

SPECTRUM OF GERMLINE PATHOGENIC
VARIANTS IN EARLY-ONSET BREAST
CANCER: A MULTICENTRE
RETROSPECTIVE STUDY
IN MALAYSIA



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F. KELULUSAN DEKAN/PENGARAH / APPROVAL FROM DEAN/DIRECTOR

Tandatangan: Signature		Tarikh: Date	26/9/26
Nama dan Cap Rasmi: Name and Official Stamp	PROF. DATIN DR. NORLINAH MOHAMED IBRAHIM TIMBALAN DEKAN (SISWAZAH) Fakulti Perubatan UKM MBBCh (Ire) BMedSci (Ire), MRCPI, FRCPE Pakar Perunding Kanan Neurologi, HCTM No. MPM: 37009	Menjalankan Tugas Dekan Fakulti Perubatan Universiti Kebangsaan Malaysia	

**G. PERAKUAN TESIS/ DISERTASI SARJANA / DOKTOR FALSAFAH
(CERTIFICATION OF MASTER'S / DOCTORAL THESIS / DISSERTATION)**

Nama penuh pengarang (Author's Full Name)	Foong Le Hui		
No. Pendaftaran Pelajar (Student's Registration No.)	P148740	Sesi Akademik (Academic Session)	2025/2026
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Nama Pelajar (Student name): Foong Le Hui No. Pendaftaran Pelajar (Student's Registration No.): P148740 Tarikh: 10th August 2026 (Date)		Nama Penyelia / Pengerusi JK Siswazah : Professor Dr. Adli bin Ali (Supervisor's / Chairperson Supervision Committee Name) ASSOCIATE PROFESSOR DR. ADLI BIN ALI MD (UKM), Dr. Paed (UKM), MSc Immunology (Oxon), DPhil (Oxon) Senior Clinical Lecturer and Consultant Pediatric Immunologist Department of Pediatric Faculty of Medicine UKM Specialist Children's Hospital (HPKK) (UKM No : 44107) (NSR No: 130739) Tarikh: 11th August 2026 (Date)	

SPECTRUM OF GERMLINE PATHOGENIC VARIANTS IN EARLY-ONSET
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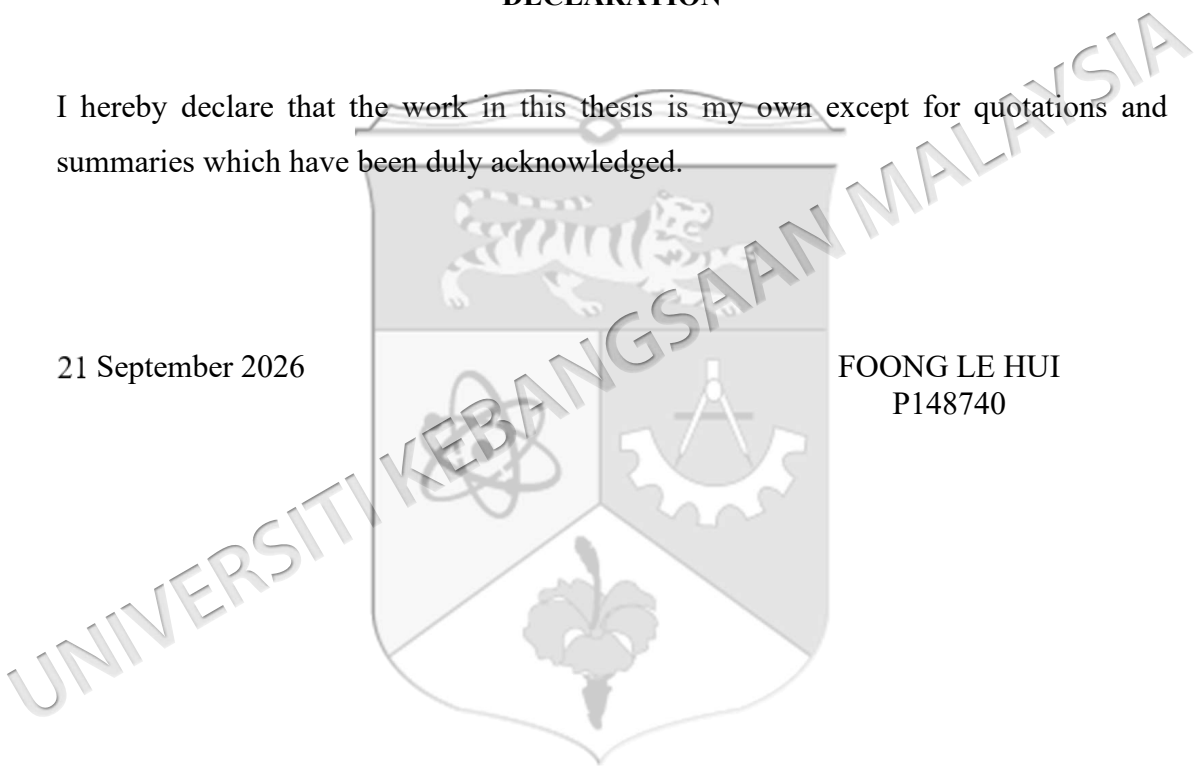
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DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged.

21 September 2026

FOONG LE HUI
P148740



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ABSTRACT

Early-onset breast cancer (EOBC) is an important clinical indication for hereditary cancer assessment, as younger patients with breast cancer (BC) have a higher likelihood of carrying germline cancer predisposition variants compared with unselected BC populations. However, local data on the gene spectrum and diagnostic yield of germline pathogenic/likely pathogenic (P/LP) variants among Malaysian EOBC patients remains limited. In particular, the contribution of non-*BRCA* cancer predisposition genes, clinicopathological predictors of germline P/LP variants, and the outcomes of different genetic testing approaches is not well characterised. This study aimed to characterise the spectrum of germline P/LP variants, examine their association with clinicopathological characteristics, and compare the diagnostic yield of *BRCA*-only and multigene panel testing among Malaysian EOBC patients. This multicentre retrospective study included EOBC patients diagnosed at ≤ 45 years of age who underwent germline genetic testing between 2020 and 2025. Data were collected from CRM, HKL and Hospital TABTAR. Demographic, clinicopathological, family history and genetic testing data were extracted from medical records. A total of 725 patients were included. Overall, 145 patients (20.0%) carried germline P/LP variants across 14 cancer predisposition genes. *BRCA1* was the most frequently identified gene (40.5%), followed by *BRCA2* (36.5%), while non-*BRCA* P/LP variants accounted for 23.0% of positive genetic findings, with *PALB2* (5.4%), *ATM* (4.1%) and *TP53* (3.4%) being the most common. In addition, triple-negative breast cancer (TNBC), bilateral BC and positive family history were identified as independent predictors of carrying germline P/LP variants. Diagnostic yield was 23.9% for *BRCA*-only testing and 19.1% for multigene panel testing. Detection of variant of uncertain significance (VUS) increased with larger panel size, highlighting the importance of careful variant interpretation and genetic counselling. These findings support EOBC as an indication for germline genetic testing and more appropriate referral for genetic counselling and testing.

ABSTRAK

Kanser payudara awal usia (*early-onset breast cancer, EOBC*) merupakan indikasi klinikal penting untuk penilaian kanser keturunan, memandangkan pesakit kanser payudara lebih muda mempunyai kebarangkalian lebih tinggi untuk membawa varian titisan germa yang meningkatkan risiko kanser berbanding populasi kanser payudara umum. Walau bagaimanapun, bukti tempatan mengenai spektrum serta kadar pengesanan varian patogenik/berkemungkinan patogenik (*pathogenic/likely pathogenic, P/LP*) titisan germa dalam kalangan pesakit EOBC di Malaysia masih terhad. Secara khususnya, peranan gen predisposisi kanser selain *BRCA*, faktor klinikopatologi yang meramalkan pengesanan varian P/LP, serta pendekatan ujian genetik optimum masih belum dicirikan. Kajian ini bertujuan untuk mencirikan spektrum varian P/LP titisan germa, menilai hubungannya dengan ciri-ciri klinikopatologi, serta membandingkan kadar pengesanan antara ujian *BRCA* sahaja dan ujian panel pelbagai gen dalam kalangan pesakit EOBC di Malaysia. Kajian retrospektif pelbagai pusat ini melibatkan pesakit EOBC yang didiagnosis pada usia ≤ 45 tahun dan menjalani ujian genetik titisan germa antara tahun 2020 hingga 2025 dari CRM, HKL, dan Hospital TABTAR. Data demografi, klinikopatologi, sejarah keluarga, dan ujian genetik diperolehi daripada rekod perubatan. Sebanyak 725 pesakit dimasukkan dan secara keseluruhannya, 145 pesakit (20.0%) membawa varian titisan germa P/LP dalam 14 gen predisposisi kanser. *BRCA1* merupakan gen yang paling kerap dikenal pasti (40.5%), diikuti *BRCA2* (36.5%). Varian P/LP bukan *BRCA* menyumbang 23.0% daripada dapatan positif, dengan *PALB2* (5.4%), *ATM* (4.1%), dan *TP53* (3.4%) paling lazim dikenal pasti. Selepas analisis multivariat, kanser payudara tiga negatif (*triple-negative breast cancer, TNBC*), kanser payudara dua belah, dan sejarah keluarga kanser positif kekal sebagai peramal bebas bagi pengesanan varian titisan germa. Kadar pengesanan varian P/LP ialah 23.9% bagi ujian *BRCA* sahaja dan 19.1% bagi ujian panel pelbagai gen. Pengesanan varian yang tidak diketahui kepentingan klinikal (*variant of uncertain significance, VUS*) meningkat dengan saiz panel lebih besar, menekankan kepentingan interpretasi varian yang teliti dan kaunseling genetik. Dapatan kajian ini menyokong EOBC sebagai petunjuk klinikal penting untuk ujian genetik titisan germa serta membantu penilaian risiko kanser keturunan, pemilihan pesakit dan kaunseling genetik yang lebih sesuai.

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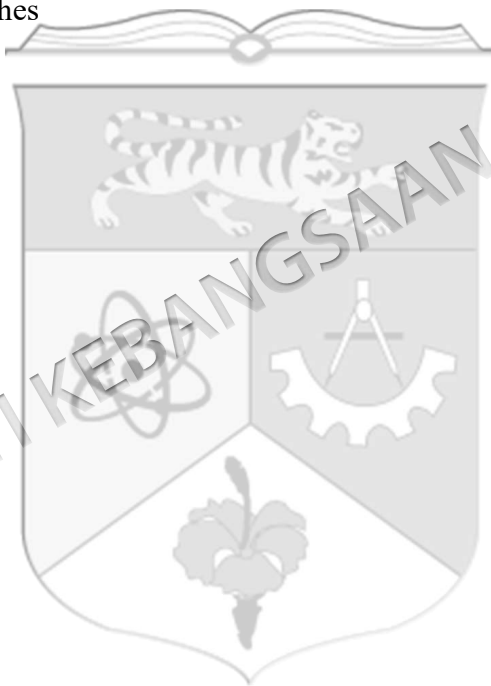
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LIST OF ABBREVIATIONS

ACMG	American College of Medical Genetics and Genomics
AJCC	American Joint Committee on Cancer
BC	Breast cancer
CI	Confidence interval
CPG	Clinical practice guideline
CRF	Case report form
CRM	Cancer Research Malaysia
EOBC	Early-onset breast cancer
ER	Oestrogen receptor
FISH	Fluorescence in situ hybridisation
HBOC	Hereditary breast and ovarian cancer
HER2	Human epidermal growth factor receptor 2
HKL	Hospital Kuala Lumpur
HPE	Histopathological examination
HR	Hormone receptor
HTABTAR	Hospital Tunku Ampuan Besar Tuanku Aishah Rohani
IHC	Immunohistochemistry
JEPUKM	Jawatankuasa Etika Penyelidikan Universiti Kebangsaan Malaysia
MNCRR	Malaysia National Cancer Registry Report
MOH	Ministry of Health
MREC	Medical Research and Ethics Committee
MRN	Medical record number
NGS	Next-generation sequencing
NMRR	National Medical Research Register
OR	Odds ratio
P/LP	Pathogenic/likely pathogenic
PR	Progesterone receptor

SPSS	Statistical Package for the Social Sciences
TAT	Turnaround time
TNBC	Triple-negative breast cancer
TNM	Tumour, node, metastasis
TPBC	Triple-positive breast cancer
VUS	Variant of uncertain significance



CHAPTER I

INTRODUCTION

1.1 Research Background

1.1.1 Breast Cancer and Early-Onset Breast Cancer

Breast cancer (BC) is the most diagnosed cancer among women worldwide and remains one of the leading causes of cancer-related mortality (Sung et al. 2021). In Malaysia, BC continues to represent a major public health burden. According to the Malaysia National Cancer Registry Report (MNCRR) 2017–2021, BC was the most common malignancy among Malaysian women, accounting for 32.4% of all female cancer cases. BC in Malaysia is also characterised by a relatively younger age distribution, with previous national registry data reporting that more than half of cases occurred among women below 50 years old, including 16.8% diagnosed before 40 years of age (Yip, Taib & Mohamed 2006). This highlights the clinical importance of early-onset breast cancer (EOBC) in the Malaysian population.

EOBC is generally defined as BC diagnosed at a young age and is commonly recognised as a high-risk group for hereditary cancer predisposition. In this study, EOBC refers to BC diagnosed at or before the age of 45 years, in line with the Malaysian Clinical Practice Guidelines (CPG), where diagnosis at this age is considered an indication for germline genetic testing (Ministry of Health 2021). Compared with BC diagnosed at older ages, EOBC is more frequently associated with inherited pathogenic or likely pathogenic (P/LP) variants in cancer predisposition genes. Therefore, identifying hereditary cancer predisposition in this group is important for guiding clinical management, surveillance, and risk assessment for both patients and their family members.

1.1.2 Hereditary Breast Cancer and Cancer Predisposition Genes

BC is a heterogeneous disease resulting from a complex interplay of environmental, hormonal, and genetic factors. Although most BC cases are sporadic, approximately 5-10% are hereditary (Hu et al. 2021; Oliveira et al. 2024), caused by inherited P/LP variants in cancer predisposition genes. These inherited variants may increase an individual's susceptibility to breast and other associated cancers.

BRCA1 and *BRCA2* are the most well-established genes associated with hereditary breast and ovarian cancer (HBOC) syndrome and are known to substantially increase the lifetime risk of both breast and ovarian cancers. In recent years, advances in genomic technologies have expanded the understanding of hereditary BC beyond *BRCA1* and *BRCA2*. Other cancer predisposition genes, including *PALB2*, *TP53*, *ATM*, *CHEK2*, *BARD1*, *RAD51C*, and *RAD51D*, have also been associated with increased BC risk. Some of these genes are considered high-penetrance genes, while others confer moderate risk or are associated with specific tumour subtypes (Dorling et al. 2021). This broader genetic landscape highlights the importance of evaluating not only *BRCA1/2* but also non-*BRCA* cancer predisposition genes, particularly among high-risk groups such as EOBC patients.

Identification of germline P/LP variants in cancer predisposition genes may influence clinical management through personalised surveillance, risk-reducing interventions, treatment selection, and family-based risk assessment. Furthermore, identification of a P/LP variant enables cascade testing, allowing at-risk relatives to undergo targeted assessment and supporting earlier implementation of preventive strategies.

1.1.3 Clinicopathological Characteristics and Germline Pathogenic Variants

Certain clinical and pathological characteristics may suggest an underlying hereditary cancer predisposition. These include young age at diagnosis, triple-negative breast cancer (TNBC), bilateral BC, high tumour grade, personal history of other cancers, and family history of breast, ovarian, pancreatic, prostate, or other hereditary cancer-related malignancies. These features are commonly considered during genetic risk

assessment and may guide referral for germline genetic testing (Akbar et al. 2022; Ow et al. 2019; Sandoval et al. 2021; Tung et al. 2016).

However, while associations between clinicopathological characteristics and germline P/LP variants have been reported across global populations, evidence from Asian cohorts remains relatively limited, and these relationships are still less well characterised among Malaysian EOBC patients. Malaysia's multi-ethnic population provides an important setting to explore whether the distribution of germline P/LP variants and their associations with clinicopathological features are consistent with those reported in other populations. Understanding these associations may improve patient selection for genetic testing and support more accurate risk assessment in clinical practice.

1.1.4 Germline Genetic Testing and Diagnostic Yield

In hereditary BC assessment, germline genetic testing refers to the analysis of an individual's DNA to identify inherited variants in cancer predisposition genes. Testing approaches vary according to clinical indication, institutional capacity, test availability, and patient affordability. Historically, *BRCA1/2* testing was the main approach used due to the strong evidence supporting its clinical actionability. However, advances in next-generation sequencing (NGS) have led to the wider use of multigene panel testing, which enables simultaneous analysis of multiple cancer predisposition genes.

In Malaysia, access to germline genetic testing differs across healthcare settings. Public hospitals may offer government-funded *BRCA1/2* testing or self-pay multigene testing, while broader multigene panel testing may be more accessible through self-funded testing, private healthcare services, or research-based settings.

Diagnostic yield refers to the proportion of tested individuals who are found to carry P/LP germline variants. In hereditary BC assessment, diagnostic yield is an important measure for evaluating the effectiveness of different genetic testing strategies, including *BRCA*-only testing and multigene panel testing. This is particularly relevant in resource-limited settings, where the balance between test

comprehensiveness, cost, accessibility, and clinical usefulness must be carefully considered. Therefore, local evidence is needed to better understand the diagnostic yield of different germline testing approaches among Malaysian EOBC patients.

1.2 Problem Statement

Despite the recognised association between EOBC and hereditary cancer predisposition, the implementation of comprehensive germline genetic testing in this group of patients in Malaysia remains limited. In public healthcare settings, government-funded germline genetic testing is largely limited to *BRCA1/2* analysis, while multigene panel testing is paid out of pocket. This may result in the under-detection of clinically relevant P/LP variants in non-*BRCA* cancer predisposition genes, particularly among patients with financial constraints.

In addition, the full spectrum of germline P/LP variants among Malaysian EOBC patients remains incompletely understood. Limited local evidence has focused primarily on *BRCA1/2*, while the contribution of other cancer predisposition genes and their associated clinical characteristics remain less well characterised. The diagnostic yield of different genetic testing approaches, including *BRCA*-only testing and multigene panel testing, also remains poorly characterised in Malaysian clinical practice. This limits the ability to optimise genetic testing strategies for EOBC patients within the local healthcare setting.

Collectively, these limitations may contribute to underdiagnosis of hereditary BC, delayed identification of at-risk individuals, and suboptimal implementation of personalised risk management. Therefore, this multicentre retrospective study was conducted to characterise the spectrum of germline P/LP variants, their clinicopathological associations, and the diagnostic yield of different genetic testing approaches among EOBC patients in Malaysia.

1.3 Research Questions

This study aims to answer the following research questions:

- a. What is the spectrum of germline P/LP variants in cancer predisposition genes among EOBC patients in Malaysia?
- b. What clinical and pathological characteristics are associated with germline P/LP variants in this cohort?
- c. What is the diagnostic yield of different germline genetic testing approaches among EOBC patients in Malaysia?

1.4 Research Objectives

1.4.1 General Objectives

To characterize the spectrum and clinicopathological association of germline P/LP variants in cancer predisposition genes among EOBC patients in Malaysia.

1.4.2 Specific Objectives

- a. To describe the spectrum of germline P/LP variants in cancer predisposition genes.
- b. To evaluate the association between clinicopathological features and germline P/LP variants.
- c. To compare the diagnostic yield of *BRCA*-only testing and multigene panel testing for identifying germline P/LP variants.

1.5 Research Hypothesis

- a. Germline P/LP variants are present in a diverse range of cancer predisposition genes among EOBC patients in Malaysia.
- b. Certain clinical and pathological features, such as TNBC, bilateral BC, family history of cancer, and high tumour grade, are associated with germline P/LP variants among EOBC patients.
- c. Broader genetic testing approaches are expected to identify additional P/LP variants beyond *BRCA1/2* compared with limited gene testing.

1.6 Significance of Study

This study aims to address important gaps in the understanding of hereditary BC among Malaysian EOBC patients, particularly regarding the contribution of non-*BRCA* cancer predisposition genes. By characterising the spectrum of germline P/LP variants, this study will provide locally relevant data on the genetic landscape of hereditary BC in this high-risk group.

From a clinical perspective, evaluating the association between germline P/LP variants and clinicopathological characteristics may improve risk assessment and patient selection for genetic testing. Furthermore, comparing the diagnostic yield of different testing approaches may provide evidence to guide genetic testing strategies within local healthcare settings.

From a genetic counselling perspective, identification of germline P/LP variants in EOBC patients has important implications for personalised risk management and family-based care, including surveillance, risk-reducing strategies and cascade testing for at-risk relatives.

Overall, this study may contribute to strengthening hereditary BC risk assessment, genetic counselling practice, and the implementation of germline genetic testing among EOBC patients in Malaysia.

CHAPTER II

LITERATURE REVIEW

2.1 Introduction

This chapter reviews the literature relevant to germline genetic testing in EOBC. It begins by defining EOBC and outlining its clinical significance, particularly in relation to its clinicopathological features, psychosocial implications, and relevance to hereditary cancer assessment. The chapter then discusses hereditary BC and the major germline cancer predisposition genes associated with BC risk, including both *BRCA1/2* and non-*BRCA* genes.

The review subsequently examines the reported spectrum and prevalence of germline P/LP variants in BC across global, Asian, Southeast Asian and Malaysian studies. This is followed by a discussion of clinicopathological factors associated with germline P/LP variants, including demographic, clinical, family history, and tumour-related factors. Germline genetic testing approaches are then reviewed, with a focus on *BRCA*-only testing, multigeneration panel testing, and diagnostic yield.

Finally, the chapter discusses a variant of uncertain significance (VUS), including their clinical and counselling implications and their relationship with panel size. The chapter concludes by identifying the research gaps in the Malaysian EOBC context and summarising the rationale for the present study.

2.2 Early-Onset Breast Cancer

EOBC is an important subgroup of BC because it differs from later-onset disease in terms of clinical presentation, psychosocial impact and hereditary cancer relevance.

This section defines EOBC, discuss the variation in age cut-offs used across studies and guidelines, and outlines its clinical significance.

2.2.1 Definition of Early-Onset Breast Cancer

EOBC generally refers to BC diagnosed at a young age. However, the age of which early-onset cases are defined differs between populations mainly due to the differences in the incidence age of each population. Different studies have used different thresholds, including diagnosis at or before 30, 35, 40 or 50 years of age (Apostolou et al. 2024; Bakhuizen et al. 2019; Lambertini et al. 2024; Shroff et al. 2023). These differences are usually related to the purpose of the study. For example, epidemiological studies may use a broader cut-off, such as below 50 years, to describe the burden of BC among younger women, while studies focusing on hereditary cancer risk may use a younger cut-off to identify patients who are more likely to carry germline pathogenic variants.

