High Prevalence of Germline Mutations in Cancer Susceptibility Genes in Thai Patients with Clinical Spectrum of Hereditary Breast-Ovarian Cancer Syndrome

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Research article
Abstract

**Background:** Germline genetic mutation plays a significant role in breast cancer susceptibility. The strength of such predisposition varies among ethnic groups across the globe, and clinical data from Asian population to develop a strategic approach to who should undergo a genetic test are lacking.

**Methods:** We performed a multigene test with next generation sequencing in our 5-year hereditary breast-ovarian cancer spectrum cohort consists of 306 breast cancer patients, 62 ovarian cancer patients, 14 pancreatic cancer patients and 7 prostatic cancer patients.

**Results:** There were 83 pathogenic/likely pathogenic (P/LP) variants identified in 104 patients, 44 of these P/LP variants were novel. We reported a high rate of germline P/LP variants in breast cancer (24%), ovarian cancer (37%), pancreatic cancer (14%), and prostatic cancer (29%). Germline P/LP variants in BRCA1 and BRCA2 accounted for 80% of P/LP variants found in breast cancer and 57% of P/LP variants found in ovarian cancer. The detection rate of NCCN 2019 guideline for genetic/familial high-risk assessment of breast and ovarian cancers was 22-40%.

**Conclusion:** Overall, the data from this study strongly support the consideration of multigene panel test as a diagnostic tool for patients with hereditary breast-ovarian cancer spectrum in Thailand.

Introduction

Breast cancer is the second most common cancer and the second leading cause of cancer-related death in the US[1]. Genetic predisposition accounts for 10–30% of breast cancer cases, and its rate of finding germline pathogenic variants in BRCA1 or BRCA2 (gBRCA) was 3–5%[2, 3]. In recent review, prevalence of BRCA1/2 status in breast cancer varied across the globe. Mutations in gBRCA were found in 3% of unselected breast cancer, while the prevalence could be above 20% in selected group[4]. Following the discovery of BRCA1 and BRCA2, several breast cancer genes with various degree of penetrance were identified[2]. BRCA1, BRCA2, CDH1, STK11 and TP53 are generally considered high-penetrance genes for breast cancer and the moderate-penetrance genes included ATM, BRIP1, CHEK2, and PALB2, though the gene lists can be dynamic [5, 6]. It is comprehensible that testing more genes could identify more patients with heritable form of breast cancer and provide benefit on cancer screening or prevention for at-risk individuals. With higher throughput and cheaper cost of next generation sequencing, multigene panel testing has been widely adopted for patients with breast cancer[7].

Though, specific guidelines for each causative gene are increasingly available, consensus on breast cancer germline testing strategy among medical community is lacking. Various approaches on test eligibility are ranging from a population-based screening campaign to an individual-based program[8]. Multiple models to estimate the likelihood of having gBRCA mutations and different testing criteria for patients with breast cancer have been used based on population data and national healthcare policies. Successful clinical implementation of germline testing also requires data from ethnically diverse
population. Unfortunately, existing models and test criteria are mostly suitable for Western population, while data on other ancestries are very limited.

This study aims to investigate prevalence and diversity of mutations from multigene panel testing of Thai patients with breast cancer and other cancer in the hereditary breast-ovarian cancer spectrum and compare the clinical phenotype of patients with detectable mutations to a widely accepted clinical guideline.

**Materials And Methods**

**Study population**

The study protocols were approved by the Siriraj Hospital Institutional Review Board Protocol No.474/2562(EC1) and 418/2562(EC2). The study was conducted according to the Good Clinical Practice and the Declaration of Helsinki. All Thai patients who were diagnosed with primary breast, ovarian, pancreas, or prostate cancers and treated at Siriraj Hospital, whose blood were sent for germline cancer susceptibility gene testing between 2016 to 2020 were included. We also included patients who had a report of pathogenic variants or likely pathogenic variants (P/LP variants) in genes for breast cancer listed in Table 1 as a secondary finding. We excluded patients with known clinical or molecular diagnosis of genetic diseases (e.g. neurofibromatosis type 1), patients referred for testing of only specific mutations, or asymptomatic individuals with known affected family members. We recruited a total of 377 unrelated patients. Three hundred and six patients had breast cancer, of which 19 of them also had primary ovarian cancer. Forty-three patients had primary ovarian cancer without breast cancer. There were 14 patients with pancreatic cancer and 7 patients with prostate cancer. Their tumor histological statuses, age of onset, and family history were comprehensively reviewed with the 2019 National Comprehensive Cancer Network (NCCN) guideline for genetic/familial high-risk assessment of breast and ovarian cancers. Descriptive statistics was used to calculate the rate of P/LP variants or variants of undetermined significance (VUS) across different indications. There were an additional of 7 patients who were recruited because they harboured P/LP variants in genes for breast cancer (either ATM, BRCA1 or BRCA2) as a secondary finding.
Table 1

