POLYMORPHISMS IN ESTROGEN METABOLIZING GENES AND THEIR ASSOCIATION WITH ESTROGEN RECEPTOR-POSITIVE BREAST CANCER AMONG PATIENTS ATTENDING AGA KHAN UNIVERSITY HOSPITAL NAIROBI, AND AFRICA INLAND CHURCH HOSPITAL KIJABE

MARY KANYIRI MURITHI

DOCTOR OF PHILOSOPHY IN BIOCHEMISTRY

JOMO KENYATTA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY

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Polymorphisms in Estrogen Metabolizing Genes and Their Association with Estrogen Receptor-Positive Breast Cancer among patients attending Aga Khan University Hospital Nairobi, and Africa Inland Church Hospital Kijabe

Mary Kanyiri Murithi

A Thesis Submitted in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy in Biochemistry of the Jomo Kenyatta University of Agriculture and Technology

2026

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

Signature……Date……...

Mary Kanyiri Murithi

This thesis has been submitted for examination with our approval as University Supervisors:

Signature……Date……...

Dr. Steven Ger Nyanjom, PhD

JKUAT, Kenya

Signature……Date……...

Dr. Francis Makokha, PhD

MKU, Kenya

Signature……Date……...

Dr. Victor Mobegi, PhD

UoN, Kenya

Signature……Date……...

Dr. Sayed Shahin, PhD

Aga Khan University Hospital, Kenya

DEDICATION

I dedicate this thesis to my beloved parents, Stephen Murithi M'Ngaruthi and Esther Mwari Murithi, whose love, wisdom, and sacrifices have shaped the person I am today. Your unwavering belief in me has been my greatest source of strength.

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ACRONYMS AND ABBREVIATIONS

A	Adenine
AAM	Age at Menarche
AIs	Aromatase Inhibitors
AKUHN	Aga Khan University Hospital Nairobi
Ala	Alanine
Arg	Arginine
ASIR	Age-Standardized Incidence Rate
Asn	Asparagine
ATM	Ataxia-Telangiectasia Mutated
BARD1	BRCA1 Associated RING Domain 1
BC	Breast Cancer
BCS	Breast-Conserving Surgery
BBD	Benign Breast Disease
BMI	Body Mass Index
BRCA1	Breast Cancer Gene 1
BRCA2	Breast Cancer Gene 2
BRIP1	BRCA1 Interacting Protein C-Terminal Helicase 1
C	Cytosine
CBE	Clinical Breast Examination
CDH1	Cadherin-1
CHEK2	Checkpoint Kinase 2
CI	Confidence Interval
COMT	Catechol-O-Methyltransferase
CT	Computed Tomography

CYP	Cytochrome P450
CYP1A1	Cytochrome P450 Family 1 Subfamily A Member 1
CYP1B1	Cytochrome P450 Family 1 Subfamily B Member 1
CYP3A5	Cytochrome P450 Family 3 Subfamily A Member 5
DCIS	Ductal Carcinoma In Situ
df	Degrees of Freedom
DNA	Deoxyribonucleic Acid
ER+	Estrogen Receptor-Positive
ER+	Estrogen Receptor-Positive
FFPE	Formalin-Fixed Paraffin-Embedded
FISH	Fluorescence In Situ Hybridization
G	Guanine
GSTs	Glutathione S-transferases
HAAs	Heterocyclic Aromatic Amines
HDI	Human Development Index
HER2+	Human Epidermal Growth Factor Receptor 2 Positive
IARC	International Agency for Research on Cancer
IC	Invasive Carcinoma
IDC	Invasive Ductal Carcinoma
IHC	Immunohistochemistry
ILC	Invasive Lobular Carcinoma
Ile	Isoleucine
IMCS	Internal Mammary Chain Sentinel Node
IGF-1	Insulin-Like Growth Factor-1
IQR	Interquartile Range
IPC	Intracystic Papillary Carcinoma

KAIC	Africa Inland Church Hospital Kijabe
LD	Linkage Disequilibrium
Leu	Leucine
Lnlike	Log-Likelihood
LR	Likelihood Ratio
LVI	Lymphovascular Invasion
MAPK	Mitogen-Activated Protein Kinase ;pathway
MEGA	Molecular Evolutionary Genetics Analysis
Met	Methionine
MC	Mucinous Carcinoma
MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribonucleic Acid
NATs	N-acetyltransferases
NST	Invasive Breast Cancer of No Special Type
OR	Odds Ratio
PAHs	Polycyclic Aromatic Hydrocarbons
PALB2	Partner and Localizer of BRCA2
PDCIS	Papillary Ductal Carcinoma In Situ
PCR-RFLP	Polymerase Chain Reaction-Restriction Fragment Length Polymorphism
PET	Positron-Emission Tomography
PTEN	Phosphatase and Tensin Homolog
RAS	Renin-Angiotensin System
rÂ²	Correlation Coefficient
Ser	Serine
SERDs	Selective Estrogen Receptor Degraders
SERMs	Selective Estrogen Receptor Modulators

SHBG	Sex Hormone-Binding Globulin
SNP	Single Nucleotide Polymorphism
SPECT	Single-Photon Emission Computed Tomography
SSA	Sub-Saharan Africa
STK11	Serine/Threonine Kinase 11
T	Thymine
TNBC	Triple-Negative Breast Cancer
TP53	Tumor Protein p53
Val	Valine
VEGF	Vascular Endothelial Growth Factor
WHO	World Health Organization

ABSTRACT

Breast cancer continues to be the most prevalent malignancy in women worldwide, with estrogen receptor-positive (ER+) tumors accounting for approximately 70% of cases. GLOBOCAN 2022 data reveal a significant global burden, with 2.3 million new cases annually, including 198,553 in Africa. Kenya reports 7,243 new cases and 3,107 deaths yearly, reflecting urgent needs for improved early detection and prevention strategies. This hospital-based case-control study aimed to determine the associations between socio-demographics, medical history, reproductive history, lifestyle factors and single nucleotide polymorphisms (SNPs) in estrogen-metabolizing genes with ER+ breast cancer risk among Kenyan women. The study compared 64 ER+ breast cancer cases with 79 benign breast disease (BBD) and 19 healthy controls from Aga Khan University Hospital Nairobi and Africa Inland Church (AIC) Kijabe Hospital. Socio-demographic and clinical data were abstracted from the questionnaires and medical records review. Estrogen plays a pivotal role in the pathogenesis of ER+ breast cancer. Individual genetic variation in estrogen-metabolizing enzymes can significantly alter the production, activity, and clearance of estrogen and its metabolites, thereby modifying cancer risk and progression. Single nucleotide polymorphisms (SNPs) in genes such as CYP1A1, CYP1B1, CYP3A5, and COMT can lead to differential enzymatic activity, influencing the critical balance between carcinogenic catechol estrogens and their detoxified forms. Consequently, these SNPs are considered key biomarkers for understanding interindividual susceptibility and prognosis in ER+ breast cancer. Five functionally relevant single nucleotide polymorphisms (SNPs) in key estrogen metabolism genes including; rs4646903 and rs1048943 of CYP1A1; rs1056836 of CYP1B1; rs776746 of CYP3A5 and rs4680 of COMT were analyzed using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). Four additional SNPs (rs10012, rs1056827, rs1056836 and rs1800440 of CYP1B1) were analyzed via Sanger sequencing methods. Key findings demonstrated that women aged 50 years and older and postmenopausal women faced significantly elevated breast cancer risk. Genetic analysis revealed complex patterns: the alternative C allele of rs4646903 in CYP1A1 showed a protective effect against ER+ cancer development (OR=0.44, 95% CI [0.19-0.99], p = 0.048), but was also paradoxically associated with increased risk of aggressive Luminal B subtypes (OR=3.83, 95% CI [1.35-10.84]). Two CYP1B1 variants – alternative C allele in rs1056836 (OR = 0.34, 95% CI [0.19–0.62], p = 0.0003) and alternative A allele in rs1056827 (OR=0.43, 95% CI [0.19-0.98], p = 0.045) – were associated with reduced likelihood of malignant transformation from benign breast disease. Linkage disequilibrium analysis revealed strong association between rs10012 and rs1056827 ($D' = 0.9466$, $r^2 = 0.5767$, $p = 1.08 \times 10^{-13}$), confirming that these loci form a haplotype block. Haplotype frequency analysis identified eight distinct CYP1B1 haplotypes, with the G–A–C–T haplotype (R–S–V–N) predominating in benign samples (42.3%) suggesting a protective role, while the G–C–C–T haplotype (G–A–V–N) showed elevated frequency in cases (12.5%) compared to controls (8.3%), indicating potential risk association. Tertiary structure modeling using AlphaFold Server revealed that the four polymorphic residues—R48G, A119S, L432V, and N453S—occupy functionally distinct domains: R48 in the N-terminal membrane

interaction region, A119 in the structural core, L432 near the substrate-binding pocket, and N453 in the heme-binding region. Comparative structural analysis of the wild-type (R–A–L–N), reference (G–S–V–N), risk-associated (G–A–V–N), and protective (R–S–V–N) haplotypes demonstrated that the protective haplotype uniquely retains the wild-type R48 residue while incorporating S119 and V432, suggesting that proper membrane anchoring may be critical for maintaining protective function. Conversely, the risk-associated haplotype combines loss of R48 with the high-activity V432 variant, potentially synergistically enhancing carcinogenic estrogen metabolism. Ramachandran plot analysis confirmed structural reliability, with >90% of residues in favored regions across all models. These findings provide important insights into the complex interplay between socio-demographic factors, genetic predisposition, and structural consequences of CYP1B1 haplotypes in ER+ breast cancer development among Kenyan women. The study's findings underscore the importance of developing population-specific risk assessment tools that incorporate both genetic and structural information to combat Kenya's disproportionate breast cancer mortality burden. Future research should focus on molecular dynamics simulations to elucidate the dynamic mechanisms underlying haplotype-specific functional differences, validation in larger cohorts, and exploration of potential clinical applications in preventive strategies and personalized treatment approaches.

CHAPTER ONE

INTRODUCTION

1.1 Background

Breast cancer continues to be the most commonly diagnosed malignancy among women worldwide. According to GLOBOCAN 2022, an estimated 2,308, 897 new cases were reported, accounting for 23.8% of all female cancer cases, with 665,684 deaths, representing 15.4% of all female cancer-related deaths. In Africa, the trend is similar, with breast cancer ranking highest in both incidence - 198,553 new cases (29.2%) and 91,252 deaths (21.9%). Breast cancer is the most common female malignancy in Sub-Saharan Africa (SSA) (133,520 annual cases; 26.5% of cancers), yet ranks second in mortality (68,036 deaths; 21.1%) after cervical cancer. Despite lower incidence than high-income countries, SSA experiences disproportionately poor survival due to advanced-stage diagnosis and limited treatment access (Parenté et al., 2025).

In Kenya breast cancer accounts for 7,243 new cases making, it the most common cancer. It is ranked second in mortalities after cervical cancer and accounts for 3,398 deaths. The incidence and mortalities represent 25.5% and 18.9% of all the female cancers, respectively (International Agency for Research on Cancer, 2022). Majority of the breast cancer cases in Kenya were reported to have late-stage disease with a 5-year relative survival of 40.1 to 64% (Ekpe et al., 2019). The low rates of survival are due to patient and health-system barriers such as low awareness, high cost of screening, diagnosis as well as treatment. The disease therefore impacts negatively on the economic growth of the country, and also leads to loss of productivity due to the cancer-related premature deaths (Hutchinson et al., 2024).

Breast cancer incidence distribution based on the four-tiers Human Development Index (HDI) countries including; very high, high, medium, and low HDI level (excluding China and India in high and medium HDI, respectively) varies (Bray et al., 2024)The age-

standardized incidence rate (ASIR) for breast cancer is 54.1 per 100,000 in transitioned (high or very high HDI) countries and 38.1 per 100,000 in transitioning (low or medium HDI) countries (Sung et al., 2021). The higher incidence in transitioned countries is associated with increased exposure to breast cancer-associated risk factors, including obstetric and gynecological factors (age at menarche, menopause, and parity), race/ethnicity, lifestyle choices, diet, physical inactivity, obesity, medical history, and genetic predispositions (Al-Shami et al., 2023; Coughlin & Smith, 2015).

Despite the higher incidence in transitioned countries, mortality rates tend to be lower due to widespread screening, early detection, and advanced treatment options. In contrast, transitioning countries in South America, Africa, and Asia continue to experience both rising incidence and high mortality rates, largely due to late-stage diagnosis, inadequate healthcare infrastructure, and limited access to treatment. In SSA, where healthcare resources remain constrained, breast cancer mortality remains disproportionately high (Bray et al., 2024).

Estrogen exposure plays a critical role in breast cancer pathogenesis, particularly in estrogen receptor-positive (ER+) breast cancer (Yager et al., 2006). Estrogens undergo metabolism through two primary pathways: the oxidative pathway, mediated by cytochrome P450 (CYP) enzymes, and the conjugation pathway, regulated by enzymes such as catechol-O-methyltransferase (COMT) (Nebert & Dalton, 2006). Genetic polymorphisms in CYP1A1, CYP1B1, CYP3A5, and COMT influence estrogen metabolism efficiency and the balance between protective (2-hydroxyestradiol) and carcinogenic (4-hydroxyestradiol) estrogen metabolites (Coughlin & Piper, 1999).

Single nucleotide polymorphisms (SNPs) in estrogen metabolizing genes may affect individuals' susceptibility to estrogen receptor-positive (ER^+) breast cancer through altered enzyme activity, promoting oxidative stress, DNA damage, and activating xenobiotics such as polycyclic aromatic hydrocarbons (PAHs) and endogenous compounds such as estrogen (Abdelmonem et al., 2024).

The CYP1A1 gene metabolizes estradiol to 2-hydroxy estradiol, a weak carcinogen. Two nonsynonymous mutations described in the CYP1A1 include; the rs4646903 thymine (T) to cytosine (C) point mutation at nucleotide 6235 in 3' untranslated region (3' UTR)) and rs1048943 adenine (A) to guanine (G) base substitution at nucleotide 2455 resulting in substitution of isoleucine (Ile) to valine (Val) at codon 462 in exon 7 of CYP1A1 (Zhan et al., 2011).

The CYP1B1 gene preferentially converts estradiol to 4-hydroxy estradiol which is highly carcinogenic. The most common CYP1B1 gene polymorphisms may influence breast cancer risk (Wen et al., 2017a). They include; rs10012 which results in substitution of arginine (Arg) to Glycine (Gly) at codon 48, rs1056827 which substitutes alanine (Ala) to serine (Ser) at codon 119, rs1056836 which substitutes leucine (Leu) to Valine (Val) at codon 432 and rs1800440 which substitutes asparagine (Asn) to serine (Ser) at codon 453 (Jiao et al., 2010)

Polymorphisms in the CYP3A gene involved in carcinogen metabolism may result in variations in breast cancer susceptibility between individuals. The CYP3A5, rs776746 is one of the predominant polymorphisms in CYP3A gene. Substitution of guanine (G) to adenine (A) in intron 3 of CYP3A5 may lead to a splice defect of the mRNA and result in the formation of nonfunctional protein (Badavi et al., 2015). Catechol-O-methyl transferase (COMT) is a phase II gene involved in conjugation and inactivation of catechol estrogens. Insufficient or lack of COMT due to transition of guanine (G) to adenine (A) of rs4680 and consequent substitution of valine (Val) to methionine (Met) amino acid at codon 158 may lead to accumulation of quinones leading to DNA adducts formation and tumor initiation (Almeida et al., 2021; Qin et al., 2012).

Understanding the role of these genetic variations in ER+ breast cancer patients in Kenya is crucial, given the regional differences in genetic predisposition and environmental exposures. This study investigated polymorphisms in low-penetrance genes involved in estrogen metabolism, specifically those in phase I (cytochrome P450 family) and phase II (catechol-O-methyltransferase) pathways. Phase I gene polymorphisms analyzed included

rs4646903 and rs1048943 in CYP1A1; rs10012, rs1056827, rs1056836, and rs1800440 in CYP1B1; and rs776746 in CYP3A5. In phase II, the rs4680 polymorphism in COMT was examined. These variants were studied in women diagnosed with estrogen receptor-positive (ER^+) breast cancer, women with benign breast disease, and healthy controls attending Aga Khan University Hospital, Nairobi, and Africa Inland Church (AIC) Kijabe Hospital between 2019 and 2021. The study also assessed associations between ER^+ breast cancer risk and demographic or probable risk factors.

1.2 Statement of the Problem

Breast cancer imposes a significant health burden in Kenya, particularly among women of reproductive and middle age. The prevalence is estimated at 34 per 100,000 women and it contributes to 23% of female cancer cases and 11% of total cancer-related mortality expected to rise (Hutchinson et al., 2024).

Early diagnosis and screening remain major challenges in low-resource settings, leading to late-stage detection when treatment is often less effective and more costly. This contributes to high mortality, psychological distress, disability, reduced productivity, and financial strain for affected individuals and their families. The increasing burden of breast cancer also strains the healthcare system, diverting government resources from prevention to expensive curative care. Households not covered by health insurance face a high economic burden due to high out-of-pocket spending. At the community level, the disease disrupts family dynamics, as many women serve as primary caregivers and income earners. Its impact on mental health is significant—higher cancer severity in Kenyan women has been linked to increased psychological distress (Ndetei et al., 2018).

Kenya has a high prevalence of risk factors for cancer including genetic predisposition, lifestyle, environmental and reproductive factors (Ministry of Health, 2022). Although understanding these factors in the local context could improve prevention, early detection, and guide treatment strategies, they remain largely unexplored.

1.3 Justification of the study

There is low uptake of screening services in Kenya with only 1% of women screened for breast cancer with mammography (Ministry of Health, 2020). There is also low awareness of risk factors, hence the need to incorporate risk factors, screening and awareness in national health surveys. This will improve on early diagnosis and will reduce on the costs of treatment by the government, reduce the emotional distress in families and improve on the quality of life for the survivors.

In Kenya, late-stage breast cancer diagnosis contributes to poor prognosis and high mortality. Understanding the risk factors associated with breast cancer is crucial for improved monitoring, evaluation. This will also guide on implementation of policies and guidelines for cancer prevention.

Estrogen metabolism plays a crucial role in breast cancer initiation and progression. This may be attributed to deregulation of genes which codify enzymes involved in estrogen metabolism and exposure to high levels of estrogen and its metabolites. However, the association between polymorphisms in estrogen-metabolizing genes and breast cancer risk remains understudied in African populations. Most genetic studies have focused on Western and Asian populations, leaving a knowledge gap in African-specific genetic risk factors. Understanding the association of polymorphisms in the local population with breast cancer may contribute to development of molecular panels that can be used for screening and early diagnosis of the disease

1.4 Research questions

i. What is the association between socio-demographics with ER+ breast cancer patients attending Aga Khan University Hospital, Nairobi and Africa Inland Church Hospital Kijabe
ii. What is the association between reproductive, medical history, lifestyle, and single nucleotide polymorphisms in estrogen-metabolizing genes with ER+ breast cancer

patients attending Aga Khan University Hospital, Nairobi and Africa Inland Church Hospital Kijabe

iii. What is the association of single nucleotide polymorphisms in estrogen metabolizing genes with the clinicopathological characteristics in estrogen receptor positive breast cancer patients attending Aga Khan University Hospital, Nairobi and Africa Inland Church Hospital Kijabe

iv. What are the structural and functional consequences of the CYP1B1 haplotypes defined by the R48G (rs10012), A119S (rs1056827), L432V (rs1056836), and N453S (rs1800440) polymorphisms as predicted by in silico approaches?

1.5 Objectives

1.5.1 General Objective

To determine association of polymorphisms in estrogen-metabolizing genes, in conjunction with socio-demographics, medical history, lifestyle and reproductive factors, with estrogen receptor-positive breast cancer in patients attending Aga Khan University Hospital, Nairobi and Africa Inland Church Hospital Kijabe

1.5.2 Specific Objectives

i. To determine the association of socio-demographics with estrogen receptor-positive breast cancer patients attending Aga Khan University Hospital, Nairobi and Africa Inland Church Hospital Kijabe

ii. To assess the relationship between medical, lifestyle, environmental, reproductive factors and polymorphisms in estrogen metabolizing genes with estrogen receptor-positive breast cancer patients attending Aga Khan University Hospital, Nairobi and Africa Inland Church Hospital Kijabe

iii. To determine the association of single nucleotide polymorphisms in estrogen-metabolizing genes on clinicopathological characteristics in estrogen receptor positive breast cancer

iv. To investigate the potential structural and functional effects of four common CYP1B1 mutations—R48G (rs10012), A119S (rs1056827), L432V (rs1056836), and N453S (rs1800440) using in-silico approaches

1.6 Scope of the Study

This study investigated the influence of socio-demographic characteristics, risk factors, and single nucleotide polymorphisms (SNPs) in estrogen-metabolizing genes on the risk and progression of estrogen receptor-positive (ER^+) breast cancer.

Specifically, it examined SNPs involved in the phase I and phase II pathways of estrogen metabolism. Phase I polymorphisms analyzed included rs4646903 and rs1048943 in CYP1A1; rs10012, rs1056827, rs1056836, and rs1800440 in CYP1B1; and rs776746 in CYP3A5. In phase II, rs4680 in the COMT gene was evaluated. The study assessed the potential associations of these variants with breast cancer susceptibility and tumor characteristics.

In addition, it explored the distribution of socio-demographic characteristics and risk factors among three groups: women diagnosed with ER^+ breast cancer, individuals with benign breast diseases, and healthy volunteers. This analysis provided insights into the prevalence and impact of modifiable and non-modifiable risk factors within the study population.

Furthermore, the study evaluated the association between the genetic polymorphisms and tumor characteristics, such as tumor stage, histological type and grade, lymph node involvement, and luminal subtype, to determine whether specific SNPs influenced disease progression and prognosis.

The study participants included women diagnosed with ER^+ breast cancer, individuals with benign breast diseases, and healthy volunteers from Aga Khan University Hospital, Nairobi, and Africa Inland Church Hospital, Kijabe, in the period between 2019 and 2021. The study period (January 2019–December 2021) was selected because this study was

nested within a larger study, hence electronic medical records and biorepository samples were consistently available and complete during this period at both sites.

1.7 Limitations of the Study

This study provides important insights into risk factors for ER+ breast cancer, though several limitations warrant consideration. The retrospective design introduces potential recall bias in self-reported exposures, mitigated through standardized data collection by trained interviewers using REDCap. Hospital-based sampling at two facilities yielded a modest sample size that precluded stratified analyses, with cases drawn from diverse regions but controls predominantly from Nairobi, introducing potential geographic bias. Funding constraints limited polymorphism coverage and prevented functional validation of findings.

Regarding the in silico structural analyses, AlphaFold 3 models represent static snapshots and do not capture dynamic conformational changes during substrate binding or catalysis; molecular dynamics simulations are required to address this limitation.

Despite these constraints, this study offers novel insights into estrogen-metabolizing gene polymorphisms, haplotype architecture, and structural consequences of CYP1B1 variants in ER+ breast cancer among Kenyan women. Future multicenter studies with population-based sampling, expanded genomic profiling, molecular dynamics simulations, and in vitro functional validation are needed to enhance generalizability and clinical utility.

CHAPTER TWO

LITERATURE REVIEW

2.1 General Overview of Cancer

Cancer is a non-communicable disease (NCD) which can be categorized based on the body part affected. It occurs when abnormal cells divide uncontrollably, infiltrate and destroy body tissues (Ministry of Health, 2020). In Kenya, Cancer is the third leading cause of NCDs after infectious diseases and cardiovascular diseases. The five common adult cancers in Kenya include cervix, breast, oesophageal, prostate and colorectal while in children the most common cancers include lymphomas, leukemias. Retinoblastomas, renal and bone (Kagiri H et al., 2020)

2.2 Overview of Breast Cancer

Breast cancer is a complex heterogenous disease characterized by the uncontrolled proliferation of mammary epithelial cells, leading to tumor formation and potential metastatic dissemination. While clinically defined by histology and receptor status, its fundamental etiology lies in the accumulation of genetic and epigenetic alterations that disrupt normal cellular homeostasis, including DNA repair, cell cycle control, and apoptotic signaling.

Globally, breast cancer represents a major public health challenge, exhibiting the highest incidence and a leading cause of cancer mortality among women (Bray et al., 2024). Although significant advancements in screening and treatment have improved outcomes in high-resource settings, the rising global burden underscores the imperative to move beyond generalized approaches and elucidate the specific molecular mechanisms driving individual risk and tumor behavior (Nolan et al., 2023).

The search for these mechanisms has historically focused on rare, high-penetrance germline mutations in genes such as BRCA1 and BRCA2, which account for a small

fraction (5-10%) of cases, predominantly within high-risk families. The vast majority of breast cancer cases are considered sporadic, with their etiology attributed to the complex interplay of common, low-penetrance genetic variants with environmental, reproductive, and lifestyle factors.

This polygenic risk architecture suggests that susceptibility is often modulated not by single, highly penetrant mutations, but by the combined effect of polymorphisms in genes governing critical biochemical pathways. A pathway of particular relevance is xenobiotic and hormone metabolism, mediated by enzymes such as the cytochrome P450 (CYP) superfamily. Polymorphisms in these genes can significantly alter enzyme activity, influencing the biotransformation of endogenous estrogens and environmental carcinogens, thereby modulating individual exposure to DNA-damaging intermediates.

Consequently, while hereditary syndromes provide crucial mechanistic insights, a comprehensive understanding of breast cancer etiology requires a shift in focus toward functional polymorphisms in metabolic genes and their interaction with environmental exposures. Elucidating these associations is critical for advancing beyond descriptive epidemiology to a mechanistic, biochemical understanding of risk, with the potential to inform more personalized strategies for risk prediction, early detection, and prevention.

2.3 Epidemiology

Breast cancer is the second most common malignancy globally after lung cancer, accounting for approximately 2,296,840 new cases annually, equivalent to 11.5% of all cancer cases (Bray et al., 2024). Among women, breast cancer ranks first in incidence at 23.8%. According to Global Cancer Statistics 2022, breast cancer was the most commonly diagnosed cancer in 158 countries (Figure 2.1). The incidence rates are notably high in both transitioned (54.1 per 100,000) and transitioning (30.8 per 100,000) countries (Sung et al., 2021).

Globally, breast cancer is responsible for approximately 666,000 deaths, constituting 6.8% of all cancer cases and 15.4% of female cancers. It was reported as the primary cause of cancer deaths in 111 of 187 countries (Figure 2.2), with the highest mortality rates observed in Melanesia, Western Africa, and Micronesia/Polynesia (Bray et al., 2024).

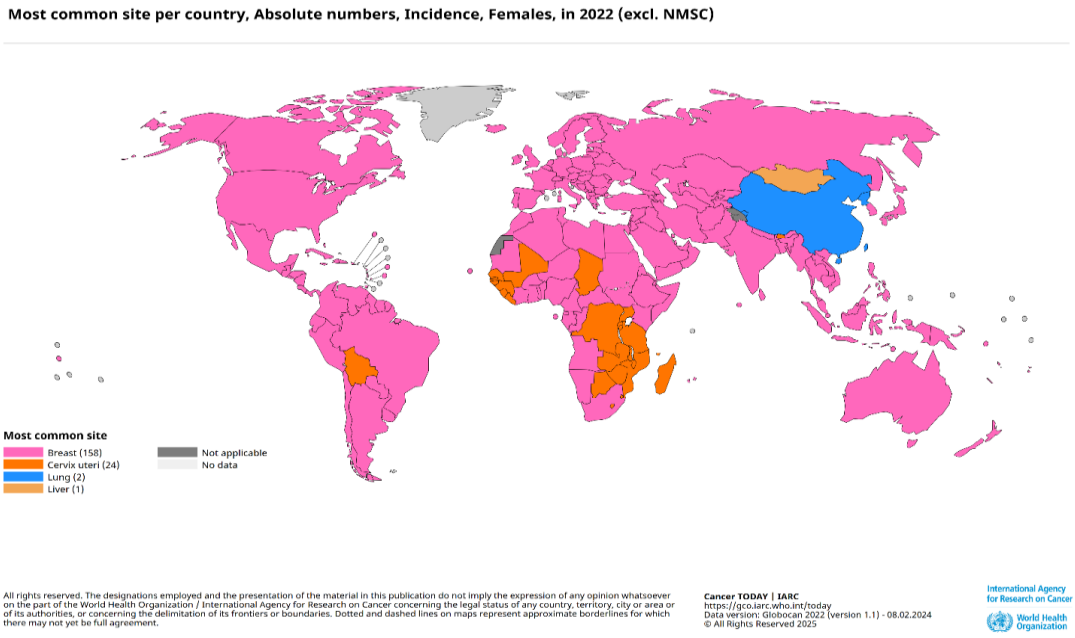

Figure 2.1: Global Map Representing Absolute Numbers of Incidence of Cancer Types in Females

Source: (Bray et al., 2024)

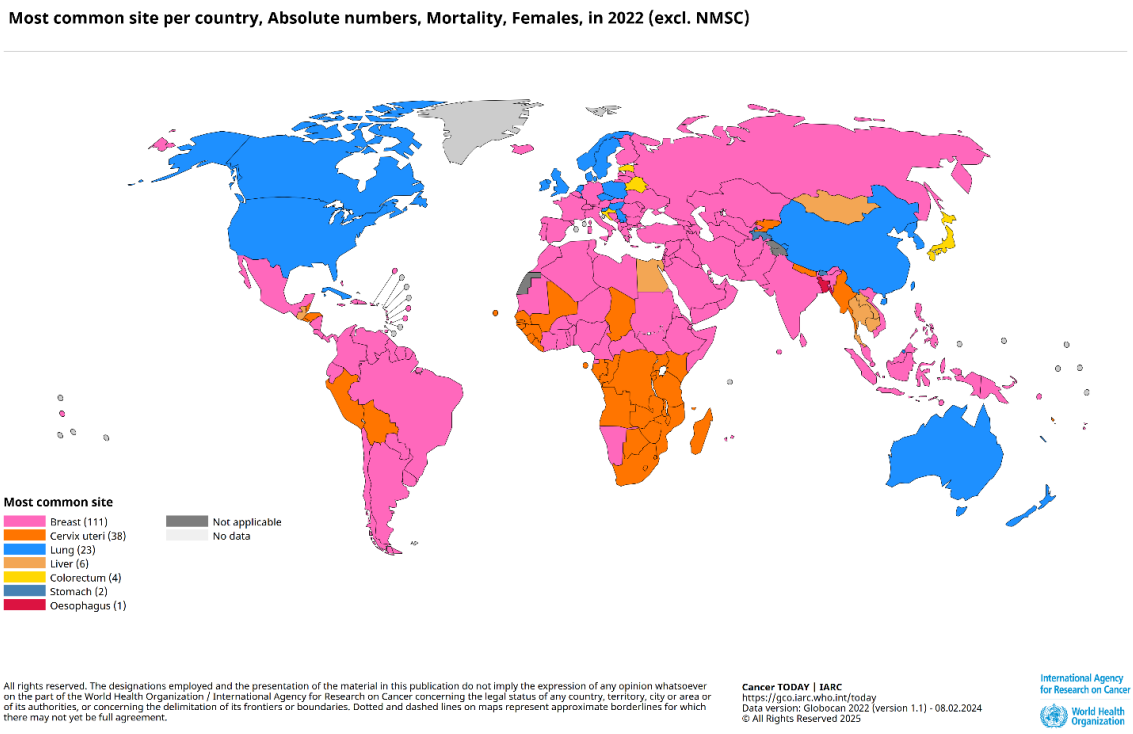

Figure 2.2: Global Map Representing Absolute Numbers of Mortalities of Cancer Types in Females

Source: (Bray et al., 2024).

In Africa, breast cancer leads in both incidence and mortality, with approximately 198,553 new cases and 91,252 deaths annually. The majority of these cases occur in Sub-Saharan Africa (SSA), where breast cancer ranks second in cancer-related mortality, with approximately 68,036 deaths (Anyigba et al., 2021). Despite a relatively low incidence compared to global figures, mortality rates remain disproportionately high, largely due to late-stage diagnosis, inadequate healthcare infrastructure, and limited access to treatment (Black & Richmond, 2019; Joko-Fru et al., 2020). The burden of breast cancer in SSA is expected to increase, highlighting the urgent need for improved screening, early diagnosis, and treatment strategies (Anyigba et al., 2021).

Breast cancer is among the five commonest cancers in Kenya. It is the most common malignancy among women with an incidence of 7243 and the second in causing deaths

with a mortality of 3398 mortalities with age standardized rates of 40.8/100000 and 19.6/100,000 respectively (International Agency for Research on Cancer, 2022). In Kenya, women are diagnosed with breast cancer at a younger age of 30-50 years compared to 50-55 years in the western countries. 7Seven out of ten breast cancers are diagnosed at late stages (stage III and IV) (Ministry of Health, 2021).

2.4 Breast Cancer Classification

2.4.1 Molecular Classification

Breast cancer is a complex disease with different molecular subtypes. Using the immunohistochemistry (IHC) technique breast cancer can be characterized by estrogen receptor (ER). Progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). Invasive breast cancer can be categorized into molecular subtypes based on mRNA gene expression profiles (Łukasiewicz et al., 2021). In 2000, Perou and colleagues initially classified breast cancer into four molecular subtypes: Luminal, HER2-enriched, Basal-like (triple-negative breast cancer), and Normal Breast-like (Prat & Perou, 2011). Further studies refined this classification by identifying two subcategories of the Luminal subtype, namely Luminal A and Luminal B (Prat et al., 2013) . The Normal Breast-like subtype was later omitted. Hence, the four widely recognized breast cancer subtypes based on mRNA gene expression are Luminal A, Luminal B, HER2-enriched, and Basal-like (triple-negative breast cancer) (Koboldt et al., 2012).

2.4.1.1 Luminal Breast Cancers

The luminal breast cancers which constitute the estrogen receptor-positive (ER+) tumors, account for nearly 70% of all breast cancer cases (Brufsky & Dickler, 2018). In ER+ breast cancer, cancer cells have receptors for the hormone estrogen, which binds to these receptors and promotes tumor growth (Murphy & Dickler, 2016). Proliferation-related and luminal-regulated pathways differentiate Luminal-like tumors into Luminal A and Luminal B subtypes; each associated with distinct clinical outcomes. The advantage of

ER+ breast cancer lies in its responsiveness to hormonal therapies, including selective estrogen receptor modulators (SERMs) and aromatase inhibitors (AIs), which block estrogen's effects on cancer cells, slowing or halting tumor progression (Murphy & Dickler, 2016).

Despite its better prognosis compared to other subtypes, ER+ breast cancer poses challenges due to potential recurrence and the need for long-term treatment, necessitating continuous research to improve treatment efficacy (Swain et al., 2023).

2.4.1.1.1 Luminal A Breast Cancer

Luminal A tumors are defined by the presence of estrogen receptor (ER) and/or progesterone receptor (PR) and the absence of HER2. These tumors exhibit low expression of proliferation-related genes (Ki-67 low of <14%), making them slow-growing with a favorable prognosis and late recurrence. Most luminal A breast cancers can be treated with endocrine therapies such as tamoxifen (Inic et al., 2014; Prat et al., 2013; Weigelt et al., 2010)

2.4.1.1.2 Luminal B Breast Cancer

Luminal B tumors are ER-positive but may be PR-negative and/or HER2-positive and Ki-67 high. The high Ki-67 has shown positive correlation with young age women, large tumors and positive lymph nodes. Hence, most Luminal B tumors are of higher grade, have high lymph node involvement, have a worse prognosis and early recurrence (Inic et al., 2014)

2.4.1.2 HER2-Enriched Breast Cancer

The HER2-enriched subtype accounts for 10–15% of breast cancer cases and is characterized by high HER2 expression in the absence of estrogen receptor (ER) and progesterone receptor (PR). This subtype is associated with aggressive tumor growth, rapid proliferation, and a poorer prognosis compared to luminal subtypes. Before the

introduction of HER2-targeted therapies, HER2-enriched breast cancer had one of the worst survival outcomes. However, advancements in targeted treatments, such as trastuzumab and pertuzumab, have significantly improved prognosis and treatment response (Łukasiewicz et al., 2021).

2.4.1.3 Basal-Like/Triple-Negative Breast Cancer

Triple-negative breast cancer (TNBC) is a highly heterogeneous group of breast cancers characterized by the absence of ER, PR, and HER2 expression. TNBC accounts for approximately 20% of all breast cancers and is more common in younger women, particularly those under 40, as well as in African-American women. Notably, around 80% of breast cancers in individuals with BRCA1 germline mutations are classified as TNBC, with 11–16% of all TNBC cases carrying BRCA1 or BRCA2 mutations. Due to its aggressive nature and lack of targeted therapies, TNBC is associated with high recurrence rates and poor prognosis, making it one of the most challenging breast cancer subtypes to treat (Plasilova et al., 2016).

2.4.2 Histopathological Classification

Breast cancer is categorized primarily by its histological appearance based on whether it arises from the ducts (ductal carcinoma) or the lobules (lobular carcinoma). The morphology is also considered based on whether the tumor is in situ (limited to the epithelial component of the breast) or invasive (invaded the stroma) (Makki, 2015).

2.4.2.1 Ductal Carcinoma In-Situ (DCIS)

DCIS is a non-invasive breast cancer characterized by proliferation of atypical epithelial cells within breast milk ducts and do not infiltrate the adjacent breast tissue. Histologically, DCIS is classified by architectural pattern (comedo, solid, cribriform, papillary, micropapillary) and nuclear grade (low, intermediate, high). Majority of the DCIS show a mixture of histological subtypes making it difficult to categorize. High grade

DCIS as well as untreated are precursors for development of invasive carcinomas (Allred, 2010).

