UNIVERSITY OF GHANA

COLLEGE OF HEALTH SCIENCES

EVALUATION OF BREAST CANCER SUSCEPTIBILITY 1 GENE (BRCA 1 GENE) POLYMORPHISM AND FINGER DERMATOGLYPHIC PATTERNS IN BREAST CANCER

BY

EMMANUEL OSEI NKANSAH

(ID NO. 10341320)

THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MPHIL HUMAN ANATOMY DEGREE.

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DECLARATION BY CANDIDATE

I hereby declare that except for references to work of other researchers, which have been duly referenced, this project is the product of my own research carried out under supervision in accordance with regulations of the School of Graduate Studies, University of Ghana. I further declare that this thesis has neither in whole nor in part been presented for another degree elsewhere, and that I am solely responsible for any flaws in this work.

Signature ……... Date……..

Name: Emmanuel Osei Nkansah

DECLARATION BY SUPERVISORS

We hereby declare that the practical work and presentation of this thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Ghana.

Principal Supervisor

…… Date……

Rev. Dr. Benjamin Arko-Boham

Co-Supervisors:

…… Date…….

Dr. John Ahenkorah

……. Date……

Dr. Emmanuel Ayitey Tagoe

……. Date……

Prof. Clegg Lamptey

DEDICATION

This work is dedicated to God for granting me knowledge and strength to pursue Master of Philosophy degree in Human Anatomy.

This work is also dedicated to my mother, Madam Juliana L. Wemegah and my uncles, Mr. Kwesi Wemegah and Mr. Hugh Kwame Wemegah as well as my only brother, Mr. Clement Owusu Nkansah who through tough moments, encouraged and supported me.

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TABLE OF CONTENTS

DECLARATION……......i

DEDICATION…….....ii

ACKNOWLEDGEMENT…….....iii

TABLE OF CONTENTS…….……iv

LIST OF TABLES…….……xiii

LIST OF FIGURES…….....xv

ABSTRACT……...xvi

CHAPTER ONE: INTRODUCTION.....1

1.1 Background…….……...1

1.1.1 Breast cancer……..……1

1.1.2 Anatomy of the breast…….……1

1.1.3 Classification of breast cancer……2

1.1.4 Breast cancer risk factors…….……..2

1.1.4.1 Modifiable risk factors……...2

1.1.4.2 Non-modifiable risk factors……...3

1.1.5 Breast cancer screening……..……..….4

1.1.6 Breast cancer treatment……..……..……5

1.1.7 Breast cancer susceptibility 1 gene (BRCA1 gene)……..…5

1.1.8 Finger dermatoglyphics……6

1.2 Problem statement……...6

1.3 Justification……7

1.4 Aim……8

1.5 Specific Objectives……8

1.6 Hypothesis……8

CHAPTER TWO: LITERATURE REVIEW……9

2.1 Breast cancer……9

2.2 Mechanism of breast cancer formation……...9

2.3 Anatomy of the breast……10

2.4 Classification of breast cancer……..12

2.4.1 Histopathological classification of breast cancer……12

2.4.1.1 Ductal carcinoma in situ……....12

2.4.1.2 Invasive ductal carcinoma……12

2.4.1.3 Invasive lobular carcinoma……..13

2.4.2 Molecular subtypes of breast cancer……13

2.4.2.1 Luminal A breast cancer……...13

2.4.2.2 Luminal B breast cancer……...13

2.4.2.3 Triple-negative/basal-like breast cancer……14

2.4.2.4 Human Epidermal Growth Factor Receptor 2 (HER2) Enriched breast cancer……..……14

2.4.2.5 Normal-like breast cancer……14

2.5 Prognosis of breast cancer……15

2.6 Microscopic grading in breast carcinoma...……..…15

2.7 Breast cancer staging……..……...17

2.7.1 TNM staging system…….....18

2.7.2 Breast cancer stage grouping…….....18

2.7.2.1 Non-invasive breast cancer……...……....18

2.7.2.2 Stage I……...…18

2.7.2.3 Stage II……..19

2.7.2.4 Stage III……..…20

2.7.2.5 Stage IV…….…...…20

2.8 Signs and symptoms of breast cancer……...21

2.9 Breast cancer risk factors……..23

2.9.1 Modifiable risk factors…….…23

2.9.2 Non-modifiable risk factors……..25

2.10 Breast cancer screening……27

2.10.1 Mammography……..……28

2.10.2 Magnetic Resonance Imaging……28

2.10.3 Ultrasonography……29

2.10.4 Clinical Breast Examination……29

2.10.5 Self-Breast Examination……29

2.11 Breast cancer treatment……30

2.11.1 Surgery……31

2.11.2 Radiation therapy……31

2.11.3 Systemic therapy……33

2.11.3.1 Chemotherapy……33

2.11.3.2 Hormonal therapy……34

2.11.3.3 Targeted therapy……35

2.12 Breast cancer susceptibility gene (BRCA gene) ……37

2.12.1 Breast cancer susceptibility 1 gene (BRCA1 gene)……37

2.12.2 Breast cancer susceptibility 2 gene (BRCA2 gene)……37

2.12.3 The structure and function of BRCA1 gene……37

2.12.3.1 Really Interesting New Gene (RING) Domain……39

2.12.3.1.1 Cancer related mutation of the RING Domain……43

2.12.3.2 Exon 11-13……43

2.12.3.3 Serine Cluster Domain……44

2.12.3.4 BRCA1 C-Terminal (BRCT) Domain……45

2.13 Gene Polymorphism……...45

2.13.1 Single Nucleotide Polymorphisms (SNPs)……47

2.13.1.1 Rs1799950……49

2.13.1.2 Rs4986850……50

2.13.1.3 Rs2227945……50

2.13.1.4 Rs16942……50

2.13.1.5 Rs1799966……51

2.14 Finger dermatoglyphics……...51

2.14.1 Development of epidermal ridges……53

2.14.2 Ridge characteristics……53

2.14.2.1 Tri radius……..53

2.14.2.2 Core……..54

2.14.3 Fingerprints……54

2.14.3.1 Visible prints……54

2.14.3.2 Latent prints……54

2.14.3.3 Impressed prints……...54

2.14.4 Fingerprint patterns……..55

2.14.4.1 Whorl……..…55

2.14.4.2 Loop……...56

2.14.4.3 Arch……...……57

2.14.5 Finger dermatoglyphic correlations……..……..57

2.14.6 Dermatoglyphics and breast cancer……...……....59

CHAPTER THREE: MATERIALS AND METHODS……..61

3.1 Study design……61

3.2 Study sites…….…….61

3.3 Study participants……62

3.4 Inclusion criteria……..62

3.5 Exclusion criteria……..62

3.6 Sample size……...62

3.7 Participants' recruitment……..64

3.8 Data collection…….…….……..….64

3.8.1 Collection of socio-demographic characteristics and clinico-pathological data…….……64

3.8.2 Procedure for data collection (finger dermatoglyphics)……..65

3.8.3 Procedure for obtaining single nucleotide polymorphism of BRCA1 gene……65

3.8.3.1 Extraction of buffy coat…….……..65

3.8.3.2 DNA extraction from buffy coat……..66

3.8.3.3 BRCA1 gene amplification……..……...67

3.8.3.4 BRCA1 gene sequencing……..……...69

3.9 Data analysis……72

3.10 Ethical consideration ……..72

CHAPTER FOUR: RESULTS……...73

4.1 Socio-demographic characteristics of study population (age)……73

4.2 Clinico-pathological characteristics……..……74

4.2.1 Site of breast cancer……..…74

4.2.2 Histopathological diagnosis of breast cancer participants…….…..75

4.2.3 Molecular subtypes of breast cancer among study participants……..76

4.2.4 Stage and Grade of breast cancer among study participants……...77

4.2.5 Risk factors of breast cancer among study participants……..…78

4.3 Finger dermatoglyphics…….…..79

4.3.1 Fingerprint patterns of the right fingers among study participants…….…79

4.3.2 Fingerprint patterns of the left fingers among study participants…….……..80

4.3.3 Mean frequency of fingerprint patterns among study participants…..……81

4.3.4 Six or more loops and six or more whorls among study participants……..…...82

4.4 Finger dermatoglyphic patterns (six or more loops) and tumour

characteristics ……..…….83

4.5 Single nucleotide polymorphism of BRCA1 gene……...…….…..…..85

4.5.1 BRCA1 gene amplification……...……85

4.5.2 Variant analysis……...……..86

4.5.3 Molecular subtypes of breast cancer and variant location of BRCA1 gene…..87

4.5.4 Fingerprint patterns (six or more loops) and predominant BRCA1 gene variants……..……89

CHAPTER FIVE: DISCUSSSION, SUMMARY OF KEY FINDINGS, CONCLUSION, RECOMMENDATION AND LIMITATIONS…….…..90

5.1 Discussion……....90

5.1.1 The predominant location of single nucleotide polymorphism and the resulting nucleotide variants of BRCA1 gene among study participants…….....90

5.1.2. The predominant finger dermatoglyphic patterns in individuals with breast cancer and individuals without breast cancer…….93

5.1.3 Tumour characteristics in individuals with breast cancer in relation to fingerprint patterns…….....95

5.1.4 The predominant variants of BRCA1 gene and the predominant finger dermatoglyphic pattern (six or more loops) among individuals with breast cancer and individuals without breast cancer……98

5.2 Summary of key findings……..……..……99

5.3 Conclusion……..……..100

5.4 Limitations……..……..101

5.5 Recommendations…….……...….101

REFERENCES……...…….……102

APPENDIX 1: Information sheet and Informed consent……..……...119

APPENDIX II: Tool for fingerprint data collection…….……...124

APPENDIX III: A picture of EDTA tube with venous blood centrifuged to obtain plasma, buffy coat and red blood cells.....125

APPENDIX IV: Forward sequences of amplified BRCA1 gene (exon 11 and surrounding introns)…….……126

APPENDIX V: A picture of exon 11 sequence of BRCA1 gene……133

APPENDIX VI: Ethical clearance from the Ethical and Protocol Review Committee of the College of Health Science, University of Ghana……..134

LIST OF TABLES

Table 1. Scores associated with the three indicators (tubule formation, nuclear pleomorphism and mitotic count) of histologic grading…..……..…...16

Table 2. Histologic grade type scoring……....17

Table 3. Reagents used for Polymerase Chain Reaction and the quantities in 25µl total mixture per reaction as well as 80 reactions and 8 negative control reactions..…...…..68

Table 4. Histopathological diagnosis of breast among study participants……....….75

Table 5. Molecular subtypes of breast cancer among study participants……...……76

Table 6. Stage and Grade of breast cancer among study participants…..……..…….77

Table 7. Risk factors of breast cancer among study participants……..……..78

Table 8. Fingerprint patterns of the right fingers of study participants……...…..79

Table 9. Fingerprint patterns of the left fingers of study participants…..……..…....80

Table 10. Mean frequency of fingerprint patterns of all ten (10) fingers among study participants……..……81

Table 11. Six or more loops and six or more whorls among study participants…….82

Table 12. Finger dermatoglyphic patterns and tumour characteristics……83

Table 13. Association between fingerprint patterns and tumour characteristics…….84

Table 14. Nucleotide variants and their location…….……86

Table 15. Molecular subtypes of breast cancer and variant location of BRCA1 gene....88

Table 16. Fingerprint patterns (six or more loops) and predominant BRCA1 gene Variants……..89

LIST OF FIGURES

Figure 1. The breast: cross-section scheme of the mammary gland……..11

Figure 2. A graph showing the three domains of BRCA1 with the highest rate of mutation generated from the breast cancer information core……39

Figure 3. BRCA1 RING Domain……..40

Figure 4. An illustration of BRCA1 exons 11-13 with multiple functions……..…….44

Figure 5. A diagram of palmar surface of the hand showing the finger dermatoglyphics……..……52

Figure 6. Pictures of fingerprints showing a whorl, loop and arch as well as the delta and a core…….…..……...…55

Figure 7. Pictures showing fingerprinting through the ink method……..…..65

Figure 8. A picture showing mismatch of nucleotides after alignment of the wild type (template) of the forward BRCA1 gene and the forward sequence of exon 11 with flanking introns of BRCA1 gene of a breast cancer participant……..…71

Figure 9. Age distribution of study participants…….…...….73

Figure 10. Site of breast cancer……..…….74

Figure 11. Image of Gel showing bands from amplicons after gel electrophoresis. ..…85

ABSTRACT

Background: Breast cancer is the commonest malignancy among women and it is the primary cause of cancer deaths among women globally. Late diagnosis and difficulty in the prediction of prognosis of breast cancer has been cited as a factor for increased mortality rate in Ghana and this could be due to insufficient preliminary screening tool for risk and prognosis assessment. The mutant form of the single nucleotide polymorphism of breast cancer susceptibility 1 gene (BRCA1 gene) usually harboured within exon 11 is reported to be associated with the risk of breast cancer. Also, studies have reported separately that six or more fingerprint whorls as well as six or more fingerprint loops are associated with women with breast cancer. Therefore, this study sought to investigate the association between finger dermatoglyphic patterns and single nucleotide polymorphism of BRCA1 gene among individuals with breast cancer.

Aim: To determine the relationship between the single nucleotide polymorphism of BRCA1 gene and finger dermatoglyphic patterns in breast cancer patients.

Methodology: The study was cross-sectional. Seventy (70) women presenting with breast cancer and seventy (70) age-matched apparently healthy females were sampled. Study participants were recruited through simple random sampling at the Department of Surgery at the Korle-Bu Teaching Hospital and its immediate environment. Data for finger dermatoglyphic analysis were collected through the ink method. From each participant, 5 ml of venous blood was collected through venipuncture into an EDTA tube. DNA was extracted from the white blood cells (buffy coat). The extracted DNA was amplified through Polymerase Chain Reaction (PCR). Amplicons were sequenced and sequencing data was retrieved and aligned with the wild type template of BRCA1 gene. Data on tumour characteristics were summarized with a bar chart, a pie chart and tables

while data on fingerprint patterns were analyzed with chi-square. The mean frequency of fingerprint patterns among study participants were analyzed with independent samples student's t-test. Six or more whorls and six or more loops were analyzed with chi square. Differences in data set with $p < 0.05$ were considered statistically significant. Association between fingerprint patterns (six or more loops) and tumour characteristics were analyzed with chi-square test.

Results: From the study, Luminal B was the predominant breast cancer molecular subtype among the patients. With regards to fingerprint patterns, the predominant fingerprint pattern among breast cancer participants was the loops. There was absence of an arch on the right ring finger of breast cancer participants and also six or more loops increased among individuals with breast cancer but $p > 0.05$. BRCA1 gene variants c.34311A>C, c.34320A>C and c.34321A>T are the predominant variants associated with breast cancer females in Ghana. Lastly, a higher frequency of the presence of six or more loops in relation to c.34311A>C were found among study participants.

Conclusion: From the study, a higher percentage frequency of presence of six or more loops in relation to c.34311A>C was observed among breast cancer females and apparently healthy females.

CHAPTER ONE

INTRODUCTION

1.1 Background

1.1.1 Breast cancer

Cancer of the breast is a malignant disease that results from abnormal proliferation of cells within the breast tissue. Advanced breast cancers usually cause metastasis into the bony skeleton, brain, lung and the liver (American Cancer Society, 2017). According to the World Health Organization 2018 report on breast cancer; breast cancer is the commonest malignancy among women; it affected 2.1 million women worldwide in 2018 and accounts for the greatest number of cancer-related deaths among women globally (World Health Organisation, 2018).

In 2018, 627,000 women died from breast cancer, representing 15% of all cancer deaths among women globally. In Africa, there is an increasing incidence of breast cancer, and most often individuals with breast cancer present late with the disease at treatment centers (World Health Organisation, 2018). In Ghana, 4,645 breast cancer cases were diagnosed in 2018, which is approximately 20.4% of all cancers diagnosed in the country (Globocan, 2018).

1.1.2 Anatomy of the human breast

The breast is made up of mammary glands surrounded by a connective tissue stroma. The breast tissue consists of ducts and secretory lobules. Each lobule has many alveoli drained by one lactiferous duct. These ducts converge at the nipple. The connective tissue stroma is made up of a fibrous and a fatty component. The fibrous stroma condenses to form suspensory ligaments of Cooper (Pearson, 2009).

1.1.3 Classification of breast cancer

There are two classifications of breast cancer: histopathological classification and molecular sub-types. The histopathological classification dwells on the origin of the cancer cells within the breast tissue; thus either originating from the epithelial lining of the ducts or from the cells within the lobules. The commonest histopathological subtype is the invasive ductal carcinoma (American Cancer Society, 2017). The molecular sub-types are specified based on statuses of the oestrogen and progesterone receptors, human epidermal growth factor receptor 2 (HER2) and Ki-67 within the luminal and myoepithelial cells. There are five molecular subtypes of breast cancer and these are Luminal A, Luminal B, Triple negative, HER 2 enriched and the normal-like breast cancers (Eliyatkın *et al.*, 2015).

1.1.4 Breast cancer risk factors

The etiology of breast cancer is not known but some predisposing factors are linked to breast cancer. These factors have been classified into modifiable and non-modifiable factors (Jung *et. al.*, 2016).

1.1.4.1 Modifiable risk factors

Excessive alcohol intake can cause a rise in the amount of oestrogen hormone in the bloodstream and this increases the risk of breast cancer (Jung *et al.*, 2016). Also, excess fatty food consumption, particularly, the saturated fat, has been linked to mortality and poor prognosis among breast cancer patients (Makarem *et al.*, 2013).

Obesity has been reported to pose a high risk of postmenopausal breast cancer (La Vecchia *et al.*, 2011). Cigarette smoke contains mutagens and exposure to these mutagens is linked to an increased risk of breast cancer (Catsburg *et al.*, 2015).

A young woman below age 30 at first full-term pregnancy or having more than one child has a decreased risk of breast cancer (Collaborative Group on Hormonal Factors in Breast Cancer, 2012). Radiation exposure or radiotherapy to the chest presents an increased risk of breast cancer (Travis *et. al.*, 2003).

1.1.4.2 Non-modifiable risk factors

Most breast cancers occur in women (American Cancer Society, 2017). Aging has been cited as a critical risk factor of breast cancer (Siegel *et al.*, 2016). Also, females with their first-degree relatives (mother or sister) affected with breast cancer have a higher chance of developing the disease (Collaborative Group on Hormonal Factors in Breast Cancer, 2012).

About 5% to 10% of all breast cancers are due to inherited mutations (Schwartz *et. al*. 2008). Breast cancer susceptibility genes, BRCA1 and BRCA2 are critical genes which are associated with an increased risk of breast cancer when mutated (Schwartz *et. al*. 2008). Also, dense breast tissue is reported to be associated with breast cancer growth (Tamimi *et al.*, 2007).

Reproductive events such as early onset of menstruation, late age at menopause and nulliparity increase oestrogen level within the bloodstream and exposes a woman to breast cancer (Soroush *et. al.*, 2016; Sun *et. al.*, 2017).

1.1.5 Breast cancer screening

Breast cancer often results in high mortality rate but it can be cured when detected early. Breast cancer is usually assessed or detected through the use of five screening modalities. These are as follows:

Mammography- It is a radiographic modality, specifically designed to assess a woman's breast for nodules which give an indication of breast cancer growth (Hela *et al.*, 2013).

Magnetic resonance imaging (MRI) is a radiographic screening modality which uses enormous magnetic field and computer-generated radio waves to produce detailed images of tissues and organs within the body (Greenwood *et. al.*, 2013).

Ultrasonography is a diagnostic modality which produces image of an individual's internal body structures using high-frequency sound waves. Ultrasound usually assesses and confirms reports from a mammogram or physical examination of the breast (Melnikow *et. al.*, 2017).

Clinical breast examination is the physical assessment of the breast of an adult female by a health professional or a clinician to ascertain the presence of lumps within the breast (Bryan & Synder, 2013).

Self-breast examination is a prescribed procedure for breast assessment to ascertain the presence of lumps within the breast (American Cancer Society, 2017).

1.1.6 Breast cancer treatment

The fundamental principles of breast cancer treatment are to lower the probability of local recurrence and metastatic spread. Surgery and radiotherapy are indicated for local control of cancer. When there is a possibility of metastasis, systemic therapy is prescribed. In locally advanced disease, a palliative systemic therapy is usually administered (Rocque *et al.,* 2018; Vande-Perre *et al.,* 2018).

1.1.7 Breast cancer susceptibility 1 gene (BRCA1 gene)

Breast cancer susceptibility (BRCA1) gene is a tumour suppressor gene located on chromosome 17q21 and it repairs the DNA, thereby preventing the growth of breast cancer (Karami & Mehdipour, 2013). A study has reported that about 72% of women who inherit a mutated BRCA1 gene will develop breast cancer by the age of 80 (Kuchenbaecker *et al.,* 2017).

Single nucleotide polymorphisms (SNPs) are genetic variations that occur at a specific locus within genomic sequence where a single nucleotide: adenine (A), thymine (T), cytosine (C) or guanine (G) is altered. Therefore, single nucleotide polymorphism of BRCA1 gene is a type of polymorphism resulting from a change of a single nucleotide within the genomic sequence of BRCA1 gene in a human population (Francies *et. al.,* 2015). The single nucleotide polymorphism of BRCA1 gene may present as either a homozygous wild, heterozygous carrier or a homozygous mutant. The mutant form of the single nucleotide polymorphism of BRCA1 gene is reported to be associated with the risk of breast cancer (Domchek & Greenberg, 2009).

1.1.8 Finger dermatoglyphics

Finger dermatoglyphics is the scientific study of the skin ridge patterns on the palmar surface of the distal end of the fingers (Eslami *et al.,* 2016). When formed, the skin ridge patterns on the palmar surface of the fingers remain stable and unique for each individual. There are three (3) types of fingerprints and they are loop, whorl and arch (Jalali & Hajian-Tilaki, 2002). Finger dermatoglyphics formation is genetically influenced and can be used as a screening tool to study the genetic patterns in an individual. A study by Chintamani *et. al.,* (2007) indicates that six or more whorls were significantly higher among females with breast cancer compared to females without breast cancer. Also, according to Natekar *et al.,* (2006), six or more loops were noticed to be significantly higher among breast cancer females compared to healthy females. Therefore, fingerprints can be used as a non-invasive anatomical marker for the assessment of risk of breast cancer.

1.2 Problem statement

Breast cancer is the commonest malignancy among women; it affected 2.1 million women worldwide in 2018 and accounts for the greatest number of cancer-related deaths among women. In 2018, it was estimated that 627,000 women died from breast cancer globally; representing 15% of all cancer deaths among women (World Health Organization, 2018). In Africa, there is an increasing incidence of the breast cancer, and most often individuals with the disease present it late at treatment centers (World Health Organization, 2018). In Ghana, 4,645 breast cancer cases were diagnosed in 2018, which was approximately 20.4% of all cancers diagnosed (Globocan, 2018). Breast cancer has a high cure rate when detected early.

In Ghana, individuals with the disease present late with the disease at treatment centers. Late diagnosis and difficulty in the prediction of prognosis of breast cancer has been cited as a factor for increased mortality rate in Ghana and this is due to insufficient preliminary screening tool for risk and prognosis. Therefore, there is the need for a preliminary tool which will adopt information of the BRCA1 gene and fingerprint patterns to predict breast cancer risk and prognosis.

1.3 Justification

Breast cancer has been reported to have the highest cancer mortality rate among women globally and in Ghana, individuals with breast cancer present late with the disease at treatment centers (World Health Organization, 2018). Therefore, it was necessary to undertake this study which provides information on a potential preliminary tool which could predict breast cancer risk and prognosis. It has been reported severally that the risk of breast cancer is associated with a mutant BRCA1 gene and also, six or more fingerprint whorls as well as six or more fingerprint loops have been reported to be associated with females with breast cancer compared to apparently healthy individuals (females). Therefore, this study sought to investigate the relationship between finger dermatoglyphic patterns and BRCA1 gene polymorphism, which can be used as a preliminary screening tool to predict breast cancer and its prognosis.

1.4 Aim

To determine the relationship between the single nucleotide polymorphism of BRCA1 gene and finger dermatoglyphic patterns in breast cancer patients.

1.5 Specific objectives

i. To determine the predominant location of single nucleotide polymorphism and the resulting nucleotide variants of BRCA1 gene (exon 11 and surrounding introns) among study participants.

ii. To determine the predominant finger dermatoglyphic patterns in individuals with breast cancer and individuals without breast cancer.

iii. To ascertain tumour characteristics in individuals with breast cancer in relation to fingerprint patterns.

iv. To assess the predominant variants of BRCA1 gene in relation to the predominant finger dermatoglyphic pattern among individuals with breast cancer and individuals without breast cancer.

1.6 Hypothesis

There is no relationship between finger dermatoglyphic patterns and BRCA1 gene polymorphism among individuals with breast cancer.

CHAPTER TWO

LITERATURE REVIEW

2.1 Breast cancer

Breast cancer is a malignant disease that is caused by abnormal proliferation of cells within the breast tissue (American Cancer Society, 2017). According to the World Health Organization 2018 report on breast cancer; breast cancer is the commonest malignancy among women, and it affected 2.1 million women worldwide in 2018. The greatest number of cancer-related deaths among women is caused by breast cancer. In 2018, it was estimated that 627,000 women died from breast cancer; approximating 15% of all cancer deaths among women (World Health Organisation, 2018). In Africa, most often individuals with breast cancer present late with the disease at treatment centers (World Health Organisation, 2018). In Ghana, 4,645 breast cancer cases were diagnosed in 2018, representing 20.4% of all cancers diagnosed (Globocan, 2018). About 70% of women diagnosed with breast cancer in Ghana present with advanced forms of the disease as a result of low awareness, resulting in high death rate (Ghartey *et al.* 2016).

2.2 Mechanism of breast cancer formation

Usually, cells undergo apoptosis when they are not required anymore. Before then, they are protected from cell suicide by certain proteins and several pathways (Cavalieri *et. al.,* 2006). Some of the protective pathways are the PI3K/AKT and RAS/MEK/ERK pathways. Occasionally, the genes involved in these protective pathways are mutated, rendering the cell incapable of undergoing apoptosis. Normally, PTEN protein deactivates the PI3K/AKT pathway when the cell is ready for apoptosis. In some breast cancers, the gene for the PTEN protein is mutated; therefore, the PI3K/AKT pathway remains turned on, and the cancer cell is unable to undergo apoptosis

(Cavalieri *et. al.*, 2006). Mutations that lead to breast cancer have been experimentally linked to oestrogen exposure. Abnormal growth factor signaling in the interaction between stromal cells and epithelial cells can facilitate malignant cell growth (Haslam & Woodward, 2003). In breast adipose tissue, over-expression of leptin leads to increased cell proliferation and cancer (Jardé *et al.*, 2011).

