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Associations Between Hereditary Breast Cancer Susceptibility Gene Alterations and Aggressive Tumor Phenotype in Women Diagnosed with Breast Cancer

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ASSOCIATIONS BETWEEN HEREDITARY BREAST CANCER SUSCEPTIBILITY GENE ALTERATIONS AND AGGRESSIVE TUMOR PHENOTYPE IN WOMEN DIAGNOSED WITH BREAST CANCER

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ABSTRACT

The first chapter of this dissertation serves as an overview of the background and significance of this study, the associations between hereditary breast cancer susceptibility gene alterations (GA) and aggressive tumor phenotypes in women with breast cancer. The body of work in all chapters focuses on the topics of breast cancer genomics, inflammatory breast cancer, the application of Protection Motivation Theory to guide prevention strategies for women with breast cancer diagnosis and positive genetic alteration, and the associations between hereditary breast cancer susceptibility GAs and aggressive tumor phenotype.

The second chapter is a review of literature to discuss breast cancer genomics, specifically two genes, Ataxia Telangiectasia Mutated (*ATM*) and the Partner and Localizer of *BRCA2* (*PALB2*). The results of the review highlight the importance of identifying two new breast cancer susceptibility genes, other than the well known *BRCA1* and *BRCA2*, and the requirement of including these genes in standard breast cancer genetic testing.

The third chapter is a review of literature to describe inflammatory breast cancer (IBC), pathogenicity of the disease and genomic investigation of IBC. IBC is an aggressive type of breast cancer with poor prognosis responsible for 2.5% of all new breast cancers. The majority of IBC patients are diagnosed with triple negative breast cancer (TNBC), which is the aggressive phenotype.

The fourth chapter provides an overview of literature that describes the Protection Motivation Theory (PMT) and how it has been applied in a variety of research settings.

A pilot study is suggested including PMT and its application for breast cancer prevention strategies uptake by patients who have a diagnosis of breast cancer. The pilot study would use a focus group of women with breast cancer to determine if the theory can guide prevention strategies for women with a mutation that causes the high risk to develop multiple types of primary cancers over their lifetime.

The fifth chapter describes the dissertation work; a quantitative study that analyzes associations between aggressive breast cancer phenotypes in a population of women at high risk for hereditary breast cancer and specific GAs.

The final chapter, is a synthesis of all manuscripts related to the breast cancer in the high risk population of women to develop this dreaded disease; breast cancer genomic investigation of *ATM* and *PALB2* genes, the aggressive IBC, PMT application for breast cancer prevention strategies uptake and association of GAs and aggressive breast cancer phenotype. The populations in all the articles were women diagnosed with breast cancer and were at high risk of hereditary breast cancer syndromes.

As a result of these manuscripts, it is expected to make suggestions for genetic testing guidelines to include multi-panel genetic testing for all eligible individuals as well as inclusion of tumor biomarkers and ethnicity in eligibility criteria. It is also recommended to apply PMT to encourage adherence to prevention strategies in order to reduce the risk of additional cancer primary.

DEDICATION

I would like to dedicate my dissertation work to my family and friends. An exceptional appreciation to my loving husband, Jahanali Oveissi whose words of inspiration helped me to go through the entire doctoral program. I also dedicate this work to my children, Ceerat Emma Oveissi and Farnam Sean Oveissi who have supported me throughout the doctoral program. I will always appreciate all they have done to support me.

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CHAPTER ONE

INTRODUCTION

The American Cancer Society predicted 231,840 new cases of invasive breast cancer and 60,290 cases of breast carcinoma *in situ* to be diagnosed in 2015 (American Cancer society, 2015). Despite an increasing body of knowledge and clear standardized recommendations, there remain a number of women who do not follow breast cancer screening (34%), prevention and early detection strategies (less than 0.2% of women in United States use Tamoxifen and approximately 3% of Australian women follow the prevention guidelines) (American Cancer Society, 2015; Cancer Genome Atlas Network, 2012; Evans, Lalloo, Shenton, Boggis, & Howell, 2001; Ralph et al., 2014).

Almost 10 % (approximately 20,000) of breast cancer cases are due to germline mutations (Tung, et al., 2015). Studies targeting tumor biology of breast cancer and epidemiology of the disease have been utilized to guide prevention, early detection and risk reduction (Cancer Genome Atlas Network, 2012; Ralph et al., 2014; Youlden et al., 2012). It is especially important that hereditary breast cancer gene mutation carriers with risk for early onset breast cancer follow strategies to detect the malignancy early (Fositra et al., 2012; Gonzalez- Angulo et al., 2011; Lee et al., 2011).

Technologic advances in human genomics, in combination with faster and less costly gene sequencing results, have enhanced the research for the advancement of targeted therapy of molecular biomarkers (Grada, & Weinbrecht, 2013; Hawthorn, Luce, Stei, & Rothschild, 2010; Staren, et al., 2014). Incorporation of genetic information using new technologies and utilizing the vast research available have given us the resources to

identify breast cancer earlier when it may be more treatable and with less invasive methods and to predict patient outcome.

Commonly found factors associated with a mutation related to a hereditary breast cancer syndrome include: 1) family history of breast cancer with close blood relatives (three generations) who are diagnosed at younger age (under the age of 50) or at least two blood relatives diagnosed with breast cancer at any age; 2) one close family member with ovarian cancer; or, 3) two blood relatives with pancreatic cancer (NCCN, 2015). Environmental factors, also known as exogenous factors (such as carcinogen exposures) as well as endogenous factors could impact breast cancer onset (See Table 1.1) (Kushi, et al., 2012; Li, 2009). Endogenous factors affecting breast cancer were the variables that were included in this study.

Table 1.1

Endogenous and exogenous factors in cancer

Endogenous Cause of Cancer	**Exogenous Cause of Cancer**
Hereditary	Environmental factors
Age	Tobacco use
Hormones	Radiation therapy
Ethnic background	UV exposure
	Diet and lifestyle
	Carcinogen exposure

Note. (Catsburg, Miller, & Rohan, 2014; Kushi et al., 2012; Li, 2009).

Breast cancer incidence and biomarkers are influenced by ethnicity and race. African American women in the United States tend to be diagnosed with a more aggressive type of breast cancer as compared to Caucasian women (Boone, et al., 2014; Iqbal, et al., 2015). African American women are diagnosed at a younger age while Caucasian women have a higher incidence after the age of 40, however, the screening and prevention guidelines is not specifically focused on this ethnic group (Gail et al., 2007; Iqbal et al., 2015; NCCN, 2015). Researchers propose that the variation could be due to their lifestyle, diet and family history (Gail et al., 2007; Iqbal et al., 2015).

Individuals, who are carriers for a mutated gene associated with one of the hereditary syndromes, consistent with the autosomal dominant inheritance pattern commonly seen in cancer, have a higher risk of breast cancer at a younger age of onset than sporadic cancer. The breast cancers associated with damaged germline DNA are often bilateral plus an increased incidence of other types of cancer (Bernstein et al., 2010; Tischkowitz et al., 2012).

There is a potential knowledge gap in applying the genetic information and in incorporating prevention strategies into practice specifically in diverse population. This knowledge gap was utilized to develop a plan of dissertation study. The primary purpose of this body of work was to look at aggressive features associated with breast cancer and to identify potential strategies to combat the troubling phenomena. This lead to the following aims:

1. Identify factors associated with the aggressive nature of inflammatory breast cancer;
2. Review the nature of two new breast cancer susceptibility genes;
3. Describe a theory that has the potential to motivate women at high risk to develop an aggressive cancer to pursue prevention and screening strategies;
4. Determine if breast cancer susceptibility genetic alterations (GA) are associated with an aggressive tumor phenotype in women with a new diagnosis of breast cancer; and,
5. Determine if the aggressive breast cancer tumor phenotype is associated with a specific gene variant(s).

It is well described that cancer is a genetic disorder caused by both acquired and inherited mutations (Knudson, 1996; Previati, et al., 2013; Rich, Woodson, Litton, & Arun, 2015; Wooster, et al., 1994). Acquired changes, such as the changes in DNA that occur over a lifetime that are caused by environmental factors including, radiation exposure, age and viruses. The acquired changes include an increase in function caused by gene fusions, insertions, duplications and translocations and alteration of tumor suppressor activity through rearrangements and deletions (Previati, et al., 2013; Sebestyen, Zawisza, & Eyras, 2015). Inherited mutations, also known as germline mutations are passed on from parents to their children and appear in all cells (Knudson, 1996; Wooster, et al., 1994). Breast cancer genetic studies have shown a correlation between *BRCA1* and *BRCA2* mutations as well as particular tumor types like triple negative breast cancer (TNBC)— an aggressive type of breast cancer (Atchley, et al.,

2008; Lee, et al., 2011; Young, et al., 2009). TNBC has also been reported in mutations of *PALB2* (Pern, et al., 2012).

Germline mutations in *BRCA1/2* genes are associated with other tissue types of cancer including ovarian cancer (*BRCA1* with an average lifetime risk of 39 % and *BRCA2* with an average risk of 45%), pancreatic cancer (*BRCA1* with relative risk (RR) of 2.3, *BRCA2* with RR of 3.5) and prostate cancer (*BRCA1* with RR of 1.8, *BRCA2* RR of 4.6) (Castro, et al., 2013; Maier, et al., 2014; Mersch, et al., 2015; Moran, et al., 2012; Petrucelli & Feldman, 2010).

