Germline Mutations in Epithelial Breast Cancers in Three Southern Border Provinces of Thailand

Panupong Sukpan

A Thesis Submitted in Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Biomedical Sciences

Prince of Songkla University

2024

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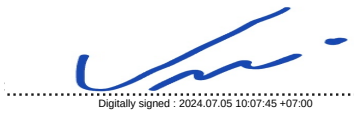
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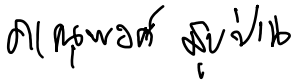
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ABSTRACT

BC ranks as the most prevalent form of cancer among women globally, encompassing Thailand. Numerous determinants augment the susceptibility to BC, including advanced age, postponed childbearing, abstaining from breastfeeding, tobacco consumption, obesity, and various other factors. Additionally, genetic mutations play a pivotal role in elevating the likelihood of BC occurrence. In accordance with the present study findings, it is discerned that approximately 10-20% of BC cases are linked to hereditary mutations transmitted across generations, with disparities evident across different ethnic populations. The data pertaining to germline genetic mutations serves as a critical guide for cancer risk mitigation, guiding both surveillance and treatment strategies.

The majority of the population of the three southern border provinces, 80%, are Muslim, use the Austronesian language family to communicate, and have similar ancestry to the population of Malaysia. Another 20% are Buddhists and use the Tai-Kadai language family to communicate, which is the same language family as the majority of the Thai population. As a result, the demographic characteristics of the three southern border provinces have specific characteristics. It has never been studied in BC before.

The first study aims to investigate BC treatment outcomes in Naradhiwas Rajanagarindra Hospital, situated in Narathiwat province. The investigation takes the form of a retrospective analysis spanning six years, encompassing the period from 2016 to 2021. A comprehensive cohort of 234 BC patients is incorporated into this inquiry. The mean age at which these individuals received their diagnosis is 52.6 years. Notably, 46.2% of these cases are characterized as locally advanced diseases, while 26.5% are categorized as metastatic diseases. The study found a five-year survival rate of 44.44% and observed that 18.7% of individuals

were diagnosed with TNBC. Based on my review of the NCCN guidelines from 2022, it appears that there may be potential benefits from germline genetic testing for hereditary cancer in approximately 48.72% of BC patients.

In the following study, the study have undertaken an analysis of 64 BC patients with the WES technique. These patients were sourced from two general hospitals located within Narathiwat province. The primary objective of this investigation is to profile and characterize germline mutations within 13 breast cancer susceptibility genes. This analysis adheres to the NCCN guidelines of 2022. The study analyzed a cohort of individuals aged 28 to 79 years, with a mean age of 51.3. Within this study annotated specific genes, namely *ATM, BARD1, BRCA1, BRCA2, CDH1, CHEK2, NF1, PALB2, PTEN, RAD51C, RAD51D, STK11*, and *TP53*. Notably, an investigation revealed that among BC patients within this cohort, 12.5% were carriers of at least one of the following genes: *ATM, BRCA1, BRCA2,* or *PALB2*, one patient carrier of two concomitant *ATM* and *BRCA2* genes who develop BC at 48 years. *BRCA1/2* are the most common truncating germline mutation, and two unrelated patients had the same novel variants in BRCA1(NM_007294.3:c.1863_1885del) who were suffering from TNBC.

The following study analyzed WES 151 BC patients from three southern border provinces of Thailand and annotated 26 BC susceptibility genes *ATM*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CASP8*, *CDH1*, *CDKN2A*, *CHEK2*, *EPCAM*, *HMMR*, *MLH1*, *MSH2*, *MSH6*, *MUTYH*, *NBN*, *NF1*, *PALB2*, *PHB1*, *PMS2*, *PTEN*, *RAD51C*, *RAD51D*, *RAD54L*, *STK11*, and *TP53* corrected by sanger sequencing. The study aimed to characterize germline mutations, specifically focusing on truncating or VUS. This investigation led to the discovery of many previously unreported genetic variants. Notably, within the cohort of BC cases studied, a substantial 15.2% harbor at least one truncating mutation in a BC susceptibility gene, with the preponderance of such mutations being identified within the BRCA1/2 genes.

In summary, the study has found that delayed presentation of BC in the deep south of Thailand strongly correlates with unfavorable survival outcomes. To address this concern, I propose that genetic germline testing could serve as a viable method for screening, thereby facilitating the development of personalized medical interventions tailored to the specific needs of this distinctive population. This study

examined both documented and undocumented germline variants within BC patients, demonstrating the need for further research to categorize these germline mutations accurately.

Keyword: breast cancer, whole exome sequencing, germline variant

ชื่อวิทยานิพนธ์ การกลายพันธุ์ตามสายเลือดของยีนซึ่งเกี่ยวข้องกับมะเร็งเซลล์บุผิวของเต้านมในสตรีสามจังหวัดชายแดนใต้

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บทคัดย่อ

มะเร็งเต้านมเป็นมะเร็งที่พบเป็นอันดับหนึ่งในผู้หญิงทั่วโลก รวมทั้งประเทศไทย มีปัจจัยต่าง ๆ มากมายที่เพิ่มความเสี่ยงต่อการเกิดมะเร็งเต้านม เช่นอายุที่มากขึ้น มีบุตรช้า ไม่ให้บุตรดื่มนมแม่ สูบบุหรี่ ภาวะอ้วน เป็นต้น อีกสาเหตุหนึ่งคือการกลายพันธ์ตามสายเลือด เพิ่มความเสี่ยงต่อการเกิดมะเร็งเต้านม จากข้อมูลการศึกษาปัจจุบันพบว่า 10-20% ของผู้ป่วยมะเร็งเต้านม นั้นสัมพันธ์กับการกลายพันธ์ตามสายเลือด ที่มีการถ่ายทอดจากรุ่นสู่รุ่น ซึ่งมีความแตกต่างกันในแต่ละกลุ่มประชากรตามชาติพันธ์ การทราบความเสี่ยงเรื่องยีนก่อมะเร็งนั้น นำมาซึ่งเป็นแนวทางในการเฝ้าระวังและสามารถรักษาเพื่อลดความเสี่ยงต่อการเกิดมะเร็งได้

ประชากรสามจังหวัดชายแดนภาคใต้นั้นส่วนใหญ่ 80% นับถือศาสนาอิสลามใช้ภาษามลายูเป็นตระกูลออสโตรนีเซียนในการสื่อสาร และมีบรรพบุรุษใกล้เคียงกับประชากรของประเทศมาเลเซีย อีกประมาณ 20% นับถือศาสนาพุทธใช้ตระกูลทางภาษา ไท-กะได ในการสื่อสาร เป็นภาษาตระกูลเดียวกับประชากรส่วนใหญ่ของไทย ทำให้ลักษณะเชิงประชากรกลุ่มสามจังหวัดชายแดนภาคใต้นั้นมีลักษณะที่จำเพาะ ไม่เคยมีการศึกษาในมะเร็งเต้านมมาก่อน

การศึกษาแรกเพื่อศึกษาผลการรักษาผู้ป่วยมะเร็งเต้านมในโรงพยาบาลนราธิวาสราชนครินทร์ซึ่งเป็นตัวแทนของโรงพยาบาลในสามจังหวัดชายแดนภาคใต้โดยการทบทวนผู้ป่วยมะเร็งเต้านมที่ได้รับการรักษาเป็นเวลา 6 ปีย้อนหลังตั้งแต่ปี พ.ศ. 2559 ถึง ปี พ.ศ. 2564 จำนวณ 234 ราย พบว่าอายุเฉลี่ยที่เป็นมะเร็งเต้านมคือ 52.6 ปี กว่า 46.2% มาพบแพทย์ในระยะลุกลาม ส่วน 26.5% มาพบแพทย์ในระยะแพร่กระจาย (ระยะที่ 4) ทำให้อัตราการรอดชีวิตที่ 5 ปีนั้นต่ำมากคือ 44.44% พบว่า 18.7% เป็นมะเร็งเต้านมชนิดที่มีความรุนแรงแบบ ทริปเปิลเนกาทีฟ หากยึดตาม NCCN guidelines 2022 พบว่า 48.72% ของผู้ป่วยมะเร็งเต้านมมีความเสี่ยงที่จะมีการกลายพันธ์ตามสายเลือดแฝงอยู่และควรได้รับการตรวจทางพันธุกรรม

ในการศึกษาต่อมาจึงมีวัตถุประสงค์เพื่อหาความชุก และลักษณะของผู้ป่วยมะเร็งเต้านมที่เป็นพาหะการกลายพันธุ์ตามสายเลือดบนยีนก่อมะเร็งเต้านม โดยการตรวจวิเคราะห์รหัสพันธุกรรมแบบ WES ตามด้วยการวิเคราะห์ทางชีวสารสนเทศสำหรับข้อมูลระดับจีโนม และเลือกวิเคราะห์ 13 ยีนตามคำแนะนำของ NCCN guidelines 2022 ดังนี้ *ATM, BARD1, BRCA1, BRCA2, CDH1, CHEK2, NF1, PALB2, PTEN, RAD51C, RAD51D, STK11, TP53* การศึกษาได้วิเคราะห์ตัวอย่างเลือดผู้ป่วยมะเร็งเต้านมในจังหวัดนราธิวาสจำนวณ 64 รายจากสองโรงพยาบาลหลักของจังหวัดนราธิวาสอายุตั้งแต่ 28-79 ปี อายุเฉลี่ย 51.3 ปี มีผู้ป่วย 8 รายคิดเป็น 12.5% มีการกลายพันธ์ตามสายเลือดบนยีน *ATM, BRCA1, BRCA2, PALB2* มีผู้ป่วยมะเร็งเต้านม 1 รายมีการกลายพันธุ์ตามสายเลือดสองยีนคือ *ATM* และ *BRCA2* ซึ่งเป็นมะเร็งเต้านมตอนอายุ 48 ปี พบว่า BRCA1/2 เป็นยีนหลักที่พบ และมีผู้ป่วยมะเร็งเต้านมสองรายเป็นมะเร็งเต้านมชนิดทริปเปิลเนกาทีฟมีการกลายพันธ์ตามสายเลือดบนยีน *BRCA1* ที่ตำแหน่งเดียวกันโดยที่ทั้งสอง ไม่ได้เป็นญาติกัน และยังพบว่าการกลายพันธุ์ตำแหน่งนี้ยังไม่เคยมีรายงานที่ไหนมาก่อน

ในการศึกษาต่อมาได้วิเคราะห์ WES ผู้ป่วยมะเร็งเต้านม 151 รายจากสามจังหวัดชายแดนภาคใต้โดยวิเคราะห์ 26 ยีนที่สัมพันธุ์กับการเกิดมะเร็งเต้านมได้แก่ *ATM*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CASP8*, *CDH1*, *CDKN2A*, *CHEK2*, *EPCAM*, *HMMR*, *MLH1*, *MSH2*, *MSH6*, *MUTYH*, *NBN*, *NF1*, *PALB2*, *PHB1, PMS2*, *PTEN*, *RAD51C*, *RAD51D*, *RAD54L*, *STK11*, และ *TP53* จุดกลายพันธุ์ของยีนยืนยันโดย sanger sequencing เพื่ออธิบายลักษณะการกลายพันธุ์ตามสายเลือดของประชากรกลุ่มนี้พบว่า 15.2% ของผู้ป่วยมะเร็งเต้านมเป็นภาหะของยีนกลายพันธุ์ที่ก่อโรคมะเร็งเต้านมบน 11 ยีน ส่วนใหญ่จะเป็น *BRCA1/2* และยังพบลักษณะการกลายพันธ์แบบแปลผลได้ไม่ชัด (VUS) อีกด้วย โดยบางการกลายพันธุ์เป็นข้อมูลใหม่ที่ไม่มีการรายงานมาก่อน

จากการศึกษาครั้งนี้สามารถสรุปได้ว่าในสามจังหวัดชายแดนภาคใต้ของไทยนั้นมะเร็งเต้านมยังเป็นปัญหาหลัก มีอัตราการรอดชีวิตที่ต่ำเนื่องมาจากการเข้ารับการรักษาที่ล่าช้า และการตรวจพันธุกรรมในผู้ป่วยมะเร็งเต้านม เพื่อหาการกลายพันธุ์ตามสายเลือดนั้นมีความสำคัญและมีประโยชน์ในเรื่องการเฝ้าระวังและป้องกันการเกิดมะเร็งต่อไป ทั้งตัวผู้ป่วยเองและเครือญาติ นอกจากนั้นการตรวจทางพันธุกรรมยังมีประโยชน์ในเรื่องการรักษามะเร็ง เพื่อให้อัตราการรอดชีวิตที่นานขึ้นต่อไป

คำสำคัญ : มะเร็งเต้านม, การกลายพันธ์ตามสายเลือด

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Finally, I would like to convey my deep appreciation to the breast cancer patients who generously contributed their blood samples in the pursuit of knowledge.