The age cut-off used is important because it can affect the characteristics of the study population and the reported diagnostic yield of genetic testing. Patients diagnosed at a very young age may have a higher possibility of an underlying hereditary cancer syndrome. For example, BC diagnosed before the age of 31 years is one of the indications for considering germline *TP53* testing in the context of Li-Fraumeni syndrome (Bakhuizen et al. 2019). In Asian studies, age cut-offs such as 35 or 40 years have also been commonly used to define EOBC in hereditary BC research (Lee et al. 2012; Toh et al. 2008). Therefore, when comparing findings across different studies, the definition of EOBC used in each study should be taken into account.

In clinical practice, age at diagnosis is also used as an important criterion for referral for genetic risk assessment and germline testing. However, the recommended threshold differs between guidelines. The NCCN uses BC diagnosed at age 50 years or younger as one of the criteria for germline testing, whereas the Malaysian CPG recommend testing for patients diagnosed with BC at age 45 years or younger.

Therefore, this study defines EOBC as BC diagnosed at age 45 years or below, as this reflects the Malaysian clinical context and is consistent with local testing criteria.

2.2.2 Epidemiology and Clinical Significance of EOBC

EOBC is clinically important because it affects patients at an earlier stage of life. Many patients diagnosed with BC at a young age may still be planning their family, raising children, building their career, or managing major financial responsibilities. As a result, the diagnosis may create additional concerns related to fertility, pregnancy, body image, employment, family roles and long-term survivorship. EOBC should therefore be viewed as a subgroup with distinct clinical and psychosocial implications, rather than simply as BC occurring at a younger age.

Several studies have reported that BC in younger patients tends to present with more aggressive clinicopathological features. Partridge et al. (2016) found that women diagnosed at age 40 years or younger were more likely to have higher-stage and higher-grade disease, as well as Luminal B, TNBC and HER2-positive subtypes. Similarly, Akakpo et al. (2023) reported that EOBC was associated with lower hormone receptor (HR)-positive status, higher TNBC frequency, larger tumour size, increased lymph node involvement and higher Ki-67 index, while Dufour et al. (2023) observed a higher proportion of aggressive molecular subtypes among very young BC patients. These less favourable tumour characteristics may contribute to poorer outcomes among younger patients. In a systematic review and meta-analysis, Basmadjian et al. (2023) reported that the pooled risk of any recurrence was significantly higher among EOBC patients compared with later-onset patients. However, outcomes in younger patients are not determined by age alone, but are also influenced by tumour stage, molecular subtype, treatment received and access to care.

EOBC is particularly relevant in Asian populations, where BC tends to occur at a younger age compared with Western populations (Kim et al. 2024). In Malaysia, previous studies have reported that a substantial proportion of BC cases occur among women below 50 years of age (National Cancer Institute Malaysia 2024). This suggests that BC among younger women is an important clinical and public health

issue in the Malaysian setting and could possibly be more carriers of inherited P/LP variants.

2.3 Hereditary Breast Cancer and Germline Cancer Predisposition

As a young age at diagnosis is an important indicator for hereditary cancer assessment, understanding the genetic basis of hereditary BC is essential. This section reviews the concept of hereditary BC, its inheritance pattern, and the main BC predisposition genes relevant to germline testing.

2.3.1 Overview of Hereditary Breast Cancer

Hereditary BC arises from inherited germline P/LP variants in cancer predisposition genes. Although most BC are sporadic, hereditary BC is estimated to account for approximately 5–10% of all BC cases (Hu et al. 2021; Momozawa et al. 2018; Oliveira et al. 2024; Tung et al. 2016). Hereditary BC is commonly suspected based on clinical and family history features. These include early age at diagnosis, bilateral BC, multiple primary cancers, male BC, and a family history of breast, ovarian or other HBOC-related cancers (Apostolou et al. 2024; Sandoval et al. 2021). Tumour characteristics may also provide additional clues, particularly TNBC and high-grade disease, which are frequently associated with *BRCA1*-related BC (Öfverholm et al. 2023; Yang et al. 2017). However, hereditary cancer risk assessment should not rely on a single feature alone, as germline P/LP variants may also be identified in patients without strong family history or classic clinical indicators.

Most hereditary BC predisposition syndromes follow an autosomal dominant pattern of inheritance. Therefore, when a germline P/LP variant is identified in an affected individual, each first-degree relative has a 50% chance of carrying the same familial variant. Nevertheless, carrying a P/LP variant does not mean that cancer will develop, as cancer risk depends on penetrance, the specific gene involved, variant type, family history and other modifying factors. This distinction is important in genetic counselling because patients and relatives need to understand the difference between inheriting a cancer predisposition and developing cancer.

2.3.2 Breast Cancer Predisposition Genes

BC predisposition genes can be broadly grouped according to their level of cancer risk, penetrance and clinical actionability. Traditionally, *BRCA1/2* have been the most established genes in hereditary BC. Both genes play important roles in homologous recombination repair, and P/LP variants in these genes are strongly associated with HBOC syndrome. *BRCA1*- and *BRCA2*-associated BC also show different clinicopathological patterns. *BRCA1*-associated cancers are more commonly diagnosed at younger ages, higher grade and are frequently associated with TNBC, whereas *BRCA2*-associated cancers are more often associated with HR-positive disease and may be relevant in male BC (Lambertini et al. 2024; Rodriguez-Hernandez et al. 2025; Yang et al. 2017). Due to their high cancer risk and established clinical management implications, *BRCA1/2* remain central to hereditary BC testing.

In Malaysian populations, *BRCA1/2* also account for a substantial proportion of detected hereditary BC predisposition. Yang et al. (2017) reported *BRCA1/2* prevalences of 2.8% and 3.2%, respectively, in an unselected Sarawak BC cohort, while Ng et al. (2016) found 12% *BRCA1/2* positivity among moderate-to-high-risk Malaysian patients. In a Malaysian early-onset cohort diagnosed at age 35 years or below, Lee et al. (2012) reported *BRCA1* and *BRCA2* variants in 11% and 6% of patients, respectively. These findings support the continued importance of *BRCA1/2* testing in Malaysian BC patients, particularly in high-risk and early-onset groups.

Beyond *BRCA1/2*, several non-*BRCA* high-penetrance genes have also been implicated in hereditary BC syndromes. Their associated syndromes and estimated lifetime BC risks are summarised in Table 2.1.

Table 2.1 Key cancer predisposition genes and their associated lifetime risks for breast cancer

Gene	Associated Syndrome	Lifetime risk for primary BC (%)
<i>BRCA1</i>	Hereditary Breast and Ovarian Syndrome	60 - 72
<i>BRCA2</i>	Hereditary Breast and Ovarian Syndrome	55 - 69
<i>TP53</i>	Li-Fraumeni Syndrome	> 60

<i>PTEN</i>	Cowden Syndrome	40 - 60
<i>CDH1</i>	Hereditary Diffuse Gastric Cancer	31 - 55
<i>STK11</i>	Peutz-Jeghers Syndrome	32 - 54
<i>PALB2</i>	-	32 - 53
<i>NF1</i>	Neurofibromatosis Type 1	20 - 40
<i>BARD1</i>	-	17 - 30
<i>CHEK2</i>	-	23 -27
<i>ATM</i>	-	21 - 24
<i>RAD51C/D</i>	-	~20

Note: Risk estimates are based on NCCN Guidelines Version 3.2026. Variations in risk may occur based on specific mutations and family history.

TP53 is associated with Li-Fraumeni syndrome, which is characterised by EOBC and a broad tumour spectrum including sarcoma, brain tumours and adrenocortical carcinoma. Several studies have reported *TP53* as one of the most frequently identified genes after *BRCA1/2*, particularly in cohorts involving high-risk or early-onset BC patients (Apostolou et al. 2024; Evans et al. 2022; Sandoval et al. 2021). *PTEN* is associated with *PTEN* hamartoma tumour syndrome, while *CDH1* is associated with hereditary diffuse gastric cancer and lobular BC. *STK11* is associated with Peutz-Jeghers syndrome, which also includes elevated BC risk. Although these genes are less frequently detected than *BRCA1/2*, they are clinically important because positive findings may lead to syndrome-specific surveillance and risk-reducing recommendations.

PALB2 has emerged as an important non-*BRCA* BC predisposition gene. As a partner and localiser of *BRCA2*, *PALB2* is involved in homologous recombination repair and has been consistently associated with increased BC risk. In Malaysian and regional data, *PALB2* variants appear to contribute a smaller but clinically relevant proportion of BC predisposition. For example, Yang et al. (2017) detected *PALB2* pathogenic variants in 0.86% of unselected Sarawak patients, while Phuah et al. (2013) reported deleterious *PALB2* variants among familial BC cases from Malaysia and Singapore. The identification of *PALB2* is clinically relevant because it may influence surveillance, risk-reducing discussions and cascade testing.

Moderate-penetrance genes, including *ATM*, *CHEK2*, *BARD1*, *RAD51C* and *RAD51D*, are increasingly identified through multigene panel testing. These genes are generally associated with lower BC risks than *BRCA1/2* or *TP53*, but their findings may still be clinically meaningful when interpreted together with personal and family history. Dorling et al. (2021) showed that protein-truncating variants in several genes, including *ATM*, *CHEK2*, *BARD1*, *RAD51C* and *RAD51D*, were associated with BC risk. Some of these genes may also show subtype-specific associations, such as stronger associations between *CHEK2* and ER-positive disease, or between *BARD1/RAD51D* and TNBC or ER-negative BC (Dorling et al., 2021).

Population-specific evidence remains important because the frequency and clinical significance of moderate-risk genes may differ across populations. For example, Mohamad et al. (2015) concluded that *CHEK2* germline mutations are rare and may play only a minor role in Malaysian BC susceptibility, whereas other international studies have reported *CHEK2* as the third most frequently identified gene after *BRCA1/2* (Rodriguez-Hernandez et al. 2025; Tung et al. 2016). Overall, BC predisposition genes represent a spectrum ranging from highly actionable genes, such as *BRCA1/2*, to moderate-risk genes with more variable management implications. Understanding this broader gene spectrum is increasingly relevant in the era of multigene panel testing.

2.4 Frequency and Spectrum of Germline P/LP Variants in Breast Cancer

The prevalence and distribution of germline P/LP variants in BC vary across populations and study settings. This section reviews the reported spectrum of germline variants in global, Asian, Southeast Asian and Malaysian studies, providing the background for interpreting findings in Malaysian EOBC patients.

2.4.1 Frequency and Spectrum of Germline Variants in EOBC

The reported prevalence of germline P/LP variants in BC varies across studies. This variation is influenced by the characteristics of the study population, including age at diagnosis, family history, bilaterality, tumour subtype, cancer spectrum, referral criteria and the number of genes included in the testing panel. Therefore, findings

from general BC cohorts, suspected hereditary cancer cohorts, bilateral BC cohorts and EOBC cohorts should be interpreted according to their clinical context.

In general, or clinically selected BC cohorts, multigene panel testing has reported P/LP variant yields ranging from approximately 10% to 20%. Tung et al. (2016) reported a P/LP yield of 10.7% among women with stage I–III BC tested using a 25-gene panel. Similarly, Rodriguez-Hernandez et al. (2025) reported a yield of 14.4% in a single-centre invasive BC cohort. In Brazilian BC cohorts, Bandeira et al. (2021) and Guindalini et al. (2022) also reported clinically relevant detection rates of germline P/LP variants using multigene approaches, approximately 20%. Öfverholm et al. (2023) reported a yield of 16.6% among individuals with breast or ovarian cancer, while Sandoval et al. (2021) reported a slightly higher yield of 20.5% among BC patients. These findings show that germline P/LP variants are detected in a clinically meaningful proportion of BC patients, particularly when testing is offered to individuals with high-risk clinical or family history features.

Higher yields have been reported in more specific high-risk subgroups. Fanale et al. (2020) reported a P/LP yield of 37.4% among patients with bilateral BC, supporting bilateral disease as an important indicator of hereditary predisposition. Diagnostic yield also appears to be influenced by the age threshold used to define EOBC. Studies involving very young patients (≤ 30 years) have reported higher detection rates, with Evans et al. (2022) reporting a yield of 38.3% and Apostolou et al. (2024) reporting a yield of 39.7% in young-onset BC patients. In contrast, studies using a broader EOBC definition, such as diagnosis ≤ 50 years, reported lower yields of 15.1% and 10.1% in Shroff et al. (2023) and Tashjian et al. (2025) respectively. These findings suggest that diagnostic yield is generally higher in more clinically selected groups, particularly very young-onset and bilateral BC patients, whereas broader EOBC definitions may include a more heterogeneous population with lower overall yield.

Overall, global evidence shows that the spectrum and prevalence of germline P/LP variants are not uniform across BC populations. Diagnostic yield is generally higher in patients with strong hereditary cancer indicators, such as very young age at

diagnosis, bilateral BC, family history of breast or ovarian cancer, or referral for suspected hereditary cancer syndrome. These findings provide an important background for interpreting germline testing results among Malaysian EOBC patients.

2.4.2 Asian and Southeast Asian Data

Evidence from Asian and Southeast Asian studies shows that germline P/LP variants are detected in a clinically significant proportion of BC patients referred for hereditary cancer assessment. Similar to global studies, the reported diagnostic yield varies widely across Asian cohorts, ranging from around 13% in larger HBOC-related cohorts to more than 40% in highly selected referred populations (Kansuttivivat et al. 2024; Lertwilaiwittaya et al. 2021; Ow, Ong & Lee 2019; Pramanik et al. 2024; Wong et al. 2016). This variation appears to be influenced by differences in referral criteria, age distribution, family history, ethnic background and the number of genes included in the testing panel.

Southeast Asian data are particularly relevant to the Malaysian context. Studies from Thailand and Singapore have shown that germline testing is useful in HBOC-related populations, but the reported yield differs depending on whether the cohort included only BC patients or a broader group of HBOC-related cancers such as ovarian, pancreatic and prostate cancer. The Singaporean multiracial cohorts are especially relevant because they include Asian ethnic groups that overlap with the Malaysian population, including Chinese, Malay and Indian individuals (Ow, Ong & Lee 2019; Wong et al. 2016). These studies support the importance of considering ethnic diversity when interpreting germline testing results in Southeast Asian populations.

When compared with European studies, Asian and Southeast Asian cohorts show a broadly similar pattern in which *BRCA1* and *BRCA2* are the dominant genes, while non-*BRCA* genes provide additional diagnostic yield through multigene testing. However, the absolute yield differs across studies and cannot be attributed to geography alone. Differences in clinical selection, age criteria, tumour characteristics, family history, cancer spectrum and panel size are likely to explain much of the

variation between studies. Therefore, although Asian and Southeast Asian studies provide useful regional evidence, their findings may not fully represent the Malaysian EOBC population.

Overall, the available Asian and Southeast Asian literature supports the clinical value of germline testing in hereditary BC assessment. However, the evidence remains heterogeneous, with differences in study design, testing criteria, ethnic composition and genes analysed. This highlights the need for Malaysian-specific data to better describe the local germline P/LP variant spectrum, particularly among patients with EOBC.

2.4.3 Malaysian Evidence and Local Knowledge Gap

Malaysian studies have provided important early evidence on hereditary BC, particularly in relation to *BRCA1* and *BRCA2*. Earlier local studies were mainly focused on *BRCA1/2* testing and often involved selected high-risk patients. For example, Toh et al. (2008) identified one P/LP *BRCA1* variant (2.7%) and two P/LP *BRCA2* variants (5.4%) among 37 early-onset patients without family history. Lee et al. (2012) later found *BRCA1* in 11%, *BRCA2* in 6%, and clinically relevant *TP53* P/LP variants in approximately 5% of 100 early-onset cases (≤ 35 years), and recommended consideration of *TP53* testing for early-onset patients. These studies showed that germline P/LP variants are present among young Malaysian BC patients, although the number of genes tested was limited compared with current multigene panel approaches.

Subsequent Malaysian studies expanded the local evidence beyond *BRCA1/2*. Yang et al. (2017) reported a P/LP prevalence of 7.2% in a Sarawak BC cohort, including *BRCA1* (2.8%), *BRCA2* (3.23%), *PALB2* (0.86%) and *TP53* (n=2), with *BRCA1* carriers more likely to present at a younger age and with TNBC. Ng et al. (2016) applied targeted NGS in moderate-to-high-risk Malaysian BC patients and reported P/LP variants in 19% of patients, with *BRCA1/2* comprising the majority and non-*BRCA* genes contributing 6%. Mohamad et al. (2015) reported *CHEK2* variants at very low frequencies across Malay, Chinese, and Indian cases, with uncertain

pathogenicity. In addition, Phuah et al. (2013) reported a low prevalence of P/LP *PALB2* mutations in multi-ethnic Malaysian and Singaporean samples.

The multi-ethnic composition of Malaysia is an important consideration in interpreting local germline data. Malaysia consists mainly of Malay, Chinese and Indian populations, and differences in genetic background may influence the distribution of variants. Some studies have explored ethnic-specific patterns, but the evidence remains insufficient to draw strong conclusions. Amini et al. (2021) noted that existing Malaysian studies often involve limited samples and overlapping patient sources, which reduces the ability to make robust conclusions about ethnic-specific prevalence.

Several methodological limitations remain in the Malaysian literature. Many primary studies were single-centre, small in scale, or based on selected high-risk groups, which limits generalisability to the wider Malaysian BC population (Lee et al. 2012; Mohamad et al. 2015; Toh et al. 2008). Gene coverage varied across studies, with some focusing only on *BRCA1/2* or selected genes, while others used broader targeted panels (Ng et al. 2016; Yang et al. 2017). Amini et al. (2021) highlighted that the Malaysian literature remains scarce and heterogeneous, with many novel or uncertain variants and a need for larger, more representative multicentre studies.

2.5 Clinicopathological Associations with Germline P/LP Variants

Clinicopathological factors are important in hereditary BC assessment because they may help identify patients who are more likely to carry germline P/LP variants. These factors include demographic characteristics, personal and family cancer history, bilaterality and tumour features. However, the strength and consistency of these associations may vary across populations, testing criteria and study designs.

2.5.1 Demographic and Clinical Factors

Age at diagnosis is one of the most consistently reported clinical factors associated with germline P/LP variants in BC. Younger patients, particularly those diagnosed at or below 30 years, have a higher likelihood of carrying germline variants compared

with patients diagnosed at older ages (Apostolou et al. 2024; Evans et al. 2022). Daly et al. (2023) reported that young age at BC diagnosis and family history are strongly associated with a higher prevalence of *BRCA1/2* pathogenic variants. In Malaysian cohorts, *BRCA1/2* variants have also been reported among women diagnosed at young ages, including early-onset cases without a strong family history, highlighting the relevance of age-based testing criteria in the local context (Lee et al. 2012; Toh et al. 2008).

Nevertheless, age alone is unlikely to fully predict germline status, particularly in studies in which the entire cohort is already selected for young age at diagnosis. In such populations, the restricted age range may reduce the ability to detect age-related differences between carriers and non-carriers. Therefore, age should be interpreted together with other clinical and pathological features, including family history, bilateral disease, and tumour subtype.

Ethnicity and population background may influence not only the overall prevalence of germline P/LP variants, but also the distribution of specific BC predisposition genes. Globally, large multigene testing cohorts have shown ancestry-related differences, with Yadav et al. (2020) reporting higher *BRCA1/2* P/LP variant frequencies among Ashkenazi Jewish and Hispanic individuals, while *CHEK2* variants were less common among Black, Hispanic and Asian women. Similar differences in P/LP variant detection rates across racial and ethnic groups were also reported by Beitsch et al. (2023).

Several Asian studies have reported patterns that may differ from Western cohorts. In Chinese BC cohorts, *BRCA2* variants have been reported to be more frequent than *BRCA1* variants, contrasting with some Western high-risk cohorts where *BRCA1* is often more prominent (Wang et al. 2018; Wu et al. 2025). In Malaysia, the multi-ethnic population provides an important context for studying hereditary BC. Local studies have reported *BRCA1/2* variants among Malay, Chinese, Indian, and indigenous populations, with some evidence suggesting population-specific mutation spectra (Wen et al. 2018; Yang et al. 2017). However, local data remain limited, particularly for non-*BRCA* genes and early-onset cohorts.

2.5.2 Personal and Family History

Family history remains a central component of hereditary BC risk assessment. A family history of breast, ovarian, pancreatic, prostate, or other hereditary cancer-related malignancies may increase the likelihood of detecting germline P/LP variants. Several studies have reported that *BRCA1/2* carriers are more likely to have a family history of breast and/or ovarian cancer compared with non-carriers (Wen et al. 2018; Wong et al. 2015). In Chinese Hakka BC patients, family history of cancer was identified as an independent predictor of *BRCA1/2* P/LP variants (Zhang et al. 2024). However, the predictive value of family history is not absolute. Germline P/LP variants can also be identified in patients without a known family history of breast or ovarian cancer (Toh et al. 2008; Wang et al. 2018). This may be explained by small family size, limited knowledge of relatives' diagnoses, paternal inheritance, reduced penetrance, early deaths in the family, or underreporting of cancer history. In some studies, family history was associated with P/LP variants in univariate analysis but lost significance in multivariable models after adjustment for other factors (Wu et al. 2025). Therefore, while family history remains clinically important, reliance on family history alone may miss some individuals with hereditary cancer predisposition, especially among EOBC patients.

Bilateral BC is another important clinical indicator of hereditary predisposition. Several studies have shown that patients with bilateral BC have a higher likelihood of carrying germline P/LP variants compared with those with unilateral disease (Rodriguez-Hernandez et al. 2025; Sandoval et al. 2021). In Chinese BC cohorts, bilateral BC has been identified as a significant predictor of germline mutations, including *BRCA1/2* P/LP variants (Wang et al. 2018; Wu et al. 2025; Zhang et al. 2024). This association is biologically plausible, as inherited defects in DNA repair pathways may increase the risk of developing multiple primary BC.

Personal history of other primary cancers may also suggest an underlying hereditary cancer syndrome, although the evidence is less consistent than for family history or bilateral BC. In a comprehensive genomic profiling study, a personal history of other cancers, defined as a second primary tumour, was not significantly

associated with germline P/LP variants (Wu et al. 2025). However, specific personal cancer histories may still be clinically relevant. Ow, Ong & Lee (2019) reported that a personal history of ovarian cancer and the presence of more than one primary cancer were associated with increased likelihood of hereditary cancer predisposition. Similarly, Lambertini et al. (2024) reported that *BRCA1* carriers had an increased risk of second primary BC and non-breast new primary malignancies.