In addition to high penetrance mutations in *BRCA1/2* (the only two genes that are tested together), *PTEN* and *TP53* (*TP53* is seen in one percent of hereditary breast cancers) (Sidransky, et al., 1992), there are other genes that have moderate to high penetrance and are included in multi-gene panel testing for breast cancer syndromes including: *CDH1, STK11, EPCAM, MLH1, MSH2, MSH6, PMS2* and *STK11* (high risk genes); *CHEK2, PALB2* and *ATM* (moderate risk genes); and also include *LH1, MSH2, MSH6, PMS2, BARD1, BRIP1, RAD51C* and *RAD51D* (NCCN, 2015; Schroeder, et al., 2015; Tung, et al., 2015).

Three manuscripts of this dissertation were developed to address the necessity of achieving a better understanding of aggressive breast cancer phenotype and its underlying genetic causes and to state the potential gap in applying the genetic information and incorporating prevention strategies into practice particularly in different ethnic backgrounds. The organization of this dissertation begins with Manuscript I (Chapter 2), "Breast Cancer Genomics." This section investigates two breast cancer susceptibility

genes, *ATM* and *PALB2* and presents updates on guidelines for genetic testing through a comprehensive review of literature. These guidelines offer options to treat women who could previously have had less than potential cure for their cancer. The results of the review highlight the importance of identifying two new breast cancer susceptibility genes, other than the well known *BRCA1* and *BRCA2,* to be included in genetic testing for hereditary breast cancer syndromes and to offer personalized treatment and disease management based on the results.

Beyond the well-known peau d'orange sign at presentation, the second Manuscript (Chapter 3), "Inflammatory Breast Cancer," discusses IBC and how GAs may affect the disease. IBC is a more aggressive type of breast cancer and commonly found to be triple negative breast cancer. This manuscript carefully describes inflammatory immune response and inflammation mechanism involved in tumorigenesis. Treatment and management of the disease is described and based on a comprehensive review of literature.

The third Manuscript (Chapter 4), entitled "Use Of Protection Motivation Theory To Guide Prevention Strategies In Women With Breast Cancer Diagnosis And a Positive Genetic Alteration," investigates literature related to PMT with application to breast cancer prevention, early detection and cancer management. This theory has previously been used in different research studies applying constructs and concepts of Roger's PMT to educate and urge women with breast cancer to follow the strategies to prevent second primaries (Bui et al., 2013; Karmakar, 2013; Lee et al., 2007; Moy, Park, Feibelmann,

Chiang, & Weissman, 2006; Ralph et al., 2014; Vogel, 2015). This manuscript proposes a pilot study to improve motivation of this population of women to follow the guidelines.

Chapter 5 describes a quantitative study, entitled "Associations Between Hereditary Breast Cancer Susceptibility GAs And Aggressive Tumor Phenotype in Women Diagnosed With Breast Cancer." The purpose of this study was to determine if genetic mutation and/or variants of unknown significance (VUS) predict aggressive breast cancer phenotype. This study enrolled 101 women with a breast cancer diagnosis and an increased risk for germline mutation(s) associated with a hereditary breast cancer syndrome. The results suggest a new use of multi-gene panel genetic testing for women with breast cancer, specifically from different ethnic backgrounds.

Chapter 6 synthesizes information presented from the body of work to present the conclusions developed by the author as guided by the chair and committee. Limitations of the study are acknowledged, implications for future research are presented and future plans for the researcher are addressed.

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CHAPTER TWO

BREAST CANCER GENOMICS

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Abstract

The American Cancer Society (ACS) predicts 231,840 new cases of invasive breast cancer and 60,290 cases of breast carcinoma in situ to be diagnosed in 2015 (American Cancer Society, 2015). Excluding non-melanoma skin cancer, breast cancer is the most common type of cancer in U.S. women; responsible for approximately 14% of new cancer diagnoses (National Cancer Institute, 2014). There are currently 28 genes included in the National Comprehensive Cancer Network (NCCN) guidelines for testing of genetic mutations associated with hereditary breast cancer, including ataxia telangiectasia mutated (*ATM*) and the partner and localizer of *BRCA2* (*PALB2*) genes (NCCN, 2015; Schroeder et al., 2015; Tung et al., 2015).

The purpose of this article is to provide an update on breast cancer genomics targeting two lesser-known pathogenic gene mutations recently added to the NCCN guidelines; *ATM* and *PALB2* (NCCN, 2015). A comprehensive review and synthesis of current literature was completed to describe recent information on *ATM* and *PALB2* and recommendations for the detection and treatment of breast cancer caused by these two gene mutations. Recent advances in genetics-related breast cancer research, testing and clinical implications are stressed. Healthcare providers play a critical role in breast cancer care and are actively engaged with patients and their families. This manuscript offers an update to information in order to understand the latest advances in breast cancer

genomics and to translate the new genomic knowledge into clinical practice in order to provide the ultimate in patient care.

Methods

A comprehensive literature search of PubMed, Google Scholar, CINAL, ProQuest Nursing and Allied Health, Cochrane Database and Web of Science was conducted using the search terms "breast cancer," "high risk breast cancer," "breast cancer genes," "*ATM*," "*BRCA1/2*," "*PALB2*," "breast cancer genetic testing," and "breast cancer genomics." The search generated 146 articles from 2009-2015. Original research articles with a focus on the genetics of *ATM* and *PALB2* genes, their variations as well as molecular pathways with comparison to other hereditary breast cancer genes were selected. Exclusion criteria included articles that were not in English, papers not related to breast cancer, research articles with a lack of evidence and animal studies. Careful evaluation of all the articles identified 26 quantitative studies relevant to the purpose of the project.

Introduction

Numerous studies have been conducted on the topic of breast cancer over the past few years. Advances in DNA sequencing have helped scientists make progress in cancer screening, prevention and treatment (Chin, Hahn, Getz, & Meyerson, 2011; Grada, & Weinbrecht, 2013; Hawthorn, Luce, Stei, & Rothschild, 2010; Staren, et al., 2014). Innovations in technology targeting human genomics (See Table 2.1) have led to superior biomedical research results via expression profiling, DNA microarray, array comparative genomic hybridization, next-generation sequencing (NGS) and massive parallel

sequencing providing valuable genetic information with detailed, faster and more reliable results. Developing a better understanding of breast cancer and reducing testing costs will help providers to implement more personalized decisions for every patient (Chin, Hahn, Getz, & Meyerson, 2011; Grada, & Weinbrecht, 2013; Hawthorn, Luce, Stei, & Rothschild, 2010; Staren, et al., 2014). There are limitations to each of these techniques. Overall massive data requires highly trained professionals to analyze the data and supercomputers to store the outcome (Grada, & Weinbrecht, 2013; Previati, et al., 2013).

The National Comprehensive Cancer Network (NCCN) guidelines recently published a new list of breast cancer susceptibility genes, including ataxia telangiectasia mutated (*ATM*) and partner and localizer of *BRCA2* (*PALB2*) genes, to their guidelines (NCCN, 2015). The guidelines addressed the updates based on current breast cancer research to provide eligibility criteria for genetic testing and new strategies for providers and education for patients. According to the newest version of the guidelines (version 2.2015 published in March), patients must meet one or more of breast cancer risk assessment criteria to be eligible for genetic testing for hereditary breast and ovarian cancer (HBOC) syndrome (NCCN, 2015). The additions of new genes lead us to the importance of a comprehensive review of literature specifically on the lesser-known genes to better detect and manage breast cancer and not to focus only on *BRCA1/2* genes. The main objective of this review is to address characteristics of the two new novel breast cancer genes with a discussion of relevance to treatment, patient education and care.

Ataxia Telangiectasia Mutated (*ATM*)

Ataxia telangiectasia mutated protein (ATM protein) is encoded by the *ATM* gene, is part of the phosphoinositide-3 kinase (PI3)/phosphoinositide-4 protein kinase (PI4) family and plays an important role in the repair of DNA double strand breaks. ATM protein kinase is an enzyme that phosphorylates proteins involved in DNA double strand damage repair in order to maintain DNA stability (Goldgar, et al., 2011). By promoting a delay in the G1 phase of the cell cycle, there is time to repair the DNA before progressing through the rest of the phases. This delay for repair prevents double strand breaks from being established in the new cells (Khoronenkova & Dianov, 2015; Goldgar, et al., 2011). The repair of double strand breaks is crucial for DNA stability. If the damage is not repaired and replication continues, uncontrolled proliferation of the damaged DNA could result in the development of cancer (Khoronenkova & Dianov, 2015).

Ionizing radiation is a carcinogen that causes a double strand break of the DNA. Therefore, patients with already mutated genes are at higher risk of cancer when exposed to ionizing radiation causing additional DNA breakage (Goldgar, et al., 2011; Bernstein, et al., 2010). There are some other carcinogens causing DNA breakage including H. Pylori, which is a bacterial carcinogen (Toller et al., 2011), chemical carcinogens such as arsenic (Litwin, Bocer, Dziadkowiec, & Wysocki, 2013) and monocrotophos that is an extremely toxic pesticide (Zhao, Wang, Zhang, Tian, Wang, & Ru, 2015).