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List of Abbreviations and Symbols

A	Adenine
ACMG	American College of Medical Genetics and Genomics
ACR	American College of Radiology
ACS	American Cancer Society
Ala	Alanine
ATM	Ataxia telangiectasia mutated gene
Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid
BARD1	BRCA1-associated ring domain 1
BC	Breast cancer
BRCA	Breast cancer 1 and 2
BRCA1	Breast cancer 1
BRCA2	Breast cancer 2
BRIP1	BRCA1 interacting helicase 1
C	Cytosine
CASP8	caspase 8
CDH1	Cadherin 1
CDKN2A	Cyclin dependent kinase inhibitor 2A
CHEK2	Checkpoint kinase 2
CI	Confidence interval
Cys	Cysteine
DCIS	Ductal carcinoma in situ
Del	Deletion mutation
DNA	Deoxyribonucleic Acid
EPCAM	Epithelial cell adhesion molecule
ER	Estrogen receptor
Fs	Frame shift
G	Guanine
Gln	Glutamine

Glu	Glutamic acid
Gly	Glycine
HBOC	Hereditary Breast and Ovarian Cancer syndrome
HER2	Human epidermal growth factor receptor-2
HGV	Human genome variation
His	Histidine
HMMR	Hyaluronan mediated motility receptor
HR	Hormone receptor
IDC	Invasive ductal carcinoma
Ile	Isoleucine
Ins	Insertion mutation
Ki67	Proliferation-related Ki-67 antigen
Leu	Leucine
LP	Likely pathogenic
Lys	Lysine
M	Metastasis stage
Met	Methionine
MLH1	mutL homolog 1
MSH2	mutS homolog 2
MSH6	mutS homolog 6
MUTYH	mutY DNA glycosylase
N	Lymph node stage
NBN	nibrin
NCCN	National Comprehensive Cancer Network
NF1	Neurofibromin 1
NGS	Next-generation sequencing
OS	Overall survival
PALB2	Partner and Localizer of BRCA2
PARP	Poly (adenosine diphosphate-ribose) polymerase
PHB1	prohibitin 1
PMS2	PMS1 homolog 2, mismatch repair system component
PR	Progesterone receptor

Pro	Proline
PTEN	Phosphatase and Tensin homolog
PV	Pathogenic
RAD51	RAD51 recombinase
RAD51C	RAD51 paralog C
RAD51D	RAD51 paralog D
RAD54L	RAD54 like
SD	Standard deviation
Ser	Serine
STK11	Serine/Threonine kinase 11
T	Tumor stage, Thymine
Ter	Termination codon
Thr	Threonine
Tis	Tumor Carcinoma in situ
TNBC	Triple negative breast cancer
TP53	Tumor Protein p53
Tyr	Tyrosine
UC	Universal Coverage
Val	Valine
VUS	Variant of uncertain significant
WES	Whole Exome Sequencing

List of Paper

This thesis is based on the following papers:

Paper 1: Panupong Sukpan, Kanyanatt Kanokwiroon, Hutcha Sriplung, Papawee Paisarn, and Surasak Sangkhathat. Survival outcomes in breast cancer patients and associated factors in a border province of Thailand: a hospital-based review on Journal of Health Science and Medical Research (JHSMR), Volume 41, Issue 3, November 2022, e2022911, (Indexed by SCOPUS and TCI, Thai-Journal Impact Factors)

Paper 2: Panupong Sukpan, Kanyanatt Kanokwiroon, Hutcha Sriplung, Wison Laochareonsuk, Pongsakorn Choochuen, Nasuha Auseng, Kasemsun Wanawanakorn, and Surasak Sangkhathat. Prevalence of Pathogenic Germline Mutations in 13 Hereditary Cancer-Related Genes in Breast Cancer Patients in Narathiwat Province, Thailand on Asian Pacific journal of cancer prevention, Asian Pacific journal of cancer prevention, Volume 24, Issue 2, February 2023, pp. 525-530, (Indexed by SCOPUS)

Introduction

BC is the predominant site-specific malignancy among women, as it holds the unfortunate distinction of being the foremost contributor to cancer-related mortality in the female population globally(1). The global prevalence of BC is on the ascent, leading to an increasing burden of the disease on a worldwide scale(2). The incidence of BC in Thai women is displaying a noteworthy and concerning surge, with projections estimating an anticipated 19,452 new cases by the year 2025(3). Several BC risk factors contribute to an increased likelihood of developing BC, including factors such as age at menarche, breastfeeding status, family history, duration of lactation, and menopausal status(4).

A germline mutation confers an elevated susceptibility to the development of BC, and the extent of this predisposition varies significantly among distinct genes. For instance, when considering the cumulative risk by the age of 80, specific gene mutations exhibit the following absolute risk percentages: *BRCA1* at 75%, *BRCA2* at 76%, *CDH1* at 53%, *PALB2* at 45%, *CHEK2* at 29%, *ATM* at 27%, and *NF1* at 26%(5). The TP53 germline mutation is an autosomal dominant genetic alteration that causes Li-Fraumeni syndrome, characterized by high penetrance. The mutation is associated with an 85% increased risk of developing BC and a significantly higher incidence of subsequent primary tumors within areas exposed to radiation(6,7). Current research indicates that over 30 cancer-associated genes are implicated in the development of BC(8). According to www.omim.org, 21 genes are directly associated with BC. Genetic modifications exhibit regional and cultural disparities, a phenomenon elucidated by variations in the ancestral gene reservoirs(9). A previous study in the United States demonstrated that 10.7% of 488 patients diagnosed with BC have a germline mutation in at least one of the 25 BC genes(10). A study from India has found that 80.9% of patients with BC were mono or biallelic for non-*BRCA2* genes, including *MUTYH, CYTB, BRIP1, MTHFR, NBN, PTEN, ATM, CDK2NA, RAD51C, STK11, MLH1, MSH2, MSH6,* and *CHEK2*(11). In a research investigation conducted in Colombia, it was determined that 22.4% of individuals afflicted with breast or ovarian cancer exhibited the presence of a detrimental germline mutation within a gene associated with cancer susceptibility(12). A previous study in central Thailand not only

identified novel germline mutations in Thai individuals but also revealed that 24% of BC patients carry such mutations(13).

In the case of individuals recently diagnosed with BC, the detection of a genetic mutation has the potential to exert a significant influence on the specific guidance and recommendations on localized therapeutic interventions(14). Clinicians should prioritize germline genetic testing for all cancer patients to maximize patient benefit(15). HBOC is classified as an autosomal dominant cancer predisposition syndrome associated with the presence of germline mutations in the *BRCA* genes(16). Proactive measures such as bilateral mastectomy and bilateral salpingo-oophorectomy serve as viable options for mitigating the risk associated with cancer development in individuals harboring *BRCA* gene mutations(17). Furthermore, Olaparib, a PARP inhibitor, enhances the survival outcomes among metastatic BC patients carrying a *BRCA* germline mutation(18). Homozygous or heterozygous germline mutations in the *ATM* gene among BC patients are not only correlated with heightened susceptibility to radiotoxicity but also elevate the risk of contralateral BC occurrence(19,20). Germline mutations in the *CHEK2* gene are associated with an elevated risk of bilateral BC. Similar to the recommendation for *BRCA* mutations, risk-reducing mastectomy is advised as it offers significant benefits in mitigating this risk(21).

The recommendations stipulated by the American Society of Breast Surgeons endorse the imperative requirement of genetic testing for individuals who possess a personal medical history of BC(22). Family members and relatives, including individuals with BC, benefit from undergoing germline genetic testing. The preceding report illustrates remarkable cost-effectiveness and received high satisfaction ratings(23). A panel testing approach encompassing mutigenes (*ATM*, *BARD1*, *BRIP1*, *CHEK2*, *NBN*, *RAD51C*, and *RAD51D*) exhibited greater cost-effectiveness compared to exclusive *BRCA* testing, as evidenced by findings from Norway(24).

The three southernmost provinces of Thailand, namely Yala, Narathiwat, and Pattani, boast a collective population of approximately 2 million inhabitants, with an overwhelming majority of 83% adhering to the Islamic faith. Most of these Muslim individuals predominantly reside within the confines of Narathiwat, Pattani, and Yala, where their cultural and religious affiliations exhibit a pronounced connection to Malay Islam, evident in the realms of ethnicity, linguistic tradition, and

religious beliefs(25). The genomic landscape of the Melayu Kelantan and Melayu Kedah populations exhibits a conspicuous resemblance to the Thai Muslim Pattani population at a genome-wide scale, marked by a noteworthy presence of genetic admixture stemming from Indian ancestral components(7). The forensics study shows populations displayed heterozygosity for the short tandem repeat loci because cultural barriers might have obstructed recent admixture between the people(26). The incidence of cancer varies notably between the Thai Muslim and Thai Buddhist populations(27). In the context of BC, when comparing the Thai Muslim and Thai Buddhist populations, significant disparities are evident. These disparities manifest as diagnoses occurring at a younger age, more advanced stages of cancer at the time of diagnosis, shorter OS durations, a higher prevalence of triple-negative tumors, notably diminished survival rates, and a clear association between aggressive disease characteristics and a less favorable prognosis(28, 29).

The advancement of DNA sequencing technologies has undergone significant progress, culminating in the remarkable capability to sequence an individual's complete genome comprehensively. The Sanger sequencing platform is considered to possess superior quality in comparison to NGS platforms due to its capability of generating shorter reads. The advancements in bioinformatics tools, driven by the need to handle abbreviated read lengths and enhanced quality requirements, have significantly improved mapping against reference genomes or facilitating de novo assembly(30). NGS represents a high-throughput DNA sequencing technology characterized by the parallel sequencing of numerous small DNA fragments, with the primary objective of elucidating DNA sequences. This innovative methodology seeks to enhance sequencing efficiency, accelerate sequencing processes, and concurrently reduce associated costs in contemporary genomics research(31). In contemporary times, the detection rate of genetic diseases has notably increased owing to the substantial volumes of sequence data generated by NGS technology(32). The precision of NGS in the acquisition of sequencing data approximates a remarkable 99.97% (33, 34).

The ACMG has formulated guidelines to discern sequence variants, categorizing them into distinct classes such as pathogenic, likely pathogenic, variants uncertain significance, likely benign, and benign. This classification is based on

systematically assessing pertinent published data to inform the interpretation process(35). Over 2,900 variants of *BRCA1* and 3,400 variants of *BRCA2* have been documented as associated with HBOC. Genetic alterations exhibit geographical or culturally isolated differences, wherein one or more ancestors carried the mutated genes(9). Most VUSs are to undergo reclassification to a lower level of uncertainty as additional data accumulates and provides stronger support for their clinical significance(36).

The thesis consists of two parts, each deeply focusing on the study of BC in the three southernmost provinces of Thailand. The first study is a retrospective review of BC treatment outcomes and the factors affecting the survival rate at Naradhiwas Rajanagarindra Hospital in Narathiwat, Thailand. An essential addition demonstrates that BC patients are at high risk for HBOC syndrome, according to NCCN guidelines, and they were screened for the risk of vertical transmission to direct relatives. Secondly, a germline genetic study of BC patients in Narathiwat province and the three southern border provinces of Thailand was conducted. The study identified BC susceptibility genes associated with HBOC syndrome. These studies are significant as they are the first to demonstrate the poor survival outcomes in BC treatment due to late-stage presentation. Moreover, they underscore the benefits of genetic testing for BC patients in the three southern border provinces of Thailand.

Objectives

1. To assess the clinical outcomes of patients diagnosed with BC.
2. To investigate the prevalence of germline mutation associated with BC in patients residing within the three southern border provinces.
3. To examine the germline genetic variation patterns exhibited by individuals diagnosed with BC within the three southern border provinces.
4. To investigate the correlation between germline genetic variations in BC patients and their clinicopathological characteristics.

