*29    | Mo Br Ca (58), Au Mat Br Ca (60), Un Mat Ga Ca (50) | 30 | 0 | 37 | 6,31 |
| 162  | F    | Br IDC               | ER+/PR+/Her2-               | 36                       | BRCA2         | c.6935delG          | Fr_del           | p.R223Q         | No FH Ca          | 1 | 1.8 | 1.7 | 6 |
| YP16b| C    | Br IDC               | ER+/PR+/Her2-               | 38                       | BRCA2         | c.7480C>T          | N                | p.R2494^         | No FH Ca          | 1 | 0.8 | 2.8 | 32 |
| 164  | C    | Br IDC, childhood acute leukemia, meningiomas |           | 32                       | BRCA2         | c.7480C>T          | N                | p.R2494^         | No FH Ca          | 1 | 0.8 | 2.8 | 32 |
| 99   | C    | Br IDC               | ER−/PR−/Her2-               | 42                       | BRCA2         | c.7522G>A          | Mis              | p.G2508S         | Mo Br Ca (80), Mo Col Ca (80), Au Mat Br Ca (70), Au Mat Ga Ca (70), Au Mat Ga Ca (70), Au Mat Ga Ca (70), Un Mat Ga Ca (70) | 2 | 0.4 | 0.5 | 33,34 |
| HR0045b| M  | Br IDC               | ER+/PR−/Her2-               | 28                       | BRCA2         | c.7613G>A          | Mis              | p.G2544D         | 14 | 7.4 | 6.8 | 5 |
| FH29  | C    | Br IDC               | ER+/PR+/Her2-               | 49                       | BRCA2         | c.7696_7697delAA    | Fr_del           | p.D2366fs*5      | Sis Br Ca (50) | 2 | 0.6 | 2.1 | 6,35 |
| LR0032 | C  | Br IDC               | ER−/PR−/Her2-               | 36                       | BRCA2         | c.8809_8891insAA    | Fr_ins           | p.A2964Kfs*54a   | Un Pat Col (40), Col Ov (30) | 10 | 1.8 | 1.7 | 6 |
| LH5  | C    | Br IDC               | ER+/PR+/Her2-               | 41                       | BRCA2         | c.8914delT          | N                | p.G2534D         | 14 | 7.4 | 6.8 | 5 |
| 104b  | C    | Br IDC               | ER+/PR+/Her2-               | 33                       | BRCA2         | c.9294C>G          | N                | p.Y3088^         | Sis Br Ca (50), Sis Br Ca (60), Un Mat Ga Ca (50) | 2 | 1.8 | 1.7 | 6 |
| 64   | C    | Br IDC/Ov Ca         | ER Unk/PR Unk/Her2 Unk      | 18                       | BRCA2         | c.7613G>A          | SE               | Deletion of Exon 19^ | 18 | Unk | Unk | 5,6 |
| YP6b  | C    | Br IDC               | ER+/PR+/Her2-               | 25                       | PALB2         | c.113C>G           | Mis              | p.A38G           | 2 Others Ca non related | 6 | Unk | Unk | 4 |
| YP59b | C    | Br IDC               | ER+/PR+/Her2-               | 34                       | PALB2         | c.113C>G           | Mis              | p.A38G           | 30 | Unk | Unk | 4 |
| 149  | IO   | Bil Serous Ov Carcinoma |                             | 59                       | PALB2         | c.3164C>T          | N                | p.Q1056fs*6      | Sis Br Ca (61), Mo Br Ca (69) | 46 | Unk | Unk | 4 |
| LR0032 | C  | Br IDC               | ER+/PR+/Her2-               | 24                       | PALB2         | c.3164C>T          | N                | p.Q1056fs*6      | Sis Br Ca (61), Mo Br Ca (69) | 46 | Unk | Unk | 4 |
| LR0026b| C  | Br IDC               | ER+/PR+/Her2-               | 29                       | PALB2         | c.3164C>T          | N                | p.Q1056fs*6      | Sis Br Ca (61), Mo Br Ca (69) | 46 | Unk | Unk | 4 |
| LR0019 | C  | Br IDC               | ER+/PR+/Her2-               | 39                       | PALB2         | c.3164C>T          | N                | p.Q1056fs*6      | Sis Br Ca (61), Mo Br Ca (69) | 46 | Unk | Unk | 4 |
| LR0009 | C  | Br IDC               | ER−/PR−/Her2-               | 26                       | TP53          | c.819delG           | Fr_del           | p.S274Afs*38     | Bro sarcoma (38), Mo Ov Ca (38), Au Pat gastric Ca (68), GM Pat Ga Ca (72) | 55 | Unk | Unk | 4 |
| 131  | C    | Br IDC               | ER+/PR+/Her2-               | 32                       | TP53          | c.616G>A           | Mis              | p.G2065          | Co Mat Br Ca (33) | 14 | Unk | Unk | 37–39 |
| 158b  | IO   | Mixed invasive       | ER+/PR−/Her2−               | 30                       | TP53          | c.356G>T           | Mis              | p.R1191^         | Mo Br Ca (49) | 18 | Unk | Unk | 4 |
| HR00054 | M | Br IDC               | ER−/PR−/Her2−               | 32                       | TP53          | c.331_343delG       | Fr_del           | p.T1116fs*16     | Mo Br Ca (34), Sis Brain tumour (10) | 22 | Unk | Unk | 4 |
| 158b  | IO   | Mixed invasive       | ER+/PR−/Her2−               | 30                       | TP53          | c.273A>G           | Mis              | p.N92S^          | Mo Br Ca (49) | 18 | Unk | Unk | 4 |
| 980221 | C  | Br IDC               | ER+/PR+Her2−                | 34                       | TP53          | c.802+1G>A         | SE               | Unk FH           | Unk | Unk | Unk | 4 |
| FH53b  | C    | Br IDC               | ER+/PR+/Her2−               | 41                       | CHEK2         | c.667C>T           | Mis              | p.R223C          | Mo Br Ca (50) | 2 | Unk | Unk | 4 |