| Phenotype                                      | Genes                                      |
|-----------------------------------------------|--------------------------------------------|
| High-penetration gene for breast cancer       | BRCA1, BRCA2, CDH1, STK11, TP53            |
| Moderate-penetration gene for breast cancer   | ATM, BRIP1, CHEK2, NF1, PALB2              |
| Possible breast cancer gene                   | BARD1, NBN, RAD50, XRCC2                  |
| Moderate-risk ovarian cancer gene             | RAD51C, RAD51D, MLH1, MSH2, MSH6, PMS2, EPCAM |
| Genes that are highly penetrance in other types of cancer | APC, AXIN2, BMPR1A, CDK4, CDKN2A, FANCC, MSH3, MUTYH, NTHL1, POLD1, POLE, PTEN, RECQL, SMAD4, VHL |

The OMIM numbers for each gene are
- BRCA1 (OMIM number 113705), BRCA2 (OMIM number 600185), CDH1 (OMIM number 192090), STK11 (OMIM number 602216), TP53 (OMIM number 191170), ATM (OMIM number 607585), BRIP1 (OMIM number 605882), CHEK2 (OMIM number 601473), NF1 (OMIM number 613113), PALB2 (OMIM number 610355), BARD1 (OMIM number 601593), NBN (OMIM number 602667), RAD50 (OMIM number 604040), XRCC2 (OMIM number 600375), RAD51C (OMIM number 602774), RAD51D (OMIM number 602954), MLH1 (OMIM number 120436), MSH2 (OMIM number 609309), MSH6 (OMIM number 600678), PMS2 (OMIM number 600259), EPCAM (OMIM number 185535), APC (OMIM number 611731), AXIN2 (OMIM number 604025), BMPR1A (OMIM number 601299), CDK4 (OMIM number 123829), CDKN2A (OMIM number 600160), FANCC (OMIM number 613899), MSH3 (OMIM number 600887), MUTYH (OMIM number 604933), NTHL1 (OMIM number 602656), POLD1 (OMIM number 174761), POLE (OMIM number 174762), PTEN (OMIM number 601728), RECQL (OMIM number 600537), SMAD4 (OMIM number 600993), VHL (OMIM number 608537)

Multigene panel test for hereditary cancer

Genomic DNA is extracted from peripheral blood. The DNA is enriched for the complete coding regions and splice junctions of the genes on this panel using custom-made targeted enrichment library. The list of genes tested in our panel is demonstrated in Table 1. All single nucleotide variants and copy number variants identified by multigene panel were validated with Sanger sequencing and Multiplex Ligation-dependent Probe Amplification (MLPA) respectively. The variants were interpreted and classified per 2015 ACMG-AMP standards and guidelines for the interpretation of sequence variants[9]. All reportable variants of each patient including pathogenic/like pathogenic variants (P/LP) and variants of undetermined significance (VUS) were manually verified. The detection rate of NCCN guideline indication fulfillment was calculated by dividing the number of patients with P/LP variants identified in each specific indication by the total number of patients who fulfilled the specific indication.

Results

There were 83 unique P/LP variants identified in 104 patients (28.1%). Seventy three of 306 patients (23.9%) with breast cancer had germline P/LP variants. 23 of 62 patients (37.1%) with ovarian cancers
carried germline P/LP variants. Two of 14 patients (14.3%) with pancreatic cancer harbored germline
P/LP variants. Two of 7 patients (28.6%) with prostate cancer were identified with germline P/LP
variants. Forty-Four out of 83 P/LP variants (53%) identified in this study have not been reported
elsewhere. Thirty-one out of 57 (54%) \textit{BRCA1} and \textit{BRCA2} P/LP variants had not been previously reported.
Meanwhile, VUS were found in 124 patients (41%) with breast cancer. Eight of them had P/LP variants.
VUS were observed in 21 patients (34%) with ovarian cancer, and six of them had P/LP variants. As for 14
patients with pancreatic cancer, 6 patients (43%) were observed to have VUS without any co-occurring
P/LP variants. Four of 7 prostatic cancer patients (57%) had VUS without any P/LP variants identified.
Note that all patients identified with VUS who also harboured P/LP variants had the criteria BP5 (variant
found in a case with an alternate molecular basis for disease) applied\[9\]. No copy number variation
(deletion/duplication) identified in this study.

