RESEARCH ARTICLE

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Prevalence of Pathogenic Germline Mutations in 13 Hereditary Cancer-Related Genes in Breast Cancer Patients in Narathiwat Province, Thailand

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Abstract

Background: *BRCA1* and *BRCA2* genes are known to increase breast cancer's lifetime risk. Early identification of women with this inherited risk can potentially reduce the risk of breast and/or ovarian cancer and, together with early screening, decrease the mortality rate. **Objective:** This study explored the frequency and distribution of genetic variants in consecutive cases of breast cancer in Narathiwat province, one of the three provinces in the southernmost Thai border. **Material & Method:** A series of 64 consecutive breast cancer patients who underwent treatment in two general hospitals in the province during the period from the year 2021 to 2022. Genotyping studies were performed using a whole exome sequencing platform. Moderate to high penetrance variants recommended by the National Comprehensive Cancer Network (NCCN) guidelines 2022 (*ATM, BARD1, BRCA1, BRCA2, CDH1, CHEK2, NF1, PALB2, PTEN, RAD51C, RAD51D, STK11, TP53*) were annotated and filtered for pathogenic, likely pathogenic, or high-impact variants. **Results:** Pathogenic germline variants were found in 8/64 cases (12.5%), namely *BRCA1* in 3 (4.7%), *BRCA2* in 4 (6.3%), *ATM* in 1 (1.6%), and PALB2 in 1 (1.6%). One patient had two concomitant germline mutations in *BRCA2* and *ATM*. **Conclusion:** This is the first study on the frequency of germline mutations in *BRCA1*/*2* and other breast cancer-predisposing genes in the southernmost provinces of Thailand. At least one pathogenic germline mutation was identified in 12.5% of the study patients, which suggests that genetic testing in this population has a high potential to provide benefits.

Keywords: Breast cancer- germline variants- hereditary cancer

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Introduction

Breast cancer (BC) is the most common organ-specific cancer in women worldwide, including in Thailand (Ghoncheh et al., 2016). In general, there are two main types of epithelial breast cancer: ductal carcinoma and lobular carcinoma. Multiple risk factors have been identified that increase the possibility of developing breast cancer, including age, exposure to certain drugs and hormones, and reproductive history (Majeed et al., 2014). Approximately 10-20% of breast cancers are hereditary, stemming from variants in the BRCA genes through vertical transmission (Tung et al., 2016; de Souza Timoteo et al., 2018). Germline pathogenic variants in *BRCA1* and/or *BRCA2* are associated with up to an 80% increased lifetime risk of BC. A recent study reported that pathogenic variants in other genes increased the absolute risk of BC by the age of 80 years at different penetrance, i.e. *BRCA1* 75%, *BRCA2* 76%, *CDH1* 53%, *PALB2* 45%, *CHEK2* 29%, *ATM* 27%, and *NF1* 26% (Thorat and Balasubramanian, 2020). Genetic alterations differ geographically and culturally, which is explained by differences in ancestral gene pools (Yoshida, 2020). For patients newly diagnosed with BC, identifying a responsible mutation may have an impact on the treatment plan, not only for the patient herself but also for close relatives (Manahan et al., 2019). Olaparib, a drug in the class of Poly (adenosine diphosphate-ribose) polymerase

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(PARP) inhibitors, has been found to increase the survival rate in metastatic BC harboring germline BRCA 1/2 mutations (Robson et al., 2017). In addition, prevention for a secondary tumor and primary prevention in siblings by early detection is advisable in cases with significant genetic risk.

The southernmost part of Thailand is located in the middle of the Malay peninsula and consists of 3 provinces, Yala, Narathiwat, and Pattani, situated along the northern border of Malaysia. The approximately 2 million people in these provinces are about 83% Muslim-Thai who share ethnicity, culture, language, and religion with the Malays (Albritton, 1999). Most people are Muslim with Malay connections, while the rest are Buddhist Thais of Chinese lineage (Thompson, 2012). A previous study found that cancer incidences were different between the Muslim-Thai and Buddhist-Thai subpopulations (Sriplung et al., 2014). Another previous study in Songkhla province, just north of Pattani, found that BC in Muslim Thais was more common in younger women, who were more likely to have a higher stage at diagnosis, higher rates of triple-negative BC, and significantly poorer survival. These data lead to speculation that BC in the Muslim-Thai majority provinces might have distinct clinical outcomes, which might be explained by either cultural or biological differences, or both.