| HR0045b| C  | Br IDC               | ER+/PR−/Her2−               | 28                       | CHEK2         | c.667C>T           | Mis              | p.R223C          | Mo Br Ca (50) | 2 | Unk | Unk | 4 |
| LR0026b| C  | Br IDC               | ER+/PR+/Her2−               | 29                       | ATM           | c.8800A>G          | Mis              | p.T2934A         | Mo Br Ca (50) | 6 | Unk | Unk | 4 |
| ID   | Race | Ca site            | Subtype                     | Age at diagnosis (years) | Affected gene | Nucleotide change | Type of mutation | Amino-acid change | Family Ca history | MC score | Bo BRCA1 | Bo BRCA2 | Ref |
|------|------|--------------------|-----------------------------|--------------------------|---------------|-------------------|------------------|------------------|------------------|----------|-----------|----------|-----|
| YP62 | C    | Br IDC             | ER+/PR+/Her2-               | 38                       | PTEN          | c.641delA        | Fr_del           | p.214Rfs*7a       | Au Mat Br (30), Un Mat Pros (60) | 22       | Unk       | Unk      | 56  |
| 146  | C    | Multifocal Ov Ca, Br IDC, Endo Ca 50 | ER+/PR+/Her2-               | 54                       | PTEN          | c.672dup         | Fr_ins           | p.Y222fs*18a      | Fa Col Ca (60), Co Mat Col Ca (30) | 1        | Unk       | Unk      | 37  |
| 60   | C    | Unk type Br Ca, Neurofibromatosis |                          | 33                       | NFI           | c.6480_6490del   | Fr_del           | p.K2160Nfs*14a    | No FH Ca | 1         | Unk       | 14 |
| 150a | B    | Br ILC             | ER−/PR−/Her2-               | 40                       | CDH1          | c.2359G>A       | Mis              | p.V787A         | Au Pat Br Ca (50), Au Pat Br Ca (59) | 10       | 4.4       | 0.9      | 36 |
| YP46  | C    | Br IDC             | ER+/PR+/Her2-               | 33                       | CDH1          | c.1888 C>G      | Mis              | p.L630V          | GF Mat Ga Ca (70), GF Mat Pros Ca (70) | 2        | Unk       | Unk      | 34 |
| 150a | B    | Br ILC             | ER−/PR−/Her2-               | 40                       | CDKN2A        | c.221A>C        | Mis              | p.D74A           | Au Pat Br Ca (50), Au Pat Br Ca (60) | 10       | 4.4       | 0.9      | 36 |
| YP43  | C    | Br IDC             | ER−/PR−/Her2-               | 31                       | MLH1          | c.2135G>T       | Mis              | p.W712L          | Au Mat Other Ca (53) | 1        | Unk       | Unk      | 40 |
| YP6b  | C    | Br IDC             | ER+/PR+/Her2-               | 25                       | MLH1          | c.1153C>T       | Mis              | p.R385C          | 2 Other Ca Unk (60) | 6        | Unk       | Unk      | 34 |
| YP28  | C    | Br IDC             | ER+/PR+/Her2-               | 39                       | MSH6          | c.2187G>A       | Mis              | p.905X          | 14.27           | Unk       | Unk       | Unk      | 34 |
| 170   | SL   | Br IDC             | ER−/PR−/Her2-               | 38                       | MSH6          | c.3227G>A       | Mis              | p.R1076H         | Mo Br Ca (39) (40) | 10       | Unk       | Unk      | 34 |
| 142   | J    | No Ca              | NA                           | 35                       | RADS1C        | c.635T>G        | Mis              | p.R212H          | 1 Unk             | 34       | Unk       | Unk      | 34 |
| 86    | C    | Unk type Br Ca, Ov Ca | ER+/PR+/Her2+               | 30                       | BARD1         | c.1298A>G       | Mis              | p.H433R         | 10 Unk             | 34       | Unk       | Unk      | 34 |
| YP44  | C    | Br IDC             | ER+/PR+/Her2-               | 37                       | BRIP1         | c.1442G>A       | Mis              | p.G481D         | 10 Unk             | 34       | Unk       | Unk      | 34 |
| 990493b | C | Br IDC with mucinous differentiation | ER+/PR+/Her2-               | 35                       | BRIP1         | c.2440 C>T      | Mis              | p.R814C         | 1 Unk             | 34       | Unk       | Unk      | 34 |
| 990493b | C | Br IDC with mucinous differentiation | ER+/PR+/Her2-               | 35                       | RADS1C        | c.635T>G        | Mis              | p.R212H         | 1 Unk             | 34       | Unk       | Unk      | 34 |
| YP5   | C    | Br IDC             | ER+/PR+/Her2-               | 38                       | RADS1D        | c.932T>A        | Mis              | p.311N           | FH not found in the Case note | Unk     | Unk       | Unk      | 34 |
| YP16b  | C    | Br IDC             | ER+/PR+/Her2-               | 38                       | RADS1D        | c.932T>A        | Mis              | p.311N           | 1 Unk             | Unk     | Unk       | Unk      | 41 |
| YP47  | C    | Br IDC             | ER+/PR+/Her2-               | 36                       | RADS1D        | c.932T>A        | Mis              | p.311N           | FH not found in the Case note | Unk     | Unk       | Unk      | 41 |
| 12522596b | C | Br IDC             | ER+/PR+/Her2-               | 32                       | RADS1D        | c.932T>A        | Mis              | p.311N           | 1 Unk             | Unk     | Unk       | Unk      | 41 |