Result and Discussion

This study represents the first investigation into the outcomes of BC treatment within the southernmost region of Thailand, characterized predominantly by a Muslim Thai population. BC diagnoses were ascertained at Naradhiwas Rajanagarindra Hospital, the primary tertiary care facility in the region, spanning from 2016 to 2021. The most recent follow-up for the record is from May 3, 2024. The age at diagnosis exhibited a spectrum spanning from 23 to 84 years, presenting an average age of 52.6 years with a standard deviation of ±12. The age at diagnosis, along with its associated standard deviation, serves as a critical parameter for determining the optimal commencement of BC screening initiatives. BC screening significantly diminishes both the mortality and morbidity associated with BC. The guidelines endorsed by prominent institutions such as the NCCN, ACS, and the ACR comprehensively advocate for the benefits of initiating annual screening commencing at the age of 40 years(37-39). Notably, a diminished five-year survival rate of 44.44% (95% CI 36.99-50.46) was observed, and this was predominantly linked to cases diagnosed in the advanced stages of the disease. In a demographic analogous to our community in Penang, Malaysia, the five-year OS rate of BC was recorded at 72.9%(28). A significant proportion, specifically 46.15%, of individuals diagnosed with BC, particularly those presenting with a breast mass exceeding 5 cm (classified as at least T3-stage), attribute their condition to a deficiency in awareness regarding the disease **Table 1**. The demographic under consideration exhibits a diminished five-year survival rate in contrast to nations characterized by higher income levels. Robust evidence derived from routine screening initiatives and comprehensive therapeutic interventions indicates that the five-year survival rate for BC in high-income countries consistently exceeds 90%(40-42). A notable observation emerges from the analysis: when BC is diagnosed at the metastatic stage, a substantial influence on survival is discerned, with a prevalence of 27.04%. In a collaborative hospital-based investigation conducted in 2011 across medical institutions in Singapore and Malaysia, noteworthy findings emerged. Following the initiation of mammographic screening, a substantial reduction in the primary tumor size was observed. Furthermore, the study revealed that a mere 10% of the patients exhibited distant metastasis upon initial presentation, a proportion that showed a declining trend

over time(43). In a comprehensive nationwide survey conducted in China in 2011, wherein a screening program had been initiated, a mere 2.4% of the participants were identified to be in metastasis of the studied medical condition(44). In this demographic cohort, the elevation of awareness and enhancement of accessibility to screening facilities have resulted in the down-staging of BC diagnoses, a phenomenon consistently observed in Malaysia(45). International recommendations for BC screening advocate either annual mammographic screening for individuals within the average-risk population aged 40 to 74 years or annual magnetic resonance imaging for those identified as high-risk populations(46).

Table 1 The study examined the distribution of clinical parameters among BC patients, followed by a univariate survival analysis employing the Log-rank test.

Parameter	N (%)	5-year OS (%)	Log rank p-value
All	234 (100)	44.44	-
Age group			
< 50 years	109 (46.58)	59.61	0.029
> 50 years	125 (53.42)	40.18	
Pathological group			
IDC	198 (84.62)	47.44	0.0215
Others	36 (15.38)	59.37	
T stage			
Tis-2	126 (58.85)	67.36	< 0.001
T3-4	108 (46.15)	29.28	
N stage			
N 0-1	119 (51.07)	71.69	< 0.001
N 2-3	114 (48.93)	28.09	
M stage			
M0	170 (73.28)	61.20	< 0.001
M1	63 (27.04)	16.89	

Parameter	N (%)	5-year OS (%)	Log rank p-value
Stage group			
Stage 0-1	25 (10.73)	77.73	< 0.001
Stage 2	78 (33.48)	71.14	
Stage 3	67 (28.76)	47.10	
Stage 4	63 (27.04)	16.89	
Estrogen receptors*			
Negative	94 (40.52)	43.77	0.0122
Positive	138 (59.48)	54.42	
Progesterone receptors*			
Negative	119 (51.29)	38.88	0.001
Positive	113 (48.71)	61.41	
Hormone receptors (any)*			
Negative	99 (38.79)	40.85	0.002
Positive	142 (61.21)	56.08	
HER2 expression*			
Negative	147 (63.91)	55.62	0.008
Positive	83 (36.09)	40.79	
TNBC*			
non-TNBC	187 (81.30)	51.40	0.249
TNBC	43 (18.70)	44.69	
KI67 index*			
< 20.0%	54 (24.43)	60.79	0.024
> 20.0%	167 (75.57)	47.56	
Hereditary risk			
No	120 (51.28)	44.05	0.454
Yes	114 (48.72)	56.10	

IDC: invasive ductal carcinoma; *: missing data

In the culmination of the stepwise regression multivariate analysis, three key factors were identified and retained within the ultimate model **Table 2**. These

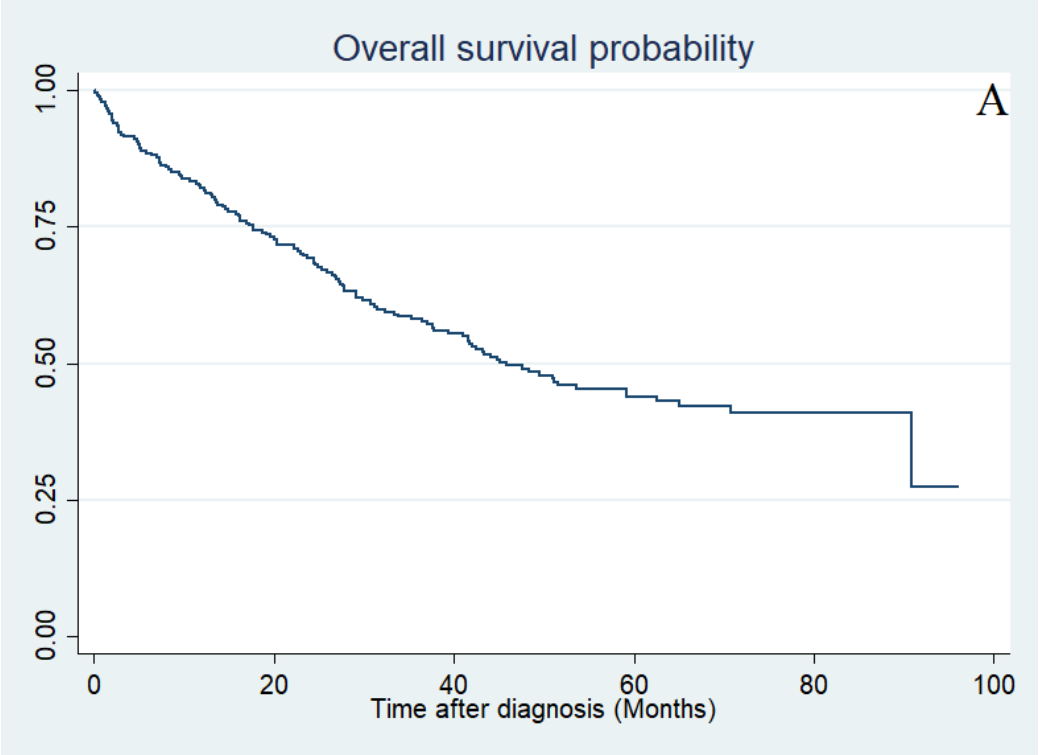
factors encompassed T stage, N stage, and metastatic status. The results of the multivariate analysis indicate that only three factors, T stage, N stage, and metastatic status, exhibit significant associations with five-year survival. In our study cohort, timely diagnosis and intervention have the potential to enhance survival outcomes for individuals with BC.

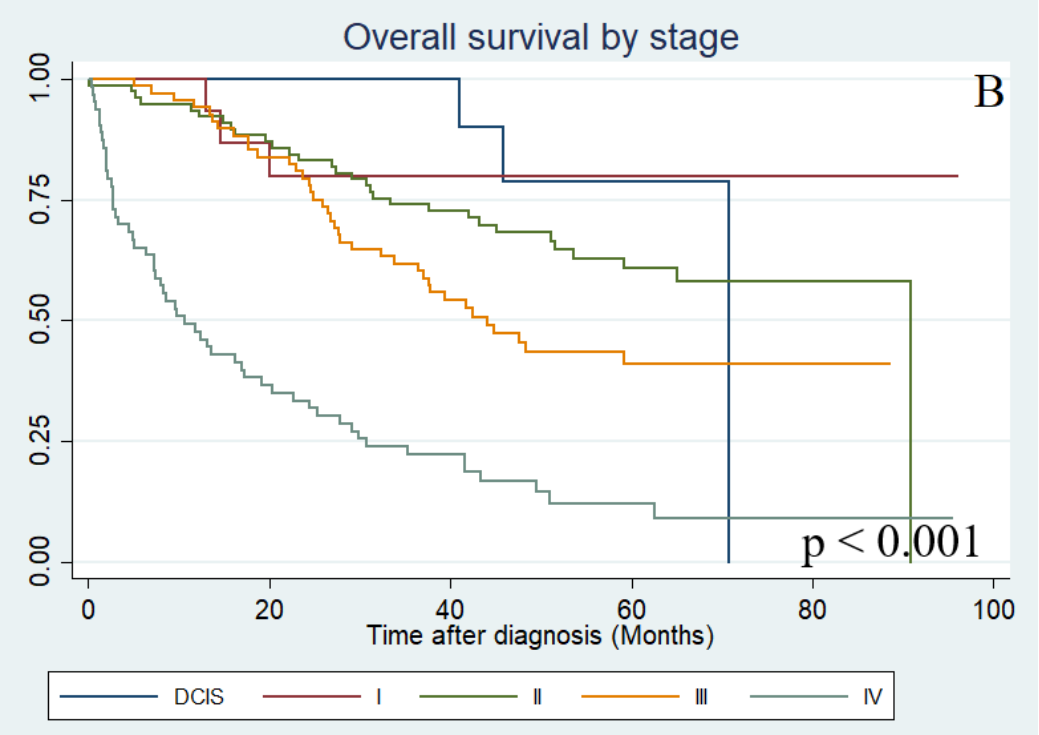
Table 2 The statistical significance was established through multivariate analysis employing stepwise regression.

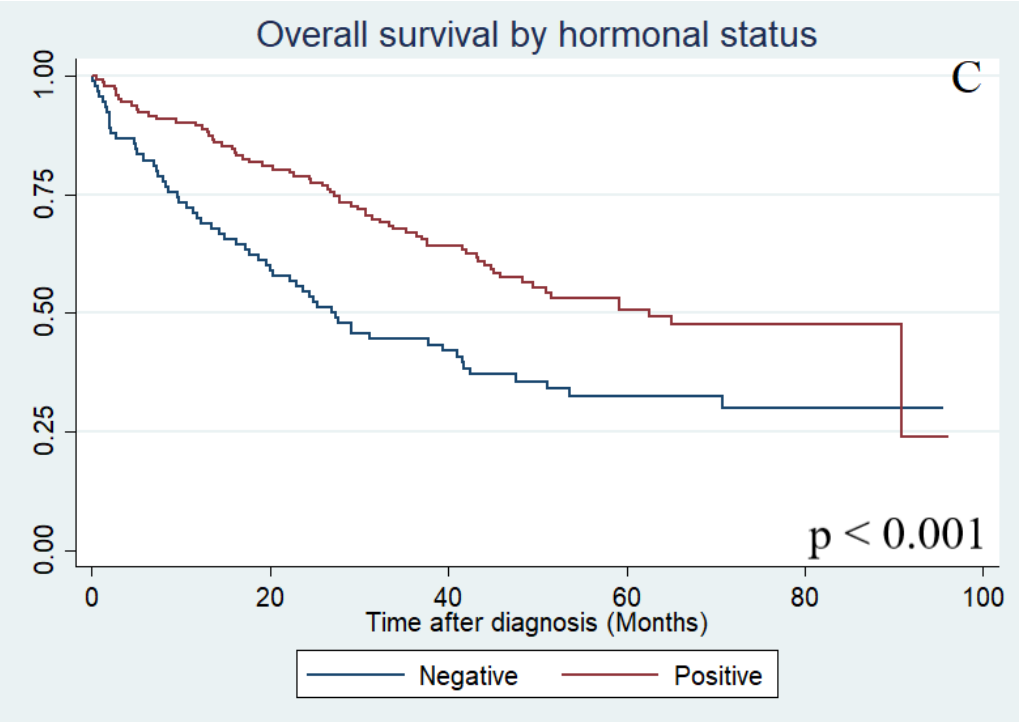
Parameters	HR	95.0% CI	Adjusted HR	95.0% CI
T stage (T3-4)	3.52	2.22 - 5.61	1.97	(1.15 3.38)
N stage (N2-3)	3.93	2.41 - 6.39	1.95	(1.05 - 3.64)
M stage	2.51	1.92 - 3.29	1.86	(1.27 - 2.71)

Remarkably, adherence to The NCCN guidelines for the year 2022 revealed distinct criteria for BC diagnosis. These criteria encompass: I. diagnosis of BC at an age below 46 years, II. diagnosis between the ages of 46-50 years with either an unknown family history or the presence of multiple primary BC, III. diagnosis at any age with at least one close family member affected by breast, ovarian, pancreatic, or prostate cancer, and IV. diagnosis at any age specifically involving TNBC. Among our cohort of 233 BC patients, 114 individuals, constituting 48.72% of the total, satisfied the established criteria warranting genetic testing for the screening of Hereditary Cancer Syndrome. It is noteworthy, however, that none of the patients within this cohort underwent the aforementioned genetic testing. The imperative and endorsement of germline genetic testing in BC patients are evident in multiple established guidelines. However, the application of germline genetic testing encounters challenges within economies characterized as low- and middle-income, exemplified by the case of Thailand(47). The American Society of Breast Surgeons underscores the imperative for genetic testing in individuals with a personal history of BC(22). Genetic testing yields substantial participant satisfaction and showcases notable cost-effectiveness(2 3) . Detecting individuals with germline mutations associated with a heightened risk of BC contributes significantly to the efficacy of cancer surveillance initiatives, thereby

promoting a secure and healthful life(48, 49). Survival outcomes in individuals who have BC are adversely affected by the presence of germline mutations in *BRCA1* or *BRCA2* genes. Enhanced and vigilant screening procedures may serve to facilitate these survival outcomes(50). Risk-reducing mastectomy and risk-reducing Salpingo-oophorectomy in the patient who carries the *BRCA* gene mutation decreases cancer-specific organs and increases OS(51).