2.4.2.2 Lobular Carcinoma in Situ (LCIS)

Histopathological examination reveals a proliferation of monomorphic, dyshesive atypical cells filling and distending the terminal duct-lobular units, with an intact basement membrane. It cannot be identified through visual examination and is found accidentally in samples or biopsies being screened for other reasons. Microscopic examination reveals intact lobular architecture (Christgen et al., 2021)

2.4.2.3 Invasive Ductal Carcinoma (IDC)

IDC are breast cancers with malignant ductal proliferation along with stromal invasion in the presence or absence of DCIS. It is the most predominant type of breast cancer constituting of 80% of all breast cases in women. They are classified based on cell type (apocrine), amount, type and location of secretion (mucinous), architectural features (papillary, tubular and micropapillary) and immunohistochemical profile (neuroendocrine). IDCs are heterogeneous and have a wide range of morphological feature variation. About 25% of invasive breast cancers are identified as 'special types,' characterized by unique growth patterns and cytological features. The specific subtypes include invasive lobular carcinoma (ILC), adenoid cystic carcinoma, apocrine carcinoma, invasive ductal carcinoma (IDC) with osteoclastic giant cells, medullary carcinoma, metaplastic carcinoma, micropapillary carcinoma, mucinous carcinoma, cribriform carcinoma, neuroendocrine carcinoma, and tubular carcinoma (Nascimento & Otoni, 2020; Weigelt et al., 2010). The majority (75%) are designated as IDC not otherwise specified (NOS) as they fail to exhibit sufficient morphological characteristics. The term no special type (NST) can also be used (Makki, 2015)

2.4.2.4 Invasive Lobular Carcinoma (ILC)

ILC is the second major invasive mammary cancer after IDC. It comprises of small, spherical, loose tumor cells growing in single files. It is characterized by unique growth patterns whereby the stromal invasion disrupts the normal architecture, loss of E-cadherin and cytoplasmic accumulation of p120 catenins (Pereslucha et al., 2023)

2.5 Benign Breast Disease

Benign breast disease (BBD) refers to a group of non-cancerous conditions that affect the breast. These conditions include fibrocystic changes, fibroadenomas, and ductal hyperplasia, all of which involve the growth of abnormal breast tissue but are not cancerous (Guray & Sahin, 2006). Benign breast disease is quite common, with many women experiencing benign breast lumps at some point in their lives (Galea, 2019).

While BBD itself does not lead to cancer, certain types, such as atypical hyperplasia, may slightly increase the risk of developing breast cancer in the future (Dyrstad et al., 2015). Despite its non-cancerous nature, BBD can have significant psychological, physical, and social impacts on women. The presence of breast lumps or abnormal tissue can lead to anxiety and uncertainty, as women often worry about the possibility of cancer (Liao et al., 2008). Additionally, the need for ongoing monitoring and, in some cases, biopsies to rule out malignancy can contribute to stress. The emotional toll is often compounded by social stigma or misconceptions surrounding breast abnormalities, leading to further isolation or concern (Srivastava et al., 2020).

2.6 Breast Cancer Risk Factors

Breast cancer is a complex disease with a multifactorial etiology, influenced by both non-modifiable and modifiable risk factors. Non-modifiable risk factors include gender, age, genetic predispositions, race or ethnicity, family history of breast cancer, and proliferative breast disease. Modifiable risk factors encompass menstrual and reproductive factors, radiation exposure, hormonal replacement therapy, alcohol consumption, smoking,

physical activity, and dietary habits, including a high-fat diet. Additionally, environmental exposures such as organochlorine chemicals and electromagnetic fields have been implicated in breast cancer risk (Łukasiewicz et al., 2021).

2.6.1 Non-Modifiable Factors

2.6.1.1 Gender

Among the non-modifiable factors, female gender is a major determinant, primarily due to heightened hormonal stimulation. Unlike men, who have negligible estrogen levels, women possess breast cells that are highly sensitive to estrogen and progesterone, making them more susceptible to hormonal imbalances that can trigger tumor development (Łukasiewicz et al., 2021). Estrogen and androgen levels have been positively correlated with breast cancer risk, and alterations in endogenous hormone levels contribute to increased susceptibility in both premenopausal and postmenopausal women (Folkerd & Dowsett, 2013). Breast cancer remains rare in men, with fewer than 1% of all cases occurring in males, translating to a lifetime risk of approximately 1 in 1,000 compared to 1 in 8 for women (Giordano, 2018).

2.6.1.2 Age

Increasing age is another well-established risk factor, with nearly 80% of breast cancer cases diagnosed in individuals over 50 and over 40% in those above 65. The risk escalates with age, from 1.5% at 40 years, to 3% at 50 years, and over 4% at 70 years. Early-onset breast cancers are often attributed to inherited factors, whereas late-onset cases result from prolonged exposure to carcinogenic influences that accumulate over time (Benz, 2008). A relationship between age and molecular subtypes of breast cancer has also been observed, with the more aggressive triple-negative breast cancer (TNBC) predominantly diagnosed in younger individuals, while the less aggressive luminal A subtypes are more prevalent in older populations (McGuire et al., 2015).

2.6.1.3 Family History

A family history of breast cancer increases risk, with 13–19% of cases linked to first-degree relatives (Łukasiewicz et al., 2021). The risk is higher when breast or ovarian cancer occurs at a younger age. While genetic predisposition plays a key role, not all individuals with a family history carry pathogenic mutations (Collaborative Group on Hormonal Factors in Breast Cancer, 2001).

2.6.1.4 Hormonal and Reproductive

Hormonal and reproductive factors are closely linked to breast cancer development. Several studies have demonstrated that prolonged exposure to estrogen and progesterone increases the risk. The timing of reproductive milestones, such as age at first menstruation, pregnancy, and menopause, plays a crucial role. Early menarche, late menopause, and a delayed first pregnancy are associated with increased risk, whereas early childbirth and prolonged breastfeeding have protective effects. Pregnancy, particularly before the age of 25, is linked to a reduced risk of breast cancer, while nulliparity is associated with a higher likelihood of developing the disease (Arnold et al., 2022).

2.6.1.5 Comorbidities

Hyperinsulinemia and type II diabetes have also been implicated in breast cancer risk due to insulin's role in cell proliferation and survival pathways (Gallagher et al., 2012). Insulin acts as an anti-apoptotic factor and promotes tumor cell invasiveness by stimulating adipokines such as leptin and vascular endothelial growth factor (VEGF). Additionally, insulin interacts with estrogen to induce adipose stromal aromatase and upregulate sex steroid hormone receptors, further increasing cancer risk (Rose & Vona-Davis, 2012). Obesity-related insulin resistance enhances mitogenic signaling via the mitogen-activated protein kinase (MAPK) pathway, thereby facilitating cancer progression. Hypertension, particularly due to the dysregulation of the renin-angiotensin system (RAS), has also been

linked to breast cancer through increased adiposity, inflammation, and angiogenesis (Draznin, 2011; Rasha et al., 2019).

2.6.2 Modifiable Factors

2.6.2.1 Alcohol Consumption

Lifestyle factors such as alcohol consumption, tobacco use, physical inactivity, and obesity significantly contribute to breast cancer risk. Alcohol is one of the primary modifiable carcinogens implicated in breast cancer development (Lachenmeier et al., 2012). The International Agency for Research on Cancer (IARC) classifies both ethanol and acetaldehyde as Group 1 human carcinogens (Tramacere et al., 2010). Alcohol influences carcinogenesis through oxidative stress, altered cellular proliferation, disruption of hormonal pathways, and DNA methylation (Freudenheim, 2020). Acetaldehyde, a major metabolite of alcohol, induces DNA adduct formation and genetic instability (Lachenmeier et al., 2010).

Alcohol consumption, even at moderate levels (15–30 grams per day), leads to elevated serum estrogen levels, which can contribute to breast cancer development (Shield et al., 2016). Additionally, alcohol impacts one-carbon metabolism and folate pathways, both of which are critical for DNA methylation and genomic stability (Shield et al., 2016). Beyond initiation, alcohol consumption may also affect breast cancer progression by promoting angiogenesis, metastasis, and the expansion of cancer stem cells (Wang et al., 2017).

2.6.2.2 Tobacco

Tobacco smoke contains more than 70 known carcinogens, including polycyclic aromatic hydrocarbons (PAHs), nitrosamines, and aromatic amines, which contribute to DNA damage and tumorigenesis (International Agency for Research on Cancer, 2004). While some studies suggest that tobacco smoking has anti-estrogenic effects that may counteract the carcinogenic potential of tobacco-related compounds, others indicate an increased risk

of breast cancer among smokers. The dual effects of tobacco on breast cancer risk appear to be influenced by menopausal status, with premenopausal women experiencing a stronger carcinogenic impact due to higher estrogen levels. Postmenopausal women may have a more balanced risk, with the anti-estrogenic effects of smoking potentially offsetting its carcinogenic properties (Cassidenti et al., 1990). Other factors influencing the impact of tobacco on breast cancer include the age at smoking initiation, duration, and intensity of use (Peñalver-Argüeso et al., 2023).

2.6.2.3 Physical Activity

Physical activity has been consistently associated with a reduced risk of breast cancer. Although the precise mechanisms remain unclear, regular exercise is believed to lower breast cancer risk by reducing exposure to endogenous sex hormones, modifying immune system responses, and lowering insulin-like growth factor-1 (IGF-1) levels (Chen et al., 2019). Obesity, on the other hand, significantly increases the likelihood of developing breast cancer, particularly in postmenopausal women.

2.7 Molecular Mechanisms of Breast Cancer

The pathogenesis of breast cancer is driven by a complex interplay of hereditary genetic defects, acquired somatic mutations, and systemic metabolic dysregulation. Understanding these molecular mechanisms is essential for elucidating disease etiology, prognostic stratification, and the development of targeted therapies.

2.7.1 Hereditary and Somatic Genetic Alterations

Hereditary breast cancer is frequently attributed to high-penetrance, loss-of-function mutations in tumor suppressor genes essential for maintaining genomic integrity. The most prominent of these are mutations in BRCA1 and BRCA2, which are critical for homologous recombination-mediated DNA double-strand break repair (Sokolova et al., 2023). While BRCA1-mutated tumors are strongly associated with the triple-negative phenotype, BRCA2 mutations are also linked to sporadic, hormone receptor-positive

cases. Although these mutations are rare in the general population (<0.1%), they account for approximately 15% of hereditary breast cancer cases (Jara et al., 2017).

Other high-penetrance genes implicated in hereditary syndromes include TP53 (Li-Fraumeni syndrome), PTEN (Cowden syndrome), CDH1 (hereditary diffuse gastric cancer syndrome), and STK11 (Peutz-Jeghers syndrome). Furthermore, moderate-penetrance genes such as PALB2, BRIP1, CHEK2, ATM, and BARD1 confer a more modest but significant increase in lifetime risk. In contrast, common low-penetrance somatic mutations and polymorphisms, particularly in genes governing estrogen metabolism (e.g., CYPs, NATs, GSTs), contribute to a polygenic risk architecture that influences overall susceptibility in the general population (Çelik et al., 2015) (Łukasiewicz et al., 2021).

2.7.2 Systemic and Metabolic Drivers: The Role of Obesity

Beyond direct genetic alterations, systemic metabolic states, particularly obesity, establish a tumor-promoting microenvironment through several integrated pathways. Obesity-induced metabolic dysregulation leads to a state of chronic low-grade inflammation, characterized by elevated levels of pro-inflammatory cytokines such as TNF-α and IL-6. Furthermore, increased aromatase activity within adipose tissue enhances the local conversion of androgens to estrogens, providing a potent mitogenic signal for hormone receptor-positive cells. Concurrent elevations in insulin and insulin-like growth factor-1 (IGF-1) levels, associated with insulin resistance, further stimulate oncogenic PI3K/AKT and MAPK signaling pathways (Rasha et al., 2019).

2.7.3 Estrogen Metabolism and Genetic Susceptibility

Estrogen and its metabolites are central to the initiation and progression of hormone-sensitive breast cancer (Yim et al., 2001). Estrogen metabolism is a two-phase enzymatic process. In Phase I, cytochrome P-450 (CYP) enzymes catalyze the hydroxylation of estrogen to form catechol estrogens, primarily 2-hydroxyestradiol (2-OHE2) and 4-

hydroxyestradiol (4-OHE2). In Phase II, conjugation and detoxification occur via enzymes such as catechol-O-methyltransferase (COMT), which methylates these reactive catechols into less genotoxic methoxyestradiols (Wen et al., 2017a). A failure in this detoxification pathway results in the accumulation of reactive quinones, elevated reactive oxygen species (ROS), and the formation of mutagenic DNA adducts, thereby initiating carcinogenesis (Cavalieri et al., 2012).

Genetic polymorphisms in the genes encoding these metabolizing enzymes are critical determinants of interindividual variation in estrogen metabolism and significantly modulate breast cancer susceptibility by altering the balance between estrogen activation and detoxification (Al-Shami et al., 2023). Key polymorphic genes include CYP1A1, CYP1B1, CYP3A5, and COMT, whose functional variants are summarized in Table 2.1.

The CYP1A1 gene encodes a Phase I enzyme involved in estrogen hydroxylation, predominantly yielding the weaker estrogenic metabolite 2-OHE2. Polymorphisms such as rs4646903 (T6235C) in the 3' UTR and the exonic rs1048943 (A4889G; Ile462Val) have been associated with increased enzyme activity and upregulated gene expression, potentially enhancing the bioactivation of procarcinogens and influencing breast cancer risk (N. Shimada et al., 2009).

Conversely, CYP1B1 catalyzes the formation of the highly reactive and carcinogenic 4-OHE2. Several functional polymorphisms, including rs10012 (G142C; Arg48Gly), rs1056827 (C355A; Ala119Ser), and the extensively studied rs1056836 (G4326C; Leu432Val), increase the enzyme's catalytic efficiency and substrate binding affinity. This leads to greater production of genotoxic 4-OHE2 and enhanced activation of environmental carcinogens such as polycyclic aromatic hydrocarbons (PAHs), thereby elevating breast cancer risk (Jiao et al., 2010; Wen et al., 2017b).

The CYP3A5 gene contributes to estrogen and tamoxifen metabolism. The common polymorphism rs776746 (G6986A) is a splicing defect that defines the CYP3A5*3 allele, resulting in a truncated, non-functional protein. Individuals homozygous for this allele

lack CYP3A5 activity, which may confer a protective effect. In contrast, carriers of the functional CYP3A5*1 allele exhibit higher enzyme expression and are potentially at increased risk (Lolodi et al., 2017).

The COMT gene encodes the critical Phase II detoxification enzyme. The seminal rs4680 (A472G; Val158Met) polymorphism results in a valine-to-methionine substitution that reduces enzyme thermostability and catalytic activity by 3- to 4-fold. Individuals homozygous for the low-activity Met/Met genotype exhibit impaired inactivation of catechol estrogens, leading to prolonged exposure to reactive metabolites, increased oxidative stress, and a well-documented elevated risk of breast cancer, with effects often more pronounced in postmenopausal women (Qin et al., 2012).

Table 2.1: Description of rs4646903, rs1048943, rs1056836, rs776746 and rs4689 SNPs

rs ID	Gene	Chromosomal Location	SNP description	Base change/position	Amino acid substitution
rs4646903	CYP1A1	15q22 - q24.1	3' UTR	T6235C	None
rs1048943	CYP1A1	15q22 - q24.1	Exon 7	A4889G	Ile462Val
rs776746	CYP3A5	7q22.1	Intron	G6986A	None
rs4680	COMT	22q11.21	Exon 4	A472G	Val158Met
rs10012	CYP1B1	2p22.2	Exon 2	G142C	Arg48Gly
rs1056827	CYP1B1	2p22.2	Exon 2	C355A	Ala119Ser
rs1056836	CYP1B1	2p22.2	Exon 3	G4326C	Leu432Val
rs1800440	CYP1B1	2p22.2	Exon 3	A4390G	Asn453Ser

Description of rs4646903, rs1048943, rs776746, rs4689, rs10012, rs1056827, rs1056836 and rs1800440 SNPs including their genes and chromosomal locations, functional regions, base change and amino acid substitutions.

2.8 Breast Cancer Screening and Diagnosis

2.8.1 Breast Cancer Screening

The World Health Organization (WHO) recommends that women at average risk for breast cancer between the ages of 50 and 69 undergo biennial mammography screening

in organized, population-based programs. However, in resource-limited settings, where late-stage diagnosis is prevalent and mammography is not cost-effective or widely accessible, early detection through clinical breast examination (CBE) and public awareness campaigns is prioritized (World Health Organization, 2014).

2.8.2 Diagnostic Imaging

Early-stage diagnosis significantly improves breast cancer prognosis. Imaging techniques such as mammography, magnetic resonance imaging (MRI), positron-emission tomography (PET), computed tomography (CT), and single-photon emission computed tomography (SPECT) are commonly used for diagnosis, screening, and monitoring therapeutic responses (Jafari et al., 2018).

Mammography remains the most widely used screening tool due to its high sensitivity, specificity, and cost-effectiveness. However, it carries risks of false positives, radiation exposure, and patient anxiety (Wellings et al., 2016). Ultrasound is often used as an adjunct to mammography, particularly in young, pregnant, or breastfeeding women, due to its high sensitivity and lack of ionizing radiation (Alcantara et al., 2014). MRI is commonly employed for high-risk patients, preoperative staging, and monitoring treatment response, although its lower specificity can result in higher false-positive rates (Sardanelli et al., 2010). Mammography combined with MRI is currently the most sensitive imaging strategy for high-risk populations (Wellings et al., 2016).

2.8.3 Molecular Testing and Biochemical Markers

While imaging techniques are useful for diagnosis and monitoring, molecular testing provides additional insights into tumor biology, prognosis, and treatment selection. Molecular markers, including DNA, mRNA, and microRNAs, have revolutionized breast cancer diagnosis and therapy by enhancing understanding of tumor heterogeneity (Duffy et al., 2017). Early-stage ER+ breast cancers are assessed using molecular tests that evaluate tumor size, nodal involvement, histological grade, proliferation markers (e.g.,

Ki-67), and hormone receptor status (ER, PR, and HER2). Immunohistochemistry (IHC) and fluorescence *in situ* hybridization (FISH) are the standard methods for determining these molecular features, which guide treatment planning and prognosis (Goldhirsch et al., 2013).

2.9 Breast Cancer Treatment

Cancer treatment is a component of cancer control aimed at curing, prolonging life and improving quality of life. Although treatment is most effective during early detection, it is also dependent on cancer type and quality of care. Cancer treatment methods include; surgery, chemotherapy (endocrine or targeted). Radiotherapy, cryotherapy, loop electrosurgical excision procedure (LEEP), brachytherapy, therapeutic nuclear medicine, end of life care and survivorship. Multi-disciplinary management is recommended over independent management for improved patient outcomes (Ministry of Health, 2017)

2.9.1 Surgery

Breast cancer surgery includes breast-conserving surgery (BCS) and mastectomy. BCS, also known as partial or segmental mastectomy, involves removing the cancerous tissue while preserving intact breast tissue, often combined with oncoplastic reconstruction techniques. Mastectomy, which entails complete removal of the breast, is typically recommended for large tumors, multicentric disease, or patients at high genetic risk. Immediate or delayed breast reconstruction is an option for women undergoing mastectomy to improve cosmetic and psychological outcomes (Keelan et al., 2021).

2.9.2 Chemotherapy

Chemotherapy is a systemic treatment administered as neoadjuvant therapy (before surgery) or adjuvant therapy (after surgery). Neoadjuvant chemotherapy is particularly beneficial for locally advanced breast cancer, inflammatory breast cancer, and triple-negative or HER2-positive subtypes, as it reduces tumor size and improves surgical outcomes. Common chemotherapeutic agents include carboplatin, cyclophosphamide, 5-

fluorouracil, taxanes (paclitaxel, docetaxel), and anthracyclines (doxorubicin, epirubicin). Although effective, chemotherapy is associated with side effects such as nausea, fatigue, hair loss, immunosuppression, and bone marrow suppression (Rouzier et al., 2005).

2.9.2.1 Endocrine Therapy

Endocrine therapy is primarily used in luminal breast cancer to block estrogen stimulation of cancer cells. It is administered as neoadjuvant, adjuvant, or metastatic treatment. Common endocrine therapy drugs include: selective estrogen receptor modulators (SERMs) like tamoxifen and toremifene, selective estrogen receptor degraders (SERDs) like fulvestrant, and aromatase inhibitors (AIs) such as letrozole, anastrozole, and exemestane. These therapies significantly reduce recurrence risk in ER+ breast cancer (Jones & Buzdar, 2004) survival (Jones & Buzdar, 2004).

2.9.2.2 Targeted Therapy

Targeted therapies, particularly for HER2-positive breast cancer, have dramatically improved patient outcomes. HER2 inhibitors such as trastuzumab, pertuzumab, lapatinib, and neratinib specifically block HER2 signaling, reducing tumor progression and recurrence risk (Ishii et al., 2019).

2.9.3 Radiotherapy

Radiation therapy is commonly used after breast-conserving surgery and/or following chemotherapy to eliminate residual cancer cells and lower recurrence risk. Despite side effects such as skin irritation, fibrosis, and fatigue, radiation therapy has significantly improved survival rates in breast cancer patients (Łukasiewicz et al., 2021).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Sites

The study participants were recruited from Aga Khan University Hospital, Nairobi (1°15'55.0"S, 36°48'48.0"E) and Africa Inland Church Kijabe Hospital (0° 56' 46" S, 36° 35' 42" E). AKUHN is a private, non-profit institution located in Parklands, Nairobi, offering both tertiary and secondary healthcare services. It attracts patients from across Kenya and diverse socio-economic backgrounds. The hospital diagnoses and treats a significant number of breast cancer cases and has a well-equipped oncology unit.

AIC Kijabe Hospital, situated in Kijabe, Kiambu County, primarily serves patients from all regions of the country. Like AKUHN, it has a diverse patient population and provides a wide range of cancer related services such as screening, chemotherapy, surgery and palliative care for different cancer types. Both hospitals have a specialized team managing breast cancer cases. They maintain extensive patient records and adhere to strict ethical guidelines, ensuring high-quality data for research.

All genomic work was conducted at the Biotechnology and Molecular Laboratory at the Pan African University of Science, Technology, and Innovation (PAUSTI), Thika.

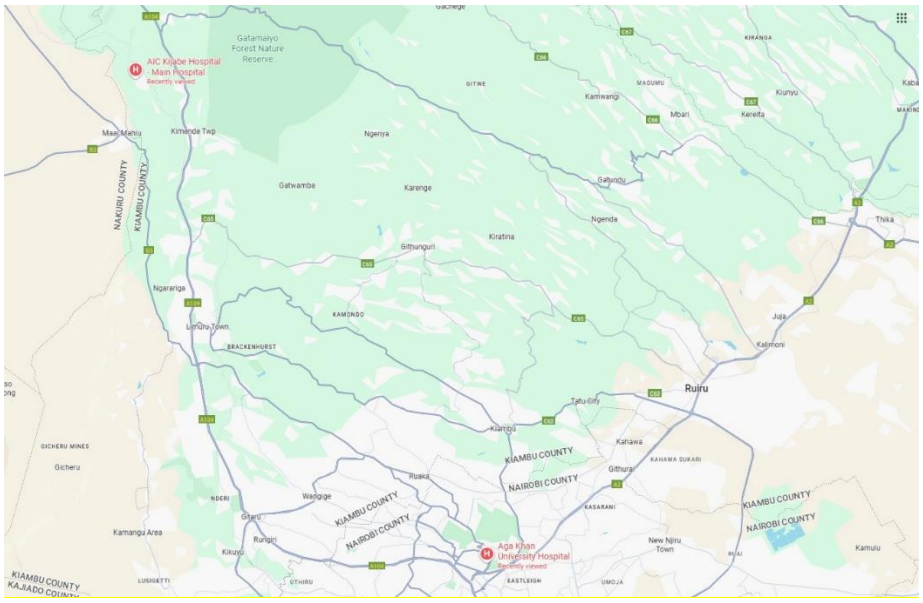

Figure 3.1: Map Showing Sampling Sites

Kenyan map showing sampling sites: Aga Khan University Hospital, Nairobi

(AKUH, N) and Africa Inland Church Kijabe Hospital (KAIC) (National Geographic Society, 2019).

3.2 Study Design

This study utilized a hospital-based, unmatched case-control design. This approach is particularly effective for retrospectively investigating associations between a defined outcome and prior exposures (Andrade, 2022). Three participant groups were established to facilitate a stratified analysis of risk: cases (women with histologically confirmed ER^+ breast cancer), women with histologically confirmed benign breast disease, BBD, and healthy controls (women with no personal history of breast cancer or any malignancy). A primary comparison of cases versus healthy controls aimed to identify factors associated with malignancy, while a secondary comparison of cases versus BBD controls enabled exploration of factors potentially distinguishing malignant progression from benign proliferative states. The inclusion of a BBD group thus allowed investigation across a

pathological continuum, refining the assessment of genetic and environmental influences on breast disease progression.

3.3 Ethical Consideration

The study protocol was reviewed and approved by the Aga Khan University Institutional Research Ethical Committee (AKU-REC) Ref: 2020/IERC-26 (v4) **(Appendix 1)**. In addition, a research permit was obtained from the National Commission for Science, Technology and Innovation (NACOSTI) (License No: NACOSTI/P/21/13890) **(Appendix 2)**. Data and biospecimens for this secondary analysis were derived from the parent study, "A Genomic Approach for Understanding Breast Cancer Progression in Kenya". All participants in the original study provided written informed consent for their samples and clinical data to be stored and used in future genetic and molecular studies **(Appendix 3).** For the current project, the institutional biobank granted access to these de-identified resources. No patient identifiers were accessible to the research team at any stage, ensuring confidentiality and anonymity throughout the current analysis.

3.4 Study Participants

The study participants comprised of the cases, individuals with benign breast diseases (BBD), and controls. Cases were women diagnosed with estrogen receptor-positive (ER+) breast cancer. For this study, 'ER^+ breast cancer' was operationally defined as a tumor showing positive immunohistochemical staining for the estrogen receptor (ER), irrespective of its progesterone receptor (PR) status. BBD participants were diagnosed with benign breast conditions, while controls were healthy volunteers working at the hospital.

3.5 Sampling Method

This study employed a purposive (non-probability) sampling strategy to select archived samples and associated clinical data that met predefined eligibility criteria. Purposive sampling was appropriate because the research specifically required samples from

individuals with defined pathological features: ER^+ breast cancer cases, benign breast disease (BBD) controls, and healthy controls.

Given the specific requirements for case confirmation (histologically verified ER^+ status) and the relative scarcity of such well-characterized samples, purposive sampling enabled the efficient selection of a focused and appropriate study cohort from the available biobank inventory. While this approach ensured the sample's relevance to the research objectives, a key limitation is the potential for selection bias and reduced generalizability, as the selected samples reflect the characteristics of the original biobanked population rather than the broader, general population.

3.6 Eligibility Criteria

The study enrolled participants from three distinct cohorts: Estrogen Receptor-Positive (ER+) Breast Cancer Cases, Benign Breast Disease (BBD) Controls, and Healthy Controls.

3.6.1 Inclusion Criteria

The study included three participant groups. For cases, eligible participants were women aged 18 years or older with a histologically confirmed diagnosis of estrogen receptor-positive breast cancer (ER/PR positive) at either AKUHN or AIC Kijabe Hospital, who provided blood samples and gave informed consent. Healthy control volunteers were required to be women aged 18 years or older with no prior diagnosis of breast cancer or any other malignancy, who provided a blood sample, gave informed consent, and were employed at AKUHN with confirmed health status at recruitment. The benign breast disease group comprised women aged 18 years or older with a histologically confirmed benign breast disease diagnosis at either hospital who provided blood samples and signed informed consent.

3.6.2 Exclusion Criteria

Patients were excluded from the cases if they had other breast cancer subtypes (such as triple-negative or HER2-positive tumors) or failed to provide a blood sample. Participants in the BBD and control groups were excluded if they did not provide a blood samples and/or did not sign the informed consent.

3.7 Sample Size Determination

The a priori sample size calculation, based on parameters from published literature (Kelsey et al., 1996), indicated a requirement of 134 participants per group to detect an odds ratio of 1.8 with 80% power. However, final recruitment yielded 64 ER^+ breast cancer cases, 79 benign breast disease (BBD) controls, and 19 healthy controls **(Appendix 5).** This shortfall was primarily due to post-COVID-19 restrictions on hospital-based recruitment and a lower-than-anticipated eligible patient flow during the study period. Consequently, the achieved statistical power for detecting the initially hypothesized effect size (OR=1.8) was reduced. To ensure rigorous analysis within this constraint, analytical methods robust to smaller sample sizes were prioritized, including exact statistical tests and careful control for confounding.

3.8 Data Collection

Existing data were abstracted from two primary sources: (1) the completed questionnaire forms from the original study database, and (2) the corresponding clinical and histopathology reports filed in the hospital records. A standardized abstraction form was used to extract the relevant variables (**Appendix 4**). No direct interaction with participants or new data collection occurred for this project. Data collected comprised of; socio-demographics, anthropometrics, reproductive and hormonal history, lifestyle factors, medical and family history, and environmental exposures. The abstraction form was used to collect verified clinical information from medical and histopathology reports. This process captured detailed clinicopathological characteristics (histology, grade, stage, and

receptor status) for breast cancer cases, and the specific benign diagnosis for BBD participants **(Appendix 6).** All collected data were de-identified using a unique study code and systematically entered into a secure electronic database for subsequent analysis.

Archived blood samples were originally collected from all participants following informed consent under the prior study protocol. For cases and BBD participants, samples were drawn during clinical visits; for healthy controls, samples were collected at a hospital occupational health unit. A standardized protocol was used: 5.0 mL of venous blood was collected into EDTA tubes, centrifuged at 744 × g for 15 minutes, with plasma and buffy coat fractions aliquoted and stored at -20°C in the institutional biobank. For this secondary analysis, de-identified buffy coat aliquots were retrieved from the biobank following ethical approval for reuse. No new samples were collected, and all samples remained anonymized throughout subsequent DNA extraction and genotyping.

3.9 DNA Extraction

Genomic DNA was isolated from buffy coat samples using the ISOLATE II Genomic DNA Kit (Bioline, Meridian Life Science Inc., USA) following the manufacturer's instructions. Briefly, 200 µL of buffy coat sample was mixed with 200 µL of lysis buffer G3 and 25 µL of Proteinase K, vortexed vigorously, and incubated at 70 °C for 10 minutes to facilitate cell lysis and protein digestion. Following incubation, 210 µL of 100% ice-cold ethanol was added, and the mixture was vortexed thoroughly to precipitate DNA. The lysate was then transferred into a 2 mL centrifuge tube containing a DNA binding column, allowed to stand for 2 minutes, and centrifuged to facilitate DNA binding.

Subsequently, the column was washed sequentially with buffers GW1 and GW2, each followed by centrifugation at 10,000 × g using a Thermo Scientific MicroCL 21 microcentrifuge with a fixed-angle rotor for 1 minute to remove contaminants. In the final step, 50 µL of Elution Buffer was added to the column, incubated at room temperature for 10 minutes, and centrifuged at 10,000 × g using a Thermo Scientific MicroCL 21 microcentrifuge with a fixed-angle rotor for 1 minute to elute the purified DNA. The

extracted DNA was dissolved in 50 µL of sterile distilled water. DNA integrity was assessed by 1.5 % agarose-1X Tris acetate-EDTA (TAE) gel stained with GelRed® nucleic acid gel stain (Biotium, Inc., Fremont, CA, USA). 8µl of the samples were resolved alongside 1 kb DNA marker (HyperLadder™ 100 bp, Bioline, Meridian Biosciences, BIO-33025). The gel was run at 70 volts 80mA and 50 Watts for one hour and the bands visualized using a UV transilluminator. The DNA samples were subsequently stored at -20 °C for downstream applications.

3.10 Polymerase Chain Reaction

Polymerase chain reaction (PCR) was used to amplify gene regions containing the polymorphic sites using specific primers designed with Primer3Plus (Untergasser et al., 2007). The primer sequences are presented in Table 3.1.

Amplifications were performed using conventional PCR in 30 µL reactions, with each reaction containing 50 ng/µL genomic DNA, 0.3 µM of each primer, 1.5 U MyTaq DNA polymerase (BIO-21105, Bioline, Meridian Life Science Inc., USA), 6 µL 5× MyTaq reaction buffer, and nuclease-free water. Thermocycling conditions consisted of an initial denaturation at 95 °C for 3 minutes, followed by 30 cycles of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 45 seconds, and extension at 72 °C for 45 seconds, with a final extension step at 72 °C for 3 minutes.

PCR reactions were conducted using a ProFlex™ 3 × 32-well PCR System (Applied Biosystems, Thermo Fisher Scientific, Waltham, UK).

Polymerase chain reaction (PCR) was used to amplify regions harboring the SNPs rs4646903 (340 bp), rs1048943 (204 bp), rs1056836 (245 bp), rs776746 (251 bp), and rs4680 (304 bp) (Table 3.1) as well as rs10012 (202 bp), rs1056827 (202 bp), rs1056836 (567 bp) and rs1800440 (567 bp) (Table 3.2). The resulting amplicons were resolved on a 2% agarose-Tris borate-EDTA (1X TBE) gel stained with GelRed® nucleic acid gel stain (Biotium, Inc., Fremont, CA, USA) alongside 100 bp DNA marker (HyperLadder™ 100

bp, Bioline, Meridian Biosciences, BIO-33056). While TAE buffer was used for initial DNA quality checks, TBE buffer was specifically selected for gels analyzing restriction digestion products and PCR amplicons due to its ability to produce sharper, better-resolved bands—a requirement for reliable genotyping interpretation. The gel was run at 100 volts 80mA and 50 Watts for 45 mins. The bands were visualized using a UV transilluminator to confirm the presence and size of the PCR products.

Table 3.1: Primers and PCR Products of the Target Regions

rs ID	Gene	Forward Primer (5' → 3')	Reverse Primer (5' → 3')	Product Size (bp)
rs4646903	CYP1A1	CAGTGAAGAGGTGTAGCCGCT	TAGGAGTCTTGTCTCATGCCT	340
rs1048943	CYP1A1	CTGTCTCCCTCTGGTTACAGGAAGC	TTCCACCCGTTGCAGCAGGATAGCC	204
rs1056836	CYP1B1	TGTCCTGGCCTTCCTTTATG	TCATCACTCTGCTGGTCAGG	245
rs776746	CYP3A5	CCATACAGGCAACATGACTT	GGTTAGAAATGACAGTAGAGCA	251
rs4680	COMT	ACAGGCAAGATCGTGGAC	CAGTGAACGTGGTGTGAAC	304
rs10012	CYP1B1	GAAACACACGGCACTCAT	ACGCTCCTGCTACTCCTGT	567
rs1056827	CYP1B1	GAAACACACGGCACTCAT	ACGCTCCTGCTACTCCTGT	567
rs1056836	CYP1B1	ATCATCACTCTGCTGGTCA	TGCCTGTCACTATTCCTCA	202
rs1800440	CYP1B1	ATCATCACTCTGCTGGTCA	TGCCTGTCACTATTCCTCA	202

The table lists the primer sequences used for PCR amplification of rs4646903, rs1048943, rs776746 and rs4680, rs10012, rs1056827, rs1056836 and rs1800440 along with the corresponding product sizes (bp).

3.11 Genotyping Using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP)

Amplified DNA fragments containing the polymorphic regions of interest namely: rs4646903, rs1048943, rs1056836, rs776746 and rs4680 were subjected to restriction enzyme digestion using the PCR-RFLP technique (Tarach, 2021). The following

restriction enzymes, obtained from New England Biolabs (UK) were used: *Msp*I (R0106) for rs4646903, *Bsr*D1/*Bse*MI (R0574) for rs1048943, *Acu*I (R0641S) for rs1056836, *Ssp*I (R3132) for rs776746 and *Nla*III (R0125S) for rs4680. Each digestion was carried out under enzyme-specific incubation conditions. The resulting restriction fragments were resolved on 3% agarose-Tris Borate EDTA (1X TBE) gels stained with GelRed. The samples were resolved resolved alongside 50 bp DNA marker (HyperLadder™ 50 bp, Bioline, Meridian Biosciences, BIO-33054). The resulting bands were visualized under a UV transilluminator. The expected fragment sizes corresponding to the wild type, heterozygous, and variant genotypes are shown in Table 3.2. A subset of samples were verified by Sanger sequencing to eliminate any ambiguity associated with gel-based fragment size discrimination.

Table 3.2: Restriction Enzymes and Conditions Used for PCR-RFLP Genotyping

SNP (rs ID)	Restriction Enzyme (NEB Catalog #)	Incubation Conditions	Fragment Sizes (bp)
rs4646903	*Msp*I (R0106)	37 °C (1hr), 80 °C (20 min)	wt: 340 het: 340,200,140 var: 200,140
rs1048943	*Bsr*D1/*Bse*MI (R0574)	65 °C (1hr), 80 °C (20 min)	wt: 150,54 het: 204,150,54 var: 204
rs1056836	*Acu*I (R0641S)	37 °C (1hr), 65 °C (20 min)	wt: 245 het: 245,176,69 var: 176,69
rs776746	*Ssp*I (R3132)	37 °C (1hr), 65 °C (20 min)	wt: 137,114 het: 251,137,114 var: 251
rs4680	*Nla*III (R0125S)	37 °C (1hr), 65 °C (20 min)	wt: 114,93,54,43 het: 304,114,93,54,43 var: 304

Summary of restriction enzymes, incubation conditions and expected fragment sizes for genotyping five SNPs using PCR-RFLP.**"wt"** = Wild type, **"het"** = Heterozygous**, "var"** = Variant

3.12 Genotyping of CYP1B1 Polymorphisms by Sanger Sequencing

Amplified PCR products for the CYP1B1 gene regions harboring the polymorphisms rs10012, rs1056827, rs1056836, and rs1800440 were purified using the QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany), according to the manufacturer's protocol, to eliminate unincorporated primers, nucleotides, and other contaminants. During purification, DNA binds to a silica membrane under high-salt conditions, while impurities pass through the column. The membrane-bound DNA was then washed thoroughly, and purified DNA was eluted using Tris buffer. The resulting clean amplicons were subjected to bidirectional Sanger sequencing by Macrogen Europe BV.