Considering that the three southern border provinces have population differences, and most of the population is Malay-Thai which has not been previously studied about genetic breast cancer, this study aimed to investigate the prevalence of pathogenic germline variations in Thai breast cancer patients in Narathiwat Province.

Materials and Methods

Study population

The study enrolled 12 consecutive cases of BC from Su-ngaikolok Hospital and 52 from Naradhiwasrajanagarindra Hospital. Patients were unselected epithelial BC or ductal carcinoma in situ patients who were treated in one of these hospitals during the years 2021-2022. BC staging followed the American Joint Committee on Cancer classification (7th Edition). The TNM system is based on tumor size and extension (T), regional lymph node metastasis (N), and evidence of distant metastasis (M). Patients were staged into different groups based on the TMN finding (CANCER, 2015). Data on the BC biomarkers in this study used the immunohistochemical subtypes estrogen receptors (ER), progesterone receptors (PR), and human epidermal growth factor receptors 2 (HER2) (Iwamoto et al., 2020). Hormonal receptors (HR) positive was defined as ER+ and/or PR+. Data on age at diagnosis, family history of cancer, and treatment received were also collected.

Following informed consent, we collected peripheral blood samples of about 10 ml per patient for genetic testing by Whole Exome Study.

Whole Exome Sequencing (WES) and Bioinformatic analysis

The main laboratory used for WES testing was the Central Research Laboratory, Translational Medicine Research Center, Faculty of Medicine, Prince of Songkla University (ISO 15189). Genomic DNA was extracted from a peripheral blood sample by using a High Pure PCR template preparation kit (Roche, Berlin, Germany). The extracted DNA was qualified and quantified using a bioanalyzer (Agilent Technologies, Santa Clara, California, United States) and nanodrop (Thermo Scientific, Delaware, United States). The qualified DNA was run on gel electrophoresis to screen for DNA fragmentation.

Exome regions were enriched by Agilent SureSelect XT V6 kit (Agilent). Paired-end exome sequencing and paired-end targeted resequencing (150 bp) were performed an Illumina Next-Seq 550 system. In our study, the average reading depth of whole exome sequencing was around 200X for approximately 9 GB/sample. All identified variants were crudely filtered using a reading depth of more than 20x and allele frequency of any individual sample of more than 25%.

FASTQ files from the sequencer were used for quality control and trimmed with FastQC and sequenced with Trimmomatic-0.38, respectively. The data were aligned with reference genome Homo sapiens assembly 38 with *BWA*-0.7.17, converted to bam files with Samtools-1.11, then sorted with Picard, indexed with Samtools-1.11, recalibrated and had variants called with GATK-4.1.2.0, and variants annotated with snpEff and snpSift. Moderate to high penetrance genes recommended by the National Comprehensive Cancer Network (NCCN) guidelines 2022 were annotated (*ATM, BARD1, BRCA1, BRCA2, CDH1, CHEK2, NF1, PALB2, PTEN, RAD51C, RAD51D, STK11, TP53*). If there is no apparent report, any effect on proteins is evaluated by the Sorting Tolerant From Intolerant (SIFT) function. Reportable variants were classified as pathogenic, likely pathogenic, or high-impact variants.

Statistical analysis

As a continuous data set, age was stratified as means with standard deviations (SD) and presented into three levels. Other categorical data are reported as frequencies and percentages. All data were analyzed against the incidence of variants by Chi-square or Fisher Exact test. A P-value of less than 0.05 was deemed to indicate statistical significance. A mutation profiling display of *BRCA1/2* by a 'Lollipop plot' was plotted by http://www.bioinformatics.com.cn/en, an online platform for data analysis and visualization.