Some gene mutations associated with breast cancer growth include p53, BRCA1 and BRCA2 mutations. These mutations are either inherited or acquired after birth. There is a strong evidence of residual risk variation that goes beyond hereditary BRCA gene mutations between carrier families. This is caused by unobserved risk factors. This implicates environmental and other causes as triggers for breast cancers (Begg *et al.*, 2008). The inherited mutation of BRCA1 or BRCA2 genes cannot repair DNA cross links and DNA double strand breaks leading to breast cancer development (Theruvathu *et al.*, 2005). Transcription factor, GATA-3, is expressed in mammary glands specifically luminal cells and directly regulates the expression of oestrogen receptors within luminal cells. GATA-3 is involved in epithelial differentiation. Therefore, absence of GATA-3 results in loss of proper epithelial differentiation and may eventually lead to breast cancer (Levin *et al.*, 2012).

2.3 Anatomy of the human breast

The breast lies between the second and sixth ribs, from the sternal edge to the mid-axillary line. The breast tissue lies anterior to the pectoralis major muscle on the chest wall and extends into the axilla as the tail of Spence. The human breast is divided into four quadrants: upper inner, upper outer, lower inner and lower outer quadrants. Usually, breast cancer occurs in the upper outer quadrant (Clegg-Lamptey, & Hodasi, 2007). The breast is composed of 15-20 lobes and each lobe is subdivided into many lobules. The lobule is the basic structural unit of the breast and is lined by epithelial cells. Each lobule is subdivided into 10 to 100 alveoli, the milk producing units of the

breast. Milk flows from the alveoli of the lobules into the ducts. The ducts gradually coalesce into 10 to 15 major ducts; each lobe containing one major duct terminating in the nipple (Figure 1) (Pearson, 2009). The human breast has blood and lymphatic vessels. Most lymphatic vessels within the breast lead to axillary lymph nodes. Some also connect to supraclavicular, infraclavicular lymph nodes, and internal mammary lymph nodes. Cancer cells may enter lymphatic vessels and spread to lymph nodes. Cancer cells may also invade blood vessels and spread to other parts of the body (Pearson, 2009). Posterior to the tissues of the breast lie the muscles on the chest wall and between the two is a fascia (a layer of connective tissue). Two layers of suspensory ligaments (Cooper's ligaments) link the breast to the fascia, providing support. As these ligaments stretch with age or weight gain, the breast loses some of its firmness (Pearson, 2009).

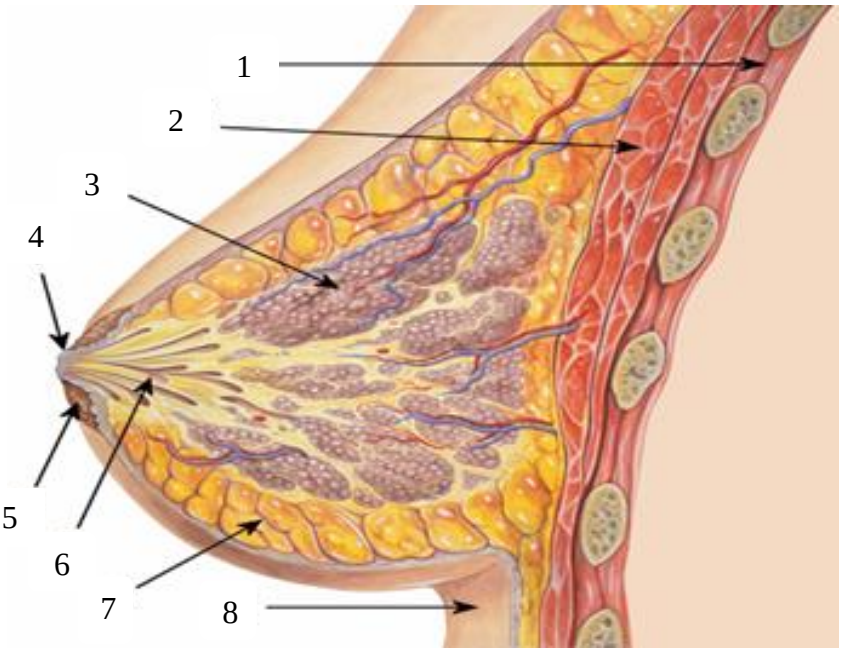


Figure 1. The Breast: cross-section scheme of the mammary gland. The labels 1, 2, 3, 4, 5, 6, 7 & 8 refers to the chest wall, pectoralis major muscle, lobules, nipple, areola, milk duct, fatty tissue and skin respectively (Pearson, 2009).

2.4 Classification of breast cancer

Breast cancer has two classifications: histopathological classification and molecular sub-types (American Cancer Society, 2017).

2.4.1 Histopathological classification of breast cancer

Histopathological classification of breast cancer is based on the origin of the cancer cells within the breast: either originating from the lining of the ducts or from the cells within the lobules of the breast. Histopathololologically, the commonest types of breast cancer are ductal carcinoma in situ, invasive ductal carcinoma, and invasive lobular carcinoma (American Cancer Society, 2017).

2.4.1.1 Ductal carcinoma in situ (DCIS)

It is a non-invasive form of breast cancer. The epithelial cells lining the lactiferous ducts transform into cancer cells without spread through the walls of the ducts into the nearby breast tissue. About 20% of breast cancers may be diagnosed as ductal carcinoma in situ and most individuals with this early staged breast cancer can be cured. Ductal carcinoma in situ can progress to invasive cancer if not treated (Allred, 2010).

2.4.1.2 Invasive ductal carcinoma

It begins to grow in the epithelial cells lining the lactiferous duct in the breast and infiltrate surrounding breast tissues through the walls of the ducts. The cancer cells are likely to metastasize to the brain, lungs, liver and the bony skeleton through either the lymphatic system or the bloodstream. About 80% of infiltrating breast cancers are invasive ductal carcinomas (American Cancer Society, 2017). Invasive ductal carcinoma is the commonest histopathological type of breast cancer in Ghana (Clegg-Lamptey & Hodasi, 2007).

2.4.1.3 Invasive lobular carcinoma

It is the abnormal proliferation of lobular cells. It begins to grow in the milk-producing glands (lobules) and metastasizes to the brain, lungs, liver and the bony skeleton. About 10% of infiltrating breast cancers are invasive lobular carcinoma (American Cancer Society, 2017).

2.4.2 Molecular subtypes of breast cancer

The molecular sub-types of breast cancer are specified based on the statuses of the oestrogen and progesterone receptors, human epidermal growth factor receptor 2 (HER2) and Ki-67 within the luminal and myoepithelial cells. There are five molecular subtypes of breast cancer and these are Luminal A and Luminal B breast cancers, Triple negative/basal-like breast cancer, Human Epidermal Growth Factor Receptor 2 (HER2) enriched breast cancer and the normal-like breast cancer (Eliyatkın *et al.,* 2015).

2.4.2.1 Luminal A breast cancer

It is hormone-receptor positive (oestrogen-receptor and/or progesterone-receptor positive), HER2 negative, and has low levels of the protein Ki-67. Luminal A cancers are low-grade, tend to grow slowly and have the best prognosis (Eliyatkın *et al.,* 2015). About 40% of breast cancers are luminal A, making it the most common breast cancer subtype (Perou & Børresen-Dale, 2011).

2.4.2.2 Luminal B breast cancer

It is hormone-receptor positive (oestrogen-receptor and/or progesterone-receptor positive), and either HER2 positive or HER2 negative with high levels of Ki-67. Luminal B breast cancers generally grow slightly faster with poorer prognosis than luminal A breast cancers *(*Eliyatkın *et al.,* 2015). About 10% to 20% of breast cancers are luminal B (Perou & Børresen-Dale, 2011).

2.4.2.3 Triple-negative/basal-like breast cancer

It is hormone-receptor negative (oestrogen-receptor and progesterone-receptor negative) and HER2 negative. This subtype of breast cancer is more common in women with BRCA1 gene mutations. Also, this type of breast cancer is common among younger and African-American women (Eliyatkın *et al.,* 2015). About 10% to 20% of breast cancers are basal-like (Voduc, *et al.,* 2010).

2.4.2.4 Human epidermal growth factor receptor 2 (HER2) enriched breast cancer

It is hormone-receptor negative (oestrogen-receptor and progesterone-receptor negative) and HER2 positive. HER2 enriched breast cancers tend to grow faster than luminal breast cancers and may have worse prognosis, but they are often successfully treated with targeted therapies aimed at the HER2 protein, such as Herceptin, Perjeta, Tykerb, Nerlynx, and Kadcyla (Eliyatkın *et al.,* 2015). About 10% of breast cancers are HER2 enriched (Blows *et al.,* 2010).

2.4.2.5 Normal-like breast cancer

It is similar to luminal A breast cancer. It is hormone-receptor positive (estrogen-receptor and/or progesterone-receptor positive), HER2 negative, and has low levels of the protein Ki-67 (which regulate how fast breast cancer cells grow). Its prognosis is slightly worse than the prognosis of luminal A breast cancers (Eliyatkın *et al.,* 2015).

2.5 Prognosis of breast cancer

The prognosis of breast cancer is dependent on the size of the tumour and the extent of spread of the breast cancer cells to other parts of the body. Therefore, a big-sized tumour with metastasis of the breast cancer cells to other parts of the body informs advanced breast cancer and thus, poor prognosis and vice versa (Edge & Compton, 2010).

2.6 Microscopic grading in breast carcinoma

One of the most significant prognostic indicators of cancers is the histologic grading. Nottingham modification of Bloom Richardson grading system has been widely used for histologic grading of breast carcinoma. It is determined based on the cyto-architectural pattern and the proliferative index of the tumour (Pradhan *et al.,* 2017). Glandular or tubular formation by the tumour cells is the first component of Nottingham modification of Bloom Richardson grading system, and it reflects the architectural pattern indicating the degree of differentiation of the tumour cells compared with that of the normal breast ducts and lobules (Table 1) (Pradhan *et al.,* 2017). Nuclear pleomorphism is the second component assessed which indicates the cytomorphology of the tumour cells (Table 1). The third component assessed is the number of mitotic figures which is a measure of proliferation of tumour cells (Table 1).

A total score is obtained by summing the scores on tubule formation, nuclear pleomorphism and mitotic count (Table 2). Histological evaluation of breast cancer provides very strong prediction for determining patient prognosis (Rakha *et al.,* 2010).

In addition, it is known that histological grading is associated with molecular changes such as oestrogen-progesterone receptor expression and HER2 amplification (Rakha *et al.,* 2010).

The tubule formation, nuclear pleomorphism and the mitotic rate which constitute the histologic grade of the breast cancer is assessed as follows:

Table 1. Scores associated with the three indicators (Tubule formation, nuclear pleomorphism and mitotic count) of histologic grading.

Tubule Formation	Nuclear Pleomorphism	Mitotic Count	Score
Tubule formation constitutes more than 75% of the breast tumour.	Differences in the nuclei shape and size of the breast cancer cells are mild.	Mild mitotic index at the periphery of the tumour at the most mitotic active areas.	1
Tubule formation constitutes 10-75% of the breast tumour.	Differences in the nuclei shape and size of the breast cancer cells are moderate.	Moderate mitotic index at the periphery of the tumour at the most mitotic active areas.	2
Tubule formation constitutes less than 10% of the breast tumour.	Differences in the nuclei shape and size of the breast cancer cells are significant.	Significant mitotic index at the periphery of the tumour at the most mitotic active areas.	3

(Eliyatkın *et al.*, 2015).

The histologic grade is determined based on the sum of the scores obtained from the tubule formation, nuclear pleomorphism and mitotic count as shown in Table 1.

Table 2. Histologic grade type scoring

Histologic Grade	Total Score
I	3-5
II	6-7
III	8-9

(Eliyatkın *et al.*, 2015).

Most Ghanaian women present with grade II and III (Mensah *et al.*, 2016). Higher tumour grade is significantly associated with low survival rate, suggesting an impact of aggressive biology at diagnosis on higher risk of cancer spread and recurrence. A wide population-based active registry is important to implement cancer control programs and improve survival in Africa (Thomas *et al.*, 2017).

2.7 Breast cancer staging

Breast cancer is commonly staged using the TNM classification system. The TNM classification of tumours is based on the following:

T refers to the tumour size and the extent of spread within the breast and to adjacent tissues. N denotes the extent of spread to the surrounding lymph nodes. M represents the presence or absence of distant metastases (Edge & Compton, 2010).

The stage describes the extent of breast cancer in the body. It is based on the size of the tumour, the involvement of regional lymph nodes and spread to other parts of the body (Hortobagyi *et al.*, 2018). The cancer's stage is one of the most important factors in determining prognosis and treatment options (Giuliano *et al.*, 2017). The most common system used to describe the stages of breast cancer is the American Joint Committee on Cancer (AJCC) TNM system.

2.7.1 TNM staging system

The letter T followed by a number from 0 to 4 describes the tumour's size and spread to the chest wall posterior to the breast. Higher T numbers indicate a larger tumour and/or wider spread to tissues near the breast (Giuliano *et al.,* 2017).

The letter N followed by a number from 0 to 3 indicates whether the cancer has spread to lymph nodes near the breast and, if so, how many lymph nodes are affected (Hortobagyi *et al.,* 2018).

The letter M followed by a 0 or 1 indicates whether the cancer has spread to distant organs. For example, the lungs or bones (Giuliano *et al*., 2017).

2.7.2 Breast cancer stage grouping

Once the T, N and M categories have been determined, this information is combined in a process called stage grouping. Cancers with similar stages tend to have a similar outlook and are often treated in a similar way. Stage is expressed in Roman numerals from stage I (the least advanced stage) to stage IV (the most advanced stage). Non-invasive cancer is listed as stage 0 (Hortobagyi *et al.,* 2018). Therefore, breast cancer stage grouping is as follows:

2.7.2.1 Non-invasive breast cancer

Sometimes, referred to as stage 0, describes breast cancer that is only in the ducts and lobules of the breast tissue and has not spread to the surrounding tissue of the breast. It is also called non-invasive cancer (T_{is}, N_0, M_0) (Hortobagyi *et al.,* 2018). Majority of women with breast cancer in Ghana present Stage III and IV of the disease (Clegg-Lamptey & Hodasi, 2007).

2.7.2.2 Stage I

Stage I has two (2) sub divisions. Thus, stage IA and IB.

Stage IA: The breast tumour is small, invasive, and has not spread to the lymph nodes (T_1, N_0, M_0) (Hortobagyi *et al.*, 2018).

Stage IB: The breast tumour has spread to the lymph nodes and the tumour in the lymph node is larger than 0.2 mm but less than 2 mm in size. There is either no evidence of a tumour in the breast or the tumour in the breast is 20 mm or smaller (either T_0 or T_1, N_1, M_0) (Giuliano *et al.*, 2017).

2.7.2.3 Stage II

Stage II has two (2) sub divisions. Thus, stage IIA and IIB.

Stage IIA refers to any one of the following conditions:

There is no evidence of a tumour in the breast, but the cancer has spread to about 1 to 3 axillary lymph nodes but has not spread to distant parts of the body. (T_0, N_1, M_0) (Hortobagyi *et al.*, 2018).

The breast tumour is 20 mm or smaller and has spread to the axillary lymph nodes (T_1, N_1, M_0) (Hortobagyi *et al.*, 2018).

The breast tumour is larger than 20 mm but less than 50 mm and has not spread to the axillary lymph nodes (T_2, N_0, M_0) (Hortobagyi *et al.*, 2018).

Stage IIB refers to either of the conditions below:

The breast tumour is larger than 20 mm but less than 50 mm and has spread to about 1 to 3 axillary lymph nodes (T_2, N_1, M_0) (Giuliano *et al.*, 2017).

The breast tumour is larger than 50 mm but has not spread to the axillary lymph nodes (T_3, N_0, M_0) (Giuliano *et al.*, 2017).

2.7.2.4 Stage III

Stage III has three (3) sub divisions. Thus, stage IIIA, IIIB and IIIC.

Stage IIIA refers to breast tumour of any size that has spread to about 4 to 9 axillary lymph nodes or to the internal mammary lymph nodes with no distant metastasis (T_0, T_1, T_2 or T_3, N_2, M_0). Stage IIIA may also refer to a breast tumour larger than 50 mm that has spread to about 1 to 3 axillary lymph nodes with no distant metastasis (T_3, N_1, M_0) (Hortobagyi *et al.*, 2018).

Stage IIIB refers to a breast tumour that has spread either to the chest wall or caused ulceration of the breast. It may or may not have spread to up to 9 axillary or internal mammary lymph nodes. However, the cancer cells has not spread to other parts of the body (T_4; N_0, N_1 or N_2; M_0) (Giuliano *et al.*, 2017).

Stage IIIC refers to a breast tumour of any size that has spread to 10 or more axillary lymph nodes, the internal mammary lymph nodes and infraclavicular lymph nodes. However, the cancer cells has not spread to other parts of the body (any T, N_3, M_0) (Giuliano *et al.*, 2017).

2.7.2.5 Stage IV

With stage IV breast cancer, the tumour can be of any size and has spread to surrounding and distant lymph nodes as well as other organs, such as the bony skeleton, lungs, brain or liver (any T, any N, M_1). Metastatic cancer cells found when the cancer is first diagnosed occurs about 6% in all diagnosed breast cancer cases. This may be called de novo metastatic breast cancer. Most commonly, metastatic breast cancer is found after a previous diagnosis of early breast cancer (Hortobagyi *et al.*, 2018).

2.8 Signs and symptoms of breast cancer

The first noticeable symptom of breast cancer is usually a lump that feels different from the rest of the breast tissue. More than 80% of breast cancer cases are discovered when the woman feels a lump. The earliest breast cancers can be detected by a mammogram (American Cancer Society, 2017).

Lumps found with lymph nodes located in the axilla can also indicate breast cancer. Other signs of breast cancer include thickening of the breast tissue, an increase in size of one of the breasts, change of position and shape of any of the nipples as well as abnormal fluid discharge from any of the nipples. Also, constant pain in any part of the breast or axilla and swelling beneath the axilla or around the clavicle are symptoms of onset of breast cancer (Watson, 2008).

Pain is an unreliable symptom in determining the presence or absence of breast cancer, but may be indicative of other breast health conditions. Inflammatory breast cancer is a particular type of breast cancer which can pose a substantial diagnostic challenge. Symptoms of inflammatory breast cancer may resemble a breast inflammation and may include itching, pain, swelling, nipple inversion, warmth and redness of the skin covering the breast, as well as an orange peel texture of the skin of the breast referred to as peau d'orange (Clegg-Lamptey & Hodasi, 2007).

Paget disease of the breast has been reported as a rare form of breast cancer. This condition manifests as skin changes such as redness, discoloration, or mild flaking of the nipple skin (Watson, 2008).

Advanced form of Paget's disease of the breast may present symptoms such as an itchy and burning sensation in the nipple as well as a discharge of fluid from the nipple. About half of women

diagnosed with Paget's disease of the breast also have a lump within the breast (American Cancer Society, 2017).

Sometimes, what initially appears as a fibroadenoma could be a phyllodes tumour. Phyllodes tumours are formed within the stroma of the breast. Phyllodes tumours are not staged but they are rather classified on the basis of their appearance under the microscope as benign, borderline, or malignant (Lacroix, 2006).

Occasionally, breast cancer presents as metastatic disease. Common sites of metastasis include the bony skeleton, liver, lung and brain. Body weight loss can sometimes indicate an occult breast cancer. Metastatic breast cancer may sometimes result in bone or joint pains (Lacroix, 2006).

Body weight loss can sometimes indicate an occult breast cancer. These are non-specific symptoms, because they could be indications of other disease conditions (Lacroix, 2006). Usually, Ghanaian women report late at health facilities due to misinterpretation of signs and symptoms, cultural influences and fear of losing their breast to surgery (Agbokey *et al.*, 2019).

2.9 Breast cancer risk factors

The etiology of breast cancer is not known. However, certain risk factors are reported to be associated with breast cancer. These risk factors have been classified into modifiable and non-modifiable factors (Knight *et al.,* 2018).

2.9.1 Modifiable risk factors

These are lifestyle related factors which can be controlled. The following are the modifiable risk factors of breast cancer:

Excessive alcohol intake can cause a rise in the amount of oestrogen hormone within the bloodstream and triggers the oestrogen receptor pathways (Jung *et al.,* 2016). Also, excess fatty food consumption, particularly, the saturated fat, is associated with mortality and poor prognosis among breast cancer patients (Makarem *et. al.,* 2013).

Obesity increases the risk of postmenopausal breast cancer. The risk of postmenopausal breast cancer is about 1.5 times higher in overweight women and about 2 times higher in obese women than in lean women (La Vecchia, *et al.,* 2011). Breast cancer risk associated with body weight gain is likely due to high oestrogen levels because fat tissue is the primary source of oestrogen in postmenopausal women. Obesity has also been reported to be a risk factor for type II diabetes, which has fairly been linked to an increased risk of breast cancer among postmenopausal women (Goodwin *et al.,* 2012). In contrast, some studies have found that obesity protects against developing breast cancer before menopause. A large meta-analysis found that among women between ages 40 and 49, the risk for developing breast cancer was about 14% lower in overweight women and about 26% lower in obese women compared to women with normal weight (Nelson *et al.,* 2012).

Cigarette smoke contains mutagens which have been reported to be associated with the risk of breast cancer (Catsburg *et al.,* 2015). The risk of breast cancer is also elevated in women who both smoke and drink alcohol (Knight *et al.*, 2018).

A young woman below age 30 at first full-term pregnancy or having more than one child has a decreased risk of breast cancer. However, there appears to be a temporal increase in breast cancer risk following a full-term pregnancy, especially among women who are older at first birth (Collaborative Group on Hormonal Factors in Breast Cancer, 2012). Pregnancy related risk factors seem to be more strongly related to positive hormone receptor than negative hormone receptor breast cancers (Yang *et al.*, 2011).

Most studies suggest that breastfeeding for a year or more slightly reduces a woman's overall risk of breast cancer (Faupel-Badger *et al.,* 2013). Also, it has been reported that the risk of breast cancer is reduced for long duration of breastfeeding (Collaborative Group on Hormonal Factors in Breast Cancer, 2012).

The use of oral contraceptives may increase the risk of breast cancer. Women who have stopped using oral contraceptives for 10 years or more have the same risk as women who never used the pill (Collaborative Group on Hormonal Factors in Breast Cancer, 2012).

The link between radiation exposure and breast cancer has been demonstrated in studies of atomic bomb survivors and women who have received high dose radiation therapy to the chest, particularly for those who were first exposed at younger ages (Travis *et al.,* 2003). Breast cancer is one of the commonest secondary cancers to occur among childhood cancer survivors. Secondary breast cancer is strongly associated with high-dose radiation therapy to the chest for women who were diagnosed of Hodgkin lymphoma in their childhood (Clemons *et al.*, 2000).

Breast cancer risk among women with such exposure begin to increase 8 years after radiation treatment and continue to be elevated for more than 25 years (Travis *et al.,* 2003).

2.9.2 Non-modifiable risk factors

Most breast cancers occur in women (American Cancer Society, 2017). According to Quayson *et al.,* 2014, approximately 3% of all breast cancers in Ghana occur among males. Also, aging is one of the critical risk factors of breast cancer; because the incidence of breast cancer is highly related to increasing age. Majority of all breast cancer-associated deaths in America were reported in women over the age of 40 (Siegel *et al.,* 2016). In Ghana, however, breast cancer mostly occur among premenopausal women and about 95% occur after age 29 (Quayson *et al.,* 2014).

First-degree relatives (mother or sister) of women with breast cancer have a higher risk of developing the disease. The risk is higher if more than one first-degree relative developed breast cancer. Compared to women without a family history, risk of breast cancer is 1.8 times higher for women with one first-degree female relative who has been diagnosed, nearly 3 times higher for women with two relatives, and nearly 4 times higher for women with three or more relatives (Collaborative Group on Hormonal Factors in Breast Cancer, 2012). The risk of breast cancer is further increased when the affected relative was diagnosed at a young age. Majority of women with affected first-degree relatives may not develop breast cancer. Also, most women who develop breast cancer may not have a family history of the disease. A family history of ovarian cancer has been linked to an increased risk of breast cancer in women (Collaborative Group on Hormonal Factors in Breast Cancer, 2012).

About 5% to 10% of all breast cancer cases are as a result of inherited mutations: breast cancer susceptibility genes BRCA1 and BRCA2 are pivotal genes associated with the risk of breast cancer when mutated (Schwartz *et al.,* 2008). The inherited mutated BRCA1 and BRCA2 genes by off-

springs are highly associated with the risk of breast cancer (Brewer *et al.*, 2017). The estimates of the risk of breast cancer in women with these mutations vary; by age 70, between 44% and 78% of women with BRCA1 mutations and between 31% and 56% of women with BRCA2 mutations will develop breast cancer (Chen & Parmigiani, 2007). Only about 15% to 20% of familial breast cancers are attributed to BRCA1 or BRCA2 gene mutations (Turnbull & Rahman, 2008). Other inherited conditions associated with smaller increased breast cancer risk include Li-Fraumeni and Cowden syndromes as well as a number of more common genetic mutations (Turnbull & Rahman, 2008). These mutations can be inherited from either parent by sons as well as daughters. In addition, low-risk variations in the genetic code may affect breast cancer risk. Scientists believe that much of the occurrence of breast cancer in families results from the interaction between lifestyle factors and these low-risk variations that may be shared within a family (Turnbull & Rahman, 2008).

Some benign breast conditions have been reported to be associated with the risk of breast cancer. Clinicians often classify these conditions into three (3) major groups, these are non-proliferative lesions, proliferative lesions without atypia and proliferative lesions with atypia (Hartmann *et al.*, 2005). Non-proliferative lesions are not associated with overgrowth of breast tissue and have little to no effect on breast cancer risk. Examples of non-proliferative lesions include fibrosis (also known as fibrocystic changes), simple cysts, and mild hyperplasia (Hartmann *et al.*, 2005). Proliferative lesions without atypia are associated with a small increase in the risk of breast cancer (1.5 to 2 times the risk of those who do not have one of these lesions) and include typical ductal hyperplasia and fibroadenoma (Hartmann *et al.*, 2005). Proliferative lesions with atypia are associated with the greatest breast cancer risk, thus, about 4 to 5 times higher than average risk.