Abbreviations: Au, aunt; B, burmese; Bi, bilateral; Bo, boadicea Score; Br, breast; Bro, brother; C, chinese; Ca, cancer; Co, cousin; Col, colorectal; Endo, endometrial; ER, oestrogen receptor; F, filipino; Fa, father; FH, family history; Fr_del, frameshift deletion; Fr_ins, frameshift Insertion; ga, gastric; GF, grandfather; GM, grandmother; GIST, gastrointestinal stromal tumour; I, indonesian; ILC, invasive ductal carcinoma; ILc, invasive lobular carcinoma; IO, indonesian; J, japanese; M, malay; Mis, missense; Mat, maternal; MC, manchester Score; Mo, mother; N, nonsense; NA, not applicable; Ov, ovarian; Pa, pancreatic; Pat, paternal; Pros, prostate; PRR, progesterone receptor; Ref, reference; SE, splice site Error; Sis, sister; SL, sri lankan; Thy, thyroid; Un, uncle; Unk, unknown; V, vietnamese.

*aUnderlined indicates novel pathogenic variants identified by our group.
*bPatients with more than one pathogenic variant.
*cPatient with male breast cancer.
variants in genes of moderate penetrance (e.g., CHEK2, ATM and BLM). There is remaining uncertainty in penetrance estimates for such variants, and, therefore, the optimal breast screening protocol and age of initiation remain unknown thus limiting the clinical utility of panel testing (for the present) to highly penetrant mutations. To better understand the role of these moderately penetrant genes will require population-based studies of mutation penetrance and clinical trials of risk-reducing interventions to guide clinical decisions. It is a major concern that while the practice of clinical cancer genetics is largely limited in developed countries to trained clinical cancer geneticists, this is not the case for the rest of the world.

