Prevalence of PALB2 Mutations in Breast Cancer Patients in Multi-Ethnic Asian Population in Malaysia and Singapore

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Abstract

**Background:** The partner and localizer of breast cancer 2 (PALB2) is responsible for facilitating BRCA2-mediated DNA repair by serving as a bridging molecule, acting as the physical and functional link between the breast cancer 1 (BRCA1) and breast cancer 2 (BRCA2) proteins. Truncating mutations in the PALB2 gene are rare but are thought to be associated with increased risks of developing breast cancer in various populations.

**Methods:** We evaluated the contribution of PALB2 germline mutations in 122 Asian women with breast cancer, all of whom had significant family history of breast and other cancers. Further screening for nine PALB2 mutations was conducted in 874 Malaysian and 532 Singaporean breast cancer patients, and in 1342 unaffected Malaysian and 541 unaffected Singaporean women.

**Results:** By analyzing the entire coding region of PALB2, we found two novel truncating mutations and ten missense mutations in families tested negative for BRCA1/2-mutations. One additional novel truncating PALB2 mutation was identified in one patient through genotyping analysis. Our results indicate a low prevalence of deleterious PALB2 mutations and a specific mutation profile within the Malaysian and Singaporean populations.

Introduction

PALB2 (PArtnet and Localizer of BRCA2) is a protein that interacts with BRCA2, stabilizing the intranuclear accumulation of BRCA2 proteins at sites of DNA damage [1]. Biallelic loss of PALB2 causes increased predisposition to cancers, increased sensitivity to DNA damaging agents and Fanconi’s Anemia [2].

Germline PALB2 mutations are rare, but have been reportedly associated with increased predisposition to breast and other cancers. In multiple-breast cancer case families, germline PALB2 mutations have been reported in 1.1% (10 out of 920), 2.7% (3 out of 113), 2.0% (1 out of 50), and 0.6% (5 out of 779) of Western European families in the United Kingdom, Finland, French-Canada, and Australasian, respectively [3–6]. Within the familial context, germline PALB2 mutations are associated with a 2.3 to 6 fold increased risk to breast cancer [3,7,8]. Germline PALB2 mutations have also been reported in unselected female breast cancer cases, but at a lower prevalence (0.9%, 18 out of 1,918 in Finnish breast cancer cases [4]; 0.5%, 2 out of 356 in French-Canadian breast cancer patients aged <50 years old [5]; and 0.4%, 5 out of 1,403 in Australian breast cancer patients [6]). Notably, deleterious germline PALB2 mutations have also been reported in pancreatic cancer cohorts from United States [9] and Europe.

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study because they were either (a) diagnosed ≤50 years old
cancer, including all third degree relatives. Mutation detection
had at least one first- or second-degree relatives with
parentage or other ethnicities (20 cases); and (d) had
study. All index patients were asked about family history of any
missing histopathology data (74 cases); (c) from mixed
study. The study was approved by the Medical Ethics
Ethics statement
All study subjects were provided written informed consent. Blood, demographic and family history data was collected from breast cancer patients who consented to participate in this study. The study was approved by the Medical Ethics Committee of University Malaya Medical Centre (UMMC).

Materials and Methods

Ethics statement
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Study subjects
(a) Familial breast cancer patients for DNA sequencing. The recruitment of breast cancer patients into the Malaysian Breast Cancer Genetic Study (MyBrCa) started in January 2003 at the University Malaya Medical Centre in Kuala Lumpur. All were histopathology-proven breast carcinoma. From January 2003 to December 2010, a total of 1,220 breast cancer patients were recruited into the MyBrCa study. All index patients were asked about family history of any cancer, including all third degree relatives. Mutation detection for germline BRCA1 and BRCA2 mutations was conducted on 402 individuals by direct DNA sequencing and multiple ligation dependent probe amplification (MLPA) as previously described [15,16]. Of these, 155 individuals were included in the PALB2 study because they were either (a) diagnosed ≤50 years old and had at least one first- or second-degree relatives with breast cancer; (b) diagnosed with bilateral breast cancer with the primary cancer at age 50 or below; (c) diagnosed with male breast cancer at any age; (d) affected with both breast and ovarian cancers; (e) affected with both breast and pancreatic cancers; or (f) diagnosed with breast cancer and had at least one first-degree relative with pancreatic cancer.