3.13 Bioinformatics Analysis of Sequences

Polymorphism analysis was performed using *Geneious Prime* 2025.1.0 software (Biomatters Ltd, Auckland, New Zealand). Raw Sanger sequencing data, obtained in .ab1 format, were imported into the software for quality assessment and processing. Chromatograms were visually inspected, and low-quality base calls at the 3′ and 5′ ends were trimmed to ensure sequence accuracy. High-quality consensus sequences were generated for each sample.

The reference sequence for the CYP1B1 gene (accession number: NG_008386.2) was retrieved from the NCBI database and imported into *Geneious Prime* for alignment. Multiple sequence alignment (MSA) was conducted in MEGA using MUSCLE program (Edgar, 2004). Single nucleotide polymorphisms (SNPs) were detected using the "Find Variations/SNPs" tool by comparing each consensus sequence to the reference. All identified SNPs were validated by cross-checking with chromatogram peaks, and filtering was applied based on base quality and allele frequency to retain high-confidence variants. The final SNP dataset was compiled in Microsoft Excel for downstream statistical analysis.

3.14 Statistical Analysis

Statistical analyses were performed using R software (Version R4.3.3) (The R Core Team, 2023) and the tidyverse package (2.0.0) (Wickham et al., 2019) for data preparation and visualization. Descriptive analysis of the independent variables among the study participants was carried out. Tests of independence were employed using Student's t or Wilcoxon rank-sum tests for categorical data and Fisher's exact, or Pearson's Chi-squared tests for continuous data.

3.15 Association Analysis

3.15.1 Association with Epidemiological and Clinical Factors

The analytical strategy employed both epidemiological and genetic association approaches to assess factors related to estrogen receptor-positive (ER+) breast cancer. Bivariate logistic regression was first conducted to explore associations between various independent variables and ER+ breast cancer case status. The variables examined included socio-demographic factors such as age, and body mass index; reproductive factors including age at menarche, menopausal status, parity, and history of contraceptive use; lifestyle factors such as alcohol consumption and tobacco use; medical history factors such as personal history of other cancers, family history of breast cancer, and comorbidities including hypertension and diabetes; and environmental exposures such as self-reported history of significant radiation. Variables with a bivariate p-value less than 0.25 were considered potential confounders and were included in subsequent multivariate logistic regression models to estimate adjusted odds ratios with 95% confidence intervals, thereby assessing the independent association of each risk factor while adjusting for potential confounding.

3.15.2 Association of Genetic Variants with Clinicopathological Characteristics

A secondary analytical objective was to evaluate the association between the genotyped polymorphisms and key clinicopathological characteristics within the ER+ breast cancer

case group. This analysis focused on tumor characteristics abstracted from histopathology reports, which were dichotomized for analysis. These included molecular subtype, categorized as Luminal A versus Luminal B based on immunohistochemistry for ER, PR, HER2, and Ki-67; cancer stage, dichotomized as Early stage (Stage I & II) versus Late stage (Stage III & IV); tumor grade, classified as Low grade (Grade 1 & 2) versus High grade (Grade 3); histopathological type, defined as Invasive Ductal Carcinoma versus other subtypes; and the presence or absence of lymphovascular invasion.

3.15.3 Genotype Association Analysis

For both epidemiological and tumor characteristic analyses involving genetic data, genotype association tests were performed. All genetic association analyses were conducted in R software (v4.3.3) using the SNPassoc package (v2.0-0). The association between each polymorphism and a binary outcome was evaluated under five genetic models—allelic, codominant, dominant, recessive, and log-additive—using unconditional logistic regression to calculate odds ratios and 95% confidence intervals. A two-sided p-value less than 0.05 was considered statistically significant.

3.16 In-Silico Analysis of CYP1B1 Haplotypes

3.16.1 Linkage Disequilibrium and Haplotype Construction

Haplotypes All statistical analyses were performed using R (v4.3.3). Pairwise linkage disequilibrium (LD) among the four CYP1B1 SNPs (rs10012, rs1056827, rs1056836, and rs1800440) was assessed using the LD function from the genetics package. The strength of association between each SNP pair was quantified using D' (D-prime) and r^2 (correlation coefficient). Statistical significance was determined using a chi-square (χ^2) test, with p-values < 0.05 considered statistically significant. These LD metrics were used to confirm the haplotype block structure and validate the co-inheritance patterns of the alleles prior to haplotype reconstruction.

Subsequently, haplotype probabilities were estimated using the haplo.em function from the haplo.stats package (Sinnwell & Schaid, 2024), which infers the most likely pair of haplotypes for each individual based on the four CYP1B1 SNPs. For each participant, the combination of alleles at these four loci was determined in the order presented (rs10012, rs1056827, rs1056836, rs1800440), and haplotypes were designated based on the specific allele present at each position. Only haplotypes with an estimated frequency greater than 1% in at least one study group (cases, controls, or benign samples) were retained for further analysis. The frequency distribution of each haplotype was calculated separately for each group and expressed as counts (n) and percentages (%).

3.16.2 Retrieval of the Wild-Type Cyp1b1 Sequence from UniProtKB and Generation of Haplotype Variants

The wild-type amino acid sequence of human cytochrome P450 1B1 (CYP1B1) was retrieved from UniProt Knowledgebase (UniProt ID: Q16678, https://www.uniprot.org/uniprotkb/Q16678/entry). This 543-amino acid sequence, representing the canonical isoform of CYP1B1, served as the template for all subsequent structural and functional analyses, including homology modeling, molecular dynamics simulations, and in silico mutagenesis. The retrieved sequence was modified using point mutations corresponding to the haplotypes. The specific amino acid substitutions were as follows: R48G, A119S, L432V, and N453S.

3.16.3 Tertiary Structure Analysis

The three-dimensional structures of wild-type CYP1B1 and its haplotypes were generated using AlphaFold Server (https://alphafoldserver.com/welcome), powered by the AlphaFold 3 model. For each haplotype, the corresponding amino acid sequence was submitted, and the top-ranked model based on the composite ranking_score was selected for visualization in PyMOL. Four representations were prepared for comparative analysis including: ribbon diagrams to illustrate the overall protein fold; surface models to visualize solvent accessibility and active site topology; colour-coded ribbon diagrams

highlighting the side chains of the four polymorphic residues (R48G, A119S, L432V, N453S); and Ramachandran plots generated using PROCHECK to assess the stereochemical quality of each model. Prediction confidence was evaluated using the pLDDT scores from AlphaFold, with regions scoring above 90 considered highly reliable.

CHAPTER FOUR

RESULTS

4.1 Genomic DNA Yield and Quality

Genomic DNA extracted from buffy coat samples of 64 ER+ breast cancer cases, 79 BBDs and 19 healthy controls showed bands of greater than 10 Kb. The yield and concentration was analysed using a NanoDrop 2000 spectrophotometer (Biospec-mini, Shimadzu Corporation, Tokyo). DNA concentration was determined spectrophotometrically, with all samples yielding sufficient quantities for downstream analysis (concentrations > 350 ng/µL). Purity was assessed by the absorbance ratio at 260 nm and 280 nm (A260/A280), which fell within the optimal range of 1.8 to 2.0 for all samples, indicating minimal protein or phenolic contamination. DNA integrity was evaluated by electrophoresis on a 1.5 % agarose gel (1X TAE buffer). The majority of samples displayed a single, high-molecular-weight band exceeding 10 kilobases, confirming high integrity. A subset of samples exhibited slight smearing below the primary band, suggesting partial shearing or degradation; however, the DNA quality in these samples was deemed acceptable for PCR amplification and subsequent genotyping analyses. (Figure 4.1)

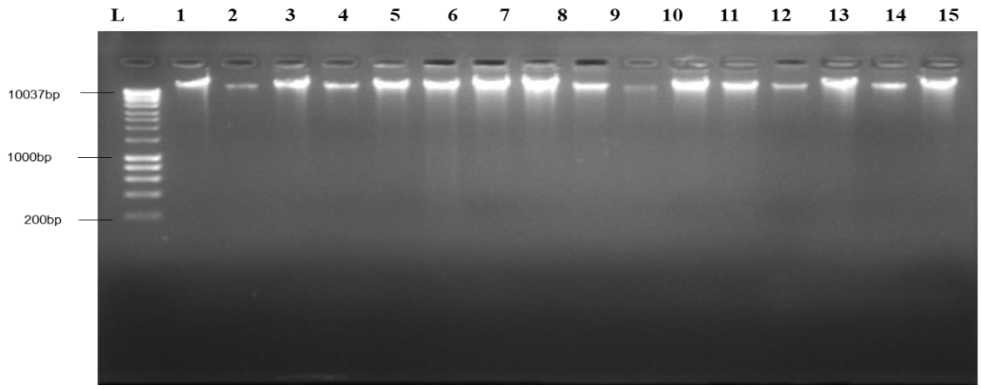

Figure 4.1: Gel Photo of Extracted Genomic DNA

Agarose gel electrophoresis of genomic DNA extracted from buffy coat samples in ER+ breast cancer cases. L: 1Kb DNA marker, 1: AKU057, 2: AKU060, 3: AKU073, 4:

AKU076, 5: AKU087, 6: AKU088, 7: AKU094, 8: AKU097, 9: AKU098, 10: AKU099, 11: AKU100, 12: AKU103, 13: KAIC005, 14: KAIC006, 15: KAIC007.

4.2 PCR Amplicons of Target Regions

Amplicons of the target gene regions were resolved on a 2% TBE agarose gel. The resulting amplicons for rs4646903 (340 bp), rs1048943 (204 bp), rs1056836 (245 bp), rs776746 (251 bp), rs4680 (304 bp), rs10012 and rs1056827 (202 bp), rs1056836 and rs1800440 (567 bp) are as shown in the agarose gel photos (Figures 4.2 – 4.8).

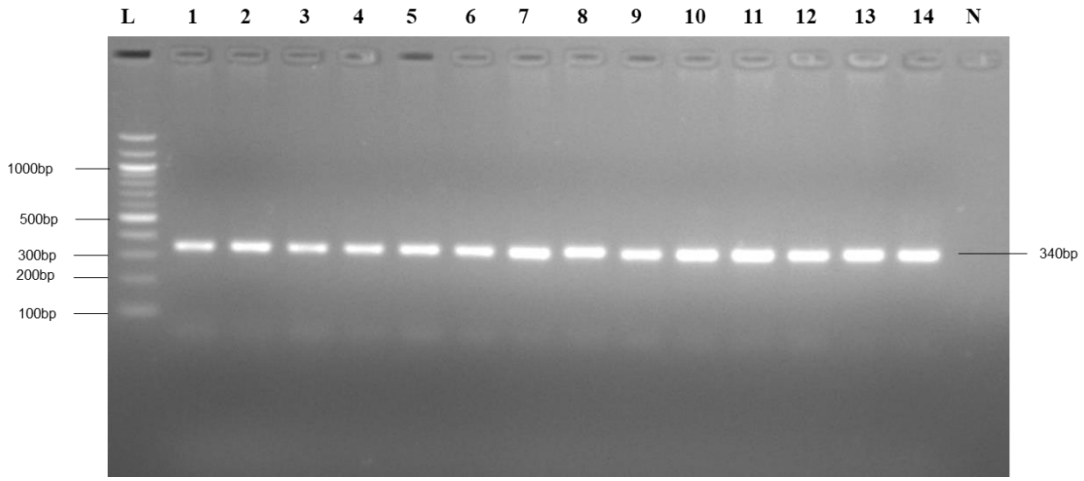

Figure 4.2: Gel Photo of rs4646903 Amplicons

Agarose gel electrophoresis of rs4646903 in ER+ breast cancer cases. L: 100 bp DNA marker, N: Negative control. 1: AKU057, 2: AKU060, 3: AKU073, 4: AKU076, 5: AKU087, 6: AKU088, 7: AKU094, 8: AKU097, 9: AKU098, 10: AKU099, 11: AKU100, 12: AKU103, 13: KAIC005, 14: KAIC006.

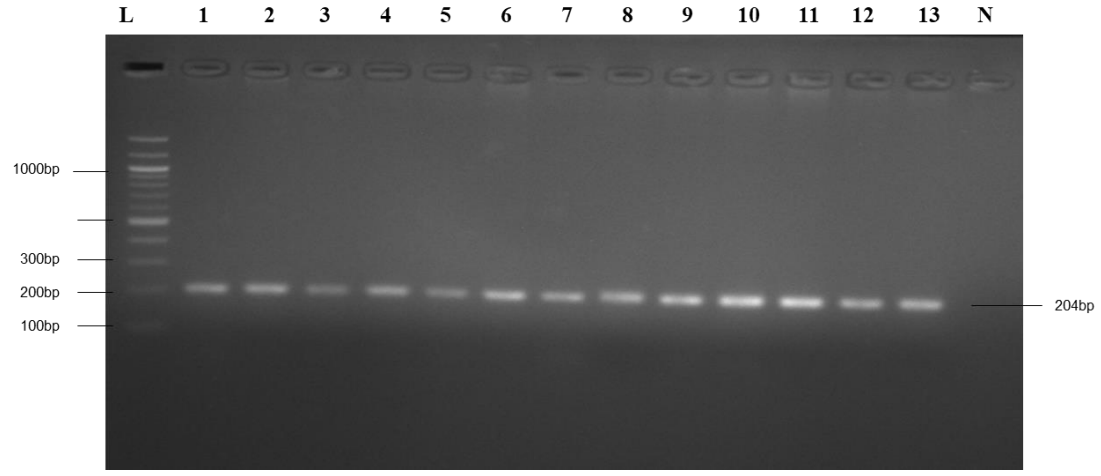

Figure 4.3: Gel Photo of rs1048943 Amplicons

Agarose gel electrophoresis of rs1048943 in ER+ breast cancer cases. L: 100 bp DNA marker, N: negative control. 1: AKU057, 2: AKU060, 3: AKU073, 4: AKU076, 5: AKU087, 6: AKU088, 7: AKU094, 8: AKU097, 9: AKU098, 10: AKU099, 11: AKU100, 12: AKU103, 13: KAIC005.

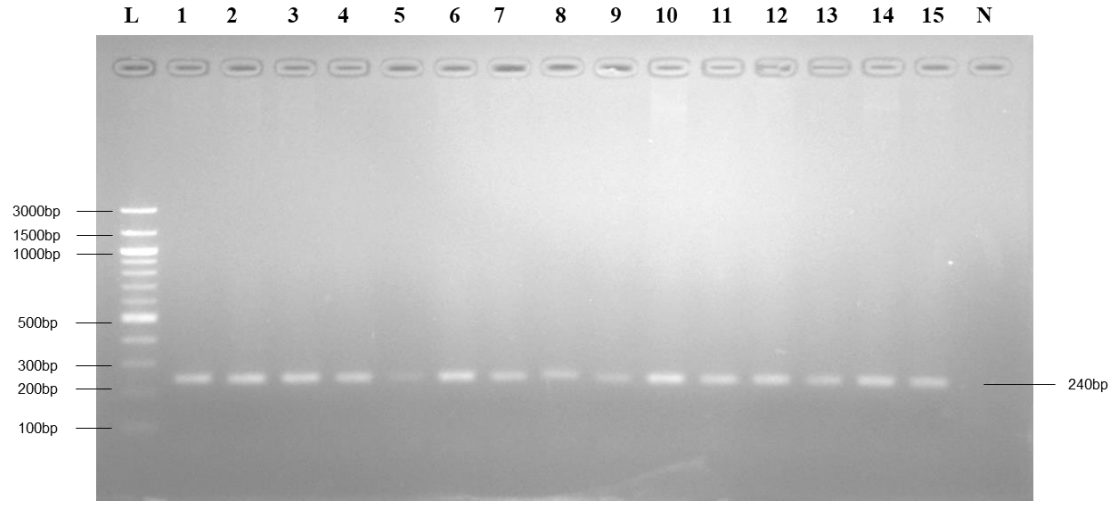

Figure 4.4: Gel Photo of rs1056836 Amplicons

Agarose gel electrophoresis of rs1056836 in ER+ breast cancer cases. L: 100 bp DNA marker, N: negative control. 1: AKU057, 2: AKU060, 3: AKU073, 4: AKU076, 5:

AKU087, 6: AKU088, 7: AKU094, 8: AKU097, 9: AKU098, 10: AKU099, 11: AKU100, 12: AKU103, 13: KAIC005, 14: KAIC006, 15: KAIC007.

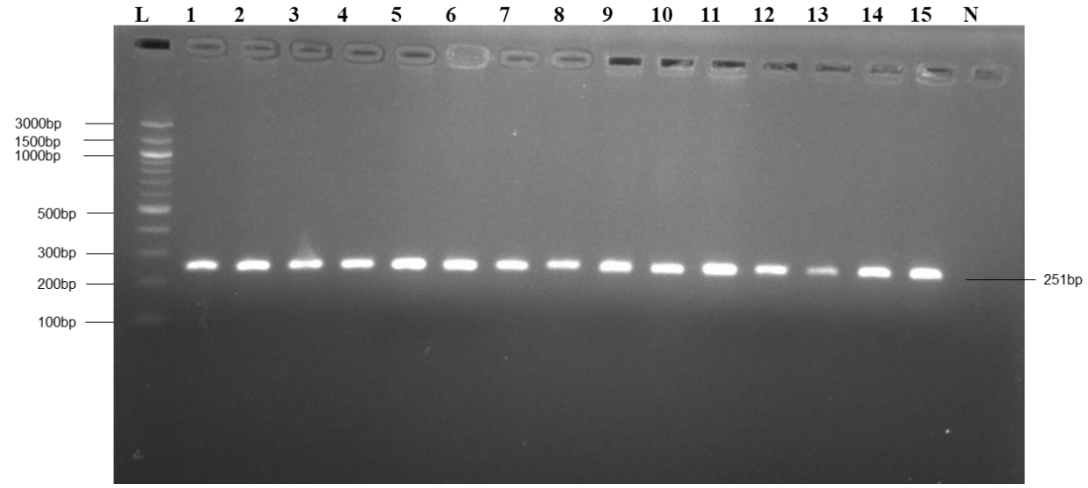

Figure 4.1: Gel Photo of rs776746 Amplicons

Agarose gel electrophoresis of rs776746 amplicons in ER+ breast cancer cases. L: 100 bp DNA marker, N: negative control. 1: AKU057, 2: AKU060, 3: AKU073, 4: AKU076, 5: AKU087, 6: AKU088, 7: AKU094, 8: AKU097, 9: AKU098, 10: AKU099, 11: AKU100, 12: AKU103, 13: KAIC005, 14: KAIC006, 15: KAIC007.

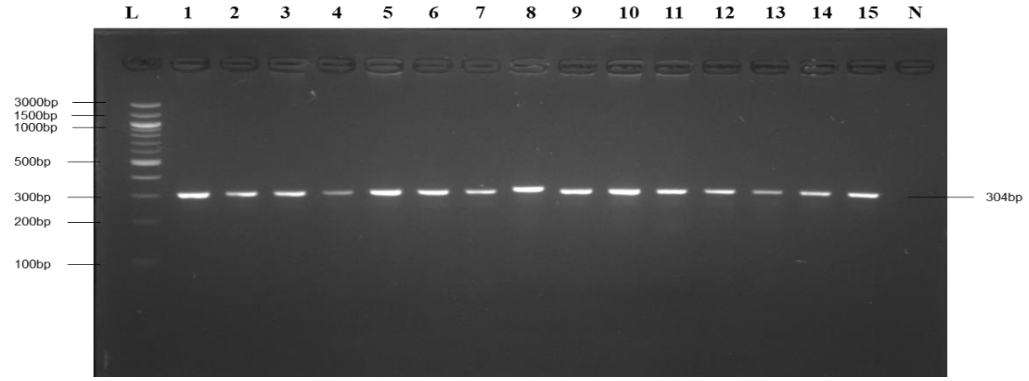

Figure 4.6: Gel Photo of rs4680 Amplicons

Agarose gel electrophoresis of rs4680 amplicons in ER+ breast cancer cases. L: 100 bp DNA marker, N: negative control. 1: AKU057, 2: AKU060, 3: AKU073, 4: AKU076, 5: AKU087, 6: AKU088, 7: AKU094, 8: AKU097, 9: AKU098, 10: AKU099, 11: AKU100, 12: AKU103, 13: KAIC005, 14: KAIC006, 15: KAIC007.

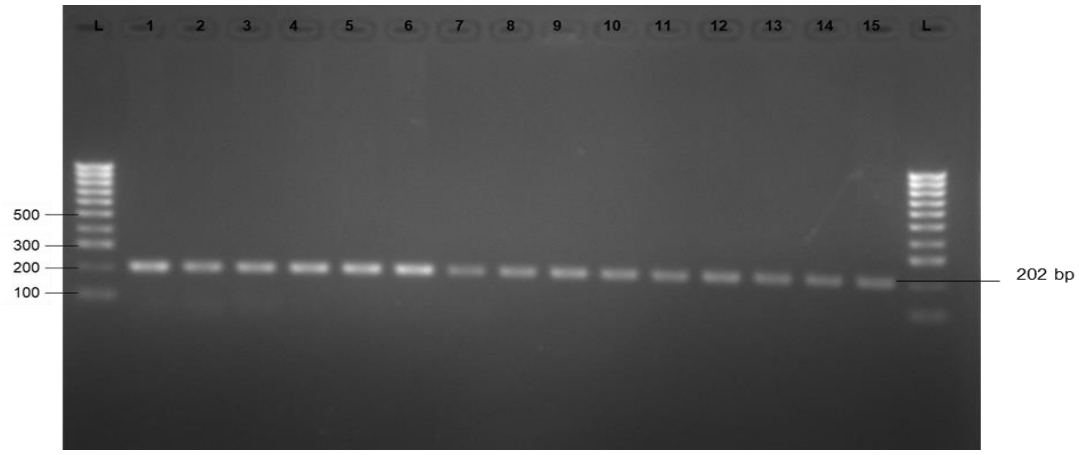

Figure 4.7: Gel photo of rs1056836 and rs1800440 Amplicons

Agarose gel electrophoresis of rs1056836 and rs1800440 target region in ER+ breast cancer cases. L: 100 bp DNA marker, 1: AKU057, 2: AKU060, 3: AKU073, 4: AKU076, 5: AKU087, 6: AKU088, 7: AKU094, 8: AKU097, 9: AKU098, 10: AKU099, 11: AKU100, 12: AKU103, 13: KAIC005, 14: KAIC006, 15: KAIC007.

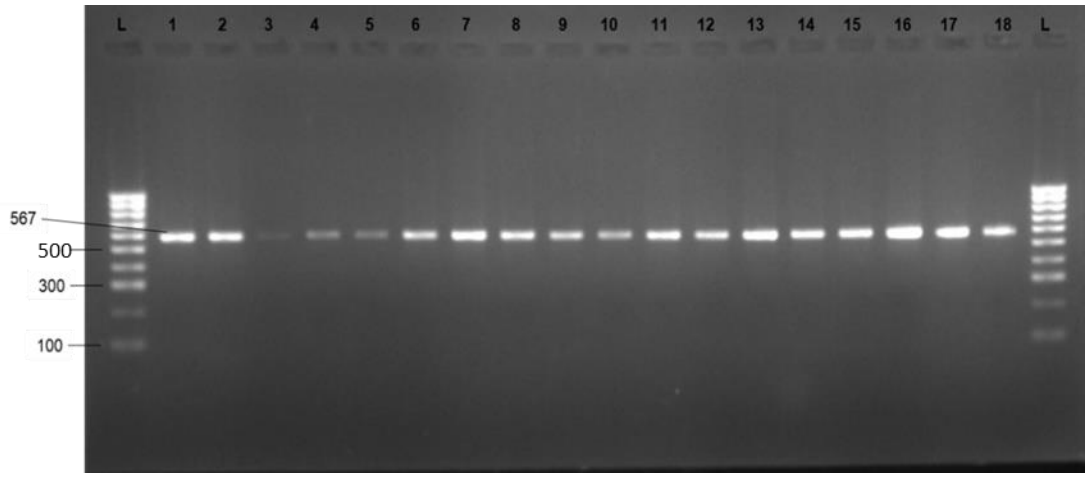

Figure 4.8: Gel Photo of rs10012 and rs1056827 Amplicons

Agarose gel electrophoresis of rs10012 and rs1056827 target region in ER+ breast cancer cases. L: 100 bp DNA marker, 1: AKU057, 2: AKU060, 3: AKU073, 4: AKU076, 5: AKU087, 6: AKU088, 7: AKU094, 8: AKU097, 9: AKU098, 10: AKU099, 11: AKU100, 12: AKU103, 13: KAIC005, 14: KAIC006, 15: KAIC007, 16: KAIC008, 17: KAIC011, 18: KAIC014.

4.3 Restriction Fragment Sizes of PCR amplicons

PCR amplicons digested with specific restriction enzymes were resolved on a 3% 1X TBE agarose gel. Agarose gel photos are shown in Figures 4.9 to 4.13. The photos project the observed banding patterns for each SNP based on restriction digestion. The complete genotype dataset is outlined in **Appendix 7**

4.3.1 Msp1 Restriction Digestion of rs4646903

Figure 4.9 displays a agarose gel photo of MspI restriction digestion for the rs4646903 (T > C) genetic variant. The T/T wild type genotype is represented by a single 340 bp fragment. The T/C heterozygous genotype is characterized by three fragments: 340 bp, 200 bp, and 140 bp. The C/C variant genotype yields two fragments: 200 bp and 140 bp. A 50 bp DNA ladder was included for size estimation.

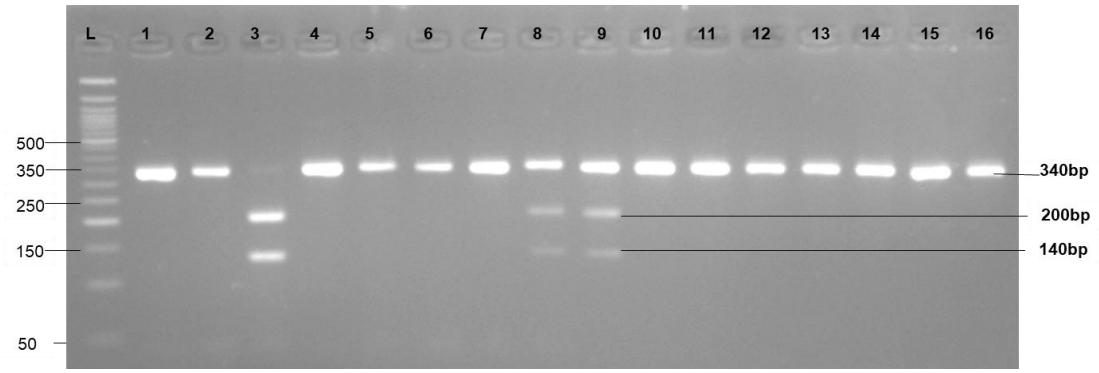

Figure 4.9: Gel Photo of PCR-RFLP Analysis for rs4646903

Agarose gel electrophoresis of MspI restriction digestion of rs4646903 in BBDs. L: 50 bp DNA marker, 1: Positive control (340 bp); 2: AKU011, 4: AKU031, 5: AKU034, 6: AKU039, 7: AKU043, 10: AKU044, 11: AKU046, 12: AKU047, 13: AKU048, 14: AKU049, 15: AKU054, and 16: AKU056 show wild type profiles (340 bp); Lanes 8: AKU059 and 9: AKU069 show heterozygous profiles (340 bp, 200 bp, 140 bp); 3: AKU196 shows a variant profile (200 bp and 140 bp).

4.3.2 BsrD1 Restriction Digestion of rs1048943

Figure 4.10 shows a gel photo of BsrDI digestion for the rs1048943 (A>G) variant. The A/A wild type genotype yields two fragments: 150 bp and 54 bp. The A/G heterozygous genotype produces three fragments: 204 bp, 150 bp, and 54 bp. The G/G variant genotype, along with the control, is represented by a single 204 bp fragment.

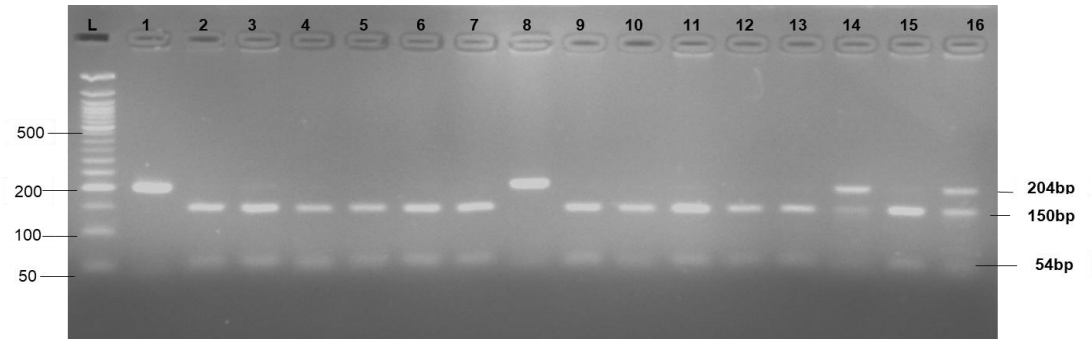

Figure 4.10: Gel Photo of RFLP-PCR for rs1048943

Agarose gel electrophoresis of BsrDI restriction digestion of rs1048943 in ER+ breast cancer cases. L: 50 bp DNA marker, 1: Undigested control (204 bp), 2: AKU055, 3: AKU073, 4: AKU104, 5: AKU105, 6: AKU110, 7: AKU112, 9: AKU116, 10: AKU122, 11: AKU124, 12: AKU127, 13: AKU128 and 15: AKU152 shows a wild type profile (150 bp and 54 bp); 14: AKU149 and 16: AKU173 show a heterozygous profile (204 bp, 150 bp, 54 bp); 8: AKU113 show a variant profile (204 bp).

4.3.3 AcuI Restriction Digestion of rs1056836

Figure 4.11 displays a gel photo of AcuI digestion for the rs1056836 (C > G) variant. The C/C wild type genotype yields a single 245 bp fragment. The C/G heterozygous genotype shows three fragments: 245 bp, 176 bp, and 69 bp. The G/G variant genotype is characterized by two fragments: 176 bp and 69 bp.

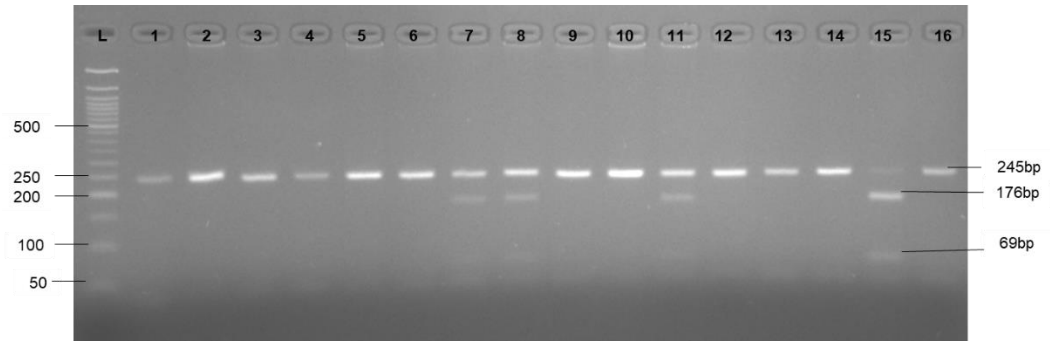

Figure 4.11: Gel Photo of RFLP-PCR for rs1056836

Agarose gel electrophoresis of AcuI restriction digestion of rs1056836 in BBDs. L: 50 bp DNA marker, 1: Undigested control (245 bp), 2: AKU083, 3: AKU187, 4: AKU107, 5: AKU119, 6: AKU126, 9: AKU139, 10: AKU146, 12: AKU153, 13: AKU166, 14: AKU170 and 14: AKU181 showed wild type profiles (245 bp); 7: AKU132, 8: AKU135 and 11: AKU148 showed heterozygous profiles (245 bp, 176 bp, 69 bp) and 15: AKU180 showed variant profile (176 bp and 69 bp).

4.3.4 SspI Restriction Digestion of rs776746

Figure 4.12 presents the gel photo of SspI digestion for rs776746 (A > G). The A/A wild type genotype produces two fragments: 137 bp and 114 bp. The A/G heterozygous genotype would be expected to show three fragments: 251 bp, 137 bp, and 114 bp. The

G/G variant genotype and the control yield a single 251 bp fragment.

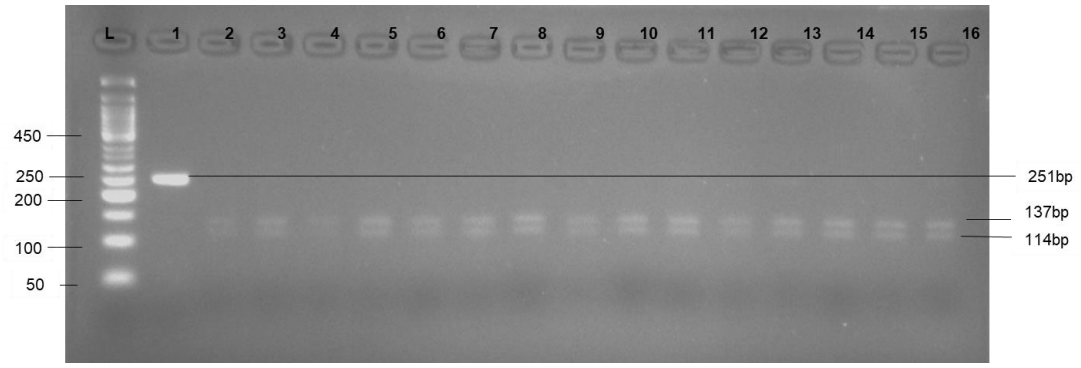

Figure 4.12: Gel Photo of PCR-RFLP for rs776746

Agarose gel electrophoresis of SspI restriction digestion of rs776746 in ER+ breast cancer cases. L: 50 bp DNA marker,1: Undigested control (251 bp), 2: AKU041, 3: AKU042, 4: AKU073, 5: AKU086, 6: AKU104, 7: AKU105, 8: AKU106, 9: AKU110, 10: AKU112, 11: AKU113, 12: AKU116, 13: AKU118, 14: AKU122, 15: AKU124 and 16: AKU127 showed wild type profiles (137 bp and 114 bp).

4.3.5 NlaIII Restriction Digestion of rs4680

Figure 4.13 shows the gel photo of NlaIII digestion for the rs4680 (A > G) variant. The A/A wild type genotype yields four fragments: 114 bp, 93 bp, 54bp and 43bp. The A/G heterozygous genotype would be expected to show all five fragments: 304,114,93,54,43. The G/G variant genotype produces one fragment: 304 bp.

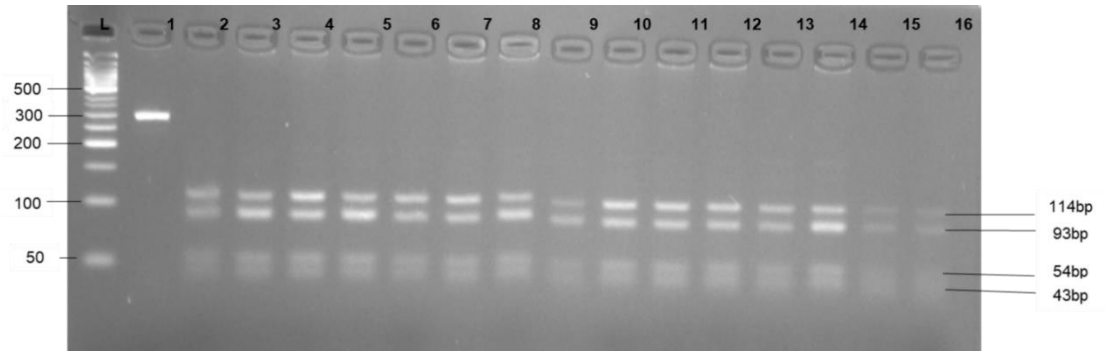

Figure 4.13: Gel Image of RFLP-PCR for rs4680

Agarose gel electrophoresis of NlaIII restriction digestion of rs4680 in BBDs. L: 50 bp DNA marker, 1: Undigested control (304 bp), 2: AKU075, 3: AKU077, 4: AKU083, 5: AKU107, 6: AKU119, 7: AKU126, 8: AKU132, 9: AKU135, 10: AKU139, 11: AKU146, 12: AKU148, 13: AKU153, 14: AKU166, 15: AKU170 and 16: AKU180 showed the wild type profiles (114 bp, 93 bp, 54 bp, and 43 bp).

4.4 Sanger Sequencing of CYP1B1 Gene

A total of 123 samples consisting of 64 cases, 19 controls and 40 benigns were sequenced.