![Figure 1. Pathogenic variants detected in 17 genes.](image)
The discovery of VUS that do not contribute to risk, may prompt anxiety and overtreatment particularly if the managing clinician is unfamiliar with genetics. Although our experience of finding ~3 VUS per gene is consistent with that from other studies, it also highlights the fact that the more we sequence, the more VUS we will unravel. This is particularly so in a population like Singapore, where we have multi-ethnic minority groups for whom there is limited publicly available sequencing data for variant reclassification. In the present study, consistent with our IRB–approved protocol, we did not re-contact any patient about VUS as there are no immediate clinical implications or recommendations to convey. In the clinical setting, where VUS results will be reported back to the patient, it is critical therefore that multigene panel testing is conducted in a dedicated genetics service with a genetics team familiar with cancer risk assessment and who are able to provide adequate pretest and post-test counselling.

This study was conducted within a formal clinical cancer genetics practice adherent to evidence-based testing guidelines, and using the definition of pathogenic variants as recommended by the American College of Medical Genetics. With the clinical availability of multiple-gene sequencing panels and the concurrent decreasing cost of panel testing, it is anticipated that an increased demand for such gene-directed risk stratification will occur. These genetic testing costs are borne by the patient and not by any third-party payer, especially in Asian countries with no insurance coverage or government subsidies for genetic testing for most countries at present. With the reducing costs of genetic testing, many of these health policies are ripe for review if we wish to harness the power of gene-enabled care.

Our study has limitations. The 25 genes that we selected reflect published literature but an optimal multiple-gene panel for routine diagnostic use remains to be defined. Patients were enrolled from within a specialized clinical cancer genetics service and do not reflect general oncology practice nor the general population at large.

### MATERIALS AND METHODS

#### Patients

We studied 220 cases referred to the Cancer Genetics Service at the National Cancer Centre Singapore. Of these, 210 had a personal history of breast and/or ovarian cancer (192 had breast cancer, 9 had ovarian cancer, and 9 had breast and ovarian cancer). The subjects fulfilled at least one of the following criteria: (1) having a family history of breast and/or ovarian cancer in first- and/or second-degree relatives; (2) having breast and ovarian cancer in the same individual or bilateral breast cancer; (3) having early-onset breast cancer or ovarian cancer (<40 years of age). Clinical information including personal and family cancer histories, cancer histology and receptor status, were retrieved from case notes and clinical databases. All patients consented to participate in this study, which was approved by the SingHealth Centralized Institutional Review Board (CIRB 2008/455/B; CIRB 2010/406/B).