\section*{Mutation Spectrum}

Among 73 breast cancer patients with detectable P/LP variants, \textit{BRCA1} and \textit{BRCA2} accounted for 58
patients (79.5%). In 23 ovarian cancer patients with detectable P/PL variants, \textit{BRCA1} and \textit{BRCA2}
accounted for 13 (56.5%) patients. \textit{BRCA2} accounted for all 2 patients (100%) in 14 pancreatic cancer
patients. \textit{BRCA1} and \textit{BRCA2} accounted for 2 patients (100%) in 7 prostate cancer patients.

Multigene panel targeted sequencing did expand spectrum of germline mutations in our database.
Besides \textit{BRCA1} and \textit{BRCA2}, \textit{ATM} (5 patients) was the most commonly mutated gene in this study
followed by \textit{PALB2} and \textit{RECQL} (3 patients each). Other mutated genes included \textit{APC, BRIP1, CHEK2,
MLH1, MSH2, PMS2, MUTYH, NBN, RAD51C, RAD51D, and TP53}.

From 219 VUS identified in this study, only 27 VUS (12.3%) were found in \textit{BRCA1} and \textit{BRCA2} while 192
VUS belonged to other genes. \textit{ATM} was the most commonly identified gene with VUS, followed by \textit{APC}
and \textit{MSH6}. There were 7 putative loss-of-function (pLOF) variants (frameshift, stop gain, start loss, and
splice site variants) in 6 genes (\textit{APC, BRCA2, MSH2, RECQL, RAD51C, and XRCC2}) with insufficient data to
be designated as P/LP.

The details of identified P/LP variants, patient's phenotype and familial history were shown in Table 2.
We also included 7 patients who had a report of P/LP variants in genes for breast cancer as a secondary
finding in Table 2. Details of VUS with putative loss-of-function prediction and its patient's phenotype
were listed in Table 3. All P/LP variants in \textit{BRCA1/2} were illustrated in a lollipop plot in Fig. 1.
Table 2
List of gene(s), variants, classification, and patient’s history