These include Atypical Ductal Hyperplasia and Atypical Lobular Hyperplasia (Hartmann *et al.*, 2005).

Reproductive events such as early menarche, late menopause, late age at first pregnancy and low parity which increases a woman's lifetime oestrogen exposure are reported to increase the risk of breast cancer (Sun *et al.*, 2017). High levels of endogenous and exogenous oestrogen are associated with the risk of breast cancer. The endogenous oestrogen is usually produced by the ovaries in premenopausal women. Also, the main sources of exogenous oestrogen are the oral contraceptives and the hormone replacement therapy (Soroush *et al.*, 2016).

Dense breast tissue has been reported to be a risk factor for breast cancer growth (Tamimi *et al.*, 2007). Breast density is affected with age, menopausal status and pregnancy. Breast density decreases with age and is further reduced by pregnancy and menopause (Boyd *et al.*, 2002). Breast density is usually lower among women with higher body weight because of the higher amount of fatty tissue (Harris *et al.*, 2011). Drugs such as tamoxifen and combined menopausal hormone therapy decreases and increases breast density respectively (Boyd *et al.*, 2011). Females with highly dense breast have increased risk of breast cancer compared to females with the less dense breasts (Boyd *et al.*, 2011). Studies have shown that unilateral breast cancer is more frequent in the left breast than in the right breast (Tulinius *et al.*, 1990; Amer, 2014).

2.10 Breast cancer screening

Most often, breast cancer metastasis causes over 90% of cancer deaths (Valastyan & Weinberg, 2012). However, if breast cancer is diagnosed early, the breast tumour can be removed by surgery and chemotherapy can work effectively. When breast cancer is detected early it can be cured (Deppen *et al.*, 2012).

The major screening methods used in the detection of breast cancer are as follows:

2.10.1 Mammography

Mammography is a radiographic modality that detects changes that may indicate the existence of breast cancer. Mammography does not provide a definitive diagnosis on the presence or absence of breast cancer but allow clinicians to see if there is a suspicion of cancer in the breast. Mammography is mostly used for women over the age of 40 years (Hela *et al.,* 2013).

The computer-aided mammographic diagnosis improves accuracy in the interpretation of mammograms and it also helps in early detection of possible tumours. For example, the presence of clusters of micro-calcifications on a mammogram shows an early sign of breast cancer. However, their detection is difficult because of their small size and their similarity to a normal breast tissue. A well-known limitation of mammography is the difficulty of its usage in the assessment of a lobular carcinoma when the breast tissue is highly dense. (Hela *et al.*, 2013).

2.10.2 Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) of the breast is a radiographic screening modality that uses powerful magnetic field, radio waves and a computer to produce detailed images of the breast tissue. It is primarily used as a supplementary tool for breast screening with mammography or ultrasound. It may be used to screen women at high risk for breast cancer, and to evaluate the extent of breast cancer seen through mammography. It is highly sensitive than mammography in high-risk women, especially in detecting invasive ductal carcinoma (Greenwood *et al.*, 2013).

2.10.3 Ultrasonography

Ultrasound is a diagnostic modality which uses high-frequency sound waves to produce an image of an individual's internal body structures. Ultrasound is used to evaluate abnormal findings from a mammogram or physical examination of the breast. Several research works have reported that ultrasound effectively detects breast cancer than mammography alone when screening women with mammographically dense breast tissue. Ultrasound is the prescribed diagnostic modality for young women. It is also useful in older women to complement mammography (Melnikow *et al.*, 2017).

2.10.4 Clinical Breast Examination

A clinical breast examination is the physical examination of the breast of an adult female by a health professional or a clinician to ascertain the presence of lumps within the breast. The American Cancer Society no longer recommends clinical breast examination for average-risk asymptomatic women based on lack of clear benefits of clinical breast examination alone or in addition to mammography. Compared to mammography alone, clinical breast examination with mammography has been reported to detect only a small additional proportion of breast tumours and increases the probability of false positives (Bryan & Synder, 2013).

2.10.5 Self-Breast Examination

Self-breast examination is a prescribed procedure for breast assessment to ascertain the presence of lumps within the breast. Although, the American Cancer Society no longer recommends that all women perform monthly breast self-exams, all women are advised to be familiar with both the appearance and feel of their breasts and report any changes promptly to their physician. Experts have concluded that self-awareness seems to be effective for early detection of breast cancer (Smith *et al.*, 2003).

2.11 Breast cancer treatment

The basic principles of breast cancer treatment are to reduce the chance of local recurrence and the risk of metastatic spread. Surgery with or without radiotherapy is indicated for local control of cancer. When there is a risk of metastatic relapse, systemic therapy such as hormonal therapy, chemotherapy, targeted therapy, or combination of any of these are prescribed. In advanced disease, systemic therapy is used as a palliative therapy with a small or no role for surgery (Rocque, *et al.,* 2018).

Treatment modalities are usually combined and classified into two broad groups (adjuvant and neo-adjuvant therapies) based on how they work and when they are used (Rocque, *et. al.,* 2018). Adjuvant therapy refers to a treatment modality which is given after the primary treatment. An example of adjuvant therapy is the additional treatment such as radiotherapy or chemotherapy usually given after surgery is done, where all detectable disease has been removed, but there remains a statistical risk of relapse due to occult disease (Rocque *et al.,* 2018).

Neoadjuvant therapy, in contrast to adjuvant therapy, is a therapy given before the main treatment is administered. For example, systemic therapy that is given before surgical removal of the breast is considered neoadjuvant chemotherapy. The most common reason for neoadjuvant therapy is to reduce the size of the tumour so as to facilitate more effective surgery (Rocque *et al.,* 2018).

There are three (3) main treatment modalities for breast cancer and these are surgery, radiation therapy and systemic therapy:

2.11.1 Surgery

This is the most common treatment for breast cancer. The subtypes of surgery for breast cancer include breast conservation surgery and mastectomy. With breast conservation surgery or lumpectomy (partial mastectomy) an operation is done to remove the gross tumour but not the entire breast tissue. Lumpectomy is usually performed together with axillary lymph node removal to remove any involved lymph nodes and reduce the probability of recurrence. Mastectomy, on the other hand is the surgical removal of the entire affected breast and it is also done with axillary lymph node removal (Vande-Perre *et al.*, 2018).

2.11.3 Radiation therapy

This involves the use of highly energetic photons (X-rays, gamma rays and electrons) to destroy cancer cells. It always follows breast conservation surgery but may be indicated after mastectomy. Morbidities of radiation are considered either acute or late effects. Acute effects occur immediately after treatment, and may include fatigue and radiation dermatitis. Late effects manifest as change in breast aesthetics and usually occur after 90 days of treatment (Vande-Perre *et al.*, 2018).

Breast cancer surgery is mostly followed by radiation therapy because it has been shown to reduce the risk of cancer recurrence and the risk of breast cancer death (Fisher *et al.*, 2002). Radiation therapy in addition to mastectomy is required if the size of the tumour is significantly larger or when cancer cells are found to have invaded surrounding lymph nodes. Radiation is also used to treat complications of advanced breast cancer, particularly, after the cancer cells have metastasized to the central nervous system or parts of the bony skeleton (Fisher *et al.*, 2002).

Radiation therapy is administered based on the type, stage, and location of the tumour, as well as the oncologist and patient's preference. Radiation therapy may be administered internally or externally. External beam radiation therapy is a radiotherapy modality for destroying cancer cells. External beam radiation therapy directs ionizing radiation from the gantry of the treatment machine (linear accelerator or a cobalt-60 teletherapy unit) which is at a specific calculated distance from the affected breast (area of treatment) after the patient has been made to lie supine or prone on the treatment machine. The area of treatment is usually the whole breast, however, depending on the extent of the cancer spread, it may be extended to include both the chest wall and axilla (Whelan *et al.*, 2010).

External beam radiation therapy is delivered daily over a period of five to six weeks. However, in recent studies, reducing the treatment period to three weeks to deliver the same amount of radiation dose which is also known as the hypofractionated radiotherapy appears to be just as effective (Whelan *et al.*, 2010).

Internal radiation therapy, also known as brachytherapy, is a form of Accelerated Partial Breast Irradiation (APBI) which uses a radioactive substance sealed in needles, seeds, wires, or catheters that are placed directly into or near the area affected with cancer. The ability to deliver radiation therapy accurately has increased dramatically in recent years and this has greatly diminished associated side effects and also reduce treatment time (Beitsch *et al.*, 2011).

2.11.3 Systemic therapy

Systemic therapy is a form of cancer treatment that runs through the bloodstream and affects all parts of the body, not only the site of the cancer. Systemic therapy is administered intravenously or orally. Systemic therapy includes chemotherapy, hormone therapy, and targeted therapy, all of which work through different means. For example, chemotherapy drugs work by attacking rapidly dividing cells. Hormone therapy either blocks the body's natural hormones or reduces the levels of those hormones that enhance cancer cell growth. Targeted therapies attack specific parts of cancer cells (Rocque *et al.,* 2018).

Systemic therapy given to patients before surgery is known neoadjuvant therapy. Neoadjuvant systemic treatment is usually administered to reduce the tumour size to enable surgical removal of tumour (Mauri *et al.,* 2005). Systemic therapy administered to patients after surgery is known as an adjuvant therapy. Adjuvant systemic therapy is used to destroy cancer cells that were left behind after surgery and had probably invaded other tissues within the body. The decision to administer adjuvant systemic therapy is basically determined by the stage of disease and the molecular subtype of breast cancer. Systemic therapy is the main treatment modality for women with metastatic breast cancer (Rocque *et al.,* 2018).

2.11.3.3 Chemotherapy

This involves the use of cytotoxic agents such as anthracycline and alkylating agents as well as antimetabolites to destroy cancer cells. However, these drugs may have mild or extreme side effects such as alopecia, nausea and vomiting. The extreme toxicity is neutropenia which leads to severe infection (Vande-Perre *et al.,* 2018).

2.11.3.4 Hormonal therapy

The ovaries produce oestrogen that enhances the growth of breast cancer. Women diagnosed with breast cancer with immunohistochemistry report of positive oestrogen or progesterone receptors are usually given hormone therapy to reduce oestrogen levels or to block the effects of oestrogen on the growth of breast cancer cells. Tamoxifen and toremifene are drugs that prevent oestrogen from binding to breast cancer cells and are effective in both postmenopausal and premenopausal women (Davies *et al.,* 2013). Treatment of oestrogen receptor positive breast cancer with tamoxifen for five years has been shown to reduce the rate of recurrence by 39% throughout the first decade and reduces breast cancer mortality by about 33% throughout the first 15 years (Davies *et al.,* 2013).

Results of the Adjuvant Tamoxifen Longer Against Shorter (ATLAS) study showed that extended use of tamoxifen (10 years versus 5 years) may further reduce the risk of breast cancer recurrence and mortality (Davies *et al.*, 2013).

Fulvestrant (Faslodex) is a novel drug that blocks oestrogen binding and lowers the number of oestrogen receptors on breast cancer cells. It is usually beneficial for postmenopausal women even when the breast cancer cells have become resistant to tamoxifen. Premenopausal women with hormone-sensitive cancer cells may also benefit from ovarian ablation (Theriault *et al.,* 2013). Oophorectomy can be done to remove the ovaries permanently. Usually, potentially reversible ovarian ablation is achieved with drugs known as Luteinizing Hormone-Releasing Hormone (LHRH) analogs: examples are goserelin (Zoladex) and leuprolide (Lupron). Studies have reported that the addition of these drugs to tamoxifen and/or chemotherapy lowers the susceptibility to breast cancer recurrence and death among premenopausal women with hormone-sensitive breast cancer (LHRH-agonists in Early Breast Cancer Overview group *et al.,* 2007).

Aromatase Inhibitors such as letrozole, anastrozole, and exemestane, are class of drugs that are used to treat hormone receptor positive breast cancer in postmenopausal women. These drugs interfere with the body's ability to produce oestrogen (Dowsett *et al.*, 2010). Aromatase Inhibitors usage are often not effective in women with functioning ovaries. Clinical trials have demonstrated a clear advantage of using either an Aromatase Inhibitors instead of tamoxifen for a total of five years or switching to an Aromatase Inhibitor after at least two to three years of tamoxifen, compared to tamoxifen alone for five years (Dowsett *et al.*, 2010).

Clinical trials continue to assess the optimal timing and duration of treatment. Although Aromatase Inhibitors have fewer serious side effects than tamoxifen, they can cause osteoporosis, joint pain, and other musculoskeletal symptoms because they completely deplete oestrogen levels in postmenopausal women (Dowsett *et al.*, 2010).

2.11.3.3 Targeted therapy

Targeted therapy is a form of systemic therapy aimed at Human Epidermal Growth Factor Receptor 2 (HER2). About 15% to 20% of breast cancers produce in excess, the growth promoting protein, HER2 (Carey *et al.*, 2006). Breast cancer cells producing excess HER2 are more likely to recur compared to those that do not overproduce HER2. Trastuzumab (Herceptin) directly targets the HER2 protein and this has been reported as the best approach to minimize the impact of HER2 (Romond *et al.*, 2005). The combined results of two large trials indicate that adding trastuzumab to standard chemotherapy for early staged HER2-positive breast cancer reduces the risk of recurrence and death by 52% and 33%, respectively, compared to chemotherapy only (Romond *et al.*, 2005).

In 2006, the United States Food and Drug Administration (FDA) sanctioned trastuzumab for all HER2 positive breast cancers (Wolff *et al.*, 2007). Also, the FDA recommended that all invasive breast cancers should be tested for the HER2 gene amplification or protein overexpression in order to identify women who would benefit from this therapy (Wolff *et al.*, 2007).

Like trastuzumab, pertuzumab is a monoclonal antibody that binds to the HER2 protein however, it binds to a different site. This drug is used to destroy HER2 positive, metastatic breast cancer cells. When pertuzumab is administered together with docetaxel (Taxotere) and trastuzumab to chemotherapy-naïve patients, it has been shown to cause tumour shrinkage compared to administering docetaxel and trastuzumab alone (Baselga *et al.*, 2012). Also, ado-trastuzumab emtansine (Kadcyla, formerly called TDM-1), was recently sanctioned by the FDA to treat HER2 positive metastatic breast cancer, and this has been shown to shrink tumours (Verma, *et. al.*, 2012). Lapatinib (Tykerb) is another drug that has been found to be effective in delaying disease progression in women with HER2 positive advanced breast cancers that have become resistant to trastuzumab (Cameron *et al.*, 2010).

Everolimus (Afinitor) is a type of targeted therapy that blocks mTOR, a protein that promotes cell growth and division. By blocking this protein, everolimus can help stop cancer cells from growing. Everolimus may also stop tumours from developing new blood vessels which also limit cancer growth (Bachelot *et al.*, 2012). Everolimus seems to improve the effectiveness of hormone therapy drugs in treating breast cancer. It was approved to treat advanced, hormone receptor positive, HER2 negative breast cancer in postmenopausal women. It is meant to be used with exemestane in women whose cancers have become worse while being treated with either letrozole or anastrozole (Bachelot *et al.*, 2012).

2.12 Breast cancer susceptibility gene (BRCA gene)

Breast cancer susceptibility genes are tumour suppressor genes which prevent the growth of breast and ovarian cancers. There are two breast cancer susceptibility genes and these are BRCA1 and BRCA2 (Kuchenbaecker *et al.*, 2017).

2.12.1 Breast cancer susceptibility 1 gene (BRCA1 gene)

Breast cancer susceptibility 1 (BRCA1) gene is a tumour suppressor gene located on chromosome 17q21. It codes for a protein containing 1863 amino acids which is responsible for the repair of the DNA, thereby preventing the growth of breast cancer (Francies *et al.*, 2015). A study has estimated that about 72% of women who inherit a mutated BRCA1 gene will develop breast cancer by the age of 80 (Kuchenbaecker *et al.*, 2017). BRCA1 gene has 24 exons and exon 11 is the site for frequent mutations in breast cancer individuals (Karami & Mehdipour, 2013).

2.12.2 Breast cancer susceptibility 2 gene (BRCA2 gene)

BRCA2 gene is located on chromosome 13q12.3. It codes for a protein with 3418 amino acids which is responsible for the repair of the DNA, thereby preventing the growth of breast cancer. BRCA2 gene has 27 exons with the largest exon being exon 11 (Karami & Mehdipour, 2013).

2.12.3 The structure and function of BRCA1 gene

There are over 1700 unique BRCA1 gene mutations within the breast cancer information core database. Of these mutations, 858 have been confirmed as being clinically significant. Clinically significant mutations cause an increased risk of breast cancer and result in a protein with reduced function or no protein product (Henderson, 2012).

Three domains of the BRCA1 protein are mutated in cancer patients with relatively high frequency. These domains include the Really Interesting New Gene (RING) domain (exons 2-7), a region encoded by exons 11-13, and the BRCA1 C-Terminal domain (exons 16-24) (Figure 2) (Clark *et al.*, 2012).

The RING domain functions as an E3 ubiquitin ligase. The amino acids encoded by exons 11-13 contain protein binding domains for a number of diverse proteins. The BRCA1 C-Terminal domain is a phosphoprotein binding domain with specificity for proteins phosphorylated by serine/threonine kinases such as ataxia-telangiectasia mutated (ATM) or ataxia telangiectasia and Rad3-related protein (ATR) kinases (Clark *et al.*, 2012).

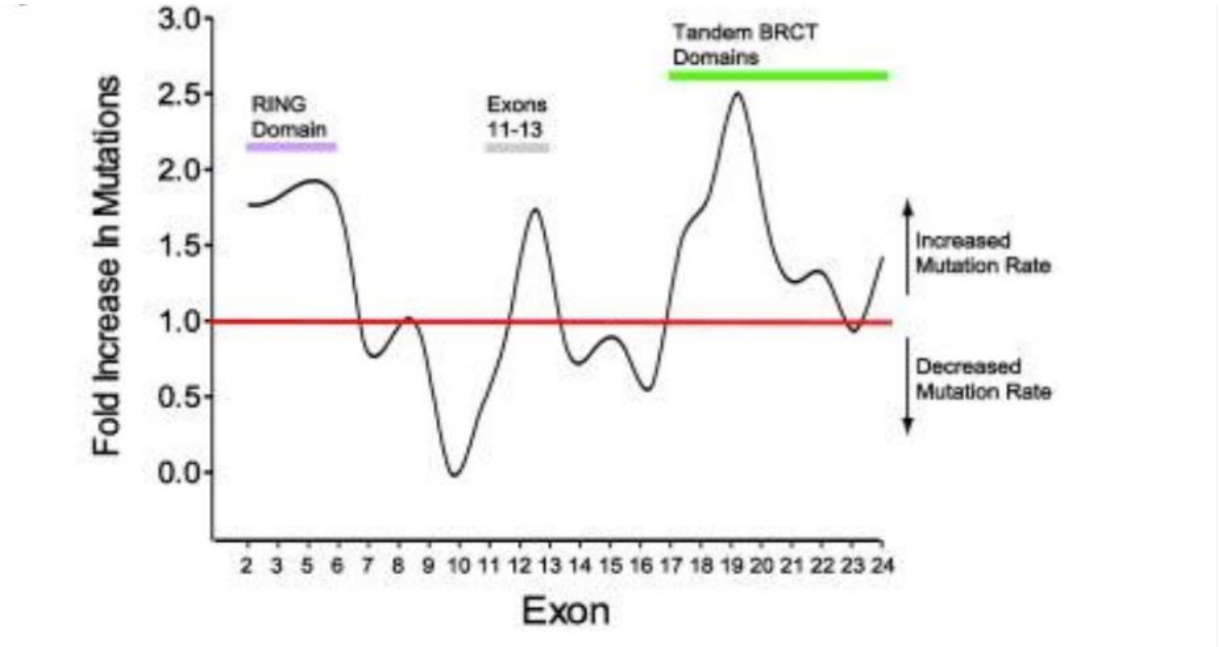


Figure 2. A graph showing the three domains of BRCA1 with the highest rate of mutation generated from the Breast Cancer Information Core. Fold increase in mutations were calculated as mutations per codon length of each exon/total mutations per total BRCA1 codons. 1.0 on the vertical axis indicates the total average mutations per codon for BRCA1 (Clark *et al.*, 2012).

2.12.3.1 Really Interesting New Gene (RING) Domain

The Really Interesting New Gene (RING) domain of BRCA1 is made up of a RING finger and two flanking alpha helices encompassing amino acids 1-109 of exons 2-7. Through seven conserved cysteine residues and one conserved histidine residue, the RING finger coordinates two Zn^{2+} atoms which stabilize the RING structure. The RING finger forms a globular structure with a core three strand β-sheet and a central helix, while the flanking helices align perpendicular to the RING finger (Figure 3) (Lipkowitz & Weissman, 2013).

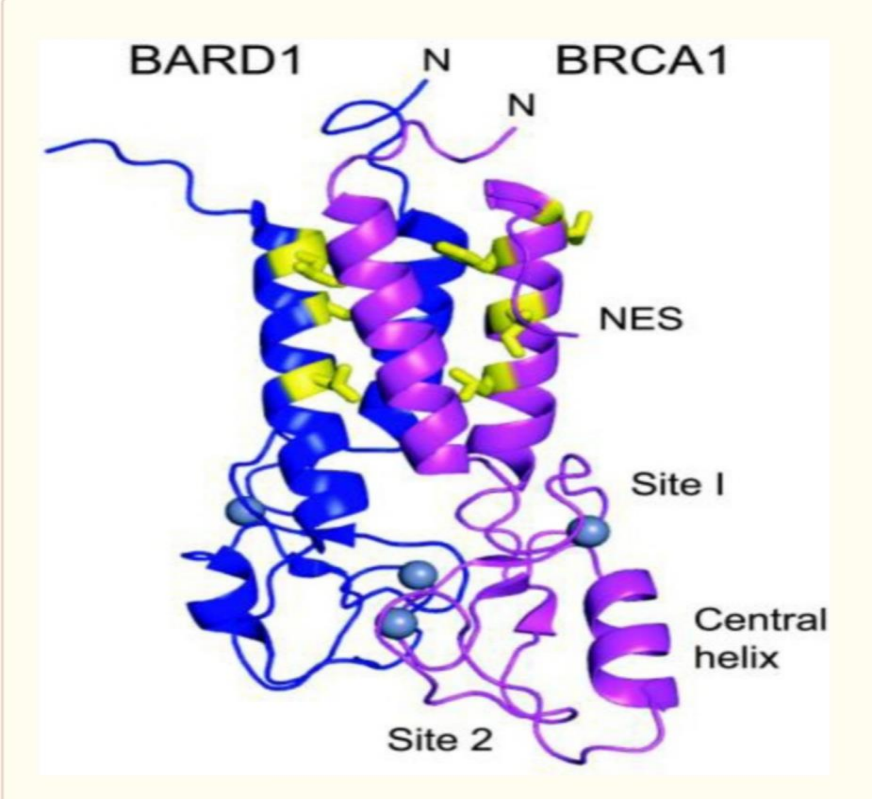


Figure 3. BRCA1 RING domain. The RING domain consists of a RING finger and two flanking alpha helices. The RING finger consists of a core of β- strands, a central helix, and two Zn^{2+} binding sites. BRCA1 (pink) forms a heterodimer with the RING domain of BRCA1-associated RING domain protein 1 (BARD1) in blue. Nuclear Export Signal (NES) residues are highlighted in yellow. N-termini of each strand are labeled (Porollo & Meller, 2007).

The RING finger, which is a highly conserved domain found in a large number of proteins, is responsible for the E3-ubiquitin ligase activity of BRCA1. The N and C-terminal helices are responsible for the interaction of BRCA1 with BARD1 (BRCA1 Associated RING Domain protein 1), a major BRCA1 binding partner that also contains a RING domain (Karami & Mehdipour, 2013).

The ubiquitin ligase activity of BRCA1 is dramatically increased by the formation of the BRCA1 or BARD1 heterodimer (Hashizume *et al.,* 2001). As with all E3-ubiquitin ligases, ubiquitination of a substrate can only occur through interaction with an E2 ubiquitin-conjugating enzyme. Ubiquitin non-covalent complex (UbcH5), as well as other E2 enzymes, binds to the surface of BRCA1 opposite the binding interface with BARD 1 (Brzovic *et al.*, 2003).

The greatest number of cancer predisposing mutations that affect the interaction of BRCA1/ BARD1 or BRCA1/UbcH5 as well as the RING E3 ligase function suggest that the ubiquitin ligase activity of BRCA1 is essential for its tumour suppressor function (Karami & Mehdipour, 2013).

BRCA1 Associated RING Domain protein 1 (BARD1) contains a RING domain with sequence and structural homology to BRCA1, including two flanking alpha helices. The N-terminal alpha-helix of BRCA1 aligns in an antiparallel fashion with the C-terminal alpha helix of BARD1. On the contrary, the C-terminal alpha-helix of BRCA1 is antiparallel to the N-terminal alpha-helix of BARD1 (Figure 3) (Karami & Mehdipour, 2013).

According to Clark *et al.,* (2012), the four helix bundle creates a large buried hydrophobic region and stabilizes the heterodimer, while interactions between the BRCA1 RING finger and the flanking alpha-helices maintain the orientation of the RING finger with respect to the flanking alpha-helices. The interaction between BRCA1 and BARD1 both increases the ubiquitin ligase activity of BRCA1 and causes the nuclear export sequence (NES), located on the C-terminal helix of the RING domain of both BRCA1 and BARD1.

In furtherance to the above, Brzovic *et al.*, (2003) argued that, the buried NES in the four helix bundle results in nuclear retention of the two proteins. The four helix bundle contains the majority of the interactions between BRCA1 and BARD1, however a few inter-RING interactions may

occur as well. Two Zn^{2+} atoms stabilize the structure within the RING finger and are coordinated by Zn^{2+} binding loops named Site I and Site II. Site I is made up of four cysteine residues, while Site II contains three cysteine residues and one histidine residue. The Zn^{2+} binding residues are highly conserved and characteristic of RING fingers found in many other proteins. Additionally, the spacing between the Zn^{2+} binding residues is conserved among many RING fingers. Conversely, a central helix is present in some RING fingers, but not all.