#### Mutation detection using next-generation sequencing (NGS)

An optimised in-house method was used to extract DNA from peripheral blood. Capture was performed using the SureSelect XT2 target enrichment kit (Agilent, Santa Clara, CA, USA), targeting 25 genes (Supplementary Table 1). The Covaris S2 system (Covaris, Woburn, MA, USA) was used to fragment the genomic DNA samples as recommended by the manufacturer. The exome-enriched libraries were sequenced on the
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Illumina HiSeq platform (San Diego, CA, USA), with 100-bp paired-end reads.

Detection of large genomic rearrangements in the BRCA1 and BRCA2 genes was done for all 220 samples using the Multiplex Ligation-dependent Probe Amplification test kits (P002-C2 BRCA1 and P045-BRCA2/CHECK2) and confirmation kits (P087-BRCA1 and P077-BRCA2; MRC-Holland, Amsterdam, Netherlands). DNA fragment analysis was performed on the ABI 3130 Genetic Analyzer (ABI-Life Technologies, Thermo Fisher Scientific Corporation, MA, USA) and analysed using the Cofalysfreeware v.131123.1303 (MRC-Holland).

Bioinformatic analysis

The raw reads were aligned to the hg19 reference genome using BWA.12 BAM files were processed to identify variants using the Genome Analysis Tool Kit. The variants were annotated using the ANNOVAR tool.25 The mean depth of coverage was \( x \times 315 \) (range: \( x^{97-858} \)). Population allele frequencies were extracted from the Exome Variant Server (http://evs.gs.washington.edu/EVS), 1000 Genomes (http://www.1000genomes.org), and dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/). Framework and nonsense mutations were considered to be deleterious. Missense variants were classified as damaged or benign using predictions from SIFT,13 PolyPhen-II HDIV,13 PolyPhen-II HVAR,16 LRT and Mutation Taster.15 If three or more of the five tools predicted the missense mutation to be damaging, then the mutation was classified as damaging. All deleterious or damaging variants were verified visually using the Integrative Genomics Viewer (IGV; Broad Institute), and collectively classified as pathogenic variants.

Variants that were synonymous, or classified as benign, unknown, uncertain or unspecified in the Breast Cancer Information Core, HGMD, ClinVar databases, were excluded. Also excluded were variants with an allele frequency greater than 1% as documented in the Exome Variant Server, 1000 Genomes, dbSNP and ExAC databases. All remaining variants were classified as VUS, and were verified visually using IGV.

Validation of variants detected by NGS

All frameshift, nonsense and damaging missense mutations were validated by Sanger sequencing. PCR amplification using HotStarTag (Qiagen, Hilden, Germany) using primers flanking mutations was performed as previously described.11 The BigDye Terminator v3.1 cycle sequencing kit (ABI-Life Technologies, Thermo Fisher Scientific Corporation) was used for the incorporation of dye-labelled dNTPs followed by Sanger sequencing using a 3130xl Genetic Analyzer (ABI-Life Technologies, Thermo Fisher Scientific Corporation). The chromatograms were visualised using the Sequenom Pro v.12 (Lasergene; DNASTAR, Madison, WI, USA) software.

Statistical analysis

Participant characteristics and sequencing results were tabulated, with descriptive statistics including medians, means and ranges.

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CONTRIBUTIONS

AL, YSY and PA conceived the study. AL and JN designed the study. PA and MHT provided genetic counselling and accrued participants for the study. EW, CC and MS contributed to acquisition of data. EW, SS, MM, CC, MS, SR, JN and AL contributed to data analysis and interpretation of data. All authors contributed to manuscript writing and approved the final version of the article. JN and AL are the guarantors of this manuscript.

COMPETING INTERESTS

The authors declare no conflict of interest.

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