Overall survival probability
A
1.00
0.75
0.50
0.25
0.00
0
20
40
60
80
100
Time after diagnosis (Months)


Overall survival by stage
B
1.00
0.75
0.50
0.25
0.00
p < 0.001
0
20
40
60
80
100
Time after diagnosis (Months)
DCIS
I
II
III
IV



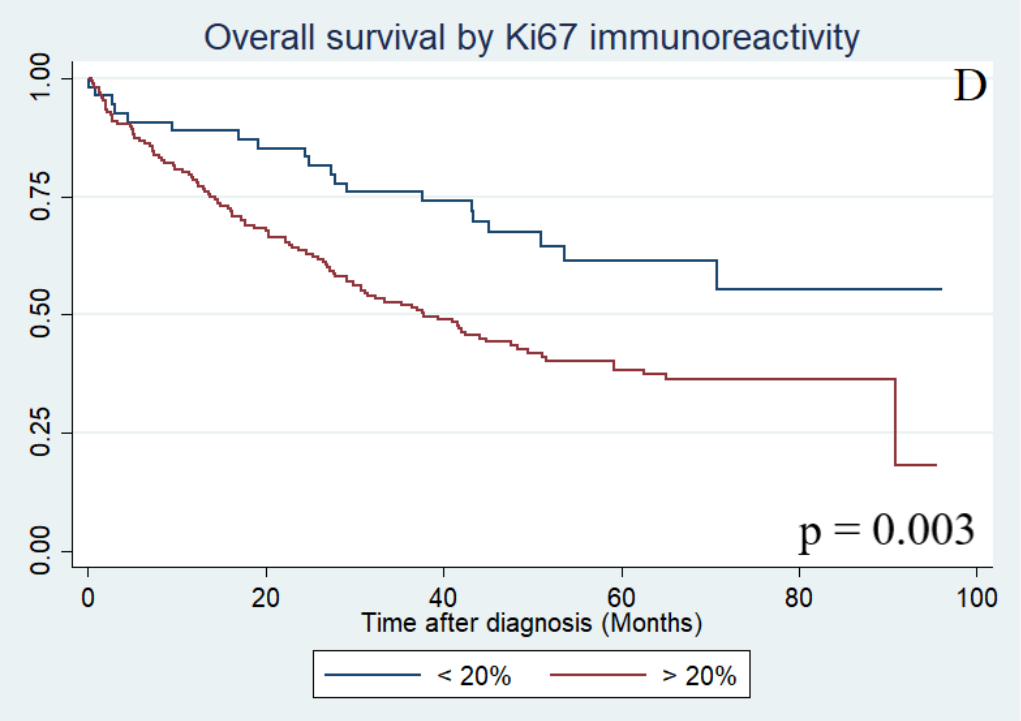


Figure 1 Survival probability of breast cancer patients in Naradhiwas Rajanagarindra Hospital. A: Overall survival B: Overall survival by stage C: Overall survival by hormonal status D: Overall survival by Ki67 status

According to the consolidated findings presented in Paper No. 1, BC patients at Naradhiwas Rajanagarindra Hospital manifest a diminished survival rate due to the disease being diagnosed at an advanced stage. The progression to an advanced stage is attributable to deficient awareness and suboptimal screening methodologies. Utilizing mammography for BC screening contributes to a reduction in BC mortality rates(52). The reduction of BC mortality is not solely attributed to mammogram interventions; instead, genetic screening also holds the potential to confer advantages within this demographic.

Germline mutations in Epithelial breast cancers

A comprehensive investigation was conducted on a cohort comprising 64 female patients who had been diagnosed with BC. The subjects were selected from Naradhiwas Rajanagarindra and Su-ngaikolok Hospitals situated in Narathiwat province. WES and subsequent bioinformatic analyses were employed to explore the genetic landscape associated with these patients. Genes characterized by moderate to high penetrance were annotated as stipulated by the 2022 NCCN guidelines. These include *ATM*, *BARD1*, *BRCA1*, *BRCA2*, *CDH1*, *CHEK2*, *NF1*, *PALB2*, *PTEN*, *RAD51C*, *RAD51D*, *STK11*, and *TP53*. Eight instances were observed among a cohort of 64 patients, constituting 12.5%, wherein a germline mutation associated with caries was identified in the genes *ATM*, *BRCA1*, *BRCA2*, and *PALB2*. Two non-related individuals presented identical novel variants in the *BRCA1* gene (NM_007294.3:c.1863_1885del, NP_009225.1:p.His621GlnfsTer7) and subsequently manifested TNBC at the ages of 28 and 51 years, respectively. A systematic review and meta-analysis revealed a correlation between germline mutations in the *BRCA1* gene and the occurrence of TNBC, accompanied by a noteworthy reduction in OS associated with the disease(50). Individuals carrying *BRCA1* mutations exhibited a cumulative risk of 65% for BC and 39% for ovarian cancer by the age of 70 years(53).

Table 3 Eight breast cancer patients in Narathiwat provinces were identified with germline mutations affecting a set of four specific genes.

Gene	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	p.His621GlnfsTer7
	c.2059C>T	Pathogenic	p.Gln689Ter
BRCA2 (NM_000059.3)	c.3680_3681del	Pathogenic	p.Leu1227GlnfsTer5
	c.5164_5165del	Pathogenic	p.Ser1722TyrfsTer4
	c.6352_6353del	Pathogenic	p.Val2118LysfsTer10
	c.7558C>T	Pathogenic	p.Arg2520Ter
PALB2 (NM_024675.3*)*	c.2257C>T	Pathogenic	p.Arg753Ter

A singular patient exhibited concurrent PV in *ATM* (NM_000051.3 c.2377-2A>G, splice acceptor) and *BRCA2* (NM_000059.3 c.7558C>T, p.Arg2520Ter), leading to the onset of BC at the age of 48. The presence of two genes elevates the likelihood of contralateral BC to a greater extent than that associated with a single gene. The available data substantiate the assertion that a polygenic predisposition contributes to an elevated risk of contralateral BC(54).

Table 4 The present study investigates the clinical and tumor characteristics, as well as the relative incidence of carrier germline mutations, among BC patients within the confines of Narathiwat province, comprising a total sample size of 64 individuals.

Study Characteristic	No.	%	No. PV/LV	p-value
Age at diagnosis, years				
Mean ± SD	51.3 ± 11.4			
Median	52			
Range	28 - 79			
≤ 55	40	62.5	8 (20.0%)	0.02
> 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%)	0.05
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%)	

Study Characteristic	No.	%	No. PV/LV	p-value
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%)	

The age at which BC is diagnosed is predominantly below 55 years, manifesting a heightened prevalence of germline mutations when compared with individuals aged 55 years and older. This discrepancy is statistically significant, as indicated by a p-value of 0.02. Utilizing age groups can serve as a valuable criterion for successfully identifying BC patients within a population who may benefit from targeted genetic testing.

A notable disparity in carrier germline mutations is observed between TNBC patients and their non-TNBC counterparts, with 31.3% of TNBC patients exhibiting such mutations in contrast to 6.3% in the non-TNBC group. This discrepancy is statistically significant, as indicated by a p-value of 0.01. TNBC is correlated with individuals harboring germline mutations and exhibits characteristics indicative of aggressive tumor subtypes(55). A comprehensive study involving 8753 TNBC patients showed that 14.4% of TNBC cases harbor germline mutations, as determined through the multigene panel test(56). The guidelines established by the NCCN advocate for genetic germline testing in individuals of all age groups diagnosed with TNBC(57). Within our studied population, 18.70% of BC patients exhibit TNBC, thereby underscoring the utility and advantages of genetic testing in this cohort.

The presence of BC in first- or second-degree relatives, characterized by a notably elevated prevalence of germline mutations, demonstrates statistical significance with a p-value of 0.01. Conversely, there is no observed correlation between the occurrence of germline mutations and solely having first-degree relatives diagnosed with BC. Individuals residing in a shared environment may also be susceptible to the development of BC. Several factors contribute to the onset of BC.

Specifically, dietary factors have been identified as correlating with an elevated risk of developing BC(58, 59). An explanatory rationale for the absence of an association between first-degree relatives diagnosed with BC and germline mutations may be considered. The enhancement of sample size can be elucidated by acquiring more precise and accurate information.

In a subset of BC patients within my investigation, a notable occurrence was observed, where 8 out of 64 individuals (12.5%) exhibited the transmission of carrier germline mutations in a vertical lineage to their descendants. *BRCA* mutations represent the predominant causative factors in hereditary BC etiology. In addition to *BRCA*, other genes, namely *ATM* and *PALB2*, have been identified in the context of hereditary BC within the Narathiwat province. Relying solely on the inspection of *BRCA* is insufficient for the comprehensive detection of hereditary cancer syndromes in BC patients. My study elucidates the factors correlated with the risk of germline mutation carriers, including individuals with TNBC and those having first- or second-degree relatives with a history of BC. In forthcoming periods, the accrual of supplementary information has the potential utility in the formulation of risk score models or for the enhancement of strategic frameworks in public health planning.

Incorporating a cohort of 151 non-discriminately selected cases, each characterized by histologically verified diagnoses of epithelial BC or DCIS. The cases were sourced from four medical institutions, namely Naradhiwas Rajanagarindra Hospital, Pattani Hospital, Yala Regional Hospital, and Sungaikolok Hospital, all situated in the southernmost provinces of Thailand. The study used second-generation sequencing through WES to annotate 26 BC susceptibility genes. Specifically, these genes are *ATM, BARD1, BRCA1, BRCA2, BRIP1, CASP8, CDH1, CDKN2A, CHEK2, EPCAM, HMMR, MLH1, MSH2, MSH6, MUTYH, NBN, NF1, PALB2, PHB1, PMS2, PTEN, RAD51C, RAD51D, RAD54L, STK11,* and *TP53*. Variants identified as warranting reporting underwent classification as either PV, LP or VUS in accordance with the 2015 guidelines established by the ACMG guidelines(35).

Among the cohort of 151 patients included in the study, nearly half of the individuals (78, constituting 51.7%) received a diagnosis of advanced-stage BC (specifically, stage III or IV). Women residing in the southernmost region of Thailand exhibit a noteworthy issue characterized by delayed diagnosis of BC and insufficient

screening measures. This constitutes a public health concern necessitating resolution. A research investigation conducted in Northern Thailand encompassed a cohort of 3,251 women diagnosed with advanced-stage BC (specifically, stage III or stage IV), constituting 34% of the studied population(60).

Table 5 The demographic profile of 151 individuals diagnosed with breast cancer within the southernmost of Thailand.

Study Characteristic (151 cases)	No (%)	No. among the 23 cases of PVs/LPs (%)	p-value
Age at diagnosis (year)			
≤ 50	91 (60.3)	19 (20.9)	0.02
> 50	60 (39.7)	4 (6.7)	
Pathological group			
Invasive ductal carcinoma (IDC)	131 (86.8)	21 (16.0)	0.13
Ductal carcinoma in situ (DCIS)	6 (4.0)	2 (33.3)	
Others	14 (9.3)	0 (0.0)	
Molecular subtypes			
Luminal A	61 (40.4)	7 (11.5)	0.05
Luminal B	32 (21.2)	4 (12.5)	
HER2-enriched	30 (19.9)	3 (10.0)	
TNBC	28 (18.5)	9 (32.1)	
Stage at diagnosis			
DCIS	6 (4.0)	2 (33.3)	0.48
Stage I	18 (11.9)	4 (22.2)	
Stage II	49 (32.5)	4 (8.2)	
Stage III	53 (35.1)	8 (15.1)	
Stage VI	25 (16.6)	5 (20.0)	
Religion			
Muslim-Thai	106 (70.2)	19 (17.9)	0.16
Buddhist-Thai	45 (29.8)	4 (8.9)	

Among the cohort of 151 individuals with a diagnosis of BC, the molecular subtype TNBC exhibits the highest percentage (32.1%) of germline mutations, p-value of 0.05. This constitutes additional substantiation affirming the elevated germline mutation incidence in TNBC, thereby accentuating the utility of germline genetic testing in this context. The germline mutation frequencies in Luminal A, Luminal B, and HER2-enriched BC subtypes are comparable, with respective incidences of 11.5%, 12.5%, and 10.0%. The prevalence of BRCA1/2 mutations among unselected patients diagnosed with TNBC ranges from 9.4% to 18.2%(61). The disparity in germline mutation prevalence does not demonstrate a statistically significant difference between individuals of Muslim-Thai and Buddhist-Thai backgrounds. While Muslim-Thai and Buddhist-Thai represent distinct ethnic backgrounds(26), there is no discernible divergence in the percentage of germline mutations between these two groups.