Results

A total of 64 female patients diagnosed with BC in Naradhiwas Rajanagarindra or Su-ngaikolok Hospitals between the years 2021 and 2022 were enrolled in the study. Age at diagnosis ranged from 28-79 years, with an average age of 51.3 years (SD 11.4 years). Twenty-two patients (34.4%) were less than 45 years old, 28 (43.8%) were in the group of age between 46-60 years old and the rest 14 patients (37.5%) were older than 60 years. The most prominent BC histopathology in our series was invasive ductal carcinoma which was diagnosed in 85.9% of the

patients. Regarding breast cancer subtypes, hormonal receptors (ER and/or PR) were positive in 60.9%, HER2 was positive in 29.7%, and 25% of the patients were triple-negative BC (Table 1). Regarding family history, 3/61 patients (4.7%) had a first-degree relative with breast cancer, and 4/60 patients (6.2%) had a second or third-degree relative with breast cancer.

On analysis of 13 BC-related genes according to the NCCN list, we found germline variants involving four genes (*ATM, BRCA1, BRCA2, PALB2*) (Table 2) in eight cases (12.5%). One patient had concomitant pathogenic variants in ATM and *BRCA2*. Two unrelated patients had the same variants in *BRCA1* (NM_007294.3:c.1863_1885del). Of these four genes, *BRCA2* had the highest frequency of pathogenic variants (4 variants), followed by *BRCA1* (2 variants in 3 patients). All *BRCA1*/2 pathogenic variants are illustrated in Figure 1.

On analysis for associations between clinicopathological parameters and pathogenic variants, the factors that significantly increased the chance of detecting at least one pathogenic variant were age 55 years or less (p-value 0.03), triple-negative BC (p-value < 0.01) and negative HER2 status (p-value 0.049). A family history of BC in first-degree relatives was associated with an increased risk of developing BC, but not at the level of statistical significance (p-value 0.26). However, when including BC history in second or third-degree relatives as a risk factor, patients with a positive family history of BC were associated with an increased risk of pathogenic variant detection (Table 3).

Discussion

BC is a disease that can be transmitted vertically to a descendant, and BRCA variants are the most common hereditary BC etiology. Carriers of pathogenic *BRCA* variants and their relatives have a very high risk of BC in a syndrome called 'hereditary breast and ovarian cancer syndrome (HBOC)'. Genetic testing for *BRCA* mutations is a standard test for high-risk BC women recommended by various standard guidelines (Valencia et al., 2017). With recent advances in genomic technology, there has been an increasing proportion of BC women tested regardless of their risk determinants (Stenehjem et al., 2021). In Thailand, the public health Universal Coverage insurance scheme incorporated BRCA testing for patients with BC into its package in 2021, although it remains unclear about the case selection and genotyping technique. Also, the coverage does not include germline variants in other hereditary cancer genes that might be responsible for BC risk.

Table 1 Clinical and Tumor Characteristics and Comparative Frequency of Pathogenic Variants in Annotated genes in Breast Cancer in This Study (N = 64)

Study Characteristic	No.	%	No. Pathogenic variants	p-value
Age at diagnosis, years				
≤ 55	40	62.5	8 (20.0%)	0.019
> 55	24	37.5	0 (0.0%)	
Pathological group				
Invasive ductal carcinoma (IDC)	55	85.9	1 (11.1%)	0.89
Others	9	14.1	7 (12.7%)	
Stage group				
Stage 0-1	10	15.6	1 (10.0%)	0.59
Stage 2	18	28.1	1(5.6%)	
Stage 3	23	35.9	4 (17.3%)	
Stage 4	13	20.3	2 (15.3%)	
Estrogen receptors (ER)				
Negative	26	40.6	5 (19.2%)	0.18
Positive	38	59.4	3 (7.9%)	
Progesterone receptors (PR)				
Negative	31	48.4	6 (19.4%)	0.11
Positive	33	51.6	2 (6.1%)	
Hormone receptors (HR)				
Negative	25	39.1	5 (20.0%)	0.15
Positive	39	60.9	3 (7.7%)	
HER2 expression				
Negative	45	70.3	8 (17.8%)	0.049
Positive	19	29.7	0 (0.0%)	
Triple-negative breast cancer (TNBC)				
non-TNBC	48	75	3 (6.3%)	0.01
TNBC	16	25	5 (31.3%)	
First-degree relative with breast cancer				
Yes	3	4.7	1 (33.3%)	0.26
No	61	95.3	7 (17.4%)	
First- or second-degree relative with breast cancer				
Yes	7	10.9	3 (42.9%)	0.01
No	57	89.1	5 (8.8%)	