Single nucleotide polymorphisms (SNPs) in CYP1B1 variants (rs10012, rs1056827, rs1056836, and rs1800440) were analyzed using *Geneious Prime* 2025.1.0 (Biomatters Ltd.). Sequence variants were mapped to the GRCh38.p14 reference genome (NCBI accession: NC_000002.12). The sequences were aligned and viewed in MEGA12. Figures 4.14–4.15. The list of Sanger sequences for are detailed in **Appendices VIII and IX.**

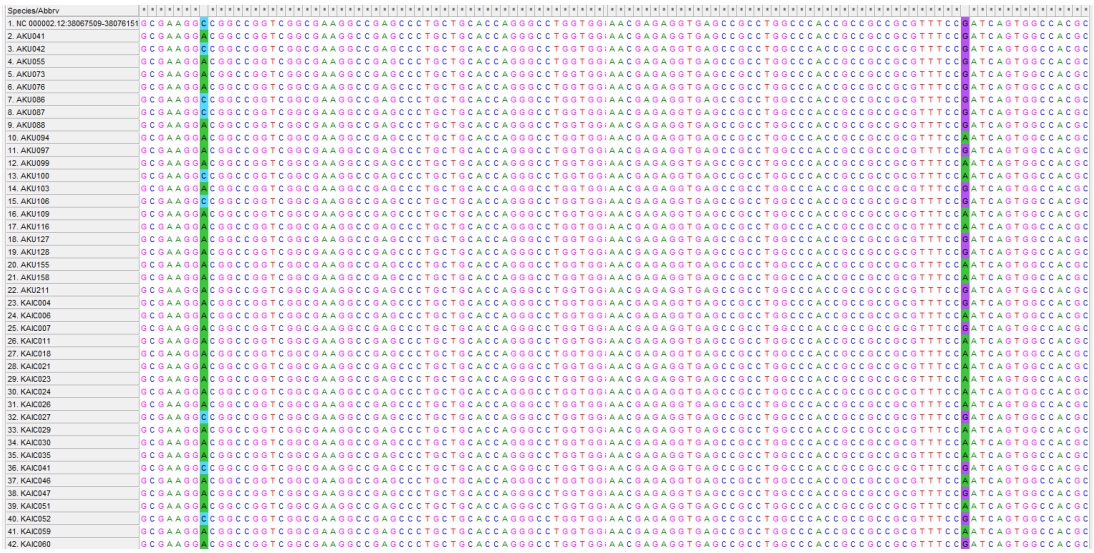

Figure 4.14: Sanger Sequences of rs10012 and rs1056827 SNPs in CYP1B1

The figure displays the CYP1B1 exon 2 sequences. Some samples exhibited wild type allele (C) and others variant allele (A) for the rs10012. For the rs1056827, some samples exhibited the wild type allele (G) and others variant allele (A).

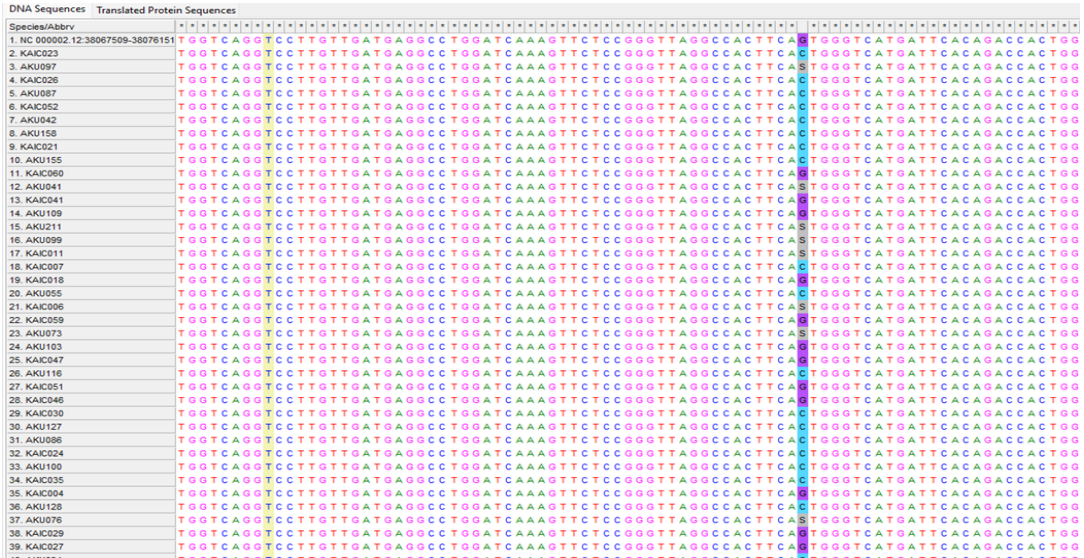

Figure 4.15: Sanger Sequences of rs1800440 and rs1056836 SNPs in CYP1B1

The figure displays the CYP1B1 exon 3 sequences. All the samples exhibited wild type allele (T) for the rs1800440. For the rs1056836 some samples exhibited wild type allele (G), majority exhibited variant allele (C) and a few heterozygous (C/G=S).

4.5 Descriptive Analysis of Socio-Demographic Characteristics, Risk Factors, and SNPs in Study Participants

The data used was obtained from Genomic Breast Cancer Progression Study. Age distribution varied significantly between the study participants. The ages of cases ranged had a median age of 52 years (IQR: 28–78), the controls had a median age of 41 years (IQR: 25–52), while that in the BBD was 42 years (IQR: 23-74). Notably, 58% of the cases were aged 50 years or older, compared to just 5.3% of controls ($p < 0.001$), and 28% of the BBDs ($p < 0.001$). indicating that ER+ breast cancer was more common among older women.

Although not statistically significant, regional distribution showed a notable trend: 84% of the controls were from Nairobi compared to 41% of the cases and 54% of the BBDs. Cases exhibited a wider geographic distribution across the seven regions while the controls and BBDs were majorly from three regions.

BMI distribution did not differ significantly between the groups. While a higher proportion of cases (52%) and BBDs (43%) were overweight or obese, the differences were not statistically significant.

Menopausal status showed a statistically significant difference between cases and controls ($p < 0.001$) as well as between cases and BBDs ($p < 0.001$). Most controls (95%) were premenopausal. This pattern may reflect the older age profile of the cases.

A family history of breast cancer was reported by 20% of cases but none for the controls ($p = 0.033$). Similarly, 33% of cases reported a history of other cancers, compared to none among controls ($p = 0.002$).

Alcohol consumption was significantly more common among controls (74%) and the BBDs (65%) compared to only 38% in the cases. The differences were statistically significant with $p = 0.005$ and $p = 0.001$ respectively. This was a potential reflection of differing lifestyle behaviors among the three groups.

For rs4646903 (CYP1A1*2A), the wild-type (TT) was the most common genotype across all groups, being most prevalent in the BBD group (77%), followed by Cases (67%), and least in Controls (47%). The heterozygous (TC) frequency was similar in Cases (28%) and Controls (37%) but lower in BBDs (22%). The variant homozygous (CC) genotype was rare overall, most frequent in Controls (16%), then Cases (4.7%), and very rare in the BBD group (1.3%).

The rs1048943 (CYP1A1*2C) SNP showed a predominant wild-type (AA) genotype in the vast majority of participants: 100% of Controls, 91% of Cases, and 87% of the BBD group. Variant genotypes were uncommon; the AG genotype was present in Cases (6.3%) and BBDs (10%) but absent in Controls, while the GG genotype was found only in Cases (3.1%) and BBDs (2.5%).

At the rs1056836 (CYP1B1*3) locus, the wild-type (GG) was most frequent in the BBD (71%) and Control (58%) groups but was less common in Cases (47%). The heterozygous (GC) frequency was similar in Cases (41%) and Controls (42%) but lower in BBDs (29%). Critically, the variant homozygous (CC) genotype was found exclusively in the Case group, present in 13% of participants and absent in both Controls and BBDs, which likely drives the significant statistical difference observed.

The analysis for rs776746 (CYP3A5*3) revealed no genetic variation in this cohort. All participants across Cases, Controls, and the BBD group (100%) were homozygous for the A allele (AA). This complete lack of polymorphism makes this SNP non-informative for case-control analysis within the studied population.

For rs4680 (COMT Val158Met), the wild-type (AA) was the predominant genotype in all groups, present in 100% of Controls and BBDs and 94% of Cases. The variant alleles were found only in the Case group: the heterozygous (AG) genotype was present in 4.7% of Cases, and the homozygous (GG) genotype was present in 1.6% of Cases. This exclusive presence in cases contributed to a statistically significant difference compared to the BBD group. (Table 4.1).

Table 4.1: Socio-Demographic Characteristics, Risk Factors, and SNPs Distributions in ER+ Breast Cancer Cases and Controls

Variable	Characteristic	Cases (n = 64[1]) n (%)	Controls (n = 19[1]) n (%)	Cases vs. Controls p-value[2]	BBD (n = 79[1]) n (%)	Cases vs. BBDs p-value[2]
Age (Years)	≥50	37 (58)	1 (5.3)	**<0.001**	18 (23)	**<0.001**
	40-49	17 (27)	10 (53)		34 (43)	
	30-39	9 (14)	4 (21)		24 (30)	
	20-29	1 (1)	4 (21)		3 (3.8)	
	median, IQR	52, 28-78	41, 25-52		42, 23-74	
Region	Nairobi	26 (41)	16 (84)	0.059	43 (54)	0.4
	Central	21 (33)	2 (11)		21 (27)	
	Rift Valley	9 (14)	1 (5.3)		6 (7.6)	
	Eastern	5 (7.8)	0 (0)		8 (10)	
	Coast	1 (1.6)	0 (0)		1 (1.3)	
	Nyanza	1 (1.6)	0 (0)		0 (0)	
	Western	1 (1.6)	0 (0)		0 (0)	
BMI (Kg/M^2)	≥30	33 (52)	6 (32)	0.2	34 (43)	0.5
	25-29	19 (30)	6 (32)		29 (37)	
	18-24	11 (17)	7 (37)		16 (20)	
	<17	1 (1)	0 (0)		0 (0)	
	median, IQR	29, 10-42	29, 19-34		29, 19-43	
Age at menarche (Years)	<12	10 (16)	6 (32)	0.2	53 (67)	0.1
	13-15	32 (50)	10 (53)		14 (18)	
	≥16	22 (34)	3 (16)		12 (15)	
	median, IQR	13, 8-22	13, 10-17		14, 11-17	
Menopausal status	No	30 (47)	18 (95)	**<0.001**	61 (77)	**<0.001**
	Yes	34 (53)	1 (5.3)		18 (23)	
Parity	No	7 (11)	6 (32)	0.065	61 (77)	0.064
	Yes	57 (89)	13 (68)		18 (23)	
Contraceptives	No	21 (33)	2 (11)	0.057	63 (80)	0.088
	Yes	43 (67)	17 (89)		16 (20)	
History of breast cancer	No	51 (80)	19 (100)	**0.033**	62 (78)	0.9
	Yes	13 (20)	0 (0)		17 (22)	
History of other cancers	No	43 (67)	19 (100)	**0.002**	60 (76)	0.2

Variable	Characteristic	Cases (n = 64[1]) n (%)	Controls (n = 19[1]) n (%)	Cases vs. Controls p-value[2]	BBD (n = 79[1]) n (%)	Cases vs. BBDs p-value[2]
	Yes	21 (33)	0 (0)		19 (24)	
Diabetes	No	57 (89)	19 (100)	0.3	72 (91)	0.7
	Yes	7 (11)	0 (0)		7 (8.9)	
Hypertension	No	44 (69)	14 (74)	0.7	61 (77)	0.3
	Yes	20 (31)	5 (26)		18 (23)	
Radiation therapy	No	63 (98)	19 (100)	>0.9	76 (96)	0.6
	Yes	1 (1.6)	0 (0)		3 (4)	
Tobacco	No	62 (97)	19 (100)	>0.9	77 (97)	>0.9
	Yes	2 (3.1)	0 (0)		2 (2.5)	
Alcohol consumption	No	40 (63)	5 (26)	**0.005**	51 (65)	**0.001**
	Yes	24 (38)	14 (74)		28 (35)	
rs4646903	TT	43 (67)	9 (47)	0.13	61 (77)	0.3
	TC	18 (28)	7 (37)		17 (22)	
	CC	3 (4.7)	3 (16)		1 (1.3)	
rs1048943	AA	58 (91)	19 (100)	0.7	69 (87)	0.7
	AG	4 (6.3)	0 (0)		8 (10)	
	GG	2 (3.1)	0 (0)		2 (2.5)	
rs1056836	GG	30 (47)	11 (58)	0.3	56 (71)	**<0.001**
	GC	26 (41)	8 (42)		23 (29)	
	CC	8 (13)	0 (0)		0 (0)	
rs776746	AA	64 (100)	19 (100)	-	79 (100)	-
rs4680	AA	60 (94)	19 (100)	>0.9	79 (100)	**0.038**
	AG	3 (4.7)	0 (0)		0 (0)	
	GG	1 (1.6)	0 (0)		0 (0)	

[1]n (%): Number of participants, percentage
[2]Fisher's exact test; Pearson's Chi-squared test
Bold: Significant p-value of <0.05

Descriptive comparison of socio-demographics, reproductive, lifestyle, and genetic characteristics in ER+ breast cancer and controls. Statistical significant differences were found in age, menopausal status, history of breast cancer, history of other cancers and alcohol consumption. No significant differences were observed in BMI, region, age at menarche, parity, contraceptive use, diabetes, hypertension, radiation therapy, tobacco use, rs4646903, rs1048943, rs1056836 and rs4680. (Data source: Genomic Breast Cancer Progression Study)

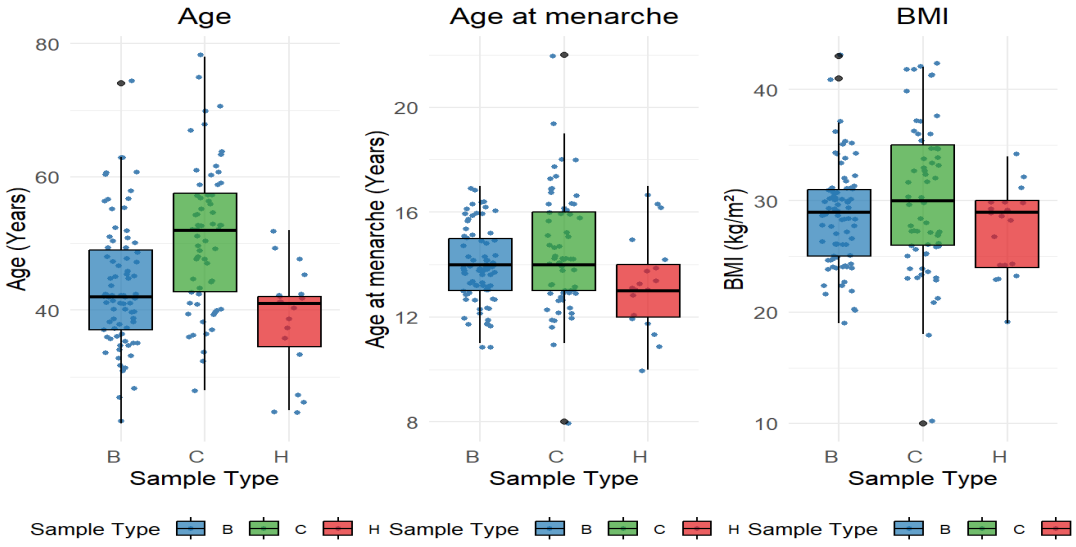

Figure 4.16: Boxplot Comparison of Age, BMI, and Age at Menarche among ER+ Breast Cancer Cases, Benign Breast Disease and Healthy Participants

The figure shows the distributions of age, age at menarche and body mass index (BMI) across the three study groups. The ER+ breast cancer cases (C) were significantly older than both benign breast disease (BBD) and healthy participants (H). No significant differences were observed in the distribution of age at menarche and BMI in the three groups.

4.6 Descriptive Analysis of CYP1B1 SNPs in ER+ Breast Cancer and Control Participants

Table 4.3 presents the distribution of CYP1B1 SNPs (rs10012, rs1056827, rs1056836, and rs1800440) among ER+ breast cancer cases and control participants. The data used was obtained from Genomic Breast Cancer Progression Study. No statistically significant associations were observed between these SNPs and breast cancer risk ($p > 0.05$ for all comparisons).

For rs10012, the heterozygous GC genotype was more prevalent in cases (55%) than controls (37%), while the variant CC genotype was more frequent in controls (53%) compared to the cases (28%). However, the differences were not statistically significant ($p = 0.300$).

For rs1056827, the variant AA genotype was predominant in both cases (72%) and controls (89%) – without significant difference ($p = 0.300$).

Similarly, for rs1056836, genotype distributions were comparable between groups, with the GG genotype slightly more frequent in controls (68%) than in cases (61%), but this difference was not significant ($p = 0.900$).

The rs1800440 SNP was monomorphic across all participants, with everyone exhibiting the TT genotype. (Table 4.2). The genotype profiles of rs10012, rs1056827, rs1056836 and rs1800440 are detailed in **Appendix 10.**

Table 4.2: Genotype Frequencies of CYP1B1 SNPs among ER+ Breast Cancer Cases and Controls

	Characteristic	Cases (n = 64[1]) n (%)	Controls (n = 19[1]) n (%)	p-value[2]
rs10012	GG	11 (17)	2 (11)	0.2
	GC	35 (55)	7 (37)	
	CC	18 (28)	10 (53)	
rs1056827	CC	12 (19)	2 (11)	0.4
	CA	6 (9.4)	0 (0)	
	AA	46 (72)	17 (89)	
rs1056836	GG	39 (61)	13 (68)	0.7
	GC	11 (17)	4 (21)	
	CC	14 (22)	2 (11)	
rs1800440	TT	64 (100)	19(100)	-

[1] n (%) frequencies and percentage
[2] Fisher's exact test; Pearson's Chi-squared test
bold high frequencies

Descriptive analysis of rs10012, rs1056827, rs1056836, and rs1800440 genotypes among ER+ breast cancer versus controls. No statistically significant differences were observed for all SNPs ($p > 0.05$). (Data source: Genomic Breast Cancer Progression Study)

4.7 Descriptive Analysis of CYP1B1 SNPs in ER+ Breast Cancer and Benign Breast Diseases Participants

Table 4.4 compares CYP1B1 genotypic distributions between ER+ breast cancer and participants with BBD. No significant differences in the distribution of the genotypes were found across the genotypes ($p > 0.05$ for all SNPs).

For rs10012, the variant CC genotype was slightly more frequent in BBD cases (37%) compared to ER+ breast cancer (28%). Conversely, the wild type GG genotype was more common in ER+ breast cancer cases (17%) than in BBD participants (8%). However, the differences were not statistically significant ($p = 0.300$).

For rs1056827, the variant AA genotype appeared more frequently in BBD participants (87%) than in breast cancer cases (72%), while the wild type CC genotype was slightly

higher in ER+ breast cancer cases (19%) compared to BBD (11%). Again, this difference was not significant (p = 0.300).

For rs1056836, genotype frequencies were similar across groups. The wild type GG genotype was observed in 63% of BBD and 61% in ER+ breast cancer cases, with no statistically significant difference (p = 0.900) (Table 4.3). The genotype profiles of rs10012, rs1056827, rs1056836 and rs1800440 are detailed in **Appendix 10.**

Table 4.3: Genotype Frequencies of CYP1B1 SNPs among ER+ Breast Cancer Cases and BBD Participants

	Characteristic	Cases (n = 64[1]) n (%)	BBD (n = 38[1]) n (%)	p-value[2]
rs10012	GG	11 (17)	3 (8)	0.4
	GC	35 (55)	21 (55)	
	CC	18 (28)	14 (37)	
rs1056827	CC	12 (19)	4 (11)	0.2
	CA	6 (9)	1 (3)	
	AA	46 (72)	33 (87)	
rs1056836	GG	39 (61)	24 (63)	>0.9
	GC	11 (17)	6 (16)	
	CC	14 (22)	8 (21)	
rs1800440	AA	64 (100)	38 (100)	

[1] n (%) Frequency and percentage
[2] Fisher's exact test; Pearson's Chi-squared test

Descriptive comparison of rs10012, rs1056827, rs1056836, and rs1800440 between ER+ breast cancer and BBDs. No statistically significant differences were observed (p > 0.05).

4.8 Tumor Characteristics in Estrogen Receptor-Positive Breast Cancer Cases

Data was obtained from "A Genomic Approach for Understanding Breast Cancer Progression in Kenya". Among the estrogen receptor positive (ER+) breast cancer participants, the predominant histological type was invasive ductal carcinoma (IDC), accounting for 76.7% of cases. Other histological types included ductal carcinoma in situ (DCIS) (6.3%), invasive carcinoma (6.3%), and invasive lobular carcinoma (ILC) (4.7%).

Most tumors were moderately differentiated (Grade 2: 62.5%), followed by poorly differentiated (Grade 3: 32.8%) and well-differentiated (Grade 1: 4.7%) subtypes

Lymph node involvement was observed in 56.2% of cases (N1-N3), with 43.8% node-negative (N0). Stage II disease was most prevalent (45.3%), followed by Stage I (28.1%) and Stage III (26.6%). Luminal A tumors comprised 82.4% of cases, while Luminal B accounted for 15.6%. (Table 4.4). The clinicalpathological characteristics of the case group participants is outlined in **Appendix 11.**

Table 4.4: Tumor Characteristics of ER+ Breast Cancer Cases

Tumor characteristic	**Levels**	**n = 64**	**%**
Histological type	IDC (Invasive ductal carcinoma)	49	76.7
	ILC (Invasive lobular carcinoma)	3	4.7
	DCIS (Ductal carcinoma in situ)	4	6.3
	IPC (Intracystic papillary carcinoma)	1	1.5
	PDCIS (Papillary ductal carcinoma in situ)	1	1.5
	MC (Mucinous carcinoma)	1	1.5
	IC (Invasive carcinoma)	4	6.3
	IMCS (Internal mammary chain sentinel node)	1	1.5
Grade	Grade 1 (Well differentiated)	3	4.7
	Grade 2 (Moderately differentiated)	40	62.5
	Grade 3 (Poorly differentiated)	21	32.8
Lymph node involvement	N0	28	43.8
	N1	18	28.1
	N2	13	20.3
	N3	5	7.8
Clinical stage	Stage I	18	28.1
	Stage II	29	45.3
	Stage III	17	26.6
Molecular subtypes	Luminal A	54	82.4
	Luminal B	10	15.6

Tumor characteristics ER+ breast cancer cases. This table summarizes the histological types, tumor grades, lymph node involvement, clinical stages, and molecular subtypes of ER+ breast cancer cases. The majority of tumors were invasive ductal carcinoma (76.7%)

and moderately differentiated (Grade 2, 62.5%). Most cases were classified as Stage II (45.3%) and belonged to the Luminal A molecular subtype (82.4%). (Data Source: "A Genomic Approach for Understanding Breast Cancer Progression in Kenya").

4.9 Association between Socio-Demographics and Risk factors with ER+ Breast Cancer Compared to Controls

In the adjusted logistic regression model, menopausal status emerged as a significant predictor of ER+ breast cancer. Postmenopausal women had significantly higher odds of developing ER+ disease compared to premenopausal women (OR = 7.16, 95% CI [1.19, 43.13], p = 0.031).

Other variables—including age category (p = 0.897), BMI (p = 0.476), parity (p = 0.430), and contraceptive use (p = 0.245)—were not significantly associated with ER+ breast cancer. However, trends suggested a possible increased risk with higher BMI and a reduced risk with parity. These findings underscore the importance of hormonal factors—particularly menopausal status—over age alone in determining susceptibility to ER+ breast cancer (Table 4.5).

Table 4.5: Association between Socio-Demographics and Risk Factors with ER+ Breast Cancer Compared to Controls

Variable		OR	95% CI	p-value
Age (years)	≤40	Ref		
	≥41	1.094	0.276-4.330	0.897
BMI (Kg/m^2)	≤24	Ref		
	≥25	1.771	0.367-8.547	0.476
Age at menarche	≥ 12	Ref		
	≤ 13	0.787	0.176-3.526	0.755
Menopausal status	No	Ref		
	Yes	7.157	1.187-43.131	**0.031**
Parity	No	Ref		
	Yes	0.453	0.063-3.233	0.430
Contraceptives use	No	Ref		
	Yes	0.306	0.042-2.256	0.245

OR: odds ratio, CI: confidence interval, bold: -statistical significance of $p < 0.05$, BMI: body mass index

Association of risk factors with ER+ Breast cancer risk compared to controls. Adjusted odds ratios (ORs), 95% confidence intervals (CIs), and p-values from multivariate logistic regression assessing associations between selected variables and ER+ breast cancer. Menopausal status was the only statistically significant predictor.

4.10 Association between Selected Variables and ER+ Breast Cancer Compared to Benign Breast Diseases

In the comparison between ER+ breast cancer cases and participants with benign breast diseases (BBD), menopausal status was again a significant risk factor. Postmenopausal women had over twice the odds of developing ER+ breast cancer (OR = 2.37, 95% CI [1.00, 5.70], p = 0.049).

Other variables—including age at menarche, parity, contraceptive use, history of other cancers, and hypertension—were not significantly associated with ER+ breast cancer. These results suggest that menopausal status is a consistent risk factor (Table 4.6).

Table 4.6: Association between Socio-Demographics and Risk Factors with ER+ Breast Cancer Compared to BBD Participants

Variable		OR	95% CI	p-value
Age (Years)	≤40	Ref		
	≥41	1.144	0.448-2.965	0.777
Age at menarche (years)	≥ 12	Ref		
	≤ 13	1.234	0.436-3.463	0.687
Menopausal status	No	Ref		
	Yes	2.367	1.000-5.698	**0.049**
Parity	No	Ref		
	Yes	0.637	0.201-1.892	0.421
Use of contraceptives	No	Ref		
	Yes	0.776	0.303-1.973	0.593
History of other cancers	No	Ref		
	Yes	1.864	0.845-4.194	0.123
Hypertension	No	Ref		
	Yes	1.166	0.501-2.676	0.717

Key: OR-odds ratio, CI- confidence interval, bold*statistical significance

Association of demographics and factors with ER+ Breast cancer compared to BBD. Adjusted odds ratios (ORs), 95% confidence intervals (CIs), and p-values from multivariate logistic regression comparing ER+ breast cancer cases and BBD participants. Menopausal status increased risk.

4.11 Association of rs4646903, rs1048943, rs1056836, rs776746 and rs4680 with ER+ Breast Cancer Compared to Controls

The rs4646903 polymorphism showed a significant association with breast cancer risk at the allele level. Specifically, the alternative C allele was found to be less frequent among ER+ breast cancer cases compared to controls, suggesting a potential protective effect (OR = 0.44, 95% CI [0.19, 0.99], p = 0.048). To further assess this association, several genotype models were tested. These included the codominant model, which compares each genotype separately (e.g., TT vs. TC vs. CC); the dominant model, which groups individuals carrying at least one copy of the variant allele (TC and CC) and compares them to wild type individuals (TT); the recessive model, which compares variant carriers (CC) to all other genotypes (TT and TC); the overdominant model, which evaluates whether heterozygous individuals (TC) differ in risk compared to both homozygotes (TT and CC); and the log-additive model, which assumes a linear change in risk with each additional copy of the variant allele. However, none of these genotype models reached statistical significance ($p > .05$), indicating that while the allele-level association is notable, the genotypic distribution did not strongly support a model-specific effect.

In contrast, for rs1056836, no statistically significant associations were observed in either allele-based or genotype-based analyses ($p > .05$), suggesting that this variant may not influence breast cancer risk in the study population. Due to limited genotype variation, model convergence issues, and lack of statistical significance, rs1048943 and rs4680 were excluded from the final genotype association analysis (Table 4.7)

Table 4.7: Genotype Frequencies and Associations of rs4646903 and rs1056836 Variants in ER+ Breast Cancer Versus Control Participants

SNP ID	Model	Variants	Cases n (%)	Controls n (%)	OR	95% CI	p-value
rs4646903	Alleles	T	104 (81.3)	25 (65.8)	Ref		
		C	24 (18.7)	13 (34.2)	0.44	0.19-0.99	**0.048**
	Codominant	T/T	43 (67.2)	9 (47.4)	Ref		
		T/C	18 (28.1)	7 (36.8)	0.54	0.17-1.67	0.283
		C/C	3 (4.7)	3 (15.8)	0.21	0.04-1.21	0.081
	Dominant	T/T	43 (67.2)	9 (47.4)	Ref		
		T/C-C/C	21 (32.8)	10 (52.6)	0.44	0.16-1.24	0.121
	Recessive	T/T-T/C	61 (95.3)	16 (84.2)	Ref		
		C/C	3 (4.7)	3 (15.8)	0.26	0.05-1.42	0.121
	Overdominant	T/T-C/C	46 (71.9)	12 (63.2)	Ref		
		T/C	18 (28.1)	7 (36.8)	0.67	0.23-1.97	0.469
	log-Additive	0,1,2	64 (77.1)	19 (22.9)	0.48	0.22-1.05	0.067
rs1056836	Alleles	G	86 (67.2)	30 (79)	Ref		
		C	42 (32.8)	8 (21)	0.54	0.23-1.29	0.169
	Codominant	G/G	30 (46.9)	11 (57.9)	Ref		
		GC	26 (40.6)	8 (42.1)	0.83	0.29-2.40	0.74
		C/C	8(12.5)	0 (0.0)	0.15	0.01-2.92	0.21
	Dominant	G/G	30 (46.9)	11(57.9)	Ref		
		G/C- C/C	34 (53.1)	8 (42.1)	0.64	0.22-1.80	0.40
	Recessive	G/G -G/C	56 (87.5)	19 (100)	Ref		
		C/C	8 (12.5)	0 (0)	0.17	0.01-3.09	0.231
	Overdominant	G/G-C/C	38 (59.4)	11 (57.9)	Ref		
		G/C	26 (40.6)	8 (42.1)	1.06	0.37-3.00	0.908
	log-Additive	0,1,2	64 (77.1)	19 (22.9)	1.82	0.76-4.31	0.298

OR: odds ratio, CI: confidence interval, bold statistical significance ($p < 0.05$)

Genotype and allele frequencies of rs4646903 and rs1056836 polymorphisms and their associations with ER+ breast cancer versus controls. The alternative C allele of rs4646903 was significantly associated with a lower risk of breast cancer (OR = 0.44, p = 0.048), suggesting a protective effect. No significant associations were observed for rs1056836 across all models.

4.12 Associations of rs4646903, rs1048943, rs1056836 SNPs with ER+ Breast Cancer Compared to Benign Breast Diseases

This analysis assessed the association of three SNPs (rs4646903, rs1048943, and rs1056836) with ER+ breast cancer in comparison to benign breast diseases (BBD). rs776746 and rs4680 were not analysed because they were monomorphic wild type in both the controls and BBDs. Among these, rs1056836 emerged as the only SNP with statistically significant associations across multiple genetic models.

In the allelic model, the alternative C allele of rs1056836 was significantly associated with reduced risk of ER+ breast cancer (OR = 0.34, 95% CI [0.19–0.62], p = 0.0003), suggesting a potential protective effect. This finding was consistent in the codominant model, where individuals with the G/C and C/C genotypes exhibited lower risk of ER+ breast cancer at OR = 0.47, 95 CI [0.23–0.96], p = 0.040 and OR = 0.03, 95 CI: 0.00–0.57, p = 0.019 respectively. Similarly, in the dominant model, carriers of either the G/C or C/C genotypes showed a significantly reduced risk compared to G/G homozygotes (OR = 0.36, 95 CI [0.18–0.72], p = 0.004). The recessive model also revealed a protective association for the C/C genotype (OR = 0.04, 95 CI [0.00–0.74], p = 0.030), and the log-additive model (per G allele) showed a dose-dependent increase in risk of ER+ breast cancer (OR = 2.94, 95% CI [1.60–5.39], p = 0.0003).

In contrast, rs4646903 and rs1048943 did not show any statistically significant associations with ER+ breast cancer or BBD. Across all tested genetic models, *p*-values exceeded the standard threshold for significance ($p > 0.05$) (Table 4.8)

Table 4.8: Genotype Frequencies and Associations of rs4646903, rs1048943 and rs1056836 variants in ER+ Breast Cancer Versus Benign Breast Disease Participants

SNP ID	Model	Variants	Cases n (%)	BBDs n (%)	OR	95% CI	p-value
rs4646903	Alleles	T	104 (81)	139 (88)	Ref		
		C	24 (19)	19 (12)	1.68	0.87 - 3.24	0.116
	Codominant	T/T	43 (67.2)	61 (77.2)	Ref		
		T/C	18 (28.1)	17(21.5)	1.50	0.70 - 3.24	0.299
		C/C	3 (4.7)	1 (1.3)	4.26	0.43-42.30	0.216
	Dominant	T/T	43 (67.2)	61(77.2)	Ref		
		T/C-C/C	21 (32.8)	18 (22.8)	1.66	0.79-3.47	0.182
	Recessive	T/T-T/C	61 (95.3)	78 (98.7)	Ref		
		C/C	3 (4.7)	1 (1.3)	3.84	0.39-37.80	0.249
	Overdominant	T/T-C/C	46 (71.9)	62 (78.5)	Ref		
		T/C	18 (28.1)	17 (21.5)	1.43	0.66-3.07	0.362
	log-Additive	0,1,2	64 (44.8)	79 (55.2)	1.66	0.87-3.18	0.121
rs1048943	Alleles	A	120 (94)	146 (92)	Ref		
		G	8 (6)	12 (8)	0.81	0.32-2.04	0.657
	Codominant	A/A	58 (90.6)	69 (87.3)	Ref		
		A/G	4 (6.2)	8 (10.1)	0.59	0.17-2.08	0.415
		G/G	2 (3.1)	2 (2.5)	1.19	0.16-8.71	0.864
	Dominant	A/A	58 (90.6)	69 (87.3)	Ref		
		A/G-G/G	6 (9.4)	10 (12.7)	0.71	0.24-2.08	0.537
	Recessive	A/A-A/G	62 (96.9)	77 (97.5)	Ref		
		G/G	2 (3.1)	2 (2.5)	1.24	0.17-9.07	0.830
	Overdominant	A/A-G/G	60 (93.8)	71 (89.9)	Ref		
		A/G	4 (6.2)	8 (10.1)	0.59	0.17-2.06	0.410
	log-Additive	0,1,2	64 (44.8)	79 (55.2	0.86	0.39-1.90	0.701
rs1056836	Alleles	G	86 (67.2)	135 (85.4)	Ref		
		C	42 (32.8)	23 (14.5)	0.34	0.19-0.62	**0.0003**
	Codominant	G/G	30 (46.9)	56 (70.9)	Ref		
		G/C	26 (40.6)	23 (29.1)	0.47	0.23-0.96	**0.040**
		C/C	8 (12.5)	0 (0)	0.03	0.00-0.57	**0.019**
	Dominant	G/G	30 (46.9)	56 (70.9)	Ref		
		G/C- C/C	34 (53.1)	23 (29.1)	0.36	0.18-0.72	**0.004**
	Recessive	G/G -G/C	56 (87.5)	79 (100)	Ref		
		C/C	8 (12.5)	0 (0)	0.04	0.00-0.74	**0.030**
	Overdominant	G/G-C/C	38 (59.4)	56 (70.9)	Ref		
		G/C	26 (40.6)	23 (29.1)	0.60	0.29-1.20	0.150
	log-Additive	0,1,2	64 (44.8)	79 (55.2)	2.94	1.60-5.39	**0.0003**

Key: OR: odds ratio, CI: confidence interval, bold: statistical significance (p=<0.05)

Association of rs4646903, rs1048943 and rs1056836 variants with ER+ breast cancer versus BBD. This table presents the distribution of genotypes and alleles for three SNPs and their association with ER+ breast cancer versus benign breast diseases, evaluated under different genetic models (allelic, codominant, dominant, recessive, overdominant,

and log-additive). Odds ratios (OR), 95% confidence intervals (CI), and p-values indicate the strength and significance of each association.

4.13 Association of CYP1B1 SNPs with ER+ Breast Cancer Risk Compared to Controls

The association between rs10012, rs1056827, and rs1056836 SNPs acquired via Sanger sequencing and estrogen receptor-positive (ER+) breast cancer risk was evaluated using multiple genetic models. The analysis revealed no statistically significant associations for any of the SNPs studied. All tested models—including allelic, codominant, dominant, recessive, overdominant, and log-additive—produced p-values greater than 0.05. Thus, none of these polymorphisms were significantly associated with ER+ breast cancer risk in this cohort (Table 4.9).