Ubiquitination of substrates occurs in a three-step process. First, an E1 ubiquitin-activating enzyme activates an ubiquitin molecule, which is transferred to an E2 ubiquitin-conjugating enzyme. The E3 brings together the E2 and substrate to complete the ubiquitination process. The human genome encodes about 40 E2 enzymes, which rely on about 1000 E3 ubiquitin ligases for their specificity (Ye & Rape, 2011).

RING E3 ubiquitin ligases, including BRCA1, act solely as scaffolds by binding to the E2 through the RING finger domain, while the substrate binds to another domain on the E3. This brings the substrate close enough to the E2 to allow for transfer of ubiquitin from the E2 to the substrate. The presence of the E2 ubiquitin conjugating enzyme, UbcH5, dramatically increases BRCA1 or BARD1 ubiquitination activity in vitro (Hashizume *et al.,* 2001). Nuclear Magnetic Resonance structures of BRCA1, BARD1 and UbcH5c show that loops of UbcH5c bind to a groove formed by the two Zn^{2+} binding sites and the central helix of the RING finger of BRCA1, and that UbcH5c has no interaction with BARD1 (Brzovic *et al.,* 2003). Targets of BRCA1 E3 ligase activity in vivo include oestrogen receptor-alpha, progesterone receptor, DNA Damage Response Protein (CtIP), and histone protein H2A with resulting alterations in gene activation, DNA repair, and DNA condensation (Calvo & Beato, 2011; Zhu *et al.,* 2012; You & Bailis, 2017).

2.12.3.1.1 Cancer related mutation of the RING Domain

Mutation of the cysteine residues that coordinate the Zn^{2+} atoms have been reported as clinically important, because its alteration correlates with an increased risk of cancer. Mutation of residues in Site I results in an altered folding of the RING domain. A thorough study of Site II residue mutations found altered structure by mass spectrometry and reduced Zn^{2+} binding at Site II (Brzovic *et al.*, 2003).

2.12.3.2 Exon 11-13

Exons 11-13 cover over 65% of the sequence of BRCA1 and encode two nuclear localization sequences (NLS) and binding sites for several proteins including retinoblastoma protein (RB), regulator genes and proto-oncogenes (cMyc), DNA repair proteins (Rad 50 and Rad 51) (Karami & Mehdipour, 2013). The amino acids encoded by these exons also contain portions of a coiled-coil domain which mediates interactions with Partner and localizer of BRCA2 (PALB 2), as well as a portion of a serine containing domain (SCD) that is phosphorylated by ATM (Figure 4). "No atomic-level structures have been determined for exons 11-13 of BRCA1. Despite the fact that exons 11-13 contain a large percentage of the clinically relevant mutations, very little is known about the structure or function of this region when compared to the RING or BRCT domains" (Karami & Mehdipour, 2013).

BRCA1 exons 11-13 binding partners are involved in a wide range of cellular pathways:

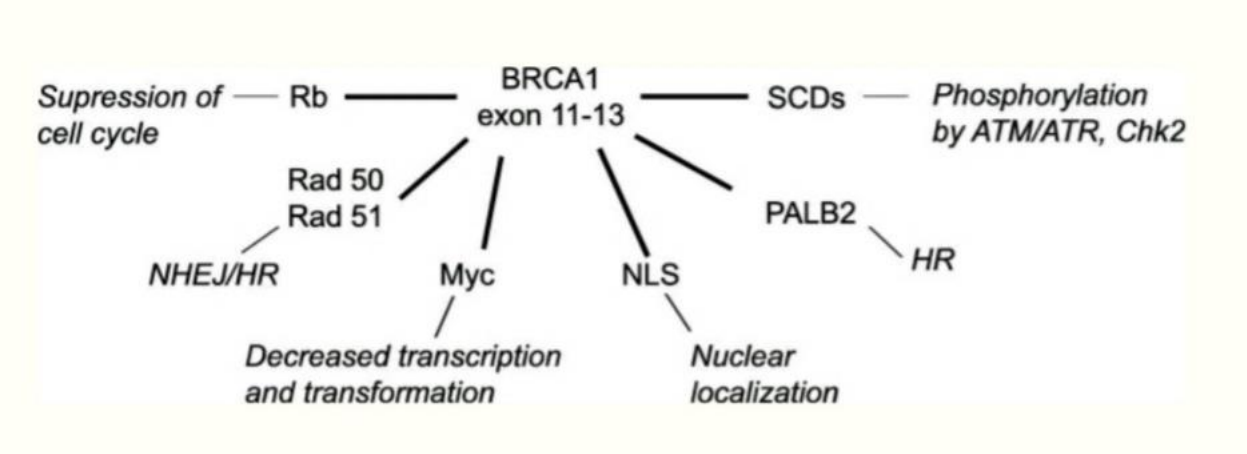


Figure 4. An illustration of BRCA1 exons 11-13 with multiple functions. The amino acids encoded by BRCA1 exons 11-13 have binding domains for several proteins including retinoblastoma (RB), Rad 50, Rad 51, c-Myc and PALB 2 (a scaffold for BRCA 2). BRCA1 exons 11-13 also contain a nuclear localization signal (NLS) and a serine cluster domain (SCD)(Clark *et al.*, 2012).

2.12.3.3 Serine Cluster Domain

BRCA1 contains a domain called the serine cluster domain. A section of the serine cluster domain of BRCA1 is located in exons 11-13 (thus, amino acids 1280-1524). The region has a concentrated amount of putative phosphorylation sites, and is phosphorylated by ATM and ATR kinases both in vitro and in vivo respectively. ATM and ATR kinases are activated by DNA damage (Karami & Mehdipour, 2013). Phosphorylation of BRCA1 causes recruitment of BRCA1 to sites of double strand breaks. Serine cluster domain of BRCA1 are common in targets of ATM/ATR Kinases including multiple DNA damage response proteins. Serines 1189, 1457, 1524, and 1542 can all be phosphorylated in vivo, while additional serines can be phosphorylated in vitro. Mutation of these

serine residues are seen clinically, and may affect localization of BRCA1 to sites of DNA damage and DNA damage response function (Karami & Mehdipour, 2013).

2.12.3.4 BRCA1 C-Terminal (BRCT) Domain

The BRCA1 C-terminal (BRCT) domain is a conserved domain mostly involved in DNA damage repair. BRCA1 C- terminal domains can occur as a single BRCT domain, as a tandem repeat (as found in BRCA1), multiple repeats, or fusions between two domains (Leung, & Glover, 2011). The BRCA1 C-terminal domain mediates phosphoprotein interactions between BRCA1 and proteins phosphorylated by ATM and ATR kinases (Singh *et al.,* 2012). BRCT domains are classified into two categories based on their ability to recognize phosphoproteins. Class I BRCT domains can recognize phosphoserine residues, while Class II BRCT domains can recognize both phosphoserine and phosphothreonine residues. While the main function of the BRCA1. BRCT domain is modulating interactions between BRCA1 and phosphoproteins, they also mediate DNA binding and non-phosphoprotein interactions (Singh *et al.,* 2012).

2.13 Gene Polymorphism

Genetic factors are the major influencers of the human phenotype variations. Whenever a comparison of the genomic DNA sequences at the same locus of chromosome of any two individuals is made, there is substantial variation noticed throughout the genome. There are many forms of these genetic variations (Weatherall, 2000).

The basic form of the variations results from a single nucleotide change from one individual to another, and is known as single nucleotide polymorphism (SNP). Many other variations may result from the insertion or deletion of a segment of DNA. The most common insertion or deletion events occur in repetitive sequences elements, where the repeated nucleotide patterns or variable number

tandem repeat polymorphisms (VNTRs) expand or contact as a result of insertion or deletion These variations in DNA sequences are sometimes described either as mutations or polymorphisms (Sripichai & Fucharoen, 2007).

A mutation is defined as any change in a DNA sequence away from normal. This implies there is a normal allele that is prevalent in the population and that the mutation changes it to a rare and abnormal variant (Sharp *et al.,* 2006).

However, a polymorphism is a variation in DNA sequence that is common in the population. In this case, no single allele is considered as the standard sequence. Instead, there are two or more equally acceptable alternatives. The arbitrary cut-off point between a mutation and a polymorphism is 1%. Thus, to be considered as a polymorphism, the variation must have a frequency of 1% or greater in a given population. If an allele occurs at a frequency lower than 1%, the allele is considered as a mutation (Sharp *et al.,* 2006).

Many clinical phenotypes observed in diseases tend to have considerable genetic components. The presence of a specific genetic variation allele may be implicated as a causative factor in human genetic disorders. Therefore, screening for such allele in an individual is likely to enable the detection of a genetic predisposition to disease (Hurles *et al.,* 2008).

Some polymorphism sequences variants may serve as a modifying risk for some phenotype. Many polymorphisms may be found within genes and may influence characteristics such as skin colour rather than medical importance while some do contribute to disease susceptibility and can influence drug responses. However, many polymorphisms are found outside the genes and are completely neutral in effect (Sripichai & Fucharoen, 2007).

2.13.1 Single Nucleotide Polymorphisms (SNPs)

Single nucleotide polymorphisms (SNPs) are genetic variations that occur when a single nucleotide: adenine (A), thymine (T), cytosine (C) or guanine (G) in the genome sequences is altered. SNPs are mostly biallelic polymorphisms, that is, the nucleotide identity at these polymorphic positions is generally constrained to one of two possibilities in human, rather than the three or four nucleotide possibilities that could occur. SNPs are the most common form of genetic variation, accounting for 90% of all human genetic variations. Their density is estimated to occur approximately every 500 - 1,000 bases in the entire human DNA sequences, leading to a total of several millions SNPs in the overall human population (Riva & Kohane, 2002).

SNPs may occur in both coding and non-coding regions of the genome. Because only about 3 to 5 percent (3-5%) of a human DNA sequences code for the production of proteins, changes in non-coding sequences is more common than coding sequences changes. Synonymous changes in coding sequence are more common than non-synonymous changes (Sripichai & Fucharoen, 2007).

The human genome contains millions of single nucleotide polymorphisms (SNPs); many of these SNPs are intronic and have unknown functional significance. SNPs occurring within intron branch point sites, especially at the adenine (A), would presumably affect splicing (Chiang *et al.*, 2017).

SNPs found within a coding sequence are of particular interest to scientists because they are more likely to alter the biological function of a protein. Occasionally, a SNP may actually cause a disease, and can be used to search for and isolate the disease-causing gene. Many SNPs have no effect on cell function, but it is believed that they could predispose people to disease or influence their response to drug. However, SNPs are not absolute indicators of disease development. A good example is the gene associated with the late onset of Alzheimer's disease, apolipoprotein (Riva &

Kohane, 2002). This gene contains two SNPs that result in three possible alleles for this gene: ε2, ε3, and ε4. An individual who inherits at least one ε4 allele will have a greater chance of getting Alzheimer's disease. In addition, those who have inherited two ε4 alleles may never develop Alzheimer's, while another who has inherited two ε2 alleles may. Most SNPs are not responsible for a disease state. Instead, they serve as biological markers for pinpointing a disease on the human genome map, because they are usually located near a gene found to be associated with certain disease (Strittmatter & Roses, 1996).

The importance of SNPs in genetic studies comes from at least three different categories. First, SNPs can be used to reconstruct the history of genome. This is due to their abundance and most are inherited from one generation to the next, evolutionary stable, making them easier to follow in population studies. Studying the frequency and distribution of SNPs can lead to information on the evolution of the species (Sripichai & Fucharoen, 2007).

Second, SNPs can be directly responsible for genetic diseases since they may alter the genetic sequence of gene or of a regulatory region. Finally, SNPs may be utilized as markers to build the high-density genetic maps needed to perform association studies, which locate and identify genes of functional importance. It has been proposed that a set of 3,000 biallelic SNP markers would be sufficient for whole-genome-mapping studies in humans; a map of 100,000 or more SNPs has been proposed as an ultimate goal to enable effective genetic mapping studies in large populations (Sripichai & Fucharoen, 2007).

SNPs may also be associated with the responses to therapeutic agent. The best examples are genetic polymorphisms of drug metabolizing enzymes, which affect about 30% of all drugs. An effective treatment proven in one patient may become ineffective in the others. Therefore, the most

appropriate drug for an individual (personalized medicine) could be determined in advance before treatment by analyzing a patient's SNP profile (Eichelbaum *et al.*, 2006).

Single nucleotide polymorphism of BRCA1 gene is a type of polymorphism resulting from a change of a single nucleotide at a specific locus in the BRCA1 gene in a human population (Figure 6). The single nucleotide polymorphism of BRCA1 gene may present as either a homozygous wild, heterozygous carrier or a homozygous mutant. The mutant type of the single nucleotide polymorphism of BRCA1 gene is associated with the risk of breast cancer (Domchek & Greenberg, 2009).

Johnson *et. al.*, (2007), reported that the overall risk of breast cancer can be estimated from alleles that by themselves would each present too small a risk to look at independently.

The single nucleotide polymorphism of BRCA1 gene considered to have risk alleles for breast cancer include the following:

2.13.1.1 Rs1799950

This single nucleotide polymorphism of BRCA1 gene, is reported to cumulatively result in a significant increased risk of breast cancer (Johnson *et al.*, 2007). It is commonly found in the Yoruba population in Nigeria (Snpedia, 2018). The risk (minor) allele is Guanine (G) and the major allele is Adenine (A). (G: G) indicates a slightly increased breast cancer risk whiles (A: A) indicates normal breast cancer risk. (A: G) also indicates a slightly increased breast cancer risk (Snpedia, 2018).

2.13.1.2 Rs4986850

This single nucleotide polymorphism of BRCA1 gene, is reported to represent independently minor, but cumulatively result in a significant increased risk of breast cancer (Johnson *et. al.*, 2007). It is commonly found in the Kenyan (specifically, Maasai and Luhya) and Nigerian (Yoruba) populations (Snpedia, 2018). The risk (minor) allele is Adenine (A) and the major allele is Guanine (G). The homozygous genotype (A: A) indicates a slightly increased breast cancer risk whiles (G: G) indicates normal breast cancer risk. The heterozygous genotype (A: G) indicates a slightly increased breast cancer risk (Snpedia, 2018).

2.13.1.3 Rs2227945

This single nucleotide polymorphism of BRCA1 gene, is reported to be associated with the risk of breast cancer (Johnson *et. al.*, 2007). It is commonly found in the Yuroba population in Nigeria (Snpedia, 2018). The risk (minor) allele is Guanine (G) and Adenine (A) is the major allele. The homozygous genotype (G: G) indicates a slightly increased breast cancer risk whiles (A: A) indicates normal breast cancer risk (Snpedia, 2018).

2.13.1.4 Rs16942

This is a single nucleotide polymorphism, a variant in the BRCA1 gene, which is reported to cumulatively result in a significant increased risk of breast cancer (Johnson *et al.*, 2007). This single nucleotide polymorphism is common in the Luhya and Maasai population in Kenya (Snpedia, 2018). Its risk (minor) allele is Guanine (G) and its major allele is Adenine (A). The homozygous genotype (G: G) indicates a slightly increased breast cancer risk whiles homozygous genotype (A: A) indicates normal breast cancer risk. (A: G) also indicates a slightly increased breast cancer risk (Snpedia, 2018).

2.13.1.5 Rs1799966

This is a single nucleotide polymorphism, a variant in the BRCA1 gene, which is reported to represent independently minor, but cumulatively result in a significant increased risk of breast cancer (Johnson *et. al.,* 2007). This single nucleotide polymorphism is common in the Luhya and Maasai population in Kenya (Snpedia, 2018). Its risk (minor) allele is Guanine (G) and its major allele is Adenine A. The homozygous genotype (G: G) indicates a slightly increased breast cancer risk whiles homozygous genotype (A: A) indicates normal breast cancer risk. (A: G) also indicates a slightly increased breast cancer risk (Snpedia, 2018).

2.14 Finger Dermatoglyphics

Finger dermatoglyphics is the scientific study of the skin ridge patterns on the palmar surface of the distal part of the fingers (Figure 5) (Gibbs 1967; Eslami, *et al.,* 2016). Finger dermatoglyphic patterns are formed during the twelfth week of embryonic development. The skin ridge patterns are genetically determined and influenced or modified by environmental factors. When formed, the skin ridge patterns on the palmar surface of the fingers remain stable and unique for each individual. The original ridge characteristics are not disturbed unless skin is damaged to the depth of 1mm. This is evident from the clear resemblance of dermatoglyphics among related persons. There are many diseases known to be caused by abnormal genes. Whenever, there is an abnormality within the genotypye of parents, it is usually inherited by the offsprings and is reflected in dermatoglyphic pattern (Bhat *et al.,* 2014).

As a result of this, finger dermatoglyphics is used as a preliminary diagnostic tool to assess genetic and congenital disease (Sontakke, Ghosh & Pal, 2010).

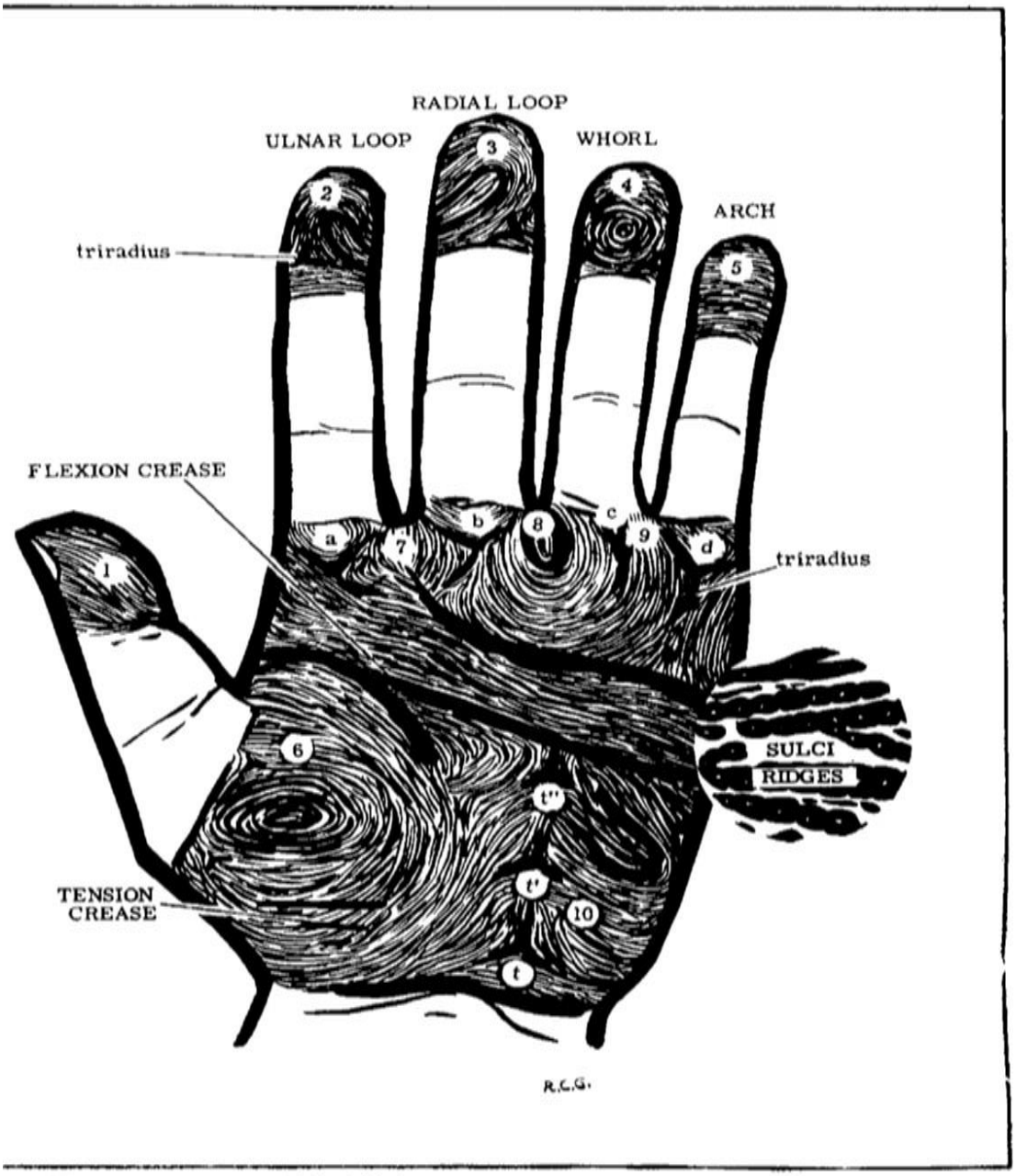


Figure 5. A diagram of palmar surface of the hand showing the finger dermatoglyphics. The first finger, second finger, third finger and fourth finger bear an ulnar loop, radial loop, whorl and an arch respectively (Gibbs, 1967).

2.14.1 Development of epidermal ridges

The development of the ridge pattern depends on the cornified layer of epithelium and dermal pattern. There is proliferation of cells in the lower layer the of epidermis which projects in the dermis as regular spaced thickenings and the dermis subsequently projects upwards in the epidermal hollows, called dermal papillae. This is followed by appearance of elevations formed by them on the skin surface which are known as epidermal ridges (Bhat *et al.*, 2014).

Differentiation of epidermal ridges takes place early in fetal development. The ridge pattern is genetically determined and is affected by environmental factors. There is a relationship between epidermal ridge and fetal volar pads, because in course of development the ridge pattern is formed at the site of these pads (Sadler & Langman, 2010). Environmental factors such as external pressure on fetal pads and embryonic fetal finger movements could influence ridge formation. Dermatoglyphics traits such as such as finger ridge count develop between 10th to 17th weeks after fertilization. Dermatoglyphic features are inherited through a polygenic system with individual genes contributing an additive genetic component. Ridge formation is influenced by individual differences in developmental stability and also, first and second trimester effect on the embryo result in dermatoglyphic changes (Sadler, & Langman, 2010).

2.14.2 Ridge characteristics

A single rolled fingerprint may have identification points, known as ridge characteristics. Most notable ridge characteristics are the core and tri radius (delta) (Bank *et al.*, 2009).

2.14.2.1 Tri radius

A tri radius is formed by the confluence of three ridge systems. Tri radius is sometimes referred as delta (Bank *et al.*, 2009).

2.14.2.2 Core

It is approximate center of the palm. The core may be of different shapes. In ridge counting, the point of core (not the whole core) is used (Bank *et al.*, 2009).

The ridge malformations may be congenital or acquired. The congenital ridge malformations are of four types. These are ridge aplasia (absence of ridge pattern), ridge hypoplasia (reduced height of ridges), ridge dissociation (breaking of ridge) and ridge of the end (vertical ridges which run off the end of the finger prints) (Bhat *et al.*, 2014).

2.14.3 Fingerprints

There are three types of finger prints: These are visible prints, latent prints and impressed prints.

2.14.3.1 Visible prints

These are also known as patent prints because these are visible to the naked eye without development. These prints are left in a medium like blood, dirt, ink or grease on the finger (Bank *et al.*, 2009).

2.14.3.2 Latent prints

These are not apparent to the naked eye and are formed from water, salt, amino acids and oils contained in the sweat. These can be made visible by dusting, fuming or chemical reagents (Bank *et al.*, 2009).

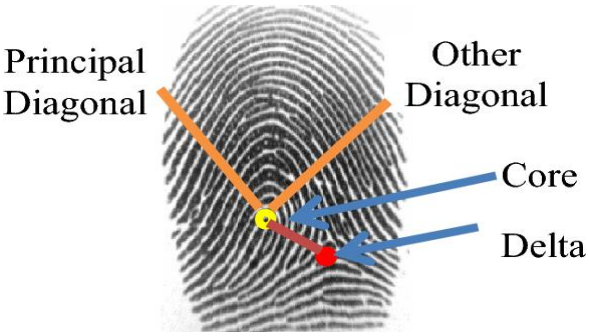
2.14.3.3 Impressed prints

These prints are indentations left on soft pliable surfaces such as clay, wax, paint or another surface that will take the impression. These are visible to the naked eye (Bank *et al.*, 2009).

2.14.4 Fingerprint patterns

There are three major types of the skin ridge patterns on the distal palmar surface of fingers. They are the whorls, loops and arches (Jalali & Hajian-Tilaki, 2002).

a. Whorl **b. Loop** **c. Arch**



d. Core and Delta

Figure 6. Pictures of fingerprints showing a whorl, loop and arch as well as the delta and a core (Jalali & Hajian-Tilaki, 2002).

2.14.4.1 Whorl

These are the second frequently occurring fingerprint patterns (Eslami *et al.*, 2016). A whorl is characterized by two (2) deltas and a core. In a whorl, some of the ridges make a turn through at

least one circuit (Figure 6a) (Jalali & Hajian-Tilaki, 2002). There are 4 types of whorls; plain whorls, central pocket whorls, double loop whorl and accidental whorl.

Plain whorl**s** have ridges which make a turn of one complete circuit with two deltas and are therefore circular or spiral in shape. This is the simplest form of whorl and also the most common (Jalali & Hajian-Tilaki, 2002).

Central pocket whorl contains a smaller pocket within a loop (Jalali & Hajian-Tilaki, 2002).

Double loop whorl consists of two separate and distinct loop formations with two separate and distinct shoulders for each core, two deltas and one or more ridges which make, a complete circuit. Between the two at least one re-curving ridge within the inner pattern area is cut or touched when an imaginary line is drawn (Jalali & Hajian-Tilaki, 2002).

Accidental whorls are derived from two distinct types of patterns that have at least two deltas. Therefore, whorls containing ridges which do not conform to the characteristics of any whorl subtypes (plain whorl, central pocket and double loop) are referred to as accidental whorls (Jalali & Hajian-Tilaki, 2002).

2.14.4.2 Loop

These are the most frequently occurring fingerprints patterns (Eslami *et al.*, 2016). They are characterized by a core and a delta (Figure 6d). In loops, the skin ridges enter on either side of the fingers, curves over the core and terminates on or in the direction of the same side, where the ridges entered (Figure 6b). In ulnar loop, the ridges open on the ulnar side and in radial loops the ridges open on the radial side (Jalali & Hajian-Tilaki, 2002).

2.14.4.3 Arch

These are the least occurring fingerprint patterns (Eslami *et al.,* 2016). Generally, they are characterized by no delta and a core. In arches, the skin ridges run from one side to another forming many spirally arranged parallel patterns, making no backward turns (Figure 6c). There are two types of arch patterns thus, plain arches and tented arches (Jalali & Hajian-Tilaki, 2002).