Table 2 List of Genes in Breast Cancer Patients/ Variant Classification/ Amino Acid Change

Gene (Reference sequence)	Variant nomenclature	Variant classification	Amino acid change
ATM (NM_000051.3)	c.2377-2A>G	Likely Pathogenic	splice acceptor
BRCA1(NM_007294.3)	c.1863_1885del*2	High-impact	NP_009225.1:p.His621GlnfsTer7
	c.2059C>T	Pathogenic	NP_009225.1:p.Gln689Ter
BRCA2(NM_000059.3)	c.3680_3681del	Pathogenic	NP_000050.2:p.Leu1227GlnfsTer5
	c.5164_5165del	Pathogenic	NP_000050.2:p.Ser1722TyrfsTer4
	c.6352_6353del	Pathogenic	NP_000050.2:p.Val2118LysfsTer10
	c.7558C>T	Pathogenic	NP_000050.2:p.Arg2520Ter
PALB2(NM_024675.3)	c.2257C>T	Pathogenic	NP_078951.2:p.Arg753Ter

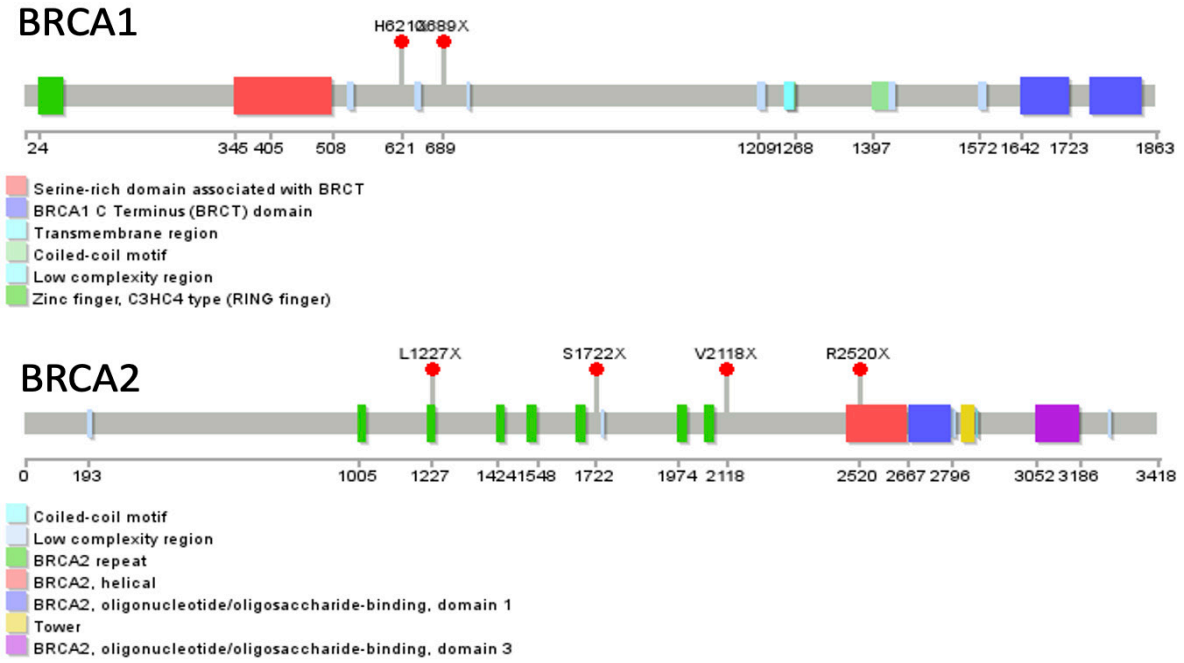

Figure 1. A Lollipop Plot of the Pathogenic Variants in *BRCA1* and *BRCA2* Detected in This Study