The *ATM* gene is involved in a series of molecular pathways associated with genes such as *TP53*, checkpoint kinase 2 (*CHEK2)* and *BRCA1*, all resulting in phosphorylation of the proteins that eventually end with apoptosis, DNA repair and cell cycle checkpoints arrest; important in DNA stability (Bernstein, et al., 2010). People with

deleterious *ATM* missense variants, such as *BRCA1/2* and *CHEK2* mutations, are also at higher risk of developing second breast cancers due to lack of repair mechanisms if they are treated with radiation (Bernstein, et al., 2010). The ATM protein is part of the signaling cascade called ATM-CHEK2-p53; CHEK2 protein and tumor protein p53 (TP53). The ATM protein activates p53 and CHEK2 proteins in response to toxic stress to the cell (Knappskog, et al., 2012). *ATM* genetic mutations have also been associated with an increased incidence of breast cancer (Knappskog, et al., 2012).

Knappskog, et al. (2012) assessed the role of *ATM* mutation in the development of chemotherapy resistance for patients with breast cancer. They conducted gene expression profiling using tumor biopsy samples from participants (N = 71) before starting a neoadjuvant chemotherapy regimen. The researchers found that the ATM-CHEK2-p53 cascade associated with DNA damage repair was responsible for chemotherapy resistance in participants receiving anthracycline/mitomycin-containing regimens.

Typically the p53 and the CHEK2-ATM pathways are responsible for repairing DNA damage due to chemotherapy; mutations or reduction of expression of any genes in the cascade may be the cause for the patient to be resistant to chemotherapy. An alternative regimen, such as vinca alkaloids, can be suggested instead of the anthracycline/mitomycin regimen that causes resistance and allows the tumor to continue to grow (Knappskog, et al., 2012). Since *ATM* is normally involved in the DNA double strand damage repair pathway, women with a pathogenic mutation will have DNA instability, due to DNA damage and cell proliferation. These individuals are at higher risk

of contralateral breast cancer diagnosis after their initial radiation therapy (Bernstein, et al., 2010).

One study identified a rare variant of the *ATM* gene (c.7271T > G) carries a 60 percent risk of breast cancer by age 80, has the same penetrance as *BRCA2* gene mutations and is significantly (p = 0.00008) associated with a breast cancer risk (Goldgar, et al., 2011). This large study (*n* = 2,570) assessed 76 variants in *ATM* among participants included in the NCI sponsored breast cancer registry in New Zealand. Sokolenko, et al. (2014) aimed to assess double heterozygosity of five hereditary breast cancer gene mutations (*BRCA1, CHEK2, ATM,* Nibrin [Nijmegen Breakage syndrome gene] (*NBN*) and Bloom syndrome (*BLM*),) in known breast cancer patients (*n* = 5931). The researchers identified 17 double heterozygotes (See Table 2.1) in the sample population, including *CHEK2* and *ATM* mutations, *BRCA1* and *ATM* mutations, *BRCA1* and *CHEK2* mutations and some with *BRCA1* and *BLM* mutations. Sokolenko's results also suggested that individuals with double heterozygosity in breast cancer predisposition genes tend to acquire the disease at a younger age (≤50) with a more aggressive phenotype (Sokolenko, et al., 2014).

Partner and Localizer of *BRCA2* (*PALB2*)

The PALB2 protein is called the 'partner and localizer of *BRCA2'* because it interacts with *BRCA2* and causes a caretaker function (Hartley, et al., 2014; Tischkowitz, et al., 2012). *PALB2* with *BRCA2* coordinates DNA stability in nuclear foci (Xia, et al., 2006) while they interact with *BRCA1* (Zhang, et al., 2009). *PALB2* is considered to act like a "bridge" and interacts with *BRCA1* and *BRCA2* building the *BRCA1-PALB2-*

BRCA2 complex (Fernandes, et al., 2014). This complex is extremely important in the DNA double strand break repair system leading to *BRCA2* mediated homologous recombination repair at the location of damaged DNA (See Table 2.2) (Fernandes, et al., 2014). If DNA double strand breaks are not repaired, they remain after replication and create established somatic mutations with uncontrolled proliferation and potential for cancer development (Fernandes, et al., 2014; Xia, et al., 2006).

According to an Australian population study of women with breast cancer and a family history of cancer, *PALB2* mutations (c.3113G > A) are associated with a high risk of breast cancer. The risk level of *PALB2* mutations (much like *ATM* mutations) is as great as *BRCA1* and *BRCA2* mutations (Southey, et al., 2010). Germline mutations in *PALB2* gene are very rare and account for approximately 1-4 percent of the breast cancer diagnoses in patients who are not *BRCA1/2* carriers (Hartley, et al., 2014).

Rahman, et al. (2006) suggested the germline mutation in *PALB2* gene should be included in the high risk category for development of breast cancer. In his study (n = 923), truncating monoallelic (See Table 2.1) *PALB2* mutations were seen in 10 patients with familial breast cancer. The researchers concluded that individuals with familial breast cancer and truncating mutations of *PALB2* had a 2.3-fold higher risk for developing breast cancer when compared to the control group with non-familial breast cancer (Rahman, et al., 2006). In a later population-based study, patients with bilateral breast cancer (n = 559) were screened and compared to patients with unilateral breast cancer (n = 565) (Tischkowitz, et al., 2012). The statistical analysis detected significant (p = 0.04) pathogenic *PALB2* mutations in patients with contralateral breast cancer when

compared to the unilateral control group. These results identify the need for genetic testing in families who are carriers of the *PALB2* germline mutations in order to guide treatment for clinicians and suggest prevention strategies from further cancer diagnoses within the families (Tischkowitz, et al., 2012).

Hartley, et al. (2014) studied participants (*n* = 17) with negative *BRCA1/2* mutations and tested them for *PALB2* mutations. They detected two deleterious mutations in two participants who also had a very strong family history of breast cancer. Although *PALB2* mutations are rare in the population, results of this study suggest this gene still has a critical role in cancer susceptibility (Hartley, et al., 2014). One of the largest cohort studies in the U.S. assessed 1,479 participants for *PALB2* mutations using Sanger sequencing and quantitative multiplex polymerase chain reaction PCR (See Table 2.1) (Fernandes, et al., 2014). The participants were divided into two groups; "high risk" and "low risk" with risk being based on calculations using age at breast cancer onset and family history of cancer. The sequencing data identified 10 pathogenic mutations (CI = 0.5-1.92) in the high risk group (*n* = 955) and two mutations (CI = -0.5-1.37) in the low risk group (*n* = 524). There were 59 samples with variants of uncertain significance (VUS) (See Table 2.2). These data suggest a low frequency of *PALB2* mutation incidence in patientts with HBOC (Fernandes, et al., 2014).

Clinical characteristics of breast cancer patients with biallelic (See Table 2.2) *BRCA2* and *PALB2* mutations are similar. These genes are part of the Fanconi anemia-breast cancer pathway and are involved in DNA double strand break repair (Adank, van Mil, Gille, Waisfisz, & Meijers-Heijboer, 2011). These gene mutations also share clinical

characteristics such as childhood tumors (biallelic), pancreatic cancer (monoallelic) and female breast cancer (monoallelic) (Adank, et al., 2011).

While several studies reviewed the prevalence of the mutations, Teo, et al. (2013) looked at the tumor morphology to predict the germline *PALB2* mutation in patients with breast cancer. The researchers compared pathology reports from 28 patients who were known carriers for the *PALB2* mutation with 828 registered breast tumors (both groups included women who were diagnosed with breast cancer before the age of 60). The researchers found minimal sclerosis (less than 20 percent) in tumors of the participants with *PALB2* mutations as opposed to *BRCA1/2* mutation carriers with extensive sclerosis; however, there was no identification of significant similar tumor morphology between *PALB2*, *BRCA1* and *BRCA2* mutation carriers. This is intriguing because *PALB2*, *BRCA1* and *BRCA2* are all part of the homologous recombination repair complex (See Table 2.2) (Teo, et al., 2013).

BRCA1 and *PALB2* are involved in transcription regulation by co-activation; they occupy large gene coding regions and are associated with RNA polymerase II (Gardini, Baillat, Cesaroni, & Shiekhatta, 2014). These genes are also involved in the retinoic acid signal, an inhibitor signal to tumor growth, so both of the PALB2 and BRCA1 proteins have critical roles in the regulation of gene expression in growth pathways causing proliferation (Gardini, et al., 2014). Loss of function mutations are also reported by a research study published in 2014 that suggests *PALB2* mutation carriers have almost the same frequency as *BRCA2* patients and recommends following the same management strategies as what is suggested for *BRCA2* mutation carriers (Antoniou et al., 2014).

Based on previously mentioned studies, although with limited sample numbers, inclusion of these new genes in breast cancer genetic testing panels can be beneficial to individuals considered at high- risk for developing breast cancer.