| Gene (Reference Sequences) | Variant Nomenclature | Variant Classification | Cancers diagnosed in the patient | Cancers diagnosed in family member(s) |
|----------------------------|----------------------|------------------------|----------------------------------|---------------------------------------|
| **APC** (NM_000038.5)     | c.1620dupA, p.Gln541Thrfs*19 | Pathogenic            | Ovary                           | -                                     |
|                            | c.2977_2980delAAGT, p.K993Ffs*11 | Likely Pathogenic    | Breast                          | Breast                               |
| **ATM** (NM_000051.3)     | c.875C>T, p.Pro292Leu | Likely Pathogenic    | Ovary                           | -                                     |
|                            | c.2086G>T, p.Gly696* | Likely Pathogenic    | Eye                             | -                                     |
|                            | c.3693_3697delATCTT, p.Leu1231Phefs*13 | Pathogenic          | Ovary                           | -                                     |
|                            | c.7519_7520delGA, p.Asp2507Argfs*8 | Likely Pathogenic  | Colon                           | -                                     |
|                            | c.8434_8435delTC, p.Ser2812Phefs*2 | Likely Pathogenic  | Colon, Common bile duct         | Stomach, Liver, Pancreas             |
| **BRCA1** (NM_007294.3)   | c.68_69delAG, p.Glu23Valfs*17 | Likely Pathogenic    | Colon                           | -                                     |
|                            | c.213-12A>G          | Pathogenic            | Breast                          | Breast                               |
|                            | c.624_625ins(20), p.Pro209Argfs*32 | Pathogenic           | Breast                          | Breast                               |
|                            | c.1265_1266dupAT, p.Ser423Ilefs*8 | Pathogenic           | Ovary                           | Ovary, Lung                           |
|                            | c.1504_1508delTTAAA, p.Leu502Alafs*2 | Pathogenic           | Breast                          | Breast, Ovary                        |
|                            | c.1889delA, p.Asn630Ilefs*2 | Pathogenic            | Breast                          | Breast                               |
|                            | c.2101_2102delAA, p.Lys701Valfs*10 | Pathogenic           | Breast                          | Breast                               |
|                            | c.3049G>T, p.Glu1017* | Pathogenic            | Prostate                        | Stomach, Prostate, Pancreas, Thyroid  |
|                            | c.3049G>T, p.Glu1017* | Pathogenic            | Breast                          | -                                     |
| Variant Nomenclature                  | Variant Classification | Cancers diagnosed in the patient | Cancers diagnosed in family member(s) |
|--------------------------------------|------------------------|----------------------------------|--------------------------------------|
| c.3181delA, p.Ile1061*               | Pathogenic             | Ovary                            | Bladder                              |
| c.3214delC, p.Leu1072*               | Pathogenic             | Breast                           | Pancreas, Ovary                      |
| c.3403C>T, p.Gln1135*                | Pathogenic             | Breast, Ovary, Thyroid           |                                      |
| c.3424delG, p.Ala1142Hisfs*13        | Pathogenic             | Breast, Ovary                    | Breast                               |
| c.3661G>T, p.Glu1221*                | Pathogenic             | Breast                           | Pancreas, Ovary, Unknown             |
| c.3748G>T, p.Glu1250*                | Pathogenic             | Breast                           | Breast, Ovary                        |
| c.3748G>T, p.Glu1250*                | Pathogenic             | Breast                           | Breast                               |
| c.3756_3759delGTCT, p.Ser1253Argfs*10| Pathogenic             | Breast, Ovary                    | Breast                               |
| c.3770_3771delAG, p.Glu1257Glyfs*9   | Pathogenic             | Ovary                            | Ovary                                |
| c.3882_3885delCTTG, p.Leu1295Phefs*11| Pathogenic             | Breast, Ovary                    | Breast                               |
| c.4327C>T, p.Arg1443*                | Pathogenic             | Breast                           | Breast, Ovary                        |
| c.4327C>T, p.Arg1443*                | Pathogenic             | Breast                           |                                      |
| c.4327C>T, p.Arg1443*                | Pathogenic             | Breast                           |                                      |
| c.4484G>A, p.Arg1516Lys              | Likely Pathogenic      | Breast                           | Breast, Colon                        |
| c.4523G>A, p.Trp1508*                | Pathogenic             | Breast, Ovary                    | Peritoneum                           |
| c.4986 + 1G>T                       | Pathogenic             | Ovary                            | Breast, Ovary                        |
| c.5030_5033delCTAA, p.T1677Ifs*2     | Pathogenic             | Breast                           |                                      |
| c.5072C>A, p.Thr1691Lys              | Likely Pathogenic      | Lung                             | Pancreas, Breast, Ovary              |
| c.5072C>A, p.Thr1691Lys              | Pathogenic             | Breast                           | Breast                               |
| Gene (Reference Sequences) | Variant Nomenclature | Variant Classification | Cancers diagnosed in the patient | Cancers diagnosed in family member(s) |
|----------------------------|----------------------|------------------------|----------------------------------|--------------------------------------|
|                            | c.5072C > A, p.Thr1691Lys | Pathogenic             | Ovary                           | Breast                               |
|                            | c.5251C > T, p.Arg1751* | Pathogenic             | Breast                           | -                                    |
|                            | c.5511G > T, p.Trp1837Cys | Likely Pathogenic      | Breast                           | -                                    |
|                            | c.5511G > T, p.Trp1837Cys | Likely Pathogenic      | Breast                           | -                                    |
|                            | c.5511G > T, p.Trp1837Cys | Likely Pathogenic      | Breast                           | -                                    |
|                            | c.5511G > T, p.Trp1837Cys | Likely Pathogenic      | Breast                           | Ovary                               |
|                            | c.5574G > T, p.Trp1858Cys | Likely Pathogenic      | Breast                           | Breast                               |
|                            | c.5574G > T, p.Trp1858Cys | Likely Pathogenic      | Breast                           | Breast                               |
|                            | c.5574G > T, p.Trp1858Cys | Likely Pathogenic      | Breast                           | Breast                               |
|                            | c.5574G > T, p.Trp1858Cys | Likely Pathogenic      | Breast                           | Breast                               |
|                            | BRCA2 (NM_000059.3)    | c.18_19delAG, p.Arg8Alafs*5 | Pathogenic                     | Breast                               |