Table 3. Details of Pathogenic Germline Mutations in This Study

ID	Gene	Age (Years)	Cancers diagnosed in family member
NA005	*BRCA1*	54	Cousin/Breast cancer
NA014	*BRCA2*	39	-
NA023	*BRCA2/ATM*	48	-
NA039	*BRCA2*	48	-
NA041	*BRCA1*	51	Aunt/Breast cancer
NA052	*BRCA1*	28	Sister/Breast cancer
KL206	*BRCA2*	48	-
KL209	*PALB2*	45	-

In our 64 unselected BC patients, 7 (10.9%) were found to be BRCA germline carriers, a finding which was comparable with 3-15 % of unselected BC patients in other systematic reviews (Armstrong et al., 2019). Five of the seven BRCA carriers presented to the hospital at the age of more than 45 years old, which is the cut-off age that the NCCN guidelines 2022 recommend that a young woman with BC should have genetic testing. One of the explanations for these late presentations might be the fact that most BC patients in the study area came to the hospital with a palpable breast mass rather than a positive mammogram. Consistent with this finding, our study found that the cut-off age for young BC that significantly increased the risk of having one or more positive germline variants was 55 years. Although a family history of cancer increased the chance of finding pathogenic variants, the difference was not statistically significant. While 25% of those with a family history of cancer had variants detected, 10% of those without a family history also had positive pathogenic variants. Interestingly, 30% of the cases in our series with triple-negative BC had BRCA germline mutations compared to only 4% of the others. Our three cases with pathogenic *BRCA1* variants were triple-negative BC which was in line with a previous study that found that BRCA variants conferred a high lifetime risk of BC and had a higher histopathological grade (Gonzalez-Angulo et al., 2011; Wendt and Margolin, 2019).

Germline variants in *BRCA1/2* have been reported in varying frequencies, depending on case selection, ethnic groups, definitions of pathogenicity, and genotyping technics. A gene array study from India that included 296 BC patients did not find any *BRCA1* pathogenic mutations, while 11% of their cases harbored *BRCA2* pathogenic variants (Kaur et al., 2018). In a study from Colombia that used protein truncation assays and mass spectrometry in 244 unselected BC, 1.2% of cases had BRCA germline mutation (Julián Esteban Londoño Hernández, 2014). The high prevalence of *BRCA1* pathogenic variants in our unselected BC patients might be explained by the characteristic of BC in this region which had a triple-negative subtype (Virani et al., 2018).

When hereditary risks are applied for patient selection, there is a higher probability of finding mutation carriers. A study from Central Thailand which sequenced BC with high genetic risk (young age, triple-negative BC, male BC, bilateral diseases) by using the next-generation sequencing method reported 18% of *BRCA1*/2 mutation carriers (Oranratnachai et al., 2022). In another study that used NCCN 2019 indication for case selection, 23.9% of BC cases harbored clinically significant mutations (Lertwilaiwittaya et al., 2021). The same study demonstrated that, with a higher number of NCCN indications, there was a higher frequency of mutation carriers.