Conclusion

Clinical genetic testing with multi-gene panels of carriers for *ATM* and *PALB2* gene mutations would be appropriate for the population of individuals considered to be "high risk" for hereditary breast cancer syndromes, even though the prevalence appears to be low. According to the genetic testing registry (GTR) at the National Center For Biotechnology Information (NCBI), there are several clinical genetic testing laboratories available for breast cancer that include *ATM* and *PALB2* mutations in their multi gene panel testing (GTR, 2014). Currently, 28 genes are included in NCCN guidelines: *APC, ATM, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, EPCA, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, PTEN, RAD50, RAD51C, RAD51D, RINT1, SMAD4, STK11, TP53* (NCCN, 2015). There are no standardized recommendations for prevention and/or screening protocols for *ATM* and *PALB2* mutation carriers. While clinicians also investigate the new research publications for guidance, many use the NCCN guidelines for genetic testing for significant family history of breast cancer or diagnosis at a young age (≤50) (NCCN, 2015).

Since most studies with a focus on *ATM* and *PALB2* genes have a low number of participants, decreasing the statistical power, the findings emphasize the importance of large studies to assess the variants of *ATM* and *PALB2* genes in order to improve screening, diagnosis, treatment and disease management. Because different disease

management strategies are available based on genetic information, genetic testing for all breast cancer susceptibility genes (not just for *BRCA1/2* genes) will guide clinicians to offer more personalized treatment based on patients' biomarkers. Healthcare providers play a critical role in patient care from screening to diagnosis and treatment of breast cancer. Current knowledge of breast cancer genomics and monitoring the rapidly changing guidelines for cancer genetics will guide clinicians to accurately offer genetic testing and implement appropriate strategies to promote better outcomes for breast cancer patients.

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Table 2.1

Technologies targeting human genomics

Technology	**Application to Cancer**
Expression Profiling by DNA Microarray	Prediction of clinical outcome
Array Comparative Genomic Hybridization	Discovery of variations in DNA copy number
Next-Generation Sequencing	Genomic profiling to guide clinical management
Massive Parallel Sequencing	Detection of cancer of unknown primary
Sanger Sequencing	DNA sequencing method using DNA primers
Quantitative Multiplex Polymerase Chain Reaction	Sequencing method using PCR to amplify DNA sequences at the same time for large genomic rearrangements

Table 2.2

Definitions

Term	**Definition**
Homologous Recombination	Exchange of similar or same nucleotides to repair DNA double strand breaks
Variants of Uncertain Significance (VUS)	A variation of genetic sequence with unknown pathogenic association
Double Heterozygosity	One person with two different gene mutations
Monoallelic Truncating Mutation	Heterozygote (single allele) mutation in one of three stop codons
Biallelic Mutation	Mutation in both alleles (both version of the same gene)

CHAPTER THREE

INFLAMMATORY BREAST CANCER

Abstract

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Inflammation is considered to be the first line of defense against tissue injury and infection. Several chronic diseases such as cancer, arthritis, cardiovascular disease, obesity, type 2-diabetes and Alzheimer's disease are associated with inflammation. Inflammatory breast cancer (IBC) is an uncommon type of breast cancer. Phenotypically it includes erythema, skin irritation and typically, no discernable tumor. The pattern of growth is aggressive, with a poor prognosis and low overall survival rate. Clinical features such as orange peel, swelling and redness diagnose IBC; signs are frequently mistaken for an abscess or mastitis. The disease is also considered to be genetically complex and heterogeneous. The purpose of this paper is to describe the evolution of inflammatory breast cancer, investigate genomic causes of the disease, explain known disease pathogenesis, evaluate different methods of diagnosis and discuss new approaches to personalized care through a comprehensive literature review of scholarly research articles.

Inflammation and Cancer

Inflammation is the first line of defense against injury and infectious factors in the body and overlaps with the immune system to play a crucial role in some human diseases, including cancer, diabetes, asthma, allergy, autoimmune disease, neurodegenerative disease, heart disease and Alzheimer's disease (Libby, 2007; Vodovotz , Bartels &

Chang, 2008). Influenced by several mechanisms affected by oxidative stress, injury, fibrosis and angiogenesis, inflammation is considered to be a protective response that also involves blood cells (platelets, leukocytes, endothelial cells) and proteins (Grivennikov, Greten & Karin, 2010; Kajihara, 2011; Libby 2007). The history of attention to inflammation begins when Aurelius Cornelius Celsus (Roman physician) first described chronic inflammation with four essential signs: pain (*dolor*), redness (*rubor*), heat (*calor*) and swelling (*tumor*) due to changes in perivasculature, hyperemia, increased blood flow and increased permeability (Libby, 2007; Scott, Khan, Cook, & Duronio, 2004).

Biological stress initiates several pathways and involves several cells, including macrophages, natural killer cells, mast cells, T- cells, B-cells and dendritic cells (See Table 3.1) (Libby, 2007; Vodovotz et al., 2010). Acute inflammation is the early reaction to tissue injury and infection and it is part of the innate immune response that has two overlapping stages: the cellular stage initiated by leucocytes movement to the affected area and the vascular stage with increased capillary permeability (Ward, 2010). Both of these stages along with released chemical mediators, such as chemokines, histamines and serotonin, to the affected area result in acute inflammation (See Figure 3.1) (Simundic, 2011; Ward, 2010). Macrophages are leukocytes leading the cell-mediated immune response by ingesting the foreign materials at the site of injury or infection through a series of chemical and cell interactions (Ward, 2010). Pattern recognition receptors (PRRs) are secreted by macrophage and (See Table 3.1) identify the initial recognition of the infection or the injury (Simundic, 2011). Some molecules are also involved in the

process, such as free radicals and cytokines (Libby, 2007; Vodovotz et al., 2010). The PRRs sense damage associated molecular patterns (DAMPs) and pathogen associated molecular patterns (PAMPs) (See Table 3.1). Inflammation initiation begins with pattern recognition receptors (expressed by macrophages) such as Toll-like receptors and NOD-like receptors (See Table 3.1) that perceive DAMPs or PAMPs. These receptors participate in the activation of signal transduction pathways, signal dependent transcription factors and eventually activation of the genes promoting inflammation (Libby, 2007; Tabas & Glass, 2013). Binding of the Toll-like receptors also increases phagocytosis, cytokine release, lipid mediators and autacoids, which expand the inflammation response (Hunter, 2012; Libby, 2007; Libby et al., 2010).

The adaptive immune response is slower than the innate response and its mechanism is more complex requiring several molecular structures (Libby, 2007; Porth, 2011; Simundic, 2011). For example, when antigens are introduced to T-cells, they are recognized causing T-reg cells to initiate several responses including cytotoxic effects by T-cells and secretion of an antibody by B-cells. There are two types of differentiated T-cells, T-helper 1 (T_h1) cells and T-helper 2 (T_h2). The T_h1 cells secrete a variety of cytokines (See Table 3.1) such as interferon-gamma (INF-γ); an important link between the innate and adaptive immune response pathways. TNF-γ induces macrophage to produce mediators and pro-inflammatory cytokines. On the other hand, T_h2 cells stimulate the humoral immune response by developing cytokines to induce B-cell antibody production. T_h2 cells can also activate mast cells that lead to chronic inflammation (See Figure 3.1) (Libby, 2007; Porth, 2011; Simundic, 2011).

Chronic inflammation lasts longer than acute inflammation and is self-perpetuating. Chronic inflammation is due to immunosuppression and is mediated by myeloid-derived suppressor cells (MDSCs) and associated with down regulation of the "cluster of differentiation" 247 (CD247) cells (See Table 3.1) (Baniyash, Sade- Feldman & Kanterman, 2014). Innate and adaptive immunity's signals couple and interact into two types of cells: mesenchymal cells (See Table 3.1) and epithelial cells .The signals induce leukocytes and eventually lead to chronic inflammation, angiogenesis, cell proliferation, extracellular remodeling and apoptosis. T_h cells are associated with several chronic inflammations seen in different organs that result in chronic hepatidites, rheumatoid synovium and atherosclerotic plaques (Grivennikov et al., 2010; Hunter, 2012; Libby, 2007; Libby et al., 2010). In the T_h2 type immune response dendritic cells (a type of macrophage) are exposed to thymic stromal lymphopoietin (See Table 3.1) (Coussens, Zitvogel & Palucka, 2013). T_h2 cells such as CD4+ T-cells produce interleukin (IL) 4 and IL13, which leads to tumorigenesis, perhaps due to direct tumor development or indirect tumor development with macrophages (Coussens et al., 2013). The apoptosis pathway alteration is a possible effect of direct tumor development whereas indirect effects include pro-angiogenic factors and an alteration of growth factors that cause CD8+ T-cell proliferation (See Figure 3.1) (Coussens et al., 2013). Moreover, macrophages can promote malignancy by tumor cell invasion, inflammation, matrix remodeling, intravasation, angiogenesis and seeding at a distant site (Condeelis & Pollard, 2006).