These findings suggest that personal cancer history should be interpreted according to the cancer type, age at diagnosis, and number of primary cancers, rather than as a single broad variable. In particular, ovarian cancer is strongly relevant to HBOC syndrome, while cancers such as sarcoma, brain tumours, thyroid, endometrial or diffuse gastric cancer may raise suspicion for other hereditary syndromes involving genes such as *TP53*, *PTEN* or *CDH1*. The limited evidence on personal cancer history beyond breast and ovarian cancer also highlights the need for more comprehensive studies examining the full cancer spectrum among germline variant carriers.

2.5.3 Tumour Characteristic

Tumour characteristics may provide additional clues to hereditary BC predisposition. Among these, HR status and molecular subtype are particularly important. *BRCA1*-associated BC are commonly associated with TNBC phenotypes (Apostolou et al. 2024; Mavaddat et al. 2022). In contrast, *BRCA2*-associated BC are more often HR-positive and may resemble sporadic BC in their receptor profile (Akita et al. 2025.; Chen et al. 2020; Rodriguez-Hernandez et al. 2025). These gene-specific tumour patterns may assist clinicians in identifying patients who are more likely to carry particular germline variants. Studies from Asian populations have reported an association between TNBC and *BRCA1* variants, although the strength of this association may vary across cohorts (Liu et al. 2017; Rashid et al. 2016). This supports the use of TNBC as an important indication for genetic testing in clinical practice. However, the association is not uniform across all populations. Some studies have reported weaker or non-significant associations, possibly due to differences in sample size, selection criteria, ethnicity, and the range of genes tested (Oranratnachai

et al. 2022; Wang et al. 2018). Therefore, TNBC should be interpreted alongside other clinical and family history factors rather than as a standalone predictor.

Beyond *BRCA1/2*, emerging evidence suggests that non-*BRCA* BC predisposition genes may also be associated with particular tumour phenotypes. *TP53*-associated BC have been reported to occur at younger ages and may show HER2 positivity in a substantial proportion of cases (Narasimhamurthy et al. 2025). *CHEK2*-associated BC are commonly HR-positive and invasive ductal carcinomas of no special type (Schwartz et al. 2024). Similarly, *ATM*-associated BC have been reported to be predominantly oestrogen receptor (ER)-positive, with luminal subtypes frequently observed (Lanier et al. 2025; Ünsal et al. 2023). These findings suggest that while *BRCA1* is strongly associated with TNBC, several moderate-penetrance genes, such as *CHEK2* and *ATM*, may be more commonly linked with HR-positive disease. However, these associations are less consistently defined than *BRCA1/2*-related patterns and may vary according to population and study design.

Tumour grade has also been associated with germline P/LP variants. Across several studies, BC patients carrying germline P/LP variants were more likely to present with high-grade or biologically aggressive tumours compared with non-carriers (Eras et al. 2024; Öfverholm et al. 2023; Tashjian et al. 2025; Tung et al. 2016). Similarly, Lambertini et al. (2024) reported clinically relevant aggressive features among germline variant carriers, including increased risks of second primary BC and non-breast primary malignancies. These findings suggest that high tumour grade may serve as a supportive clinicopathological indicator of hereditary predisposition. However, tumour grade should not be interpreted in isolation, as its association with germline status may vary according to tumour subtype, age at diagnosis, family history, and the specific gene involved.

The relationship between tumour stage and germline variant status is less consistent. Some studies suggest that *BRCA* mutation carriers may present with more advanced disease or lymph node involvement, while others show no clear difference in stage distribution by mutation status (Akakpo et al. 2023; Rodriguez-Hernandez et al. 2025; Wang et al. 2018). These inconsistencies may reflect differences in screening

practices, access to care, age at diagnosis, and study population. Histological subtype is also less consistently associated with germline status, as invasive ductal carcinoma remains the most common histological subtype in both hereditary and sporadic BC (Apostolou et al. 2024). Overall, tumour subtype, HR status and grade appear to be more informative than stage or histology in predicting germline P/LP variants.

In summary, germline P/LP variants in BC are associated with several clinicopathological features, particularly younger age at diagnosis, family history of breast or ovarian cancer, bilateral BC, TNBC, and high tumour grade. However, these associations are not always consistent across populations and may differ according to ethnicity, testing criteria, panel composition, and study design. In the Malaysian EOBC context, evaluating these associations is important to determine whether predictors reported in international studies apply to local patients and to support more tailored genetic testing and counselling strategies.

2.6 Germline Genetic Testing Approaches and Diagnostic Yield

Germline genetic testing for hereditary BC has evolved from targeted *BRCA1/2* testing to broader multigene panel testing. Earlier testing strategies focused mainly on *BRCA1/2* because these genes account for a substantial proportion of HBOC and have well-established clinical actionability. However, the recognition of non-*BRCA* predisposition genes, together with the wider availability of NGS, has expanded the use of multigene panels in hereditary BC assessment.

2.6.1 *BRCA*-Only Testing and Multigene Panel Testing

BRCA-only testing remains clinically relevant because *BRCA1/2* variants have clear implications for treatment, surveillance, risk-reducing surgery and cascade testing. In settings where genetic testing resources are limited, *BRCA*-only testing may also be more accessible and cost-effective, particularly for patients with clinical features strongly suggestive of HBOC syndrome. However, this approach is limited by its inability to detect clinically relevant P/LP variants in non-*BRCA* genes.

Multigene panel testing addresses this limitation by analysing multiple cancer predisposition genes simultaneously. Several studies have shown that a proportion of clinically relevant findings occur outside *BRCA1/2*. For example, Fanale et al (2020) reported that 14.4% of P/LP variants in patients with bilateral BC would have been missed by *BRCA*-only testing. Similarly, Akbar et al. (2022) found that nearly half of patients with P/LP variants carried variants in genes outside *BRCA1/2*, supporting the added value of broader testing. Öfverholm et al. (2023) also demonstrated that expanding testing beyond *BRCA1/2* increased diagnostic yield by 7.7% in a suspected HBOC cohort. In addition, Crawford et al. (2017) identified P/LP variants in non-*BRCA* genes among high-risk women who had previously tested negative for *BRCA1/2*, suggesting that panel testing may be useful when *BRCA*-only testing is uninformative despite persistent clinical suspicion.

Despite this added value, multigene panel testing introduces interpretation challenges. Broader panels may increase the detection of VUS and may include genes with variable penetrance or less clearly established management recommendations. Therefore, the clinical utility of multigene panel testing depends not only on the number of genes tested but also on the actionability of the genes included, the quality of variant interpretation, and the availability of genetic counselling support.

2.6.2 Diagnostic Yield of Different Testing Approaches

In hereditary BC testing, diagnostic yield of identifying germline P/LP variants varies according to patient selection, age at diagnosis, family history, tumour subtype, ethnicity, testing setting and panel composition. Therefore, it should not be interpreted as a fixed measure, but as a context-dependent outcome.

The literature generally shows that multigene panel testing can improve diagnostic yield by identifying non-*BRCA* P/LP variants. Tung et al. (2016) identified deleterious variants in non-*BRCA* genes among consecutive BC patients undergoing multigene panel testing, while Evans et al. (2022) reported an additional diagnostic yield of 4.35% from extended panel testing following negative initial screening of *BRCA1/2*, *TP53* and *CHEK2*. These findings suggest that broader testing may be

particularly useful when the clinical phenotype is not specific to *BRCA1/2* or when *BRCA*-only testing is negative despite a strong suspicion of hereditary predisposition.

However, comparing the yield of *BRCA*-only testing and multigene panel testing is not always straightforward. *BRCA*-only testing may show a high diagnostic yield when offered to highly selected patients with strong hereditary features, such as very young age at diagnosis, TNBC, bilateral disease or strong family history. In contrast, multigene panel testing may be used in broader or more heterogeneous cohorts, which may reduce the overall percentage of positive findings despite detecting a wider range of genes. Therefore, differences in diagnostic yield must be interpreted in relation to testing eligibility, referral pathways and clinical selection criteria.

In resource-limited healthcare settings, this distinction is particularly important. Although multigene panel testing can detect variants beyond *BRCA1/2*, cost and access barriers may limit its routine use. Conversely, *BRCA*-only testing may be more feasible but may miss non-*BRCA* hereditary cancer predisposition. This creates a need for local evidence on the diagnostic yield of available testing approaches. In Malaysia, where access to genetic testing differs across public, private and research-based settings, evaluating the yield of *BRCA*-only testing and multigene panel testing among EOBC patients is important for informing practical and equitable testing strategies.

2.7 Variant of Uncertain Significance

VUS are genetic variants for which the available evidence is insufficient to determine whether they are pathogenic or benign. In hereditary BC testing, VUS are frequently encountered, particularly with the increasing use of multigene panel testing. Although VUS are not considered clinically actionable in the same way as P/LP variants, they remain important because they create uncertainty in result interpretation, genetic counselling, and patient understanding.

2.7.1 Clinical / Counselling Implications of VUS

The main clinical challenge of VUS is that they should not be used to guide major medical management decisions. Unlike P/LP variants, VUS do not provide sufficient evidence to confirm hereditary cancer predisposition. Therefore, risk-reducing surgery, intensified surveillance, or predictive testing for relatives should not be based on a VUS result alone. Instead, clinical management should continue to be guided by the patient's personal cancer history, family history, tumour characteristics, and existing clinical guidelines (Makhnoon et al. 2021).

From a genetic counselling perspective, VUS results can be difficult for patients to understand because the result is neither clearly positive nor clearly negative. Previous studies have shown that patients may experience uncertainty, anxiety or worry after receiving a VUS, and some may have difficulty understanding or recalling the meaning of the result (Amano et al. 2022; Makhnoon et al. 2021; Mighton et al. 2021). This uncertainty may be particularly challenging in EOBC patients, who may already have concerns about future cancer risk, treatment decisions, and implications for family members. Therefore, pre-test counselling should include discussion of the possibility of receiving a VUS result, while post-test counselling should clearly explain that a VUS is not equivalent to a positive result.

Another important issue is that VUS interpretation may change over time as more evidence becomes available. Some VUS may later be reclassified as benign or likely benign, while a smaller proportion may be upgraded to P/LP. Makhnoon et al. (2021) reported that 8.1% of individuals with VUS experienced reclassification, with most reclassified variants downgraded to benign or likely benign (87.1%) and a smaller proportion upgraded to P/LP (12.9%). The median time to reclassification also decreased over time, from 3.08 years in 2014 to 0.91 years in 2019. Although only a minority of reclassified VUS led to clinically actionable findings, some resulted in changes to clinical management, including intensified screening or prophylactic surgery. These findings highlight the importance of periodic review, laboratory updates and clear communication with patients regarding the limitations of current genetic knowledge. However, until reclassification occurs, a VUS should be managed

cautiously and should not be used as the basis for predictive testing in relatives or irreversible clinical decisions.

2.7.2 Panel Size and VUS Detection

The likelihood of detecting VUS increases as the number of genes included in a testing panel expands. This was demonstrated by Alem et al. (2025), who reported that a 144-gene panel identified a higher proportion of VUS compared with smaller 20- or 23-gene panels, with VUS rates of 56.3%, 23.9% and 31.0%, respectively. Similarly, Ow, Ong & Lee (2019) showed that while testing beyond *BRCA1/2* influenced clinical management in 15.9% of carriers, extensive panel testing also identified VUS in up to 44.5% of probands. Importantly, they also found that restricting testing to a guideline-based 20-gene panel, including Lynch syndrome genes, detected 94.6% of mutation carriers while reducing the VUS rate to 21.5%.

These findings highlight the balance between increasing diagnostic scope and minimising uncertain results. Although larger panels may identify additional clinically relevant variants, they also increase the burden of VUS, which may complicate counselling and result interpretation. This issue is particularly important in multigene panel testing for hereditary BC. While broader panels may improve the detection of non-*BRCA* P/LP variants, they may also increase the burden of uncertain findings. Therefore, the clinical value of expanding panel size should be balanced against the possibility of generating results that are difficult to interpret or not clinically actionable. This has direct implications for genetic counselling, as patients need to understand that a larger panel does not always mean a more useful result.

VUS rates may also differ across populations, with higher rates generally reported among non-European groups. This has been partly attributed to the underrepresentation of diverse ancestral populations in genetic databases, which limits the evidence available for variant classification. Caswell-Jin et al. (2018) reported a higher VUS rate among non-white patients compared with white patients undergoing hereditary cancer testing (36% vs. 27%), with the highest rate observed among Asian patients. Similarly, Ndugga-Kabuye & Issaka (2019) found that Asian and Pacific

Islander individuals had higher VUS rates than Europeans for HBOC/Lynch syndrome genes (16.6% vs. 6.1%) and other cancer genes (24.4% vs. 12.2%). In Asian cohorts, Wong et al. (2016) reported that 46.8% of participants had at least one VUS, while Togashi et al. (2025) reported a VUS rate of 41.7% in a Japanese BC cohort. These findings suggest that limited ancestry-specific and regional variant data may contribute to greater uncertainty in variant interpretation among Asian populations. This is relevant to Malaysia, where the multi-ethnic population remains underrepresented in many genetic databases. Therefore, Malaysian patients undergoing broader panel testing may have a higher likelihood of receiving uncertain results, particularly in less well-characterised non-*BRCA* genes.

Overall, VUS findings are an unavoidable part of hereditary BC genetic testing, particularly with larger multigene panels. Although VUS are not clinically actionable, they have important implications for counselling, patient understanding, and follow-up. The relationship between panel size and VUS detection is especially relevant in resource-limited and multi-ethnic settings, where broader testing must be balanced against interpretive uncertainty. Therefore, evaluating VUS patterns alongside diagnostic yield is important when assessing the clinical utility of different genetic testing approaches.

2.8 Research Gap

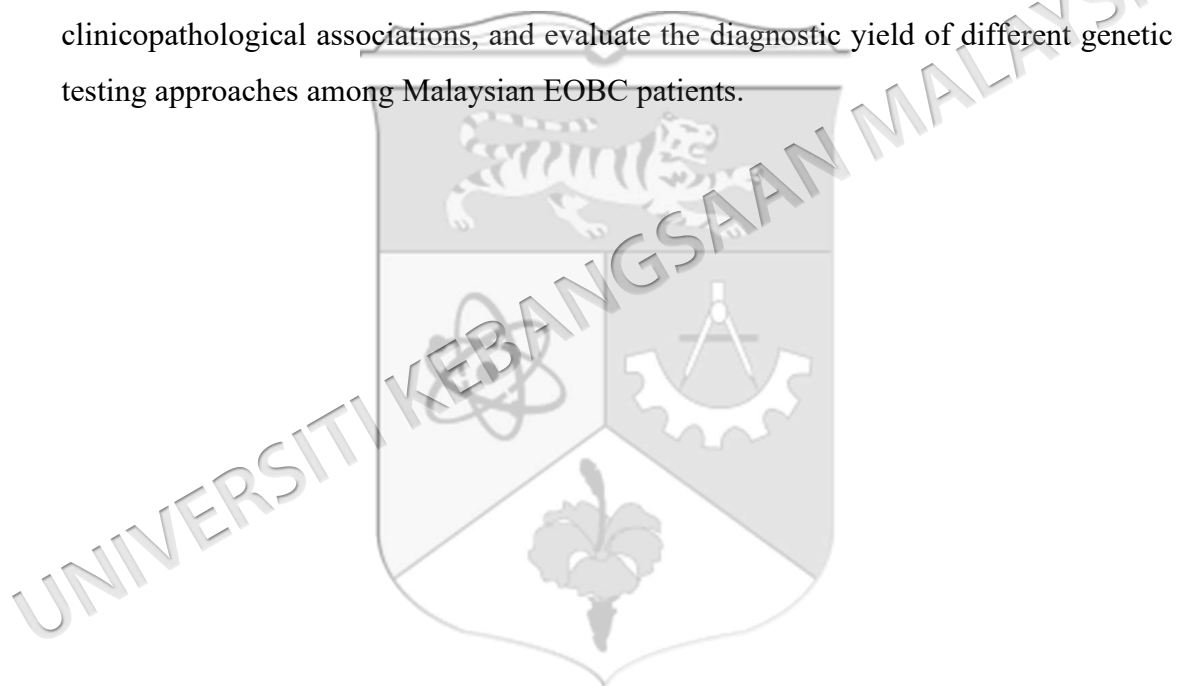
Despite growing evidence on hereditary BC, several gaps remain in the Malaysian context. First, existing local studies have mainly focused on *BRCA1/2* or selected cancer predisposition genes, often involving relatively small or single-centre cohorts. Consequently, the broader spectrum of germline P/LP variants identified through multigene panel testing particularly among patients with EOBC, remains insufficiently characterised.

Second, although numerous international studies have identified clinicopathological characteristics associated with germline P/LP variants, evidence from Malaysia remains limited. Existing local studies have largely described mutation

frequencies, with limited evaluation of the relationship between germline P/LP variants and clinicopathological factors.

Third, while multigene panel testing has become increasingly adopted in hereditary BC assessment, few Malaysian studies have directly evaluated its diagnostic performance relative to *BRCA*-only testing. Consequently, the additional diagnostic yield of multigene panel testing in detecting clinically actionable non-*BRCA* variants among Malaysian EOBC patients remains unclear.

Taken together, these gaps highlight the need for a comprehensive multicentre study to characterise the spectrum of germline P/LP variants, examine their clinicopathological associations, and evaluate the diagnostic yield of different genetic testing approaches among Malaysian EOBC patients.



CHAPTER III

METHODOLOGY

3.1 Study Design

This was a retrospective, cross-sectional study conducted across multiple centres in Malaysia. All clinical, pathological, molecular and genetic testing data were extracted from existing medical records and analysed.

3.2 Study Population and Sampling Strategy

3.2.1 Target Population

The target population consisted of all Malaysian BC patients diagnosed 45 years and below who underwent germline genetic testing for cancer predisposition genes between 2020 and 2025. Predictive testing or familial variant testing of asymptomatic clients was not included in this study.

3.2.2 Study Sites

Eligible participants were identified from three major types of healthcare institutions in Malaysia:

- Hospital Kuala Lumpur (HKL)
- Hospital Tunku Ampuan Besar Tuanku Aishah Rohani (HTABTAR)
- Cancer Research Malaysia (CRM)

These three sites were selected to ensure broad representation of the Malaysian population across different healthcare settings. HKL, a major public hospital, serves as

a central genetic referral centre in Malaysia. HTABTAR represents the university hospital setting. CRM is a non-profit research organisation with a wholly-owned subsidiary to provide genetic counselling and testing. It is a single-site referral centre with patients referred from both the private and public sector. Together, these sites encompass public, private-referral, and academic healthcare systems, providing a more comprehensive representation of the Malaysian population.

3.2.3 Sampling Method and Sampling Frame

This study applied purposive sampling to achieve a near-census of eligible EOBC patients who had undergone germline genetic testing at the participating centres within the study period. No formal sample-size calculation was performed; the sampling frame was based on the availability of existing, retrievable medical records and laboratory reports within the clinical and research databases of HKL, HTABTAR, and CRM. As the sampling frame relied on existing institutional records, the final sample size was determined by the number of patients who met the inclusion criteria, rather than by probability-based sampling. Patients identified in the records who did not meet the eligibility criteria were excluded from the analysis.

Inclusion Criteria

- Malaysian BC patient.
- Age at diagnosis between 18 and 45 years old.
- Completed germline genetic testing for cancer predisposition genes within the study period (2020–2025).
- Availability of more than 50% of the required clinical, pathological, and molecular data in the medical records.

Exclusion criteria

- Non-Malaysian BC patient.
- Age at diagnosis greater than 45 years.

- Availability of less than 50% of required clinical, pathological, and molecular data (i.e., insufficient information for meaningful analysis).

3.2.4 Study Size and Case Selection

A total of 725 patients met these criteria. For patients with bilateral or recurrent BC, eligibility was retained if the age at diagnosis of the first primary BC was 45 years or younger. To ensure consistency in clinicopathological association analysis, only the characteristics of the first primary BC were recorded and used in the analysis.

3.3 Data Collection

Clinical data were collected retrospectively by reviewing the medical records. Data were extracted using a standardised case report form (CRF) developed specifically for this multicentre study and piloted at one site (HTABTAR) to ensure clarity, consistency, and feasibility across institutions. All data were de-identified before transfer and analysis; unique anonymised study identifiers were assigned to each patient. Extracted data were entered into a centralised Google Sheet-based template standardised across sites, incorporating predefined dropdown lists to minimise spelling errors, ensure uniform variable coding and formatting, and harmonise variable definitions (e.g., ethnicity, tumour stage, and receptor status). Access to the database was restricted to authorised research team members, and regular data quality checks were performed throughout the data collection process. Data collection procedures differed slightly across centres depending on the availability and format of medical records.

3.3.1 Hospital Kuala Lumpur

At HKL, data were extracted from physical patient files in the Genetic Clinic as oncology records were not fully integrated into an electronic medical record system. More than 1,400 cancer genetics files were initially screened. Eligible cases were then filtered to identify BC patients diagnosed at ≤ 45 years who had completed germline genetic testing between 2020 and 2025. This yielded 283 potentially eligible EOBC patients; after data cleaning and verification, 262 were included in the final analysis.

3.3.2 Hospital Tunku Ampuan Besar Tuanku Aishah Rohani

At HTABTAR, cases were initially identified from a list provided by the centre's genetic counsellor, which included patients' medical record numbers (MRNs) and genetic testing status in 2025. To capture all eligible cases from 2020 to 2025, genetic testing records on the testing provider platform were retrospectively reviewed to identify BC patients who had undergone germline cancer genetic testing. Once MRNs were obtained, additional clinical and demographic data were extracted from the hospital's electronic medical record system. In total, 25 patients were initially identified, of whom 16 met the inclusion criteria after data validation and cleaning.

3.3.3 Cancer Research Malaysia

At CRM, data were initially retrieved from an existing research masterlist comprising 1,277 cancer cases referred for genetic counselling and genetic testing. After filtering for BC patients aged ≤ 45 years who had completed germline genetic testing between 2020 and 2025, 503 cases were identified. Following data cleaning and completeness assessment, 447 patients were included in the final analysis. The cases in CRM which had incomplete histopathological data, missing pathological variables were coded as "unknown" to indicate data unavailability rather than absence.

Overall, this multi-source data collection approach, incorporating physical record review at HKL, electronic extraction at HTABTAR, and research database utilisation at CRM, enabled comprehensive capture of eligible EOBC patients, enhancing the representativeness of the study population in Malaysia. The flow chart of the data collection process is summarised in Figure 3.1.