Table 4.9: Genotype Frequencies and Associations of rs10012, rs1056827 and rs1056836 Variants in ER+ Breast Cancer Versus Control Participants

SNP ID	Model	Variants	Controls	Cases	OR	95% CI	p-value
rs10012	Alleles	G	11(28.9)	57(44.5)	Ref		
		C	27(71.7)	71(55.5)	0.5	0.23 - 1.11	0.09
	Codominant	G/G	2 (10.5)	11(17.2)	Ref		
		G/C	7 (36.8)	35(54.7)	0.91	0.16 - 5.03	0.913
		C/C	10 (52.6)	18 (28.1)	0.33	0.06 - 1.78	1.196
	Dominant	G/G	2 (10.5)	11 (17.2)	Ref		
		G/C-C/C	17 (89.5)	53 (82.8)	0.57	0.11 - 2.81	0.488
	Recessive	G/G-G/C	9 (47.4)	46 (71.9)	Ref		
		C/C	10 (52.6)	18 (28.1)	0.35	0.12 - 1.01	0.052
	Overdominant	G/G-C/C	12 (63.2)	29 (45.3)	Ref		
		G/C	7 (36.8)	35(54.7)	2.07	0.72 - 5.94	0.176
	log-Additive	0,1,2	19 (22.9)	64 (77.1)	0.49	0.21 - 1.1	0.073
rs1056827	Alleles	C	34 (89.5)	98 (76.6)	Ref		
		A	4 (10.5)	30 (23.4)	2.6	0.85 - 7.92	0.09
	Codominant	C/C	17 (89.5)	46 (71.9)	Ref		
		C/A	0 (0.0)	6 (9.4)	4.89	0.26 - 91.49	0.288
		A/A	2 (10.5)	12 (18.8)	2.22	0.45 - 10.95	0.328
	Dominant	C/C	17 (89.5)	46 (71.9)	Ref		
		C/A-A/A	2(10.5)	18 (28.1)	3.33	0.7 - 15.88	0.131
	Recessive	C/C-C/A	17 (89.5)	52 (81.2)	Ref		
		A/A	2 (10.5)	12 (18.8)	1.96	0.4 - 9.66	0.41
	Overdominant	C/C-A/A	19 (100)	58 (90.6)	Ref		0.328
			0 (0.0)	6 (9.4)		0	
	log-Additive	0,1,2	19 (22.9)	64 (77.1)	1.73	0.74 - 4.04	0.373

SNP ID	Model	Variants	Controls	Cases	OR	95% CI	p-value
rs1056836	Alleles	G	30 (78.9)	89 (69.5)	Ref		
		C	8 (21.1)	39 (30.5)	0.60	0.25-1.44	0.260
	Codominant	G/G	13 (68.4)	39 (60.9)	Ref		
		G/C	4 (21.1)	11(17.2)	1.09	0.29-4.02	0.896
		C/C	2 (10.5)	14 (21.9)	0.42	0.09-2.14	0.302
	Dominant	G/G	13 (68.4)	39 (60.9)	Ref		
		G/C-C/C	6 (31.6)	25 (39.1)	0.72	0.24-2.14	0.550
	Recessive	G/G-G/C	17 (89.5)	50 (78.1)	Ref		
		C/C	2 (10.5)	14 (21.9)	0.42	0.09-2.04	0.280
	Overdominant	G/G-C/C	15 (78.9)	53 (82.8)	Ref		
		C/G	4 (21.1)	11 (17.2)	1.28	0.35-4.62	0.701
	log-Additive	0,1,2	19 (22.9)	64 (77.1)	0.68	2.79	

Key: OR: odds ratio, CI: confidence interval

This table presents the distribution of alleles and genotypes for rs10012, rs1056827 and rs1056836 and their association with ER+ breast cancer cases versus controls. The odds ratios (OR), confidence intervals (CI), and p-values for various models are reported for various genetic models. No statistically significant associations were observed for any of the SNPs assessed.

4.14 Association of CYP1B1 SNPs with ER+ Breast Cancer Risk Compared to Benign Breast Diseases

This study investigated the association between three polymorphisms - rs10012, rs1056827, and rs1056836 - with the risk of ER+ breast cancer risk compared to BBD.

No significant association was observed for rs10012.Although the C allele appeared more frequently in BBD (64.5%) than in breast cancer cases (55.5%), the difference was not statistically significant. Across various genetic models (codominant, dominant, recessive, overdominant, and log-additive), the associations remained non-significant ($p > 0.05$), indicating no clear link between rs10012 and ER+ breast cancer risk.

Similarly, rs1056836 showed no statistically significant associations across all genetic models ($p > 0.05$), suggesting that this SNP does not contribute meaningfully to differentiating breast cancer from BBD.

However, rs1056827 demonstrated a potential protective effect. The A allele was significantly associated with a reduced risk of ER+ breast cancer (OR = 0.43, 95% CI [0.19 – 0.98], p = 0.045). Although the recessive model (A/A vs. C/C-C/A) also showed a trend toward a protective effect, this was not statistically significant. These findings suggest that rs1056827, particularly the A allele, may be linked to a lower risk of developing ER+ breast cancer. (Table 4.10).

Table 4.10: Genotype Frequencies and Associations of rs10012, rs1056827 and rs1056836 Variants in ER+ Breast Cancer Versus Benign Breast Disease Participants

SNP ID	Model	Variants	BBD	Cases	OR	95% CI	p-value
rs10012	Alleles	G	27 (35.5)	57 (44.5)	Ref		
		C	49 (64.5)	71 (55.5)	0.68	0.382 - 1.232	0.207
	Codominant	G/G	3 (7.9)	11 (17.2)	Ref		
		G/C	21 (55.3)	35 (54.7)	0.45	0.11 - 1.82	0.265
		C/C	14 (36.8)	18 (28.1)	0.35	0.08- 1.50	0.158
	Dominant	G/G	3 (7.9)	11 (17.2)	Ref		
		G/C-C/C	35 (92.1)	53 (82.8)	0.41	0.11 - 1.59	0.197
	Recessive	G/G-G/C	24 (63.2)	46 (71.9)	Ref		
		C/C	14 (36.8)	18 (28.1)	0.67	0.29 - 1.58	0.360
	Overdominant	G/G-C/C	17 (44.7)	29 (45.3)	Ref		0.954
		G/C	21 (55.3)	35 (54.7)	0.98	0.44 - 2.19	0.955
	log-Additive	0,1,2	38 (37.3)	64 (62.7)	0.65	0.34 - 1.22	0.172
rs1056827	Alleles	C	9 (11.8)	30 (23.4)	Ref		
		A	67 (88.2)	98 (76.6)	0.43	0.19 - 0.98	**0.045**
	Codominant	C/C	4 (10.5)	12 (18.8)	Ref		
		C/A	1 (2.6)	6 (9.4)	2.00	0.18 - 22.06	0.571
		A/A	33 (86.8)	46 (71.9	0.46	0.14 - 1.57	0.216
	Dominant	C/C	4 (10.5)	12 (18.8)	Ref		
		C/A-A/A	34 (89.5)	52 (81.2)	0.51	0.15 - 1.71	0.275
	Recessive	C/C-C/A	5 (13.2)	18 (28.1)	Ref		
		A/A	33 (86.8)	46 (71.9)	0.39	0.13 - 1.15	0.087
	Overdominant	C/C-A/A	37 (97.4)	58(90.6)	Ref		
		C/A	1(2.6)	6 (9.4)	3.83	0.44- 33.08	0.222
	log-Additive	0,1,2	38 (37.3)	64 (62.7)	0.63	0.34 - 1.16	0.115
rs1056836	Alleles	G	54 (75)	89 (69.5)	Ref		
		C	18 (25)	39 (30.5)	0.76	0.39-1.46	0.411
	Codominant	G/G	24 (63.2)	39 (60.9)	Ref		
		G/C	6 (15.8)	11 (17.2)	0.88	0.29-2.70	0.832
		C/C	6 (21.1)	14 (21.9)	0.69	0.23-2.05	0.512
	Dominant	G/G	24 (63.2)	39 (60.9)	Ref		
		G/C-C/C	14 (36.8)	25 (39.1)	0.91	0.39-2.08	0.823
	Recessive	G/G-G/C	30 (78.9)	50 (78.1)	Ref		
		C/C	8 (21.1)	14 (21.9)	0.95	0.35-2.53	0.922
	Overdominant	G/G-C/C	32 (84.2)	53 (82.8)	Ref		
		C/G	6 (15.8)	11 (17.2)	0.90	0.30-2.67	0.854
	log-Additive	0,1,2	38 (37.3)	64 (62.7)	1.05	0.64-1.71	0.855

Key: OR-odds ratio, CI- confidence interval, bold*statistical significance

This table presents OR, 95% CI, and p-values for rs10012, rs1056827, and rs1056836. While rs10012 and rs1056836 showed no significant associations ($p > 0.05$), the rs1056827 A allele was linked to a lower breast cancer risk (OR = 0.43, $p = 0.045$), suggesting a possible protective effect.

4.15 Association of rs4646903, rs1048943, rs1056836, rs776746 and rs4680 SNPs with ER+ Breast Cancer Tumor Characteristics

The rs4646903, rs1048943, rs1056836, and rs4680 polymorphisms were genotyped using PCR-RFLP. Their associations with tumor characteristics in ER+ breast cancer were evaluated using five genetic models: allelic, dominant, recessive, additive, and codominant. This section specifically focusses on the relationship between rs4646903, rs1048943, and rs1056836 and key tumor characteristics, including molecular subtype, grade, histopathological type, lymphovascular invasion (LVI), and cancer stage. However, only the results for rs4646903 are presented, as it was the only polymorphism that showed statistically significant associations with tumor features.

The alternative allele C of rs4646903 was significantly associated with an increased risk of the Luminal B subtype (OR=3.83, 95 CI [1.35–10.84], $p = 0.011$). In the codominant model, the heterozygous T/C genotype was significantly associated with an increased risk for the Luminal B subtype (OR=6.67, 95 CI [1.45–30.75], $p = 0.015$). Similarly, the dominant model (T/C-C/C genotypes) indicated a significantly increased risk of the Luminal B subtype (OR = 6.67, 95 CI [1.51–29.38], $p = 0.012$). The overdominant model (T/T-C/C vs. T/C genotypes) showed significant association with Luminal B (OR = 5.25, 95 CI [1.27–21.69], $p = 0.022$). The log-additive model also revealed a statistically significant association between rs4646903 and the Luminal B subtype (OR=3.7, 95 CI [1.23–11.07], $p = 0.017$). These findings suggest that the T/C genotype of rs4646903 may predispose individuals to a higher likelihood of developing Luminal B subtype ER+ breast cancer.

Significant associations were also observed between rs4646903 and breast cancer stage. In both codominant and dominant models, the T/C genotype was significantly associated with an increased risk of late-stage disease (codominant model: OR = 3.50, 95% CI [1.05–11.69], p = 0.041; dominant model: OR = 3.28, 95% CI [1.03–10.43], p = 0.044). The overdominant model showed a similar trend (OR = 3.29, 95% CI [1.01–10.71], p = 0.048), indicating that the T/C variant may be linked to a higher risk of progression to advanced-stage ER+ breast cancer.

In contrast, rs4646903 did not show significant associations with tumor grade, histopathological type (IDC vs. other subtypes), or LVI. Across all genetic models, p-values for these features were >0.05, indicating that rs4646903 may not influence these tumor characteristics in ER+ breast cancer. Table 4.11.

Furthermore, no statistically significant associations were found between rs1048943 and rs1056836 polymorphisms and any of the tumor characteristics assessed. Across all models, p-values were >0.05 for tumor grade, histopathological type, and LVI (data not shown), suggesting these polymorphisms do not significantly impact these aspects in the study population.

Table 4.11: Frequencies and Association of rs4646903 Variants with Tumor Characteristics in ER+ Breast Cancer Patients

Association of rs4646903 with molecular subtype in ER+ breast cancer						
Model	**Variants**	**lum A n (%)**	**lum B n (%)**	**OR**	**95% CI**	**p-value**
Allele	T	92 (85)	12 (60)	Ref		
	C	16 (15)	8 (40)	3.83	1.35- 10.84	**0.011**
Codominant	T/T	40 (74.1)	3 (30)	Ref		
	T/C	12 (22.2)	6 (60)	6.67	1.45-30.75	**0.015**
	C/C	2 (3.7)	1 (10)	6.67	0.46-96.44	0.164
Dominant	T/T	40 (74.1)	3 (30)	Ref		
	T/C-C/C	14 (25.9)	7 (70)	6.67	1.51-29.38	**0.012**
Recessive	T/T-T/C	52 (96.3)	9 (90)	Ref		
	C/C	2 (3.7)	1 (10)	2.89	0.24-35.28	0.406
Overdominant	T/T-C/C	42 (77.8)	4 (40)	Ref		
	T/C	12 (22.2)	6 (60)	5.25	1.27-21.69	**0.022**
log-Additive	0,1,2	54 (84.4)	10 (15.6)	3.7	1.23-11.07	**0.017**

Association of rs4646903 with stage of ER+ breast cancer						
Model	**Variants**	**early stage n (%)**	**late stage n (%)**	**OR**	**95% CI**	**p-value**
Allele	T	80 (77)	24 (70.6)	Ref		
	C	24 (23)	10 (29.4)	1.38	0.58 - 3.30	0.457
Codominant	T/T	35 (74.5)	8 (47.1)	Ref		
	T/C	10 (21.3)	8 (47.1)	3.50	1.05-11.69	**0.041**
	C/C	2 (4.3)	1 (5.9)	2.19	0.18-27.2	0.542
Dominant	T/T	35 (74.5)	8 (47.1)	Ref		
	T/C-C/C	12 (25.5)	9 (52.9)	3.28	1.03-10.43	**0.044**
Recessive	T/T-T/C	45 (95.7)	16 (94.1)	Ref		
	C/C	2 (4.3)	1 (5.9)	1.41	0.12-16.58	0.786
Overdominant	T/T-C/C	37 (78.7)	9 (52.9)	Ref		
	T/C	10(21.3)	8 (47.1)	3.29	1.01-10.71	**0.048**
log-Additive	0,1,2	47 (73.4)	17 (26.6)	2.28	0.9-5.78	0.082
Association of rs4646903 with grade in ER+ breast cancer						
Model	**Variants**	**high grade n (%)**	**low grade n (%)**	**OR**	**95% CI**	**p-value**
Allele	T	99 (81)	5 (83)	Ref		
	C	23 (19)	1 (17)	1.16	0.12 - 10.42	0.893
Codominant	T/T	41 (67.2)	2 (66.7)	Ref		
	T/C	17 (27.9)	1 (33.3)	0.82	0.07 - 9.76	0.881
	C/C	3 (4.9)	0 (0)	0.42	0.01 - 10.62	0.599
Dominant	T/T	41 (67.2)	2 (66.7)	Ref		
	T/C-C/C	20 (32.8)	1 (33.3)	0.97	0.09 - 11.99	0.984
Recessive	T/T-T/C	58 (95.1)	3 (100)	Ref		
	C/C	3 (4.9)	0 (0)	0.418	0.01 - 9.80	0.588
Overdominant	T/T-C/C	44 (72.1)	2(66.7)	Ref		
	T/C	17 (27.9)	1 (33.3)	0.77	0.06 - 9.08	0.837
log-Additive	0,1,2	61 (95.3)	3 (4.7)	0.87	0.1 - 7.25	1
Association of rs4646903 with histopathology type in ER+ breast cancer						
Model	**Variants**	**IDC n (%)**	**Others n (%)**	**OR**	**95% CI**	**p-value**
Allele	T	80 (81.6)	24 (80)	Ref		
	C	18 (18.4)	6 (20)	0.90	0.32 - 2.52	0.841
Codominant	T/T	34 (69.4)	9 (60)	Ref		
	T/C	12 (24.5)	6 (40)	1.89	0.55-6.43	0.308
	C/C	3 (6.1)	0 (0)	0.51	0.02-10.94	0.673
Dominant	T/T	34 (69.4)	9 (60)	Ref		
	T/C-C/C	15 (30.6)	6 (40)	1.51	0.46-5.01	0.499
Recessive	T/T-T/C	46 (93.9)	15 (100)	Ref		
	C/C	3 (6.1)	0 (0)	0.42	0.02-8.76	0.582
Overdominant	T/T-C/C	37 (75.5)	9 (60)	Ref		
	T/C	12 (24.5)	6 (40)	2.06	0.61-6.97	0.248
log-Additive	0,1,2	49 (76.6)	15 (23.4)	1.1	0.41-2.97	0.418

Association of rs4646903 with lymphovascular invasion (LVI) in ER+ breast cancer						
Model	**Variants**	**LVI absent n (%)**	**LVI present n (%)**	**OR**	**95% CI**	**p-value**
Allele	T	46 (82)	48 (77)	Ref		
	C	10 (18)	14 (23)	1.34	0.54- 3.32	0.525
Codominant	T/T	20 (71.4)	23 (63.9)	Ref		
	T/C	6 (21.4)	12 (33.3)	1.74	0.55-5.49	0.345
	C/C	2 (7.1)	1 (2.8)	0.43	0.04-5.16	0.509
Dominant	T/T	20 (71.4)	23 (63.9)	Ref		
	T/C-C/C	8 (28.6)	13 (36.1)	1.41	0.49-4.1	0.524
Recessive	T/T-T/C	26 (92.9)	35 (97.2)	Ref		
	C/C	2 (7.1)	1 (2.8)	0.37	0.03-4.32	0.413
Overdominant	T/T-C/C	22 (78.6)	24 (66.7)	Ref		
	T/C	6 (21.4)	12 (33.3)	1.83	0.59-5.72	0.297
log-Additive	0,1,2	28 (43.8)	36 (56.2)	1.1	0.46-2.62	0.825

Key: OR-odds ratio, CI- confidence interval, bold*statistical significance

This table presents the associations between rs4646903 genotypes and key tumor characteristics—molecular subtype (Luminal A vs. Luminal B), stage (early vs. late), and grade (high vs. low)—under different genetic models (allelic, codominant, dominant, recessive, overdominant, and log-additive). Odds ratios (ORs), 95% confidence intervals (CI), and p-values are provided for each model.

4.16 Association of CYP1B1 SNPs with ER+ Breast Cancer Tumor Characteristics

The rs10012, rs1056827, and rs1056836 polymorphisms were genotyped using Sanger sequencing, and their association with tumor characteristics in ER+ breast cancer were evaluated under five genetic models: allelic, dominant, recessive, additive, and codominant. Although CYP1B1 plays a recognized role in estrogen metabolism, no statistically significant associations were observed between these SNPs and tumor size, histological grade, lymph node involvement, or other clinicopathological features. These findings suggest that rs10012, rs1056827, and rs1056836 may not independently influence tumor characteristics in ER+ breast cancer among the study participants. However, further research with larger and more diverse populations is warranted to validate these findings and clarify the role of CYP1B1 polymorphisms in breast cancer progression (data not shown).

4.17 In-Silico Analysis of CYP1B1 Haplotypes

4.17.1 Linkage Disequilibrium Analysis

The pairwise linkage disequilibrium (LD) analysis of three genetic variants (rs10012, Pairwise linkage disequilibrium (LD) analysis of the three CYP1B1 variants (rs10012, rs1056827, and rs1056836) revealed distinct patterns of association among the loci (Table 4.12). Strong and statistically significant LD was observed between rs10012 and rs1056827, with a D' value of 0.9466 and a moderate correlation ($r^2 = 0.5767$). The chi-square test for this pair yielded a highly significant p-value (1.08×10^{-13}), confirming that these two loci are strongly linked and likely inherited together with minimal recombination.

In contrast, the LD analysis between rs10012 and rs1056836 revealed very weak association (D' = 0.0498, $r^2 = -0.026$, p = 0.7369), indicating that these loci segregate independently. Similarly, rs1056827 and rs1056836 showed weak LD (D' = 0.3416, $r^2 = -0.109$, p = 0.1604), with no statistical significance.

These findings confirm that rs10012 and rs1056827 form part of the same haplotype block, while rs1056836 appears to be independent of these two loci, validating the haplotype structure used for subsequent frequency and structural analyses.

Table 4.12: Summary of Pairwise Linkage Disequilibrium among rs10012, rs1056827 and rs1056836 Polymorphisms

SNP Pair	D′	r^2	Chi-square (X^2)	P-value	LD Strength
rs10012 – rs1056827	**0.9466**	**0.5767**	**55.22**	**1.08×10^{-13}**	**Strong LD (Highly significant)**
rs10012 – rs1056836	0.0498	-0.0260	0.11	0.7369	Weak LD (Not significant)
rs1056827 – rs1056836	0.3416	-0.1090	1.97	0.1604	Weak LD (Not significant)

4.17.2 Haplotype Frequencies of CYP1B1 Polymorphisms

Following confirmation of the linkage patterns, eight distinct CYP1B1 haplotypes were identified across the study groups, with notable differences in distribution observed among breast cancer cases (n=40 chromosomes), healthy controls (n=12 chromosomes), and benign breast disease participants (n=26 chromosomes) (Table 4.13).

The C–A–C–T haplotype (reference) was most frequent in cases (25.0%), while G–A–C–T (protective) predominated in benign samples (42.3%). The wild-type haplotype (G–C–G–T) exhibited a frequency of 5.0% in cases and 3.8% in benign samples, but was absent in controls. Notably, three haplotypes—C–A–G–T, G–C–G–T (wild-type), and G–A–S–T—were absent in controls but collectively accounted for 20.0% of case chromosomes. The risk-associated haplotype (G–C–C–T) showed its highest frequency in cases (12.5%) compared to controls (8.3%) and benign samples (3.8%).

Table 4.13: Haplotype Frequencies of CYP1B1 SNPs in Cases, Controls, and Benign Samples

Haplotype (rs10012–rs1056827–rs1056836–rs1800440)	Amino acids (48-119-432-453)	CASES n (%)	CONTROLS n (%)	BENIGNS n (%)
C – A – C – T**	G-S-V-N	10 (25.0%)	3 (25.0%)	9 (34.6%)
G – A – G – T	R-S-L-N	7 (17.5%)	2 (16.7%)	3 (11.5%)
C – A – S – T	G-S-?-N	6 (15.0%)	3 (25.0%)	2 (7.7%)
G – C – C – T***	R-A-V-N	5 (12.5%)	1 (8.3%)	1 (3.8%)
G – A – C – T****	R-S-V-N	4 (10.0%)	3 (25.0%)	11 (42.3%)
C – A – G – T	G-S-L-N	4 (10.0%)	0 (0%)	2 (7.7%)
G – C – G – T *	R-A-L-N	2 (5.0%)	0 (0%)	1 (3.8%)
G – A – S – T	R-S-?-N	2 (5.0%)	0 (0%)	0 (0%)

Key: *=wild-type haplotype, **=most common haplotype, ***=risk-associated haplotype, ****=protective haplotype, R; Arginine, A; Alanine, V; Valine, N; Asparagine, ?; Leucine or Valine. (ambiguous call); [1]The ambiguous S–?–N indicates that the rs1056836 genotype could not be definitively assigned to either L432 or V432.

4.17.3 Wild-Type Sequence Retrieval and Haplotype Variant Generation

The human CYP1B1 wild-type amino acid sequence (NP_000095.2) was successfully retrieved from the NCBI database and verified against the UniProtKB canonical sequence (Q16678). The 543-amino acid sequence corresponds to the wild-type haplotype (G–C–G–T) identified in the frequency analysis.

Domain mapping confirmed the functional relevance of the four polymorphic residues targeted in this study, as summarized in Table 4.14. Residue R48 is located in the N-terminal hinge region involved in membrane interaction and protein orientation, while A119 resides in the N-terminal structural region critical for protein folding and integrity. Residue L432 is positioned near the substrate-binding pocket, where it influences substrate recognition and binding, and N453 is located in the C-terminal heme-binding region, essential for heme coordination and electron transfer.

Table 4.14: Functional Domain Mapping of Target Residues

Residue Position	Amino Acid	Domain/Region	Functional Role
48	Arginine (R)	Hinge/flexibility region	Membrane interaction and protein orientation
119	Alanine (A)	N-terminal / structural region	Protein folding and structural integrity
432	Leucine (L)	Near substrate-binding pocket	Substrate recognition and binding
453	Asparagine (N)	C-terminal / heme-binding region	Heme coordination and electron transfer

Based on the haplotype frequency data, four representative haplotypes were selected for structural analysis, each defined by a specific combination of amino acids at positions 48, 119, 432, and 453. The wild-type haplotype (G–C–G–T) contains R48, A119, L432, and N453 (R–A–L–N). The reference haplotype (C–A–C–T), which was most common in cases (25.0%), contains G48, S119, V432, and N453 (G–S–V–N). The risk-associated haplotype (G–C–C–T), elevated in cases (12.5%) compared to controls (8.3%) and benign samples (3.8%), contains G48, A119, V432, and N453 (G–A–V–N). The protective

haplotype (G–A–C–T), which predominated in benign samples (42.3%), contains R48, S119, V432, and N453 (R–S–V–N).

The complete amino acid sequences for these four haplotypes, generated through in silico mutagenesis by introducing the corresponding substitutions into the wild-type template, are presented in Figure 4.19. The four polymorphic residues are highlighted in bold and underlined at their respective positions.

A. Wild-Type (R–A–L–N) Sequence

MGTSLSPNDPWPLNPLSIQQTTLLLLLSVLATVHVGQRLL**R**QQRRRQLRSAPPG
PFAWPLIGNAAAVGQAAHLSFARLARRYGDVFQIRLGSCPIVVLNGERAIHQAL
VQQGSAFADRPAFASFRVVSGGRSMAFGHYSEHWKVQRR**A**AHSMMRNFFTRQ
PRSRQVLEGHVLSEARELVALLVRGSADGAFLDPRPLTVVAVANVMSAVCFGC
RYSHDDPEFRELLSHNEEFGRTVGAGSLVDVMPWLQYFPNPVRTVFREFEQLNR
NFSNFILDKFLRHCESLRPGAAPRDMMDAFILSAEKKAA**L**GDSHGGGARLDLEN
VPATITDIFGASQDTLSTALQWLLLLFTRYPDVQTRVQAELDQVVGRDRLPCMG
DQPNLPYVLAFLYEAMRFSSFVPVTIPHATTANTSVLGYHIPKDTVVFVNQWSV
NHDPLKWPNPENFDPARFLDKDGLINKDLTSRVMIFSVGKRRCIGEELSKMQLF
LFISILAHQCDFRANPNEPAKMNFSYGLTIKPKSFKVNVTLRESMELLDSAVQNL
QAKETC**N**Q

B. Reference (G–S–V–N) Haplotype Sequence

MGTSLSPNDPWPLNPLSIQQTTLLLLLSVLATVHVGQRLL**G**QQRRRQLRSAPPG
PFAWPLIGNAAAVGQAAHLSFARLARRYGDVFQIRLGSCPIVVLNGERAIHQAL
VQQGSAFADRPAFASFRVVSGGRSMAFGHYSEHWKVQRR**S**AHSMMRNFFTRQ
PRSRQVLEGHVLSEARELVALLVRGSADGAFLDPRPLTVVAVANVMSAVCFGC
RYSHDDPEFRELLSHNEEFGRTVGAGSLVDVMPWLQYFPNPVRTVFREFEQLNR
NFSNFILDKFLRHCESLRPGAAPRDMMDAFILSAEKKAA**V**GDSHGGGARLDLEN
VPATITDIFGASQDTLSTALQWLLLLFTRYPDVQTRVQAELDQVVGRDRLPCMG

DQPNLPYVLAFLYEAMRFSSFVPVTIPHATTANTSVLGYHIPKDTVVFVNQWSV
NHDPLKWPNPENFDPARFLDKDGLINKDLTSRVMIFSVGKRRCIGEELSKMQVF
LFISILAHQCDFRANPNEPAKMNFSYGLTIKPKSFKVNVTLRESMELLDSAVQNL
QAKETC**N**Q

C. Risk-Associated (G–A–V–N) Haplotype Sequence

MGTSLSPNDPWPLNPLSIQQTTLLLLLSVLATVHVGQRLL**G**QQRRRQLRSAPPG
PFAWPLIGNAAAVGQAAHLSFARLARRYGDVFQIRLGSCPIVVLNGERAIHQAL
VQQGSAFADRPCFASFRVVSGGRSMAFGHYSEHWKVQRR**A**AHSMMRNFFTRQ
PRSRQVLEGHVLSEARELVALLVRGSADGAFLDPRPLTVVAVANVMSAVCFGC
RYSHDDPEFRELLSHNEEFGRTVGAGSLVDVMPWLQYFPNPVRTVFREFEQLNR
NFSNFILDKFLRHCESLRPGAAPRDMMDAFILSAEKKAA**V**GDSHGGGARLDLEN
VPATITDIFGASQDTLSTALQWLLLLFTRYPDVQTRVQAELDQVVGRDRLPCMG
DQPNLPYVLAFLYEAMRFSSFVPVTIPHATTANTSVLGYHIPKDTVVFVNQWSV
NHDPCKWPNPENFDPARFLDKDGLINKDLTSRVMIFSVGKRRCIGEELSKMQVF
LFISILAHQCDFRANPNEPAKMNFSYGLTIKPKSFKVNVTLRESMELLDSAVQNL
QAKETC**N**Q

D. Protective (R–S–V–N) Haplotype Sequence

MGTSLSPNDPWPLNPLSIQQTTLLLLLSVLATVHVGQRLL**R**QQRRRQLRSAPPG
PFAWPLIGNAAAVGQAAHLSFARLARRYGDVFQIRLGSCPIVVLNGERAIHQAL
VQQGSAFADRPAFASFRVVSGGRSMAFGHYSEHWKVQRR**S**AHSMMRNFFTRQ
PRSRQVLEGHVLSEARELVALLVRGSADGAFLDPRPLTVVAVANVMSAVCFGC
RYSHDDPEFRELLSHNEEFGRTVGAGSLVDVMPWLQYFPNPVRTVFREFEQLNR
NFSNFILDKFLRHCESLRPGAAPRDMMDAFILSAEKKAA**V**GDSHGGGARLDLEN
VPATITDIFGASQDTLSTALQWLLLLFTRYPDVQTRVQAELDQVVGRDRLPCMG
DQPNLPYVLAFLYEAMRFSSFVPVTIPHATTANTSVLGYHIPKDTVVFVNQWSV
NHDPLKWPNPENFDPARFLDKDGLINKDLTSRVMIFSVGKRRCIGEELSKMQVF

LFISILAHQCDFRANPNEPAKMNFSYGLTIKPKSFKVNVTLRESMELLDSAVQNL
QAKETC**N**Q

Figure 4.17: CYP1B1 Haplotype Amino Acid Sequences

The four haplotypes selected for structural analysis are shown with the polymorphic residues (positions 48, 119, 432, 453) highlighted in bold. Substitutions relative to the wild-type sequence are highlighted in bold and underlined. A; Wild-type (R–A–L–N), B. Reference (G–S–V–N), C. Risk-associated (G–A–V–N) and D. Protective (R–S–V–N)

4.17.4 Tertiary Structure Analysis

The ribbon structures, surface models and colour coded ribbons diagrams of the four haplotype models were visualized in PyMol. Ramachandran plot analysis was performed to evaluate the stereochemical quality of the wild-type and variant CYP1B1 models. All the models demonstrated >90% of residues in favored regions, with no significant outliers in disallowed regions, confirming the structural reliability of the generated models for subsequent MD simulations.

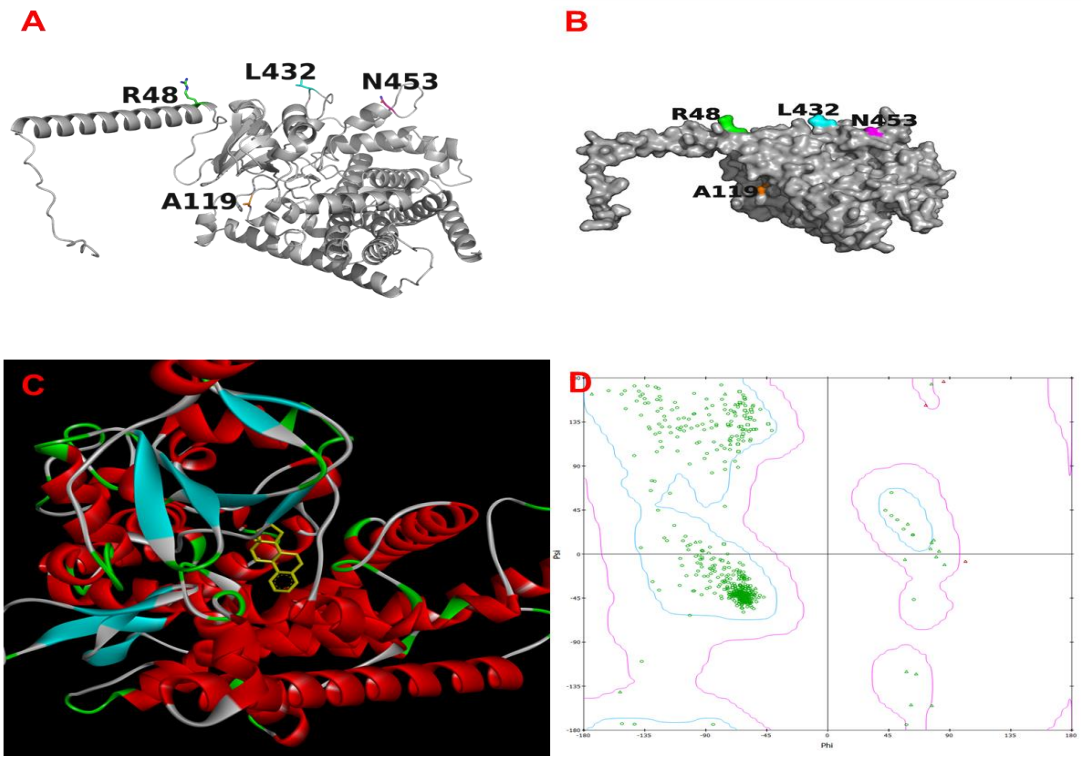

Figure 4.18: Tertiary Structures and Ramachandran Plot of CYP1B1 Highlighting the R48, A119, L432 and N453 Residues.