Dermatoglyphic patterns are analyzed in various ways, such as quantitative analysis of finger prints (loops, arches and whorls), total finger ridge count, absolute finger ridge count, position of axial tri radii, total number of palmar tri radii, A-B ridge count and ATD angle (Bank *et al.*, 2009).

2.14.7 Finger dermatoglyphic correlations

Cummins was the first individual to study clinical medicine with dermatoglyphics. Since then, a considerable improvement has been achieved with regards to the association between finger dermatoglyphics and some health disorders. The current status of dermatoglyphics is such that, it serves as a preliminary tool for diagnosis of some health disorders (Bhat *et al.,* 2014).

Every individual has a unique finger print pattern and this is dependent on the genetic characteristics of each individual. In view of this, dermatoglyphics is used to make predictions about various diseases. Also, dermatoglyphics is used in identification of an individual. A set of physical, functional or psychic, normal or pathological characteristics defines an individual (Hassan *et al.,* 2011).

Due to uniqueness of finger prints, they can be used to identify criminals at crime scene, dead or unconscious person in injuries or mass disasters, accidental exchange of new born babies, prevention of impersonation of cheques, bank notes and even for national identification exercises (Hassan *et al.,* 2011).

Dermatoglyphics has been used to study temperaments, characters, potentials and talents of humans. A whorl is associated with stubbornness, sign of faithless and unreliable character. A loop is associated with lack of perseverance and an arch is associated with merciless and crude behaviour (Bhat *et al.*, 2014). An individual with ulnar loop on all fingers is associated with clear spirited, mild mannered and strong willed person (melancholic), cool in judgment and ruthless in business. An individual having whorls on all fingers is restless, doubting, sensitive, clever, eager for action and inclined to crime. A mixture of whorls and loops is associated with a neutral character, kind, obedient, truthful but often undecided and impatient. Arches and radial loops is associated with a person who is ambitious, cool, stubborn, disobedient, defiant and rebellious (Bhat *et al.*, 2014).

There is a correlation between cephalic size, form of an individual and type of finger print pattern. In Chinese (brachycephalic) there is increased frequency of whorls and arches, in English (dolichocephalic) there is reduction of whorls and increase in arches. There is an association between whorls and blood group B, a loop and blood group A, person with blood group O have more loops and fewer whorls. In general, females have narrow ridges, more arches and fewer whorls. The finger prints of imbecile and idiots are similar to the finger prints of monkeys. In these people palmar hypothenar pattern is dominant, arches are more, axial tri radius located centrally and simian crease is present. In imbecile persons, there is great reduction of whorls in the right index and ring fingers (Bhat *et al.*, 2014).

2.14.8 Dermatoglyphics and breast cancer

Over the past three decades, research in dermatoglyphics and breast cancer has been explored. The following are some of the notable studies on dermatoglyphics and breast cancer:

Seltzer *et al.,* (1982), conducted their study with fingerprints taken from 119 study participants; out of which 34 were histopathologically diagnosed of breast carcinoma and 53 were participants with high risk of breast cancer and 32 healthy participants (controls). The difference in fingerprint pattern frequencies between cases and controls were significant. Out of the participants with 6 or more whorls, 95% participants were either breast cancer or in the group of high risk of breast cancer.

Huang & Mi, (1987), conducted a study on 570 breast cancer patients (cases) and 570 age matched healthy participants (controls). The dermatoglyphic patterns from both cases and controls groups were taken. There was increased frequencies of ulnar loops were observed on the left fingers of case group of 32 premenopausal women. However, in postmenopausal women, there was an increased frequency of radial loops were on the left fingers of case group.

Bierman *et al.,* (1988), obtained dermatoglyphic patterns from 200 women with histopathologically confirmed breast cancer (case group) and 138 healthy women without any history of malignant disease (control group). They found that ulnar and radial loops were associated with breast cancer.

Abbasi, *et al.,* (2006), studied finger print patterns in 616 women in three groups. Out of which, 154 were breast cancer patients, 154 were women with increased risk of breast cancer and 308 were healthy women (control). In breast cancer patients, 6 or more digital whorls were noticed as compared to the control group. The whorls were also found to be more in women with increased

risk of breast cancer compared to control group. There was no significant increase of patterns between group of breast cancer patients and women with increased risk of breast cancer.

Natekar *et al.*, (2006), conducted a study on finger print patterns of 100 breast cancer females and 100 matched controls accordingly. A pattern of six or more digital loops were identified more frequently in women with breast cancer than those without the disease ($p < 0.01$). The presence of more than 6 whorls significantly associated negatively with carcinoma of breast ($p < 0.01$).

Chintamani *et al.,* (2007) conducted on 60 histopathologically confirmed breast cancer patients as the case group and their dermatoglyphic patterns were studied in comparison to finger prints from 60 age matched healthy participants (controls). The findings were; 6 or more whorls were statistically significant among breast cancer patients when compared to the controls. The mean pattern intensity index in cases was 12.91 and in controls, 11.33, $p < 0.03$. Also, 6 or more digital whorls within the case group in comparison to the control group caused a statistically significant difference, $p < 0.02$. Whorls were commonly observed in right ring finger of the case group as compared to the control group, $p < 0.02$. Whorls were commonly observed in right little finger of the case group compared to controls, $p < 0.01$.

Studies have reported that breast cancer has a significant causal genetic component and evidence is available suggesting that six or more whorls have been frequently observed in females with breast cancer (Chintamani *et al.*, 2007). Also, pattern of six or more digital loops were identified more frequently in women with breast cancer than those without the disease ($p < 0.01$). Finger dermatoglyphics formation is genetically influenced. After birth, the finger dermatoglyphic patterns remain constant and hence may serve as a screening tool to study the genetic patterns in any individual. The fingerprints can therefore, represent a non-invasive anatomical marker of breast cancer risk (Chintamani *et al.*, 2007).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design

The study was a quantitative cross-sectional design which analyzed data from a population of female breast cancer patients and age-matched apparently healthy females at the Department of Surgery of the Korle-Bu Teaching Hospital and the immediate environment.

3.2 Study sites

The study was conducted at the Department of Surgery of the Korle-Bu Teaching Hospital and the immediate environment. Korle-Bu Teaching Hospital is the leading referral hospital in Ghana and it is located in the Accra Metropolitan District, Accra. Korle-Bu Teaching Hospital is affiliated to the University of Ghana, Medical School and it has several departments which offer health services to individuals with various disease conditions. One of these departments which is concerned with the management of cancers is the Department of Surgery. The Department of Surgery has an Oncology Unit which offers chemotherapy and surgical treatment to individuals with cancer. In a month, the Oncology Unit offers chemotherapy and surgical treatments to an average number of fifty (50) cancer patients out of which about fifteen (15) are usually breast cancer patients.

Apparently healthy participants were recruited from the immediate environment of the Korle-Bu Teaching Hospital. The bench work which entailed DNA extraction and amplification was carried out at the Virology laboratory at the Department of Biochemistry, Cell and Molecular Biology of the University of Ghana, Legon. The Virology laboratory is well resourced to carry out such procedures and investigations.

3.3 Study participants

Females clinically diagnosed with breast cancer and undergoing chemotherapy at the Oncology Unit of the Department of Surgery, Korle-Bu Teaching Hospital who met the inclusion criteria were recruited. Also, apparently healthy females within the immediate environment of the Korle-Bu Teaching Hospital who met the inclusion criteria were recruited.

3.4 Inclusion criteria

Females clinically diagnosed with breast cancer at the study site and age-matched apparently healthy females were considered for the study. Also, breast cancer females with no apparent or debilitating comorbidities were considered for the study.

3.5 Exclusion criteria

Breast cancer patients with any of the fingers amputated were excluded from the study. Also, breast cancer patients with mutilated finger dermatoglyphic patterns were excluded from the study. Individuals with multiple cancers and those with skin diseases such as fingertip eczema, pompholyx, pyoderma, cellulite of the finger tip, pitted keratolysis, Lichen planus and acanthosis nigricans were excluded from the study.

3.6 Sample size

A total of seventy (70) breast cancer patients (females) undergoing chemotherapy at the Oncology Unit of the Department of Surgery, Korle-Bu Teaching Hospital were randomly recruited. Also, seventy (70) age-matched apparently healthy females who have not been diagnosed of breast cancer were randomly selected at the immediate environs of the Korle-Bu Teaching Hospital for the study. The sample size was estimated using the formula below:

$N=\frac{2\,(Z_{\alpha}+Z_{\beta})^{2}\sigma}{\Delta^{2}}$ (Habib *et al.*, 2014).

N= Total number of participants (sample size)

(α predicts the probability of making a Type I error and β predicts the probability of making a Type II error)

Where α is the Significance level, α (for this study) = 0.05.

Z_{α} = A standardized normal deviate value that correspond to a level of statistical significance (0.05) equals 1.96, $Z_{\alpha} = 1.96$

β = Power, Probability of detecting a significant result (typically 80%, 90%)

β (for this study) is 80% = 0.20,

Z_{β}= probability of detection or power (80%), Z_{β}=0.84.

σ is the standard deviation for the data (in similar studies, $\sigma = 1$)

Δ= the Effect Size

Effect sizes as small, moderate, and large (0.2, 0.5, and 0.8 for two-group comparisons)

$N=\frac{2\,(1.96+0.84)^{2}\times 1}{0.5^{2}}$

$N=\frac{15.68}{0.25}$

N= 62.72, thus, N=63.

The total number of breast cancer participants was rounded to seventy (70) with equal number of apparently healthy participants.

3.7 Participants' recruitment

Breast cancer patients (females) undergoing chemotherapy at the Oncology Unit of the Department of Surgery, Korle-Bu Teaching Hospital were approached, and the aim and objectives of the study as well as data collection procedures for the study were made known to them after which those who gave their consent to partake in the study (Appendix I) were randomly selected through simple random sampling. Also, age-matched apparently healthy females, who had not been diagnosed with breast cancer were approached, and the aim and objectives of the study as well as the data collection procedures for the study were made known to them after which those who gave their consent to partake in the study (Appendix I) were randomly selected through simple random sampling at the immediate environs of the Korle-Bu Teaching Hospital.

3.8 Data collection

3.8.1 Collection of socio-demographic characteristics and clinico-pathological data

Data on socio-demographics (sex and age) and clinico-pathological data including exposed risk factors, sites of breast cancer, stage of breast cancer, histopathological diagnosis, grade of breast cancer and molecular sub-types were obtained from the clinical notes within the hospital folders of each consented breast cancer participant.

3.8.2 Procedure for data collection (finger dermatoglyphics)

Data for finger dermatoglyphic patterns were obtained using the ink method (Figure 7). Each participant was directed appropriately to dip the palmar surface of distal part of the fingers of each of the ten (10) fingers in dry ink. The ten (10) inked fingers of each participant were then pressed on a white paper (Appendix II), one finger at a time. The resulting fingerprints captured were then identified.

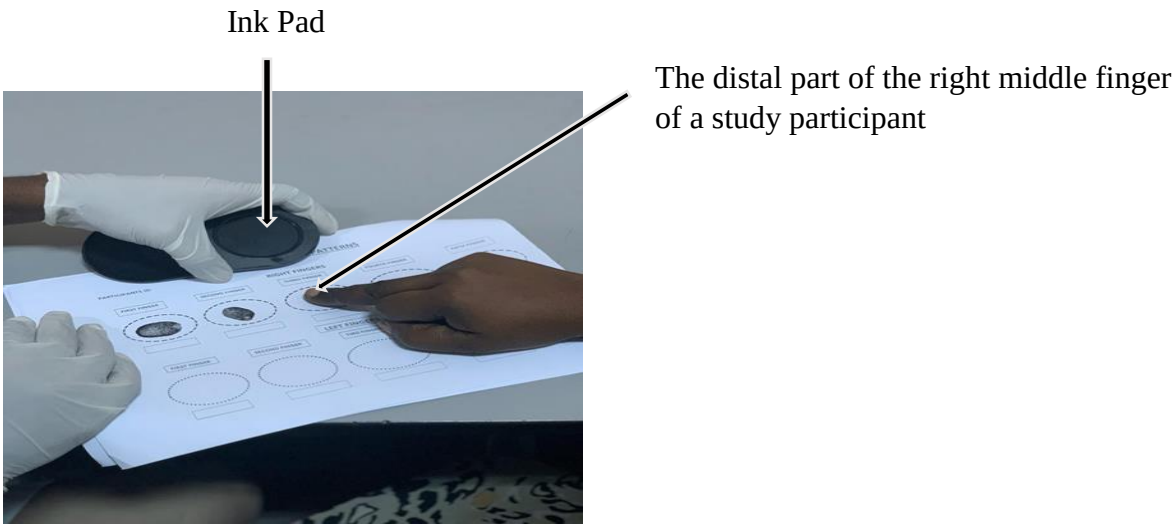


Figure 7. Picture showing fingerprinting through the ink method. The fingerprint pattern of the right middle finger of a study participant is generated by pressing the inked finger on a paper.

3.8.3 Procedure for obtaining single nucleotide polymorphism of BRCA1 gene

3.8.3.1 Extraction of buffy coat

Five (5) milliliters of peripheral blood was collected from each study participant (breast cancer females and apparently healthy females) through venipuncture of the median cubital vein and placed in EDTA coated test tube.

Blood collected into EDTA tubes were centrifuged at a speed of 3000rpm for a duration of 5 minutes to separate the blood components into layers (Appendix III). The plasma was gently

aspirated into an Eppendorf tube to make the buffy coat accessible. The buffy coat was then aspirated using a Pasteur pipette into a fresh Eppendorf tube. The buffy coat for all participants were then stored in a refrigerator at a temperature of -20°C. The Eppendorf tubes containing the buffy coat were placed in a cryo- box before storing within the refrigerator.

3.8.3.2 DNA extraction from buffy coat

DNA was extracted from the white blood cells within the buffy coat using the Zymo Research Kit [Inqaba Biotechnical Industries (PTY) Limited, South Africa].

Five hundred (500) microliters of Genomic lysis buffer [Inqaba Biotechnical Industries (PTY) Limited, South Africa] was added to 150 µl of the buffy coat from blood. The mixture was vortexed for 5 seconds and then let to stand for 10 minutes at room temperature.

The mixture was transferred to a Zymo-Spin column [Inqaba Biotechnical Industries (PTY) Limited, South Africa] placed in a collection tube and centrifuged at 10,000 xg for one minute. The collection tube together with the flow through were discarded. The Zymo-Spin column was transferred to a new collection tube. Then, 200 µl of DNA pre-wash buffer [Inqaba Biotechnical Industries (PTY) Limited, South Africa] was added to the spin column and centrifuged at 10,000 xg for one minute.

Five hundred (500) microliters of g-DNA wash buffer [Inqaba Biotechnical Industries (PTY) Limited, South Africa] was then added to the spin column and centrifuged at 10,000 xg for one minute. The spin column was then transferred to a clean micro centrifuge tube. 40 µl of DNA elution buffer [Inqaba Biotechnical Industries (PTY) Limited, South Africa] was added to the spin column and incubated for 5 minutes and then centrifuged at 10,000 xg for 30 seconds to elute the DNA.

The purity and concentration of the eluted DNA were assessed using the Nano Drop Spectrophotometer. The purity of 1.8-2.0 (A_{260} /A_{280}) and concentration of ≥20 ng/µl were required to qualify the eluted DNA as good. The extracted DNA served as template DNA for the Polymerase Chain Reaction.

3.8.3.3 BRCA1 gene amplification

Polymerase Chain Reaction (PCR) was used for amplifying the DNA extracted using appropriate primers.

PCR was carried out with 25 µl total mixture per reaction for optimization (to ascertain the appropriate temperature conditions for the PCR) and substantive PCRs using the thermocycler (Prime thermal cycler, Bibby Scientific Limited). The quantities of reagents used for 25 µl total mixture per reaction as well as 80 reactions and 8 negative controls reactions as presented in Table 3.

Table 3. Reagents used for Polymerase Chain Reaction and the quantities in 25 µl total mixture per reaction as well as 80 reactions and 8 negative control reactions.

Reagents	Quantities per reaction	Quantities for 80 reactions	Quantities for negative control (8 reactions)
One Taq Quick load Master Mix with standard buffer	12.5 µl	1000 µl	100 µl
BRCA1 forward primer – 5'CACACAGCTAGGACGTCATC-3'	0.5 µl	40 µl	4 µl
BRCA1 reverse primer - 5'TCCATCAAGGTGCTTACAGTC-3'	0.5 µl	40 µl	4 µl
Nuclease free water	10.5 µl	840 µl	84 µl
Template DNA (extracted DNA)	1 µl	1 µl per a reaction	Nuclease free water

The reaction mixture was placed in a thermocycler (Prime thermal cycler, Bibby Scientific Limited) and ran for 29 cycles with the following reaction conditions:

Initial denaturation, resulting in separation of DNA strands was achieved at 94°C for 5 minutes per cycle and denaturation was achieved at 94°C for 1 minute per cycle. Annealing of primer to target sequence was achieved at 62.5°C for 3 minutes, extension of DNA strand was achieved at 72°C for 3 minutes and finally final extension was achieved at 72°C for 3 minutes

The PCR products were subjected to Agarose gel electrophoresis to visualize and confirm correct amplification.

The processes involved in the Gel Electrophoresis are as follows:

Agarose gel (1.5%) was prepared by weighing 1.5 g of Agarose powder [Inqaba Biotechnical Industries (PTY) Limited, South Africa] after which 100 ml of Tris-acetate-EDTA (TAE) buffer was added to dilute it. The 1.5% Agarose mixture was heated till boiling on a hot plate. The beaker was covered with aluminium foil to prevent evaporation of the mixture during heating. The mixture was then removed from the hot plate and allowed to cool. Two microliters (2 µl) of Ethidium Bromide [Inqaba Biotechnical Industries (PTY) Limited, South Africa] was added to the Agarose mixture. The mixture was then swirled and poured into a casting tray (with a comb fitted) and allowed to solidify.

The electrophoresis tank was disinfected with 70% ethanol before 1XTAE (running buffer) was poured into the tank. The solid gel which was within the casting tray was placed in the electrophoresis tank. Approximately 8 µl of PCR product from each sample was loaded into separate wells in the gel. A 100bp DNA ladder [Inqaba Biotechnical Industries (PTY) Limited, South Africa] was used as a molecular marker. Gel electrophoresis was ran for 45 minutes at 100V. After gel electrophoresis, the gel with the amplicons were visualized using an Amersham imager (General Electric Healthcare Manufacturing Company, USA)

3.8.3.4 BRCA1 gene sequencing

Twenty-six (26) amplified DNA samples (PCR products) from study participants (21 breast cancer participants and 5 apparently healthy participants) were sent to Inqaba Biotechnical Industries (PTY) Limited, South Africa for BRCA1 gene sequencing. The forward strand of the amplified DNA (BRCA1 gene) samples specifically exon 11 with upstream and downstream introns were sequenced through Sanger Sequencing. The results were received. Molecular Evolutionary

Genetics Analysis software (MEGA X) was used to retrieve forward sequences of BRCA1 gene exon 11 and surrounding introns (Appendix IV). The molecular location of BRCA1 is 43,044,295 to 43,125,364 on chromosome 17 (Homo sapiens GRCh38). BRCA1-005 (ENS00000471181, CCDS11456) has 81189 base pair and has 24 exons. Exon 11 starts from base pair number 34452 and ends at 34540 (Appendix V). Alignment of the forward sequence of BRCA1 gene (template or wild type) and the forward sequences of amplified BRCA1 gene from study participants was done using benchling software (Figure 8). After alignment of the forward sequences, nucleotides present on the amplified BRCA1 gene which differed from the template sequence at specific locations were noted.

Figure 8. A picture showing mismatch of nucleotides after alignment of the wild type (template) of the forward BRCA1 gene and the forward sequence of exon 11 with flanking introns of BRCA1 gene of a breast cancer participant. The red arrow shows that exon 11 with surrounding introns of BRCA1 gene was amplified.

3.9 Data analysis

Socio-demographic data (age), clinico-pathological (tumour characteristics) data and data on fingerprint patterns were captured and cleaned using Statistical Package for Social Sciences (SPSS) version 20. Data on tumour characteristics were summarized using tables and a pie chart. Also, data on fingerprint patterns were summarized with tables and analyzed with chi-square to assess difference within fingerprint patterns among study participants. The mean frequency of fingerprint patterns among study participants were analyzed with independent samples student's t-test using Graph pad prism version 8 software. Six or more whorls and six or more loops were analyzed with chi square. Normality test was done to confirm normal distribution of data before conducting independent samples t-test. Differences in data set with $p < 0.05$ were considered statistically significant. Association between fingerprint patterns (six or more loops) and tumour characteristics were analyzed with chi-square test.

3.10 Ethical consideration

Ethical approval with Protocol Identification Number CHS-Et/M2 – 5.6/2019-2020 was sought from the Ethics and Protocol Review Committee of the College of Health Sciences, University of Ghana (Appendix VI). Participation in this study was made absolutely voluntary to study participants. Participants were given the liberty to withdraw from the study at any time with no consequences. Consent was sought from all participants.

CHAPTER FOUR

RESULTS

4.1 Socio-demographic characteristics of study population (age)

Breast cancer females were age matched with apparently healthy females, therefore, the age distribution were same for both breast cancer participants and apparently healthy participants (Figure 9). Most of the study participants were pre-menopausal (Figure 9). The minimum age of study participants (breast cancer females and apparently healthy females) was twenty-nine (29) years and the maximum age of study participants was eighty-five (85) years. The modal age was 47 years. The age range of study participants is shown in Figure 9.

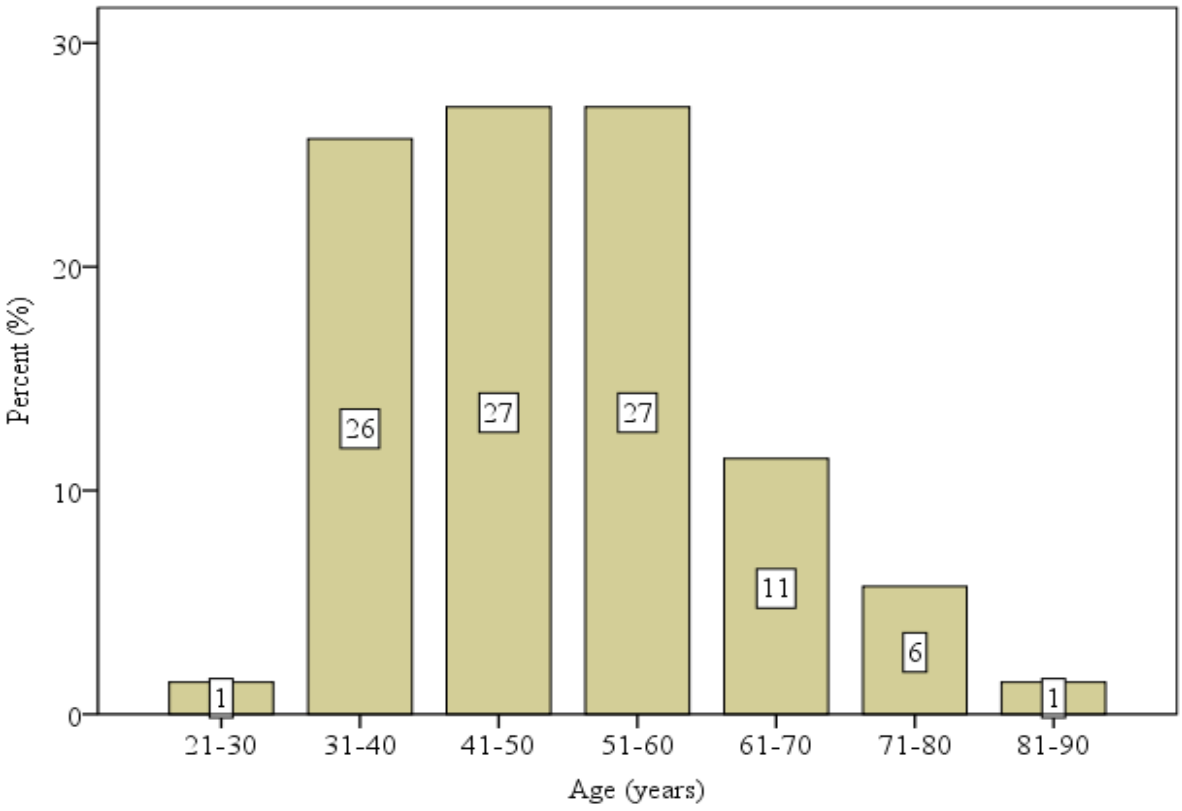


Figure 9. Age distribution of study participants.

4.2 Clinico-pathological characteristics of breast cancer patients

4.2.1 Site of breast cancer

In 50% of the breast cancer participants, the pathology affected the left breast, while in 47% of the cases, it was the right breast. In about 3% of the cases, the pathology was bilateral (Figure 10).