(b) Malaysian breast cancer cases and controls for genotyping. All women with invasive breast cancer who participated in the MyBrCa study between January 2003 and December 2010 were included in hospital-based cohort of breast cancer cases. Of the 1,220 breast cancer patients, 195 individuals were excluded from the study if they were either (a) diagnosed with non-invasive carcinoma (51 cases); (b) had missing histopathology data (74 cases); (c) from mixed parentage or other ethnicities (20 cases); and (d) had insufficient or low quality genomic DNA samples (52 cases). Of the remaining individuals, a total of 874 breast cancer cases consisting of 528 (out of 621) Chinese, 180 (out of 224) Malay and 1166 (out of 180) Indian cases were included in the study.

Women with no breast cancer were selected from two sources. The first was a cohort of 270 healthy individuals attending the hospital for non-cancer related ailments and who had no family history of cancer in first or second degree relatives (270 healthy individuals comprising 90 Chinese, 90 Malays and 90 Indians). The second cohort comprised 1,072 women attending an opportunistic mammographic screening programme from October 2011 to September 2012.

(c) Singaporean breast cancer cases and controls for genotyping. An additional 532 breast cancer cases from Singapore Breast Cancer Cohort Project and 541 age-matched healthy controls from the Multi-ethnic Cohort in Singapore Consortium of Cohort Studies (SCCS) were analysed in this study. The recruitment of Singapore Breast Cancer Cohort Project started in April 2010 at National University Hospital and included both prevalent and incident breast cancer cases diagnosed from 1988 onwards. 740 patients have been recruited between April 2010 and March 2011 and 75% of total participants donated blood or saliva. Cases were excluded from current analysis due to (a) from mixed parental or other ethnicities (13 cases), (b) male gender (1 case) and (c) had insufficient or low quality of genomic DNA samples (7 cases). The Multi-ethnic Cohort in SCCS consisted of approximately 12,000 community-dwelling individuals who are Singaporean or Singaporean Permanent Residents, 21 years and older. Exclusion criteria were a medical history of cancer, acute myocardial infarction or stroke, or major psychiatric morbidity including schizophrenia, psychotic depression, and advanced Alzheimer’s Disease. 77% of the participants who were interviewed provided a blood sample.

PALB2 analysis
Sequencing of all the intron-exon junctions and exonic sequences was conducted using Sanger sequencing of genomic DNA extracted from peripheral white blood cells using standard methods. Primer sequences were adapted from several publications (Table S1) [2,6,17].

PALB2 Genotyping
Multiplex genotyping of seven PALB2 mutations identified in exon 4 in this study and an additional two Asian recurrent mutations were performed using high-throughput Sequenom MassARRAY iPLEX platform (Sequenom Inc., San Diego, California, USA). Mutations in other exons were identified after the completion of the genotyping study and were therefore excluded from analyses. All mutations identified were confirmed by direct sequencing in an independent DNA sample.

In silico analysis
In silico analysis of the effect of missense mutations on protein function was determined using Polyphen-2 (Polymorphism Phenotyping version 2) [18] and SIFT (Sorting Tolerant from Intolerant) [19,20].
developed invasive ductal carcinoma at age 38, which was positive for the expression of both estrogen- and progesterone-receptors and had amplification of HER-2 receptor. Her mother was affected with breast cancer at age 51 (Figure 1 B).