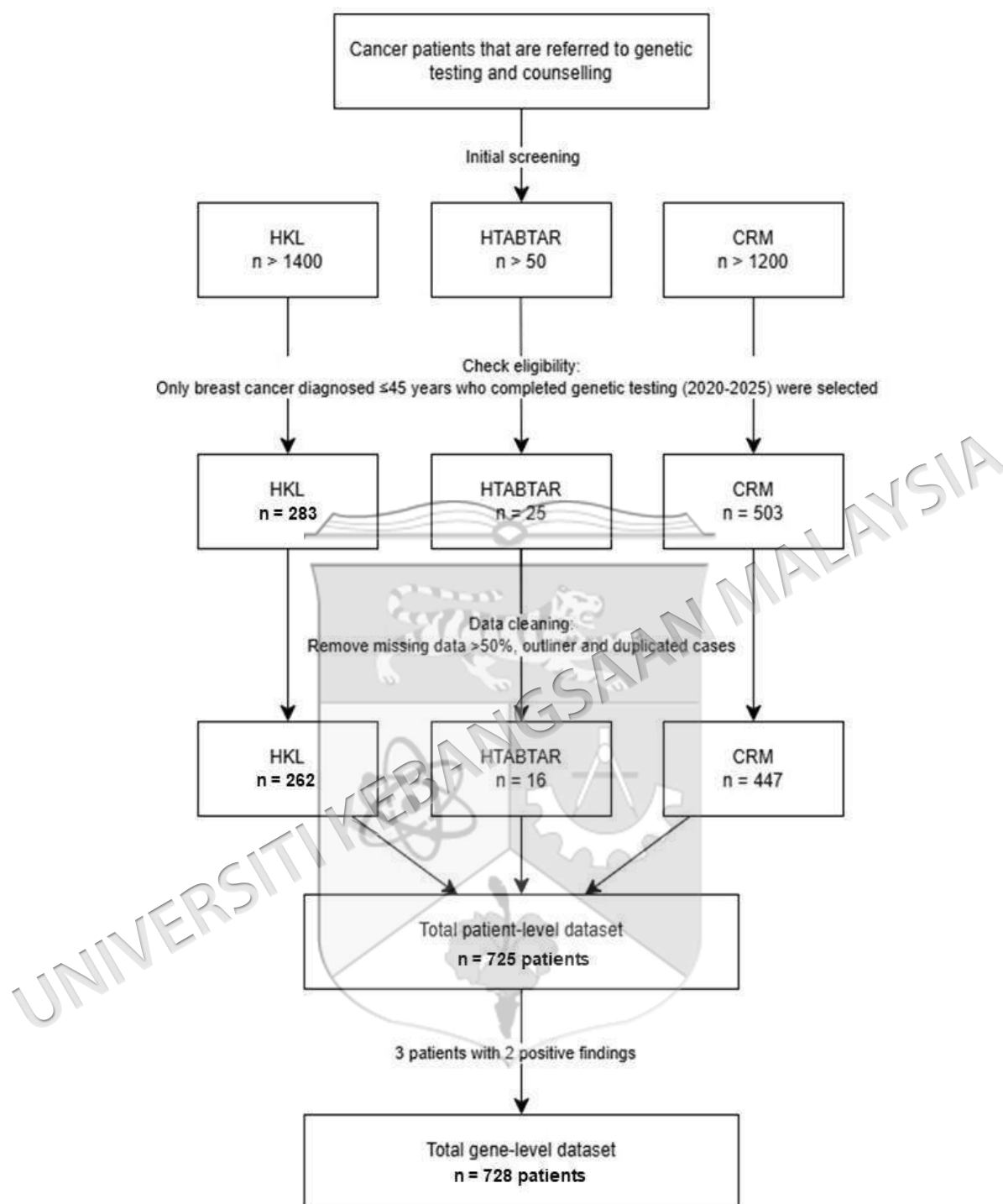


Figure 3.1 Flow diagram of patient selection

The following data were systematically extracted for each eligible patient:

- Demographic and clinical data
 - Age at diagnosis (numerical)

- Ethnicity (Malay, Chinese, Indian, Others)
- BC laterality (unilateral, synchronous bilateral, metachronous bilateral)
- Parity (nulliparous, parous)
- Personal history of other primary cancers (yes, no)
- Family history of cancer (up to third-degree relatives)
- Number of affected family members (if applicable)
- Pathological data
 - Tumour histological subtype (invasive ductal carcinoma, invasive lobular carcinoma, ductal carcinoma in situ, others)
 - Tumour grade (Nottingham grading system; Grade 1–3)
 - Tumour stage (AJCC TNM staging; Stage 0–IV)
 - Tumour immunohistochemical profile, including oestrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2)
- Molecular and genetic data
 - Genetic test result (positive, VUS, negative)
 - Gene(s) identified
 - Variant classification (P, LP, VUS)
 - Molecular consequence (e.g., missense, nonsense, frameshift, splice-site)
- Genetic testing information
 - Type of germline test performed (*BRCA*-only testing, multigene panel)
 - Number of genes analysed in multigene testing (numerical)
 - Name of gene panel used
 - Testing laboratory
 - Year of testing

Demographic and clinical information were abstracted mainly from referral letters, clinical notes and hospital electronic medical records. Pathological data were obtained from histopathology (HPE) and immunohistochemistry (IHC) reports. Molecular data and germline genetic findings were extracted directly from the original genetic test reports issued by the testing laboratories.

3.4 Genetic Testing Methods and Technology

Germline genetic testing results in this cohort were obtained from clinically accredited diagnostic laboratories. The two main testing approaches included, *BRCA*-only testing and multigene panel testing. Variant interpretation and classification followed the American College of Medical Genetics and Genomics (ACMG) guidelines, and final clinical reports were used as the source of genetic testing data for this study.

3.4.1 Current Clinical Practice for Genetic Test Selection

The choice of genetic testing approach varied according to healthcare setting, clinical indication, patient preference and affordability. In private healthcare (HTABTAR) and research settings (CRM), multigene panel testing was generally offered as the primary testing approach. In the public healthcare setting (HKL), patients were offered either government-funded *BRCA1/2* testing or self-funded multigene panel testing. Although multigene panel testing was encouraged due to its broader coverage, affordability influenced the final testing decision.

3.4.2 *BRCA*-only Testing

BRCA-only testing was performed through the local hospital laboratory using full-gene sequencing and copy number analysis where indicated. The number of government-funded *BRCA1/2* tests available each year was limited by funding allocation, and these tests usually came with a longer turnaround time (TAT) of approximately 6 months. These results were included in the analysis and compared with multigene panel findings. Patients with negative *BRCA1/2* results were offered the option of further genetic evaluation through multigene panel testing, particularly when clinical suspicion for hereditary cancer predisposition remained. The decision to

proceed with expanded testing was based on clinical assessment, patient preference, and financial considerations.

3.4.3 Multigene Panel Testing

Multigene panel testing was performed through a clinically accredited external diagnostic laboratory using NGS-based technology. Panel selection was individualised according to the patient's personal and family history, clinical indication, and the discussion between both the clinician and patient. Available multigene panels ranged from hereditary BC-targeted panels to broader hereditary cancer panels with varying gene coverage, including:

- STAT panel (9 genes)
- Hereditary Breast Cancer panel (18 genes)
- Hereditary Breast and Gynae Cancer guidelines-based panel (19 genes)
- Hereditary Breast and Gynae Cancer panel (28 genes)
- Common Hereditary Cancers panel (48 genes)
- Multicancer panel (70 genes)

Although the number of genes analysed differed between panels, the core hereditary BC susceptibility genes were included in the smallest panel, while larger panels expanded coverage to additional cancer predisposition genes associated with other hereditary cancer syndromes. Some patients initially underwent smaller panels and subsequently received expanded testing due to additional family history or persistent clinical suspicion. For analysis, the total number of genes tested per patient was collapsed into a composite “total genes analysed” variable, enabling assessment of diagnostic yield according to the breadth of gene coverage rather than panel name alone.

3.5 Operational Definitions and Categorisation

For statistical analysis, key variables were grouped into mutually exclusive categories to facilitate meaningful comparisons while preserving clinical and epidemiological

relevance. Definitions and categorisations were informed by standard clinical practice, Malaysian demographic conventions, and guideline-based classifications where applicable.

3.5.1 Ethnicity

Ethnicity was categorised as Malay, Chinese, Indian, and Others, reflecting the conventional ethnic categories commonly used in Malaysian population statistics and healthcare settings. The “Others” category included smaller ethnic groups such as Iban, Kadazan, other indigenous communities, and minority ethnicities not captured under the three major groups.

3.5.2 Age at Diagnosis

Age at diagnosis was analysed as a continuous variable and was also categorised into two groups for categorical analyses: 18–30 years and 31–45 years. This grouping was defined for analytical purposes to distinguish very young BC patients from the broader EOBC group, while ensuring sufficient case numbers within each category.

3.5.3 Family History of Cancer

Family history of cancer was recorded for first-, second-, and third-degree relatives. First-degree relatives included parents, siblings, and children; second-degree relatives included grandparents, grandchildren, aunts, uncles, nieces, nephews, and half-siblings; while third-degree relatives included first cousins, great-grandparents, and great-grandchildren. For each affected relative, the type of cancer and relationship to the patient were documented as raw data. These data were then grouped into four mutually exclusive categories for analysis: negative family history, breast/ovarian cancer only, HBOC-related cancers, and other cancers. HBOC-related cancers included breast, ovarian, prostate, and pancreatic cancers, in line with cancers commonly considered in HBOC risk assessment, while breast/ovarian cancer only included families with only either breast or ovarian. The number of affected first-, second-, and third-degree relatives were also recorded separately for breast, ovarian, pancreatic, and prostate cancers to allow more detailed familial risk assessment.

3.5.4 Personal History of Other Primary Cancer

Personal history of other primary cancer was defined as the presence of non-breast primary malignancies diagnosed in the patient, either before or after the index BC diagnosis. The index BC was excluded from this variable to avoid double-counting and to distinguish primary BC from additional primary malignancies.

3.5.5 Tumour Stage and Grade

Tumour stage was defined according to the American Joint Committee on Cancer (AJCC) tumour, node and metastasis (TNM) staging system (8th edition), to ensure consistency with international BC staging standards (Amin et al. 2017). Tumour grade was recorded using the Nottingham grading system (Grade 1 to 3), where available. If the pathology report described grade qualitatively, the categories were converted numerically as follows: low grade = Grade 1, intermediate grade = Grade 2, and high grade = Grade 3.

3.5.6 Hormone Receptor and HER2 Status

HR and HER2 status were abstracted from IHC and/or HPE reports. ER and PR status were recorded as positive or negative based on standard IHC criteria, where $\geq 1\%$ of invasive tumour nuclei showing positive staining was considered positive (Allison et al. 2020). HER2 status was classified as positive, negative, or equivocal according to local institutional reporting and ASCO/CAP HER2 testing guidance. Cases reported as HER2 equivocal without further confirmatory testing, such as fluorescence in situ hybridisation (FISH), were coded as unknown in the study dataset.

For analytical purposes, combinations of HR and HER2 status were further grouped into five clinically relevant subtypes as follows:

- HR-positive/HER2-negative (HR+/HER2-): ER and PR positive, HER2 negative.
- HR-negative/HER2-positive (HR-/HER2+): ER and PR negative, HER2 positive.

- Triple-negative breast cancer (TNBC): ER negative, PR negative, HER2 negative.
- Triple-positive breast cancer (TPBC): ER positive, PR positive, HER2 positive.
- Other combinations: All remaining discordant or mixed patterns (e.g., ER-/PR+/HER2-, ER+/PR-/HER2+, ER-/PR+/HER2+, ER+/PR-/HER2-). Grouped under “Other” to capture heterogeneous receptor profiles without diluting the main predefined subtypes.

This five-group classification allowed meaningful stratification of tumour subtypes for association analyses with germline P/LP variants, while preserving commonly used clinical and epidemiological categories such as HR+/HER2-, HR-/HER2+, TNBC, and TPBC.

3.5.7 Genetic Variant Classification

Genetic test results were categorised based on the final clinical reports issued by the respective testing laboratories. Variants were classified according to ACMG guidelines into five categories: pathogenic (P), likely pathogenic (LP), VUS, likely benign, and benign (Richards et al. 2015). Pathogenic variants refer to variants with sufficient evidence to support a disease-causing role, while likely pathogenic variants are those with strong but not definitive evidence of pathogenicity. VUS are variants for which available evidence is insufficient or conflicting, and therefore their clinical significance cannot be clearly determined. Likely benign and benign variants are variants with evidence suggesting no clinically significant disease-causing effect.

For this study, P/LP variants were grouped as positive findings, as these variants are clinically actionable. VUS were reported descriptively but were not considered positive findings. Benign and likely benign variants were treated as negative findings. For inferential analyses, VUS and negative results were grouped as non-positive findings.

3.6 Data Preparation and Cleaning

3.6.1 Data Entry and Storage

Raw data were initially recorded into a password-protected Google Sheet, which served as a secure platform for collaborative data management. Each participating centre maintained a separate worksheet with a standardised variable structure. Variables were recorded in their original descriptive form where appropriate, with predefined dropdown lists were used for selected variables such as tumour subtype, ethnicity, and receptor status to minimise entry errors. Following data collection, all site-specific worksheets were merged into a single master file containing eligible patients from HKL, HTABTAR, and CRM. Site-specific identifiers, including hospital MRNs, were replaced with anonymised study IDs to maintain patient confidentiality.

3.6.2 Data Cleaning

Missing data were reviewed and coded systematically. For categorical variables, missing data were coded as “unknown” to distinguish unavailable information from valid negative responses. Free-text entries were standardised to ensure consistency across the dataset. Spelling errors and typographical inconsistencies were corrected according to predefined coding rules documented in the data dictionary. Tumour subtype, receptor status, and family history of cancer were then regrouped into standard analytical categories. Outliers and inconsistent entries were identified through range checks, descriptive review, and assessment of clinical plausibility before numeric coding and Statistical Package for Social Sciences (SPSS) import.

3.6.3 Numeric Coding and SPSS Import

After data cleaning was completed, categorical variables were converted into numeric codes in the master worksheet to ensure compatibility with IBM SPSS Statistics. Only the final cleaned, and numerically coded dataset was exported from Google Sheets and imported into SPSS for analysis.

3.7 Data Management and Confidentiality

The research team implemented strict procedures to protect participants' privacy and confidentiality. All data handling, storage, and access were conducted in accordance with the approved data-management plan and relevant ethical and institutional requirements.

3.7.1 Data De-identification

To protect participant confidentiality, direct personal identifiers, including names, dates of birth, and identification card numbers, were not included in the central research dataset. MRNs were initially recorded during data collection to enable traceability and verification across clinical records but were replaced with unique anonymised study IDs before data analysis. Any temporary linkage between MRNs and study IDs was stored separately at the respective clinical sites and was not transferred to the central dataset.

3.7.2 Data Storage and Access

All research data were managed securely in an electronic spreadsheet format. The primary dataset was stored in a password-protected computer that was secured with a strong operating system password. To ensure data continuity and backup, the dataset was also stored on a password-protected cloud service (e.g., Google Sheets, secured with strong login credentials and two-factor authentication). Access to the anonymised dataset was limited to authorised members of the research team, including the Principal Investigator and Co-Investigators. No identifiable patient information was shared outside the respective clinical sites.

3.7.3 Data Retention and Destruction

The anonymised dataset was retained securely for one year following completion of the study, such as thesis submission, thesis defence, or publication, in accordance with institutional requirements. After the retention period, the electronic research dataset will be permanently destroyed by securely deleting the password-protected files from the primary storage device and cloud backup platform.

3.8 Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics. Descriptive analyses were used to summarise demographic, clinical, pathological, and molecular characteristics of the study cohort. Categorical variables were presented as frequencies and percentages, whereas continuous variables were summarised using means with standard deviations or medians with interquartile ranges, depending on data distribution. Genetic findings were classified as positive, VUS, or negative based on the categories defined in Section 3.5.7.

3.8.1 Spectrum of Germline P/LP Variants in Cancer Predisposition Genes

The proportion of positive, VUS, and negative genetic findings was summarised. Among patients with positive genetic findings, the frequency and distribution of germline P/LP variants across cancer predisposition genes were analysed. Detailed information on individual P/LP variants, including gene name, variant classification, and molecular consequence, was compiled and presented in the appendix.

3.8.2 Association Between Germline P/LP Variants and Clinicopathological Features

The clinicopathological characteristics among patients with *BRCA1*, *BRCA2*, and non-*BRCA* germline P/LP variants was evaluated descriptively. The association between positive genetic findings and clinicopathological characteristics were assessed using chi-square analysis. Variables assessed included age group, ethnicity, laterality, parity, personal history of other primary cancer, family history of cancer, tumour grade, tumour stage, and hormone subtype. Variables with a p-value < 0.05 were considered statistically significant.

Subsequently, variables with a p-value ≤ 0.25 in the association analysis were considered for logistic regression analysis. Univariate logistic regression was performed to estimate the crude odds ratio (OR), 95% confidence interval (CI), and p-value. Variables significant in the univariate analysis were then included in the multivariable logistic regression model to identify independent factors associated with positive genetic findings.

For regression analysis, selected variables were regrouped into binary categories where appropriate. For example, family history of cancer was dichotomised into yes and no, whereby the “yes” category included patients with a family history of breast, ovarian, pancreatic and prostate cancers. Hormone subtype was regrouped into TNBC and non-TNBC.

3.8.3 Evaluation of Diagnostic Yield Across Germline Genetic Testing Approaches

The distribution of *BRCA*-only testing and multigene panel testing across participating centres was summarised. Diagnostic yields were calculated as the proportion of positive genetic findings identified by each testing approach. The additional contribution of multigene panel testing was assessed based on the identification of non-*BRCA* P/LP variants.

Furthermore, trends in genetic testing practices over time were analysed by examining the number of genetic tests performed across different years. The association between the number of genes analysed and VUS detection was also evaluated using chi-square analysis.

3.9 Ethical Considerations

This study involved retrospective review and analysis of existing clinical and genetic data from EOBC patients. No direct patient contact or intervention was performed; the cohort was limited to adult patients (aged 18–45 years), with no paediatric or minor participants included. All data were de-identified before analysis, and no personal identifiers were retained in the research dataset. Access to the anonymised dataset was restricted to members of the core research team, and all data were handled in accordance with institutional policies to ensure participant confidentiality.

Ethical approval was obtained from the *Jawatankuasa Etika Penyelidikan Universiti Kebangsaan Malaysia* (JEPUKM), before data collection commenced. As this study involved Ministry of Health (MOH) hospitals, additional approvals were obtained through the National Medical Research Register (NMRR) and the Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia.

CHAPTER IV

RESULT

4.1 Study Population

The study analysed a cohort of 725 Malaysian patients diagnosed with BC at ≤ 45 years of age between the years 2020 and 2025. Data was pooled from three major institutions: CRM, $n = 447$, HKL, $n = 262$, and HTABTAR, $n = 16$. The baseline demographic and clinicopathological characteristics of the study population are described in Table 4.1.

Table 4.1 Baseline demographic and clinicopathological characteristics of study cohort (N= 725)

Variable	Category	Frequency (n)	Percentage (%)
Age at diagnosis (years)	Median (IQR)	37 (33-42)	
	Range	18-45	
Age group (years)	18-30	111	15.3
	31-45	614	84.7
Gender	Female	723	99.7
	Male	2	0.3
Ethnicity	Malay	307	42.3
	Chinese	363	50.1
	Indian	43	5.9
	Others	12	1.7

Laterality	Unilateral	674	93.0
	Synchronous bilateral	15	2.0
	Metachronous bilateral	36	5.0
Parity	Nulliparous	255	35.2
	Parous	456	62.9
	Unknown	14	1.9
Personal history of cancer	Yes	20	2.8
	No	705	97.2
Family history of cancer (Up to 3rd degree relatives)	Negative	178	24.6
	Breast/Ovarian cancer	298	41.1
	HBOC-related cancer	91	12.6
	Other cancer	152	21.0
	Unknown	6	0.8
Tumour histology	IDC	282	38.9
	ILC	3	0.4
	DCIS	23	3.2
	Others	27	3.7
	Unknown	390	53.8
Tumour grade	Grade 1	34	4.7
	Grade 2	120	16.6
	Grade 3	133	18.3
	Unknown	438	60.4
Tumour stage	Stage 0	20	2.8
	Stage I	106	14.6
	Stage II	222	30.6
	Stage III	161	22.2
	Stage IV	27	3.7

	Unknown	189	26.1
Hormone receptor status	TNBC	214	29.5
	TPBC	48	6.6
	HR+, HER2-	178	24.6
	HR-, HER2+	56	7.7
	Others	37	5.1
	Unknown	192	26.5

Notes: Percentages (%) were calculated based on the total cohort (N = 725). Missing data are presented as unknown.

Abbreviations: IQR, interquartile range IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; DCIS, ductal carcinoma in situ; TNBC, triple negative breast cancer; TPBC, triple positive breast cancer; HR, hormone receptor; HER2, human epidermal growth factor receptor 2.

The median age at diagnosis of the cohort was 37 years (IQR: 33-42). The vast majority fell into the 31-45 age group (84.7%), while 15.3% were diagnosed at a very young age (18-30). The cohort was almost exclusively female (99.7%), with only 2 male patients (0.3%) represented. Chinese patients made up the largest segment (50.1%), followed closely by Malay patients (42.3%), Indian patients (5.9%), and other minor ethnic groups (1.7%).

In addition, 93% presented with unilateral BC, 7% presented with bilateral BC, divided into synchronous (2%) and metachronous (5%) bilateral presentations. In terms of personal and family history, 2.8% of patients had a personal history of other primary cancers (including pancreatic, thyroid, colorectal, endometrial, ampullary, ovarian, liver, and lung malignancies). Crucially, 74.6% of the cohort possessed a positive family history of cancer up to third-degree relatives. Breast/ovarian cancer families accounted for 41.1% of the total cohort, while HBOC-related malignancies (breast, ovarian, prostate, pancreatic) accounted for 12.6%.

For the pathological characteristics, a considerable proportion of missing data was observed across variables, including tumour histology, grade, stage, and HR status. This was mainly attributable to incomplete HPE reports from one of the participating centres, the CRM cohort, which contributed the majority of cases in this

study. Nevertheless, pathological information was available for a substantial proportion of patients and was included in the analysis where available.