Inflammation is associated with the four steps of tumorigenesis: initiation, promotion, invasion and metastasis (Hunter, 2012). It can be the result of infections due

to *Helicobacter pylori* (gastric cancer), Hepatitis B or C (hepatocellular carcinoma), gram negative bacteria such as the *Bacteroides* (colon cancer) and *Schistosoma* (bladder cancer) (Grivennikov, Greten & Karin, 2010). Chronic inflammation is responsible for 20% of all cancers (Grivennikove & Karin, 2011; Mantovani et al., 2008). The Ras protein is involved in inflammation and plays an important role in ethiopathogenesis of epithelial ovarian cancer (Liu et al., 2004). IL-1β, IL-6 and tumor necrosis factor α (TNF-α) cytokines promote epithelial ovarian cancer (Maccio & Madeddu, 2012) while expression of the Toll-like receptor-4 gene is associated with colon cancer (Fukata et al., 2007). Pathophysiology of endometriosis suggests over-expression of several markers potentially involved in Toll-like receptor dependent inflammation. Both Toll-like receptors and oxidative stress pathways are activated during the process of the chronic inflammation (Kajihara et al., 2011).

Inflammatory Breast Cancer

Inflammatory Breast Cancer (IBC) is a very rare type of invasive breast cancer, accounting for approximately 2.5 percent of new breast cancer diagnoses in the United States. It has specific features that are different from those of invasive breast cancer with IBC aggressively developing within a few weeks or months (Fernandez, et al., 2013; Makower & Sparano, 2013; Robertson, et al., 2010; Shkurnikov, et al, 2013). The most distinctive physical symptoms include edema, erythema and swelling of the breast with an overall dimpling that makes the skin appear much like an orange-peel (peau d' orange). Commonly, no tumor is identifiable via breast or diagnostic examinations. IBC is frequently characterized by hormone receptor negative status, rapid propagation and

early metastatic evolution resulting in a poor prognosis (Makower & Sparano, 2013). Currently, IBC has been described as a distinct entity from other types of breast cancer and mostly attacks females; however, it has been reported to affect men as well (Robertson, et al., 2010). The disease regularly occurs in people who are under the age of 50 due to premenopausal carcinogenesis and it is usually misdiagnosed because of its typical appearance, which includes swelling, erythema and orange peel skin (Fernandez, et al., 2013).

The incidence of the disease varies among different populations; in Japan, IBC is responsible for 0.09 to 2.9 percent of all breast cancer diagnoses, whereas Tunisia has an incidence of 5.7 percent and Egypt an11.1 percent (Dawood, et al., 2011b). Patients who are diagnosed with IBC have a higher risk of mortality when compared to patients with non-IBC (non inflammatory breast cancer). This is probably due to the invasive nature of the disease (Dawood, et al., 2011b). Statistics show that the incidence of this type of breast cancer is increasing on a global scale (Fernandez, et al., 2013). Patients who have been diagnosed with IBC have unknown metastases, though many are identified at the time of diagnosis (Van Laere, et al., 2013). IBC has similar symptoms to diagnoses of mastitis, erysipelas (a mammary gland inflammatory disease), or abscess and this is the main concern when it comes to early and proper detection. This can interfere with the time-to-diagnosis of IBC, as the patient is treated initially with at least a 7-10 day cycle of antibiotics (Dawood et al., 2010). The extended time could allow the IBC to develop to the point of metastases since even at the earliest stages this is an extremely fast growing malignancy (Shkurnikov, et al, 2013).

In a large population study performed by Dawood, et al. (2011b), breast cancer-specific survival was compared between stages IIIB/C IBC and locally advanced non-IBC (LABC) participants. The statistical analysis revealed a 43% increased risk (p=0.008) of death for the stage IIIB/C IBC patients as opposed to LABC patients emphasizing the importance of new and effective treatments to increase participant outcomes (Dawood, et al., 2011b).

Histological Features of IBC

The majority of patients diagnosed with IBC have estrogen and progesterone receptor negative (ER/PR negative) and human epidermal growth factor receptor 2 (HER2) negative biomarkers though HER2/neu receptors can also be positive. Like other types of breast cancer diagnoses, IBC patients with hormone receptor positive tumors have a better prognosis due to available treatments that target those receptors (Makower & Sparano, 2013). Other biomarkers are also used for molecular analysis in patients with IBC such as oncosuppressors genes (e.g., p53) and certain growth factors such as epidermal growth factors (Shkurnikov, et al, 2013). In a study done by Cairo University, 27 tumors were assessed from patients with IBC and compared with non-IBC tumors. Women diagnosed with IBC were also diagnosed with at least four positive lymph nodes (LN) with positive tumor emboli as opposed to non-IBC patients with no positive LNs at diagnosis (Mohamed, et al., 2014).

The most distinctive physical symptoms are hypothesized to be the result of dermal lymphatic (very small vessels in dermis) invasion by tumor emboli obstructing the dermal lymphatic vessels; the clinical symptom of inflammation is initiated by the

obstruction (Makower & Sparano, 2013; Mohamed, et al., 2014; Tomasevic & Kolarevic, 2012). Tumor emboli are able to cause metastasis and ultimately organ failure—due to being obstructive, extremely angiogenic and angioinvasive (Tsoi, et al., 2010).

Diagnosis

IBC can be difficult to diagnose and this could be due to a variety of complications. As previously noted, symptoms of IBC can be confused with the signs of mastitis as they are both associated with inflammation of the breast tissue (Dawood, et al., 2011a). Pain and firm breast tissue associated with inflammation makes it difficult to palpate breast lesions and frequently there is no breast lump that can be palpated or visualized through the use of technology (Dawood et al.). In addition, many young women diagnosed with this disease already have dense breast tissue, making it even more challenging for mammography to identify the presence of the tumor (Dawood, et al., 2011a).

In 2008, the first international conference on IBC was held and included a panel of global IBC experts including oncologists, radiologists, surgeons and pathologists operating in the breast cancer arena. The panel was brought together with the purpose of designing comprehensive guidelines. A consensus statement was developed based on their extensive research of the studies focused on IBC. The panel also identified criteria for the diagnosis and treatment of this aggressive breast cancer, including requirements for early detection, diagnosis, staging and treatment approaches (Dawood, et al., 2011a; Van Laere, et al, 2013). The first step in diagnosing IBC, based on the panel of expert recommendations, suggests checking for required minimum clinical criteria (Dawood, et

al., 2011). These characteristics may be present for up to six months, cover most of the breast and be accompanied by other breast abnormalities but without discernable lumps (Dawood, et al., 2011a). Clinical examinations and tissue biopsy should follow histology examination and biomarkers. Biomarkers such as hormone receptors and HER2 status can be evaluated to confirm invasive breast carcinoma and a diagnosis of IBC (Dawood et al.).

Radiological tests such as ultrasound and Magnetic Resonance Imaging *(*MRI) are included to identify breast parenchymal lesions that have not been previously detected by ultrasound and/or mammography. In some advanced cases, a Computed Tomography (CT) scan can verify whether the disease has spread to other parts of the body. Positron Emission Tomography (PET) scan is not recommended for staging of IBC. This is due to the lack of adequate data to support the necessity and IBC panel recommendations (Dawood, et al., 2011a; Scotti, et al., 2013).

Ultimately the panel suggested following the American Joint Committee on Cancer (AJCC) criteria when staging for IBC. This staging categorizes IBC as T4D and defines IBC as stage IIIB (including some additional qualities not found in stage IIIB) or higher (Edge & Compron, 2010).

Biomarker evaluation is necessary to confirm the tissue subtype, tumor grade, ER/PR and HER2/neu status when guiding the type and schedule of systemic chemotherapy (Dawood, et al., 2011a). Vascular endothelial growth factors (VEGF) such as VEGF-C, VEGF-D or C-fos-induced growth factor (FIGF) and VEGFR-3 or Fms-Related Tyrosine Kinase 4 (FLT4), responsible for lymphoangiogenesis, angiogenesis,

vasculogenesis, proliferation and metastasis, are increased in patients with IBC (Lerebours, et al., 2013; Dawood, et al., 2011a). Other markers, such as the *p53* mutation, have been linked to IBC and associated with resistance to therapy and overall diminished survival rate (Dawood, et al., 2011a; Gonzales-Angulo, et al., 2004). C-X-C chemokine receptor type 4 (CXCR4) is a neoangiogenesis mediator associated with upregulation of VEGF and chemokine (C-C motif) Receptor 7 (CCR7) is associated with differentiation of T-Cells and enables the disease to spread into lymph nodes (Cabioglu, et al., 2007). A poor prognosis was found to be associated with the chemokine receptors, CXCR4 and CCR7; responsible for metastasis (Cabioglu, et al., 2007).

Genomic Investigation of the Cause of IBC

Genome-wide association studies were conducted to address molecular mechanisms of IBC through expression profiling (Van Laere, et al., 2013). Although several genes and gene products are found to be associated with IBC, because of the heterogeneous nature of the disease, clinical diagnosis criteria are required to confirm the diagnosis. According to National Center for Biochemical Information (NCBI), several different genes such as: forkhead box P3 (*FOXP3), ras* homolog gene family member A (*RHOA), ras* homolog gene family member C (*ARHC*) and *WNT1* Inducible Signaling Pathway Protein 3 (*WISP3)* are responsible for the phenotypic characteristics of the disease (Van Laere, et al.).