A; Ribbon structure, B; Surface model, C; Colour coded ribbon diagram and D; Ramachandran plot

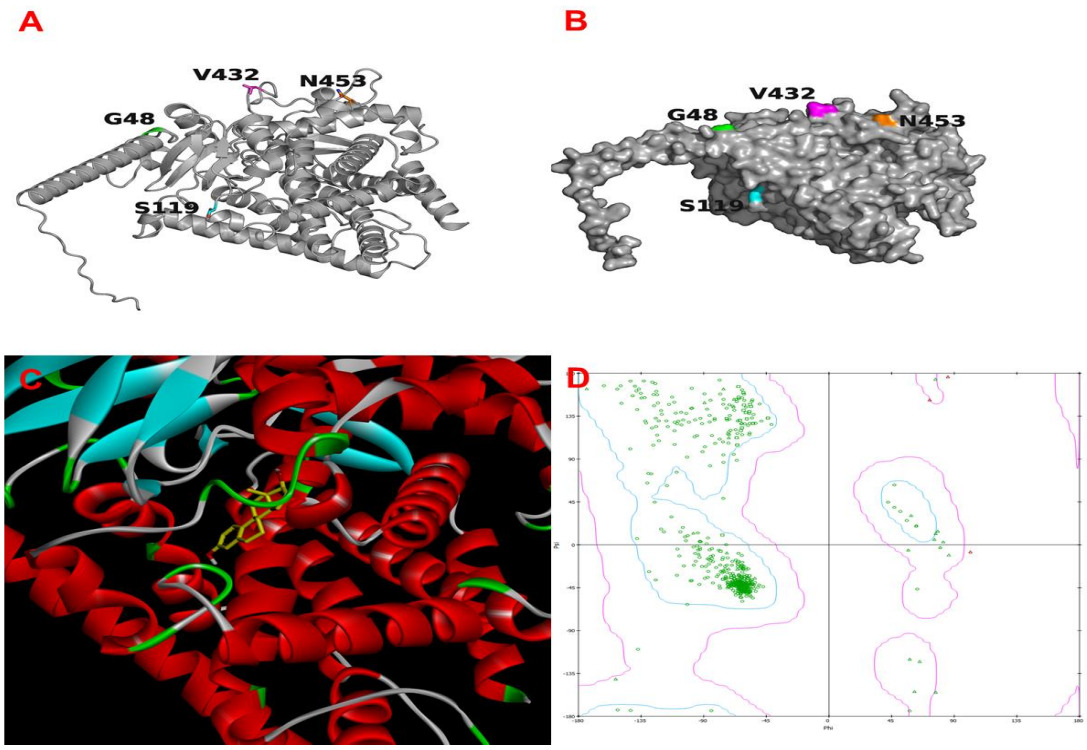

Figure 4.19: Tertiary Structures and Ramachandran Plot of CYP1B1 Highlighting the G48, S119, V432 and N453 Residues

A; Ribbon structure, B; Surface model, C; Colour coded ribbon diagram and D; Ramachandran plot

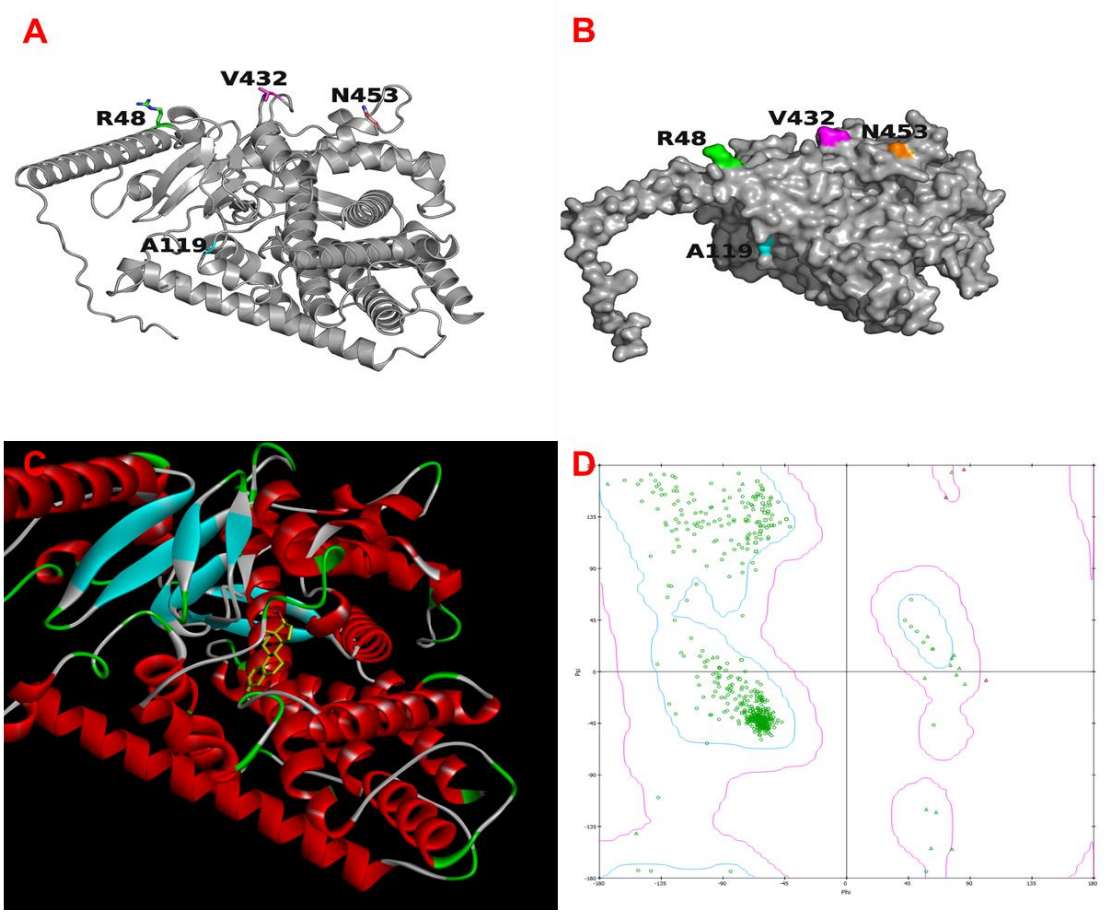

Figure 4.20: Tertiary Structures And Ramachandran Plot of CYP1B1 Highlighting the R48, A119, V432 and N453 Residues

A; Ribbon structure, B; Surface model, C; Colour coded ribbon diagram and D; Ramachandran plot

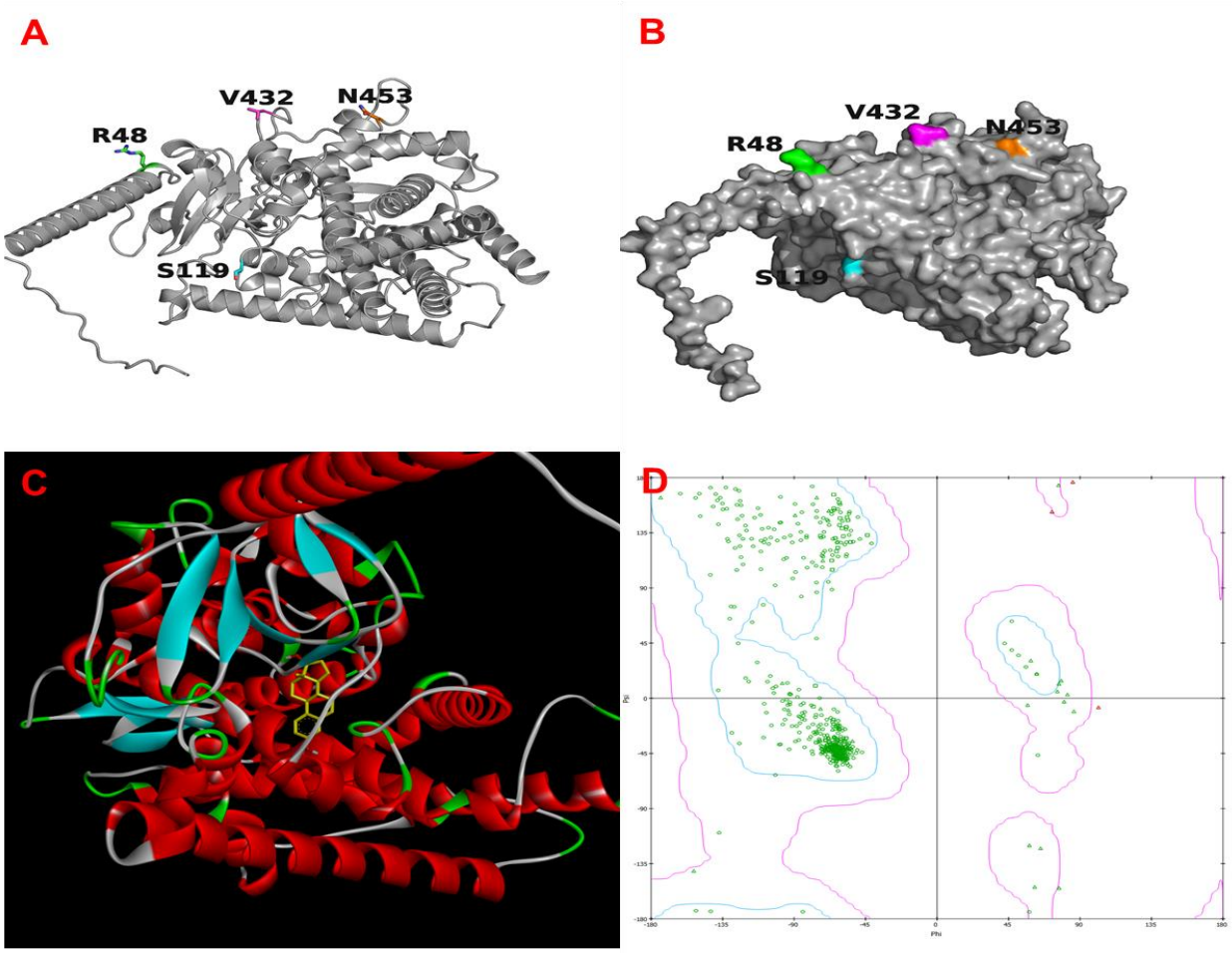

Figure 4.21: Tertiary Structures and Ramachandran Plot of CYP1B1 Highlighting the R48, S119, V432 and N453 Residues

A; Ribbon structure, B; Surface model, C; Colour coded ribbon diagram and D; Ramachandran plot

CHAPTER FIVE

DISCUSSION

5.1 Overview

The increasing global burden of breast cancer, driven by rising incidence and mortality rates in developing countries, underscores its significance as a public health challenge (Cai et al., 2025). Estrogen receptor-positive (ER+) breast cancer accounts for 70–80% of all breast cancer cases and relies on estrogen for tumor growth (Özdemir et al., 2018). This type of breast cancer may be influenced by genetic variations involved in estrogen metabolism. Polymorphisms in genes such as CYP1A1, CYP1B1, CYP3A5, and COMT can alter estrogen metabolism efficiency, therefore potentially modulating the risk of developing ER+ breast cancer (Tsuchiya et al., 2005). However, associations between these polymorphisms and breast cancer risk remain inconsistent, possibly due to differences in population-specific genetic susceptibility, environmental factors, lifestyle choices and reproductive histories (Sun et al., 2015). In addition to genetic influences, socio-demographic disparities may further contribute to risk variability. Thus, investigating these polymorphisms alongside reproductive, lifestyle, and socio-demographic factors is essential to understanding the multifaceted etiology of estrogen ER+ breast cancer in the local population.

This study examined the associations between socio-demographic characteristics, medical history, lifestyle behaviors, and genetic polymorphisms specifically rs4646903 and rs1048943 in CYP1A1, rs10012, rs1056827, rs1056836, and rs1800440 in CYP1B1, rs776746 in CYP3A5, and rs4680 in COMT. These genes were selected based on their previously reported roles in breast cancer susceptibility, with breast cancer risk. The analysis involved a comprehensive approach, including descriptive statistics, association analysis, and haplotype analysis. The comparisons were conducted between ER+ breast cancer cases and two reference groups - healthy controls and individuals with benign

breast disease (BBD). These comparisons are critical for identifying factors that may influence breast cancer development and progression.

Notably, comparing ER+ breast cancer cases with the BBD group provides distinct insights. BBD shares several clinical and pathological features with breast cancer but carries a distinct risk profile. It is well established that women diagnosed with BBD are at an elevated risk of developing breast cancer (Figueroa et al., 2021). Supporting this link, Schilling and Silva (2020) reported a significant association between family history of breast cancer and BBD, reinforcing the importance of genetic and familial factors in breast cancer susceptibility (Schilling & Silva, 2020).

5.2 Prevalence of the Socio-Demographics, Risk Factors and Single Nucleotide Polymorphisms in the Study Participants

Age is a well-documented risk factor for ER+ breast cancer. These findings in this study are consistent with existing evidence stating that advancing age, particularly postmenopausal status, increases the risk of ER+ breast cancer (Diab et al., 2000).

Globally, the median age at breast cancer diagnosis varies across regions. Studies in Ghana, Saudi Arabia, and Egypt reported higher median ages - 55 years, 53.7 years, and 50–59 years, respectively (Bosompem et al., 2024; El-Moselhy, 2017; Khabaz, 2014) - aligning with the findings of this study. In contrast, breast cancer in Sub-Saharan Africa, North Africa, and parts of Asia tends to affect younger populations. For instance, in Kenya, the most affected age group was 45–49 years (Makanga et al., 2013), with other studies reporting median ages of 48 and 47.5 years (Sayed et al., 2014, 2018). In Western Kenya, a median age of 48.5 years was reported (Sawe et al., 2016). Similarly, studies from Uganda (Galukande et al., 2014), Libya (Abousahmeen et al., 2023) and Eastern Morocco (Errahhali et al., 2017) reported median ages ranging between 47.3 and 48.7 years. A recent study examining a heterogeneous African population (Nigeria, Cameroon, and Uganda), reported a median age of 48 years, compared to 31 years in the BBD group (Omoleye et al., 2023).

These age disparities likely reflect regional differences in population demographics, with younger population structures in Sub-Saharan Africa, as well as possible variations in genetic susceptibility, environmental exposures, and access to diagnostic services (Errahhali et al., 2017). In contrast, Western countries tend to report older ages at diagnosis of breast cancer, which may be attributed to higher life expectancy and later onset of menopause (Leong et al., 2010).

The majority of ER+ breast cancer cases in this study were postmenopausal. This aligns with previous findings that identify menopause as a significant risk factor for the disease (Collaborative Group on Hormonal Factors in Breast Cancer, 2001). The increased risk in postmenopausal women is largely attributed to hormonal changes, particularly the shift from ovarian estrogen production to peripheral estrogen synthesis in adipose tissue (Cleary & Grossmann, 2009) . Postmenopausal breast cancer risk is closely linked to increased adiposity, which not only elevates circulating estrogen levels but also reduces sex hormone-binding globulin (SHBG), leading to higher bioavailable estrogen. This hormonal imbalance fosters carcinogenic processes, including enhanced cell proliferation, pro-angiogenesis, and suppression of apoptosis, all of which contribute to tumor development and progression (Rinaldi et al., 2006).

The association between menopausal status and BBD differs from its link with ER+ breast cancer. While postmenopausal women are at a higher risk of developing ER+ breast cancer, BBD is more common in younger, premenopausal women (Hasan et al., 2020). In this study, the majority of BBD cases occurred in premenopausal women, reinforcing the idea that age and hormonal fluctuations influence both benign and malignant breast conditions, but in distinct ways.

This contrast suggests that menopause is more strongly associated with an increased risk of breast cancer, whereas BBD tends to occur earlier in life when hormonal fluctuations are more frequent (Johansson et al., 2021). These findings highlight the importance of age-specific screening and risk assessment to distinguish benign from malignant conditions effectively.

Family history remains an important risk factor for breast cancer. While previous studies show that 10–30% of patients have affected relatives (Brewer et al., 2017). This study highlights that family history of breast or other cancers influences breast cancer risk. This suggests broader genetic or shared lifestyle factors could play a role in disease development.

Similar trends have been observed in previous studies. In Morocco, Tazzite et al. (2013) found that 18.4% of breast cancer patients had a family history of the disease (Tazzite et al., 2013). In the United States, Haber et al. (2012) reported that 11.3% of women with breast cancer had a family history of breast cancer, while 46% had a family history of other cancers (Haber et al., 2012). In China, Kilfoy et al. (2008) found an increased risk of breast cancer in individuals with a family history, although no association was observed between a history of other cancers and breast cancer risk (Kilfoy et al., 2008). However, a subgroup analysis by Zhou et al. (2014) in Chinese women linked a family history of lung cancer to an increased risk of ER+ breast cancer (Zhou et al., 2014).

Findings from Kenya also align with this study. Ekpe et al. (2019) reported that 23% of breast cancer patients had a family history of cancer, closely matching 20% prevalence of familial breast cancer cases in this study (Ekpe et al., 2019). While family history suggests a combination of genetic and environmental influences, the precise mechanisms linking breast cancer with other familial cancers remain unclear (Kilfoy et al., 2008).

Moderate alcohol consumption has been associated with a 30–50% increased risk of breast cancer, a relationship consistently observed in both case-control and cohort studies (McDonald et al., 2013). However, in this study, alcohol intake was assessed only over the past 12 months, which may not fully capture the cumulative effects of long-term alcohol consumption. Individuals in the ER+ breast cancer group may have reduced their alcohol intake after diagnosis due to health concerns or treatment side effects, potentially leading to lower reported consumption compared to the control and BBD groups. The 12-month assessment period may not accurately reflect the drinking patterns that contribute to breast cancer development over the long term. Alcohol's carcinogenic effects

accumulate over time, suggesting that past consumption, beyond the last year, could play a more significant role in breast cancer risk. Additionally, the type of alcoholic beverage consumed may also influence disease development (Liu et al., 2015). Given these factors, further research is needed to explore the timing, duration, and patterns of alcohol consumption in relation to breast cancer risk.

This study characterized the baseline distribution of key polymorphisms in estrogen metabolism genes, including CYP1A1 (rs4646903 and rs1048943), CYP1B1 (rs10012, rs1056827, rs1056836, and rs1800440), CYP3A5 (rs776746), and COMT (rs4680), across breast cancer cases, benign breast disease controls, and healthy controls. These specific variants were selected based on their established roles in estrogen biosynthesis and metabolism pathways. Notably, variation in rs1056836 distribution was observed between the cases and the controls. This suggests possible effects that may require consideration in subsequent analyses. The findings contribute to building a more complete understanding of the genetic architecture underlying estrogen metabolism in breast cancer pathogenesis

5.3 Prevalence of Tumor Characteristics

Breast cancer is a heterogeneous disease with significant variability in tumor characteristics across different ethnic and geographic populations. These tumor features influence disease progression, treatment response, and overall prognosis, making them essential for clinical decision-making. Key tumor characteristics include histological grade, histological type, lymph node status, molecular subtype, and disease stage, all of which play a critical role in determining patient outcomes (Abousahmeen et al., 2023).

With regards to histological subtype, this study established invasive ductal carcinoma (IDC) to be the most prevalent. It accounted for 84.2% of ER+ breast cancer cases. This finding is consistent with the report by Sayeed et al. (2014) (Sayed et al., 2014). Similarly, studies conducted in Western Kenya and Eastern Morocco have also identified IDC as the predominant histological subtype (Errahhali et al., 2017; Sawe et al., 2016).

The predominance of invasive ductal carcinoma (IDC) among ER+ breast cancer cases in this cohort aligns with established global patterns, as demonstrated in studies from North Africa (Errahhali et al., 2017), East Africa (Sawe et al., 2016; Sayed et al., 2014). This consistent histological distribution across diverse populations reinforces the importance of prioritizing IDC-focused research in both basic and translational settings. Particularly for ER+ disease, understanding the interplay between ductal morphology and hormone receptor signaling may yield clinically actionable insights.

The high frequency of lymph node involvement observed in this study reinforces its established role as a critical prognostic indicator in breast cancer. This pattern aligns with findings from Kenya (Sayed et al., 2014), Libya (Abousahmeen et al., 2023), and Morocco (Errahhali et al., 2017), though with notable variations that likely reflect differences in tumor biology, stage at diagnosis, and healthcare access. The particularly high rates reported in these African studies - compared to global averages - may indicate regional challenges in early detection systems. Research from Eastern India further confirms that tumor characteristics including grade, stage and lymphovascular invasion remain universal predictors of nodal metastasis, regardless of geographical setting (Chakraborty et al., 2016). These collective findings highlight the need for both improved diagnostic infrastructure and tailored treatment approaches across diverse healthcare environments.

Research from Kenya, Uganda, and Libya consistently shows that breast cancer is often detected at advanced stages in these regions (Abousahmeen et al., 2023; Galukande et al., 2014; Sayed et al., 2014). This pattern likely results from two key factors: the types of breast cancer being studied and differences in healthcare systems. Studies that include aggressive subtypes like triple-negative breast cancer naturally report more late-stage diagnoses, as these tumors progress rapidly. In contrast, ER-positive breast cancers—which tend to grow more slowly—are more likely to be caught earlier when screening is available (Nakashima et al., 2019). Healthcare access plays an equally important role; regions with established screening programs and greater public awareness typically detect cancers at earlier, more treatable stages (Hoveling et al., 2025). Together, these biological

and systemic factors explain why diagnosis timing varies significantly across different populations.

The predominance of luminal A breast cancer observed in this study aligns with findings from other studies, including research from Kenya (Sayed et al., 2014) and Uganda (Galukande et al., 2014). However, significant regional variations exist, as demonstrated by a Libyan study reporting luminal B as the most frequent subtype (Abousahmeen et al., 2023). A recent systematic review of African breast cancer phenotypes noted that luminal subtypes collectively represent the most common classification across the continent, with Kenya showing particularly high frequencies of luminal A tumors (Onyia et al., 2023).

These discrepancies in subtype distribution may reflect both true biological differences and methodological challenges in molecular classification. Historically, inconsistent biomarker panels and varying laboratory protocols have complicated reliable distinction between luminal A and B subtypes in many settings. This technical variability underscores the need for standardized molecular profiling approaches in breast cancer research, particularly for hormone receptor-positive disease where accurate subtyping carries significant therapeutic implications.

5.4 Associations of Socio-Demographics and Risk Factors with ER^+ Breast Cancer

While demographic characteristics provide a foundation for understanding disease patterns, identifying other factors associated with ER+ breast cancer is crucial for improving risk assessment and prevention strategies.

Existing literature consistently demonstrates that postmenopausal status significantly elevates risk for hormone receptor-positive breast cancer, primarily through prolonged estrogen exposure and altered hormonal metabolism (Johansson et al., 2021; Rinaldi et al., 2006). This biological mechanism is particularly relevant in ER+ tumors, where estrogen signaling plays a central role in tumorigenesis. This biological mechanism is central to ER+ tumorigenesis. In our study, this association was strongly supported.

Postmenopausal status was significantly more prevalent in ER+ breast cancer cases compared to the controls. The odds of an individual being a case was seven times that of control. This finding aligns with the established literature, reinforcing the role of menopausal status as a key risk determinant in our study population. Furthermore, it underscores the relevance of endocrine factors in the pathogenesis of ER+ breast cancer within the local context, consistent with global epidemiological patterns.

The relationship between benign breast disease (BBD) and subsequent cancer risk warrants particular attention. Proliferative breast lesions, especially those with atypia, are established risk factors (Alamri et al., 2020), yet the underlying mechanisms remain incompletely understood. The high prevalence of BBD compared to breast cancer (Hatim et al., 2017) underscores the importance of better characterizing these lesions for risk stratification.

These findings collectively emphasize the multifactorial nature of ER+ breast cancer etiology, where biological, behavioral, and healthcare access factors interact. Moreover, even though the participants were purposively selected, some participants suffered from other complications such as diabetes and hypertension that might have influenced the results.

5.5 Associations between Single Nucleotide Polymorphisms and ER^+ Breast Cancer

Research on estrogen-related gene variants reveals striking geographic and ethnic variations in breast cancer risk associations. The CYP1A1 rs4646903 variant demonstrates particularly divergent effects, showing risk associations in Korean and Mexican populations (Moreno-Galván et al., 2018; Shin et al., 2007), while exhibiting no significant effects in Iraqi populations (Ibrahem et al., 2021). Similarly, large-scale analyses of Caucasian and Chinese groups found no overall risk associations (Economopoulos & Sergentanis, 2010), highlighting how genetic background modifies variant effects. These population-specific patterns likely stem from differences in allele frequencies, local environmental exposures, and variations in linkage disequilibrium

patterns across ethnic groups. In the present study we observed a significant protective association of the alternative allele C of the rs4646903. There was a reduced odds of individuals association with ER+ breast cancer risk by 56%. Mechanistically, this may be explained by the enzyme's dual function; CYP1A1 contributes to breast cancer risk through a complex balance between the activation of procarcinogens and the detoxification of harmful compounds (Androutsopoulos et al., 2009). In our cohort, the variant may favor detoxification pathways or interact with local environmental factors in a way that attenuates risk, aligning it with the subset of populations where non-risk or protective effects have been suggested.

The influence of rs1056836 on breast cancer risk demonstrates striking variability across populations and disease stages. While this variant has been associated with increased risk in Iranian (Adab et al., 2017), Nigerian (Okobia et al., 2009), and Han Chinese populations (Jiao et al., 2010), protective effects during cancer initiation were observed in Chinese women (Qiu et al., 2018), with neutral effects reported in Southern Indian (Francis et al., 2019), Iraqi (Ibrahem et al., 2021), and Egyptian cohorts (El-Moselhy, 2017). In the present study, the CYP1B1 rs1056836 polymorphism exhibited distinct associations depending on the control group. While it showed no significant association when cases were compared to healthy controls, it demonstrated a protective effect against ER+ breast cancer when cases were compared to the Benign Breast Disease (BBD) group. This suggests that rs1056836 may influence later stages of carcinogenesis, potentially affecting the progression from benign proliferative states to invasive malignancy rather than initial cancer development. Such stage-specific influences could explain the variability across studies, as the relative importance of genetic factors may differ between initial transformation and malignant progression. These patterns highlight the complex interplay between genetic background, disease stage, and susceptibility mechanisms. Further research is needed to clarify whether these population differences reflect true biological variation or methodological factors in study design.

The current understanding of rs1056827's role in breast cancer risk remains inconclusive, with existing literature suggesting potential modulation of estrogen metabolism that may

influence carcinogenesis. The variant's effects appear to be highly context-dependent, with studies reporting varying associations across different populations. This variability extends to other CYP1A1 and CYP1B1 polymorphisms, where ethnic differences in allele frequencies, lifestyle factors, and environmental exposures may collectively contribute to divergent risk profiles. This study observed a protective association of the ER+ breast cancer compared to the BBDs.

The potential for gene-environment interactions further complicates risk prediction, particularly regarding exposures to endocrine-disrupting compounds or hormonal therapies that may differentially affect individuals based on their genetic background. Such interactions could explain why certain variants demonstrate protective effects in some populations but neutral or even risk-enhancing effects in others. These observations underscore the importance of considering both genetic and environmental contexts when evaluating breast cancer susceptibility markers.

5.6 Associations between Single Nucleotide Polymorphisms and Tumor Characteristics

This study identified significant associations between the rs4646903 variant and more aggressive ER+ breast cancer characteristics, specifically Luminal B molecular subtype and late-stage disease. These findings contrast with reports from Iraqi populations where no significant associations were observed for these variants (Ibrahem et al., 2021). The connection between rs4646903 and advanced disease in this study aligns with documented relationships to high-grade tumors in Southern Indian women (Francis et al., 2019), while differing from patterns seen in Brazilian cohorts where certain variants occurred more frequently in low-grade (I-II) versus high-grade (III) tumors (Martins de Oliveira et al., 2015).

The population-specific nature of these genetic effects may reflect variations in allele frequencies or environmental exposures. This study found no significant associations for rs1048943 or rs1056836 with tumor characteristics, suggesting variant-specific rather

than generalized effects across estrogen metabolism genes. The consistent observation of rs4646903 associations with aggressive disease features across multiple studies warrants further investigation into its potential role in tumor progression pathways. Additional research is needed to elucidate the biological mechanisms underlying these population-dependent patterns and their clinical relevance for risk stratification.

5.7 In-Silico Analysis of CYP1B1 Haplotypes

5.7.1 Linkage Disequilibrium

The analysis of CYP1B1 haplotypes provides valuable insights into ER+ breast cancer susceptibility patterns. The strong linkage between rs10012 and rs1056827 suggests these variants are typically inherited together and may function as a combined genetic unit, while rs1056836 appears to segregate independently. These findings align with previous cancer research demonstrating that haplotype effects often differ from individual variant associations, (Trubicka et al., 2010; Yu et al., 2015). The moderate r^2 value of 0.5767 suggests that while these two SNPs are strongly linked, some degree of historical recombination has occurred, which is typical for genes with moderate recombination rates (Ardlie et al., 2002). The independent segregation of rs1056836 (L432V) aligns with its location approximately 1.3 kb downstream, outside the core haplotype block, indicating that this variant may have arisen on a different evolutionary background (Hanna et al., 2000).

5.7.2 Haplotype Distribution and Breast Cancer Risk

The identification of eight distinct haplotypes across the study groups reflects the genetic diversity of CYP1B1 in the population studied. The wild-type haplotype (G–C–G–T) characterized by the combination of R48 (from rs10012), A119 (from rs1056827), L432 (from rs1056836) and N453 (from rs1800440) was notably absent in controls but present at 5.0% in cases, suggesting that individuals carrying this haplotype may have been underrepresented in the control group due to selection or sampling bias. Alternatively, this

distribution may indicate that the wild-type haplotype is not necessarily protective, a finding that contrasts with some previous studies where the wild-type allele has been associated with reduced risk (Sissung et al., 2006).

The risk-associated haplotype (G–C–C–T) is characterized by the combination of R48 (from rs10012), A119 (from rs1056827), V432 (from rs1056836) and N453 (from rs1800440) demonstrated a progressive increase in frequency from benign samples (3.8%) to controls (8.3%) to cases (12.5%), consistent with a dose-dependent effect on breast cancer risk. Notably, the V432 variant has been previously associated with increased catalytic activity toward estradiol, leading to elevated 4-hydroxyestradiol production, which is a known genotoxic metabolite (Hanna et al., 2000). The combination of this high-activity variant with R48 and A119 may further modulate enzyme function through altered membrane association and protein stability (Banday et al., 2014).

5.7.3 Functional Domain Mapping of Polymorphic Residues

The domain mapping analysis placed the four polymorphic residues within functionally critical regions of CYP1B1, providing a structural rationale for their potential impact on enzyme activity. R48 is located in the hinge region between the N-terminal membrane anchor and the globular catalytic domain. This position is critical for proper membrane insertion and orientation, and the R48G substitution may alter membrane binding affinity, potentially affecting subcellular localization (Sissung et al., 2006). The A119S substitution resides in a structural core region; while alanine to serine is a conservative substitution, the introduction of a hydroxyl group at this position could subtly alter hydrogen bonding patterns within the protein core, affecting overall stability (Banday et al., 2014). The R48G and A119S substitutions located near the hinge region of CYP1B1 are not known to directly impact CYP1B1 catalytic activity when assessed in isolation; rather, their functional significance is likely mediated through synergistic interactions with other polymorphic residues within the haplotype structure (Sissung et al., 2006)

L432 is positioned near the substrate-binding pocket, a region that directly influences substrate specificity and catalytic efficiency. The L432V substitution has been extensively studied and is known to alter the regiospecificity of estradiol hydroxylation, shifting the balance toward the genotoxic 4-hydroxyestradiol pathway (Hanna et al., 2000). This substitution may also affect the binding of other endogenous substrates and procarcinogens(T. Shimada et al., 1999). The L432V and N453 substitutions encodes the heme-binding domain, the region critical to the catalytic function of CYP1B1 (Bailey et al., 1998). N453 is located in the C-terminal region near the heme-binding domain. While the N453S substitution is relatively conservative, its proximity to the heme prosthetic group suggests it could influence electron transfer efficiency from cytochrome P450 reductase, thereby modulating overall catalytic turnover (Gajjar et al., 2012).

5.7.4 Tertiary Structure Analysis

The generation of high-confidence three-dimensional models for the four haplotypes using AlphaFold Server represents a significant advancement in our ability to visualize the structural consequences of these polymorphisms. The AlphaFold 3 model demonstrated excellent prediction quality, as evidenced by the high pLDDT scores (>90) for the ordered regions of all models, consistent with the model's strong performance on single-chain protein predictions (Abramson et al., 2024). The Ramachandran plot analysis confirmed the stereochemical quality of all models, with >90% of residues in favored regions, validating their suitability for downstream molecular dynamics simulations.

Visual inspection of the ribbon structures and surface models revealed that the four polymorphic residues are distributed across distinct structural domains, with R48 located in an extended loop region, A119 and L432 buried within the hydrophobic core and substrate-binding pocket respectively, and N453 positioned near the C-terminal helix in proximity to the heme-binding site. The surface models suggest that while R48 and A119 are relatively solvent-exposed, L432 and N453 are more buried, consistent with their predicted roles in substrate binding and heme coordination (Sissung et al., 2006)

The colour-coded ribbon diagrams allowed for direct comparison of the local environments surrounding each polymorphic residue. The wild-type haplotype (R–A–L–N) exhibited the expected distribution of charged and hydrophobic residues, with R48 potentially forming electrostatic interactions with membrane phospholipids (McLellan et al., 2000). In the reference haplotype (G–S–V–N), the R48G substitution removes a positive charge, potentially reducing membrane affinity, while the A119S substitution introduces a potential hydrogen bond donor in the structural core. The risk-associated haplotype (G–A–V–N) combines the loss of membrane interaction (R48G) with the highly active V432 variant, potentially creating a "gain-of-function" phenotype through enhanced substrate access and reduced membrane tethering (Banday et al., 2014). The protective haplotype (R–S–V–N) retains the wild-type R48 residue, which may maintain proper membrane localization, while incorporating both S119 and V432, suggesting that the R48 residue may be critical for maintaining the protective phenotype despite other substitutions (Sissung et al., 2006).

The tertiary structure analysis revealed that the protective haplotype (R–S–V–N) maintains the wild-type R48 residue while incorporating S119 and V432. This suggests that the presence of R48 may be critical for proper membrane anchoring, potentially outweighing any deleterious effects of the other substitutions. Conversely, the risk-associated haplotype (G–A–V–N) combines the loss of R48 with the high-activity V432 variant, which may synergistically enhance carcinogenic estrogen metabolism while reducing proper subcellular localization (Hanna et al., 2000). These structural observations provide a mechanistic framework for understanding the differential breast cancer risk associated with these haplotypes.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study established that:

1. ER+ breast cancer cases predominantly affect older, postmenopausal women and presents primarily as invasive ductal carcinomas (76.7%) with Luminal A features (82.4%), frequently at Stage II (45.3%) with moderate differentiation (Grade II: 62.5%) and lymph node involvement (56.2%).
2. The study revealed low odds of CYP1A1 rs4646903-C allele against overall ER+ cancer development (OR=0.44, p=0.048) and CYP1B1 rs1056836-C (OR=0.34, p=0.0003) and rs1056827-A (OR=0.43, p=0.045) against malignant transformation from benign lesions. On the other hand, G-A-C haplotype (rs10012-G/rs1056827-A/rs1056836-C) conferred 4.75-fold increased ER+ cancer risk (p=0.046)
3. The rs4646903-C allele was associated with Luminal B subtypes (OR=3.83) and late tumor stage
4. Tertiary structure modeling revealed that the four polymorphic residues occupy functionally critical domains: R48 in the membrane interaction region, A119 in the structural core, L432 near the substrate-binding pocket, and N453 in the heme-binding region.

6.2 Recommendations

Based on the findings, the following recommendations are proposed:

1. Develop targeted screening and educational policies for postmenopausal women and those with a family history of breast cancer.

2. Investigate the mechanistic basis of rs4646903's dual role—protective against ER+ breast cancer yet associated with aggressive Luminal B subtypes—to determine its clinical utility.
3. Perform molecular dynamics simulations to elucidate the dynamic conformational changes, active site flexibility, and substrate binding affinities of the four CYP1B1 haplotypes (R–A–L–N, G–S–V–N, G–A–V–N, R–S–V–N).
4. Develop population-specific risk assessment tools integrating demographic risk factors with validated genetic markers and haplotype structural information.

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APPENDICES

Appendix I: Ethical Approval

THE AGA KHAN UNIVERSITY

Ref: 2020/IERC-26 (v2)
March 3, 2020

Dr. Shahin Sayed, Faculty & AKU Site Investigator
Department of Pathology, Aga Khan University

Ms. Mary Muriithi – Doctor of Philosophy Degree
Jomo Kenyatta University of Science and Technology
Nairobi, Kenya

Dear Dr. Shahin,

RE: Genetic Polymorphisms in Estrogen Metabolising Enzymes in Patients Diagnosed with Estrogen Receptor Positive Breast Cancer at the Aga Khan University Hospital, Nairobi

The Aga Khan University, Nairobi (AKUN), Institutional Ethics Review Committee (IERC) is in receipt of your protocol and application form resubmitted to the Research Office on March 2, 2020. With reference to the AKU-IERC letter Ref: 2020/IERC-26 (v1) dated February 19, 2020, the committee notes that the researcher has addressed concerns earlier raised. This is a retrospective study that seeks to profile genetic polymorphisms (SNPs) in estrogen metabolizing enzymes and determine possible association with the prognosis and outcome in breast cancer patients diagnosed at Aga Khan University Hospital, Nairobi. Data will be collected from retrospective review of patient's charts, clinical notes and through molecular experiments for the period of June 2012 and June 2015. There will be no patient contact.

As per the AKU IERC regulations, the committee records that this study qualifies for exemption from a full committee review process. The committee has thus approved your exemption request *{as per attached official stamped protocol and attachments - version 2020/IERC-26 (v2)}* This approval is valid from **March 3, 2020** to **March 2, 2021.** As applicable, prior to export of biological specimens, ensure an MTA is in place as well as seek shipment authority/permit from the relevant government ministry.

To maintain anonymity and confidentiality of data no patient identifiers will be collected. The study should be conducted in full accordance with all the applicable institutional policies of the AKUHN, especially with regards to **patient records management**. You should notify the IERC immediately of any changes that may affect your project. Further, you must provide an interim report **60 days before expiration** of the validity of this approval and request extension if additional time is required for study completion. You must advise the IERC when this study is completed or discontinued and a final report submitted to the Research Office. The hospital management should be notified of manuscripts emanating from this work.

If you have any questions, please contact Research Office - research.supportea@aku.edu or call 020-366 2148/1136.

With best wishes,

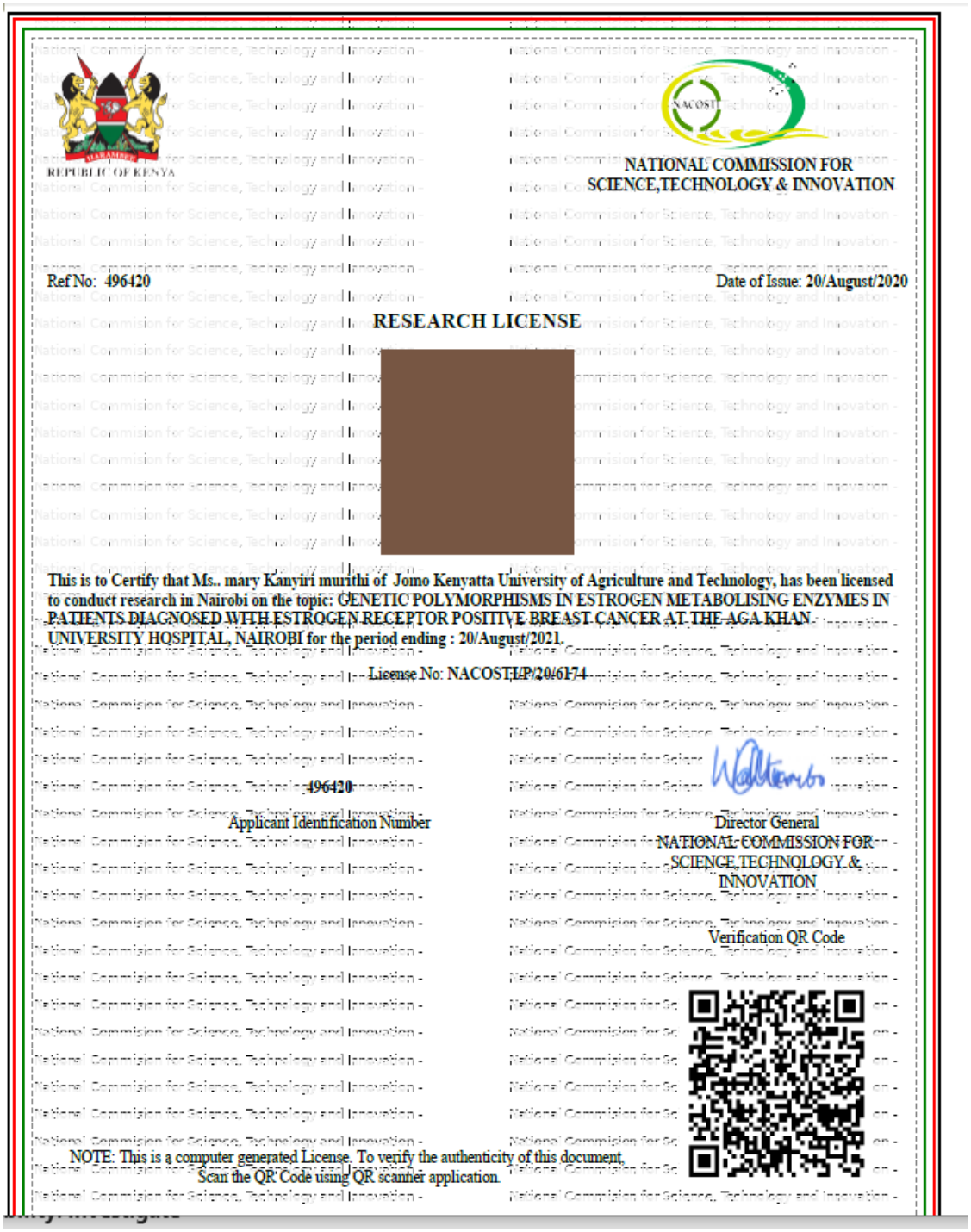
Wangari.