|                            |                       | c.22_23delAG, p.Arg8Alafs*5 | Pathogenic                     | Breast                               |
|                            |                       | c.22_23delAG, p.Arg8Alafs*5 | Pathogenic                     | Breast, Ovary                        |
|                            |                       | c.22_23delAG, p.Arg8Alafs*5 | Pathogenic                     | Breast                               |
|                            |                       | c.22_23delAG, p.Arg8Alafs*5 | Pathogenic                     | Pancreas                             |
|                            |                       | c.157A > T, p.Lys53*    | Pathogenic                     | Breast                               |
|                            |                       | c.346delA, p.Ser116Valfs*5 | Pathogenic                     | Breast                               |
|                            |                       | c.755_758delACAG, p.Asp252Valfs*24 | Pathogenic | Nasopharynx | Breast, Ovarian, Pancreas |
|                            |                       | c.1399_1402delAAGA, p.Lys467Glufs*17 | Pathogenic | Breast     |
| Gene (Reference Sequences) | Variant Nomenclature | Variant Classification | Cancers diagnosed in the patient | Cancers diagnosed in family member(s) |
|----------------------------|----------------------|------------------------|----------------------------------|--------------------------------------|
| c.1813delA, p.Ile605Tyrfs*9 | Pathogenic | Colon                  | Colon, Breast, HCC                |
| c.2327delA, p.Lys776Argfs*7 | Pathogenic | Ovary                  | -                                 |
| c.2372C>G, p.Ser791*       | Pathogenic | Breast                 | -                                 |
| c.2808_2811delACAA, p.Ala938Profs*21 | Pathogenic | Breast | Male breast, Ovary |
| c.3716_3717delAA, p.Lys1239Thrfs*3 | Pathogenic | Breast | Breast, Leukemia, Prostate |
| c.3716_3717delAA, p.Lys1239Thrfs*3 | Pathogenic | Colon                  | Lung                              |
| c.3847_3848delGT, p.Val1283Lysfs*2 | Pathogenic | Breast, Ovary | Peritoneum |
| c.3865_3868delAAAT, p.Lys1289Alafs*3 | Pathogenic | Breast | Breast |
| c.5645C>A, p.Ser1882*      | Pathogenic | Breast, Thyroid        | Prostate                          |
| c.5645C>A, p.Ser1882*      | Pathogenic | Breast                 | Breast                            |
| c.6298_6299insA, p.Asn2101Lysfs*10 | Pathogenic | Breast | Breast, Endometrium, Pancreas |
| c.6405_6409delCTTAA, p.Asn2135Lysfs*3 | Pathogenic | Breast | - |
| C6486_6489delACAA, p.Lys2162Asnfs*5 | Pathogenic | Breast | Breast |
| c.6532dupC, p.His2178Profs*11 | Pathogenic | Breast                 | Breast, Prostate, Colon           |
| c.6673delA, p.Thr2225Glnfs*4 | Pathogenic | Breast                 | Breast                            |
| c.6777_6778delTG, p.N2259Kfs*33 | Pathogenic | Breast, Ovary | Colon, Endometrium |
| c.6896delA, p.Asn2299Ilefs*6 | Pathogenic | Breast                 | -                                 |
| Gene (Reference Sequences) | Variant Nomenclature | Variant Classification | Cancers diagnosed in the patient | Cancers diagnosed in family member(s) |
|---------------------------|----------------------|------------------------|----------------------------------|--------------------------------------|
| BRIP1 (NM_032043.2)       | c.1343G > A, p.Try448* | Pathogenic             | Breast                           | -                                    |
|                           | c.2431_2432dupCT, p.Pro812Tyrfs*15 | Likely Pathogenic | Breast                           | -                                    |
| CHEK2 (NM_007194.3)       | c.1008 + 2T > A       | Likely Pathogenic      | Breast                           | -                                    |
| MLH1 (NM_000249.3)        | c.790 + 1G > A        | Pathogenic             | Ovary, Endometrium               | Breast, Endometrium                  |
| MSH2 (NM_000251.2)        | c.811_814delTCTG, p.Ser271Argfs*2 | Pathogenic             | Breast                           | -                                    |
| Gene (Reference Sequences) | Variant Nomenclature                                       | Variant Classification | Cancers diagnosed in the patient | Cancers diagnosed in family member(s) |
|----------------------------|------------------------------------------------------------|------------------------|----------------------------------|---------------------------------------|
| MUTYH (NM_001128425.1)     | c.1237C > T, p.Gln413*                                     | Pathogenic             | Ovary                            | Endometrium, Breast                   |
| NBN (NM_002485.4)          | c.934-2A > G                                               | Likely Pathogenic      | Breast                           | -                                     |
| PALB2 (NM_024675.3)        | c.89delA, p.Asn30Thrfs*5                                   | Likely Pathogenic      | Breast                           | -                                     |
|                            | c.2968G > T, p.Glu990*                                     | Pathogenic             | Breast                           | Breast                                |
|                            | c.3267_3268delGT, p.Phe1090Serfs*6                         | Pathogenic             | Ovary                            | Lung                                  |
|                            | c.3426_3429delAAGT, p.Leu1142Phefs*20                       | Pathogenic             | Breast                           | Breast                                |
| PMS2 (NM_000535.6)         | c.325dupG, p.Glu109Glyfs*30                                | Pathogenic             | Ovary                            | -                                     |
|                            | 706-1G > T                                                 | Pathogenic             | Breast                           | Breast                                |
| RAD51C                     | c.905-2A > C                                               | Likely Pathogenic      | Breast                           | Breast                                |
| RAD51D (NM_002878.3)       | c.270_271dupTA, p.Lys91Ilefs*13                            | Pathogenic             | Ovary                            | -                                     |
|                            | c.270_271dupTA, p.Lys91Ilefs*13                            | Pathogenic             | Breast                           | Male Breast                           |
| RECQL (NM_002907.3)        | c.796C > T, p.Gln266*                                      | Pathogenic             | Breast, Ovary                     | Breast                                |
|                            | c.796C > T, p.Gln266*                                      | Pathogenic             | Breast                           | Ovary                                 |
|                            | c.1217-2A > C                                              | Likely Pathogenic      | Breast, Ovary                     | -                                     |
| TP53 (NM_000546.5)         | c.96 + 1 G > A                                             | Pathogenic             | Breast, Brain                     | Breast, Lung                          |
|                            | c.1024C > T, p.Arg342*                                     | Pathogenic             | Breast                           | -                                     |
Multigene panel testing in breast cancer categorized by NCCN 2019 indication fulfillment