The genomic investigation of the disease using the NCBI database shows that several different genes and gene products are associated with IBC in humans. Some of the designated genes are also responsible for other diseases, including different types of

breast cancer (NCBI, 2014a). The identified gene products participate in different pathways such as, v-akt murine thymoma viral oncogene homolog 1 (*AKT1*) that encodes for serine-threonine protein kinase. This kinase is responsible for regulation of cell proliferation, cell differentiation and apoptosis. Mutations in *AKT1* are also associated with Proteus syndrome, Cowden disease 6, familial breast cancer, familial colorectal cancer, neoplasm of the ovary and schizophrenia (NCBI, 2014b).

Cadherin 1, type 1 (*CDH1*), is a gene from the cadherin super family and encodes for a calcium dependent protein; a cell-cell adhesion glycoprotein used to keep cellular structure and prevent the cells from spreading. Mutations that cause a loss of function of this gene are responsible for invasiveness and metastasis characteristics of several cancer types (thyroid, colorectal, gastric and ovarian) in addition to breast (NCBI, 2014c).

The *Ras* homolog family member A (*RHOA*) is a guanosine triphosphatase (GTP) enzyme responsible for the regulation of actin cytoskeleton, cell adhesion and proliferation (Gilbert-Ross, Marcus, & Zhou, 2015; NCBI, 2014d). Cytoskeleton forms the cells and stabilizes the tissue to avoid cell deformation and migration (Wickstead & Gull, 2011). The *RHOA* mutation is associated with oncogenesis of the mammary glands (Wu, et al., 2010). Its cell matrix adhesion functions are essential for cell stability, mortality and invasion and if mutated, this results in oncogenesis (Wu, et al., 2010).

FOXP3 is a transcriptional regulator found in the cell nucleus. Over expression of the FOXP3 gene product is responsible for immune response via the regulatory T-cells (T-regs) (See Table 3.1) (Nair, et al., 2013). The overexpression is linked to the

recurrence of IBC, which means T-regs are increased due to the immune system response to cancer (Nair, et al., 2013; Zhang & Zhao, 2007).

Pathogenesis

The complex pathophysiology of IBC is the primary reason for the lack of knowledge about the pathogenicity of this disease. Multiple studies have used *in vivo* and *in vitro* methods to understand the mechanisms and to be able to guide the treatment; some of them are discussed in this paper.

In a study done by Bieche, et al. (2004), 36 IBC patients were compared to non-IBC women at stage IIb and III. Researchers found genes located on chromosome 6p21 that were upregulated in IBC patients. These upregulated genes were: genes coded for growth factors including *VEGF, IGFBP7, DTR/HB-EGF, EREG, IL6, CCL3/MIP1A, ANGP2* and *CCL5/RANTES*; genes coded for transcription factors including *EGR1, JUN, FOS, JUNB, MYCN, SNAIL1* and *FOSB*; and genes coded for growth factor receptors *ROBO2, TBXA2R* and *TNFRSF10A/TRAILR1* (Bieche, et al., 2004). This shows the heterogeneic nature of the disease and indicates why it has been difficult to identify unique treatment to offer a better prognosis.

Fernandez, et al. (2013) used the triple negative breast cancer (TNBC) cell line FC-IBC02, originally from an inflammatory breast cancer patient's pleural effusion (excess fluid accumulation in the pleural cavity), as a model of IBC. The cells were full-grown in both non-adherent (for 14 days) and adherent conditions. These cells formed mammospheres, a mass of mammary gland cells, after 14 days and then the cells were transferred to regular culture in a suspension of the adhesion molecules E-cadherin,

TSPAN24 and β-catenin (See Table 3.1). Adhesion molecules are associated with invasiveness and migration of cancer cells. Cell-to-cell adhesion is very important for cell stability and if this contact is impaired, cells will have the capability to migrate and metastasize. FC-IBC02 cells were also injected into severe combined immunodeficiency (SCID) mice, without functional T-cells and B-cells, for *in vivo* studies that led to complete tumor growth (Fernandez, et al., 2013). Xenografts grew rapidly in a statistically significant manner with the mice showing metastases in LNs and lungs (Fernandez, et al., 2013). Due to this research a new IBC cell line was developed with the same biomarkers and gene signature as the original cell collected from pleural effusion of a patient with IBC. The results from this study, the new IBC cell line with TNBC features, will allow researchers to conduct more research to understand the disease pathogenesis and study new treatments for IBC.

Woodward, et al. (2013) compared the gene expression profiles of their samples from IBC, non-IBC and normal healthy controls matched for ER and HER2 status using RNA probes. They failed to find statistically significant specific biomarker signatures (important for understanding the disease and guiding treatment) for IBC when compared to non-IBC samples (Woodward, et al., 2013).

Macrophages are important in inflammation in breast tumors and have a critical role in metastasis (Condeelis & Pollard, 2006; Mukhtar, et al., 2011; Pollard, 2008). Approximately 50% of the IBC tumors contain leukocytes, mainly macrophages and lymphocytes. The increased number of macrophages in the tumor is consistent with invasive tumor and poor prognosis (Sica, Allavena, & Mantovani, 2008). Tumor-

associated macrophages (TAMs) (Sica et al., 2008) are the regulatory factors in the relationship between cancer and inflammation and also classified as M1, M2 and regulatory microphages (Mohamed, et al., 2014; Wang, Liang, & Zen, 2014) (See 3.1). M1 secretes anti- proliferation cytokines whereas M2 secretes cytokines to enhance proliferation and is activated by the pro-inflammatory components INF-γ and TNF-α. The M2 usually is in response to interleukin-4 (IL-4) and interleukin-3 (IL-3) (Mohamed et al., 2014; Wang et al., 2014). The regulatory microphages secrete cytokines to promote anti-inflammation and cause tumor growth, metastasis and invasion (Mohamed, et al., 2014). The results from this study indicated a higher number of macrophage differentiation markers when compared to non-IBC patients; perhaps a useful marker for the diagnosis of IBC.

Small non-coding RNAs known as microRNAs (miRNAs) are responsible for deregulation of gene expression. In several cancer types, including breast cancer, miRNAs are deregulated either by epigenetic changes or genomic alterations. Several miRNAs are considered to have good prognostic and diagnostic capabilities because they are seen in tumor tissues and associated with invasiveness in IBC. Moreover, they can be predictive, which makes miRNAs expression analyses a potentially critical analytic and prognostic marker for IBC (Lerebours, et al., 2013; Volinia, et al., 2012). Lerebours, et al. (2013) examined miRNA expression profiles in patients with IBC by screening 804 miRNAs. Deregulation of 13 miRNAs was found in IBC patient samples as opposed to non-IBC samples. Seven miRNAs were found to be specifically upregulated in the IBC samples. All miRNAs except for miR-133 were upregulated in IBC patient samples. The

data obtained from this study were anticipated because of the nature of miRNAs. They may be a component of either oncogenes or tumor suppressor genes (Lerebours, et al., 2013).

The FOXP3 protein controls the development, differentiation and function of the T-regs (Samstein et al., 2012). As noted previously, T-regs are increased in the blood and tumor microenvironment of patients with cancer because they suppress anti-tumor activity of the immune system (Facciabene, Motz, & Coukos, 2012). According to the Genetic Association Database (GAD), *FOXP3* is associated with several other disorders such as vitiligo, Graves' disease, leukemia, hay fever, asthma, diabetes type 1, sarcoidosis, juvenile arthritis and celiac disease (GAD, 2014). Nair, et al. (2013) suggested that over expression of *FOXP3* is linked to the recurrence of TNBC in IBC patients; therefore, the FOXP3 protein could be an immunotherapeutic target against IBC cells (Nair, et al., 2013).

Metastasis and invasive activity of IBC is related to two signal pathways affected by the *ras* homolog family member C (*ARHC)* and *WNT-1* induced secreted protein 3 (WISP3) genes. *WISP3* is involved in *ARHC* expression in IBC cells and these two genes act together in the aggressive type of IBC (Kleer, et al., 2004). A current assay from a whole transcriptome analysis performed by researchers Shkurnikov, et al. (2013), found 137 mRNAs that expressed differentially in the tumor tissue samples of the patients with IBC (17 downregulated and 120 upregulated genes) which shows the necessity of assessment of regulatory genes involved in the disease pathogenesis and metastasis (Shkurnikov, et al., 2013). There are three main biological processes directed by a variety

of genes involved in IBC including: 1) inflammation (*ERBB2IP*, *IGF2*, *CHST2*, *CX3CL1*, *GRIN2B*, *IL1RL2*, *SAA1*, *SAA2*, *SAA4* and *DEFB131)*; 2) transcription (*SOX8*, *SOX9*, *ETV4*, *NFIB* and *MAFG),*; 3) chemotaxis (*CCL28*, *CX3CL1*, *EFNA5*, *CMTM7*, *GRIN2B*, *IL28A*, *PROM1* and *TSLP)*. For example, overexpression of the v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2 interactive protein (*ERBB2IP)* gene in IBC patients is associated with the inhibition of nucleotide-binding oligomerization domain containing 2 (NOD2) signal pathways. This pathway is responsible for initiation of inflammation by neutrophils and macrophages. (Shkurinkov, et al., 2013). The study also found higher expression in *CX3CL1* and *CCL28* genes responsible for tumors spreading in cancer patients. IBC expression profiles indicate genes involved in regulation of signaling transduction processes and transcription processes as well as cell adhesion, apoptosis and chemotaxis (Shkurinkov, et al.).