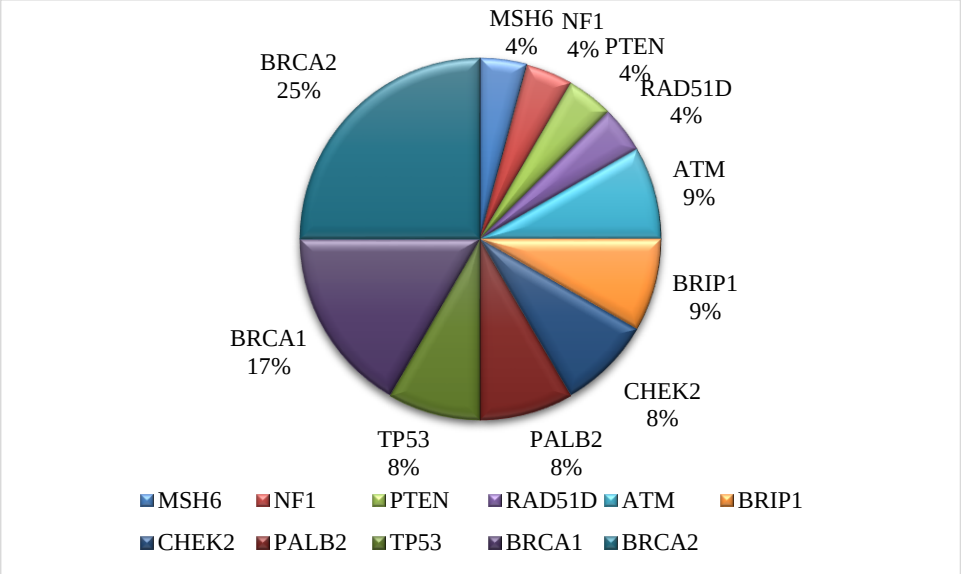


Figure 2 The distribution of germline mutations across the 26 annotated genes associated with breast cancer susceptibility revealed the presence of mutations in 11 genes.

In my series, I found that 15.2% of patients with BC carried at least one gene germline mutation. Interestingly, I observed that the frequency of mutations in my study aligns with findings from a prior investigation conducted within the Taiwanese population. Notably, there are variations in the inclusion criteria between the two studies. The preceding research selected participants based on hereditary risk, revealing a 13.5% carrier rate of pathogenic germline mutations(62). In a substantial investigation involving 8,085 unselected Chinese BC patients, findings indicated a germline mutation rate of 9.2%(63). A study encompassing 35,000 women diagnosed with BC in the United States, subjected to testing utilizing a 25-gene panel targeting hereditary cancer genes, revealed that 9.3% of the cohort exhibited at least one germline mutation. Moreover, discernible disparities in genetic prevalence were observed in relation to both ancestral background and the TNBC status, suggesting a noteworthy influence on the prevalence of specific genes(64).

The predominant germline mutation is observed in *BRCA2,* followed by *BRCA1* as the secondary mutation. *BRCA1/2* constitutes the predominant germline mutation, accounting for 42% of germline mutation cases in the etiology of Hereditary Breast-Ovarian Cancer Syndrome. The majority, comprising 58%, of cases involving germline mutations involve genes other than *BRCA*. Among the cohort of 151 non-selected BC cases, the presence of the *BRCA* germline mutation was identified in 10 instances, representing a prevalence of 6.62%. In a conducted study, cancer multigene panel tests were administered to 296 female BC patients from the Malwa region of Punjab, India. The findings from these tests did not indicate the existence of a *BRCA1* germline mutation(11). A study conducted on 467 BC patients in Sarawak, Malaysia, which is adjacent to Thailand's three southern borders, identified PVs in the *BRCA1* and *BRCA2* genes in 2.8% and 3.2% of the patients, respectively(65). In an investigation encompassing patients from central Thailand diagnosed with BC and categorized as high-risk for HBOC according to the 2019 NCCN guidelines, it was determined that 29 out of the total 309 individuals (constituting 9.5% of the patient cohort) possessed germline *BRCA1* mutations(13). The Thailand Universal Coverage (UC) policy in 2023 extends coverage exclusively to germline genetic testing for *BRCA* genes. Based on available information, genetic testing for genes beyond *BRCA* confers greater genetic advantages in the context of cancer. Multigene panel testing,

encompassing a spectrum of cancer susceptibility genes beyond BRCA, is a robust and highly accurate method for identifying a range of HBOC susceptibility genes from low to high risk(66).

I identified novel germline mutations in *BRIP1, CHEK2, MSH6, PALB2*, and *PTEN* genes, all of which fall within the classification criteria outlined by the ACMG as either PV or LP variants. (**Table 6)** In an individual diagnosed with HER2-enriched BC at the age of 40, a novel variant in the *BRIP1* gene, specifically identified as c.195_196insC, resulting in the amino acid change p.Ser66fs, was detected. The *BRIP1* gene, encoding BRIP1 proteins, engages in interactions with BRCA1 proteins during the routine repair of double-strand breaks in a normal cellular context. Additionally, a germline mutation in BRIP1 increases the risk of hereditary ovarian cancer(67). A Luminal B BC patient aged 57 harboring a novel germline mutation in the *CHEK2* gene (c.1178C>G, p.Pro393Arg). A previous study within a Malaysian population identified a rare germline variant in the *CHEK2* gene(68). The CHEK2 gene exhibited an association with an approximately two-fold elevation in the risk of contralateral BC among individuals diagnosed with BC(69). MSH6 mutations have been implicated in Lynch Syndrome, a hereditary condition characterized by an increased susceptibility to colorectal and various other cancers(70). Within the scope of my investigation, I have identified a previously unreported loss-of-function germline mutation in *MSH6* (c.4003del, p.Glu1335LysfsTer11), shedding light on its potential significance in the context of BC predisposition. In the dataset featuring individuals with MSH6 germline mutations, a BC patient experienced the onset of TNBC at the age of 44. The Partner and Localizer of *BRCA2 (PALB2)* constitutes a pivotal component in the cellular response to DNA damage and is acknowledged as a gene associated with BC predisposition(71). Individuals carrying loss-of-function mutations in *PALB2* face a 9.5-fold elevated risk of developing BC in comparison to those who do not have such mutations(72). In an investigation of the populations in Malaysia and Singapore focusing on a rare condition, analysis of germline mutations in *PALB2* uncovered a restricted incidence of *PALB2* mutations (2 out of 122) within patients exhibiting BC and a familial history of BC(73). *PALB2* mutations were observed in 2 out of 151 cases. Additionally, a hitherto unreported *PALB2* variant (c.2101delT, p.Ser701fs) was identified, demonstrating an association with increased susceptibility

to BC. Furthermore, I identified a novel large insertion in the *PTEN* gene at position c.154_154+1 insGG, leading to the mutation p.His53GlyfsTer10. This variant is linked to Luminal A subtype of BC. Recent investigations have underscored distinctions in the HER2-overexpressing carcinoma profile(74), deviating from the findings in my dataset. Identifying a discernible mutation in the *PTEN* gene has been demonstrated to elevate the risk of BC by 85%(75). Consequently, surveillance protocols for individuals harboring *PTEN* variants are recommended to be on par with those advised for individuals carrying mutations in the *BRCA1/2* genes(76).

Significantly, juxtapose the germline mutation spectrum of the preceding investigation with that of central Thailand. In an analysis comprising 389 patients diagnosed with cancer who met the criteria for hereditary cancer and underwent multigene panel testing, only a PV identified in TP53 c.1024C>T (p.Arg342Ter) exhibited congruence with the mutations observed in my study(13).

A study revealed a distinctive variant spectrum among a cohort of 12 individuals harboring germline mutations in *BRCA1/2*(77). Among the cohort of 112 patients diagnosed with non-mucinous epithelial ovarian cancer, twenty-six exhibited disparities in the germline mutation spectrum in contrast to the outcomes documented in my study(78).

Table 6 Within the list of 26 genes associated with susceptibility to breast cancer, mutations in the germline were discerned in 11 specific genes. These genes encompass *ATM, BRCA1, BRCA2, BRIP1, CHEK2, MSH6, NF1, PALB2, PTEN, RAD51D*, and *TP53*.

Gene	HGVS	dbSNP ID
ATM	c.8373C>A(p.Tyr2791Ter) c.2377-2A>G(p.Splicing)	rs1060504292 rs1057516553
BRCA1	c.2059C>T(p.Gln689Ter) c.1863_1885del(p.His621GlnfsTer7) * 2 cases c.5068A>C(p.Lys1690Gln)	rs273898674 Novel rs397507239
BRCA2	c.6352_6353del(p.Val2118LysfsTer10) c.5164_5165del(p.Ser1722TyrfsTer4) c.7558C>T(p.Arg2520Ter) c.3680_3681del(p.Leu1227GlnfsTer5) c.3283C>T(p.Gln1095Ter) c.262_263del(p.Leu88AlafsTer12)	rs80359576 rs80359490 rs80358981 rs80359395 rs397507662 rs276174825
BRIP1	c.2990_2993del(p.Thr997ArgfsTer61) c.195_196insC(p.Ser66fs)	rs771028677 Novel
CHEK2	c.1178C>G(p.Pro393Arg) c.246_260del(p.Asp82_Glu86del)	Novel rs587780181
MSH6	c.4003del(p.Glu1335LysfsTer11)	Novel
NF1	c.6592_6593del(p.Asp2198fs)	rs765010702
PALB2	c.2257C>T(p.Arg753Ter) c.2101delT(p.Ser701fs)	rs180177110 Novel
PTEN	c.154_154+1insGG(p.His53GlyfsTer10)	Novel
RAD51D	c.680C>T(p.Ser227Leu)	rs370228071
TP53	c.818G>A(p.Arg273His) c.1024C>T(p.Arg342Ter)	rs28934576 rs730882029

Table 7 List of variants of uncertain significance as detected by the American College of Medical Genetics and Genomics guideline, 2015.

Gene	HGVS	SNP ID
ATM	c.8797A>G(p.Lys2933Glu)	rs587779875
	c.2944C>T(p.Arg982Cys)	rs587779830
	c.9124C>G(p.Pro3042Ala)	Noval
BARD1	c.1174_1194delTTGCCTGAATGTTCTTCACCA (p.Leu392_Pro398del)	Noval
	c.538T>C (p.Tyr180His)	rs1060501311
	c.127C>T (p.Arg43Cys)	rs752871324
BRCA1	c.3625T>G (p.Leu1209Val)	rs273900711
BRCA2	c.4089C>G (p.Asn1363Lys)	Noval
	c.5299_5307del (p.Lys1767_Asp1769del)	rs80359504
	c.5218_5223del (p.Leu1740_Ser1741del)	rs397507775
BRIP1	c.67C>T (p.Pro23Ser)	Noval
	c.1912A>G (p.Asn638Asp)	Noval
CDH1	c.2603G>A (p.Arg868His)	rs369126891
CHEK2	c.1231G>A (p.Asp411Asn)	rs755127902
	c.976C>T (p.Pro326Ser)	rs1555917036
MLH1	c.1730C>T (p.Ser577Leu)	rs56185292
	c.290A>G (p.Tyr97Cys)	rs773647920
MSH2	c.2435C>G (p.Thr812Ser)	rs1553369826
	c.2439G>T (p.Met813Ile)	rs587781678
MUTYH	c.398A>G (p.Asn133Ser)	rs771641237
	c.158-2A>T(Splicing)	Noval
	c.892-2A>G(Splicing) *5 cases	rs77542170
NF1	c.6743G>A (p.Arg2248His)	rs562367786
PMS2	c.752T>A (p.Val251Glu)	Noval
RAD51C	c.722T>C (p.Val241Ala)	rs1555599127
RAD54L	c.1862T>C (p.Leu621Pro)	rs768472959
	c.1850A>C (p.Tyr617Ser)	Noval
TP53	c.7G>A (p.Glu3Lys)	Noval

The categorization of variants as "Variant of Uncertain Significance" (VUS), according to the ACMG, designates a subset of genetic alterations that fall within a gray area, rendering it inconclusive to ascertain their role in the development of cancer definitively(35). Within the scope of this investigation, VUS were discerned in 15 genes, as elucidated in **Table 7**. Furthermore, previously unreported VUS were detected within the genetic sequences of *ATM, BARD1, BRCA2, BRIP1*, *MUTYH, PMS2, RAD54L*, and *TP53*. Significantly, a substantial proportion of instances classified as VUS undergo reclassification, trending towards designations indicative of likely benign or unequivocally benign variants(79, 80). VUS necessitates additional investigation to facilitate reclassification in health provider datasets. It is imperative to communicate this information to the patient.