Overall, patients who met at least one indication in 2019 NCCN guideline have P/LP variant detection rate varying from 22 to 40%. The most frequent indication is early onset breast cancer (age of diagnosis less than 45 years). One hundred and ninety-eight patients (64.7%) fit this indication and had 27% P/LP variant detection rate. Since each patient could fulfill more than one indication in the guideline, we found that patients who matched more than one indication had higher likelihood of detecting P/LP variants. Detection rate was also increased with number of indications Fig. 2. We found that patients matched 4 and 5 indications in 2019 NCCN guidelines had 54.6% and 75% detection rate respectively. Interestingly, four of 18 patients (22%) who had breast cancer with second primary cancer outside hereditary breast-ovarian cancer spectrum carried germline P/LP variants. The 4 breast cancer patients had P/LP variants in BRCA1 (with ovarian cancer and thyroid cancer), BRCA2 (with endometrium cancer), BRCA2 (with thyroid cancer) and TP53 (with brain tumor).

Multigene panel testing in patients with primary ovarian cancer, pancreatic cancer and prostate cancer

Overall, 62 patients with primary ovarian cancer had fulfilled the NCCN 2019 guideline by its specific tumor type. We further divided patients with ovarian cancer into 2 groups by the presence of breast cancer. We found that 15 of 43 patients (34.8%) in primary ovarian cancer without breast cancer harboured P/LP variants. Meanwhile, 8 of 19 patients (42.1%) with both primary ovarian cancer and breast cancer were tested positive for P/LP variants. We also found that 12 of 19 patients tested positive for P/LP variants had their tumor histopathology read as high-grade serous cystadenoma.