Treatment

Management protocols for IBC suggest a multidisciplinary route. The most common treatment of inflammatory breast cancer continues to be the use of systemic neoadjuvant chemotherapy (Scotti, et al., 2013). The National Comprehensive Cancer Network (NCCN) guidelines recommend neoadjuvant chemotherapy for IBC patients (NCCN Guidelines, 2014). The study done by Gianni, et al. (2010) suggested the addition of neoadjuvant trastuzumab to the anthracycline-taxane regimen, followed by one year of treatment with adjuvant trastuzumab. This regimen leads to a better pathologic complete response (pCR) and an improvement in overall survival rates (Gianni, et al., 2010). ER

positive women with IBC should also be offered hormonal therapy (Tamoxifen or aromatase inhibitor) after chemotherapy and a mastectomy (Carlson, et al., 2011).

The treatments followed by immediate surgery and radiation therapy helped to diminish any remaining tumors (Dawood, et al., 2011a). If the tumor was HER2 positive, anti-HER2 therapy treatment was suggested with Tamoxifen and aromatase inhibitors by the IBC panel of experts (Dawood, et al., 2011a). Higher pCR has been reported with the chemotherapy regimen that includes taxane, trastuzumab and anthracyclines in women with HER2 positive biomarkers (Makower & Sparano, 2013; NCCN Guidelines, 2014).

Once systemic chemotherapy is completed, traditional breast reconstruction surgery is not recommended immediately after the mastectomy for IBC patients due to the necessity of radiation therapy, however, delayed reconstruction is reported in some research studies (Dawood, et al., 2011a). Radiation therapy follows after surgery in order to eliminate any remaining tumor cells in the tumor area (Dawood, et al., 2011a; Scotti, et al, 2013). Women with IBC who were treated with a multimodal approach responded well to therapy, resulting in longer survival (Dawood, et al., 2011a).

Discussion

The literature reveals that IBC is clinically unique and develops rapidly. Inflammation is the first line of defense in the body after skin, with several different mechanisms to protect the body. Cellular and vascular stages and their chemical mediators such as histamines and chemokines are involved in the inflammation process (Simundic, 2011; Ward, 2010). Inflammation involved in tumorigenesis and chronic inflammation is associated with 20% of cancers (Grivennikove & Karin, 2011; Hunter,

2012; Mantovani et al., 2008). IBC will advance as malignant cells infiltrate the lymphatic and blood vessels, further enhancing the invasive nature of IBC. A majority of patients diagnosed with IBC have pathology indicating TNBC, the most aggressive type of breast cancer. Recent management guidelines suggest an aggressive personalized approach of multi-modal therapy including several types of neoadjuvant chemotherapy followed by mastectomy, anti-hormone therapy (for patients with positive hormone receptors) and radiation therapy (Dawood, et al., 2011a).

The panel of IBC experts suggests standard guidelines for diagnosis, treatment and patient education regarding their risk factors, prevention and lifestyle changes (Dawood et al., 2011a). The goal of these guidelines is to improve the survival rate. The objectives were created to develop and promote physician and patient education as well as encourage the development of clinical trials and international collaboration on IBC research (Dawood, et al., 2011a).

The genetic alterations mentioned in this article suggest that IBC has a heterogeneous and complex nature. Furthermore, it emphasizes the necessity of further investigation and new personalized drug developments in order to achieve better prognosis and overall survival rate.

Improving awareness plus educating the general public about the disease symptoms could offer earlier diagnosis, treatment, follow up and ultimately improve the prognosis. Further collaborative interdisciplinary studies are needed to decipher the complex molecular mechanism of IBC. These strategies would also lead to better prognosis and improved survival rate.

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Table 3.1

Definitions

Term	Definition
Macrophages[1, 2]	Type of leukocytes involved in ingestion of foreign materials, they are activated and deactivated during inflammation; initiation, maintenance and resolution
Mast Cells or mastocytes[3]	Are derived from myeloid stem cells and mediating allergy reactions and in general, inflammatory responses
Natural killer cells (NK Cells) [2,4]	Cytotoxic lymphocytes involved in innate immune response
Dendritic cells [2,4]	Cells that present antigens to T-cells at cell surface
Toll-like receptors (TLRs) [5]	These receptors are involved in innate immunity and expressed in dendritic cells and macrophages
Cytokines [2,4]	Small proteins secreted by cells for cell to cell communication and are responsible for inflammatory response
Interferon [2,4]	Part of cytokines proteins and secreted in response to pathogens
T-helper cells [4,5,6]	Involved in adaptive immunity involved in B-cells, T-cells and macrophages activities
Thymic stromal lymphopoietin	Cytokine proteins involved in T-cell maturation
Myeloid-derived suppressor cells (MDSCs) [1]	Myeloid immune cells involved in cancer and chronic infections
Cluster of differentiation (CD) [7]	A protocol used to determine cell surface proteins involved in cell signaling and act like ligands or receptors (CD1 through CD335)
Mesenchymal cells[2,4,7]	Stem cells with capability of differentiation to any cell type
Pattern recognition receptors (PRRs) by DNA Microarray [6]	The initial recognition of the infection or the injury
Pathogen associated molecular patterns (PAMPs) [6]	Group of pathogenic molecules that are recognizable by immune system such as TLRs and PRRs
Damage associated molecular patterns (DAMPs) [8]	Initiate immune inflammatory response (noninfectious)
NOD-like receptors[9]	Part of PRRs and associated with innate immune response
Regulatory T-cells (T-regs) [10]	Regulate the immune system and is part of self check of immune system to avoid unnecessary

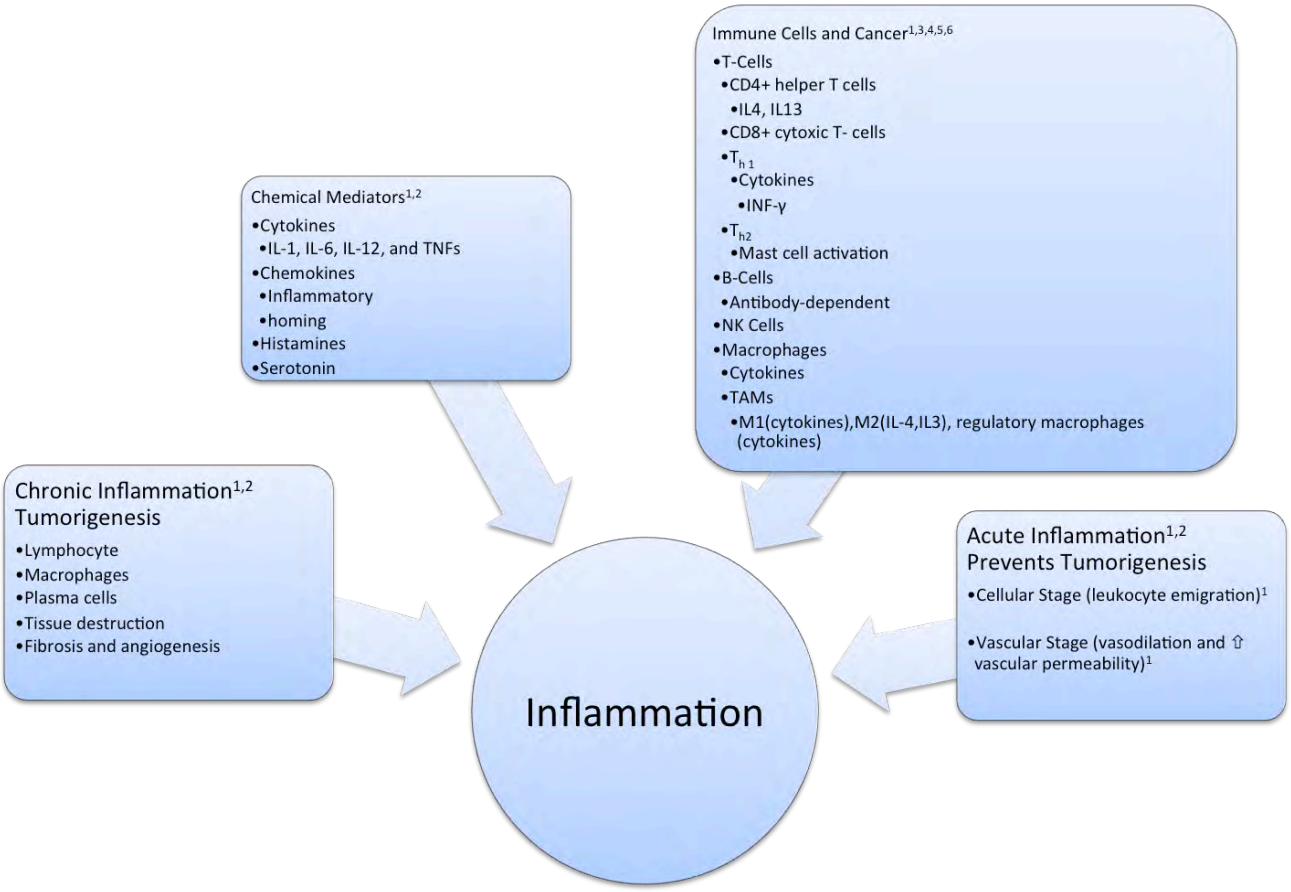
	immune response
E-cadherin [2, 7]	Cell to cell adhesion molecule involved in tissue development and cancer suppression
TSPAN24 [11]	Tetraspanin 24 are transmembrane 4 super family and has an important role in cell adhesion
β-catenin [12]	A protein that regulates cell to cell adhesion and transcription
Tumor associated macrophage (TAM)[16]	Regulatory factors of the link between inflammation and cancer
M1[16]	M1 macrophage secrete cytokines to inhibit proliferation
M2[16]	M2 macrophages secrete cytokines to promote proliferation
Regulatory macrophages[17]	Regulate inflammatory response

Sources

1. Baniyash, et al., 2014; 2. Porth, 2011; 3. Jung, et al., 2013; 4. Ward, 2010; 5. Libby, 2007; 6. Simundic, 2011; 7. Chan & Hui, 1988; 8. Krysco, et al., 2011 9. Chen, Shaw, Kim, & Nuñez, 2009; 10. Nair, et al., 2013; 11. Fernandez, et al., 2013. 12. MacDonald, Tamai, & He, 2009; 13. Sica, et al., 2008; 14. Fleming & Mosser, 2011.