In a study of 8,085 unselected Chinese BC patients by target-sequencing on a 62-gene panel, pathogenic germline mutations in *BRCA1*/2 were identified in 5.3% of patients (Sun et al., 2017). For those without a *BRCA1*/2 mutation in that study, 3.4% had at least one pathogenic variant in 16 DNA repair genes. Among the 13 BC-related genes in our study, a pathogenic variant in PALB2 (partner and localizer of *BRCA2*) was found in a patient who presented with BC at the age of 45 years. In other reports, the frequency of PALB2 mutations was 7-15% among BC genes (Tung et al., 2015), and another study reported the cumulative risk of BC for females carrying a PALB2 mutation was estimated to be 35% by 70 years of age (Antoniou et al., 2014). A germline mutation of ATM (ataxia telangiectasia mutated) was found to be a likely pathogenic variant in one of our cases. ATM is a moderate-risk gene for BC, but more importantly, ATM mutation carriers were found to be at risk of radiation toxicity (Stucci et al., 2021). From Utah, a study of 35,000 women with BC using a 25-gene panel of hereditary cancer genes found that 9.3% carried at least one pathologic variant, and the study found differences in the number of pathogenic variants among different ancestries. In the same study, triple-negative BC appeared to have a significant impact on the prevalence of individual genes (Buys et al., 2017).

Genetic testing can have a notable impact on the primary prevention of BC through the identification of more hereditary cases. Germline genetic testing not only provides benefits for cancer surveillance and prophylaxis but may also help in precision cancer therapies (Piccinin et al., 2019). The American Society of Breast Surgeons currently recommends germline genetic testing for all BC patients (Manahan et al., 2019). Multigene Panel Testing for Hereditary Breast and Ovarian Cancer was proven to be cost-effective when considering the chance to detect significant variants other than *BRCA1*/2 (Asphaug and Melberg, 2019; Catana et al., 2019).

In conclusion, BC patients in Narathiwat province had a relatively high prevalence of pathogenic germline variants in *BRCA1*, *BRCA2*, and other BC-related genes, including ATM and PALB2. The study suggests that genetic testing in BC patients should be implemented into the clinical practice guideline for this population.

Author Contribution Statement

All authors contributed equally in this study.

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Ethical Declaration

The study protocol was approved by the Human Research Ethics Committee of Naradhiwas Rajanagarindra Hospital (REC 001/2564). The study was conducted according to good clinical practice and the Declaration of Helsinki.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Conflicts of interest

The authors do not hold any conflicts of interest.

References

Albritton RB (1999). Political diversity among Muslims in Thailand. *Asian Studies Rev*, **23**, 233-46.

Antoniou AC, Casadei S, Heikkinen T, et al (2014). Breast-cancer risk in families with mutations in PALB2. *N Engl J Med*, **371**, 497-506.

Armstrong N, Ryder S, Forbes C, et al (2019). A systematic review of the international prevalence of BRCA mutation in breast cancer. *Clin Epidemiol*, **11**, 543-61.

Asphaug L, Melberg HO (2019). The Cost-Effectiveness of Multigene Panel Testing for Hereditary Breast and Ovarian Cancer in Norway. *MDM Policy Pract*, **4**, 2381468318821103.

Buys SS, Sandbach JF, Gammon A, et al (2017). A study of over 35,000 women with breast cancer tested with a 25-gene panel of hereditary cancer genes. *Cancer*, **123**, 1721-30.

Catana A, Apostu AP, Antemie RG (2019). Multi gene panel testing for hereditary breast cancer - is it ready to be used?. *Med Pharm Rep*, **92**, 220-5.

de Souza Timoteo AR, Goncalves A, Sales LAP, et al (2018). A portrait of germline mutation in Brazilian at-risk for hereditary breast cancer. *Breast Cancer Res Treat*, **172**, 637-46.

Ghoncheh M, Pournamdar Z, Salehiniya H (2016). Incidence and Mortality and Epidemiology of Breast Cancer in the World. *Asian Pac J Cancer Prev*, **17**, 43-6.

Gonzalez-Angulo AM, Timms KM, Liu S, et al (2011). Incidence and outcome of BRCA mutations in unselected patients with triple receptor-negative breast cancer. *Clin Cancer Res*, **17**, 1082-9.

Iwamoto T, Kajiwara Y, Zhu Y, et al (2020). Biomarkers of neoadjuvant/adjuvant chemotherapy for breast cancer. *Chin Clin Oncol*, **9**, 27.