Invasive carcinoma of no special type (NST) or IDC was present in 282 patients (38.9%), followed by DCIS (23, 3.2%), other histological subtypes (27, 3.7%), and ILC (3, 0.4%). Histological data were unavailable for 390 patients (53.8%). High-grade tumours (grade 3) represented 18.3% (133) of the cases, followed by Grade 2 (120, 16.6%) and Grade 1 (34, 4.7%). 438 cases (60.4%) had no description of tumour grade in the records. Furthermore, the largest proportion of patients were diagnosed at Stage II (222, 30.6%), followed by Stage III (161, 22.2%), Stage I (106, 14.6%), Stage IV (27, 3.7%), and Stage 0 (20, 2.8%). Staging information was unavailable for 189 patients (26.1%). For HR status, TNBC was highly prevalent in this early-onset population, making up 29.5% of total cases. This was followed by HR+/HER2- (24.6%), HR-/HER2+ (7.7%), and TPBC (6.6%). The HR status of 192 cases was not available in the medical records.

4.2 Frequency and Spectrum of Germline P/LP Variants

Out of the 725 tested individuals, 145 patients (20.0%) carried at least one germline P/LP variant across 14 distinct cancer predisposition genes (positive result). Of these, three patients harboured dual P/LP variants, resulting in a total of 148 distinct P/LP variant findings evaluated at the gene level. A further 175 patients (24.1%) carried at least one VUS in the absence of a P/LP variant, while 405 patients (55.9%) did not harbour any reported germline P/LP variants (negative result). The overall genetic testing outcomes are illustrated in Figure 4.1.

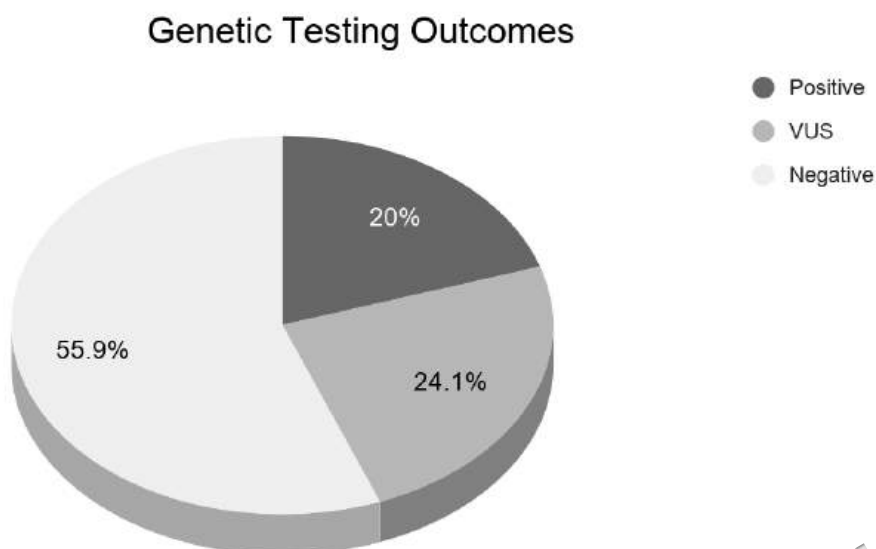


Figure 4.1 Overall genetic testing outcomes (N = 725)

Positive genetic findings were overwhelmingly driven by traditional HBOC syndrome genes, with *BRCA1* (40.5%) and *BRCA2* (36.5%) together accounting for 77.0% (114/148) of all positive findings. Non-*BRCA* genes constituted the remaining 23.0% (34/148), with *PALB2* (5.4%), *ATM* (4.1%) and *TP53* (3.4%) being the most frequently identified contributors. Other P/LP variants were detected in *CDH1*, *RAD51C*, *RAD51D*, *MUTYH*, *BARD1*, *BLM*, *LZTR1*, *MSH6*, and *PTEN* at a lower frequency. These findings demonstrate that while *BRCA1/2* remained the predominant contributors, clinically relevant P/LP variants were also identified across multiple non-*BRCA* cancer predisposition genes. The frequency and distribution of P/LP variant findings across genes is shown in Figure 4.2. Detailed information on the individual P/LP variants detected is provided in Appendix A.

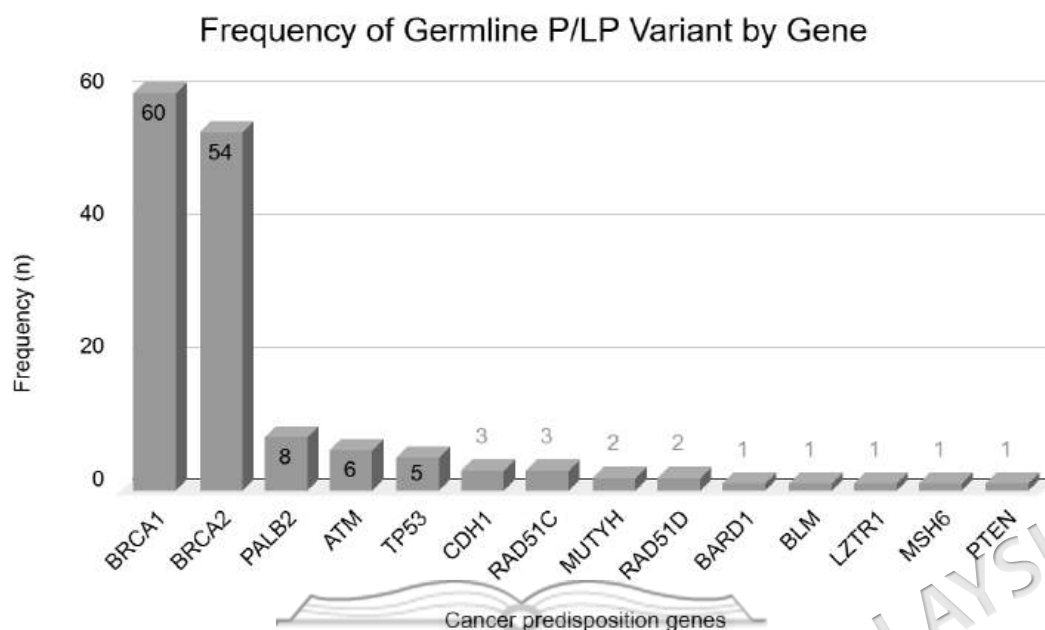


Figure 4.2 Distribution of germline P/LP variants across cancer predisposition genes (N=148)

Among the 34 P/LP variants identified in non-*BRCA* genes, 29 of them were found in established BC susceptibility genes, comprising 17 variants in high-penetrance genes (*PALB2*, *TP53*, *CDH1*, and *PTEN*) and 12 variants in moderate-penetrance genes (*ATM*, *RAD51C*, *RAD51D*, and *BARD1*). The remaining five variants occurred in genes for which the association with hereditary BC is less well established, namely *MUTYH*, *MSH6*, *BLM* and *LZTR1*.

4.3 Clinicopathological Characteristics and Predictors of Germline P/LP Variants

Given the distinct contribution of *BRCA1*, *BRCA2*, and non-*BRCA* genes to hereditary BC susceptibility, the clinicopathological characteristics of these P/LP variants were further compared to explore potential genotype–phenotype patterns (Table 4.2).

Table 4.2 Comparison of clinicopathological characteristics of *BRCA1*, *BRCA2*, and non-*BRCA* germline P/LP variants (N=148)

Variable	Category	<i>BRCA1</i> , n=60 (%)	<i>BRCA2</i> , n=54 (%)	Non- <i>BRCA</i> , n=34 (%)
Age group	18-30	16 (26.7)	4 (7.4)	8 (23.5)
	31-45	44 (73.3)	50 (92.6)	26 (76.5)

Ethnicity	Malay	31 (51.7)	26 (48.1)	10 (29.4)
	Chinese	22 (36.7)	24 (44.4)	20 (58.8)
	Indian	7 (11.7)	1 (1.9)	3 (8.8)
	Others	0 (0.0)	3 (5.6)	1 (2.9)
Laterality	Unilateral	53 (88.3)	44 (81.5)	31 (91.2)
	Bilateral	7 (11.7)	10 (18.5)	3 (8.8)
Parity	Nulliparous	19 (32.2)	14 (26.4)	14 (42.4)
	Parous	40 (67.8)	39 (73.6)	19 (57.6)
Personal history of cancer	Yes	0 (0.0)	5 (9.3)	6 (17.6)
	No	60 (100.0)	49 (90.7)	28 (82.4)
Family history of cancer	Negative	6 (10.2)	9 (16.7)	6 (17.6)
	Breast/Ovarian cancer	30 (50.8)	29 (53.7)	14 (41.2)
	HBOC-related cancer	18 (30.5)	9 (16.7)	5 (14.7)
	Other cancer	5 (8.5)	7 (13.0)	9 (26.5)
Tumour grade	Grade 1	1 (5.6)	1 (4.5)	1 (6.3)
	Grade 2	2 (11.1)	11 (50.0)	7 (43.8)
	Grade 3	15 (83.3)	10 (45.5)	8 (50.0)
Tumour stage	Stage I	6 (17.6)	11 (28.2)	2 (8.3)
	Stage II	13 (38.2)	17 (43.6)	9 (37.5)
	Stage III	12 (35.3)	9 (23.1)	13 (54.2)
	Stage IV	3 (8.8)	2 (5.1)	0 (0.0)
Hormone status	TNBC	40 (78.4)	11 (28.2)	6 (26.1)
	TPBC	1 (2.0)	5 (12.8)	2 (8.7)
	HR+, HER2-	2 (3.9)	19 (48.7)	9 (39.1)
	HR-, HER2+	2 (3.9)	2 (5.1)	4 (17.4)
	Others	6 (11.8)	2 (5.1)	2 (8.7)

Note: Data are presented as n (%). n represents the number of patients with available data for each variable within each gene group. Percentages were calculated based on available data, with missing data excluded from percentage calculations.

Abbreviations: TNBC, triple-negative breast cancer; TPBC, triple-positive breast cancer; HR, hormone receptor; HER2, human epidermal growth factor receptor 2.

BRCA1 variants tended to occur in younger patients, with a higher proportion diagnosed between 18–30 years compared with *BRCA2* variants (26.7% vs 7.4%). Furthermore, *BRCA1* and *BRCA2* variants demonstrated similar distribution among Malay and Chinese ethnicity, whereas non-*BRCA* variants were more frequently observed among Chinese (58.8%). Histopathological patterns also differed across variant groups, with *BRCA1* variants showed a higher proportion of grade 3 tumours (83%) compared with *BRCA2* (45.5%) and non-*BRCA* variants (50.0%). Furthermore, TNBC was predominant among *BRCA1* variants (78.4%), whereas HR-positive, HER2-negative tumours were more commonly observed among *BRCA2* (48.7%) and non-*BRCA* variants (39.1%).

Overall, these findings demonstrate distinct genotype–phenotype patterns across germline P/LP variant groups, with *BRCA1* showing a phenotype associated with younger age at diagnosis, higher tumour grade, and TNBC subtype, whereas *BRCA2* and non-*BRCA* variants displayed more heterogeneous clinicopathological characteristics.

Beyond describing genotype–phenotype patterns among different genes, clinicopathological characteristics associated with the overall likelihood of identifying a germline P/LP variant (positive genetic findings) were subsequently evaluated by comparing patients with positive and non-positive genetic findings (Table 4.3).

Table 4.3 Association between clinicopathological characteristics and positive genetic findings

Variable	Category	Positive, n (%)	Non-positive, n (%)	p-value
Age group (n=725)	18-30	27 (24.3)	84 (75.7)	0.216
	31-45	118 (19.2)	496 (80.8)	
Ethnicity (n=725)	Malay	66 (21.5)	241 (78.5)	0.385
	Chinese	65 (17.9)	298 (82.1)	
	Indian	10 (23.3)	33 (76.7)	
	Others	4 (33.3)	8 (66.7)	

Laterality (n=725)	Unilateral	125 (18.5)	549 (81.5)	<0.001*
	Bilateral	20 (39.2)	31 (60.8)	
Parity (n=711)	Nulliparous	47 (18.4)	208 (81.6)	0.442
	Parous	95 (20.8)	361 (79.2)	
Personal history of other primary cancer (n=725)	Yes	10 (50)	10 (50)	0.002*
	No	135 (19.1)	570 (80.9)	
Family history of cancer (n=719)	Negative	21 (11.8)	157 (88.2)	<0.001*
	Breast/Ovarian cancer	71 (23.8)	227 (76.2)	
	HBOC-related cancer	32 (35.2)	59 (64.8)	
	Other cancer	20 (13.2)	132 (86.8)	
Tumour grade (n=287)	Grade 1	3 (8.8)	31 (91.2)	0.114
	Grade 2	20 (16.7)	100 (83.3)	
	Grade 3	31 (23.3)	102 (76.7)	
Tumour stage (n=536)	Stage 0	0 (0)	20 (100)	0.296
	Stage I	19 (17.9)	87 (82.1)	
	Stage II	38 (17.1)	184 (82.9)	
	Stage III	32 (19.9)	129 (80.1)	
	Stage IV	5 (18.5)	22 (81.5)	
Hormone receptor status (n=533)	TNBC	56 (26.2)	158 (73.8)	0.058
	TPBC	8 (16.7)	40 (83.3)	
	HR+, HER2-	30 (16.9)	148 (83.1)	
	HR-, HER2+	7 (12.5)	49 (87.5)	
	Others	10 (27)	27 (73)	

*Note: Data are presented as n (%). n represents the number of patients with available data for each variable. Percentages were calculated based on available data, with missing data excluded from percentage calculations. *Significant association ($p < 0.05$).*

Abbreviations: TNBC, triple-negative breast cancer; TPBC, triple-positive breast cancer; HR, hormone receptor; HER2, human epidermal growth factor receptor 2.

Patient with positive genetic findings were more likely to have bilateral BC than unilateral BC (39.2% vs 18.5%, $P < 0.001$). Similarly, positive genetic findings were more frequent among patients with a personal history of other primary cancers than among those without (50.0% vs 19.1%, $p = 0.002$). A significant association was also observed with family history of cancer ($p < 0.001$), with the highest proportion of positive genetic findings identified among patients with a family history of HBOC-related cancers (35.2%), followed by those with a family history of breast or ovarian cancer (23.8%). In contrast, no significant associations were observed between positive genetic findings and age group (18-30 vs 31-45), ethnicity, parity, tumour stage, tumour grade and HR status within this specific, age-restricted cohort.

To control for confounding variables, clinicopathological characteristics with a p -value < 0.25 were considered clinically relevant and entered into the multivariable logistic regression model to identify independent predictors of positive genetic findings. Variables were regrouped into binary categories for regression analysis, where appropriate. The results of the multivariable logistic regression analyses are presented in Table 4.4.

Table 4.4 Independent factors associated with positive genetic findings

Variable	Univariate		Multivariable	
	Crude OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Bilateral BC (Yes/No)	2.834 (1.563-5.136)	$<0.001^*$	3.823 (1.570-9.310)	0.003^*
Personal history of other primary cancer (Yes/No)	4.222 (1.723-10.347)	$<0.001^*$	2.923 (0.621-13.764)	0.175
Family history of cancer (Yes/No)	2.539 (1.707-3.776)	$<0.001^*$	3.213 (2.006-5.144)	$<0.001^*$
TNBC (Yes/No)	1.701 (1.117-2.592)	0.013^*	2.069 (1.325-3.230)	0.001^*
Tumour grade	1.647 (1.024-2.648)	0.039^*	-	-
Age group	0.740 (0.459-1.194)	0.217	-	-

Reference categories: unilateral BC, no family history of cancer, no personal history of other cancer, non-TNBC, and low tumour grade. * p -value < 0.05 was considered statistically significant. Abbreviations: OR, odds ratio; CI, confidence interval; TNBC, triple-negative breast cancer

After adjustment, three features emerged as independent predictors of positive genetic findings, including bilateral BC, a positive family history of cancer, and TNBC. Patients with bilateral BC had approximately 3.8-fold higher odds of carrying a germline P/LP variant than those with unilateral disease (adjusted OR = 3.823, 95% CI: 1.570–9.310, p = 0.003). Similarly, patients with a positive family history of cancer had over three-fold higher odds of a positive genetic finding than those without a family history (adjusted OR = 3.213, 95% CI: 2.006–5.144, p < 0.001). TNBC was also independently associated with positive genetic findings, with approximately two-fold higher odds of harbouring a germline P/LP variant compared with non-TNBC subtypes (adjusted OR = 2.069, 95% CI: 1.325–3.230, p = 0.001).

4.4 Genetic Testing Approaches and Diagnostic Yield

The study highlights variation in germline genetic testing approaches across the participating centres. Overall, 81% (587/725) of patients underwent multigene panel testing, while 19% (138/725) received *BRCA*-only testing. The distribution of the genetic testing according to the study site is summarised in Table 4.5.

Table 4.5 Distribution of genetic testing approaches across the three participating centres

Study centre	<i>BRCA</i> -only testing, n (%)	Multigene panel testing, n (%)
CRM (n=447)	0 (0)	447 (100.0)
HKL (n=262)	138 (52.7)	124 (47.3)
HTABTAR (n=16)	0 (0)	16 (100.0)
Total	138 (19.0)	587 (81.0)

Abbreviations: CRM, Cancer Research Malaysia; HKL, Hospital Kuala Lumpur; HTABTAR, Hospital Tunku Ampuan Besar Tuanku Aishah Rohani.

Multigene panel testing was exclusively utilised at CRM and HTABTAR. In contrast, HKL offered both government-funded *BRCA*-only testing and self-funded multigene panel testing, resulting in a nearly equal distribution of testing approaches (52.7% *BRCA*-only vs 47.3% multigene panel). Among the 124 HKL patients who

underwent multigene panel testing, 22 had previously received *BRCA*-only testing without significant findings and subsequently proceeded to multigene panel testing. For patients who did not proceed to larger panel testing after negative *BRCA* result, documented reasons included lack of interest and financial constraints, while no reason was recorded for some patients.

Furthermore, the annual number of germline cancer genetic tests performed from 2020 to 2025 is illustrated in Figure 4.3. An increasing trend in genetic testing uptake was observed throughout the study period, reflecting the mainstreaming of germline genetic testing in BC care.

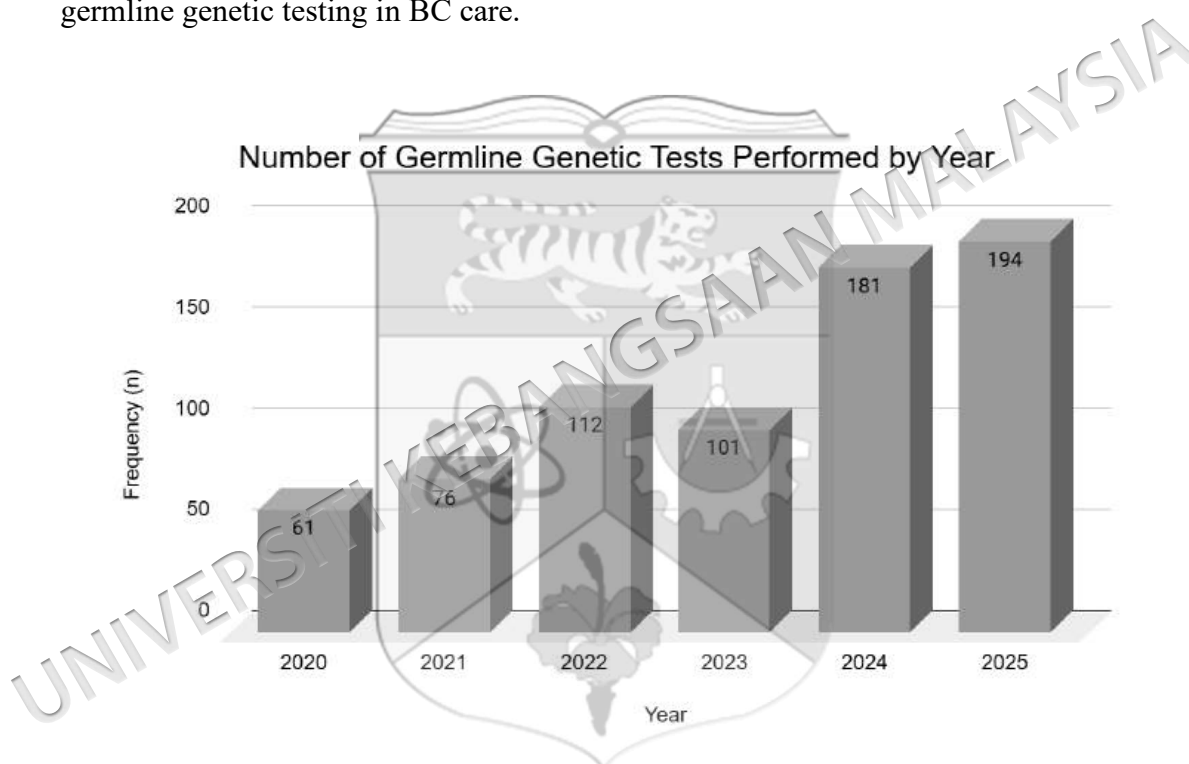


Figure 4.3 Number of germline genetic tests performed by year (2020-2025)

The diagnostic yields of *BRCA*-only testing and multigene panel testing for identifying germline P/LP variants are summarised in Table 4.6. Among patients who underwent *BRCA*-only testing (n=138), 33 (23.9%) had positive genetic findings, 98 (71.0%) had negative findings and 7 (5.1%) had VUS findings. In comparison, among patients who underwent multigene panel testing (n=587), 112 (19.1%) had positive genetic findings, 307 (52.3%) had negative findings, and 168 (28.6%) had VUS findings.

Table 4.6 Diagnostic yields of *BRCA*-only testing and multigene panel testing

Testing method	Negative, n (%)	VUS, n (%)	Positive, n (%)
<i>BRCA</i> -only (n=138)	98 (71.0)	7 (5.1)	33 (23.9)
Multigene panel (n=587)	307 (52.3)	168 (28.6)	112 (19.1)

Abbreviations: VUS, variant of uncertain significance

Although *BRCA*-only testing demonstrated a slightly higher percentage of P/LP variant detection compared with multigene panel testing (23.9% vs 19.1%), this should be interpreted cautiously within the context of centre-specific testing availability and patient-related factors. Among the 145 patients with positive genetic findings in this cohort, 34 (23%) carried P/LP variants in non-*BRCA* susceptibility genes, which would not have been captured by *BRCA1/2*-only testing.

Additionally, multigene panel testing was associated with a higher proportion of VUS findings compared with *BRCA*-only testing, as illustrated in Figure 4.4. This observation reflects the expected increase in VUS detection with broader gene analysis and prompted further evaluation of the association between the number of genes analysed and VUS detection.