In this study, we identified a VUS denoted as *MUTYH* c.892-2A>G (splicing) (rs77542170) in five individuals diagnosed with BC. Mutations in the *MUTYH* gene give rise to an autosomal recessive syndrome characterized by significantly elevated penetrance, manifesting as intestinal adenomatous polyposis and an escalated susceptibility to colorectal cancer, along with a high risk of other cancer types. Within a case-control investigation involving Jewish populations in North Africa, it was observed that the monoallelic mutation G396D in the *MUTYH* gene exhibited an association with an increased risk of BC (Odds Ratio: 1.86, 95% CI: 1.02-3.39; P-value: 0.039)(81). The investigation encompasses 225 monoallelic *MUTYH* mutations sourced from cohorts in the United States, Australia, New Zealand, and Canada. The calculated cumulative risks for females to develop BC by the age of 70 stands at 11% (95% CI: 8.3–16), indicating a marginally increased incidence of BC development(82). In a comprehensive Italian Multicenter Study involving 560 male BC cases and 1,540 male controls, NGS was applied to examine 50 cancer susceptibility genes, including the *MUTYH* gene. The findings indicate that the presence of biallelic or monoallelic *MUTYH* PV is associated with an elevated risk of male BC(83). Evidence indicates that monoallelic and biallelic germline mutations in *MUTYH* significantly elevate susceptibility to BC. This investigation identified five BC patients with VUS in *MUTYH* c.892-2A>G (splicing) (rs77542170), underscoring the imperative for additional research to establish the association between these mutations and BC definitively.

Among a cohort of 151 BC patients, four individuals were found to harbor VUS in the *BRCA1/2* genes. The presence of pathogenic or likely pathogenic germline variants in the *BRCA1/2* genes is associated with a markedly elevated risk of BC, ranging from 10 to 20 times higher in carriers of these mutations compared to individuals without *BRCA* mutations(84). Nevertheless, VUS in the *BRCA1/2* genes does not provide conclusive evidence regarding the risk of developing BC. The clarification of functional assays is essential for proper disclosure, as they significantly influence the therapeutic benefits for patients(85). The interpretation and communication of *BRCA* VUS necessitate careful consideration, particularly by genetic counselors or healthcare providers(86). The clinical significance of VUSs in *BRCA1/2* genes is subject to evolution over time. Individuals harboring VUSs should undergo regular follow-ups, employing personalized screening and prevention strategies(87). VUSs do not influence therapeutic or prophylactic surgical decisions, in underscoring their utmost importance averting unnecessary medical procedures(88).

In summary, the evolution of sequencing technology is progressing swiftly, with concurrent reductions in cost and enhanced accessibility. In high-income countries, there is greater accessibility to cancer genetic testing. In Thailand, access to cancer genetic tests is constrained within the context of middle-income countries. The study of comprehensive exome sequencing in individuals diagnosed with BC reveals a considerable prevalence of germline mutations within a cohort of unselected BC patients. Germline mutations were identified in the context of cancer susceptibility, encompassing genes with varying degrees of penetrance, ranging from low to high. Furthermore, the investigation revealed the absence of previously documented germline variants classified as pathogenic, likely pathogenic, or VUS.

Concluding

BC represents a substantial health problem among women residing in the three Southern border provinces of Thailand. The specific survival rate for BC is diminished, with an overall 5-year survival rate of 50%, primarily attributable to delayed diagnosis and treatment interventions. The singular challenge may lie in the augmentation of health awareness and the facilitation of access to BC screening services. The prognosis for survival in cases of BC may significantly enhance through early diagnosis and prompt initiation of treatment. In conjunction with breast ultrasonography and mammography, germline genetic testing assumes a pivotal role in the assessment of individuals diagnosed with BC. The investigation indicates that approximately 50% of individuals diagnosed with BC exhibit a heightened susceptibility to hereditary cancer syndromes.

NGS represents a high-throughput method of DNA sequencing, characterized by the concurrent sequencing of numerous small DNA fragments. This technique is employed to elucidate the sequence of genetic material, with a primary aim to enhance sequencing efficiency, expedite the process, and concurrently reduce associated costs in the contemporary landscape. This investigation stands as the first study endeavor to employ high-throughput technology through WES on an unselected cohort of 151 BC patients situated in three Southern border provinces of Thailand. The study revealed that out of the 151 patients diagnosed with breast cancer (BC), twenty-three individuals (15.2%) exhibited the presence of at least one germline mutation in a gene recognized for its association with heightened susceptibility to cancer. In the course of my study, it was discerned that *BRCA2* manifested as the primary occurrence, with *BRCA1* subsequently identified as the second in sequence. VUSs were identified in certain instances of BC, presenting a challenge in briefly summarizing the correlation with cancer development. The investigation unveiled numerous novel variants, necessitating additional research to corroborate their correlation with cancer development. The investigation of germline mutations in the central region of Thailand has revealed notable divergences in the spectrum of genetic variations when contrasted with prior research findings.

My study's primary limitation is the sample size of 151 breast cancer cases. Increasing the sample size would enhance the accuracy of the findings. Thailand, categorized as a middle-income nation, currently faces constraints in the accessibility of genetic testing services within its territorial confines. Due to constraints inherent in our data-collection procedures, I found myself unable to undertake a subsequent analysis of treatments with the specific aim of monitoring and evaluating survival rates. WES is limited in its ability to detect genomic rearrangements, large deletions, large insertions, and copy number variations. Investigating areas beyond the limitations of WES is of significant interest. Concerning the intron region, a non-protein coding segment, it is essential to acknowledge that mutations within this domain can impact gene expression and potentially instigate carcinogenic processes. However, it is crucial to underscore that a comprehensive examination of this particular region has not been conducted thus far. The intron stands as a compelling area for investigation, necessitating more extensive scrutiny in future research endeavors.

The genomic foundation of the populace confers advantages pertinent to the strategic formulation of public health planning initiatives. The newly identified germline variant, ascertained through this investigation, necessitates a comprehensive functional analysis to elucidate its potential implications in the pathogenesis of cancer. Other methods are gene editing, case-control study, segregation study, germline comparison to somatic mutation, and in vivo or in vitro analysis. Moreover, investigations involving survival analysis and cost-benefit analysis are of particular interest. The exploration of genomic rearrangements and intron studies is imperative for this specific population.

The *MUTYH* c.892-2A>G (splicing) variant (rs77542170) was identified in five patients diagnosed with BC and elucidated as a VUS. Further investigation is warranted to elucidate the association of this variant with BC and other malignancies.

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Appendix

Paper 1 (Survival Outcomes in Breast Cancer Patients and Associated Factors in a Border Province of Thailand: A Hospital-based Review)

Paper 2 (Prevalence of Pathogenic Germline Mutations in 13 Hereditary Cancer-Related Genes in Breast Cancer Patients in Narathiwat Province, Thailand)

Utilized primers

Paper I

Sukpan P, Kanokwiroon K, Sriplung H, Paisarn P, Sangkhathat S. Survival outcomes in breast cancer patients and associated factors in a border province of Thailand: a hospital-based review. *J Health Sci Med Res*. 2023;41(3):e2022911.

Original Article

JHSMR Journal of Health Science and Medical Research

Survival Outcomes in Breast Cancer Patients and Associated Factors in a Border Province of Thailand: A Hospital-based Review

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Abstract:

Objective: To evaluate the outcomes of breast cancer (BC) patients in Naradhiwas Rajanagarindra Hospital and factors associated with the outcomes.

Material and Methods: A hospital-based retrospective review of patients who were diagnosed with BC in a tertiary-level hospital in a Thailand border province was performed. Familial histories were obtained by telephone interviews.

Results: There were 234 female patients diagnosed with BC during the 6-year study period. The mean age at diagnosis was 52.6 years. At presentation, 46.2% of the patients had locally advanced disease and 26.5% already had distant metastasis. Invasive ductal carcinoma (IDC) was the most common pathological subtype (84.6%) and 4.3% were ductal carcinoma in-situ. The five-year overall survival (OS) was 54% (95% confidence interval (CI) 40.25%-58.15%). On univariate analysis, factors associated with poorer survival were age >50 years, IDC pathology, T stage 3-4, N stage 2-3, distant metastasis, negative estrogen receptors, negative progesterone receptors, or both, positive human epidermal growth factor receptors 2, and Ki67 immunoreactivity >20%. Multivariate analysis showed that advanced T stage (adjusted HR1.97 95% CI 1.15-3.38), N stage (adjusted HR1.95 95% CI 1.05-3.64) and distant metastasis (HR1.86 95% CI 1.27-2.71). Forty-nine percent of the BC women met the hereditary cancer criteria.

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Conclusion: The survival outcome of BC in Narathiwat province was poorer than that reported in other parts of the country. Delayed presentation might be an explanation for this disparity and a BC screening program is recommended to improve early detection.

Keywords: breast cancer, prognosis, rural area, survival outcome

Introduction

Breast cancer (BC) is the most common site-specific cancer in women and the leading cause of cancer-related deaths worldwide.[1] While the incidence of BC is declining in high-income countries, it is increasing in low/middle-income countries.[2] In Thailand, BC ranks the first among cancers in women and the incidence is rapidly increasing, with over 19,400 new cases expected in 2025.[3] Although the southern part of the country had the lowest incidence of BC in the past, the figure is predicted to increase continuously.[4]

The southernmost part of Thailand is located in the middle of the Malay peninsula, consisting of 3 provinces, Yala, Narathiwat, and Pattani, situated close to the northern border of Malaysia. Political unrest has been ongoing for the last 3 decades in this area. The population of this area is approximately 2 million, of whom 83% are Muslim, sharing ethnicity, culture, language and religion with the Malays[5] while the remainders are mostly Chinese-Thai who are closer in ethnicity to Thais in other areas of the country[6] who are Asian immigrants, known as Buddhist-Thai. A previous study found that cancer incidences were different between the Muslim-Thai and Buddhist-Thai subpopulations.[7] Another previous study in Songkhla province, just north of Pattani, found that BC in Muslim-Thais was more common in younger age 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. In high-income societies, current presentation of BC is mostly abnormal mammogram. However, without the availability of mammograms or other effective screening programs, patients with BC often only seek medical attention when there is a palpable lump in their breast. Although BC screening is included in the Thailand universal health coverage program, the availability of mammogram machines is still limited in many rural areas of Thailand, including the 3 southernmost provinces. In addition, the compliance with standard management recommendations in Muslims, especially surgery, could be an issue precluding effective management of BC in the southern region. The same problems of late presentation and relatively poor adherence to modern management have also been reported in case series from other countries in the same region.[8,9]

This study aimed to address the clinical outcomes and factors associated with the outcomes of BC in Narathiwat province. The study was also interested in exploring the incidence of BC patients in the region who may be indicated for genetic testing and counselling. These data could be useful in planning appropriate strategies for disease screening and therapy in these provinces.

Material and Methods

The study retrospectively reviewed the medical records of female patients diagnosed with epithelial BC from Naradhiwas Rajanagarindra Hospital, the main tertiary care hospital in the area, during the period from June 2016 to May 2021. Qualifying patients were identified using the hospital database through the ICD10 code C50.0-C50.9 (malignant neoplasm of breast). Extracted data included demographic data such as age at diagnosis, BC-specific

data including stage at diagnosis and histopathology and family history of cancer. BC staging was based on the American Joint Committee on Cancer classification (7th edition). The TNM system was based on tumor size and extension (T), regional lymph node metastasis (N), and evidence of distant metastasis (M). The group of TNM staging data summarized the pathologic stage group.[10] In cases of BC-related death, mortality date was taken from the death register of the local government office. In cases with no clear familial history information, the data was obtained by telephone interview.

Treatment of BC in Naradhiwas Rajanagarindra Hospital followed the Clinical Practice Guideline of the National Institute of Cancer (NCI) of Thailand. The hospital provides primary surgical treatment or surgical treatment after neoadjuvant chemotherapy. BC patients who need postoperative adjuvant treatment are referred to a regional center or the large university hospital in Southern Thailand. Surgical treatment was the first step for almost all patients diagnosed with breast cancer in this series.

Data on BC biomarkers in this study used the immunohistochemical subtypes estrogen receptors (ER), progesterone receptors (PR), and human epidermal growth factor receptors 2 (HER2).[11] Hormonal receptors (HR) positive were defined as ER+ and/or PR+. Ki67 is a biomarker that evaluates the proliferative index in breast cancer, grouped to more than 20% and less than 20%.[12] BC patients were defined as having a genetic risk when they met the criteria of the National Comprehensive Cancer Network (NCCN) guidelines 2022, which included I. diagnosis of BC at age less than 46 years, II. diagnosis of BC at age 46-50 years with an unknown family history or with multiple primary breast cancers. III. diagnosis of BC at any age with at least one close family member with breast, ovarian, pancreatic, or prostate cancer. IV. diagnosis at any age with triple-negative breast cancer (TNBC).