Evaluation of variants pathogenicity through case-control analysis

Rare mutations detected from the sequencing of exon 4 of PALB2 (n=7) and deleterious mutations that were reported to be recurrent in other Asian populations (Chinese PALB2 c.751 C>T and PALB2 c.1250_1251 delAAinsTCT) [13] were tested for in additional cohorts of breast cancer cases and controls by genotyping (Table 3). The cases comprised a hospital-based cohort of 874 breast cancer cases from Malaysia and 532 breast cancer cases from Singapore. The unaffected controls comprised 270 women attending non-cancer related procedures at the same hospital, 1072 women attending mammogram screening and 541 population-based controls from Singapore.

(a) Deleterious mutations. One of the three deleterious mutations evaluated was detected in the Malaysian breast cancer cases and none were identified in the Malaysian unaffected women, Singaporean breast cancer cases or unaffected women (Table 3). Sequence analysis after genotyping also revealed a novel PALB2 frameshift mutation, PALB2 c.1050 delAAinsTCT occurring at the same amino acid as the tested mutation, PALB2 c.1050 delAAinsTCT.

The patient with this novel deleterious mutation was a Chinese woman who developed infiltrating ductal carcinoma at age 62, which was positive for the expression of the estrogen receptor and negative for the expression of the progesterone- and HER2 receptors. Her paternal uncle was diagnosed with nasopharyngeal cancer in his 70s (Figure 1 C).

(b) Missense variants. PALB2 Q559R was determined to be common in the cases and controls (33% and 27% respectively) and this variant was also found in five homozygote unaffected controls (data not shown) showing that this variant is a polymorphism.

Both PALB2 I309V and PALB2 D498Y variants were detected in more than 1% of unaffected women from the Malaysian and Singaporean control cohorts suggesting that these are polymorphisms. Notably, PALB2 I309V was predicted to be benign based on SIFT and Polyphen-2 in-silico predictions whereas PALB2 D498Y was suggested to be possibly damaging in these in-silico analysis. The remaining three missense mutations were determined to be rare. PALB2 H553Q, predicted to be benign by Polyphen-2 and SIFT, was found in one Malaysian and one Singaporean breast cancer cases (0.1% and 0.2% respectively) and none in the controls. PALB2 P249L and PALB2 E352Q were each found in one breast cancer case and one control. In-silico predictions suggest that P249L may be benign while E352Q is probably damaging.

Table 1. Characteristics of 122 individuals tested for PALB2 germline mutations.

| Characteristics | No | % |
|-----------------|----|---|
| **Ethnicity**   |    |   |
| Chinese         | 82 | 67.2 |
| Malay           | 25 | 20.5 |
| Indian          | 12 | 9.8 |
| Others          | 3  | 2.5 |
| **Female breast cancer** |    |   |
| Chinese         | 121 | 99.2 |
| Malay           | 1   | 0.8 |
| **Male breast cancer** |    |   |
| Chinese         | 1  | 0.8 |

| Age at diagnosis | No | % |
|------------------|----|---|
| ≤ 35             | 28 | 23.0 |
| 36-40            | 31 | 25.4 |
| 41-50            | 55 | 45.1 |
| 51-60            | 5  | 4.1 |
| > 60             | 3  | 2.5 |

| Personal cancers | No | % |
|------------------|----|---|
| Bilateral cancer: |    |   |
| 1 ≤ 50 years     | 21 | 17.2 |
| Ovarian cancer    | 6  | 4.9 |
| Pancreatic cancer | 1  | 0.8 |
| **Manchester Score** |    |   |
| < 15             | 83 | 68.0 |
| ≥ 15             | 39 | 32.0 |

| Family history of cancers (up to 3rd degree) | No | % |
|---------------------------------------------|----|---|
| Breast cancer                               | 100 | 82.0 |
| Ovarian cancer                              | 3  | 2.5 |
| Pancreatic cancer                           | 2  | 1.6 |
| Prostate cancer                             | 3  | 2.5 |
| **No family history of either breast, ovarian, pancreatic and prostate cancers** | 20 | 16.4 |

In total, 122 breast cancer patients who were tested negative for germline mutations in BRCA1 and BRCA2 were analyzed for germline mutations in PALB2 by Sanger sequencing. Table 1 shows the distribution of index patients according to their ethnicity, age at diagnosis and family history characteristics.