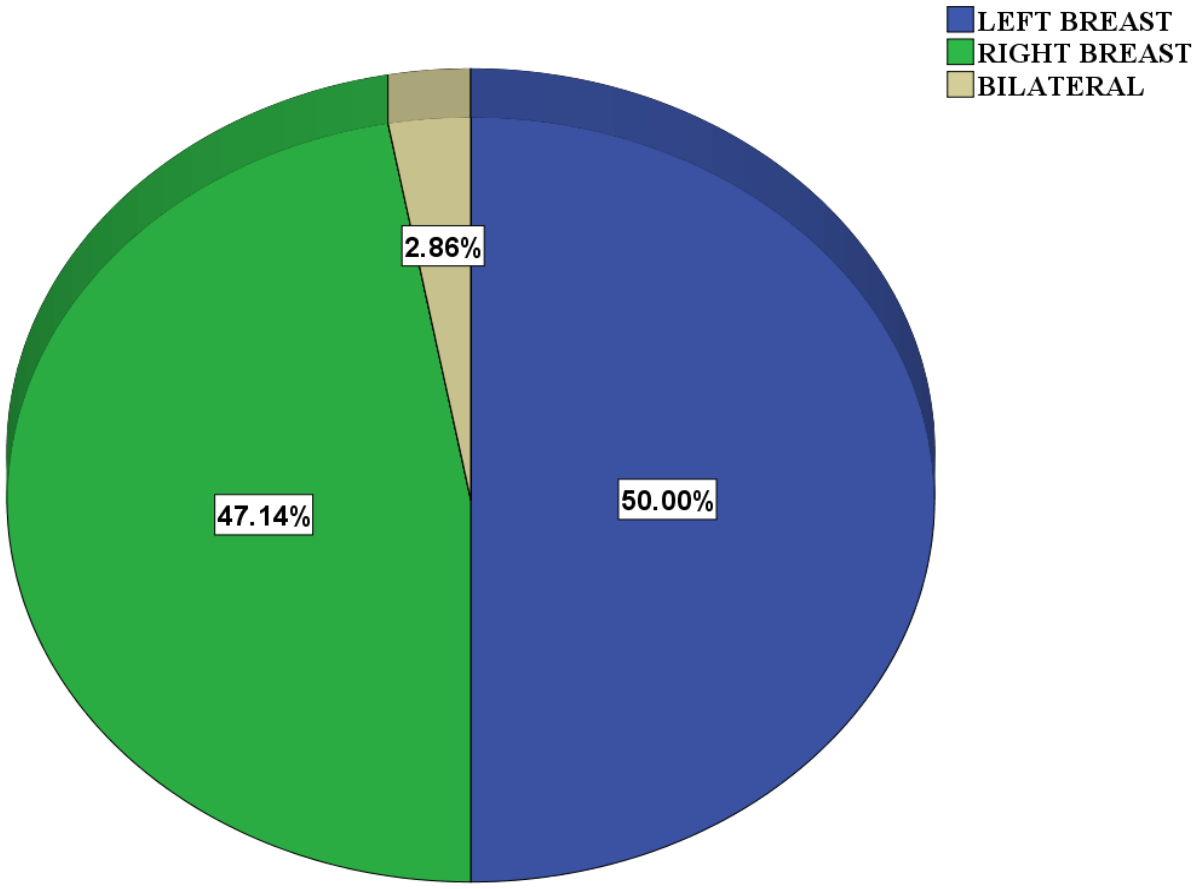


Figure 10. Site of breast cancer.

4.2.2 Histopathological diagnosis of breast cancer participants

As presented in Table 4, 49% of the breast cancer participants were diagnosed with invasive carcinoma of no special type, 18.6% with invasive ductal carcinoma and 4.3% with mixed carcinoma (mucinous carcinoma and invasive carcinoma of no special type). Other less frequent histopathological subtypes are shown in Table 4.

Table 4. Histopathological diagnosis of breast cancer among participants.

Histopathological Diagnosis	Frequency (%)
Invasive Carcinoma of no Special Type	49 (70.0)
Invasive Ductal Carcinoma	13 (18.6)
Mixed Carcinoma (Mucinous Carcinoma and Invasive Carcinoma of no Special Type)	3 (4.3)
Multinodular Carcinoma	1 (1.4)
Invasive Cribriform Carcinoma with Extensive In Situ Component	1 (1.4)
Intracystic Papillary Carcinoma	1 (1.4)
Extensive High Grade DCIS with Stromal Invasion and Lymph Node Metastasis	1 (1.4)
Invasive Lobular Carcinoma	1 (1.4)

4.2.3 Molecular subtypes of breast cancer among study participants

In this study, Luminal B was the predominant breast cancer molecular subtype among the patients (Table 5). Twenty-five (25) out of the 70 breast cancer patients representing 35.7% were diagnosed as Luminal B (Table 5). This was followed by the triple negative subtype (25.7%) and then HER2 enriched subtype (21.4%) as shown in Table 5. The least frequently diagnosed molecular subtype was Luminal A. For two of the cases, information on the molecular subtype was not available (Table 5).

Table 5. Molecular subtypes of breast cancer among study participants.

Molecular subtypes of breast cancer	Frequency (%)
Luminal B	25 (35.7)
Triple Negative	18 (25.7)
HER2 Enriched Breast Cancer	15 (21.4)
Normal-like Breast Cancer	9 (12.9)
Luminal A	1 (1.4)
Not Available	2 (2.9)

4.2.4 Stage and Grade of breast cancer among study participants

The data on breast cancer stage of the participants is illustrated in Table 6. Stage IIIB was the commonest (34.3%) followed by stage IIIA (21.4%) and then stage IIB (14.3%). Participants with stage IV were 8.6% (Table 6).

Also, in this study about 48.6% of the breast cancer participants were diagnosed with Grade II, 35.7% of the breast cancer participants were diagnosed with Grade III and about 12.9% of the breast cancer participants were diagnosed of Grade I (Table 6).

Table 6. Stage and Grade of breast cancer among study participants.

Stage and Grade of breast cancer	Frequency (%)
Stage	
IA	2 (2.9%)
IIA	5 (7.1%)
IIB	10 (14.3%)
IIIA	15 (21.4%)
IIIB	24 (34.3%)
IIIC	4 (5.7%)
IV	6 (8.6%)
Right breast-IIIB and Left breast-IIIC	1 (1.4%)
Not available	3 (4.3%)
Grade	
I	9 (12.9%)
II	34 (48.6%)
III	25 (35.7%)
Not available	2 (2.9%)

4.2.5 Risk factors of breast cancer among study participants

Exposed risk factors among the breast cancer participants are summarized in Table 7. The commonest exposed risk factor was late menarche (18.6%), followed by family history of breast cancer (11.4%) and alcohol intake (7.1%). Among the 70 participants, 31.4% did not record any association with the known exposed risk factors (Table 7).

Table 7. Risk factors of breast cancer among study participants.

Risk Factors	Frequency (%)
None	22 (31.4)
Late Menarche	13 (18.6)
Family History	8 (11.4)
Alcohol Intake	5 (7.1)
Late Menopause	3 (4.3)
Null parity	2 (2.9)
Oestrogen Contraceptives Usage	2 (2.9)
Late Menarche And Null parity	2 (2.9)
Late Menarche And Alcohol Intake	2 (2.9)
Family History And Alcohol Intake	2 (2.9)
Late Menarche And Contraceptives Usage	2 (2.9)
Alcohol Intake And Smoking	1 (1.4)
First Child Birth After Age 30	1 (1.4)
First Child Birth At Age 13	1(1.4)
Family History And Null parity	1 (1.4)
Late Menarche And Family History of Other Cancers	1(1.4)
Early Menopause, Alcohol And Family History	1 (1.4)
Late Menarche And First Child Birth After Age 30	1 (1.4)

4.3 Finger Dermatoglyphics

4.3.1 Fingerprint patterns of the right fingers among study participants

Generally, loop was the predominant fingerprint pattern on all right fingers of breast cancer particpants. In apparently healthy participants, loop fingerprint pattern had the highest frequency on the right index finger, right middle finger, right ring finger and right little finger, however, there were more whorls than loops on the right thumb (Table 8). The right ring fingers of breast cancer participants did not have an arch fingerprint pattern. The difference in frequencies of occurrence of fingerprint patterns were not statstically significant (Table 8).

Table 8. Fingerprint patterns of the right fingers of study participants

	Fingerprint Patterns								
	Frequency of Loop (%)			Frequency of Whorl (%)			Frequency of Arch (%)		
Right finger types	Breast Cancer Participants N = 70	Apparently Healthy Participants N = 70	p-value	Breast Cancer Participants N = 70	Apparently Healthy Participants N = 70	p-value	Breast Cancer Participants N = 70	Apparently Healthy Participants N = 70	p-value
Thumb	38 (54.3%)	27 (38.6%)	0.12	24 (34.3%)	31 (44.3%)	0.26	8 (11.4%)	12 (17.1%)	0.26
Index	47 (67.1%)	39 (55.7%)	0.32	14 (20%)	21 (30%)	0.16	9 (12.9%)	10 (14.3%)	0.85
Middle	58 (82.9%)	55 (78.6%)	0.75	7 (10%)	9 (12.9%)	0.53	5 (7.1%)	6 (8.6%)	0.62
Ring	47 (67.1%)	40 (57.1%)	0.37	23 (32.9%)	28 (40%)	0.41	0 (0%)	2 (2.9%)	-
Little	62 (88.6%)	60 (85.7%)	0.82	6 (8.6%)	8 (11.4%)	0.66	2 (2.9%)	2 (2.9%)	1.00

4.3.2 Fingerprint patterns of the left fingers among study participants

From Table 9, loop was the predominat fingerprint pattern among breast cancer participants. In apparently healthy participants, loop fingerprint pattern had the highest frequency of occurrence on the left index fingers, left middle fingers, left ring fingers and the left little fingers, however, there were more whorls than loops on the left thumb (Table 9). The difference in the loop fingerprint pattern on the left thumbs among study participants was statistically significant, $p < 0.05$.

Table 9. Fingerprint patterns of the left fingers of study participants

Left finger types	Fingerprint Patterns								
	Frequency of Loop (%)			Frequency of Whorl (%)			Frequency of Arch (%)		
	Breast Cancer Participants N = 70	Apparently Healthy Participants N = 70	p -value	Breast Cancer Participants N = 70	Apparently Healthy Participants N = 70	p-value	Breast Cancer Participants N = 70	Apparently Healthy Participants N = 70	p-value
Thumb	37 (52.9%)	24 (34.3%)	0.04*	22 (31.4%)	28 (40%)	0.29	11 (15.7%)	18 (25.7%)	0.12
Index	44 (62.9%)	34 (48.6%)	0.19	15 (21.4%)	22 (31.4%)	0.17	11 (15.7%)	14 (20%)	0.51
Middle	50 (71.4%)	47 (67.1%)	0.73	11 (15.7%)	12 (17.1%)	0.86	9 (12.9%)	11 (15.7%)	0.58
Ring	44 (62.9%)	38 (54.3%)	0.41	22 (31.4%)	28 (40%)	0.29	4 (5.5%)	4 (5.5%)	1.00
Little	59 (84.3%)	58 (82.9%)	0.94	5 (7.1%)	9 (12.9%)	0.18	6 (8.6%)	3 (4.3%)	0.17

4.3.3 Mean frequency of fingerprint patterns among study participants

The mean frequency of loops for breast cancer participants was higher compared to that of apparently healthy participants though the difference was not statistically significant (Table 10). Also, mean frequency of whorls for apparently healthy participants was higher compared to that of breast cancer participants, however, the difference was not statistically significant (Table 10). Lastly, apparently healthy participants had higher mean frequency of arches compared to that of breast cancer participants, however, the difference was not statistically significant (Table 10).

Table 10. Mean frequency of fingerprint patterns of all ten (10) fingers among study participants.

Fingerprint Patterns	Mean frequency of fingerprint patterns		p-value
	Breast cancer participants	Apparently healthy participants	
Loop	48.6±8.6	42.2±12.5	0.201
Whorl	14.9±7.5	19.6±9.2	0.227
Arch	6.5±3.8	8.2±5.6	0.435

4.3.4 Six or more loops and six or more whorls among study participants

Table 11 shows a comparison of the means of six or more loops among breast cancer participants and apparently healthy participants as well as the means of six or more whorls among breast cancer participants and apparently healthy participants. Even though, six or more loops was higher among breast cancer participants compared to apparently healthy participants, the difference was not statistically significant since $p > 0.05$ (Table 11). Also, six or more whorls had higher frequency among apparently healthy participants compared to breast cancer participants however, the difference was not statistically significant, since $p > 0.05$ (Table 11).

Table 11. Six or more loops and six or more whorls among study participants.

Fingerprint patterns	Frequency of fingerprint patterns (%)		p-value
	Breast cancer participants N =70	Apparently healthy participants N = 70	
Six or more loops	51 (72.9%)	47 (67.1%)	0.61
Six or more whorls	6 (8.6%)	10 (14.3%)	0.30

4.4 Finger dermatoglyphic patterns (six or more loops) and tumour characteristics

Most study participants diagnosed with luminal B had six or more loops (Table 12). Participants with unilateral breast cancer had more six or more loops. Also, most participants diagnosed with stage III and grade II breast cancers had six or more loops (Table 12). Since, characteristics of luminal A and normal-like breast cancers are very similar, they were combined in assessing the association of six or more loops and the molecular subtypes of breast cancer (Table 13).

Table 12. Finger dermatoglyphic patterns and tumour characteristics.

Tumour characteristics	Frequency of presence of six or more loops (%) N = 51	Frequency of absence of six or more loops (%) N = 19
Molecular subtypes		
Luminal A	7 (10.3%)	3 (4.4%)
Luminal B	19 (27.9%)	6 (8.8%)
Triple Negative	13 (19.1%)	5 (7.4%)
HER 2 Enriched	10 (14.7%)	5 (7.4%)
N = 68		
Site of breast cancer		
Left breast	25 (35.7%)	10 (14.3%)
Right breast	25 (35.7%)	8 (11.4%)
Bilateral	1 (1.4%)	1 (1.4%)
N = 70		
Stage		
I	1 (1.5%)	1 (1.5%)
II	12 (17.9%)	3 (4.5%)
III	32 (47.8%)	12 (17.9%)
IV	4 (6%)	2 (3%)
N = 67		
Grade		
I	6 (8.8%)	3 (4.4%)
II	26 (38.2%)	8 (11.8%)
III	17 (255)	8 (11.8%)
N = 68		

Table 13 shows the association between fingerprint patterns and tumour characteristics among breast cancer participants. From Table 13, Cramer's V which is a measure of the strength of association was approaching 0 (for all variables) which indicates a weak association between fingerprint patterns and tumour characteristics. The p values from Table 13 are all greater than 0.05 which also indicate statistically insignificant association between variables.

Table 13. Association between fingerprint patterns and tumour characteristics.

Variables	Cramer's V	p-value
Six or more loops vs Molecular subtypes of breast cancer N = 68	0.080	0.934
Six or more loops vs Site of breast affected N = 70	0.100	0.703
Six or more loops vs Stage of breast cancer N = 67	0.124	0.793
Six or more loops vs Grade of breast cancer N = 68	0.099	0.718

4.5 Single nucleotide polymorphism of BRCA1 gene

4.5.1 BRCA1 gene amplification

Figure 11 shows a gel with bands (representing amplicons) of amplified DNA samples from study participants after gel electrophoresis. The average size of the bands was 450 base pairs.

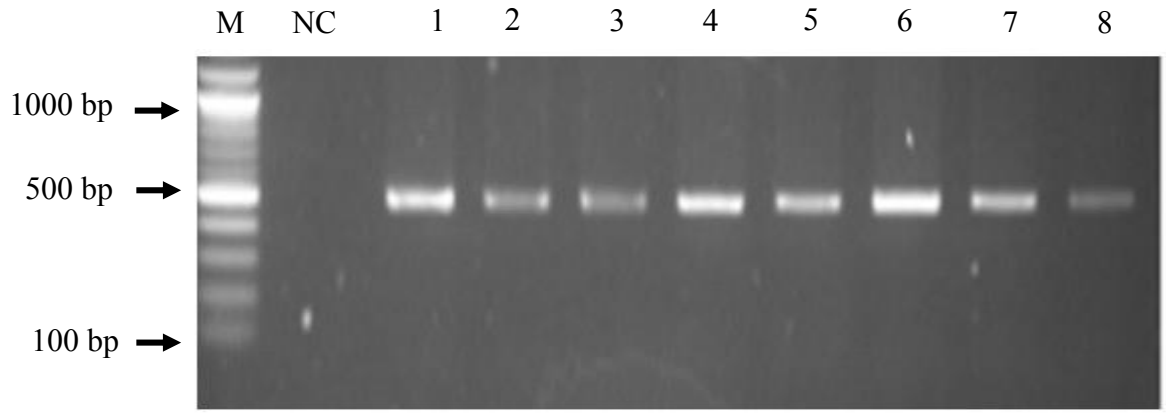


Figure 11. Image of Gel showing bands from amplicons after gel electrophoresis. M is 100bp molecular ladder. Lanes 1,2,3,4,5,6,7 and 8 have bands showing amplification of BRCA1 gene of 450 base pairs. NC is the negative control.

4.5.2 Variant analysis

After alignment of the forward sequence of the amplified BRCA1 gene to the forward sequence of the wild type of BRCA1 gene, a heterozygous allele, c.34311A>C had the highest frequency of occurrence in both breast cancer participants and apparently healthy participants (Table 14). Variant location c.34320 was the second predominant location of nucleotide mismatch having two heterozygous allele c.34320A>C (major) and c.34320A>T (minor) among study participants. The third predominant variant location was c.34321 and the resulting variant was c.34321A>T (Table 14). These variants c.34311A>C, c.34320A>C (major) and c.34320A>T (minor), c.34321A>T are introns located upstream of exon 11.

Table 14. Nucleotide variants and their location.

Study participants	Nucleotide sequence	Location	Variants	Resulting Amino acid	Frequency (%)
Breast cancer participants N = 21	Upstream Intron	c.34311	A>C	None	15 (71.4%)
	Upstream Intron	c.34320	A>T	None	6 (28.6%)
			A>C	None	1 (4.8%)
	Upstream Intron	c.34321	A>T	None	5 (23.8%)
	Upstream Intron	c.34322	A>T	None	1 (4.8%)
	Downstream Intron	c.34598	G>C	None	1 (4.8%)
			G>A	None	1 (4.8%)
	Downstream Intron	c.34596	T>G	None	1 (4.8%)
	Upstream Intron	c.34402	A>C	None	1 (4.8%)
	Downstream Intron	c.34629	A>C	None	1 (4.8%)
	Exon	c.34474	G>A	p. Arg1373Lys	1 (4.8%)
Apparently healthy participants N = 5	Upstream Intron	c.34311	A>C	None	3 (60%)
	Upstream Intron	c.34320	A>T	None	3 (60%)
	Upstream Intron	c.34321	A>T	None	3 (60%)
	Upstream Intron	c.34322	A>T	None	1 (20%)
	Upstream Intron	c.34374	A>C	None	1 (20%)
	Downstream Intron	c.34632	A>C	None	1 (20%)

4.5.3 Molecular subtypes of breast cancer and variant location of BRCA1 gene.

Out of the seventy (70) samples from breast cancer participants, 21 amplified DNA samples from breast cancer participants were selected through simple random sampling and sequenced. Out of the 21 breast cancer participants, 5 participants were diagnosed with luminal B breast cancer, 5 participants had triple negative breast cancer, 5 participants had HER2 enriched breast cancer, 5 participants had normal-like breast cancer and a participant was diagnosed with luminal A breast cancer (Table 15).

From Table 15, all 5 participants with HER2 enriched breast cancer had c.34311 as the predominant variant location, 4 participants with triple negative breast cancer had c.34311 as the predominant variant location, 3 participants with normal-like breast cancer had c.34311 as the predominant variant location and 2 participants with luminal B breast cancer had c.34311 as the predominant variant location. The participant with luminal A breast cancer had c.34311, c.34320 and c.34321 as variant locations (Table 15) however, out of the seventy (70) breast cancer participants only one was diagnosed with Luminal A.

Table 15. Molecular subtypes of breast cancer and variant location of BRCA1 gene.

Molecular subtype of breast cancer	Variant Location	Frequency	Total Frequency
Luminal A	c.34311	1	
N = 1	c.34320	1	3
	c.34321	1	
Luminal B	c.34311	2	
N = 5	c.34320	1	4
	c.34629	1	
Triple negative	c.34311	4	
N = 5	c.34320	2	**7**
	c.34321	1	
HER2 Enriched breast	c.34311	5	
cancer	c.34320	2	10
N = 5	c.34321	2	
	c.34474	1	
Normal-like breast cancer	c.34311	3	
N = 5	c.34320	1	
	c.34321	1	8
	c.34322	1	
	c.34402	1	
	c.34598	1	

4.5.4 Fingerprint patterns (six or more loops) and predominant BRCA1 gene variants

Forty-eight percent (48%) of breast cancer participants had six or more loops and c.34311A>C (Table 16). Also, 60% of apparently healthy participants had six or more loops and c.34311A>C (Table 16). However, the difference in the frequency of occurrence of six or more loops and c.34311A>C among study participants was not statistically significant (Table 16).

Table 16. Fingerprint patterns (six or more loops) and predominant BRCA1 gene variants

Fingerprint patterns in relation to BRCA1 gene	Breast cancer participants (N = 21)	Apparently healthy participants (N =5)	p -value
Six or more loops and c.34311A>C	10 (48%)	3 (60%)	0.25
Six or more loops and c.34320A>T	3 (14%)	3 (60%)	<0.001*
Six or more loops and c.34321A>T	2 (9%)	3 (60%)	<0.001*

CHAPTER FIVE

DISCUSSSION, SUMMARY OF KEY FINDINGS, CONCLUSION, RECOMMENDATION AND LIMITATIONS

5.1 Discussion

This study generated data on the single nucleotide polymorphism of BRCA1 gene and finger dermatoglyphic patterns in relation to tumour characteristics presented by breast cancer participants. The discussion here is presented to address the specific objectives below.

5.1.1 The predominant location of single nucleotide polymorphism and the resulting nucleotide variants of BRCA1 gene among study participants.

The specific objective 1 was to determine the predominant location of single nucleotide polymorphism (variant location) and the resulting nucleotide variants of BRCA1 gene among study participants.

The molecular location of BRCA1 gene is 43,044,295 to 43,125,364 on chromosome 17 (Homo sapiens GRCh38). BRCA1-005 (ENS00000471181, CCDS11456) has 81189 base pairs and has 24 exons. Exon 11 starts from base pair number 34452 and ends at 34540. Exon 11 is made up of 89 base pairs and codes for 29 amino acids.

From Figure 11, the band size of the amplified DNA (BRCA1 gene) was roughly 450 base pairs. The forward strands of the amplified DNA containing BRCA1 gene from study participants were sequenced and the resulting nucleotides constituted upstream introns, exon 11 and downstream introns.

After alignment of the forward sequence of the amplified BRCA1 gene and the forward sequence of the wild type of BRCA1 gene, nucleotide variation occurred more within the intronic sequence. Variant location c.34311 with a heterozygous allele c.34311A>C had the highest frequency of occurrence in both breast cancer participants and apparently healthy participants (Table 14). Variant location c.34320 was the second predominant location of nucleotide mismatch having two heterozygous alleles c.34320A>T (major) and c.34320A>C (minor). The third predominant variant location was c.34321 and the resulting variant was c.34321A>T (Table 14). These variants c.34311A>C, c.34320A>C, c.34320A>T and c.34321A>T were all within introns located upstream of exon 11.

According to Sripichai & Fucharoen, (2007), exons are the coding sequence of a genome whereas introns are usually the non-coding sequence of a genome and they are both involved in gene expression. Single nucleotide polymorphism may occur in both exons and introns but alterations in the intronic sequences are more common than in exons (Sripichai & Fucharoen, 2007). These polymorphic intronic sequences have unknown functional significance. However, these single nucleotide variants occurring within intron branch point sites, especially at the position with adenine (A), would presumably affect splicing (Chiang *et al.,* 2017).

The findings of this study are consistent with the findings of Sripichai & Fucharoen, (2007) since most nucleotide variations occurred within the intronic sequence (Table 14). Also, in this study, the predominant variant occurred at positions where adenine was supposed to be present in comparison with the wild type. Therefore, if c.34311 was a branch point site then splicing would be affected as argued by Chiang *et al.,* (2017).

Furthermore, promoter regions which are sites for initiation of transcription of RNA are located upstream of exons. Promoter sites usually contain TATA sequences in eukaryotes and these are

bound by TATA binding proteins to initiate transcription (Hurles *et al.*, 2008). From Table 14, the three predominant variants c.34311A>C, c.34320A>C and c.34321A>T had adenine (A) replaced with cytosine (C), cytosine (C) and thymine (T) respectively and therefore, transcription of RNA from BRCA1 gene would be affected if any of the predominant variant locations were to be part of the promoter sites.

Exon 11 of BRCA1 gene is the site for frequent mutations in breast cancer individuals (Karami & Mehdipour, 2013). Karami & Mehdipour, (2013) reported on the global spectrum of mutations within BRCA1 gene among breast cancer participants in Africa focusing on populations in Nigeria, Egypt, Tunisia, Morocco, Algeria and South Africa. Several studies have reported on the single nucleotide polymorphism or mutations of BRCA1 gene with regards to individuals with breast cancer in many countries. However, information on single nucleotide polymorphism or mutations of BRCA1 gene among individuals with breast cancer in Ghana is unavailable.

Table 14 showed that within exon 11 specifically c.34474, a variant, c.34474G>A was observed in a study participant with HER2 enriched breast cancer which resulted in an amino acid alteration; p. Arg1373Lys. The resulting amino acid, lysine is a basic amino acid just as arginine (the default amino acid) as such protein folding would not be adversely affected since lysine and arginine have similar qualities.

In summary, introns play a role in the regulation of gene expression and alternative splicing. Variations in intronic sequences may cause down regulation of gene expression and eventually lead to neoplasms (Sripichai & Fucharoen, 2007). However, c.34311A>C which was predominant in both breast cancer females and apparently healthy females gives an indication that the variant may be a naturally occurring variant. Therefore, a similar study with a larger sample size may

provide relevant information on the variations of introns and their association with neoplasms among Ghanaians.

5.1.2. The predominant finger dermatoglyphic patterns in individuals with breast cancer and individuals without breast cancer.

The specific objective 2 was to determine the predominant finger dermotoglyphic patterns among individuals with breast cancer and individuals without breast cancer.

There are three major types of the skin ridge patterns on the distal palmar surface of fingers. They are the loops, whorls and arches (Jalali & Hajian-Tilaki, 2002).

For both right and left thumbs, apparently healthy participants had more whorls than loops compared to breast cancer participants (Tables 8 and 9). The predominant fingerprint pattern of the right and left index fingers of study participants was loop (Tables 8 and 9). Also, the predominant fingerprint pattern of the right and left middle fingers of study participants was loop (Tables 8 and 9). The predominant fingerprint pattern of the right and left ring fingers of study participants was loop (Tables 8 and 9). From table 8, there was no arch on the right ring fingers of breast cancer participants. The predominant fingerprint pattern of the right and left little fingers of study participants was loop (Tables 8 and 9). The difference in the loop fingerprint pattern on the left thumbs among study participants was statistically significant, $p < 0.05$ (Table 9).

According to Eslami *et al.,* (2016), loops are the most frequently occurring fingerprints patterns. The study by Eslami *et al.,* (2016), recruited 323 patients with malocclusion (107 males, 228 females) who were referred to Orthodontic Department of Mashhad Dental School in Iran. In the study, participants differed from the present study in terms of characteristics of study participants, gender and sample size.