Julián Esteban Londoño Hernández ML, Gonzalo Vásquez P, Juan David F, et al (2014). Prevalence of BRCA1 and BRCA2 mutations in unselected breast cancer patients from medellín, Colombia. *Hereditary Cancer Clin Pract*, **12**.

Kaur RP, Shafi G, Benipal RPS, et al (2018). Frequency of pathogenic germline mutations in cancer susceptibility genes in breast cancer patients. *Med Oncol*, **35**, 81.

Lertwilaiwittaya P, Roothumnong E, Nakthong P, et al (2021). Thai patients who fulfilled NCCN criteria for breast/ovarian cancer genetic assessment demonstrated high prevalence of germline mutations in cancer susceptibility genes: implication to Asian population testing. *Breast Cancer Res Treat*, **188**, 237-48.

Majeed W, Aslam B, Javed I, et al (2014). Breast cancer: major risk factors and recent developments in treatment. *Asian Pac J Cancer Prev*, **15**, 3353-8.

Manahan ER, Kuerer HM, Sebastian M, et al (2019). Consensus Guidelines on Genetic` Testing for Hereditary Breast Cancer from the American Society of Breast Surgeons. *Ann Surg Oncol*, **26**, 3025-31.

Oranratnachai S, Yamkaew W, Tunteeratum A, et al (2022). Characteristics of breast cancer patients tested for germline BRCA1/2 mutations by next-generation sequencing in Ramathibodi Hospital, Mahidol University. *Cancer Rep (Hoboken)*, **2022**, e1664.

Piccinin C, Panchal S, Watkins N, et al (2019). An update on genetic risk assessment and prevention: the role of genetic testing panels in breast cancer. *Expert Rev Anticancer Ther*, **19**, 787-801.

Robson M, Im SA, Senkus E, et al (2017). Olaparib for Metastatic Breast Cancer in Patients with a Germline BRCA Mutation. *N Engl J Med*, **377**, 523-33.

Sriplung H, Bilheem S, Kuntipundee T, et al (2014). Differences in cancer incidence among predominantly Muslim and Buddhist subpopulations in Songkhla. *Asian Pac J Cancer Prev*, **15**, 9979-83.

Stenehjem DD, Telford C, Unni SK, et al (2021). BRCA testing and outcomes in women with breast cancer. *Breast Cancer Res Treat*, **186**, 839-50.

Stucci LS, Interno V, Tucci M, et al (2021). The ATM Gene in Breast Cancer: Its Relevance in Clinical Practice. *Genes (Basel)*, **12**.

Sun J, Meng H, Yao L, et al (2017). Germline Mutations in Cancer Susceptibility Genes in a Large Series of Unselected Breast Cancer Patients. *Clin Cancer Res*, **23**, 6113-9.

Thompson EC (2012). Anthropology in Southeast Asia: National Traditions and Transnational Practices. *Asian J Soc Sci*, **40**, 664-89.

Thorat MA, Balasubramanian R (2020). Breast cancer prevention in high-risk women. *Best Pract Res Clin Obstet Gynaecol*, **65**, 18-31.

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

Tung N, Lin NU, Kidd J, et al (2016). Frequency of Germline Mutations in 25 Cancer Susceptibility Genes in a Sequential Series of Patients With Breast Cancer. *J Clin Oncol*, **34**, 1460-8.

Valencia OM, Samuel SE, Viscusi RK, et al (2017). The Role of Genetic Testing in Patients With Breast Cancer: A Review. *JAMA Surg*, **152**, 589-94.

Virani S, Wetzel EC, Laohawiriyakamol S, et al (2018). Ethnic disparity in breast cancer survival in southern Thai women. *Cancer Epidemiol*, **54**, 82-9.

Wendt C, Margolin S (2019). Identifying breast cancer susceptibility genes - a review of the genetic background in familial breast cancer. *Acta Oncol*, **58**, 135-46.

Yoshida R (2020). Hereditary breast and ovarian cancer (HBOC): review of its molecular characteristics, screening, treatment, and prognosis. *Breast Cancer*, **2020**.