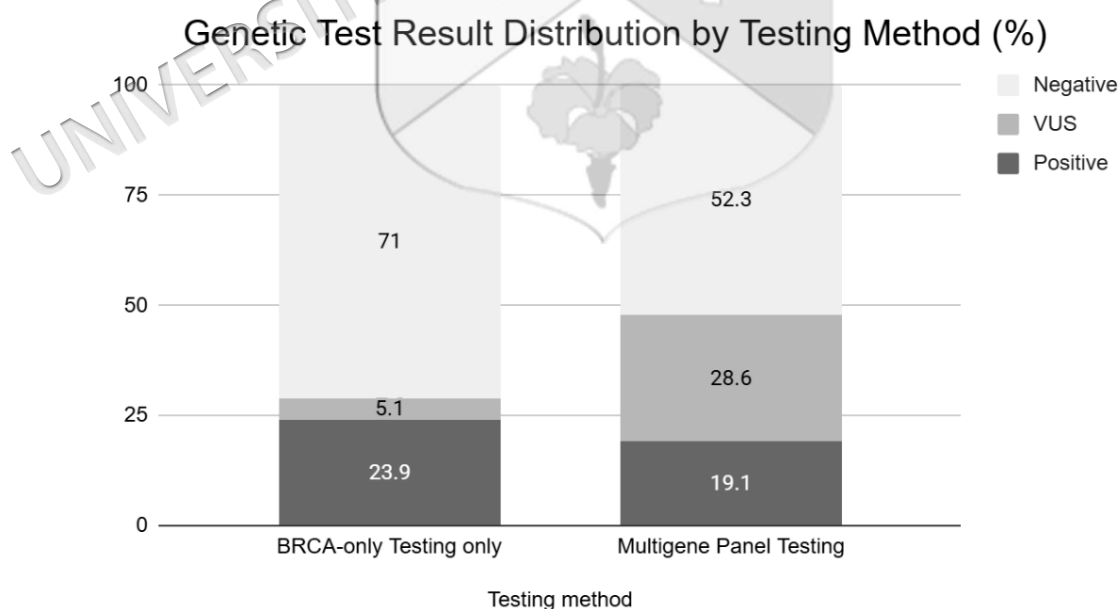


Figure 4.4 Distribution of genetic test results by genetic testing approaches

4.5 Variant of Uncertain Significance Detection and Impact of Panel Size

Among the 725 patients, 175 (24.1%) had at least one VUS identified. The rate of VUS detection differed according to testing approach and number of genes analysed, with VUS identified in 7 of 138 patients (5.1%) who underwent *BRCA*-only testing and 168 of 587 patients (28.6%) who underwent multigene panel testing.

Multigene panel size was categorised into four study-specific groups based on the number of genes analysed in the test: 1–10 genes, 11–30 genes, 31–60 genes, and >60 genes. These categories were not intended to represent equal interval widths but to broadly reflect increasing test breadth, ranging from targeted testing to more comprehensive multigene panels. The association between number of genes analysed and VUS detection is summarised in Table 4.6.

Table 4.7 Association between number of genes analysed and VUS detection

Number of genes analysed	Non-VUS, n (%)	VUS, n (%)	p-value
1-10 genes (n=183)	174 (95.1)	9 (4.9)	<0.001*
11-30 genes (n=362)	282 (77.9)	80 (22.1)	
31-60 genes (n=95)	59 (62.1)	36 (37.9)	
>60 genes (n=85)	35 (41.2)	50 (58.8)	

Notes: Chi-square test was used to assess the association between number of genes analysed and VUS detection. * $p < 0.05$ was considered statistically significant.

Abbreviations: VUS, variant of uncertain significance

A significant association was observed between the number of genes analysed and VUS detection ($p < 0.001$). The proportion of VUS findings increased progressively with increasing number of genes analysed, from 4.9% among patients tested with 1–10 genes to 58.8% among those tested with >60 genes.

These findings demonstrate that broader multigene panels were associated with a substantially increased likelihood of VUS detection. Although expanded panels enable assessment of more cancer predisposition genes, they may also increase the complexity of genetic result interpretation and counselling. Appropriate pre- and post-test genetic counselling remains essential, as VUS findings should not be used to guide clinical management or predictive testing decisions.

CHAPTER V

DISCUSSION

5.1 Spectrum and Yield of Germline P/LP Variants

5.1.1 Overall Diagnostic Yield of P/LP Variants in Malaysian EOBC

In the present study, the overall diagnostic yield of germline P/LP variants was 20% among Malaysian patients with EOBC, indicating that approximately one in five patients carried a germline P/LP variant in a cancer predisposition gene.

The yield observed in this study is consistent with previous studies involving EOBC cohorts, where germline P/LP variant detection has been reported at approximately 10–40%, depending on the definition of young age, testing criteria and genes analysed. Studies using stricter age cut-offs, such as diagnosis at ≤ 30 years, tend to report higher yields, with some very early-onset cohorts reporting P/LP variant rates of approximately 20–40% (Apostolou et al. 2024; Bakhuizen et al. 2019; Evans et al. 2022). A similar pattern was observed in this study, where patients diagnosed before 30 years had a slightly higher P/LP yield than those diagnosed between 31 and 45 years (24.3% vs. 19.2%). Although the difference was modest, it is consistent with the overall trend that a younger age at diagnosis may be associated with a higher likelihood of detecting a germline P/LP variant. In contrast, studies using broader EOBC definitions, such as ≤ 50 years, generally report lower but still clinically meaningful yields, often around 10–20% (Shroff et al. 2023.; Tashjian et al. 2025). Thus, the 20% diagnostic yield observed in the present study falls within the expected range for EOBC, particularly given that this study included patients diagnosed at ≤ 45 years.

The yield is also comparable to clinically selected or high-risk BC cohorts, including patients suspected of HBOC or those meeting NCCN testing criteria, where P/LP yields are often reported around 15–30% (Akbar et al. 2022; Öfverholm et al. 2023; Rodriguez-Hernandez et al. 2025; Sandoval et al. 2021; Togashi et al. 2025). This comparison is relevant because the present cohort consisted of patients who underwent germline testing through clinical and research genetic testing pathways, rather than an unselected population-based BC cohort.

In the Malaysian context, the present finding is broadly consistent with previous local studies involving early-onset or clinically selected BC patients. Ng et al. (2016) identified deleterious variants in approximately 19% of moderate- to high-risk Malaysian BC patients, including about 13% in *BRCA1/2* and 6% in non-*BRCA* genes. Similarly, Lee et al. (2012) reported P/LP variants in approximately 21% of Malaysian EOBC patients diagnosed before 35 years of age, including 11% in *BRCA1*, 6% in *BRCA2*, and 5% in *TP53*. Thus, the 20% yield observed in the present study is consistent with findings from Malaysian EOBC and high-risk BC cohorts while remaining higher than that reported in unselected local BC populations.

Taken together, these findings suggest that variation in P/LP yield across studies is largely influenced by differences in study population and testing approach. Cohorts restricted to younger patients or enriched for hereditary cancer features, such as family history, bilateral BC or TNBC, are expected to show higher yields than unselected BC cohorts. Differences in ethnic composition, referral pathways and access to genetic testing may further contribute to this variation, particularly in settings where testing availability depends on institutional resources, research programmes or patient affordability. This is especially relevant in Malaysia, where cost, limited genetic testing infrastructure, service delivery models and willingness to pay have been identified as factors influencing genetic testing uptake (Htay, Su & Donnelly 2024; Padmanabhan et al. 2026; Yoon et al. 2011).

Overall, the identification of germline P/LP variants in 20% of Malaysian EOBC patients reinforces EOBC as an important indication for germline genetic testing. It is consistent with the expected range for EOBC and clinically selected

hereditary cancer testing cohorts. From a genetic counselling perspective, this finding is clinically meaningful because identifying a germline P/LP variant can guide personalised cancer management, inform surveillance, risk-reducing strategies, and enable cascade testing for at-risk relatives. Therefore, the substantial yield observed in this study supports the continued integration of germline genetic testing into the care pathway for Malaysian patients with EOBC.

5.1.2 Spectrum of Germline P/LP Variants in Malaysian EOBC

Following the overall diagnostic yield, the distribution P/LP variants identified across genes in this study provides further insight into the hereditary cancer predisposition profile of Malaysian patients with EOBC. Together, *BRCA1/2* accounted for approximately three-quarters of positive genetic findings, indicating that they remain the major contributors to hereditary BC predisposition in Malaysian EOBC patients.

This predominance of *BRCA1/2* is consistent with previous local and international studies, which have shown that *BRCA1/2* remain the most commonly identified BC predisposition genes in early-onset, familial, and clinically selected BC cohorts (Evans et al. 2022; Lee et al. 2012; Ng et al. 2016; Sandoval et al. 2021; Tung et al. 2016). As the genes most frequently associated with HBOC syndrome, *BRCA1/2* have well-established implications for cancer management, surveillance, risk-reducing options and cascade testing for at-risk relatives (NCCN 2025).

Nevertheless, non-*BRCA* genes also contributed a clinically meaningful proportion of germline P/LP variants. In the present study, approximately one-quarter of positive genetic findings were detected in non-*BRCA* genes with *PALB2*, *ATM* and *TP53* among the more frequently observed genes following *BRCA1/2*. This is broadly consistent with multigene panel literature, where *PALB2* and *ATM* are commonly reported as important non-*BRCA* BC predisposition genes, while *TP53* is particularly relevant in very young-onset BC or cases suggestive of Li-Fraumeni syndrome (Apostolou et al. 2024; Evans et al. 2022; Lee et al. 2012; Sandoval et al. 2021). Most of these non-*BRCA* genes are clinically actionable, with guideline-based recommendations available for risk management. These genes differ in penetrance,

associated cancer spectrum and management implications. For example, *PALB2* is associated with a high risk of BC, *ATM* is generally considered a moderate-penetrance BC predisposition gene, whereas *TP53* has broader implications due to the risk of multiple cancers and the requires syndrome-specific surveillance (NCCN 2025). Therefore, identifying P/LP variants beyond *BRCA1/2* may provide management-relevant information that can guide gene-specific surveillance, risk-reducing strategies and cascade testing for at-risk relatives.

These findings add to the limited local evidence on the spectrum of germline P/LP variants among Malaysian patients with EOBC. Earlier Malaysian studies have mainly focused on *BRCA1/2* or selected high-risk groups, while fewer studies have described the broader contribution of non-*BRCA* genes using multigene panel testing (Ng et al. 2016). Given Malaysia's multi-ethnic population, local data are important to improve understanding of hereditary BC predisposition patterns. However, the number of germline P/LP variants identified in individual non-*BRCA* genes was small; therefore, gene-specific conclusions should be interpreted cautiously.

Overall, the spectrum of germline P/LP variants identified in this study supports a balanced interpretation of hereditary BC testing in Malaysian EOBC patients. Although *BRCA1/2* remained the major contributors and continue to justify their central role in hereditary BC assessment, clinically relevant P/LP variants were also identified in several non-*BRCA* genes.

5.2 Clinicopathological Characteristics and Predictors of Germline P/LP Variants

5.2.1 Clinicopathological Characteristics of *BRCA1/2* and Non-*BRCA* Germline P/LP Variants

The clinicopathological characteristics observed among *BRCA1* variants in this study reflected the established phenotype of *BRCA1*-associated BC. The predominance of TNBC among *BRCA1* carriers is consistent with previous reports (Apostolou et al. 2024; Lambertini et al. 2024; Mavaddat et al. 2022; Tahsjan et al. 2025; Wang et al. 2018) and may be attributed to impaired homologous recombination repair resulting in genomic instability and basal-like tumour characteristics. The association between

BRCA1 P/LP variants and younger age at diagnosis (18-30 years) further supports the importance of early genetic evaluation among young patients presenting with aggressive tumour features.

In contrast, *BRCA2*-associated BC showed a different clinicopathological profile, with a predominance of HR-positive, HER2-negative tumours, consistent with previous reports (Akita et al. 2025; Chen et al. 2020; Rodríguez-Hernandez et al. 2025). Compared with *BRCA1*, *BRCA2*-associated tumours generally exhibit greater phenotypic variability, which was also observed in this cohort.

Due to the limited number of carriers for individual non-*BRCA* genes, gene-specific clinicopathological analyses were not feasible. Therefore, non-*BRCA* P/LP variants were analysed as a combined group. The non-*BRCA* group showed heterogeneous clinicopathological features, reflecting diverse underlying genes and cancer risks. Unlike *BRCA1*-associated BC, no consistent tumour phenotype was observed to guide gene-specific suspicion, limiting the use of clinicopathological features alone for identifying non-*BRCA* carriers. Notably, a higher proportion of non-*BRCA* variants was observed among Chinese patients in this cohort, consistent with Ow et al. (2019), although this finding should be interpreted cautiously due to the small sample size.

Overall, these findings demonstrate that while *BRCA1* and *BRCA2* exhibit recognisable clinicopathological patterns, non-*BRCA* susceptibility genes represent a heterogeneous group that may not be readily distinguished based on phenotype alone, highlighting the complexity of hereditary BC predisposition in Malaysian EOBC patients.

5.2.2 Predictors of Positive Genetic Findings

In this study, positive family history of cancer, bilateral BC, and TNBC subtype were identified as independent predictors of germline P/LP variant detection among Malaysian EOBC patients. Although young age at diagnosis was the primary inclusion criterion, these additional familial and tumour-related characteristics further enriched the likelihood of identifying a germline P/LP variant.

A positive family history of cancer was one of the key predictors of germline P/LP variant detection in this study. This finding expected and consistent with previous studies (Akbar et al. 2022; Öfverholm et al. 2023; Sandoval et al. 2021; Tung et al. 2016), as hereditary cancer predisposition is often reflected by clustering of related cancers within families. In the context of HBOC syndrome, a family history of breast, ovarian, pancreatic, or prostate cancer may suggest shared inherited susceptibility, supporting the continued importance of detailed three-generation family history assessment during genetic counselling. However, the association between family history and positive genetic findings should be interpreted carefully, as the absence of reported family history does not exclude hereditary cancer predisposition. This study showed that 11.8% of patients without a reported family history still had a positive genetic finding, suggesting that reliance on family history alone may miss individuals with clinically significant germline variants. Family history may also be underestimated due to incomplete information, limited knowledge of relatives' diagnoses, or small family structures. Therefore, family history remains an important component of risk assessment, but should be interpreted with tumour characteristics and age at diagnosis rather than used as the sole criterion for genetic testing.

Bilateral BC was also independently associated with germline P/LP variant detection. This finding is clinically plausible, as bilateral disease may indicate an underlying predisposition to develop multiple primary BC. Germline P/LP variants in high-risk BC predisposition genes, particularly *BRCA1* and *BRCA2*, are associated with an increased risk of contralateral BC. Previous studies have also shown that P/LP variants in genes such as *BRCA1*, *BRCA2*, *CHEK2*, *PALB2*, and *ATM* may contribute to contralateral BC risk, although the magnitude of risk differs by gene (Yadav et al. 2020). Therefore, the finding in this study reinforces bilateral BC as an important clinical marker for hereditary cancer evaluation among EOBC patients.

TNBC subtype was another independent predictor of germline P/LP variant detection in this cohort. The higher proportion of positive genetic findings observed among patients with TNBC compared with other tumour subtypes is consistent with previous studies demonstrating that TNBC is enriched for germline P/LP variants, particularly in *BRCA1* and other homologous recombination-related genes (Engel et

al. 2018; Shimelis et al. 2018). These findings support the role of TNBC as an important tumour characteristic for identifying EOBC patients who may benefit from germline genetic testing.

Overall, these findings suggest that germline P/LP variant detection among EOBC patients is influenced by a combination of familial, tumour-related, and clinical features. A positive family history of cancer, bilateral BC, and TNBC may help refine risk assessment within young-onset populations; however, the absence of these features should not preclude genetic testing. A comprehensive assessment incorporating age at diagnosis, family history, tumour characteristics, and personal cancer history remains important for identifying patients who may benefit from germline evaluation.

5.3 Genetic Testing Strategies and Diagnostic Yield

5.3.1 Interpretation of *BRCA*-only and Multigene Panel Yield

In this study, two main germline testing strategies were used: *BRCA*-only testing and multigene panel testing. The diagnostic yield was slightly higher among patients who underwent *BRCA*-only testing compared with those who underwent multigene panel testing (23.9% vs. 19.1%). However, this finding should be interpreted cautiously, and should not be taken to suggest that *BRCA*-only testing is superior to multigene panel testing.

The slightly higher yield in the *BRCA*-only group may be explained the predominance of *BRCA1/2* variants in this EOBC cohort, allowing *BRCA*-only testing to capture the majority of identified P/LP variants. However, this comparison between testing strategies is limited by differences in testing pathways as patients with negative *BRCA1/2* results did not routinely proceed to broader multigene panel testing. Therefore, some non-*BRCA* P/LP variants may have remained undetected in this group. Furthermore, in the Malaysian clinical setting, the selection of testing approach may be influenced by factors such as funding availability and patient affordability, rather than clinical risk alone.

Therefore, the slightly higher *BRCA*-only yield likely reflects the predominance of *BRCA1/2* variants and limitations of sequential testing pathways, rather than the overall sufficiency of *BRCA*-only testing for all EOBC patients.

5.3.2 Clinical Value of Multigene Panel Testing Beyond *BRCA1/2*

Although *BRCA1/2* remained the most commonly identified genes in this EOBC cohort, non-*BRCA* P/LP variants accounted for 23.0% (34/148) of all positive genetic findings. These variants would not have been detected through *BRCA*-only testing, demonstrating the added value of multigene panel testing in identifying hereditary cancer predisposition beyond *BRCA1/2*.

The clinical relevance of these findings is supported by the diverse hereditary cancer syndromes associated with the identified non-*BRCA* genes. Among the 34 non-*BRCA* P/LP variants, 30 were associated with established NCCN-based surveillance and risk-management recommendations, including genes such as *PALB2*, *ATM*, *TP53*, *CDH1*, *RAD51C*, *RAD51D*, *BARD1*, *PTEN*, and *MSH6*. Therefore, identification of these variants may provide clinically actionable information beyond diagnosis, including personalised surveillance, risk-reducing strategies, and cascade testing for relatives.

Among patients who underwent multigene panel (n=587), non-*BRCA* P/LP variants were identified in 31 patients, representing an additional diagnostic yield of 5.3% (31/587) beyond *BRCA1/2*. This finding is consistent with previous studies reporting additional diagnostic yields of approximately 3–11% from multigene panel testing among individuals with suspected hereditary BC (Ece Solmaz et al., 2021; Kapoor et al., 2015; Lincoln et al., 2015; Park et al., 2022). Together, these findings support the clinical utility of multigene panel testing into hereditary risk assessment for selected EOBC patients.

Overall, these findings support the clinical value of multigene panel testing in Malaysian EOBC patients, as a meaningful proportion of germline P/LP variants were detected in non-*BRCA* genes. Nevertheless, in resource-limited settings, *BRCA*-only testing remains a practical approach for identifying the majority of P/LP findings. A

stepwise testing strategy may therefore be considered, whereby *BRCA*-only testing is prioritised when broader testing is not feasible. Multigene panel testing is always offered to patients with negative *BRCA1/2* results who remain clinically high-risk or when additional gene information may influence clinical management.

5.3.3 Access, Uptake and Mainstreaming of Genetic Testing in Malaysia

The choice between *BRCA*-only testing and multigene panel testing in this study also reflects real-world differences in genetic testing access across healthcare settings. In the public hospital setting, approximately half of the patients underwent *BRCA*-only testing while the other half underwent multigene panel testing. This likely reflects differences in test availability, funding and patient affordability. Local government-funded *BRCA*-only testing is more accessible in public hospital pathways, whereas multigene panel testing is often outsourced and may require self-payment.

In Malaysia, the choice of genetic testing approach may be influenced not only by clinical indication, but also by institutional resources, public-private healthcare pathways, availability of testing services and patient affordability. Limited access to genetic testing facilities and lack of insurance coverage remain important barriers to wider implementation of hereditary cancer testing (Htay, Su & Donnelly 2024). Cost may also substantially affect testing uptake, as willingness to undergo genetic testing does not always translate into actual testing when out-of-pocket payment is required (Padmanabhan et al. 2026). Therefore, improving access to multigene panel testing may require better funding support, increased availability of local genetic testing services, and efforts to make genetic testing more affordable.

At the same time, genetic testing uptake appears to be increasing and becoming more mainstream in Malaysia. In this study, the increasing number of BC patients undergoing germline genetic testing over the study period suggest growing awareness and adoption of genetic testing. This trend may reflect increasing clinician awareness, decreasing sequencing costs, greater availability of commercial multigene panels, and expanding clinical utility of genetic results for treatment and surveillance. Internationally, mainstreaming models have been proposed to improve access by

integrating genetic testing into oncology care pathways, while maintaining appropriate counselling and referral support (Dubsky et al. 2024).

Local evidence also supports the feasibility alternative approaches to expand genetic testing access in Malaysia. The Malaysian MaGiC study demonstrated comparable genetic testing uptake between oncologist and the genetics-led *BRCA* counselling model (Yoon et al. 2022). Although this evidence is from ovarian cancer, it suggests that oncology-based approaches may be considered to improve access to genetic testing in BC care, provided that appropriate genetic counselling support, referral pathways and systems for managing complex results are maintained.

5.4 Variant of Uncertain Significance and Genetic Counselling Implications

5.4.1 VUS Yield and Panel Size

In this study, VUS were reported in 24.1% of genetic findings among Malaysian EOBC patients, indicating that nearly one-quarter of variants were uncertain at the time of reporting. The VUS rate observed in this study is within the range reported in hereditary cancer genetic testing, where VUS rates commonly vary from approximately 10–40%, depending on the study population, testing criteria, number of genes analysed and variant interpretation practices (Chrysafi et al. 2023). Therefore, the VUS rate in the present cohort may be considered clinically meaningful but not unexpected, particularly given the use of multigene panel testing in a large proportion of patients.

A key finding in this study was the significant association between VUS detection and the number of genes analysed. Patients tested using larger multigene panels were more likely to receive a VUS result compared with those tested using targeted panels because larger panels analyse more genes and genomic regions, increasing the probability of detecting rare variants with insufficient evidence for classification. Therefore, the significant association between number of genes analysed and VUS detection supports the need for careful panel selection, as broader testing may increase uncertainty without always producing a proportional increase in clinically actionable findings.

The VUS rate in this study is also relevant in the context of Asian and other underrepresented populations. Previous studies have consistently shown that VUS rates tend to be higher among non-European populations, including Asian individuals, largely due to limited ancestry-specific data in variant databases (Caswell-Jin et al. 2018; Chen et al. 2023; Ndugga-Kabuye & Issaka 2019). This issue is particularly relevant in Malaysia, where the population is multi-ethnic and local variant data remain limited. As more Malaysian and regional data are collected and shared, some variants currently classified as uncertain may eventually be reclassified.