Before analysis, the data were re-checked and cleaned in Microsoft-Excel. Mean with standard deviation (S.D.) or median with interquartile range were used as representative values for parametric data. Survival outcomes were presented as overall survival using death as a failure criterion and presented as survival probability with a 95% confidence interval (95% CI) Comparisons used t-test, Mann-Whitney U test or chi-square test as appropriate. Univariate survival analysis used log-rank test and Kaplan-Meier survival plots with cancer-related death as a censor in overall survival (OS) analysis. The beginning date was defined as the diagnosis date and survival status was as of December 2021. Multivariate analysis used Cox's proportional hazard analysis. All data were analyzed with Statistical Package Stata 14.0 (Stata Corporation, TX). A p-value of less than 0.05 was considered statistically significant.

Results

A total of 234 female patients were diagnosed with BC in Naradhiwas Rajanagarindra Hospital during the 6-year study period. Age at diagnosis ranged from 23-84 years, with an average age of 52.6 years (S.D. 12.0 years). Regarding the primary tumor, 103/234 cases (46.2%) had locally advanced disease at the diagnosis and 62/234 cases (26.5%) had metastasis. 123/234 cases (53.0%) received primary surgical treatment while 178/234 cases (76.1%) received chemotherapy.

The largest BC histopathology finding in our cases was invasive ductal carcinoma which was diagnosed in 84.6% of the patients, followed by ductal carcinoma in-situ in 4.3% and 11.1% with other pathologies (Table 1). Regarding breast cancer subtypes, hormonal receptors (ER and/or PR) were positive in 61.2%, HER2 was positive in 36.1% and 18.7% of patients were TNBC. Considering the Ki67 proliferative index, 162 (75.6%) of the cases were

reported as more than 20% positive. When considering family history related to genetic risk, 114/233 patients met at least one criterion according to the NCCN guideline, with age being the most common item recommending BC patients for genetic testing.

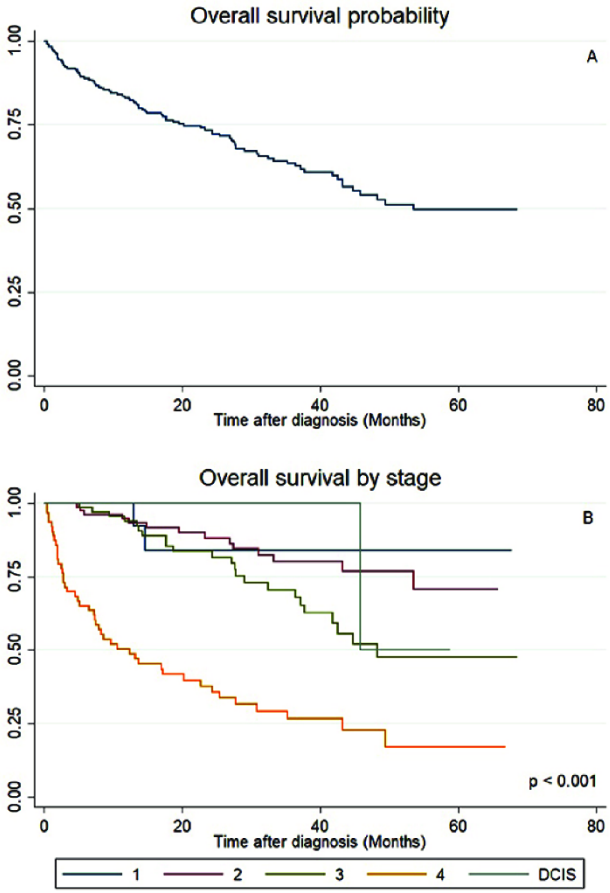
Table 1 Distribution of clinical parameters in breast cancer patients in this study and univariate survival analysis with Log-rank test

Parameter	N (%)	5-year OS (%)	Log-rank p-value
All	234 (100)	49.54	-
Age group			
<50 years	109 (46.58)	59.61	0.029
>50 years	125 (53.42)	40.18	
Pathological group			
IDC	198 (84.62)	47.44	0.0215
Others	36 (15.38)	59.37	
T stage			
Tis-2	126 (58.85)	67.36	<0.001
T3-4	108 (46.15)	29.28	
N stage			
N 0-1	119 (51.07)	71.69	<0.001
N 2-3	114 (48.93)	28.09	
M stage			
M0	170 (73.28)	61.20	<0.001
M1	63 (27.04)	16.89	
Stage group			
Stage 0-1	25 (10.73)	77.73	<0.001
Stage 2	78 (33.48)	71.14	
Stage 3	67 (28.76)	47.10	
Stage 4	63 (27.04)	16.89	
Estrogen receptors*			
Negative	94 (40.52)	43.77	0.012
Positive	138 (59.48)	54.42	
Progesterone receptors*			
Negative	119 (51.29)	38.88	0.001
Positive	113 (48.71)	61.41	
Hormone receptors (any)*			
Negative	99 (38.79)	40.85	0.002
Positive	142 (61.21)	56.08	
HER2 expression*			
Negative	147 (63.91)	55.62	0.008
Positive	83 (36.09)	40.79	
TNBC*			
Non-TNBC	187 (81.30)	51.40	0.249
TNBC	43 (18.70)	44.69	
KI67 index*			
<20%	54 (24.43)	60.79	0.024
>20%	167 (75.57)	47.56	
Hereditary risk			
No	120 (51.28)	44.05	0.454
Yes	114 (48.72)	56.10	

*with missing data

IDC=invasive ductal carcinoma, HER2=human epidermal growth factor receptors 2, TNBC=triple-negative breast cancer

Two-year and five-year overall survival (OS) were 73.45% (95% CI 77.7%-87.49%) and 54% (95% CI 40.25%-58.15%), respectively (Figure 1). On univariate analysis of factors associated with survival after diagnosis (Table 1), older age (>50 years), IDC pathology, higher stage, expression of hormone receptors, expression of HER2 and high Ki67 proliferative index (>20%) were found to have significant associations with poorer OS (Table 1). On univariate logistic regression, N stage had the highest hazard ratio (HR 3.93, 95% CI 2.41-6.39), followed by T stage (HR 3.52, 95% CI 2.22-5.61) and IDC pathology (HR 2.77, 95% CI 1.12-6.84). However, when stepwise regression was conducted, 3 factors were retained in the final model, T stage, the N stage and metastatic status, with T stage having the highest adjusted HR at 1.97 (95% CI 1.15-3.38) (Table 2).



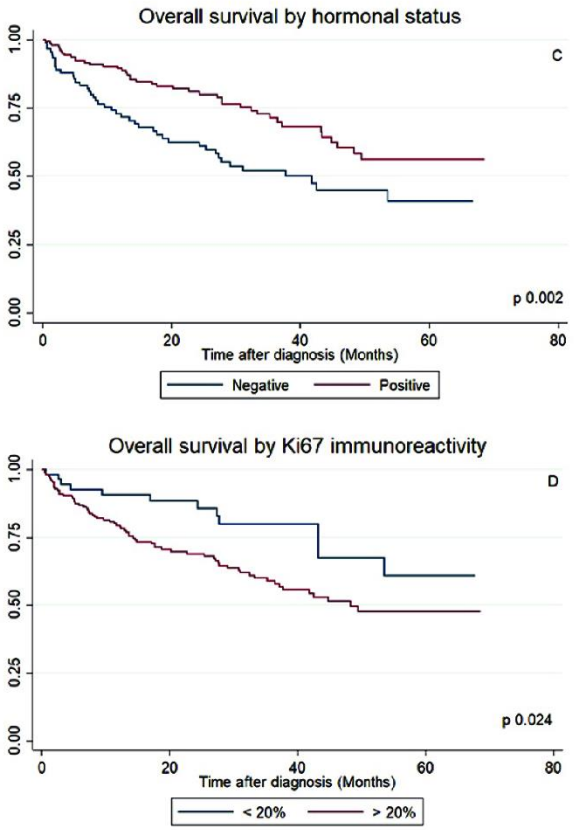


Figure 1 Survival probability of breast cancer patients in this study.

A: Overall survival

B: Overall survival by stage

C: Overall survival by hormonal status

D: Overall survival by Ki67 status

Table 2 Univariate Cox proportional hazard analysis of factors associated with survival in breast cancer patients in Narathiwat and multivariate analysis by stepwise regression

Parameters	Hazard ratio	95% confidence interval	Adjusted Hazard ratio	95% confidence interval
Age >50 years	1.63	1.05–2.54		
Invasive ductal carcinoma	2.77	1.12–6.84		
T stage (T3–4)	3.52	2.22–5.61	1.97	(1.15–3.38)
N stage (N2–3)	3.93	2.41–6.39	1.95	(1.05–3.64)
M stage	2.51	1.92–3.29	1.86	(1.27–2.71)
Estrogen receptors	1.72	1.12–2.63		
Progesterone receptors	2.05	1.31–3.22		
HER2 expression	1.77	1.52–2.73		
KI67 >20%	2.00	1.08–3.69		

HER2=human epidermal growth factor receptors 2

Discussion

With standard screening and therapeutic programs, 5-year survival of BC in high-income countries has been reported at more than 90%.[13–15] In Thailand, Chitapanarux I and colleagues reported a study of BC outcomes in the Northern part of the country during 2006–2015, in which OS was at 75% and survival in localized disease (stages I and II) was 85–94%.[16] In our 234 BC patients, the average age of diagnosis at 53 years was comparable to other reports from Asian countries including Thailand. The 5-year OS in our patients at 54% was obviously lower than in other reports from countries with comparable economic levels[17,18] which might partly be explained by the fact that nearly half (46%) of our group had locally advanced disease at presentation, 62% of them had lymph node metastasis and 27% had evidence of distant metastasis. Our patients had significantly poorer survival outcomes because of advanced-stage disease. In addition, when compared stage-by-stage, overall mortality was similar between our and other Asian studies.[17,19] In a cooperative hospital-based study from Singapore and Malaysia reported in 2011, the size of the primary tumor significantly decrease after the implementation of mammographic screening and the same study showed that only 10% of the patients had distant metastasis at presentation and the figure decreased with time.[20] A nation-wide survey in China in 2011, which had begun a screening program, reported that 18.7% of their BC patients were in stage III, and only 2.4% were in stage IV.[21] A study in the Northeastern provinces of Thailand found that factors associated with delayed treatment in BC included poor socioeconomic status and prolonged referral duration.[22] The evidence from our study suggests that early screening might be a key strategy to reduce BC-related deaths in our population. When the other part of the country has embraced modern screening methods such as radiologic and genetic screening, implementation of these technologies in rural areas like the 3 border provinces of Thailand still has a long journey to go.

Currently, there are 2 effective strategies for detecting patients at risk of developing BC, mammograms and genetic screening. Mammograms have been reported to change the stage at diagnosis to earlier stages and reduce cancer-specific mortality in population-based studies in Europe and America.[23–25] In our community, the availability of this technology is still limited to private hospitals and breast centers situated hours away from the province. A

mammogram is thus not a generally accessible technology at this time but should be a developmental goal. According to the NCCN guideline, 48.7% of our patients should be screened for risk of vertical transmission. Genetic testing for BRCA gene mutation has been included in the Thailand Universal Coverage (UC) scheme since 2021, which may have some impact on the primary prophylaxis of BC through the identification of more hereditary cases. Germline genetic tests not only provide benefits for cancer surveillance and prophylaxis but may also help in precision cancer therapies.[26] Recently, the American Society of Breast Surgeons recommended germline genetic testing for all breast cancer patient.[27]

Our study found similar frequencies of hormone receptor positivity in our BC patients when compared to the Western series.[28,29] Hormonal profiles, including ER and PR, are predictive and prognostic markers for hormonal therapy. As in other studies, our patients who had positive ER or PR or both markers had significantly better survival, while patients with positive HER2 had poorer outcomes, possibly because of limitations in access to trastuzumab in most of our cases. However, the levels of hormonal receptors and HER2 positivity reflect an acceptable standard of pathological studies in our BC patients.

The main limitation of this study was in the nature of retrospective and hospital-based studies as there were missing data, especially in the details of the management of each patient. However, we were certain of the demographic, pathological and survival data retrieved from the well-established registry.

Conclusion

The study reviewed BC in a tertiary care level hospital in Narathiwat province in Thailand and found that delayed presentation is associated with poor survival outcomes. Early detection of the disease by an effective screening program including mammography and genetic studies should be included in the government healthcare policy for this patient group.

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Conflict of interest

We declare no conflicts of interest.