Also, Sontakke *et al.*, (2010), found a higher frequency of whorls compared to loops in 60 patients with klinefelter syndrome compared to 60 healthy participants in India.

Furthermore, Chintamani *et al.*, (2007) reported that whorls were commonly observed in the right ring fingers and the right little fingers of the case group (60 breast cancer participants) as compared to the control group (60 healthy participants) in India, $p < 0.02$. These findings were observed differently in the present study (Table 8).

Ultimately, the predominant fingerprint pattern for all right and left fingers of both breast cancer participants and apparently healthy participants was loop (Table 10). This study adopted and analyzed parameters such as six or more loops from Natekar *et al.*, (2006) and six or more whorls from Chintamani *et al.*, (2007). Even though, six or more loops were higher among breast cancer participants compared to apparently healthy participants, the difference was not statistically significant since $p > 0.05$ (Table 11). Also, six or more whorls had higher frequency among apparently healthy participants compared to breast cancer participants however, the difference was not statistically significant, since $p > 0.05$ (Table 11).

Chintamani *et al.*, (2007), observed that six or more whorls in the fingerprint pattern were statistically significant among 60 breast cancer participants as compared to 60 healthy participants. Also, Natekar *et al.*, (2006), reported that six or more loops were found to have higher frequency among breast cancer participants compared to healthy participants, with $p < 0.01$. The finding by Chintamani *et al.*, (2007) was differently observed in the present study (Table 11).

Even though, fingerprint patterns are formed before the onset of diseases, they can be used to predict the risk of contracting diseases per the trends reported in various studies. In this case, breast cancer participants had no arch on the right ring fingers and also six or more loops were found to

be predominant among breast cancer participants. This could be explored further through a similar study with a larger sample size in order to adopt fingerprint patterns (presence of six or more loops and the absence of an arch on the right ring finger) as a preliminary prediction tool for risk and prognosis of breast cancer.

In subsequent sections of this chapter, six or more loops will be used to represent fingerprint patterns in assessing breast cancer because loop was the predominant fingerprint pattern among study participants and also because six or more loops incorporates both right and left fingers of study participants.

5.1.3 Tumour characteristics in individuals with breast cancer in relation to fingerprint patterns.

The specific objective 3 was to ascertain tumour characteristics in individuals with breast cancer in relation to fingerprint patterns.

Data on tumour characteristics provides information on affected breast, molecular subtype of breast cancer, histopathological diagnosis of breast cancer, stage and grade of breast cancer.

In 50% of the breast cancer participants, the pathology affected the left breast, while 47% of the case, it was the right breast (Figure 10). In about 3% of the cases, the pathology was bilateral.

Studies by Tulinius *et al.*, (1990) and Amer, (2014).reported that unilateral breast cancer is more frequent in the left breast than in the right breast. However, reasons for such observation could not be ascertained.

With regards to the association between fingerprint patterns and the affected breast; even though more patients with cancer of the unilateral left breast and the unilateral right breast had six or more

loops (table 12), Cramer's V was 0.100 and $p > 0.05$ (Table 13), which indicate a weak association between the two variables. Perhaps, a larger sample size could have confirmed association between the two variables. In that case, the presence of six or more loops may be used to predict the risk of being diagnosed of a unilateral breast cancer.

In this study, Luminal B was the predominant breast cancer molecular subtype among the patients followed by triple negative (Table 5). The prognosis for this molecular subtype is worse (Eliyatkın *et. al.*, 2015). This may suggest why most study participants were clinically diagnosed with breast cancer of late stage and high grade (Table 6). A study by Perou & Børresen-Dale (2011), reported that the commonest molecular subtype of breast cancer is luminal A. Molecular subtypes of breast cancer dwell on gene expression. Different populations may have varying genetic compositions and therefore may account for such different observations.

Considering the association between fingerprint patterns and the molecular subtype of breast cancer; even though there was a higher frequency of presence of six or more loops in relation to all the molecular subtypes of breast cancer, luminal B patients had a higher frequency of six or more loops (table 12), however, Cramer's V was 0.080 and $p > 0.05$ (Table 13), which indicate a weak association between the two variables (six or more loops and molecular subtypes of breast cancer). In view of this, a similar study with larger sample size could provide a more substantive information about the association between the two variables. In that case, the presence of six or more loops could be adopted in the preliminary assessment of the prognosis of breast cancer among patients.

From Table 15, all 5 participants with HER2 enriched breast cancer had c.34311 as the predominant variant location. Eliyatkın *et al.*, (2015), reported that triple negative breast cancer is more common in women with BRCA1 gene mutations. This study assessed single nucleotide

polymorphism at specified locations within BRCA1 specifically exon 11 and its upstream and downstream introns. Even though the sample size is small, all 5 breast cancer participants with HER2 enriched breast cancer had c.34311 as the predominant variant location.

As presented in Table 4, 70% of the breast cancer participants were diagnosed with invasive carcinoma of no special type, 18.6% with invasive ductal carcinoma and 4.3% with mixed carcinoma (mucinous carcinoma and invasive carcinoma of no special type).

Histopathololologically, the commonest types of breast cancer are ductal carcinoma in situ, invasive ductal carcinoma, and invasive lobular carcinoma (American Cancer Society, 2017). Invasive ductal carcinoma is the commonest histopathological type of breast cancer and about 80% of infiltrating breast cancers are invasive ductal carcinomas (American Cancer Society, 2017). The findings of this study are consistent with the findings of the American Cancer Society (2017). This is because invasive carcinoma of no special type was previously known as invasive ductal carcinoma. Data on histopathological diagnosis in relation to fingerprint patterns could not be analysed with chi-square due to the low frequencies of the various histopathological diagnosis of breast cancer participants.

At the time of data collection, most breast cancer participants were clinically diagnosed with late stage and their hispathological reports indicated high grade breast cancers (Table 6). Unfortunately, date of initial diagnosis of breast cancer for participants could not be ascertained in order to track aggressiveness of the disease.

According to Edge & Compton, (2010), the prognosis of breast cancer is dependent on the size of the tumour and the extent of spread of the breast cancer cells to other parts of the body (stage) and

the grade. Therefore, a big-sized tumour with metastasis of the breast cancer cells to other parts of the body informs advanced breast cancer and thus, poor prognosis.

From Table 12, participants with stage III and grade II had higher frequencies of the presence of six or more loops, however, there was a weak association between fingerprint patterns (six or more loops) and tumour characteristics (stage and grade) (Table 13).

5.1.4 The predominant variants of BRCA1 gene and the predominant finger dermatoglyphic pattern (six or more loops) among individuals with breast cancer and individuals without breast cancer.

The specific objective 4 was to assess the predominant variants of BRCA1 gene in relation to the predominant finger dermatoglyphic pattern (six or more loops) among breast cancer participants.

From Table 16, apparently healthy participants had a higher percentage frequency of six or more loops in relation to c.34311A>C compared to breast cancer participants. Even though, apparently healthy participants had a higher percentage frequency of six or more loops in relation to the c.34311A>C, the difference was statistically insignificant (Table 16). Also, the higher percentage frequencies of six or more loops in relation to c.34320A>T and c.34321A>T among apparently healthy participants compared to breast cancer participants was noticed and this observation was due to the inconsistencies in the sample size of sequenced BRCA1 gene samples from breast cancer participants and apparently healthy participants.

Therefore, this information is insufficient to be used to explore risk and prognosis of breast cancer, because six or more loops and the predominant BRCA1 gene variant, c.34311A>C were present in both breast cancer participants and apparently healthy participants and the difference in frequency of occurrence among study participants was not statistically significant.

5.2 Summary of key findings

In this study, Luminal B was the predominant breast cancer molecular subtype among the patients. With regards to fingerprint patterns, the predominant fingerprint pattern among breast cancer participants was the loop. Apparently healthy participants had more whorls than loops on the right and left thumbs, the rest of the fingers had loop as the predominant fingerprint pattern. The absence of an arch on the right ring fingers of breast cancer participants was noticed. The difference in the loop fingerprint pattern on the left thumbs among study participants was statistically significant, $p < 0.05$.

Variant location c.34311 with a heterozygous allele c.34311A>C had the highest frequency of occurrence among study participants. Variant location c.34320 was the second predominant location of nucleotide mismatch having two heterozygous alleles c.34320A>T (major) and c.34320A>C (minor). The third predominant variant location was c.34321 and the resulting variant was c.34321A>T. These variants c.34311A>C, c.34320A>C, c.34320A>T and c.34321A>T are introns located upstream of exon 11.

Lastly, a higher percentage frequency of six or more loops in relation to the predominant BRCA1 gene variant, c.34311A>C was observed in apparently healthy participants compared to breast cancer participants, however, the difference in the frequency of occurrence of six or more loops and c.34311A>C among study participants was statistically insignificant.

5.3 Conclusion

This study explored the characteristics of breast cancer in relation to BRCA1 gene variants and fingerprint patterns. The aim of this study was to analyze the relationship between the single nucleotide polymorphism of BRCA1 gene and finger dermatoglyphic patterns in breast cancer patients and how this information can be used to assess risk and prognosis of breast cancer.

From the study, there was absence of arch fingerprint pattern on the right ring finger of breast cancer participants. Also, six or more loops had higher frequency among females with breast cancer compared to apparently healthy females. BRCA1 gene variant c.34311A>C was the predominant variant associated with breast cancer females and apparently healthy females. Lastly, a higher percentage frequency of six or more loops in relation to c.34311A>C was observed in apparently healthy females compared to breast cancer females.

This study sought to provide preliminary tools for early assessment of risk and prognosis of breast cancer, however, the results of the study are inconclusive to permit the adoption of the presence of six or more loops and BRCA1 gene variant c.34311A>C to assess risk and prognosis of breast cancer since six or more loops and BRCA1 gene variant c.34311A>C were predominant in both breast cancer participants and apparently healthy participants. The hypothesis, H_o, was rejected since a higher frequency of six or more loops and c.34311A>C were noticed within individuals with breast cancer.

5.4 Limitations

This study explored exon 11 of BRCA1 gene and fingerprint patterns in females with breast cancer. The following were the shortfalls of this study:

1. Due to the small sample size of the sequenced BRCA1 gene from study participants (as a result of financial constraints), there was greater potential for type one error.
2. Further details of fingerprint patterns such as subtypes of loops, whorls and arches as well as ridge counts were not studied.

5.5 Recommendations

Future studies should focus on the following:

1. A larger sample size is recommended to provide more relevant information about variables.
2. Subtypes of the fingerprint patterns and ridge counts in order to obtain pertinent information on finger dermatoglyphics in relation to breast cancer in Ghana.

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APPENDIX I

INFORMATION SHEET

Participant Number: ……..……

CONSENT TO PARTICIPATE IN A RESEARCH PROJECT

Before you agree to participate in this research study, it is important that you read and understand the study. This statement describes the aim, procedure, risks, discomforts and precautions as well as your right to withdraw from the study at any time.

You are being invited to participate in a research project titled: '**EVALUATION OF BREAST CANCER SUSCEPTIBILITY GENE 1 (BRCA1 GENE) POLYMORPHISM AND FINGER DERMATOGLYPHIC PATTERNS IN BREAST CANCER'**.

The aim of this study is to analyze the relationship between the single nucleotide polymorphic status of BRCA1 gene and finger dermatoglyphic patterns in breast cancer patients.

Explanation of Procedures

The approach of the research is through the use of your demographic and tumour characteristics data which will be obtained from the clinical notes in your folder, also fingerprint analysis and BRCA1 gene polymorphism analysis.

Data for fingerprint analysis will be collected using the ink method. You will place the palmar surface of your distal fingers in a dry ink. All your ten (10) fingers will be placed in the dry ink. The ten (10) inked fingers will then be pressed on a white paper with one finger at a time. The resulting fingerprints captured will then be identified.

Data for BRCA1 gene polymorphism analysis will be obtained through the following procedures:

Five milliliters (5 ml) of your blood will be collected through venipuncture and placed in an EDTA coated test tube. DNA will then be extracted from the white blood cells in the blood collected from you and then BRCA1 gene segments will then be amplified using appropriate primers. Finally, the variants in BRCA1 will be analyzed using Benchling software after sequencing of the BRCA1 gene has been done.

Possible Risks and Discomforts

You will not be at any risk when participating in this research, though you may experience a minor discomfort during the blood collection due to the prick of the needle used.

Confidentiality

All the information that will be obtained from you and from the analysis of your blood sample will be handled confidentially and used for the purpose stated for the study only. Your identity as a participant will not be disclosed to any unauthorized persons; only the researcher will have access to the research materials. The study participants will be coded with the code known only to the principal investigator.

Costs and Compensation for Participation in Research

This is a purely voluntary participation that is required of you and no monetary compensation is available. There will also be no costs for participating in the research.

Withdrawal from Project

Participation in this study is voluntary; refusal to participate will not involve any penalty. You are free to withdraw consent and discontinue participation in this project at any time without you being victimized. Withdrawal or refusal to participate will not affect the care you receive from this treatment unit.

INFORMED CONSENT FORM

Principal Researcher: Emmanuel Osei Nkansah

Name of Institution: Department of Anatomy, University of Ghana Medical School.

Supervisory Team

Dr. Benjamin Arko Boham

Dr. John Ahenkorah

Mr. Emmanuel Tagoe

Prof. Clegg Lamptey

Project Title: 'EVALUATION OF BREAST CANCER SUSCEPTIBILITY GENE 1 (BRCA1 GENE) POLYMORPHISM AND FINGER DERMATOGLYPHIC PATTERNS IN BREAST CANCER'

I have been invited to take part in this study for the research titled above. My role in this study is to allow my demographic and tumour characteristics data to be used for the study and to provide my finger dermatoglyphic patterns. Lastly, I am to allow my blood sample to be taken to be used for the study.

I acknowledge that the purpose, research procedures, risk and discomforts as described above have been explained to me fully and that any questions that I have asked have been explained to my satisfaction.

I have been informed of the alternatives of participation in this study including the right to not participate. I also understand that I may not benefit directly from this research and that my

participation is totally voluntary and I have also been given enough time and opportunity to consider taking part in this study.

I have also been informed that the information I will provide will be safeguarded and that my privacy and anonymity will be ensured in the collection, storage, and publication of the research material.

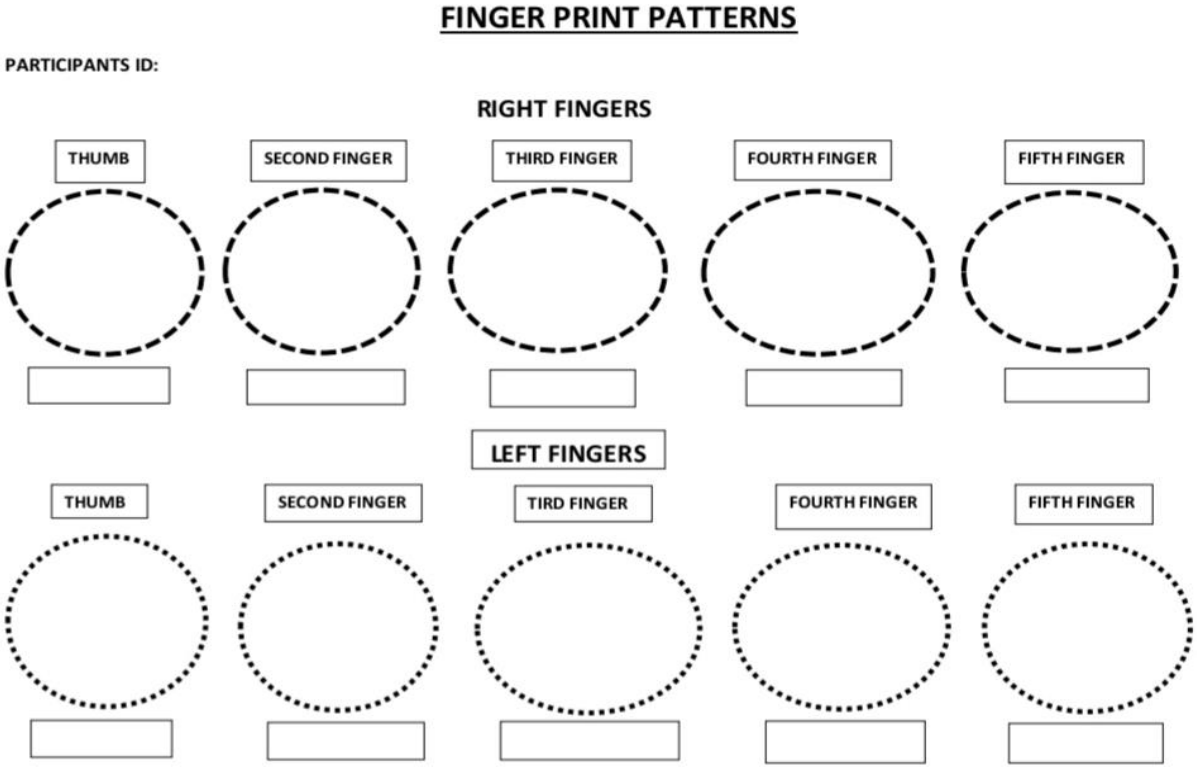
I, ……... have fully understood the aim, methods and conditions to participate in this study and I therefore, consent to my participation.

…… **……**

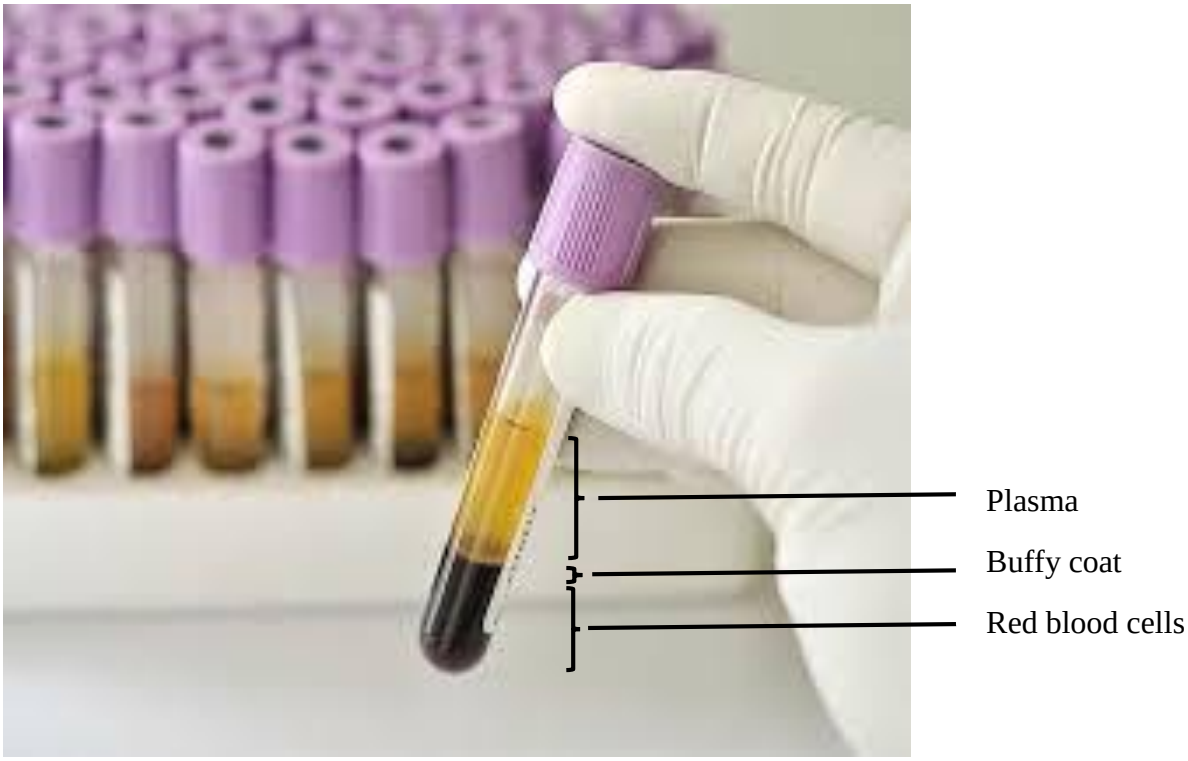
Participant's signature/Thumb-print **Date**

APPENDIX II

Tool for fingerprint data collection



APPENDIX III



A picture of EDTA tube with venous blood centrifuged to obtain plasma, buffy coat and red blood cells.

APPENDIX IV

Forward sequences of amplified BRCA1 gene (exon 11 and surrounding introns)

STUDY PARTICIPANTS (SAMPLE NUMBER)	SEQUENCE OF AMPLIFIED DNA
BREAST CANCER PARTICIPANT (8)	5'ATTWMMATYCCTAATTACTGGTGGACTTACTTCTGGTTTCATTTTATAAA AGCAAATCCCGGTGTCCCWWWKCAAGGAATTTAATCATTTTGTGTGACAT GAAAGTAAATCCAGTCCTGCCAATGAGAAGAAAAAGACACAGCAAGTTGC AGCGTTTATAGTCTGCTTTTACATCTGAACCTCTGTTTTTGTTATTTAAGGTG AAGCAGCATCTGGGTGTGAGAGTGAAACAAGCGTCTCTGAAGACTGCTCA GGGCTATCCTCTCAGAGTGACATTTTAACCACTCAGGTAAAAAGCGTGTGT GTGTGTGCACATGCGTGTGTGTGGTGTCCTTTGCATTCAGKAGTATGTATCC CACATTCTTAGGTTTGCTGACATCATCTCTTTGAATTAATGGCACAATTGTT TGTGGTTCATTGTCTCCTTAAATTARACTGWAAGCACCTTGATGG-3'
BREAST CANCER PARTICIPANT (12)	5'ATCCCTTWAAWTTMCTGGGTGGACTTACTTCTGGTTTCATTTTATAAAAG CAAATCCCGGTGTCCCWAAGCAAGGAATTTAATCATTTTGTGTGACATGAA AGTAAATCCAGTCCTGCCAATGAGAAGAAAAAGACACAGCAAGTTGCMGC GTTTATAGTCTGCTTTTACATCTGAACCTCTGTTTTTGTTATTTAAGGTGAA GCAGCATCTGGGTGTGAGAGTGAAACAAGCGTCTCTGAAGACTGCTCAGG GCTATCCTCTCAGAGTGACATTTTAACCACTCAGGTAAAAAGCGTGTGTGT GTGTGCACATGCGTGTGTGTGGTGTCCTTTGCATTCAGTARTATGTATCCCA CATTCTTAGGTTTGCTGACATCATCTCTTTGAATTAATGGCACAATTGTTTG TGGTTCATTGTCTCCTTAAATTAGACTGTAARCACCTTGATGGAA-3'
BREAST CANCER PARTICIPANT (17)	5'CTTMMMTYCCCTAATTACTGGTGGACTTACTTCTGGTTTCATTTTATAAA AGCAAATCCCGGTGYCCYWWWGCAAGGAATTTAATCATTTTGTGTGACAT GAAAGTAAATCCAGTCCTGCCAATGAGAAGAAAAAGACACAGCAAGTTGC AGCGTTTATAGTCTGCTTTTACATCTGAACCTCTGTTTTTGTTATTTAAGGTG AAGCAGCATCTGGGTGTGAGAGTGAAACAAGCGTCTCTGAAGACTGCTCA GGGCTATCCTCTCAGAGTGACATTTTAACCACTCAGGTAAAAAGCGTGTGT GTGTGTGCACATGCGTGTGTGTGGTGTCCTTTGCATTCAGTARTATGTATCC CACATTCTTAGGTTTGCTGACATCATCTCTTTGAATTAATGGCACAATTGTT TGTGGTTCATTGTCTCCTTAAATTAGACTGTAAGCACCTTGATGGAA-3'

BREAST CANCER PARTICIPANT (25)	5'ACCCTAATTACTGGTGGACTTACTTCTGGTTTCATTTTATAAAAGCAAATC CCGGTGTCCCWWAGCAAGGAATTTAATCATTTTGTGTGACATGAAAGTAA ATCCAGTCCTGCCAATGAGAAGAAAAAGACACAGCAAGTTGCAGCGTTTA TAGTCTGCTTTTACATCTGAACCTCTGTTTTTGTTATTTAAGGTGAAGCAGC ATCTGGGTGTGAGAGTGAAACAAGCGTCTCTGAAGACTGCTCAGGGCTATC CTCTCAGAGTGACATTTTAACCACTCAGGTAAAAAGCGTGTGTGTGTGTGC ACATGCGTGTGTGTGGTGTCCTTTGCATTCAGTAGTATGTATCCCACATTCT TAGGTTTGCTGACATCATCTCTTTGAATTAATGGCACAATTGTTTGTGGTTC ATTGTCTCCTTAAATTAGACTGTAAGCACCTTGATGGAA-3'
BREAST CANCER PARTICIPANT (27)	5'TYCCCTAATTACTGGTGGACTTACTTCTGGTTTCATTTTATAAAAGCAAAT CCCGGTGTCCCWWAGCAAGGAATTTAATCATTTTGTGTGACATGAAAGTA AATCCAGTCCTGCCAATGAGAAGAAAAAGACACAGCAAGTTGCAGCGTTT ATAGTCTGCTTTTACATCTGAACCTCTGTTTTTGTTATTTAAGGTGAAGCAG CATCTGGGTGTGAGAGTGAAACAAGCGTCTCTGAAGACTGCTCAGGGCTAT CCTCTCAGAGTGACATTTTAACCACTCAGGTAAAAAGCGTGTGTGTGTGTG CACATGCGTGTGTGTGGTGTCCTTTGCATTCAGTAGTATGTATCCCACATTC TTAGGTTTGCTGACATCATCTCTTTGAATTAATGGCACAATTGTTTGTGGTT CATTGTCTCCTTAAATTAGACTGTAAGCACCTTGATGGA-3'
BREAST CANCER PARTICIPANT (28)	5'GACCTTTAMATCCTAATTACTGGTGGACTTACTTCTGGTTTCATTTTATAA AAGCAAATCCMGGTGTCCCAAAGCAAGGAATTTAATCATTTTGTGTGACAT GAAAGTAAATCCAGTCCTGCCAATGAGAAGAAAAAGACACAGCAAGTTGC AGCGTTTATAGTCTGCTTTTACATCTGAACCTCTGTTTTTGTTATTTAAGGTG AAGCAGCATCTGGGTGTGAGAGTGAAACAAGCGTCTCTGAAGACTGCTCA GGGCTATCCTCTCAGAGTGACATTTTAACCACTCAGGTAAAAAGCGTGTGT GTGTGTGCACATGCGTGTGTGTGGTGTCCTTTGCATTCAGTAGTATGTATCC CACATTCTTAGGTTTGCTGACATCATCTCTTTGAATTAATGGCACAATTGTT TGTGGTTCATTGTCTCCTTAAATTAGACTGTAAGCACCTTGATGGAA-3'
BREAST CANCER PARTICIPANT (29)	5'TCCTTTAMMTCCTAATTACTGGTGGACTTACTTCTGGTTTCATTTTATAAA AGCAAATCCMGGTGTCCCWAAGCAAGGAATTTAATCATTTTGTGTGACAT GAAAGTAAATCCAGTCCTGCCAATGAGAAGAAAAAGACACAGCAAGTTGC AGCGTTTATAGTCTGCTTTTACATCTGAACCTCTGTTTTTGTTATTTAAGGTG AAGCAGCATCTGGGTGTGAGAGTGAAACAAGCGTCTCTGAAGACTGCTCA GGGCTATCCTCTCAGAGTGACATTTTAACCACTCAGGTAAAAAGCGTGTGT GTGTGTGCACATGCGTGTGTGTGGTGTCCTTTGCATTCAGTAGTATGTATCC CACATTCTTAGGTTTGCTGACATCATCTCTTTGAATTAATGGCACAATTGTT TGTGGTTCATTGTCTCCTTAAATTAGACTGTAAGCACCTTGATGGAA-3'