5.4.2 Challenges of VUS in Genetic Counselling

From a genetic counselling perspective, VUS results present several challenges. A VUS should not be used to guide major clinical decisions, risk-reducing surgery, or predictive testing of unaffected relatives. The ACMG variant interpretation guideline clearly states that a VUS should not be used in clinical decision-making (Richards et al. 2015). Instead, management should generally be based on the patient's personal and family history while awaiting possible variant reclassification.

Uncertain results may also create communication and psychosocial challenges in genetic counselling. Previous studies have shown that VUS results can be difficult for patients to understand and may contribute to uncertainty in decision-making, expectation management and follow-up care (Amano et al. 2022; Medendorp et al. 2020). This may be particularly relevant in Asian populations, where patients may already face additional challenges related to limited ancestry-specific variant data and uncertainty around result interpretation (Ishak et al. 2024). Therefore, clear pre-test and post-test counselling is essential to help patients understand that a VUS is not a positive result, avoid inappropriate management decisions, and remain informed about the possibility of future reclassification.

Overall, the VUS rate observed in this study reflects the combined influence of number of genes analysed and population-specific challenges in variant interpretation. These findings highlight the importance of careful test selection and genetic

counselling to support appropriate understanding and management of uncertain results among Malaysian EOBC patients.

5.5 Residual Risk Assessment Following Absence of Germline P/LP Variants

More than half of the study cohort (56.0%) had negative genetic findings. However, this should not be interpreted as the absence of hereditary or familial cancer risk, particularly in EOBC where young age at diagnosis itself is a recognised indicator of potential hereditary cancer predisposition. Among patients with non-positive genetic findings (n=580), 519 had available clinical information for assessment of additional hereditary cancer risk features. Of these, 76.6% (402/519) presented with at least one additional feature beyond young age at diagnosis, including TNBC, bilateral BC, or positive family history of cancer. This highlights that many patients without identified germline P/LP variants may remain clinically high-risk and required further residual risk assessment.

Several factors may explain why clinically high-risk patients may not have identifiable germline P/LP findings. First, this may reflect limitations of current testing approaches. Patients who underwent *BRCA*-only testing may still carry P/LP variants in non-*BRCA* cancer predisposition genes that were not assessed, while multigene panels may differ in gene content, panel size and technical coverage. Second, hereditary cancer risk may involve novel genes or gene–disease associations that remain unidentified or are not included in current clinical panels. Third, some patients may have an elevated risk due to polygenic, multifactorial or shared environmental factors rather than a single detectable high-penetrance variant. This is supported by multifactorial BC risk models such as BOADICEA, which incorporate genetic, familial and lifestyle factors to estimate BC risk (Lee et al. 2019; Walker et al. 2024). Therefore, the absence of germline P/LP variants may reflect current limitations in genetic testing and knowledge, rather than the true absence of hereditary or familial risk.

From a genetic counselling and risk management perspective, patients with no identified germline P/LP variants should continue to be managed according to their

residual clinical risk. Post-test counselling should address the limitations of genetic testing, clarify that negative results reduce but do not eliminate hereditary risk, and reinforce the importance of personal history, family history and tumour characteristics in guiding management. For patients with negative *BRCA1/2* but remain clinically high-risk, further multigene panel testing may be considered where available and affordable. For those with negative multigene panel results, ongoing surveillance and family counselling should still be guided by clinical and familial risk factors rather than genetic findings alone. This approach helps avoid false reassurance and supports more appropriate risk management for Malaysian EOBC patients.

5.6 Limitations of the Study

This study has several limitations. First, as this was a retrospective study, the analysis was dependent on the completeness and accuracy of existing clinical and genetic records. Some clinicopathological information was incomplete or unavailable, particularly among CRM cases as CRM mainly receives referrals for genetic counselling and testing rather than serving as the primary treating hospital. Consequently, complete clinical and histopathological records were not always available in CRM records. Although the available tumour characteristic data were sufficient for analysis, missing data for variables such as tumour grade, tumour stage and receptor status may have reduced statistical power and limited the precision of association estimates. Nevertheless, the available data were systematically extracted using a standardised data collection sheet to improve consistency across centres.

Second, the findings should be interpreted in the context of possible cohort selection bias. This study included only EOBC patients who had undergone germline genetic testing; therefore, the cohort may not fully represent all Malaysian patients with EOBC. Patients who are referred for or proceed with genetic testing may be more likely to have stronger hereditary cancer features, such as suggestive personal or family history, bilateral BC, TNBC, or greater clinician awareness and access to testing. Conversely, some eligible EOBC patients may not have undergone testing because of cost, limited test availability, personal preference, or other barriers. As a result, the observed P/LP yield may reflect the diagnostic yield among a clinically

tested EOBC cohort rather than the true prevalence of germline P/LP variants among all Malaysian EOBC patients. Nevertheless, the inclusion of multiple centres with different service settings provides a broader view of real-world genetic testing practice than would be captured in a single-centre study.

Third, the ethnic distribution of the cohort should be interpreted with caution. Chinese patients made up the largest proportion of the study population, partly because the majority of cases were contributed by CRM, with a higher proportion of Chinese patients. Therefore, ethnicity-related findings were interpreted cautiously and should not be overgeneralised to the wider Malaysian EOBC population.

Fourth, genetic testing methods were not uniform across all patients. The type of test, laboratory used, panel size and the number of genes analysed varied between centres and individual patients. Some patients underwent *BRCA*-only testing, while others had multigene panel testing of different sizes and gene content. These differences may affect direct comparison of diagnostic yield and VUS detection across testing strategies, as broader panels are more likely to detect both non-*BRCA* P/LP variants and VUS. In addition, gene-specific associations were not examined due to the small number of P/LP variants identified in non-*BRCA* genes. As such, the associations reported in this study reflect predictors of overall germline P/LP variant detection rather than gene-specific variant patterns.

Lastly, this study did not include longitudinal follow-up of clinical outcomes after genetic testing. Therefore, it was not possible to assess uptake of risk-reducing strategies, surveillance adherence, cascade testing among relatives, psychosocial outcomes, or VUS reclassification over time. These areas are important for understanding the broader clinical and genetic counselling impact of germline testing among Malaysian EOBC patients.

5.7 Strengths of the Study

This study has several strengths. First, it included a relatively large, multi-ethnic cohort of Malaysian patients with EOBC who underwent germline genetic testing. The inclusion of 725 patients comprising Malay, Chinese, Indian and other ethnic groups

provide a substantial local dataset for describing the spectrum of germline P/LP variants and evaluating their associations with clinicopathological characteristics. To our knowledge, this represents one of the largest Malaysian EOBC cohorts reporting germline genetic testing data beyond *BRCA1/2*, which is important given the limited local evidence on hereditary BC predisposition in this population.

Second, this was a multicentre study involving participating sites representing different service settings in Malaysia. HKL is a major public hospital and one of the main referral centres for genetic services in the country, while CRM receives referrals mainly from both public and private hospitals for cancer genetic counselling and testing. Together with HTABTAR, these centres allowed the study to capture variation in hereditary BC genetic testing practices across different healthcare settings.

Overall, these strengths support the value of this study in providing local, multicentre evidence on germline P/LP variants among Malaysian patients with EOBC, particularly in relation to genes beyond *BRCA1/2* and real-world genetic testing practices.

5.8 Recommendations For Future Research

Larger multicentre studies are needed to further define the contribution of non-*BRCA* genes among Malaysian patients with EOBC. In the present study, P/LP variants were identified in several non-*BRCA* genes, including *PALB2*, *ATM*, *TP53*, *CDH1*, *RAD51C* and *RAD51D*. However, the number of variants detected in each non-*BRCA* gene was small, limiting gene-specific analysis. Future studies with larger cohorts or gene-focused approaches could further evaluate genotype–phenotype associations and determine whether specific genes are associated with distinct clinicopathological features, tumour characteristics or familial cancer patterns.

Longitudinal studies are also needed to assess the clinical and genetic counselling impact of germline testing. Future research could evaluate uptake of enhanced surveillance, risk-reducing strategies, treatment-related decisions and cascade testing among relatives after positive genetic findings. In addition, monitoring

VUS reclassification over time would be valuable, particularly given the substantial proportion of VUS detected in this cohort.

Finally, collaborative efforts to develop Malaysian or Southeast Asian variant databases should be encouraged to improve ancestry-specific variant interpretation and reduce uncertainty in VUS classification. Such efforts are particularly relevant in multi-ethnic populations such as Malaysia, where local genetic data remain limited.



CHAPTER VI

CONCLUSION

6.1 Introduction

This study was undertaken to address the limited local data regarding the spectrum of germline P/LP variants in cancer predisposition genes, associated clinicopathological characteristics, and the diagnostic yield of different germline genetic testing approaches among Malaysian patients with EOBC. By addressing these research questions, the study aimed to generate local evidence to support hereditary BC risk assessment and optimise genetic testing strategies in Malaysia. This chapter concludes the study by synthesising the key findings, highlighting the study's contributions, and presenting the overall conclusions and implications for hereditary BC assessment among Malaysian patients with EOBC.

6.2 Summary of Key Findings

This study identified germline P/LP variants in 20% of EOBC patients in Malaysia, indicating that approximately one in five patients in this cohort had an underlying hereditary cancer predisposition. The identified P/LP variants were detected across 14 cancer predisposition genes, with *BRCA1* and *BRCA2* representing the predominant genetic contributors and accounting for the majority of positive findings. Nevertheless, P/LP variants were also identified in several non-*BRCA* genes, particularly *PALB2*, *ATM* and *TP53*, demonstrating that hereditary BC susceptibility among Malaysian EOBC patients is not limited to *BRCA1/2* alone. These findings highlight the genetic heterogeneity of EOBC and support the consideration of broader genetic evaluation beyond *BRCA1/2*.

This study also characterised the clinicopathological characteristics observed among EOBC patients with different germline P/LP variant profiles. *BRCA1*-associated variants were more frequently observed among patients with TNBC, higher tumour grade and younger age at diagnosis, whereas *BRCA2*-associated variants were more commonly identified among patients with HR-positive disease. Non-*BRCA* P/LP variants demonstrated diverse clinical presentations, including a higher proportion observed among Chinese patients in this cohort, reflecting the heterogeneous phenotypic spectrum of cancer predisposition genes beyond *BRCA1/2*.

In addition, several clinicopathological characteristics were associated with positive genetic findings, including personal history of other primary cancer, bilateral BC, and family history of cancer. After adjustment for potential confounding factors, TNBC, bilateral BC, and positive family history of cancer remained independent factors associated with germline P/LP variant detection. These findings suggest that tumour phenotype, disease presentation, and family cancer background are important indicators for identifying patients with a higher likelihood of carrying P/LP variants.

Comparison of genetic testing approaches demonstrated that multigene panel testing enables the identification of additional germline P/LP variants beyond *BRCA1/2*. Although the observed diagnostic yield was slightly higher in the *BRCA*-only testing group, this difference should be interpreted cautiously due to the predominant contribution of *BRCA1/2* variants and the potential for non-*BRCA* variants to remain undetected among patients undergoing *BRCA*-only testing. These findings highlight the clinical value of identifying non-*BRCA* variants as part of comprehensive hereditary cancer assessment, with implications for personalised risk assessment, clinical management and familial counselling. However, broader panel testing was also associated with increased VUS detection, emphasising the importance of appropriate pre-test counselling, careful variant interpretation, and comprehensive post-test management.

6.3 Contribution and Significance of The Study

This study contributes important local evidence to the field of hereditary BC genetics by characterising the spectrum of germline P/LP variants among Malaysian patients with EOBC. By addressing the limited understanding of germline susceptibility patterns in this cohort, particularly regarding the contribution of non-*BRCA* cancer predisposition genes, this study provides a more comprehensive characterisation of the genetic landscape underlying EOBC in Malaysia. The identification of P/LP variants across both *BRCA* and non-*BRCA* genes expands current knowledge of hereditary BC susceptibility and supports the consideration of a wider range of genes in hereditary cancer assessment.

The study findings may also be applied in clinical practice to support hereditary cancer risk assessment and referral for genetic testing. The identification of TNBC, bilateral BC and positive family history of cancer as independent predictors of germline P/LP variant detection may assist clinicians and genetic counsellors in recognising individuals with a higher likelihood of hereditary cancer predisposition. This is particularly relevant for patient selection and prioritisation of genetic testing, especially in settings where testing resources are limited. Therefore, this study supports a more comprehensive assessment that integrates age at diagnosis, tumour characteristics, disease presentation and family history when evaluating patients for germline genetic testing.

In addition, this study provides evidence to inform genetic testing strategies for hereditary BC assessment in Malaysia. The findings regarding the distribution of germline P/LP variants across both *BRCA* and non-*BRCA* cancer predisposition genes provide valuable information for guiding the design and selection of appropriate multigene panels. Furthermore, these findings may support healthcare planning, including resource allocation, funding considerations and the development of accessible genetic testing services to facilitate comprehensive hereditary cancer assessment among EOBC patients in Malaysia.

This study also highlights the impact of germline genetic findings on genetic counselling practice. Identification of germline P/LP variants can guide personalised risk management, including surveillance, risk-reducing strategies, treatment considerations and cascade testing for at-risk relatives. This is particularly relevant for EOBC patients, who may face unique survivorship considerations, including longer-term health management, family planning decisions and psychosocial challenges. Furthermore, the detection of VUS through broader genetic testing approaches emphasises the importance of pre-test counselling, careful variant interpretation and residual risk assessment among patients with uncertain or negative findings. These findings reinforce the role of genetic counsellors in supporting informed decision-making and integrating genetic information into patient and family care.

6.4 Concluding Remarks

In conclusion, this study provides a comprehensive characterisation of the germline genetic landscape of hereditary BC among Malaysian patients with EOBC and demonstrates the clinical relevance of integrating genetic findings with clinicopathological characteristics in hereditary cancer assessment. The findings strengthen the evidence supporting genetic evaluation in this high-risk population and underscore the importance of adopting a comprehensive approach to genetic testing, risk assessment and genetic counselling. Collectively, this study provides a foundation for improving hereditary BC care in Malaysia and contributes valuable evidence to support future advances in clinical practice, genetic testing strategies and healthcare service development for patients with EOBC and their families.

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APPENDIX A

LIST OF PATHOGENIC / LIKELY PATHOGENIC VARIANTS

Sr No.	Study ID	Gene	Variant details	Molecular consequences	Variants classification
1	A003	<i>BRCA1</i>	c.4508C>A (p.Ser1503*)	Nonsense	Pathogenic
2	A004	<i>BRCA1</i>	c.5074+3A>G (Intronic)	Splice site	Likely Pathogenic
3	A020	<i>BRCA1</i>	c.2635G>T (p.Glu879*)	Nonsense	Pathogenic
4	A032	<i>BRCA1</i>	c.2726dup (p.Asn909Lysfs*6)	Frameshift (CNV-dup)	Pathogenic
5	A036	<i>BRCA1</i>	c.213-12A>G (Intronic)	Splice site	Pathogenic
6	A041	<i>BRCA1</i>	c.2059C>T (p.Gln687*)	Nonsense	Pathogenic
7	A044	<i>BRCA1</i>	c.3770_3771del (p.Glu1257Glyfs*9)	Frameshift (CNV-del)	Pathogenic
8	A048	<i>BRCA1</i>	c.1961dup (p.Tyr655Valfs*18)	Frameshift (CNV-dup)	Pathogenic
9	A052	<i>BRCA1</i>	c.3214del (p.Leu1072*)	Deletion	Pathogenic
10	A053	<i>BRCA1</i>	c.66dup (p.Glu23Argfs*18)	Frameshift (CNV-dup)	Pathogenic
11	A072	<i>BRCA1</i>	c.541G>T (p.Glu181*)	Nonsense	Pathogenic
12	A079	<i>BRCA1</i>	Exons 20-22 (deletion)	Deletion	Pathogenic
13	A092	<i>BRCA1</i>	c.4467del (p.Glu1490Asnfs*15)	Frameshift (CNV-del)	Pathogenic
14	A109	<i>BRCA1</i>	Deletion (Exon12)	Deletion	Pathogenic
15	A133	<i>BRCA1</i>	c.5072C>A (p.Thr169Lys)	Missense	Likely Pathogenic
16	A155	<i>BRCA1</i>	c.3499del (p.Glu1167Lysfs*43)	Frameshift (CNV-del)	Pathogenic

17	A162	<i>BRCA1</i>	c.3333del (p.Glu1112Asnfs*5)	Frameshift (CNV-del)	Pathogenic
18	A180	<i>BRCA1</i>	c.68_69del (p.Glu23Valfs*17)	Frameshift (CNV-del)	Pathogenic
19	A230	<i>BRCA1</i>	c.2110_2111del (p.Asn704Cysfs*7)	Frameshift (CNV-del)	Pathogenic
20	A247	<i>BRCA1</i>	c.3083del (p.Arg1028Leufs*20)	Frameshift (CNV-del)	Pathogenic
21	A254	<i>BRCA1</i>	c.1961dup (p.Tyr655Valfs*18)	Frameshift (CNV-dup)	Pathogenic
22	A265	<i>BRCA1</i>	Exons 20-22	Deletion	Pathogenic
23	A300	<i>BRCA1</i>	c.182G>A (p.Cys61Tyr)	Missense	Pathogenic
24	A354	<i>BRCA1</i>	c.5521A>C (p.Ser1841Arg)	Missense	Likely Pathogenic
25	A365	<i>BRCA1</i>	Exons 1-15	Deletion	Pathogenic
26	A373	<i>BRCA1</i>	Exons 16-19	Deletion	Pathogenic
27	A375	<i>BRCA1</i>	c.3214del (p.Leu1072*)	Nonsense	Pathogenic
28	A398	<i>BRCA1</i>	c.5153-1G>A (Splice acceptor)	Splice site	Pathogenic
29	A422	<i>BRCA1</i>	c.4999A>T (p.Lys1667*)	Nonsense	Pathogenic
30	A428	<i>BRCA1</i>	c.213-12A>G (Intronic)	Splice site	Pathogenic
31	B019	<i>BRCA1</i>	c.2766del (p.Val1923LeufsTer77)	Frameshift	Pathogenic
32	B035	<i>BRCA1</i>	c.5328dup (p.Thr1777Hisfs*53)	Frameshift (CNV-dup)	Pathogenic
33	B042	<i>BRCA1</i>	c.5335del (p.Gln1779AsnfsTer14)	Frameshift (CNV-del)	Pathogenic
34	B053	<i>BRCA1</i>	c.1204G>T (p.Glu402Ter)	Nonsense	Pathogenic
35	B058	<i>BRCA1</i>	c.3607C>T (p.Arg1203*)	Nonsense	Pathogenic
36	B076	<i>BRCA1</i>	c.3856del (p.Ser1286ValfsTer21)	Frameshift (CNV-del)	Pathogenic

37	B084	<i>BRCA1</i>	c.4467_4470del (p.Lys1489Asnfs*15)	Frameshift (CNV-del)	Pathogenic
38	B085	<i>BRCA1</i>	c.5328dup (p.Thr1777HisfsTer53)	Frameshift (CNV-dup)	Pathogenic
39	B091	<i>BRCA1</i>	c.4689C>G (p.Tyr1563Ter)	Nonsense	Pathogenic
40	B092	<i>BRCA1</i>	c.5509T>C (p.Trp1837Arg)	Missense	Pathogenic
41	B111	<i>BRCA1</i>	c.3607C>T (p.Arg1203Ter)	Nonsense	Pathogenic
42	B130	<i>BRCA1</i>	c.5328dup (p.Thr1777HisfsTer53)	Frameshift (CNV-dup)	Pathogenic
43	B141	<i>BRCA1</i>	c.205pC>T (p.Gln687Ter)	Nonsense	Pathogenic
44	B145	<i>BRCA1</i>	c.2398_2401 (p.Lys800Valfs*2)	Frameshift (CNV-del)	Pathogenic
45	B149	<i>BRCA1</i>	c.709G>T (p.Glu237*)	Nonsense	Pathogenic
46	B156	<i>BRCA1</i>	c.3856del (p.Ser1286Valfs*21)	Frameshift (CNV-del)	Pathogenic
47	B169	<i>BRCA1</i>	c.598G>T (p.Gly200Ter)	Nonsense	Likely Pathogenic
48	B178	<i>BRCA1</i>	c.470_471del (p.Ser157Ter)	Frameshift (CNV-del)	Pathogenic
49	B180	<i>BRCA1</i>	c.1115T>C (p.Cys39Arg)	Missense	Pathogenic
50	B186	<i>BRCA1</i>	c.3893C>A (p.Ser1298*)	Nonsense	Pathogenic
51	B187	<i>BRCA1</i>	c.3856del (p.Ser1286Valfs*21)	Frameshift (CNV-del)	Pathogenic
52	B201	<i>BRCA1</i>	c.5328dupC (p.Thr1777HisfsTer53)	Frameshift (CNV-dup)	Pathogenic
53	B219	<i>BRCA1</i>	c.68_69del (p.Glu23Valfs*17)	Frameshift (CNV-del)	Pathogenic
54	B220	<i>BRCA1</i>	c.5251C>T (p.Arg1751Ter)	Nonsense	Pathogenic
55	B224	<i>BRCA1</i>	c.3381T>A (p.Tyr1127Ter)	Nonsense	Pathogenic
56	B227	<i>BRCA1</i>	c.5213_5215delGAG (p.Gly1738del)	Frameshift (CNV-del)	Likely Pathogenic