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Paper II

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Pathogenic Germline Mutations Breast Cancer Patients in Narathiwat Province

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

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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

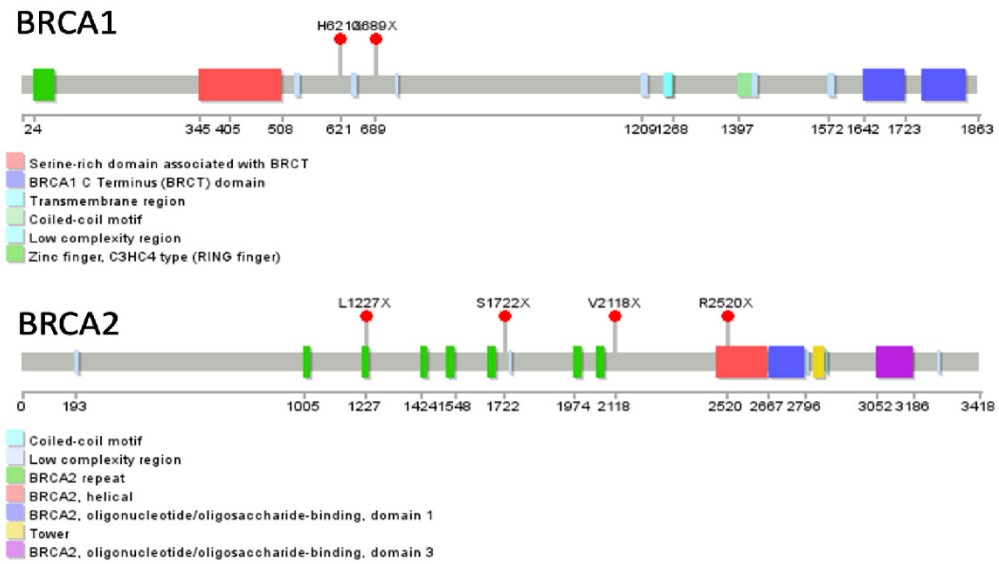


Figure 1. A Lollipop Plot of the Pathogenic Variants in *BRCA1* and *BRCA2* Detected in This Study

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).

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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.

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The primers employed in the Sanger sequencing methodology.

Gene	
ATM: NM_000051.3	
c.8373C>A	F 5'-TCAGCGAAGTGGTGTTCTTG-3'
	R 5'-TGGGTGTCACTCACCATCAT-3'
C.2377-2A>G	F 5'-AAGATGTGTTTTTGAAGCAGCA-3'
	R 5'-ATCGCAGGAAAAAGCCAGAT-3'
c.8797A>G	F 5'-CGTTGACAACATTGGTGTGT-3'
	R 5'-TGAAGCAGTGCTCTTCACATC-3'
c.2944C>T	F 5'-GCCAATGGAAGATGTTCTTGA-3'
	R 5'-CAGAGTCCATATTGCTTTGACCT-3'
c.9124C>G	F 5'-AGGAGTGGAAGAAGGCACTG-3'
	R 5'-AAGGCTGAATGAAAGGGTAATTC-3'
BARD1: NM_000465.4	
c.1174_1194del	F 5'-AACGGTGCCCTCAGAAAATA-3'
	R 5'-CCATATTGGGCAACAGCTTC-3'
c.538T>C	F 5'-GCCCTCTCAGAAACATCTGC-3'
	R 5'-TGTGGTTTAGCCCTCGAAGT-3'
c.127C>T	F 5'-CCCAAGAAGCTCCGTCTTTA-3'
	R 5'-GAGGAGCCTTTCATCCGAAG-3'
BRCA1: NM_007294.3	
c.2059C>T	F 5'-GATCTTTGGGGTCTTCAGCA-3'
	R 5'-GCAACTGGAGCCAAGAAGAG-3'
c.1863_1885del	F 5'-TGCTTTCAAAACGAAAGCTG-3'
	R 5'-CCTGACTGGCATTTGGTTGT-3'
c.5068A>C	F 5'-TTTATGCAGCAGATGCAAGG-3'
	R 5'-TGTAGAACGTGCAGGATTGC-3'
c.3625T>G	F 5'-AAGTGTTGGAAGCAGGGAAG-3'
	R 5'-CAAAAGCGTCCAGAAAGGAG-3'
BRCA2: NM_000059.3	
c.6352_6353del	F 5'-TGAGCATAGTCTTCACTATTCACCT-3'
	R 5'-CCAACTGTTGTTTGTCTTGTTGA-3'
c.5164_5165del	F 5'-CTGTCAATCCAGACTCTGAAGAA-3'
	R 5'-TTGACACTTGGGTTGCTTGT-3'

c.7558C>T	F 5'-GGTTGTGCTTTTTAAATT TCAATTTTATTTTTGCT-3' R 5'-GTCATAAAAGCCATCAG TATTGTAGACAAAC-3'
c.3680_3681del	F 5'-GACAGCAGCAAGCAATTTGA-3' R 5'-CCTCTGCAGAAGTTTCCTCAC-3'
c.3283C>T	F 5'-AACTGAGCAAGCCTCAGTCAA-3' R 5'-TTCTGCCTTTTGGCTAGGTG-3'
c.262_263del	F 5'-AATCAGCTGGCTTCAACTCC-3' R 5'-GGCCAGAGAGACTGATTTGC-3'
c.4089C>G	F 5'-GAATTTGATGGCAGTGATTCAA-3' R 5'-CTTGAGCTTTCGCAACTTCC-3'
c.5299_5307del	F 5'-CAGCTATTCCTACCATTCTGATGA-3' R 5'-CACAAGTTCCTCAACGCAAA-3'
c.5218_5223del	F 5'-TTTGATGGTCAACCAGAAAGAA-3' R 5'-TGGCTCAATACCAGAATCAAGTT-3'
BRIP1: NM_032043.2	
c.2990_2993del	F 5'-ATTGAGGGCATGATCCAAAC-3' R 5'-GATCCACAAGCCCAACTTTC-3'
c.195_196insC	F 5'-GTCCCACAGGAAGTGGAAAA-3' R 5'-ACAGCATGGCTGAACCAGTC-3'
c.67C>T	F 5'-CAATTGGTGGGGTGAAGATT-3' R 5'-TCTTCCAAGTGAACCCAGAAA-3'
c.1912A>G	F 5'-GGCAAAGTTCAGACCATTGTT-3' R 5'-TTACTTGCTGGCACTTCAGG-3'
CDH1: NM_004360.5	
c.2603G>A	F 5'-GAAAGCGGCTGATACTGACC-3' R 5'-TCTCTCGAGTCCCCTAGTCG-3'
CHEK2: NM_001005735.2	
c.1178C>G	F 5'-CATGCCTGCCTTTCTGTGTA-3' R 5'-TCCTTTTTACGCTCCCACTTT-3'
c.246_260del	F 5'-CAAGATTGGCAAATCCATCC-3' R 5'-CAAGGCTCCTCCTCACAGTC-3'

c.1231G>A	F 5'-TGTCTTCTTGGACTGGCAGA-3'
	R 5'-GCACGGTTATACCCAGCAGT-3'
c.976C>T	R 5'-GGATGAGAAAGGCAAGCCTAC-3'
	F 5'-CCCTCTGGGCAGATGTTCTA-3'
MLH1: NM_000249.3	
c.1730C>T	F 5'-TTCTCCCATTTTGTCCCAAC-3'
	R 5'-TGGAGAGCTACTATTTTCAGAAACG-3'
c.290A>G	F 5'-TGGGAATTCAAAGAGATTTGG-3'
	R 5'-TGACAGACAATGTCATCACAGG-3'
MSH2: NM_000251.2	
c.2435C>G	F 5'-CCTTGGCCAATCAGATACCA-3'
	R 5'-TCCTACCCCCAGACTGTGAA-3'
c.2439G>T	F 5'-TGTCACAGCACTCACCACTG-3'
	R 5'-TCCTACCCCCAGACTGTGAA-3'
MSH6: NM_000179.2	F 5'-GGAAGGGATGATGCACTATGA-3'
c.4003del	R 5'-CCACCTTTGTCAGAAGTCAACTC-3'
MUTYH: NM_001048171.1	
c.398A>G	F 5'-TCTCTACACCCACCCCAAAG-3'
	R 5'-CCAGTGTGGGTCTCAGAGGT-3'
c.158-2A>T	F 5'-GCCAGAAACTTAGCCACAGC-3'
	R 5'-GGAGACAGAGGCCTGCAATA-3'
c.892-2A>G	F 5'-ACCACACTCCTCCACGTCA-3'
	R 5'-CACAGTGTGTACCCCACAGC-3'
NF1: NM_000267.3	
c.6592_6593del	F 5'-TTGCATGGACTGTGTTATTGG-3'
	R 5'-GGACATACCTTTGAGCTAGTTCTG-3'
c.6743G>A	F 5'-ATAATCCATCCCTGCAACCA-3'
	R 5'-ATTTCATTGACCTCAAATTTAAACG-3'
PALB2: NM_024675.3	
c.2257C>T	F 5'-CACTTGCAGGGTGGTATGTG-3'
	R 5'-CACCTGCTTTCCCCATCTTA-3'
c.2101delT	F 5'-CGCATGGATACAGAAATGGA-3'
	R 5'-TAAGATGGGGAAAGCAGGTG-3'

PMS2: NM_000535.7 c.752T>A	F 5'-AAGACACGAAACTATTAGCCTTAGA-3' R 5'-GTCCACTCTGTCTTTATTAGGAAGT-3'
PTEN: NM_001304717.5 c.154_154+1insGG	F 5'-GAGTCGCCTGTCACCATTTC-3' R 5'-GACGAATAATCCTCCGAACG-3'
RAD51C: NM_058216.3 c.722T>C	F 5'-AGGTCCCTGCTCTCTTGGAG-3' R 5'-TTTGCAAGGCTGATCATTTG-3'
RAD51D: NM_001142571.1 c.680C>T	F 5'-CCCACATTCCTTCAGGTGAC-3' R 5'-GCCAGAGACCAGACTCCAGA-3'
RAD54L: NM_001142548.1 c.1862T>C c.1850A>C	F 5'-CTAACCGGCTGGTCATGTTT-3' R 5'-TCCATGACCCACCCCTACTA-3'
TP53: NM_000546.5 c.818G>A c.1024C>T c.7G>A	F 5'-TGCTTACCTCGCTTAGTGCTC-3' R 5'-CAAGGGTGGTTGGGAGTAGA-3' F 5'-ACTTCTCCCCCTCCTCTGTT-3' R 5'-GAAGGCAGGATGAGAATGGA-3' F 5'-ATCCCCACTTTTCCTCTTGC-3' R 5'-TCCCACAGGTCTCTGCTAGG-3'

Primer sequences targeting germline variants were crafted utilizing Primer3Plus, a software tool based in Boston, United States. These primers were subsequently employed in the Sanger sequencing process.

VITAE

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Educational Attainment

Degree	Name of Institution	Year of Graduation
Training Curriculum in Genetic Counseling	Ramathibodi Hospital	2023
Diploma of the Thai Board of Surgery	Bhumibol Adulyadej Hospital	2018
Doctor of Medicine	Princess of Naradhiwas University	2013

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List of Publication and Proceedings :

List of Publication

1. **Sukpan P**, Kanokwiroon K, Sriplung H, Paisarn P, Sangkhathat S. Survival outcomes in breast cancer patients and associated factors in a border province of Thailand: a hospital-based review. *J Health Sci Med Res*. 2023;41(3):e2022911.

2. **Sukpan P**, Kanokwiroon K, Sriplung H, Laochareonsuk W, Choochuen P, Auseng N, Wanawanakorn K, Sangkhathat S. Prevalence of Pathogenic Germline Mutations in 13 Hereditary Cancer-Related Genes in Breast Cancer Patients in Narathiwat Province, Thailand. Asian Pac J Cancer Prev. 2023;24(2),525-530.

3. **Sukpan P**, Sangkhathat S, Sriplung H, Laochareonsuk W, Choochuen P, Auseng N, Khoonjan W, Salaeh R, Thangnaphadol K, Wanawanakorn K. Exome Sequencing Reveals Novel Germline Variants in Breast Cancer Patients in the Southernmost Region of Thailand. Journal of Personalized Medicine. 2023;13(11):1587.

List of Presentation

1. **Oral presentation**, Topic **'**Pathogenic Germline mutation in 13 cancer genes A case series in Narathiwat Province patients with non-selective breast cancer.' 47th Annual Scientific Congress Organizing Committee of The Royal College of Surgeons of Thailand at Ambassador City Jomtien Hotel, Pattaya, Thailand from July 13 - 16, 2022.

2. **Oral presentation**, Topic **'**Survival outcomes in breast cancer patients and associated factors in a border province of Thailand: a hospital-based review.' 1st Annual Health Research Conference 2022 (AHR-iCON 2022) at Conference Center and Health Science Library Building, Faculty of Medicine, Prince of Songkla University, Songkla, Thailand from August 1-2, 2022.

3. **Poster presentation**, Topic **'**Exome sequencing suggested novel pathogenic variant candidates in breast cancer susceptibility genes in Thais.' 82nd Annual Meeting of the Japanese Cancer Association (82nd JCA) at Pacifico Yokohama, Yokohama, Japan from September 21-23, 2023.