BREAST CANCER PARTICIPANT (30)	5'ACCCTAATTACTGGTGGACTTACTTCTGGTTTCATTTTATAAAAGCAAATC CCGGTGTCCCAAAGCAAGGAATTTAATCATTTTGTGTGACATGAAAGTAAA TCCAGTCCTGCCAATGAGAAGAAAAAGACACAGCAAGTTGCAGCGTTTAT AGTCTGCTTTTACATCTGAACCTCTGTTTTTGTTATTTAAGGTGAAGCAGCA TCTGGGTGTGAGAGTGAAACAAGCGTCTCTGAAGACTGCTCAGGGCTATCC TCTCAGAGTGACATTTTAACCACTCAGGTAAAAAGCGTGTGTGTGTGTGCA CATGCGTGTGTGTGGTGTCCTTTGCATTCAGTAGTATGTATCCCACATTCTT AGGTTTGCTGACATCATCTCTTTGAATTAATGGCACAATTGTTTGTGGTTCA TTGTCTCCTTAAATTAGACTGTAAGCACCTTGATGGAA-3'
BREAST CANCER PARTICIPANT (31)	5'GTTWMMTCCCTWAATTACTGGTGGACTTACTTCTGGTTTCATTTTATAAA AGCAAATCCMGGTGTCCCWWAGCAAGGAATTTAATCATTTTGTGTGACAT GAAAGTAAATCCAGTCCTGCCAATGAGAAGAAAAAGACACAGCAAGTTGC AGCGTTTATAGTCTGCTTTTACATCTGAACCTCTGTTTTTGTTATTTAAGGTG AAGCAGCATCTGGGTGTGARAGTGAAACAAGCGTCTCTGAAGACTGCTCA GGGCTATCCTCTCAGAGTGACATTTTAACCACTCAGGTAAAAAGCGTGTGT GTGTGTGCACATGCGTGTGTGTGGTGTCCTTTGCATTCAGTAGTATGTATCC CACATTCTTAGGTTTGCTGACATCATCTCTTTGAATTAATGGCACAATTGTT TGTGGTTCATTGTCTCCTTAAATTAGACTGTAAGCACCTTGATGGAA-3'
BREAST CANCER PARTICIPANT (32)	5'CYCCCTWAATTACTGGTGGACTTACTTCTGGTTTCATTTTATAAAAGCAA ATCCCGGTGTCCCWWWGCAAGGAATTTAATCATTTTGTGTGACATGAAAG TAAATCCAGTCCTGCCAATGAGAAGAAAAAGACACAGCAAGTTGCAGCGT TTATAGTCTGCTTTTACATCTGAACCTCTGTTTTTGTTATTTAAGGTGAAGC AGCATCTGGGTGTGAGAGTGAAACAAGCGTCTCTGAAGACTGCTCAGGGCT ATCCTCTCAGAGTGACATTTTAACCACTCAGGTAAAAAGCGTGTGTGTGTG TGCACATGCGTGTGTGTGGTGTCCTTTGCATTCAGTAGTATGTATCCCACAT TCTTAGGTTTGCTGACATCATCTCTTTGAATTAATGGCACAATTGTTTGTGG TTCATTGTCTCCTTAAATTAGACTGTAAGCACCTTGATGGAA-3'
BREAST CANCER PARTICIPANT (40)	5'TCCTAAATTACTGGTGGACTTACTTCTGGTTTCATTTTATAAAAGCAAATC CCGGTGTCCCAAAGCAAGGAATTTAATCATTTTGTGTGACATGAAAGTAAA TCCAGTCCTGCCAATGAGAAGAAAAAGACACAGCAAGTTGCAGCGTTTAT AGTCTGCTTTTACATCTGAACCTCTGTTTTTGTTATTTAAGGTGAAGCAGCA TCTGGGTGTGAGAGTGAAACAAGCGTCTCTGAAGACTGCTCAGGGCTATCC TCTCAGAGTGACATTTTAACCACTCAGGTAAAAAGCGTGTGTGTGTGTGCA CATGCGTGTGTGTGGTGTCCTTTGCATTCAGTAGTATGTATCCCACATTCTT AGGTTTGCTGACATCATCTCTTTGAATTAATGGCACAATTGTTTGTGGTTCA TTGTCTCCTTAAATTAGACTGTAAGCACCTTGATGGAA-3'

BREAST CANCER PARTICIPANT (43)	5'ATCCGTAATTACTGGTGGACTTACTTCTGGTTTCATTTTATAAAAGCAAAT CCAGGTGTCCCAAAGCAAGGAATTTAATCATTTTGTGTGACATGAAAGTAA ATCCAGTCCTGCCAATGAGAAGAAAAAGACACAGCAAGTTGCAGCGTTTA TAGTCTGCTTTTACATCTGAACCTCTGTTTTTGTTATTTAAGGTGAAGCAGC ATCTGGGTGTGAGAGTGAAACAAGCGTCTCTGAAGACTGCTCAGGGCTATC CTCTCAGAGTGACATTTTAACCACTCAGGTAAAAAGCGTGTGTGTGTGTGC ACATGCGTGTGTGTGGTGTCCTTTGCATTCAGTAGTATGTATCCCACATTCT TAGGTTTGCTGACATCATCTCTTTGAATTAATGGCACAATTGTTTGTGGTTC ATTGTCTCCTTAAATTAGACTGTAAGCACCTTGATGGAA-3'
BREAST CANCER PARTICIPANT (44)	5'TCCTAATTACTGGTGGACTTACTTCTGGTTTCATTTTATAAAAGCAAATCC MGGTGTCCCWWAGCAAGGAATTTAATCATTTTGTGTGACATGAAAGTAAA TCCAGTCCTGCCAATGAGAAGAAAAAGACACAGCAAGTTGCAGCGTTTAT AGTCTGCTTTTACATCTGAACCTCTGTTTTTGTTATTTAAGGTGAAGCAGCA TCTGGGTGTGAGAGTGAAACAAGCGTCTCTGAAGACTGCTCAGGGCTATCC TCTCAGAGTGACATTTTAACCACTCAGGTAAAAAGCGTGTGTGTGTGTGCA CATGCGTGTGTGTGGTGTCCTTTGCATTCAGTAGTATGTATCCCACATTCTT AGGTTTGCTGACATCATCTCTTTGAATTAATGGCACAATTGTTTGTGGTTCA TTGTCTCCTTAAATTAGACTGTAAGCACCTTGATGGAA-3'
BREAST CANCER PARTICIPANT (46)	5'CCCCGTAAATTACTGGTGGACTTACTTCTGGTTTCATTTTATAAAAGCAAA TCCCGGTGTCCCAAAGCAAGGAATTTAATCATTTTGTGTGACATGAAAGTA AATCCAGTCCTGCCAATGAGAAGAAAAAGACACAGCAAGTTGCAGCGTTT ATAGTCTGCTTTTACATCTGAACCTCTGTTTTTGTTATTTAAGGTGAAGCAG CATCTGGGTGTGAGAGTGAAACAAGCGTCTCTGAAGACTGCTCAGGGCTAT CCTCTCAGAGTGACATTTTAACCACTCAGGTAAAAAGCGTGTGTGTGTGTG CACATGCGTGTGTGTGGTGTCCTTTGCATTCAGTAGTATGTATCCCACATTC TTAGGTTTGCTGACATCATCTCTTTGAATTAATGGCACAATTGTTTGTGGTT CATTGTCTCCTTAAATTAGACTGTAAGCACCTTGATGGAA-3'
BREAST CANCER PARTICIPANT (47)	5'TACCTWMMATCCTAATTACTGGTGGACTTACTTCTGGTTTCATTTTATAA AAGCAAATCCMGGTGTCCCAWWGCAAGGAATTTAATCATTTTGTGTGACA TGAAAGTAAATCCAGTCCTGCCAATGAGAAGAAAAAGACACAGCAAGTTG CAGCGTTTATAGTCTGCTTTTACATCTGAACCTCTGTTTTTGTTATTTAAGGT GAAGCAGCATCTGGGTGTGAGAGTGAAACAAGCGTCTCTGAAGACTGCTC AGGGCTATCCTCTCAGAGTGACATTTTAACCACTCAGGTAAAAAGCGTGTG TGTGTGTGCACATGCGTGTGTGTGGTGTCCTTTGCATTCAGTAGTATGTATC CCACATTCTTAGGTTTGCTGACATCATCTCTTTGAATTAATGGCACAATTGT TTGTGGTTCATTGTCTCCTTAAATTAGACTGTAAGCACCTTGATGGAA-3'

BREAST CANCER PARTICIPANT (48)	5'TACTTAMATCCTAATTACTGGTGGACTTACTTCTGGTTTCATTTTATAAAA GCAAATCCAGGTGTCCCAAAGCAAGGAATTTAATCATTTTGTGTGACATGA AAGTAAATCCAGTCCTGCCAATGAGAAGAAAAAGACACAGCAAGTTGCAG CGTTTATAGTCTGCTTTTACATCTGAACCTCTGTTTTTGTTATTTAAGGTGAA GCAGCATCTGGGTGTGAGAGTGAAACAAGCGTCTCTGAAGACTGCTCAGG GCTATCCTCTCAGAGTGACATTTTAACCACTCAGGTAAAAAGCGTGTGTGT GTGTGCACATGCGTGTGTGTGGTGTCCTTTGCATTCAGTAGTATGTATCCCA CATTCTTAGGTTTGCTGACATCATCTCTTTGAATTAATGGCACAATTGTTTG TGGTTCATTGTCTCCTTAAATTAGACTGTAAGCACCTTGATGGAA-3'
BREAST CANCER PARTICIPANT (52)	5'ACCCTAATTACTGGTGGACTTACTTCTGGTTTCATTTTATAAAAGCAAATC CMGGTGTCCCAAAGCAAGGAATTTAATCATTTTGTGTGACATGAAAGTAAA TCCAGTCCTGCCAATGAGAAGAAAAAGACACAGCAAGTTGCAGCGTTTAT AGTCTGCTTTTACATCTGAACCTCTGTTTTTGTTATTTAAGGTGAAGCAGCA TCTGGGTGTGAGAGTGAAACAAGCGTCTCTGAAGACTGCTCAGGGCTATCC TCTCAGAGTGACATTTTAACCACTCAGGTAAAAAGCGTGTGTGTGTGTGCA CATGCGTGTGTGTGGTGTCCTTTGCATTCAGTAGTATGTATCCCACATTCTT AGGTTTGCTGACATCATCTCTTTGAATTAATGGCACAATTGTTTGTGGTTCA TTGTCTCCTTAAATTAGACTGTAAGCACCTTGATGGAA-3'
BREAST CANCER PARTICIPANT (53)	5'CCTTTMMATCCCTAATTACTGGTGGACTTACTTCTGGTTTCATTTTATAAA AGCAAATCCCGGTGTCCCAAAGCAAGGAATTTAATCATTTTGTGTGACATG AAAGTAAATCCAGTCCTGCCAATGAGAAGAAAAAGACACAGCAAGTTGCA GCGTTTATAGTCTGCTTTTACATCTGAACCTCTGTTTTTGTTATTTAAGGTGA AGCAGCATCTGGGTGTGAGAGTGAAACAAGCGTCTCTGAAGACTGCTCAG GGCTATCCTCTCAGAGTGACATTTTAACCACTCAGGTAAAAAGCGTGTGTG TGTGTGCACATGCGTGTGTGTGGTGTCCTTTGCATTCAGTAGTATGTATCCC ACATTCTTAGGTTTGCTGACATCATCTCTTTGAATTAATGGCACAATTGTTT GTGGTTCATTGTCTCCTTAAATTAGACTGTAAGCACCTTGATGGAA-3'
BREAST CANCER PARTICIPANT (59)	5'TCCCTWAATTWACTGGGTGGACTTACTTCTGGTTTCATTTTATAAAAGCA AATCCAGGTGYCCCWTWWKCAAGGAATTTAATCATTTTGTGTGACATGAA AGTAAATCCAGTCCTGCCAATGAGAAGAAAAAGACACAGCAAGTTGCAGC GTTTATAGTCTGCTTTTACATCTGAACCTCTGTTTTTGTTATTTAAGGTGAA GCAGCATCTGGGTGTGAGAGTGAAACAAGCGTCTCTGAAGACTGCTCAGG GCTATCCTCTCAGAGTGACATTTTAACCACTCAGGTAAAAAGCGTGTGTGT GTGTGCACATGCGTGTGTGTGGTGTCCTTTGCATTCAGTAGTATGTATCCCA CATTCTTAGGTTTGCTGACMTCATCTCTTTGAATTAATGGCACAATTGTTTG TGGTTCATTGTCTCCTTAAATTAGACTGTAAGCACCTTGATGGAA-3'

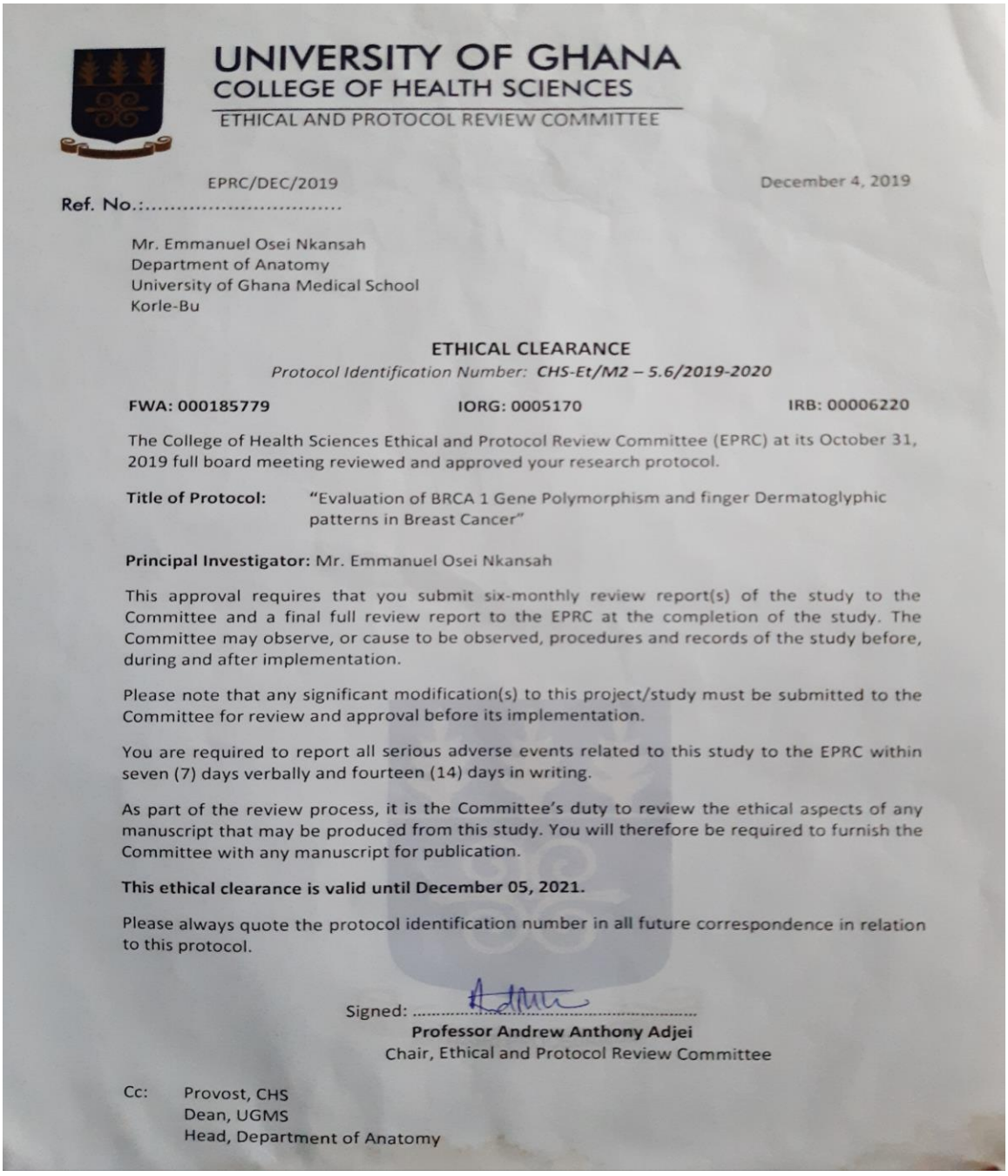
BREAST CANCER PARTICIPANT (61)	5'CTTTAMATCCTAATTACTGGTGGACTTACTTCTGGTTTCATTTTATAAAAG CAAATCCMGGTGTCCCAAAGCAAGGAATTTAATCATTTTGTGTGACATGAA AGTAAATCCAGTCCTGCCAATGAGAAGAAAAAGACACAGCAAGTTGCAGC GTTTATAGTCTGCTTTTACATCTGAACCTCTGTTTTTGTTATTTAAGGTGAA GCAGCATCTGGGTGTGAGAGTGAAACAAGCGTCTCTGAAGACTGCTCAGG GCTATCCTCTCAGAGTGACATTTTAACCACTCAGGTAAAAAGCGTGTGTGT GTGTGCACATGCGTGTGTGTGGTGTCCTTTGCATTCAGTAGTATGTATCCCA CATTCTTAGGTTTGCTGACATCATCTCTTTGAATTAATGGCACAATTGTTTG TGGTTCATTGTCTCCTTAAATTAGACTGTAAGCACCTTGATGGAA-3'
BREAST CANCER PARTICIPANT (66)	5'CTGCTTWAMATCCTAATTACTGGTGGACTTACTTCTGGTTTCATTTTATAA AAGCAAATCCMGGTGTCCCWAAGCAAGGAATTTAATCATTTTGTGTGACA TGAAAGTAAATCCAGTCCTGCCAATGAGAAGAAAAAGACACAGCAAGTTG CAGCGTTTATAGTCTGCTTTTACATCTGAACCTCTGTTTTTGTTATTTAAGGT GAAGCAGCATCTGGGTGTGAGAGTGAAACAAGCGTCTCTGAAGACTGCTC AGGGCTATCCTCTCAGAGTGACATTTTAACCACTCAGGTAAAAAGCGTGTG TGTGTGTGCACATGCGTGTGTGTGGTGTCCTTTGCATTCAGTAGTATGTATC CCACATTCTTAGGTTTGCTGACATCATCTCTTTGAATTAATGGCACAATTGT TTGTGGTTCATTGTCTCCTTAAATTAGACTGTAAGCACCTTGATGGAA-3'
APPARENTLY HEALTHY PARTICIPANT (1)	5'CTTTWMATCCTAATTACTGGTGGACTTACTTCTGGTTTCATTTTATAAAAG CAAATCCCGGTGTCCCWWAGCAAGGAATTTAATCATTTTGTGTGACATGA AAGTAAATCCAGTCCTGCCAATGAGAAGAAAAAGACACAGCAAGTTGCAG CGTTTATAGTCTGCTTTTACATCTGAACCTCTGTTTTTGTTATTTAAGGTGAA GCAGCATCTGGGTGTGAGAGTGAAACAAGCGTCTCTGAAGACTGCTCAGG GCTATCCTCTCAGAGTGACATTTTAACCACTCAGGTAAAAAGCGTGTGTGT GTGTGCACATGCGTGTGTGTGGTGTCCTTTGCATTCAGTAGTATGTATCCCA CATTCTTAGGTTTGCTGACATCATCTCTTTGAATTAATGGCACAATTGTTTG TGGTTCATTGTCTCCTTAAATTAGACTGTAAGCACCTTGATGGAA-3'
APPARENTLY HEALTHY PARTICIPANT (2)	5'CCCCTAATTACTGGTGGACTTACTTCTGGTTTCATTTTATAAAAGCAAATC CCGGTGTCCCWWAGCAAGGAATTTAATCATTTTGTGTGACATGAAAGTAA ATCCAGTCCTGCCAATGAGAAGAAAAAGACACAGCAAGTTGCAGCGTTTA TAGTCTGCTTTTACATCTGAACCTCTGTTTTTGTTATTTAAGGTGAAGCAGC ATCTGGGTGTGAGAGTGAAACAAGCGTCTCTGAAGACTGCTCAGGGCTATC CTCTCAGAGTGACATTTTAACCACTCAGGTAAAAAGCGTGTGTGTGTGTGC ACATGCGTGTGTGTGGTGTCCTTTGCATTCAGTAGTATGTATCCCACATTCT TAGGTTTGCTGACATCATCTCTTTGAATTAATGGCACAATTGTTTGTGGTTC ATTGTCTCCTTAAATTAGACTGTAAGCACCTTGATGGAA-3'

APPARENTLY HEALTHY PARTICIPANT (3)	5'GCCCTWAAATTTACTGGTGGACTTACTTCTGGTTTCATTTTATAAAAGCA AATCCCGGTGYCCCCMAWKCAAGGAATTTAATCATTTTGTGTGACATGAA AGTAAATCCAGTCCTGCCAMTGAGAAGAAAAAGACACAGCAAGTTGCAGC GTTTATAGTCTGCTTTTACATCTGAACCTCTGTTTTTGTTATTTAAGGTGAA GCAGCATCTGGGTGTGAGAGTGAAACAAGCGTCTCTGAAGACTGCTCAGG GCTATCCTCTCAGAGTGACATTTTAACCACTCAGGTAAAAAGCGTGTGTGT GTGWGCACATGCRTGTG-3'
APPARENTLY HEALTHY PARTICIPANT (6)	5'CTTWMATCCTATTACTGGTGGACTTACTTCTGGTTTCATTTTATAAAAGCA AATCCCGGTGTCCCWWWGCAAGGAATTTAATCATTTTGTGTGACATGAAA GTAAATCCAGTCCTGCCAATGAGAAGAAAAAGACACAGCAAGTTGCAGCG TTTATAGTCTGCTTTTACATCTGAACCTCTGTTTTTGTTATTTAAGGTGAAGC AGCATCTGGGTGTGAGAGTGAAACAAGCGTCTCTGAAGACTGCTCAGGGCT ATCCTCTCAGAGTGACATTTTAACCACTCAGGTAAAAAGCGTGTGTGTGTG TGCACATGCGTGTGTGTGGTGTCCTTTGCATTCAGTAGTATGTATCCCACAT TCTTAGGTTTGCTGACATCATCTCTTTGAATTAATGGCACAATTGTTTGTGG TTCATTGTCTCCTTAAATTAGACTGTAAGCACCTTGATGGAA-3'
APPARENTLY HEALTHY PARTICIPANT (8)	5'GCCCCTAAATTWMCTGGGTGGACTTACTTCTGGTTTCATTTTATAAAAGC AAATCCMGGTGTCCCAWWKCAAGGAATTTAATCATTTTGTGTGACATGAA AGTAAATCCAGTCCTGCCAATGAGAAGAAAAAGACACAGCAAGTTGCAGC GTTTATAGTCTGCTTTTACATCTGAACCTCTGTTTTTGTTATTTAAGGTGAA GCAGCATCTGGGTGTGAGAGTGAAACAAGCGTCTCTGAAGACTGCTCAGG GCTATCCTCTCAGAGTGACATTTTAACCACTCAGGTAAAAAGCGTGTGTGT GTGTGCACATGCGTGTGTGTGGTGTCCTTTGCATTCAGTAGTATGTATCCCA CATTCTTAGGTTTGCTGACATCMTCTCTTTGAATTAATGGCACAATTGTTTG TGGTTCATTGTCTCCTTAAATTAGACTGTAAGCACCTTGATGGA-3'

APPENDIX V

A picture of exon 11 sequence of BRCA1 gene

APPENDIX VI



UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES
ETHICAL AND PROTOCOL REVIEW COMMITTEE

Ref. No.: EPRC/DEC/2019 December 4, 2019

Mr. Emmanuel Osei Nkansah
Department of Anatomy
University of Ghana Medical School
Korle-Bu

ETHICAL CLEARANCE

Protocol Identification Number: ***CHS-Et/M2 – 5.6/2019-2020***

FWA: 000185779 **IORG: 0005170** **IRB: 00006220**

The College of Health Sciences Ethical and Protocol Review Committee (EPRC) at its October 31, 2019 full board meeting reviewed and approved your research protocol.

Title of Protocol: "Evaluation of BRCA 1 Gene Polymorphism and finger Dermatoglyphic patterns in Breast Cancer"

Principal Investigator: Mr. Emmanuel Osei Nkansah

This approval requires that you submit six-monthly review report(s) of the study to the Committee and a final full review report to the EPRC at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study before, during and after implementation.

Please note that any significant modification(s) to this project/study must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the EPRC within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

This ethical clearance is valid until December 05, 2021.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed: .....
Professor Andrew Anthony Adjei
Chair, Ethical and Protocol Review Committee

Cc: Provost, CHS
Dean, UGMS
Head, Department of Anatomy

Ethical clearance from the Ethical and Protocol Review Committee of the College of Health Science, University of Ghana