Results

Prevalence of PALB2 germline mutations in familial breast cancer patients

Of the 155 high risk breast cancer patients selected for this study, 20 had germline deleterious mutations in BRCA1 and 13 in BRCA2. The remaining 122 individuals tested negative for BRCA mutations were analyzed for PALB2 germline mutations by direct Sanger sequencing (Table 1) and 13 genetic variants were identified (Table 2). These included two deleterious mutations (mutation prevalence of 1.6%), ten missense variants of unknown clinical significance and one synonymous variant.

(a) Mutation c.1037_1041 delAAGAA; STOP 358. This truncating mutation was found in a Chinese woman who developed invasive ductal carcinoma at age 38, which was negative for the expression of the estrogen- and HER-2 receptors and positive for the expression of the progesterone-
Four other missense mutations (PALB2 T300I, L763F, A1017T and E1018D) were not tested in the case-control analysis. Variants pathogenicity prediction using in-silico tools suggest that PALB2 T300I is benign while Polyphen-2 predicted PALB2 L763F, PALB2 A1017T and PALB2 E1018D to be probably damaging.

Discussion

In this study, we have analysed the prevalence of PALB2 mutations in a cohort of 122 familial breast cancer cases who do not have alterations in BRCA1 or BRCA2 and found 2 (1.3%) were PALB2 carriers. Notably, we did not examine large chromosomal rearrangements which have previously been reported in other studies [21,22] and it is possible that these rare genetic changes could have account for additional PALB2 carriers in our population. Although the patients in this study comprised 3 ethnic groups (Chinese, Malay and Indian), all 3 individuals with PALB2 deleterious mutations are Chinese. There was only 4 missense variants found in the Malay and Indian women in this study (compared to 7 in the Chinese women) and this is likely to be because the majority of the women in this study are Chinese. The rarity of germline PALB2 mutations is consistent with that reported in other populations where a mutation prevalence of 0-2% has been reported [17,23–25].

Our study also identified 10 PALB2 missense variants, of which 5 are novel [25,26]. Of these, 6 were tested in a case-control analysis and our results suggest that 5 are likely to be either benign (PALB2 Q559R, I309V, D498Y, P249L and E352Q) or associated with a low increased risk of breast cancer. PALB2 H553Q was found in 2 women affected with breast cancer but not in 1,875 controls. Further analysis of this and other variants [T300I, L763F, A1017T, E1018D] require other methods, including in vitro functional characterization [27] and co-segregation analyses.

Our study uncovered an unusual situation where genotyping of a deleterious mutation (PALB2 c.1050 delAAinsTCT) [13] revealed a distinct mutation (PALB2 c.1050 delAAACA) at the same site. Sequence alteration caused by deletion of AACA resulted in mutant SNP that is similar to the interrogated SNP and was therefore regarded as a positive screen. This highlights the limitation of genotyping assay in distinguishing distinct mutations which share a common mutant SNP and therefore, confirmation by conventional sequencing is necessary.

Notably, of the 3 individuals with germline deleterious PALB2 mutations, 2 have moderate family history with Manchester score of 12 and 14 respectively, whereas one developed breast cancer at late age in the absence of any family history of cancer (Manchester score of 2). Indeed, by contrast to BRCA1 and BRCA2 mutation status, we found that there was no association between PALB2 mutation status and family history of breast, ovarian, prostate or pancreatic cancer (data not shown). This is consistent with other studies that have also shown no familial clustering of breast cancer in PALB2 families compared to non-BRCA carrier families [3,14,23]. The family members of the PALB2 carrier families were not available for co-segregation analysis and therefore it was not possible to determine the increased risk caused by germline mutation of PALB2 in our population.