Table 3
List of putative loss-of-function VUS (frameshift deletion, stop gain, start loss, splice site variant)

| Gene (Reference Sequences) | Variant Nomenclature | Variant Classification | Cancers diagnosed in the patient |
|---------------------------|----------------------|------------------------|---------------------------------|
| APC                       | c.1A > G, p.Met1Val   | Start loss             | Ovary                           |
| BRCA2                     | c.8954-5_8954-2delAACA| Splice variant         | Ovary                           |
|                           | c.7617 + 2dupT        | Splice variant         | Primary Peritoneal              |
| MSH2                      | c.792 + 3A > T        | Splice variant         | Breast                          |
| RECQL                     | c.2T > C, p.Met1Thr   | Start loss             | Breast                          |
| RECQL                     | c.2T > C, p.Met1Thr   | Start loss             | Breast                          |
| RAD51C                    | c.571 + 5G > A        | Splice variant         | Breast                          |
| XRCC2 (NM_005431.1)       | c.832G > T, p.Glu278* | Stop gain             | Breast                          |
Patients with pancreatic cancer were also sufficient for NCCN 2019 guideline fulfilment. Only one of 14 pancreatic cancer patients have Colonic cancer as another primary malignancy. Only one of 14 pancreatic cancer patients had their histopathology read as neuroendocrine tumor. 2 Patients with BRCA2 P/LP variants were sporadic cases of adenocarcinoma of pancreatic cancer.

Prostate cancer patients that warranted further genetic test by NCCN 2019 guideline were described as metastatic prostate cancer or having high-grade prostate cancer (Gleason score \( \geq 7 \)) with family history of certain cancer. In our 7 patients with prostate cancer, there were 3 patients who had evidence of metastasis, and 4 patients who had Gleason score \( \geq 7 \)). Patient with BRCA1 P/LP variants had Gleason score of 6 with familial history of gastric, thyroid, prostate and pancreas. The other patient with BRCA2 P/LP variants had prostate cancer Gleason score of 9, with another primary cancer included male breast cancer and squamous cell carcinoma of esophagus. He had no familial history of cancer.

**Discussion**

Breast cancer is one of the common cancers associated with heritable mutations. Identifying germline mutation in those patients provides great benefit on treatment selection, prophylactic and screening options for both the patients and their at-risk family members. For the first time, this study provided prevalence and landscape of germline P/LP variants among Thai patients with breast-ovarian cancer spectrum who were clinically indicated for genetic test. Germline P/LP variants were detected in 24% of breast cancer and 37% of ovarian cancer patients.

The prevalence observed in our breast cancer patients was significantly higher than Western patients who underwent genetic testing with similar clinical indication. In 2018, the rate of P/LP variants from multigene-sequencing performed by 4 laboratories across the US in “higher-risk” patients filtered by National Comprehensive Cancer Network (NCCN) guideline was 12.5%[7]. There are some explanations for this double in detection rate in Thai population. First, as both genetic and lifestyle factors are associated with an increased risk of breast cancer, differences in lifestyle could affect the rate. Many lifestyle factors such as hormonal use, obesity, and alcohol consumption among Asian population are less prevalent than Western counterpart. It is therefore possible that genetic factor could play more role on cancer susceptibility in Thai patients whose clinical phenotypes were not totally conformed to common sporadic cancer. Next, it had been noted that approximately half of the patients in our study fulfilled more than one NCCN 2019 indication. Our cohort may represent easily recognized patients with higher risk profile than previous studies. Data of multigene-sequencing in breast cancer in Asian population was limited, and most publication did not recruit patients based on NCCN guideline. Nevertheless, there was one multicentre study in Chinese breast cancer patient that recruited patients using the Breast Cancer Diagnosis and Treatment Guidelines and Standards (Chinese Cancer Society, V2015), of which the criteria were slightly more stringent than the NCCN 2019. The detection rate of germline P/LP observed was comparable to our finding (23.8% vs 23.9% in our study)[10], as well as the proportion of gBRCA (71% vs 79.5% in our study).
The overall rate of P/LP germline mutation in ovarian cancer was 37% (N = 62). gBRCA accounted for 23% in this cancer group (57.5% of overall P/LP variants). The rate was considerably higher than observed ovarian cancer patients tested with multigene panel sequencing in the US laboratory[11] (overall rate of 13.4%, 50.5% of this were gBRCA; N = 663). Data of multigene-sequencing in ovarian cancer in Asian population was also limited. When consider only gBRCA in an Asian population, our rate was comparable to the rate of 14.7% for gBRCA in Japanese ovarian cancer cohort[12] and 22.4% in Chinese cohort[13]. Expansion of genetic test beyond BRCA1/2 nearly double the rate of finding germline P/LP variants, thus multigene panel approach should be fostered in ovarian cancer. The pathological report of high-grade serous cystadenoma contributed to 63.2% of all patients with positive P/LP variants. We support the idea that multigene panel test should be carried out in all patients with ovarian cancer regardless of their pathological finding[12].