Dr. Wangari Waweru-Siika,
Chair - Institutional Ethics Review Committee (IERC)
Aga Khan University, (Kenya)

3rd Floor, Park Place Building, 2nd Parklands Avenue, Off Limuru Road
P. O. Box 30270, GPO 00100, Nairobi, Kenya
Tel: +254 20 366 1200; Website: www.aku.edu

AK 958

Appendix II: NACOSTI Permit



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This is to Certify that Ms.. mary Kanyiri murithi of Jomo Kenyatta University of Agriculture and Technology, has been licensed to conduct research in Nairobi on the topic: GENETIC POLYMORPHISMS IN ESTROGEN METABOLISING ENZYMES IN PATIENTS DIAGNOSED WITH ESTROGEN RECEPTOR POSITIVE BREAST CANCER AT THE AGA KHAN UNIVERSITY HOSPITAL, NAIROBI for the period ending : 20/August/2021.

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Appendix III: Consent Page

Confidential

Introduction and Consent

Record Number	_____
Facility/Institution	◯ AKU ◯ KAIC ◯ ACC
Study ID	_____ (This is the study ID such as AKU001)
Date of Interview	_____ (DDMMYYY)

Hello my name is ..... I am here on behalf of ..... As you know, you have been asked to participate in a study, and your doctor/nurse has explained the study to you. This study involves your breast tissue which will be used for testing and research. You were given the opportunity to sign the INFORMED CONSENT, and you willingly signed the INFORMED CONSENT and agreed to participate in the study. Today, I would like to invite you to participate in filling out this important questionnaire which is related to the study. I will be asking you questions on your socio-demographics, medical history, reproductive history and other health behaviours. I will also ask you which ethnicity you belong to. This will not in any way affect the management and treatment of your condition. In addition to these questions, we will also collect some blood, saliva and tissue samples for further testing in our laboratory. No HIV testing will be performed as part of this study. Your participation is voluntary, and if you choose not to participate, you will not be treated with prejudice. Please note that if at any point you feel uncomfortable, you can stop the interview at any time if you do not want to continue. Your answers to the questions asked will be completely confidential and will be used for research purposes only. Your responses and those of others who agree to participate in the study will be used by researchers for this study, and other partner organisations to understand the problem of breast cancer in Kenyan women and how this may differ among the three major ethnic groupings.

Do you have any questions about the survey?	◯ Yes ◯ No
Do I have agreement to proceed?	◯ Yes ◯ No
Completed Consent Document	
Does the respondent agree to be interviewed?	◯ Yes ◯ No
Interviewer's Signature	

Appendix IV: Data Abstraction Form

The standardized form below was used to systematically record data regarding the participants. The form has seven section capturing information on: sample and identification, demographic and anthropometric, reproductive and hormonal history, medical and family history, lifestyle and environmental exposures, tumor clinicopathology data (cases only) and genetic data

	Variable Name	**Description/ Definition**	**Data Type / Categories**
I. SAMPLE AND IDENTIFICATION	Sample id	Unique study identification code	Text (Alphanumeric)
	Sample type	Participant group classification	1 = Case (ER+ Breast Cancer), 2 = BBD, 3 = Healthy Control
II. DEMOGRAPHIC & ANTHROPOMETRIC DATA	Age	Age at recruitment (years)	Continuous (Integer)
	Body mass index (BMI)	Body Mass Index (kg/m^2)	Continuous (Decimal)
III. REPRODUCTIVE & HORMONAL HISTORY	Age at menarche	Age at first menarche (years)	Continuous (Integer)
	Menopausal status	Menopausal status at diagnosis/recruitment	1 = Premenopausal, 2 = Postmenopausal
	Parity	Number of live births	Continuous (Integer)
	Hormonal contraceptives	Ever use of hormonal contraceptives	0 = No, 1 = Yes
IV. MEDICAL & FAMILY HISTORY	Family history of breast cancer	First-degree relative with breast cancer	0 = No, 1 = Yes
	Family history of other cancers	First-degree relative with other cancer	0 = No, 1 = Yes
	Diabetes	Diagnosed with diabetes mellitus	0 = No, 1 = Yes
	Hypertension	Diagnosed with hypertension	0 = No, 1 = Yes
V. LIFESTYLE & ENVIRONMENTAL EXPOSURES	Tobacco	Ever smoked tobacco	0 = No, 1 = Yes
	Alcohol	Ever consumed alcohol	0 = No, 1 = Yes
	Radiation therapy	History of therapeutic radiation exposure	0 = No, 1 = Yes
VI. TUMOR CLINICOPATHOLOGY DATA (Cases only)	Histological type	Primary histopathological diagnosis	1 = Invasive Ductal Carcinoma, 2 = Other

	Grade	Tumor histological grade (Nottingham)	1 = Grade 1, 2 = Grade 2, 3 = Grade 3
	Stage	Clinical/pathological TNM stage (simplified)	1 = Stage I/II (Early), 2 = Stage III/IV (Late)
	Lymph node invasion	Presence of lymph node metastasis	0 = No, 1 = Yes
	Molecular subtype	Immunohistochemistry-based subtype	1 = Luminal A, 2 = Luminal B
VII. GENETIC DATA	rs4646903	CYP1A1 rs4646903 genotype	0 = Wild type, 1 = Heterozygous, 2 = Variant
	rs1048943	CYP1A1 rs1048943 genotype	0 = Wild type, 1 = Heterozygous, 2 = Variant
	rs776746	CYP3A5 rs776746 genotype	0 = Wild type, 1 = Heterozygous, 2 = Variant
	rs4680	COMT rs4680 genotype	0 = Wild type, 1 = Heterozygous, 2 = Variant
	rs1056827	*CYP1B1* rs1056827 genotype	0 = Wild type, 1 = Heterozygous, 2 = Variant
	rs10012	*CYP1B1* rs10012 genotype	0 = Wild type, 1 = Heterozygous, 2 = Variant
	rs1800440	*CYP1B1* rs1800440 genotype	0 = Wild type, 1 = Heterozygous, 2 = Variant
	rs1056836	*CYP1B1* rs1056836 genotype	0 = Wild type, 1 = Heterozygous, 2 = Variant

Appendix V: Participants Group Classification and Identifiers

The table below lists the 162 participants group classification (sample type) along with their unique sample identifiers. Coding: **C** = Case (ER+ Breast Cancer), **B** = Benign Breast Disease (BBD), **H** = Healthy Control. Samples are grouped by classification.

	BBD Group (B)	**Case Group (C)**	**Healthy Control Group (H)**
1.	AKU011	AKU041	AKU 182
2.	AKU025	AKU042	AKU 183
3.	AKU026	AKU057	AKU 191
4.	AKU031	AKU060	AKU 193
5.	AKU034	AKU073	AKU 194
6.	AKU039	AKU076	AKU 195
7.	AKU043	AKU086	AKU 196
8.	AKU044	AKU087	AKU 197
9.	AKU046	AKU088	AKU 198
10.	AKU047	AKU094	AKU 199
11.	AKU048	AKU097	AKU 200
12.	AKU049	AKU099	AKU 201
13.	AKU054	AKU100	AKU 202
14.	AKU056	AKU103	AKU 204
15.	AKU059	AKU104	AKU 205
16.	AKU061	AKU105	AKU 206
17.	AKU062	AKU106	AKU 207
18.	AKU063	AKU109	AKU 208
19.	AKU064	AKU110	AKU 209
20.	AKU067	AKU112	
21.	AKU069	AKU113	
22.	AKU070	AKU116	
23.	AKU071	AKU118	
24.	AKU075	AKU122	
25.	AKU077	AKU124	
26.	AKU078	AKU127	
27.	AKU081	AKU128	
28.	AKU083	AKU133	
29.	AKU107	AKU142	
30.	AKU115	AKU149	
31.	AKU119	AKU152	
32.	AKU126	AKU158	
33.	AKU132	AKU164	
34.	AKU135	AKU173	
35.	AKU136	AKU211	
36.	AKU139	AKU155	
37.	AKU144	KAIC004	
38.	AKU145	KAIC005	
39.	AKU146	KAIC006	

40.	AKU148	KAIC007	
41.	AKU151	KAIC008	
42.	AKU153	KAIC011	
43.	AKU154	KAIC016	
44.	AKU159	KAIC018	
45.	AKU162	KAIC019	
46.	AKU163	KAIC021	
47.	AKU165	KAIC023	
48.	AKU166	KAIC024	
49.	AKU167	KAIC025	
50.	AKU170	KAIC026	
51.	AKU172	KAIC027	
52.	AKU175	KAIC029	
53.	AKU176	KAIC030	
54.	AKU177	KAIC032	
55.	AKU180	KAIC033	
56.	AKU181	KAIC035	
57.	AKU182	KAIC041	
58.	AKU183	KAIC046	
59.	AKU185	KAIC047	
60.	AKU187	KAIC051	
61.	AKU188	KAIC052	
62.	AKU189	KAIC057	
63.	AKU190	KAIC059	
64.	AKU191	KAIC060	
65.	AKU192		
66.	AKU194		
67.	AKU196		
68.	AKU197		
69.	AKU198		
70.	AKU200		
71.	AKU202		
72.	AKU204		
73.	AKU205		
74.	AKU206		
75.	AKU207		
76.	AKU208		
77.	AKU210		
78.	AKU212		
79.	AKU213		

Appendix VI: Participants Demographic, Clinical and Lifestyle Characteristics

This table presents the coded values for key demographic, reproductive, and clinical variables collected from study participants. Variable abbreviations are defined as follows: Demographic/Clinical: Participant Identifier (PID), Body Mass Index (BMI), Diabetes Mellitus (DM), Hypertension (HTN); Reproductive: Menopausal Status (Meno_Stat), Hormonal Contraceptive Use (Horm_Contracept), Parity (Parity), Age at Menarche (Menarche_Age); Family History: Family History of Breast Cancer (FH_BC), Family History of Other Cancers (FH_OtherCa); Lifestyle/Exposure: Smoking (Smoking), Alcohol Consumption (Alcohol), Radiation Therapy (RTx).

PID	Age	Menarche_Age	BMI	Meno_Stat	Parity	Horm_Contracept	FH_BC	FH_OtherCa	DM	HTN	Smoking	Alcohol	RTx
AKU041	40	13	27	No	Yes	Yes	No	Yes	No	No	Yes	Yes	No
AKU042	57	12	40	Yes	Yes	No	No	Yes	Yes	Yes	No	No	No
AKU057	52	12	25	No	Yes	No	No	No	No	No	No	Yes	No
AKU060	28	14	18	No	No	No	No	No	No	No	Yes	Yes	No
AKU073	43	14	33	No	Yes	Yes	No	Yes	Yes	Yes	No	No	No
AKU076	42	12	32	No	Yes	Yes	No	Yes	No	No	No	No	No
AKU086	52	12	21	Yes	Yes	No	No	Yes	No	No	No	Yes	No
AKU087	63	14	34	Yes	Yes	No	No	No	No	No	No	Yes	No
AKU088	34	14	21	No	No	No	Yes	Yes	No	No	No	No	No
AKU094	56	16	24	Yes	Yes	Yes	No	Yes	No	No	No	No	No
AKU097	32	14	23	No	No	Yes	No	No	No	No	No	Yes	No
AKU099	60	13	27	Yes	Yes	No	No	No	No	No	No	No	No
AKU100	75	16	37	Yes	Yes	No	No	No	No	No	No	Yes	No
AKU103	64	13	23	Yes	Yes	No	Yes	No	No	Yes	No	No	No
AKU104	57	14	33	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes	No
AKU105	41	17	30	No	Yes	Yes	Yes	Yes	No	No	No	Yes	No
AKU106	48	8	27	No	Yes	Yes	No	Yes	No	No	No	Yes	No
AKU109	52	15	41	No	Yes	Yes	No	No	No	No	No	Yes	No

AKU110	44	11	26	No	No	No	No	Yes	No	Yes	No	Yes	No
AKU112	57	13	36	Yes	Yes	Yes	No	No	No	Yes	No	No	No
AKU113	39	14	25	No	Yes	Yes	No	Yes	No	No	No	Yes	No
AKU116	40	16	28	No	Yes	Yes	Yes	No	No	Yes	No	Yes	No
AKU118	70	13	25	Yes	Yes	Yes	Yes	No	No	Yes	No	Yes	No
AKU122	53	16	33	Yes	Yes	Yes	Yes	No	No	No	No	No	No
AKU124	62	14	30	Yes	Yes	Yes	No	Yes	Yes	Yes	No	Yes	No
AKU127	49	14	32	Yes	No	Yes	No	No	No	Yes	No	Yes	Yes
AKU128	53	17	27	Yes	Yes	Yes	No	Yes	Yes	Yes	No	Yes	No
AKU133	78	17	26	Yes	Yes	Yes	No	No	No	Yes	No	No	No
AKU142	51	16	27	Yes	Yes	Yes	Yes	No	No	No	No	Yes	No
AKU149	54	15	36	Yes	Yes	Yes	No	Yes	No	No	No	Yes	No
AKU152	44	15	26	No	Yes	Yes	No	No	No	No	No	Yes	No
AKU158	54	16	27	Yes	Yes	Yes	Yes	No	Yes	Yes	No	No	No
AKU164	38	17	36	Yes	Yes	Yes	No	No	No	No	No	Yes	No
AKU173	48	13	30	Yes	Yes	Yes	No	Yes	No	No	No	No	No
AKU211	55	16	35	No	Yes	Yes	Yes	No	No	No	No	No	No
AKU155	53	13	41	Yes	Yes	Yes	Yes	No	No	No	No	No	No
KAIC004	59	15	34	Yes	Yes	Yes	No	Yes	No	No	No	No	No
KAIC005	55	14	42	Yes	Yes	No	No	No	No	No	No	No	No
KAIC006	36	13	26	No	Yes	Yes	No	Yes	No	No	No	No	No
KAIC007	50	14	23	Yes	Yes	No	No	No	No	No	No	No	No
KAIC008	71	13	33	Yes	Yes	No	No	No	Yes	Yes	No	No	No
KAIC011	68	14	37	Yes	Yes	No	No	No	No	Yes	No	No	No
KAIC016	67	17	10	Yes	Yes	Yes	No	No	No	Yes	No	No	No
KAIC018	43	16	42	No	Yes	Yes	No	No	No	No	No	No	No
KAIC019	41	13	32	No	Yes	Yes	No	No	No	No	No	No	No
KAIC021	56	18	23	No	Yes	Yes	No	No	No	No	No	No	No
KAIC023	45	18	23	No	Yes	Yes	No	No	No	No	No	No	No

KAIC024	57	18	35	Yes	Yes	Yes	No	No	No	No	No	No	No
KAIC025	47	17	32	No	Yes	No	No	No	No	No	No	No	No
KAIC026	39	16	35	No	No	No	No	No	No	No	No	No	No
KAIC027	37	12	42	No	Yes	No	No	No	No	No	No	No	No
KAIC029	59	19	26	No	Yes	Yes	No	No	No	Yes	No	No	No
KAIC030	59	22	28	Yes	Yes	Yes	Yes	No	No	No	No	No	No
KAIC032	53	12	27	Yes	Yes	Yes	No	Yes	No	No	No	Yes	No
KAIC033	60	12	24	Yes	Yes	No	No	No	No	No	No	No	No
KAIC035	61	13	33	Yes	Yes	Yes	No	No	Yes	Yes	No	No	No
KAIC041	49	16	38	Yes	Yes	No	No	Yes	No	Yes	No	No	No
KAIC046	53	13	42	No	Yes	Yes	Yes	No	No	Yes	No	No	No
KAIC047	36	14	28	No	Yes	Yes	No	No	No	No	No	No	No
KAIC051	50	12	35	No	Yes	Yes	No	No	No	No	No	No	No
KAIC052	36	13	24	No	No	Yes	No	No	No	No	No	Yes	No
KAIC057	48	16	26	No	Yes	No	No	Yes	No	No	No	No	No
KAIC059	61	15	30	Yes	Yes	No	No	No	No	No	No	No	No
KAIC060	40	15	35	No	Yes	Yes	No	No	No	No	No	No	No
AKU011	51	15	22	Yes	Yes	Yes	No	No	No	No	No	No	No
AKU025	42	14	32	No	Yes	Yes	No	Yes	No	Yes	No	Yes	No
AKU026	47	14	28	No	Yes	Yes	No	Yes	No	No	No	Yes	No
AKU031	28	13	27	No	No	No	Yes	Yes	No	No	Yes	Yes	No
AKU034	35	13	35	No	Yes	Yes	No	No	No	No	No	Yes	No
AKU039	42	13	30	No	Yes	Yes	No	No	No	No	No	No	No
AKU043	34	14	19	No	Yes	No	Yes	Yes	No	No	No	Yes	No
AKU044	40	14	26	No	Yes	Yes	No	No	No	No	No	No	No
AKU046	55	16	24	Yes	Yes	No	No	No	No	No	No	Yes	No
AKU047	57	14	22	Yes	Yes	No	No	No	No	No	No	Yes	No
AKU048	60	12	32	Yes	Yes	Yes	No	No	No	Yes	No	Yes	Yes
AKU049	61	15	28	Yes	Yes	No	No	No	No	No	No	Yes	No

AKU054	40	12	41	No	Yes	Yes	Yes	Yes	No	No	No	Yes	No
AKU056	42	14	24	No	No	No	Yes	No	No	No	No	No	No
AKU059	74	16	26	Yes	No	No	Yes	Yes	No	Yes	No	No	No
AKU061	57	16	24	Yes	Yes	Yes	No	No	No	No	No	No	No
AKU062	61	16	34	Yes	Yes	Yes	No	Yes	No	No	No	No	No
AKU063	51	16	28	No	Yes	Yes	No	No	Yes	Yes	No	Yes	No
AKU064	63	14	24	Yes	Yes	Yes	No	No	No	No	No	No	Yes
AKU067	42	16	33	No	Yes	Yes	No	No	No	No	No	No	No
AKU069	42	14	30	No	Yes	Yes	No	Yes	No	No	No	No	No
AKU070	45	14	30	Yes	Yes	Yes	Yes	No	No	No	No	Yes	No
AKU071	38	13	22	No	No	Yes	Yes	No	No	No	No	No	No
AKU075	48	15	35	No	Yes	Yes	No	No	No	No	No	No	No
AKU077	41	14	20	No	No	Yes	Yes	No	No	No	No	Yes	No
AKU078	52	16	35	Yes	Yes	Yes	No	No	No	No	No	Yes	No
AKU081	47	16	25	No	Yes	Yes	No	Yes	No	Yes	No	Yes	No
AKU083	50	12	28	No	Yes	Yes	No	No	No	No	No	Yes	No
AKU107	31	13	34	No	Yes	Yes	Yes	No	Yes	Yes	No	Yes	No
AKU115	45	15	25	Yes	Yes	Yes	No	No	No	No	No	No	No
AKU119	35	14	25	No	Yes	Yes	No	No	No	No	No	Yes	No
AKU126	46	14	31	No	Yes	Yes	No	No	No	No	No	Yes	No
AKU132	38	15	31	No	Yes	Yes	No	Yes	No	No	No	Yes	No
AKU135	35	16	31	No	Yes	Yes	Yes	No	No	No	No	No	No
AKU136	36	14	37	No	Yes	Yes	No	No	Yes	Yes	No	No	No
AKU139	36	14	34	No	No	No	Yes	Yes	No	No	No	Yes	No
AKU144	35	13	43	No	No	Yes	No	No	Yes	Yes	No	Yes	No
AKU145	27	14	30	No	No	Yes	No	No	No	No	No	Yes	No
AKU146	33	12	29	No	No	Yes	Yes	Yes	No	No	Yes	Yes	No
AKU148	23	13	24	No	No	No	No	No	No	No	No	Yes	No
AKU151	44	17	31	No	Yes	Yes	No	No	No	No	No	No	Yes

AKU153	49	13	36	Yes	Yes	Yes	Yes	Yes	No	No	No	Yes	No
AKU154	41	13	29	No	Yes	Yes	No	No	No	No	No	No	No
AKU159	37	13	30	No	No	No	No	No	No	No	No	Yes	No
AKU162	63	11	28	No	Yes	No	No	No	No	No	No	No	No
AKU163	55	14	31	Yes	Yes	No	Yes	No	Yes	Yes	No	Yes	No
AKU165	45	13	22	No	Yes	Yes	No	No	No	No	No	No	No
AKU166	32	12	25	No	No	Yes	No	Yes	No	No	No	Yes	No
AKU167	47	15	31	No	Yes	Yes	Yes	No	No	No	No	Yes	No
AKU170	45	14	26	No	Yes	Yes	Yes	No	No	No	No	Yes	No
AKU172	35	14	25	No	No	Yes	No	No	No	No	No	Yes	No
AKU175	31	14	20	No	No	No	No	No	No	No	No	No	No
AKU176	40	14	30	No	Yes	Yes	No	Yes	No	No	No	Yes	No
AKU177	36	15	26	No	Yes	Yes	No	Yes	No	Yes	No	Yes	No
AKU180	37	14	28	No	Yes	Yes	No	No	No	No	No	Yes	No
AKU181	49	14	29	No	Yes	Yes	No	No	No	No	No	Yes	No
AKU182	37	13	29	No	Yes	Yes	No	No	No	No	No	No	No
AKU183	39	16	31	No	Yes	Yes	No	No	No	No	No	No	No
AKU185	33	11	30	No	No	Yes	No	No	No	Yes	No	No	No
AKU187	42	12	31	No	Yes	Yes	No	No	No	No	No	Yes	No
AKU188	38	14	24	No	Yes	Yes	No	Yes	No	No	No	No	No
AKU189	39	13	23	No	Yes	Yes	No	Yes	No	No	No	Yes	No
AKU190	41	12	26	No	Yes	Yes	No	No	No	No	No	Yes	No
AKU191	42	13	34	No	Yes	Yes	No	No	No	No	No	Yes	No
AKU192	56	15	30	Yes	Yes	No	No	No	Yes	Yes	No	Yes	No
AKU194	42	13	27	No	Yes	Yes	No	No	No	No	No	No	No
AKU196	49	16	30	No	Yes	Yes	No	No	No	No	No	Yes	No
AKU197	42	14	24	No	Yes	Yes	No	No	No	Yes	No	Yes	No
AKU198	48	13	24	No	Yes	Yes	No	No	No	Yes	No	Yes	No
AKU200	41	14	30	No	Yes	Yes	No	No	No	Yes	No	No	No

AKU202	52	17	30	Yes	Yes	Yes	No	No	No	Yes	No	Yes	No
AKU204	45	12	29	No	Yes	Yes	No	No	No	No	No	Yes	No
AKU205	41	15	29	No	No	No	No	No	No	Yes	No	Yes	No
AKU206	42	12	28	No	No	Yes	No	No	No	No	No	Yes	No
AKU207	40	13	32	No	Yes	Yes	No	No	No	No	No	Yes	No
AKU208	36	13	29	No	Yes	Yes	No	No	No	No	No	Yes	No
AKU210	50	14	26	No	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No
AKU212	58	16	25	Yes	Yes	Yes	No	No	No	No	No	Yes	No
AKU213	34	12	27	No	No	No	No	No	No	No	No	No	No
AKU 182	37	13	29	No	Yes	Yes	No	No	No	No	No	No	No
AKU 183	39	16	31	No	Yes	Yes	No	No	No	No	No	No	No
AKU 191	42	13	34	No	Yes	Yes	No	No	No	No	No	Yes	No
AKU 193	26	11	24	No	No	Yes	No	No	No	No	No	Yes	No
AKU 194	42	13	27	No	Yes	Yes	No	No	No	No	No	No	No
AKU 195	27	14	23	No	No	No	No	No	No	No	No	No	No
AKU 196	49	16	30	No	Yes	Yes	No	No	No	No	No	Yes	No
AKU 197	42	14	24	No	Yes	Yes	No	No	No	Yes	No	Yes	No
AKU 198	48	13	24	No	Yes	Yes	No	No	No	Yes	No	Yes	No
AKU 199	33	12	23	No	Yes	Yes	No	No	No	No	No	Yes	No
AKU 200	41	14	30	No	Yes	Yes	No	No	No	Yes	No	No	No
AKU 201	25	11	23	No	No	Yes	No	No	No	No	No	Yes	No
AKU 202	52	17	30	Yes	Yes	Yes	No	No	No	Yes	No	Yes	No
AKU 204	45	12	29	No	Yes	Yes	No	No	No	No	No	Yes	No
AKU 205	41	15	29	No	No	No	No	No	No	Yes	No	Yes	No
AKU 206	42	12	28	No	No	Yes	No	No	No	No	No	Yes	No
AKU 207	40	13	32	No	Yes	Yes	No	No	No	No	No	Yes	No
AKU 208	36	13	29	No	Yes	Yes	No	No	No	No	No	Yes	No
AKU 209	25	10	19	No	No	Yes	No	No	No	No	No	Yes	No

Appendix VII: Genotypes for rs4646903, rs1048943, rs1056836, rs7767746 and rs4680

PID	rs4646903	rs1048943	rs1056836	rs776746	rs4680	PID	rs4646903	rs1048943	rs1056836	rs776746	rs4680
AKU041	TT	AA	GG	GG	AA	**AKU133**	CC	AA	GC	GG	AA
AKU042	TT	AA	GG	GG	AA	**AKU142**	TT	AA	CC	GG	AA
AKU057	TT	AA	GC	GG	AA	**AKU149**	TT	AG	GG	GG	AA
AKU060	TC	AA	CC	GG	AA	**AKU152**	TT	AA	GG	GG	AA
AKU073	TC	AA	GC	GG	AA	**AKU158**	TT	AA	GC	GG	AA
AKU076	TT	AA	GC	GG	AA	**AKU164**	TT	GG	GG	GG	AA
AKU086	TC	AA	GG	GG	AA	**AKU173**	TT	AG	GG	GG	AA
AKU087	TC	AA	GG	GG	AA	**AKU211**	TC	AA	GC	GG	AA
AKU088	TT	AA	CC	GG	AA	**AKU155**	TT	AA	GC	GG	AA
AKU094	TT	AA	GC	GG	AA	**KAIC004**	CC	AG	GC	GG	AA
AKU097	TT	AA	GG	GG	AA	**KAIC005**	TT	AA	GG	GG	AA
AKU099	TC	AA	GC	GG	AA	**KAIC006**	TT	AA	GG	GG	AA
AKU100	TT	AA	GC	GG	AA	**KAIC007**	TC	AA	GG	GG	AA
AKU103	TT	AA	CC	GG	AA	**KAIC008**	TC	AA	GC	GG	AA
AKU104	TT	AA	GG	GG	AA	**KAIC011**	TT	AA	GG	GG	AA
AKU105	TC	AA	GC	GG	AA	**KAIC016**	TT	AA	GG	GG	AA
AKU106	TC	AA	GG	GG	AA	**KAIC018**	TT	AG	GG	GG	AA
AKU109	TC	AA	CC	GG	AA	**KAIC019**	TT	AA	GC	GG	AA
AKU110	TT	AA	GC	GG	AA	**KAIC019**	TT	AA	GC	GG	AA
AKU112	TT	AA	GG	GG	AA	**KAIC021**	TT	AA	GG	GG	AG
AKU113	TT	GG	GG	GG	AA	**KAIC023**	TC	AA	GG	GG	AG
AKU116	TT	AA	GG	GG	AA	**KAIC024**	TT	AA	GC	GG	AA
AKU118	CC	AA	GC	GG	AA	**KAIC025**	TT	AA	GG	GG	AA
AKU122	TC	AA	GC	GG	AA	**KAIC026**	TC	AA	GG	GG	AA
AKU124	TT	AA	GG	GG	AA	**KAIC027**	TC	AA	GC	GG	AA
AKU127	TT	AA	GG	GG	AA	**KAIC029**	TT	AA	GC	GG	AA

AKU128	TT	AA	GC	GG	AA	**KAIC030**	TT	AA	GG	GG	AA
PID	**rs4646903**	**rs1048943**	**rs1056836**	**rs776746**	**rs4680**	**PID**	**rs4646903**	**rs1048943**	**rs1056836**	**rs776746**	**rs4680**
KAIC032	TC	AA	GC	GG	AA	**AKU064**	TT	AA	GG	GG	AA
KAIC033	TT	AA	GC	GG	AA	**AKU067**	TT	AA	GG	GG	AA
KAIC035	TC	AA	GG	GG	AA	**AKU069**	TC	AA	GG	GG	AA
KAIC041	TT	AA	GC	GG	AA	**AKU070**	TT	AA	GG	GG	AA
KAIC046	TT	AA	GC	GG	GG	**AKU071**	TC	GG	GC	GG	AA
KAIC047	TT	AA	CC	GG	AA	**AKU075**	TT	AA	GG	GG	AA
KAIC051	TT	AA	CC	GG	AA	**AKU077**	TT	AA	GC	GG	AA
KAIC052	TT	AA	GC	GG	AG	**AKU078**	TC	AA	GC	GG	AA
KAIC057	TC	AA	GG	GG	AA	**AKU081**	TC	AA	GG	GG	AA
KAIC059	TT	AA	CC	GG	AA	**AKU083**	TT	AA	GG	GG	AA
KAIC060	TT	AA	GG	GG	AA	**AKU107**	TT	AA	GG	GG	AA
AKU011	TT	AG	GC	GG	AA	**AKU115**	TT	AA	GG	GG	AA
AKU025	TC	AA	GC	GG	AA	**AKU119**	TT	AA	GG	GG	AA
AKU026	TC	AA	GG	GG	AA	**AKU126**	TT	AA	GG	GG	AA
AKU031	TT	AG	GG	GG	AA	**AKU132**	TC	AA	GC	GG	AA
AKU034	TT	AA	GC	GG	AA	**AKU135**	TT	AA	GC	GG	AA
AKU039	TT	AA	GG	GG	AA	**AKU136**	TT	AG	GC	GG	AA
AKU043	TT	AA	GG	GG	AA	**AKU139**	TT	AG	GG	GG	AA
AKU044	TT	AA	GG	GG	AA	**AKU144**	TC	AA	GC	GG	AA
AKU046	TT	AA	GG	GG	AA	**AKU145**	TT	AA	GG	GG	AA
AKU047	TT	AA	GG	GG	AA	**AKU146**	TT	AA	GG	GG	AA
AKU048	TT	AA	GG	GG	AA	**AKU148**	TT	AA	GC	GG	AA
AKU049	TT	AA	GG	GG	AA	**AKU151**	TT	AA	GG	GG	AA
AKU054	TT	AA	GG	GG	AA	**AKU153**	TT	AA	GG	GG	AA
AKU056	TT	AA	GG	GG	AA	**AKU154**	TT	AA	GC	GG	AA
AKU059	TC	AA	GG	GG	AA	**AKU159**	TT	AA	GC	GG	AA
AKU061	TT	AA	GG	GG	AA	**AKU162**	TT	AA	GC	GG	AA

AKU062	TT	AA	GG	GG	AA	**AKU163**	TT	AA	GG	GG	AA
AKU063	TT	AA	GG	GG	AA	**AKU165**	TT	AA	GC	GG	AA

PID	rs4646903	rs1048943	rs1056836	rs776746	rs4680	PID	rs4646903	rs1048943	rs1056836	rs776746	rs4680
AKU166	TT	AG	GG	GG	AA	**AKU208**	TC	AA	GC	GG	AA
AKU167	TT	AA	GG	GG	AA	**AKU210**	TT	AG	GG	GG	AA
AKU170	TT	AA	GG	GG	AA	**AKU212**	TT	AA	GC	GG	AA
AKU172	TT	AA	GC	GG	AA	**AKU213**	TT	AA	GG	GG	AA
AKU175	TC	AA	GG	GG	AA	**AKU 182**	TT	AA	CC	GG	AA
AKU176	TT	AA	GG	GG	AA	**AKU 183**	TC	AA	CC	GG	AA
AKU177	TC	AA	GG	GG	AA	**AKU 191**	TT	AA	GC	GG	AA
AKU180	TT	AG	GC	GG	AA	**AKU 193**	TT	AA	CC	GG	AA
AKU181	TT	AA	GG	GG	AA	**AKU 194**	TT	AA	CC	GG	AA
AKU182	TT	AA	GG	GG	AA	**AKU 195**	CC	AA	GC	GG	AA
AKU183	TC	AG	GG	GG	AA	**AKU 196**	CC	AA	GC	GG	AA
AKU185	TT	AA	GC	GG	AA	**AKU 197**	CC	AA	CC	GG	AA
AKU187	TC	AA	GG	GG	AA	**AKU 198**	TC	AA	CC	GG	AA
AKU188	TT	AA	GG	GG	AA	**AKU 199**	TT	AA	GC	GG	AA
AKU189	TT	AA	GG	GG	AA	**AKU 200**	TC	AA	GC	GG	AA
AKU190	TT	AA	GG	GG	AA	**AKU 201**	TT	AA	GC	GG	AA
AKU191	TT	AA	GC	GG	AA	**AKU 202**	TT	AA	CC	GG	AA
AKU192	TT	AA	GG	GG	AA	**AKU 204**	TT	AA	CC	GG	AA
AKU194	TT	AA	GG	GG	AA	**AKU 205**	TC	AA	GC	GG	AA
AKU196	CC	AA	GC	GG	AA	**AKU 206**	TT	AA	GC	GG	AA
AKU197	TC	GG	GG	GG	AA	**AKU 207**	TC	AA	CC	GG	AA
AKU198	TT	AA	GC	GG	AA	**AKU 208**	TC	AA	CC	GG	AA
AKU200	TC	AA	GC	GG	AA	**AKU 209**	TC	AA	CC	GG	AA
AKU202	TT	AA	GG	GG	AA						
AKU204	TT	AA	GG	GG	AA						
AKU205	TT	AA	GG	GG	AA						
AKU206	TT	AA	GC	GG	AA						
AKU207	TC	AA	GG	GG	AA						

Appendix VIII: Sanger Sequences for rs1800440 and rs1056836 in exon 3 of CYP1B1

The sequences below contain the forward (F) and reverse (R) raw Sanger sequencing reads of the rs1800440 and rs1056836 SNPs in the CYP1B1 gene. These sequences were obtained from case, control, and benign group participants, with all variants located in exon 3 of CYP1B1.

rs1800440 and rs1056836 sequences of the case group

AKU103_F

CTATCCAAGTTCTCCGGGTTAGGCCACTTCAGTGGGTCATGATTCACAGACCACTGGTTGACAAAAACCACAGT
GTCCTTGGGAATGTGGTAGCCCAAGACAGAGGTGTTGGCAGTGGTGGCATGAGGATAGGTGACAGGCAAAATG
TTTATCTTGTGATCTGTGTCTGAGGAAACTGCTCGCAGTGTGTCACCATGAGATGCTGTTTGTGTTTTT

AKU103_R

GTCGGTTTTTGTCAACCAGTGGTCTGTGAATCATGACCCACTGAAGTGGCCCTAACCCGGAGAACTTTGATCCA
GCTCGATTCTTGGACAAGGACGGCCTCATCAACAAGGACCTGACCAGACAGTGATGATAGAGGATCTTTCGGC
TCTTTACTTTACCATCCGATCTGATAGACGATAACCCTGATAAACCCTTCACCCTCTTGGTGGCTTCCTGCTCCT
ATCCTGCACGGACCAAGTTATTGTAGATATCC

AKU104_F

TTATTCCACAGACCACTGGTTAGACAAAAACCACAGTTGTCCTTCGGGAATGTGGTAGCCCAAGACAAGAGGT
GTTGGGCAGTGGTGGCATGAGGATAAGTGACAGGCTCA

AKU104_R

GTCGCGTTTTTGTCAACCAGTGGTCTGTGAATCATGACCCACTGAAGTGGCCCTAACCCGGAGAACTTTGATCC
AGCTCGATTCTTGGACAAGGATGGCCTCATCAACAAGGACCTGACCAGAGAGTGATGATAGACTGCG

AKU105_F

CCCATTCATTGGGTTTCATGGATTTCACAGACCACTGGTTAGACAAAAACCACAAGTGTCCTTGGGAATGTGGT
AGCCCAAGACAGAGGTGTTGGCAGTGGTGGCATGAGGATAGGTGACAGGCAAA

AKU105_R

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AKU106_F

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GAATGTGGTAGCCCAAGACAGAGGTGTTGGCAGTGGTGGCATGAGGATAAGTGACAGGCAAAGGA

AKU106_R

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GCTCACCTCATGGTTTACTGCTGGCTATAAAGGATAAATCTGAGACAAATTCACCCTCTTGATGGCTTCCTGCTC
CTATCTCTGACTGACCCACTGATTATAGATATAATTCTTCCCTCCC

AKU109_F

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GGCA

AKU109_R

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AACCCGGAGAACTTTGATCCAGCTCGATTCTTGGACAAGGACGGCCTCATCAACAAGGACCTGACCAGCAGGA
GTGATGATCGCAACAATCTCCTGCTCCAACTGCTGCCCACAGATGCCCCTCGGGCTCCCTGCTACACTGGTGCC
ACTTGTCATATTT

AKU110_F

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AGTGGTGGCATGAGGATAAGTTGACAGGCAAAACA

AKU110_R

CACGGACACTGTGGTTTTTGTCAACCAGTGGTCTGTGAATCATGACCCACTGAAGTGGCCCTAACCCGGAGAAC
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AKU112_F