In summary, we found 2 germline PALB2 deleterious mutation carriers in 122 high risk non-BRCA1 or BRCA2 breast cancer patients by DNA sequencing and 1 germline carrier in 1,875 controls. Further analysis of this and other variants requires other methods, including in vitro functional characterization and co-segregation analyses.

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Figure 1. Pedigrees of families with germline mutations in PALB2. (A) Family pedigree of BRC945, carrier of c.1037_1041delAAGAA [STOP358]. (B) Family pedigree of BRC859, carrier of c.2606delC [S869X]. (C) Family pedigree of BRC1126, carrier of c.1050delAACA [STOP353]. Index patients are indicated with an arrow while individuals affected with breast cancer are indicated with filled symbol. Date of birth (DOB) and age of diagnosis (in bracket) for affected individuals are indicated. Deceased individuals are indicated with a slash.

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Table 3. Case-control analysis of nine PALB2 variants identified from exon 4 sequencing (n=7) and Asian populations (n=2).

| Exon | Type | Mutation/Variant | Malaysia Case-control | Singapore Case-Control |
|------|------|------------------|-----------------------|------------------------|
|      |      |                  | Cases (874) | Controls (270) | Controls (1072) | Cases (532) | Controls (541) |
|      | Exonic | HGVS | AA change | (%) | (%) | (%) | (%) |
| 4   | Deletion | delAAAGA | c.1037_1041 | STOP | 358 | 1/868 | 0.1 | 0/264 | 0.0 | 0/1058 | 0.0 | 0/519 | 0.0 | 0/536 | 0.0 |
| 4   | Missense | A>G | c.1676 A>G | Q559R | 286/871 | 32.8 | 70/257 | 27.2 | na | na | na | na | na | na |
| 4   | Missense | A>G | c.925 A>G | I309V | 7/870 | 0.8 | 2/270 | 0.7 | 16/1079 | 1.5 | 14/507 | 2.8 | 13/519 | 2.5 |
| 4   | Missense | G>T | c.1492 G>T | D498Y | 5/845 | 0.6 | 1/225 | 0.4 | 13/1060 | 1.2 | 5/526 | 1.0 | 10/528 | 1.9 |
| 4   | Missense | C>T | c.745 C>T | P249L | 1/872 | 0.1 | 2/1070 | 0.7 | 0/1002 | 0.0 | 0/529 | 0.0 | 0/536 | 0.0 |
| 4   | Missense | G>C | c.1054 G>C | E352Q | 1/870 | 0.1 | 0/262 | 0.0 | 0/1079 | 0.0 | 0/527 | 0.0 | 1/535 | 0.2 |
| 4   | Missense | C>A | c.1659 A>G | H553Q | 1/870 | 0.1 | 0/269 | 0.0 | 0/1078 | 0.0 | 1/514 | 0.2 | 0/528 | 0.0 |
| 4   | Chinese deletion | C>T | c.751 C>T | Q251X | 0/870 | 0.0 | 0/265 | 0.0 | 0/1051 | 0.0 | 0/523 | 0.0 | 0/536 | 0.0 |
| 4   | Chinese deletion | delAAinsTCT | c.1050_1051 | STOP | 353 | 1/873 | 0.1 | 0/267 | 0.0 | 0/1059 | 0.0 | 0/519 | 0.0 | 0/538 | 0.0 |

Table S1. Amplification primers used for the sequencing of PALB2 gene.

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Author Contributions

Conceived and designed the experiments: SYP SYL SHT. Performed the experiments: SYP SYL PK INK. Analyzed the data: SYP. Contributed reagents/materials/analysis tools: SYL MKT MH JHS CHY NAMT. Wrote the manuscript: SYP SHT.
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