An observed rate of 14.3% in pancreatic cancer (N = 14) was also comparable with previous study in pancreatic cancer (10.5%)[11]. We reported gBRCA as a secondary finding in patients in 3 patients with colon cancer, 1 patient with nasopharyngeal cancer and 1 patient with lung cancer in Table 2. Previous report from US laboratory estimated the yield of gBRCA in colon/stomach cancer to be 1.6% and colon/endometrial cancer to be 2.9%[11]. The yield of secondary finding of genes for hereditary breast-ovarian cancer spectrum in colorectal cancer patients should be reviewed in the future when the number of testing is sufficient.

The overall rate of VUS in our breast cancer patients was as high as 40%. This rate fell between observed rates of VUS among different ethnics in the US laboratories[7] (23.7% in white, 44.5% in African-American, and 50.9% in Asian). When looking into specific genes, the prevalence of VUS in BRCA1/2 in our cohort was lower than previous report[14] (7.2% compare to 15% in European laboratories, and 21% in African-American population). However, there was a report of 0% rate of VUS in BRCA1/2 in Asian population by the US laboratories[7]. The decrease in rate of VUS in our database was likely contributed from the availability of genomic data in Asian population, and the increase of functional studies in recent year[15]. Previous study from South Korea showed that most of VUS in BRCA1/2 (57%) remained unchanged and only 2.7% of the VUS was reclassified as likely pathogenic[16]. The reclassification of identified variants in this study remains to be seen. We have selected variants that almost fulfill the ACMG 2015 guideline for P/LP variant classification[9]. There were 8 variants with putative loss of function in Table 3 which included start codon loss in APC and RECQL. Although there were many reports of start codon loss in other diseases[17, 18], initiation codon loss in APC, a well-known gene, had never been reported in colorectal cancer cases[19]. Additional genomic data and functional validation might help reclassification of VUS in our study.

Absence of copy number variations of BRCA1 and BRCA2 in our cohort may suggest that prevalence of large deletion/duplication in gBRCA among Thai patients is not as common as other population[20].

Utilization Of Nccn Guideline In Thai Population
The prevalence of finding positive germline variants in each specified indication from NCCN guideline 2019 ranged between 27%-40% (Fig. 1). Among breast cancer patients, the rate was highest (38%) in breast cancer patient with personal or familial history of primary malignancy in hereditary breast ovarian cancer spectrum. Our breast cancer patients with another primary cancer not in hereditary breast ovarian cancer spectrum also yielded the rate of germline P/LP variants of 22% (N = 18). This scenario could be added as testing indication in Thai population as multigene panel sequencing in patients with pretest probability over 10% was proposed to be cost-effective in US and UK population[21]. The rate of finding P/LP variants did positively correlate with number of indications fulfilled (Fig. 2). This warrants a strong recommendation of providing germline genetic test in patient with multiple indication fulfilled.

**Conclusion**

We reported a high diagnostic yield of P/LP variants from multigene panel sequencing in Thai patients with breast cancer (24%), or ovarian cancer (37%) that fulfilled NCCN 2019 indication for germline genetic testing. The rate of VUS and the number of identified novel variants were high and reflected the need to include more Asian or Thai dataset in genomic database. The results from our study warrant the incorporation of multigene panel sequencing in management of breast cancer and ovarian cancer in Thailand.

**Abbreviations**

*BRCA1/2*
*BRCA1* and *BRCA2*
gBRCA
germline pathogenic variants in *BRCA1* or *BRCA2*

*NCCN*
National Comprehensive Cancer Network

P/LP variants
pathogenic variants or likely pathogenic variants

VUS
variants of undetermined significance

**Declarations**

- Ethical Approval and Consent to participate:

The study protocols were approved by the Siriraj Hospital Institutional Review Board Protocol No.474/2562(EC1) and 418/2562(EC2).

- Consent for publication:

Not Applicable
- Availability of supporting data:

The data that support the findings of this study are available from the corresponding author upon reasonable request.

- Competing interests:

The authors declare that they have no competing interests.

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- Authors’ contributions:

MP, CL and PL contributed to subject recruitment. ER, PN, PD, CM, WTa, KP, SW, SC, MP, WTi and WTh contributed to the design and laboratory work. CL, MP, PL, ER, PN, PD, CM, WTa, KP, SW, WTi and WTh contributed to data interpretation. PL, MP and SC provided the statistical analysis, writing and editing manuscripts. All authors read and approved the final manuscript.

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