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GGCAGTGGTGGCATGAGGATAATTGACAGGCACAACAAAAATACCAAGACCTATTTCTGAGGATTTTGCTGGT
TTTTTAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

AKU112_R

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AKU113_F

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GGAATGTGGTAGCCCAAGACAGAGGTGTTGGCAGTGGTGGCATGAGGATAAGTGACAGGCAAAAAAAG

AKU113_R

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CTTTGATCCAGCTCGATTCTTGGACAAGGATGGCCTCATCAACAAGGACCTGACCAGAAAAGTGATGA

AKU116_F

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A

AKU116_R

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CAGCTCGATTCTTGGACAAGGATGGCCTCATCAACAAGGACCTGACCAGAGAGTGATGATTGCTTCC

AKU118_F

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CAAGACAGAGGTCGTTGGCAGTGGTGGCATGAGAGAATTGACAGGCGAAAGCCCGGAAAAAAAAAAAAAAAA
AAAAAAA

AKU118_R

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GTTTTTTACAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAACAAAAAAGAAAGT
ATTAGAAACAATCCCGGGATTTCGTTTGGGAAATAAGAAAGTCGTGAGTGTAGTTTC

AKU122_F

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ATA

AKU122_R

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AGCTCGATTCTTGGACAAGGACGGCCTCATCAACAAGGACCTGACCAGAGAGTGATGTTAAACGTGTGCCACT
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CTCTGAA

AKU124_F

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AKU124_R

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TATAAAG

AKU127_F

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AKU127_R

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AKU128_F

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AKU128_R

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AKU133_F

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AGTGACAGGCAA

AKU133_R

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AKU142_F

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AKU142_R

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AKU149_F

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AKU149_R

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TGTTTGTTTTTTTTTAGGAAAAAAATAAAAAGGAGGGTTGATAAATGGGATGAAGAAAAGTAATAAGAGGGGGG
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AKU152_F

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AACAGG

AKU152_R

AAGGACATTGTGTTTTTGTCAACCAGTGGTCTGTGAATCATGACCCAGTGAAGTGGCCTAACCCGGAGAACTTT
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AKU155_F

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TTTT

AKU155_R

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TTTTT

AKU158_F

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CAA

AKU158_R

ACTTCCCAGGACACTGTGTTTTTAGGCAACCAGTGGTCTGTGAATCATGACCCAGTGAAGTGGCCTAACCCGGA
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AKU164_F

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AKU164_R

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AKU173_F

TCCTGTCCAGAATCCGAGCTGGGACAAAGTTCTCCGGGTTAGGCCACTTCACTGGGTCATGATTCACAGACCAC
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AKU173_R

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AKU211_F

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CACAGTGTCCTTGGGAATGTGGTAGCCCAAGACAGAGGTGTTGGCAGTGGTGGCATGAGGAATAGTGACAGGC
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AKU211_R

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KAIC004_F

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AAATATTATCAAATAAAGTCTATACATGAGGAACTTGCAGAAAACTTACCTCCATGAAGAAGGCTGAATGGTTT
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KAIC004_R

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GAGAACTTTGATCCAGCTCGATTCTTGGACAAGGACGGCCTCATCAACAAGGACCTCGACTCAGAAAAAGTGA
TGAACCAAATGTGATAGCGCTGTATCGAGGTCTTTTGCCGGCCAGATAGACGATAAAGCTCATAGACGTTTCGC
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KAIC005_F

CGCGATCAAGTTCTCCGGGTTAGGCCACTTCACTGGGTCATGATTCACAGACCACTGGTTGACAAAAACCACAG
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KAIC005_R

ACACTGGGGGGTTTTTATTCAACCAGTGGTCTGTGAATCATGACCCAGTGAAGTGGCCTAACCCGGAGAACTTT
GATCCAGCTCGATTCTTGGACAAGGATGGCCTCATCAACAAGGACCTGACCACAGGAGTGATGATAAGACGGG
AGCTTTTGTGTTTTTTTCTCCCATCGATCAATAGTCATAACATCATAAGATACCCTCTGAGCATTGCTTTTTTTTT
TGTTTTTACCAAAAGCGTGTATGGCCTGAGCTGCCTACTTAT

KAIC006_F

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AAAAACCACAGTGTCCCTTCGGGAATGTGGTAGCCCAAGACAGAGGTGTTGGCAGTGGTGGCATGAGGATAAG
TGACAGGCAAATTGTTTATCTTATACCTCTGTGATATGAGAGAAATTGCTCAGCAGGCCTGACACGGCTGAGGA
TGATGTT

KAIC006_R

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AGAACTTTGATCCAGCTCGATTCTTGGACAAGGATGGCCTCATCAACAAGGACCTGACCAGAGAGTGATGTTA
GATAGCATCTTTTGTCTCTTAACTTTACCATCCGATCTCATATCGCTAAAGCGCCGAACCCCTTAACTCTCCTTG
TTTCACATTGCACTCCTCCTGCAGGGACTCAGTGATTGTAGATATAG

KAIC007_F

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AGTGTCCTTGGGAATGTGGTAGCCCAAGACAGAGGTGTTGGCAGTGGTGGCATGAGGATAAGTGACAGGCAAA
ATATTTTTCTTATCCCTCACAAAACCGCTGCTCCTCCCCTGAGGCCGGATGGGGATGAGGAGTTTTTTTTTTTTT
TTTTT

KAIC007_R

CACAGACACTGTGGTTTTTGTCAACCAGTGGTCTGTGAATCATGACCCAGTGAAGTGGCCTAACCCGGAGAACT
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TTCTTCTCTTCGTTTATTCCGATCGATAGAGGATAAACCTCCTAAAGCTTCCCCCTCCCGATGTCGTTTGCTCCCA
ACAGCAGTGAAAGTGATTGTAGATATCCGTCGTCGCTCATGCACTAGATTGCACGTGTCTCCTATGACAAACTC
AGAAGTCCCAGAGGTCCAGCTCAAGATGGGGGGGCCG

KAIC008_F

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KAIC008_R

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AAAAAAGTAGTTGTCTATGAGACCGCCTCGCCTCCCGCCCGCTTCTCATCAGGATTACGTGAATTCTTGTGCTT

KAIC011_F

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AGGCAAAATAACTTTTCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
CAAAAAAA AAAAAAAAA

KAIC011_R

CACTACCCAGGACACTGTGGTTTTTGTCAACCAGTGGTCTGTGAATCATGACCCACTGAAGTGGCCTAACCCGG
AGAACTTTGATCCAGCTCGATTCTTGGACAAGGATGGCCTCATCAACAAGGACCTGACCAGAGAGTGATGATC
AA

KAIC012_F

AATCGAGCTGGATCAAGTTCTCCGGGTTAGGCCACTTCACTGGGTCATGATTCACAGACCACTGGTTGACAAAA
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TTGACAGGCTAAGA

KAIC012_R

CACCACATTTCCCAAGGACACTGTGGTTTTTGTCAACCAGTGGTCTGTGAATCATGACCCACTGAAGTGGCCCT
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GTGATGATAGAAAGATTGATTTCTTCCTGCTCTTTTTCCCGCACAGAGACGATAACCCTCATGACCATTCCTCTC
TTTTTTCATTTGCTTT

KAIC014_F

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ACAGTGTCCTTCGGGAATGTGGTAGCCCAAGACAGAGGTGTTGGCAGTGGTGGCCATGAGGATAAGTGACAGG
CAAAATTTTTTTTTTTTCCCCCCCCCCCCCCCCCCCCCCCCTCCCCGAACCCTCTTCAAACATGTTTTTTTTTTTTTT
TT

KAIC014_R

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TTTTT

KAIC016_F

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TGTCCTTCGGGAATGTGGTAGCCCAAAGACAGAGGTGTTGGCAGTGGTGGCATGAGGTAGTAAGTGACAGGCA

AGAAAGTTTATCCTTATGCTCTCCCCCTCCCAGCTCCTCCCTCGCACCCCCTGCTCGCGCCCCGAACAACGATAT
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KAIC016_R

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CCTCAGGATCATTTTCTCCTAGTGTTGCATGCATTGAATTTACTAAAATAAACTTATTCCCCAGTGTAGTTCCGG
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KAIC018_F

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AAACCACAGTGTCCTTGGGAATGTGGTAGCCCAAGACAGAGGTGTTGGCAGTGGTGGCATGAGGAATAGTGAC
AGGCAACCTTTATCCTAGGACTCTATAATATGAGGAATAGTGACAGGCAAA

KAIC018_R

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GATAAA

KAIC021_F

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CAGTGTCCTTGGGAATGTGGTAGCCCAAGACAGAGGTGTTGGCAGTGGTGGCATGAGGAATAGTGACAGGCAA
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KAIC021_R

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KAIC023_F

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KAIC023_R

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ATGATAGA

KAIC011_R

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AA

KAIC012_F

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KAIC012_R

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TTTTTTCATTTGCTTT

KAIC014_F

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CAAAATTTTTTTTTTTTCCCCCCCCCCCCCCCCCCCCCCCCCTCCCCGAACCCTCTTCAAACATGTTTTTTTTTTTTTT
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KAIC014_R

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AKU081_F

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ATAAGTGACAGGCAAG

AKU081_R

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CCAGAGGAGTGATGATAAACATTTCCCCCTGAAAGGGGATTCTTGGTGGAAAGAGATTGTGGGGGGGGGGGGG
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AKU132_F

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TAAGTGACAGGCAAGATCATTCCCCCCCCAGTGAAGGCCTGAATCTAATTGCCAGGGTCTTTCTACTGTTTTTTT
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AKU132_R

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CTGACCAGAGAGTGATGATAGAGTAGCCGTCCTTGTCCAATATCGAGCTGCAGGAAAGTTCTACGGAAAAAGC
CCAATAGGGGGGAGAGCCCCTAAAAGGCGTTAAACACCCCCCCTTGCCTGGGAAAATTTTTTTATACCACAAA
AAATTTTTTTTTGCCGGTGTTTCTGGGGAACAAAATGGGACTGGGGAAAAAAGT

AKU135_F

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TGAGGAATAGTGACAGGCAAATAAATA

AKU135_R

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CTGACCAGAGAGTGATGATAG

AKU136_F

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ATAGTGACAGGCAAAAATAAGATCTCTTAAAGTAGTGGTTGTGGGTGTTGGTGGTTGGGGGGGGGGGCTTTTTT
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AKU136_R

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GACCAGAGAAGTGATGATACAAA

AKU144_F

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GACAGAGCAAAATCAATTTCCCCCCCC

AKU144_R

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GATGAAAAA

AKU148_F

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CCCTTTTTTTTTTTTTTTTTTTTTT

AKU148_R

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AAAGTGATGATCA

AKU159_F

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AGGCAAAACAGGGGGGCCCCAAAAAAAAAAAAAAAAAA

AKU159_R

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AKU163_F

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AKU163_R

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AKU180_F

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TTTTTT

AKU180_R

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AKU182_F

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CAGGCAAA

AKU182_R

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AKU185_F

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CAAA

AKU185_R

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TGTGTTTTTT

AKU191_F

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AKU191_R

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AKU196_F

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AKU196_R

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AKU198_F

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AKU198_R

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T

AKU200_F

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AKU200_R

GATTACCCAGGACACTGTGGTTTTTGTCAACCAGTGGTCTGTGAATCATGACCCACTGAAGTGGCCTAACCCGG
AGAACT

Appendix IX: Sanger Sequences for rs10012 and rs1056827 exon 2 of CYP1B1

The sequences below contain the forward (F) and reverse (R) raw Sanger sequencing reads of the rs1056827 and rs10012 SNPs in the CYP1B1 gene. These sequences were obtained from case, control, and benign group participants, with all variants located in exon 2 of CYP1B1.

rs1056827 and rs10012 sequences of the case group

AKU041_F

GGTACCCGAACATATAGCGGCCCTCGGGTCGAGGAGGCGCCGTCCGCGCTGCCGCGCACCAGCCGCGCCACCA
GCTCGCGCGCCTCGCTCAGCACGTGGCCCTCGAGGACTTGGCGGCTGCGCGGCTGGCGCGTGAAGAAGTTGCG
CATCATGCTGTGGGCTGCGCGCCGCTGCACCTTCCAGTGCTCCGAGTAGTGGCCGAAAGCCATGCTGCGGCCGC
CGGACACCACACGGAAGGAGGCGAAGGACGGCCGGTCGGCGAAGGCCGAGCCCTGCTGCACCAGGGCCTGGT
GGATGGCGCGCTCGCCATTCAGCACCACTATGGGGCAGCTGCCCAGGCGGATCTGGAAAACGTCGCCGTAGCG
CCGCGCCAGGCGAGCGAACGAGAGGTGAGCCGCCTGGCCCACCGCCGCCGCGTTTCCGATCAGTGGCCACGCA
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CCAGCACCGACAGGAGTAGCAAGGAGCGTA

AKU041_R

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GGCGCTGCTGGTGCGCGGCAGCGCGGACGGCCGCCTTCCTCGACCCGAGGCCGCTGACCGTCGTGGCCGTGGC
CAACGTCATGAGTGCGGGGGGGGTTTAA

AKU042_F

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CACCGACAGGATAGACAAAGGGGAGCGTA

AKU042_R

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ACGTTTTCCAGATCCGCCTGGGCAGCTGCCCCATAGTGGTGCTGAATGGCGAGCGCGCCATCCACCAGGCCCTG
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GGTGCGCGGCAGCGCGGACGGCGCCTTCCTCGACCCGAGGCCGCTGACCGTCGTGGCCGTGGCCAACGTCATG
AGTGCGGGGGGGGGGTTTAAAGAA

AKU055_F

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AKU055_R

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AKU057_F

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CTCCGTTGCCTCAGCAGCCGCTGGCCCACATGCACAGTGGCCAGCACCGACGGAGAGCCAGGGGGGGAGTTTA
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AKU057_R

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GTTTCAAA

AKU060_F

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AKU060_R

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AKU073_F

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AKU073_R

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AKU076_F

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AKU076_R

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AKU086C_F

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AKU086C_R

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rs1056827 and rs10012 sequences of the control group

AKU182_F

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AKU182_R

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rs1056827 and rs10012 sequences for the benign group

AKU011_F

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GCGCGGCAGCGCGGACGGCGCCTTCCTCGACCCGAGGCCGCTGACCGTGGTGGCCGTGGCCAACGTCATGAGT
GCGTTGTGTTTCA

AKU144_F

TTAGGCGGGCCTCGGGTCGAGGAGGCGCCGTCCGCGCTGCCGCGCACCAGCGCGCCCCAGCTCGCGCGCCTCG
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GCGCGCCGCTGCACCTTCCAGTGCTCCGAGTAGTGGCCGAAAGCCATGCTGCGGCCGCCGGACACCACACGGA
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CATTCAGCACCACTATGGGGCAGCTGCCCAGGCGGATCTGAAAAACGTCGCCGTAGCGCCGCGCCAGGCGAGC
GAACGAGAGGTGAGCCGCCTGGCCCACCGCCGCCGCGTTTCCGATCAGTGGCCACGCAACGGGCCCGGGGGCG
CGGACCCGAGCTGCCGCCTCCGTTGCCTCAGCAGCCGCTGGCCCACATGCACAGTGGCCAGCACCGACAGGAG
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AKU144_R

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ACGCGGCGGCGGTGGGCCAGGCGGCTCACCTCTCGTTTCGCTCGCCTGGCGCGGCGCTACGGCGACGTTTTCCA
GATCCGCCTGGGCAGCTGCCCCATAGTGGTGCTGAATGGCGAGCGCGCCATCCACCAGGCCCTGGTGCAGCAG
GGCTCGGCCTTCGCCGACCGGCCGTCCTTCGCCTCCTTCCGTGTGGTGTCCGGCGGCCGCAGCATGGCTTTCGGC

CACTACTCGGAGCACTGGAAGGTGCAGCGGCGCGCAGCCCACAGCATGATGCGCAACTTCTTCACGCGCCAGC
CGCGCAGCCGCCAAGTCCTCGAGGGCCACGTGCTGAGCGAGGCGCGCGAGCTGGTGGCGCTGCTGGTGCGCGG
CAGCGCGGACGGCGCCTTCCTCGACCCGAGGCCGCTGACCGTGGTGGCCGTGGCCAACGTCATGAGTGCGGTG
GTGTTTCAG

AKU148_F

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CTTCCAGTGCTCCGAGTAGTGGCCGAAAGCCATGCTGCGGCCGCCGGACACCACACGGAAGGAGGCGAAGGAC
GGCCGGTCGGCGAAGGCCGAGCCCTGCTGCACCAGGGCCTGGTGGATGGCGCGCTCGCCATTCAGCACCACTA
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CCGCCTGGCCCACCGCCGCCGCGTTTCCAATCAGTGGCCACGCAACGGGCCCGGGGGCGCGGACCCGAGCTGC
CGCCTCCTTTGCCTCAGCAGCCGCTGGCCCACATGCACAGTGGCCAGCACCGACGGGAGTAGCAAGGAGCGTA
TAATAAGGTTTAGTGGGGGGGCTGTGGTGGTGAGGTGTGGAAAGGGTTAGGAGGAGGGGTAGGAGAGGGGGG
GGGGGGGGGGGGGGAGGGGGGGGGGGGGAGAGGGTATGGTAGGGTTTGGTGTATGAGAATGTGGGGTGGGGG
GTGGGTGAGAGGGGGAGGAGGGTTTGGGGGTTGGGTGGGGGTGTGGGCGGGTGTTGATGCTTGGGTGCGGGG
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AKU148_R

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GCCTGGGCAGCTGCCCCATAGTGGTGCTGAATGGCGAGCGCGCCATCCACCAGGCCCTGGTGCAGCAGGGCTC

GGCCTTCGCCGACCGGCCGTCCTTCGCCTCCTTCCGTGTGGTGTCCGGCGGCCGCAGCATGGCTTTCGGCCACTA
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AGCCGCCAAGTCCTCGAGGGCCACGTGCTGAGCGAGGCGCGCGAGCTGGTGGCGCTGCTGGTGCGCGGCAGCG
CGGACGGCGCCTTCCTCGACCCGAGGCCGCTGACCGTGGTGGCCGTGGCCAACGTCATGAGTGCGGTGGGGGT
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AKU159_F

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GTAGCACGAGCGTAAGTGGGAGGAGGGGGGGGAGGAGGGGAAGGGGGGGAAGGAAAGGGAAGGAGGAGAG
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GGGAAGAGGGAGGGGAGGAGGGGGGGAGGGGGGGGGGGGGGGGGGGGAGGGGAGGGAAAGAAGAAAAG
AAGGAAAAAGAAAAGAAGAGGGGAAGAGAGAAGAGAGGGAGGAGGAGGAGGGGGGGGAAAGAGAGGGAAG
GG

AKU159_R

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GCGCAGCCGCCAAGTCCTCGAGGGCCACGTGCTGAGCGAGGCGCGCGAGCTGGTGGCGCTGCTGGTGCGCGGC
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TGTTTCAGAA

AKU163_F

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GCGCCGCTGCACCTTCCAGTGCTCCGAGTAGTGGCCGAAAGCCATGCTGCGGCCGCCGGACACCACACGGAAG
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AKU163_R

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GCCGCGCAGCCGCCAAGTCCTCGAGGGCCACGTGCTGAGCGAGGCGCGCGAGCTGGTGGCGCTGCTGGTGCGC
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TAGTGTTTCAGATTTG

AKU180_F

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GTGCTCCGAGTAGTGGCCGAAAGCCATGCTGCGGCCGCCGGACACCACACGGAAGGAGGCGAAGGACGGCCG
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CCGTTGCCTCAGCAGCCGCTGGCCCACATGCACAGTGGCCAGCACCGACGGATATGCCAGGGGGAGCGTTATT
TCTTTTTATACATACCAGCCAAACTTGACTAAGACTTTTAATCGAGAATTCACTGCGGGGGGAGGTGGTGCAGG
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CGGGTGCTGGCGTTAGGTGATTTTGATTTTGTTATTTTTGTCTT

AKU180_R

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GCCTTCGCCGACCGGCCGTCCTTCGCCTCCTTCCGTGTGGTGTCCGGCGGCCGCAGCATGGCTTTCGGCCACTAC
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GCCGCCAAGTCCTCGAGGGCCACGTGCTGAGCGAGGCGCGCGAGCTGGTGGCGCTGCTGGTGCGCGGCAGCGC
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CAAA

AKU182_F

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GCTGCGCGGCTGGCGCGTGAAGAAGTTGCGCATCATGCTGTGGGCTGCGCGCCGCTGCACCTTCCAGTGCTCCG
AGTAGTGGCCGAAAGCCATGCTGCGGCCGCCGGACACCACACGGAAGGAGGCGAAGGCCGGCCGGTCGGCGA
AGGCCGAGCCCTGCTGCACCAGGGCCTGGTGGATGGCGCGCTCGCCATTCAGCACCACTATGGGGCAGCTGCC
CAGGCGGATCTGGAAAACGTCGCCTTAGCGCCGCGCCAGGCGAGCGAACGAGAGGTGAGCCGCCTGGCCCACC
GCCGCCGCGTTTCCAATCAGTGGCCACGCAGACGGGCCCGGGGGCGCGGACCGGAGCTGCCGCCTCCTTTGCCT
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AKU182_R

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CCCATAGTGGTGCTGAATGGCGAGCGCGCCATCCACCAGGCCCTGGTGCAGCAGGGCTCGGCCCTCGCCGACC
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CCTCGACCCGAGGCCGCTAACCGTCGTGGCCGTGGCCAACGTCATGAAGTGCGTTGAAGGTTCGAGA

AKU185_F

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TTTTTT

AKU185_R

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GGCTCGGCCTTCGCCGACCGGCCGTCCTTCGCCTCCTTCCGTGTGGTGTCCGGCGGCCGCAGCATGGCTTTCGGC
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CGCGCAGCCGCCAAGTCCTCGAGGGCCACGTGCTGAGCGAGGCGCGCGAGCTGGTGGCGCTGCTGGTGCGCGG
CAGCGCGGACGGCGCCTTCCTCGACCCGAGGCCGCTGACCGTCGTGGCCGTGGCCAACGTCATGAGTGCGCGG

GGGGTGTTTAAAAAAGACGGCCCATTTTTAAAAAAAAGGCCTTGGACTTTCCGGAGAGTGTAGAGGGGGGGTG
TGATCCTGGCCGGAAAAATTGTTGAATTAAACCCCCCGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG
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AKU191_F

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CCAGTGCTCCGAGTAGTGGCCGAAAGCCATGCTGCGGCCGCCGGACACCACACGGAAGGAGGCGAAGGACGG
CCGGTCGGCGAAGGCCGAGCCCTGCTGCACCAGGGCCTGGTGGATGGCGCGCTCGCCATTCAGCACCACTATG
GGGCAGCTGCCCAGGCGGATCTGGAAAACGTCGCCGTAGCGCCGCGCCAGGCGAGCGAACGAGAGGTGAGCC
GCCTGGCCCACCGCCGCCGCGTTTCCGATCAGTGGCCACGCAAACGGGCCCGGGGGCGCGGACCCGAGCTGCC
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AKU191_R

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TTTTCCAGATCCGCCTGGGCAGCTGCCCCATAGTGGTGCTGAATGGCGAGCGCGCCATCCACCAGGCCCTGGTG
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GCGCGGCAGCGCGGACGGCGCCTTCCTCGACCCGAGGCCGCTGACCGTCGTGGCCGTGGCCAACGTCATGAGT
GCGGTGTGTTTCAGA

AKU196_F

TTAGCGGCCTCGGGTCGAGGAGGCGCCGTCCGCGCTGCCGCGCACCAGCAGCGCCACCAGCTCGCGCGCCTCG
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CTGCGCGCCGCTGCACCTTCCAGTGCTCCGAGTAGTGGCCGAAAGCCATGCTGCGGCCGCCGGACACCACACG
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GCGAACGAGAGGTGAGCCGCCTGGCCCACCGCCGCCGCGTTTCCGATCAGTGGCCACGCAAACGGGCCCGGGG
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AKU196_R

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TTCCAGATCCGCCTGGGCAGCTGCCCCATAGTGGTGCTGAATGGCGAGCGCGCCATCCACCAGGCCCTGGTGCA
GCAGGGCTCGGCCTTCGCCGACCGGCCGTCCTTCGCCTCCTTCCGTGTGGTGTCCGGCGGCCGCAGCATGGCTT
TCGGCCACTACTCGGAGCACTGGAAGGTGCAGCGGCGCGCAGCCCACAGCATGATGCGCAACTTCTTCACGCG
CCAGCCGCGCAGCCGCCAAGTCCTCGAGGCCCACGTGCTGAGCGAGGCGCGCGAGCTGGTGGCGCTGCTGGTG
CGCGGCAGCGCGGACGGCGCCTTCCTCGACCCGAGGCCGCTGACCGTCGTGGCCGTGGCCAACGTCATGAGTG
CGTGGGGGGTTCAAGAA

AKU198_F

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AGGACTTGGCGGCTGCGCGGCTGGCGCGTGAAGAAGTTGCGCATCATGCTGTGGGCTGCGCGCCGCTGCACCTT
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GCCTGGCCCACCGCCGCCGCGTTTCCAATCAGTGGCCACGCAAACGGGCCCGGGGGCGCGGACCGGAGCTGCC
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TAT

AKU198_R

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AKU200_F

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GCCGCCTGGCCCACCGCCGCCGCGTTTCCGATCAGTGGCCACGCAAACGGGCCCGGGGGCGCGGACCGGAGCT
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TAT

AKU200_R

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GGCGCCTTCCTCGACCCGAGGCCGCTGACCGTCGTGGCCGTGGCCAACGTCATGAGTGCGTGGGGGGGTTCAA
GAAA

Appendix X: Genotypes for rs1800440, rs1056836, rs1056827 and rs10012

PID	rs1800440	rs1056836	rs1056827	rs10012	PID	rs1800440	rs1056836	rs1056827	rs10012
AKU041C	GG	GC	CA	GC	**AKU142C**	AA	CC	CA	GC
AKU042C	AA	CC	CA	GC	**AKU149C**	AA	CC	CC	GC
AKU057C	AA	CC	AA	GC	**AKU152C**	AA	CC	AA	GC
AKU060C	GG	GG	AA	GC	**AKU155C**	AA	CC	AA	GC
AKU073C	AG	GC	AA	CC	**AKU158C**	AA	CC	AA	GC
AKU076C	AG	GC	AA	CC	**AKU164C**	AA	CC	AA	CC
AKU086C	AA	CC	CA	GC	**AKU173C**	AA	CC	AA	GC
AKU087C	AA	CC	CC	GG	**AKU211C**	AG	GC	AA	GC
AKU088C	AA	CC	AA	GC	**KAIC004C**	AG	GG	AA	GC
AKU094C	AA	CC	AA	CC	**KAIC005C**	AA	CC	CC	GG
AKU097C	AA	GC	AA	GC	**KAIC006C**	AA	GC	AA	CC
AKU099C	AG	GC	AA	GC	**KAIC007C**	AG	CC	AA	CC
AKU100C	AA	CC	CA	GC	**KAIC008C**	AA	CC	CA	GC
AKU103C	AG	GG	AA	GC	**KAIC011C**	AG	GC	AA	CC
AKU104C	AA	CC	CC	GG	**KAIC014C**	GG	GC	CC	GG
AKU105C	AA	CC	AA	GC	**KAIC016C**	AA	CC	AA	CC
AKU106C	AA	CC	CC	GG	**KAIC018C**	GG	GG	AA	GC
AKU109C	AA	CC	AA	GC	**KAIC021C**	AA	CC	AA	GC
AKU110C	AA	CC	AA	CC	**KAIC023C**	AA	CC	AA	GC
AKU112C	AA	CC	CC	GG	**KAIC024C**	AA	CC	AA	GC
AKU113C	AA	GC	CC	GG	**KAIC025C**	AA	CC	AA	CC
AKU116C	AA	CC	AA	GC	**KAIC026C**	AA	CC	AA	GC
AKU118C	AA	CC	AA	CC	**KAIC027C**	AG	GG	CC	GG
AKU122C	AA	CC	AA	GC	**KAIC029C**	GG	GG	AA	CC
AKU124C	AA	CC	CC	GG	**KAIC030C**	AA	CC	AA	CC
AKU127C	AG	CC	AA	GC	**KAIC032C**	AG	GG	AA	CC

AKU128C	AA	CC	AA	GC	**KAIC033C**	AG	GC	AA	GC
AKU133C	AG	GG	AA	CC	**KAIC035C**	AA	CC	AA	CC
PID	**rs1800440**	**rs1056836**	**rs1056827**	**rs10012**	**PID**	**rs1800440**	**rs1056836**	**rs1056827**	**rs10012**
KAIC041C	AG	GG	CC	GG	**AKU077B**	AG	GC	AA	CC
KAIC046C	AG	GG	AA	CC	**AKU081B**	AG	GC	AA	GC
KAIC047C	GG	GG	AA	CC	**AKU132B**	GG	CC	AA	CC
KAIC051C	GG	GG	AA	GC	**AKU135B**	AG	CC	AA	GC
KAIC052C	AG	CC	CC	GG	**AKU136B**	AG	GG	AA	GC
KAIC057C	AA	CC	AA	GC	**AKU144B**	GG	GG	AA	CC
KAIC059C	AG	GG	AA	GC	**AKU148B**	AG	GG	AA	CC
KAIC060C	GG	GG	AA	GC	**AKU159B**	AA	GC	AA	CC
AKU011B	AG	GG	AA	GC	**AKU163B**	AA	CC	AA	CC
AKU025B	GG	GG	CC	GG	**AKU180B**	GG	GG	AA	CC
AKU026B	AG	CC	AA	CC	**AKU182B**	AA	CC	CC	GG
AKU031B	AA	CC	CA	GC	**AKU185B**	AG	GG	AA	CC
AKU034B	AG	GC	AA	GC	**AKU191B**	AA	CC	AA	CC
AKU039B	AG	CC	AA	GC	**AKU196B**	AG	CC	AA	CC
AKU043B	AA	CC	AA	GC	**AKU198B**	AA	CC	AA	GC
AKU044B	AG	GC	AA	CC	**AKU200B**	AG	GG	AA	GC
AKU046B	AA	CC	AA	GC	**AKU182N**	AA	CC	CC	GG
AKU047B	AG	CC	AA	GC	**AKU183N**	AA	CC	AA	CC
AKU048B	AA	CC	CC	GG	**AKU191N**	AG	GC	AA	GC
AKU049B	AA	CC	CC	GC	**AKU193N**	AA	CC	AA	CC
AKU054B	AG	CC	AA	GC	**AKU194N**	AA	CC	CC	GG
AKU056B	AA	CC	AA	GC	**AKU195N**	AA	CC	AA	CC
AKU059B	AA	CC	AA	GC	**AKU196N**	AG	CC	AA	CC
AKU061B	AA	CC	AA	GC	**AKU197N**	AA	CC	AA	GC
AKU062B	AG	GC	AA	CC	**AKU198N**	AA	CC	AA	GC
AKU063B	AA	CC	AA	CC	**AKU199N**	AA	CC	AA	CC

AKU064B	AA	CC	AA	GC	**AKU200N**	AG	GG	AA	GC
AKU067B	AG	CC	AA	GC	**AKU201N**	AG	GC	AA	CC
AKU069B	AA	CC	AA	GC	**AKU202N**	AG	GC	AA	CC
AKU070B	AA	CC	AA	GC	**AKU204N**	AG	GC	AA	GC
PID	**rs1800440**	**rs1056836**	**rs1056827**	**rs10012**					
AKU205N	AA	CC	AA	GC					
AKU206N	GG	GG	AA	GC					
AKU207N	AA	CC	AA	CC					
AKU208N	AA	CC	AA	CC					
AKU209N	AA	CC	AA	CC					

Appendix XI: Clinicopathological Characteristics of Case Group Participants

This table presents the detailed histopathological and molecular profile data for participants in the case group (ER+ breast cancer).

PID	Histo_Type	Grade	Stage	LN_Status	Mol_Sub type	PID	Histo_Type	Grade	Stage	LN_Status	Mol_Sub type
AKU041	IDC	3	1	N0	lum A	**AKU118**	IDC	2	2	N0	lum A
AKU042	IDC	2	3	N2	lum A	**AKU122**	IDC	2	2	N0	lum A
AKU057	IDC	2	2	N0	lum A	**AKU124**	IDC	2	2	N1	lum A
AKU060	IDC	2	3	N3	lum B	**AKU127**	ILC	1	2	N1	lum A
AKU073	IPC	3	1	N1	lum A	**AKU128**	IDC	2	1	N0	lum A
AKU076	DCIS	3	3	N2	lum A	**AKU133**	IDC	2	3	N2	lum B
AKU086	IDC	2	1	N0	lum A	**AKU142**	IDC	2	1	N0	lum A
AKU087	ILC	2	2	N0	lum A	**AKU149**	IDC	3	2	N1	lum A
AKU088	IDC	1	1	N0	lum A	**AKU152**	IC	2	1	N0	lum A
AKU094	DCIS	2	1	N0	lum A	**AKU158**	IDC	2	1	N0	lum A
AKU097	IDC	2	1	N0	lum A	**AKU164**	IDC	2	1	N0	lum A
AKU099	ILC	3	3	N2	lum A	**AKU173**	IC	2	1	N0	lum A
AKU100	IDC	3	2	N1	lum A	**AKU211**	MC	2	1	N0	lum A
AKU103	IC	3	2	N0	lum A	**AKU155**	IDC	2	1	N0	lum A
AKU104	IDC	3	2	N1	lum A	**KAIC004**	IDC	2	1	N0	lum A
AKU105	IDC	1	2	N0	lum A	**KAIC005**	IDC	2	2	N1	lum B
AKU106	IC	2	1	N0	lum B	**KAIC006**	IDC	3	2	N1	lum A
AKU109	IDC	3	3	N1	lum A	**KAIC007**	IDC	3	2	N1	lum A
AKU110	DCIS	2	2	N3	lum A	**KAIC008**	IDC	2	3	N2	lum A
AKU112	IDC	2	2	N1	lum A	**KAIC011**	PDCIS	2	2	N0	lum A
AKU113	IDC	2	1	N0	lum A	**KAIC016**	IDC	3	3	N2	lum A

AKU116	IDC	3	2	N1	lum A	**KAIC018**	IDC	2	3	N2	lum A

PID	**Histo_Type**	**Grade**	**Stage**	**LN_Status**	**Mol_Subtype**
KAIC019	IDC	3	3	N2	lum A
KAIC021	IDC	3	3	N3	lum A
KAIC023	IDC	3	3	N3	lum B
KAIC024	IDC	2	2	N1	lum A
KAIC025	IDC	2	3	N2	lum A
KAIC026	IDC	2	2	N1	lum B
KAIC027	IDC	2	3	N2	lum B
KAIC029	IDC	2	2	N2	lum A
KAIC030	IDC	3	2	N0	lum A
KAIC032	IDC	3	2	N1	lum A
KAIC033	IDC	2	2	N1	lum A
KAIC035	IDC	3	3	N3	lum A
KAIC041	IDC	2	3	N2	lum A
KAIC046	IDC	2	2	N0	lum B
KAIC047	DCIS	2	1	N1	lum A
KAIC051	IDC	2	2	N1	lum A
KAIC052	IDC	2	2	N0	lum B
KAIC057	IMCS	2	3	N2	lum B
KAIC059	IDC	3	2	N0	lum A
KAIC060	IDC	3	2	N0	lum A

Appendix XII: Publication Abstracts

Association of rs4646903 and rs1048943 CYP1A1 estrogen-metabolizing gene polymorphisms with estrogen receptor-positive breast cancer in Kenyan women

Mary Murithi[1,2,*], Steven Nyanjom[1], Victor Mobegi[3], Sayed Shahin[4] and Francis Makokha[5]

[1]*Biochemistry Department, Jomo Kenyatta University of Agriculture and Technology,* 62000-00200, Nairobi, Kenya
[2]*Pre-Clinical Department, Kabarak University,* Private Bag-20157, Nakuru, Kenya
[3]*Department of Biochemistry, University of Nairobi, 30197-00100*, Nairobi, Kenya
[4]*Department of Pathology, Aga Khan University Hospital,* 30270-00100, Nairobi, Kenya
[5]*Directorate of Research and Innovation, Mount Kenya University,* 342-01000, Thika, Kenya

***Corresponding author:** murithimary11@gmail.com

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Abstract: Breast cancer is the most prevalent neoplasm and the second leading cause of death among females in Kenya. Estrogen and its metabolites are known risk factors for breast cancer. Polymorphisms in these genes and breast cancer susceptibility are unique among different populations. This study aimed to determine the probable associations between estrogen-metabolizing gene variations and other risk factors for breast cancer risk in Kenyan women. Buffy coat samples were obtained from patients diagnosed with estrogen receptor-positive breast cancer, benign breast disease, and healthy volunteers. Genotyping of target polymorphisms was conducted using polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) analysis. The rs4646903 variant genotype CC was associated with breast cancer in the case-control model (P=0.001); the heterozygous genotype TC (P=0.01) and the luminal B molecular subtype (P=0.02) showed increased odds of late-stage breast cancer. The rs1048943 variant genotype GG was associated with breast cancer in the case-benign model (P=0.04), whereas CG was associated with breast cancer in the case-control model (P=0.02). These findings imply that the rs4646903 and rs1048943 variant genotypes are involved in breast cancer risk in Kenyan women. Hence, they may be explored further as potential markers for the disease.

Keywords: breast cancer; gene polymorphisms; estrogen metabolizing gene; polymerase chain reaction-restriction fragment length polymorphism; genotype