57	B232	<i>BRCA1</i>	c.68_69delAG (p.Glu23ValfsTer17)	Frameshift (CNV-del)	Pathogenic
58	B247	<i>BRCA1</i>	c.150del (p.Lys50Asnfs*19)	Frameshift (CNV-del)	Pathogenic
59	B256	<i>BRCA1</i>	c.5095_5105del (p.Ard1699IlefsTer13)	Frameshift (CNV-del)	Pathogenic
60	B261	<i>BRCA1</i>	Deletion (Exons 20-21)	Deletion	Pathogenic
61	A009	<i>BRCA2</i>	c.3957_3958del (p.Asn1319Lysfs*3)	Frameshift (CNV-del)	Pathogenic
62	A017	<i>BRCA2</i>	c.3002C>G (p.Ser1001*)	Nonsense	Pathogenic
63	A084	<i>BRCA2</i>	c.1796_1800del (p.Ser599*)	Nonsense	Pathogenic
64	A095	<i>BRCA2</i>	c.262_263del (p.Leu88Alafs*)	Frameshift (CNV-del)	Pathogenic
65	A113	<i>BRCA2</i>	c.480_487delinsGGGG (p.Cys161Glyfs*10)	Nonsense	Pathogenic
66	A158	<i>BRCA2</i>	c.1454delinsTGTATT (p.Lys485Metfs*26)	Nonsense	Pathogenic
67	A159	<i>BRCA2</i>	c.7878G>A (p.Trp2626*)	Nonsense	Pathogenic
68	A167	<i>BRCA2</i>	c.3922G>T (p.Glu1308*)	Nonsense	Pathogenic
69	A172	<i>BRCA2</i>	c.1813dup (p.Ile605Asnfs*11)	Frameshift (CNV-dup)	Pathogenic
70	A177	<i>BRCA2</i>	c.3125T>A (p.Leu1042*)	Nonsense	Pathogenic
71	A208	<i>BRCA2</i>	c.2808_2811del (p.Ala938Profs*21)	Frameshift (CNV-del)	Pathogenic
72	A223	<i>BRCA2</i>	c.6591_6592del (p.Glu2198Asnfs*4)	Frameshift (CNV-del)	Pathogenic
73	A234	<i>BRCA2</i>	c.7976+5G>A (intronic)	Splice site	Likely Pathogenic
74	A235	<i>BRCA2</i>	c.3125del (p.Leu1042*)	Frameshift (CNV-del)	Pathogenic
75	A244	<i>BRCA2</i>	c.5967dup (p.Asp1990Argfs*13)	Frameshift (CNV-dup)	Pathogenic
76	A245	<i>BRCA2</i>	c.9117G>A (Silent)	Silence	Pathogenic

77	A266	<i>BRCA2</i>	c.6152del (p.Asn2051Ilefs*19)	Frameshift (CNV-del)	Pathogenic
78	A268	<i>BRCA2</i>	c.5164_5165del (p.Ser1722Tyrfs*4)	Frameshift (CNV-del)	Pathogenic
79	A279	<i>BRCA2</i>	c.9097del (p.Thr3033Leufs*29)	Frameshift (CNV-del)	Pathogenic
80	A296	<i>BRCA2</i>	c.2808_2811del (p.Ala938Profs*21)	Frameshift (CNV-del)	Pathogenic
81	A299	<i>BRCA2</i>	c.2808_2811del (p.Ala938Profs*21)	Frameshift (CNV-del)	Pathogenic
82	A306	<i>BRCA2</i>	c.3267_3268del (p.Gln1089Hisfs*9)	Frameshift (CNV-del)	Pathogenic
83	A335	<i>BRCA2</i>	Exons 22-24	Deletion	Pathogenic
84	A352	<i>BRCA2</i>	c.7999del (p.Ser2667Alafs*6)	Frameshift (CNV-del)	Pathogenic
85	A366	<i>BRCA2</i>	Exons 14-16	Deletion	Pathogenic
86	A380	<i>BRCA2</i>	c.6450dup (p.Val2151Serfs*25)	Frameshift (CNV-dup)	Pathogenic
87	A382	<i>BRCA2</i>	c.2808_2811del (p.Ala938Profs*21)	Frameshift (CNV-del)	Pathogenic
88	A384	<i>BRCA2</i>	c.5967dup (p.Asp1990Argfs*13)	Frameshift (CNV-dup)	Pathogenic
89	A385	<i>BRCA2</i>	c.262_263del (p.Leu88Alafs*12)	Frameshift (CNV-del)	Pathogenic
90	A394	<i>BRCA2</i>	c.7880T>A (p.Ile2627Asn)	Missense	Likely Pathogenic
91	A438	<i>BRCA2</i>	c.3680_3681del (p.Leu1227Glnfs*5)	Frameshift (CNV-del)	Pathogenic
92	A440	<i>BRCA2</i>	c.3109C>T (p.Gln1037*)	Nonsense	Pathogenic
93	A442	<i>BRCA2</i>	c.6468_6469del (p.Gln2157Ilefs*18)	Frameshift (CNV-del)	Pathogenic
94	B025	<i>BRCA2</i>	c.5967dup (p.Asp1990ArgfsTer13)	Frameshift (CNV-dup)	Pathogenic
95	B032	<i>BRCA2</i>	c.6778G>T (p.Glu2260Ter)	Nonsense	Likely Pathogenic
96	B034	<i>BRCA2</i>	c.5967dup (p.Asp1990Argfs*13)	Frameshift (CNV-dup)	Pathogenic

97	B067	<i>BRCA2</i>	c.5595_5596del (p.Phe1866TyrfsTer6)	Frameshift (CNV-del)	Pathogenic
98	B071	<i>BRCA2</i>	c.7234del (p.Thr2412LeufsTer55)	Frameshift (CNV-del)	Pathogenic
99	B079	<i>BRCA2</i>	c.262_263del (p.Leu88AlafsTer12)	Frameshift (CNV-del)	Pathogenic
100	B094	<i>BRCA2</i>	c.9380G>A (p.Trp3127Ter)	Nonsense	Pathogenic
101	B102	<i>BRCA2</i>	c.5576_5579del (p.Ile1859Lysfs*3)	Frameshift (CNV-del)	Pathogenic
102	B121	<i>BRCA2</i>	c.5967dup (Asp1990ArgfsTer13)	Frameshift (CNV-dup)	Pathogenic
103	B124	<i>BRCA2</i>	2.33_23del (p.Arg8AlafsTer5)	Frameshift (CNV-del)	Pathogenic
104	B133	<i>BRCA2</i>	c.682-2A>G	Splice site	Pathogenic
105	B151	<i>BRCA2</i>	c.2339C>G (p.Ser780Ter)	Nonsense	Pathogenic
106	B155	<i>BRCA2</i>	c.262_263del (p.Leu88AlafsTer12)	Frameshift (CNV-del)	Pathogenic
107	B165	<i>BRCA2</i>	c.5482_5486del (p.Lys1828ValfsTer4)	Frameshift (CNV-del)	Pathogenic
108	B183	<i>BRCA2</i>	c.5967dupA (p.Asp1990ArgfsTer13)	Frameshift (CNV-dup)	Pathogenic
109	B225	<i>BRCA2</i>	c.4872del (p.Tyr1625Ilefs*8)	Frameshift (CNV-del)	Pathogenic
110	B229	<i>BRCA2</i>	c.4037_4038del (p.Thr1346Serfs*5)	Frameshift (CNV-del)	Pathogenic
111	B249	<i>BRCA2</i>	c.3109C>T (p.Gln1037*)	Nonsense	Pathogenic
112	B257	<i>BRCA2</i>	c.8210_8219delinsG (p.Leu2737Ter)	Nonsense	Likely Pathogenic
113	C008	<i>BRCA2</i>	c.2808_2811del (p.Ala938Profs*21)	Frameshift (CNV-del)	Pathogenic
114	C009	<i>BRCA2</i>	c.2808_2811del (p.Ala938Profs*21)	Frameshift (CNV-del)	Pathogenic
115	A019	<i>PALB2</i>	c.426_428delins	Deletion-Insertion	Pathogenic
116	A065	<i>PALB2</i>	c.2336C>G (p.Ser779*)	Nonsense	Pathogenic

117	A186	<i>PALB2</i>	c.3469C>T (p.Gln1157*)	Nonsense	Pathogenic
118	A225	<i>PALB2</i>	c.3267_3268del (p.Phe1090Serfs*6)	Frameshift (CNV-del)	Pathogenic
119	A326	<i>PALB2</i>	c.538G>T (p.Glu180*)	Nonsense	Pathogenic
120	A374	<i>PALB2</i>	c.1400del (p.Gly467Alafs*18)	Frameshift (CNV-del)	Pathogenic
121	A436	<i>PALB2</i>	c.2607del (p.Val870*)	Nonsense	Pathogenic
122	B001	<i>PALB2</i>	c.658_659del (p.Ser220Cysfs*14)	Frameshift (CNV-del)	Pathogenic
123	A005	<i>ATM</i>	c.7307+1G>T (splice donor)	Splice site	Likely Pathogenic
124	A151	<i>ATM</i>	c.3732del (p.Ile1244Metfs*12)	Frameshift (CNV-del)	Pathogenic
125	A308	<i>ATM</i>	c.5692C>T (p.Arg1898*)	Nonsense	Pathogenic
126	A359	<i>ATM</i>	c.7928-2A>G (Splice acceptor)	Splice site	Likely Pathogenic
127	B254	<i>ATM</i>	c.9139C>T (p.Arg3047*)	Nonsense	Pathogenic
128	C010	<i>ATM</i>	c.6913C>T (p.Gln2305*)	Nonsense	Pathogenic
129	A040	<i>TP53</i>	c.375+1G>A (splice donor)	Splice site	Pathogenic
130	A272	<i>TP53</i>	c.464C>A (p.Thr155Asn)	Missense	Pathogenic
131	A308	<i>TP53</i>	c.304del (p.Thr102Profs*21)	Frameshift	Pathogenic
132	B103	<i>TP53</i>	Deletion (Exon10)	Deletion	Pathogenic
133	B211	<i>TP53</i>	c.722C>A (p.Ser241Tyr)	Missense	Pathogenic
134	A314	<i>CDH1</i>	c.2194C>T (p.Arg732Trp)	Missense	Likely Pathogenic
135	A389	<i>CDH1</i>	c.1003C>T (p.Arg335*)	Nonsense	Pathogenic
136	C007	<i>CDH1</i>	c.1003C>T (p.Arg335*)	Nonsense	Pathogenic

137	A174	<i>RAD51C</i>	c.934C>T (p.Arg312Trp)	Missense	Likely Pathogenic
138	A283	<i>RAD51C</i>	c.394dup (p.Thr132Asnfs*23)	Frameshift (CNV-dup)	Pathogenic
139	A288	<i>RAD51C</i>	c.394dup (p.Thr132Asnfs*23)	Frameshift (CNV-dup)	Pathogenic
140	B004	<i>MUTYH</i>	c.325C>T (p.Arg109Trp)	Missense	Pathogenic
141	C012	<i>MUTYH</i>	c.1435G>T (p.Glu479*)	Nonsense	Pathogenic
142	A319	<i>RAD51D</i>	c.270_271dup (p.Lys91Ilefs*13)	Frameshift (CNV-dup)	Pathogenic
143	A436	<i>RAD51D</i>	c.270_271dup (p.Lys91Ilefs*13)	Frameshift	Pathogenic
144	A258	<i>BARD1</i>	c.1904-1G>A (splice acceptor)	Splice site	Pathogenic
145	A310	<i>BLM</i>	c.320dup (p.Leu107Phefs*36)	Frameshift (CNV-dup)	Pathogenic
146	A314	<i>MSH6</i>	c.1509_1615del (p.Lys504Serfs*2)	Frameshift (CNV-del)	Pathogenic
147	A392	<i>LZTR1</i>	c.1768C>T (p.Gln590*)	Nonsense	Pathogenic
148	B055	<i>PTEN</i>	c.1003C>T(p.Arg335*)	Nonsense	Pathogenic

APPENDIX B

DATA COLLECTION SHEET

Demographic and Clinical Data								
No	Study ID	Study Site	Gender	Ethnicity	Laterality	Age Onset	Personal History of Other Primary Cancers (If yes, specify)	Parity

Family History of cancers					Pathological Data			
Family history of cancers	Breast Cancer	Ovarian Cancer	Pancreatic Cancer	Prostate Cancer	Tumour Histology	Tumour Grade	Tumour Stage	Hormone Receptors Status

Molecular Data					Genetic Testing				
Genetic Results	Gene Identified	Variant Classification	Molecular Consequences	Variant Details	Testing Method	Name of Testing	Number of Genes Tested	Testing Lab	Year of Testing

APPENDIX C

JEPUKM ETHICS APPROVAL



SEKRETARIAT ETIKA PENYELIDIKAN UKM • UKM RESEARCH ETHICS SECRETARIAT

Reference: UKM PPI/111/8

Date : 07 January 2026

Associate Professor Dr. Adli Ali
Department of Paediatrics
Faculty of Medicine UKM

Dear Professor/Datuk/Dato'/Datin/Sir/Madam,

ETHICAL APPROVAL TO CONDUCT RESEARCH IN THE NATIONAL UNIVERSITY OF MALAYSIA

Research title : Spectrum Of Germline Pathogenic Variants In Early-Onset Breast Cancer: A Multicentre Retrospective Study In Malaysia

Ethics Ref. No. : JEP-2025-746

Approval period : 31 December 2025 – 30 February 2027

Study Site : 1. Hospital Canselor Tuanku Muhriz
2. Cancer Research Malaysia (CRM)
3. Hospital Kuala Lumpur (HKL)
4. UKM Specialist Children's Hospital (HPPK)

Sample Size : N/A

With reference to the above,

2. The Research Ethics Committee (Health and Life Sciences), The National University of Malaysia (RECUKM) has provided ethical approval for above study. Please be reminded permission from the Deputy Dean of research of the faculty or Director of Institute/Centre and all relevant heads of departments / units where the study will be carried out must be obtained prior to the study. You are required to follow and comply with their decision and all other relevant regulations.

3. Please note that the investigator's responsibility is to ensure that:

- i. All Adverse Events should be reported to RECUKM as soon as possible.
- ii. Progress report should be submitted every 6 Months.
- iii. All changes / amendments to the study documents / study sites / study team must be notified to the RECUKM. All changes must be approved by RECUKM before continuation of the study.
- iv. Application for renewal of the approval has to be submitted to RECUKM within one month (1 month) prior to the expiry of ethical approval.
- v. Final Report should be provided to RECUKM when the project is complete.
- vi. Please take note that all records and data are to be kept strictly CONFIDENTIAL and can only be used for the purpose of this study. All precautions are to be taken to maintain data confidentiality.

Required forms can be obtained from the The Research Ethics Committee (Health and Life Sciences), The National University of Malaysia (RECUKM) website: <https://www.ukm.my/jepukm>

Thank you.

Sekretariat Etika Penyelidikan Universiti Kebangsaan Malaysia
Tingkat 1, Blok Klinik, Hospital Canselor Tuanku Muhriz, Pusat Perubatan UKM, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras Kuala Lumpur.
Telefon: +603-9145 5046 / 5048
Email: sepukm@uam.edu.my Web: <https://www.ukm.my/jepukm/>

ILMU, MUTU DAN BUDI



SEKRETARIAT ETIKA PENYELIDIKAN UKM • UKM RESEARCH ETHICS SECRETARIAT

Yours sincerely,

PROFESOR DR MOHD SHAHRIR MOHAMED SAID
Chairman
Research Ethics Committee (Health and Life Sciences)
The National University Of Malaysia

- c.c.
- **Director**
Hospital Canselor Tuanku Muhriz
 - **Deputy Dean (Research & Innovation)**
Faculty of Medicine, UKM
 - **Head Department of Paediatrics**
Faculty of Medicine UKM
 - **Mrs. Sharifah Azween Syed Omar**
 - **Foong Le Hui (P148740 – Master Candidate)**
Department of Paediatrics
Faculty of Medicine UKM
 - **Dr. Winnie Ong Peitee**
Hospital Kuala Lumpur
 - **Ms. Yoon Sook-Yee**
Cancer Research Malaysia
 - **Approval letter FHS 2025**



Sekretariat Etika Penyelidikan Universiti Kebangsaan Malaysia
Tingkat 1, Blok Klinikal, Hospital Canselor Tuanku Muhriz, Pusat Perubatan UKM, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras Kuala Lumpur.
Telefon: +603-9145 5046 / 5048
Email: sepukm@ukm.edu.my Web: <https://www.ukm.my/sepukm/>

ILMU, MUTU DAN BUDI



SEKRETARIAT ETIKA PENYELIDIKAN UKM • UKM RESEARCH ETHICS SECRETARIAT

NAME OF ETHICS COMMITTEE/IRB: Research Ethics Committee, The National University of Malaysia	ETHICS COMMITTEE/IRB REF NO : JEP-2025-746
PROTOCOL TITLE: Spectrum Of Germline Pathogenic Variants In Early-Onset Breast Cancer: A Multicentre Retrospective Study In Malaysia	
PRINCIPAL INVESTIGATOR: Associate Professor Dr. Adli Ali Department of Paediatrics Faculty of Medicine UKM	

The following items ☒ have been received and reviewed in connection with the above study to be conducted by the above investigator.

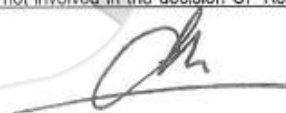
Documents

- ☒ Research Application Form (UKM-JEP-BO01)
☒ Review Form (UKM-JEP-BO02)
☒ Non-Disclosure Agreement (UKM-JEP-BO03)
☒ Conflict Of Interest Form
☒ Research Proposal / Protocol
☐ Participant Information Sheet:
☐ Malay ☐ English ☐ Others :
☐ Participant Consent Form:
☐ Malay ☐ English ☐ Others :
☐ Questionnaire:
☐ Malay ☐ English ☐ Others :
☒ Curriculum Vitae of Researchers:
☒ Principal ☒ Co-researcher ☒ Student
☒ Good Clinical Practice Certificate (GCP)
☐ Project Agreement

The Research Ethics Committee (Health and Life Sciences), The National University of Malaysia operates in accordance to the International Conference of Harmonization Good Clinical Practice Guidelines.

Comments (if any): Associate Professor Dr. Adli Ali is the principal investigator for this study and also Member Of Research Ethics Committee. He strictly was not involved in the decision Of Research Ethics Committee to approve this study.

Date of Approval: 31 December 2025


PROFESSOR DR. MOHD SHAHRIR MOHAMED SAID
 Chairman
 Research Ethics Committee (Health and Life Sciences)
 The National University of Malaysia

APPENDIX D

NMRR / MREC ETHICS APPROVAL



JAWATANKUASA ETIKA & PENYELIDIKAN PERUBATAN
(Medical Research & Ethics Committee)
KEMENTERIAN KESIHATAN MALAYSIA
 d/a Kompleks Institut Kesihatan Negara
 Blok A, No 1, Jalan Setia Murni U13/52,
 Seksyen U13, Bandar Setia Alam,
 40170 Shah Alam, Selangor.



Tel: 03-3362 8888/8205

Ruj.Kami: 25-03410-JK6 (1)
 Tarikh : 27-November-2025

AP ADLI BIN ALI
PUSAT PERUBATAN UNIVERSITI KEBANGSAAN MALAYSIA (PPUKM)

Dr/ Tuan/ Puan,

ETHICS INITIAL APPROVAL: NMRR ID: 25-03410-JK6
SPECTRUM OF GERMLINE PATHOGENIC VARIANTS IN EARLY-ONSET BREAST CANCER: A
MULTICENTRE RETROSPECTIVE STUDY IN MALAYSIA

Dengan hormatnya perkara di atas adalah dirujuk.

2. Bersama dengan surat ini dilampirkan surat kelulusan saintifik dan etika bagi projek ini. Segala rekod dan data subjek adalah SULIT dan hanya digunakan untuk tujuan kajian dan semua isu serta prosedur mengenai data confidentiality mesti dipatuhi. Kebenaran daripada Pengarah Hospital / Institusi di mana kajian akan dijalankan mesti diperolehi terlebih dahulu sebelum kajian dijalankan. Dato' / Tuan / Puan perlu akur dan mematuhi keputusan tersebut dan undang-undang lain yang berkaitan.

3. Penyelidik- penyelidik dan lokasi kajian yang terlibat ialah:

HOSPITAL PAKAR KANAK-KANAK UKM

AP Adli Bin Ali (Principal / Coordinating Investigator)
 Foong Le Hui (Co / Sub Investigator at the site)
 Sharifah Azween Binti Syed Omar (Co / Sub Investigator at the site)
 Shyahlini Devi A/P Rajendran (Co / Sub Investigator at the site)

HOSPITAL KUALA LUMPUR

Winnie Ong Peitee (Principal Investigator at the site)

CANCER RESEARCH MALAYSIA

Yoon Sook-Yee (Principal Investigator at the site)

4. Dokumen kajian yang berikut telah diterima dan dinilai:

Documents received and reviewed with reference to the above study:

1. Study Protocol Version 2.0, dated 20-November-2025
2. Cover Letter to MREC Version 2.0, dated 20-November-2025
3. Declaration of Conflict of Interest (COI form) Version 2.1, dated 11-August-2025
4. Clinical Form Report / Data Collection Form Version 2.1, dated 20-November-2025
5. Investigator's documents : Declaration of Conflict of Interest (COI), IA-HOD-IA, and CV;
 - a) AP Adli Bin Ali (Principal / Coordinating Investigator)
 - b) Winnie Ong Peitee (Principal Investigator at the site)
 - c) Yoon Sook-Yee (Principal Investigator at the site)
 - d) Foong Le Hui (Co / Sub Investigator at the site)
 - e) Sharifah Azween Binti Syed Omar (Co / Sub Investigator at the site)
 - f) Shyahlini Devi A/P Rajendran (Co / Sub Investigator at the site)

5. Adalah dimaklumkan bahawa kelulusan ini adalah sah sehingga **26-November-2026**. Tuan/Puan perlu menghantar dokumen-dokumen seperti berikut selepas mendapat kelulusan etika. Borang-borang berkaitan boleh dimuat turun daripada laman web *National Medical Research Registry (NMRR)*.
 - i. *Continuing Review Form* selewat-lewatnya dalam tempoh 2 bulan (60 hari) sebelum tamat tempoh kelulusan ini bagi memperbaharui kelulusan etika.
 - ii. *Study Final Report* pada penghujung kajian.
 - iii. Mendapat kelulusan etika sekiranya terdapat pindaan ke atas sebarang dokumen kajian / lokasi kajian / penyelidik. Pihak JEPP mempunyai hak untuk menarik balik kelulusan etika sekiranya terdapat perubahan dokumen kajian yang tidak diisytiharkan.
6. Kajian tersebut hanya melibatkan pengumpulan data melalui:
 - i. Retrospektif
 - ii. Rekod perubatan
7. Sila ambil maklum bahawa sebarang urusan surat-menyurat berkaitan dengan penyelidikan ini haruslah dinyatakan nombor rujukan surat ini untuk melicinkan urusan yang berkaitan.

Sekian terima kasih.

Komen (Jika ada) : NIL

Lokasi Kajian:

HOSPITAL PAKAR KANAK-KANAK UKM
HOSPITAL KUALA LUMPUR
CANCER RESEARCH MALAYSIA

Keputusan Jawatankuasa Etika dan Penyelidikan Perubatan:

(☒) Lulus
(☐) Tidak Lulus

Tarikh kelulusan etika : 27-November-2025

"MALAYSIA MADANI"

"BERKHIDMAT UNTUK NEGARA"

Saya yang menjalankan amanah,


DR NURAIN BINTI MOHD NOOR
Pengerusi
Jawatankuasa Etika & Penyelidikan Perubatan
Kementerian Kesihatan Malaysia
No. MPM: 31576

//Approval2024/Mrecshare