Figure 3.1

Inflammation and Cancer: An Overview



Sources

1. Porth, 2011*;* 2. Ward, 2010; 3. Libby, 2007; 4. Simundic, 2011; 5. Coussens, et al., 2013; 6. Mohamed, et al., 2014

CHAPTER FOUR

USE OF PROTECTION MOTIVATION THEORY TO GUIDE PREVENTION STRATEGIES IN WOMEN WITH BREAST CANCER DIAGNOSIS AND POSITIVE GENETIC ALTERATION

Abstract

The American Cancer Society and the National Comprehensive Cancer Network (NCCN) frequently update the guidelines for breast cancer detection, prevention and risk reduction (American Cancer Society, 2015, NCCN, 2015 a). However, the numbers of individuals who follow the guidelines are low (less than 0.2% of women in United States use Tamoxifen and approximately 3% of Australian women follow the prevention guidelines) (Ralph et al., 2014). The numbers to follow prevention strategies are even lower in minority ethnicities (American Cancer Society, 2015). In order to improve the number of individuals who follow the detection, prevention and risk reduction guidelines, the Protection Motivation Theory (PMT) is applied to identify and promote the use of the motivators to help individuals to understand and follow the risk management strategies for breast cancer.

The purpose of this paper is to discuss the components of PMT and how this theory can be beneficial in breast cancer prevention studies, specifically among different ethnic groups. A comprehensive review and synthesis of recent literature from scholarly journals will be used to direct the use of PMT in a suggested pilot study utilizing a focus group of women with a diagnosis of breast cancer and identified mutation in a susceptibility gene.

Purpose

The purpose of the proposed research is to determine if the Protection Motivation Theory can guide prevention education for women who have a breast cancer diagnosis plus are positive for a mutation in a susceptibility gene that makes them at higher risk for another cancer.

Methodology

A focus group research pilot study is suggested to be conducted using high risk breast cancer patients who have already been diagnosed with breast cancer and completed genetic testing. The PMT would be used to guide the discussion about experiences with genetic testing in order to develop prevention education for these women.

Background

Breast cancer is projected to be responsible for 14% of all cancer deaths in the United States in 2015 (National Cancer Institute [NCI], 2015) with almost 10% of breast cancer cases associated with germline mutations (Tung et al., 2015). Too many studies to be counted have been conducted on the topic of breast cancer over the past decade and with advances in DNA sequencing, progress has been made in cancer screening, prevention, diagnosis and treatment. The National Comprehensive Cancer Network (NCCN) guidelines suggest different risk reduction strategies for women with a mutation in a susceptibility gene including mastectomy, bilateral salpingo oophorectomy and agents such as estrogen receptor modulators (NCCN, 2015b; Vogel, 2015; Zhang, Simondsen, & Kolesar, 2012). Although there are several suggested strategies for reducing breast cancer susceptibility in women at high risk, current use of the procedures

with or without the agents is very limited (Vogel, 2010; 2015). Disparities and lower socioeconomic status among different ethnic groups is a challenge for cancer prevention and screening (American Cancer Society, 2015). The Protection Motivation Theory (PMT) model is one option that can be applied to the motivation of women at high risk to participate in prevention and risk reduction protocols.

Protection Motivation Theory

Rogers originally suggested the PMT in 1975 with evolution throughout the years (See Figure 4.1) (Maddux & Rogers, 1983; Milne, Sheeran, & Orbell, 2000; Rogers, 1975). Based on a social cognitive theory, the PMT describes how fear appraisals impact behavior (Bui, Mullan, & McCaffery, 2013; Rogers, 1975). Fear and threats are unpleasant emotions, so any circumstances that induce fear can change behavior and attitudes specifically if it depends upon an individual's aim to lessen the emotional impact of the fear or threat (Maddux & Rogers, 1983). This theory is a very reliable predictor of the existing behavior but is limited in future prediction (Milne et al., 2000).

The PMT model adapted from Lee et al. (2007) demonstrated two main constructs of the theory: threat appraisal and coping appraisal. Threat appraisal is threat and fear of someone's health status, so interventions can be taken to prevent or lower the impact of the threat such as health education (e.g., training for women's self-breast exam) and screening methods (e.g., mammography); however, it is difficult to initiate interventions to change health behavior based on fear (Lee et al.). Rogers later suggested coping appraisal as a better predictor for prevention studies (Maddux & Rogers, 1983; Milne et al., 2000).

The PMT consists of series of interrelated concepts: severity of the health problem, vulnerability or risk of the threat, self-efficacy and response to the protective measures (See Figure 4.1) (Helmes, 2002). These four concepts of the theory have the advantage to promote protective behavior when compared to other models, such as the theory of planned behavior and the health belief model (Ralph et al., 2014). However, some of variables in the theoretical model are difficult to measure, while other variables such as severity, susceptibility, self-efficacy and response efficacy are well defined (Milne et al., 2000). The characteristics of this theory make it suitable for research studies related to health and well-being of individuals who could benefit from its application in clinical practice and patient care.

Research, PMT and Practice

Research on using PMT and its constructs has been conducted in different topics of health-related issues. Grindley et al. (2008) focused on physical activity and exercise using PMT to predict adherence to rehabilitation programs. They used PMT as a screening tool for patients who are suffering from orthopedic disorders; fear resulted from mobility complications, pain and also prescribed rehabilitations. The study found that using the PMT model in their screening tool could help clinicians to find out if their patient was at higher risk of poor adherence (Grindley, Zizzi, & Nasypany, 2008).

A skin cancer screening tool based on PMT was used in a research study focusing on patient education of skin cancer prevention strategies such as using sunscreen and avoiding tanning beds (Baghianimoghadam,, Mohammadi, Noorbala, & Mahmoodabad, 2011). The PMT was also used by Katz et al. (2009) to create an educational video for

colorectal cancer screening. The training video was evaluated and suggested changes to some of the components of the video were made in order to help patients better follow the guidelines for screening (Katz et al.).

Helms (2002) applied PMT and its components (See Figure 4.1) to discuss the predictors of intention and motivation of women to consider breast cancer genetic testing. The study found that women who were more worried about breast cancer (perceived risk) believed it is an advantage to go forward with genetic testing and wanted to take benefit of testing for an inherited mutation. This theory helped Helms to determine the important factors in decision-making in regard to genetic testing for mutations in women at low or moderate risk for breast cancer (Helms, 2002).

Another group of researchers looked at the effect of personalized genetic risk information on perceived efficacy of the helpfulness of the prevention, early detection and treatment protocols (Collins, Wright, & Marteau, 2011). They systematically reviewed studies that assessed the effectiveness of genetic risk information on changing health behavior (obesity, heart disease, diabetes and depression). Of the 5 (out of 1340) selected articles reviewed, only one study showed effective results on perceived effectiveness of medical intervention in terms of personalized genetic information (Collins et al.). On the other hand, patients who have done obesity risk check, showed intention to initiate a healthy lifestyle when they understood their high risk of becoming obese or overweight by using PMT for intention to change behavior (Frosch, Mello, & Lerman, 2005). This intention for behavioral change in patients indicates the importance

of perceived behavioral control and intention to change risky habits based on their genetic susceptibility to obesity (Fosch et al.).

PMT and Breast Cancer Screening/Prevention

The American Cancer Society (ACS) predicts 231,840 new cases of invasive breast cancer and 60,290 cases of breast carcinoma in situ to be diagnosed in 2015 (American Cancer Society, 2015). According to the American Cancer Society, the lower the socioeconomic status, the higher the breast cancer mortality; this is regardless of race. The ACS also reported that individuals with 12 or fewer years of education tend to have triple the mortality rate compared to those with higher levels of education (American Cancer Society, 2015). Their results also indicated that cancer mortality rates in non-Hispanic black individuals are higher than in other ethnic groups; disparities are due to lack of high-quality prevention, diagnosis and treatment services (American Cancer Society, 2015).

A large number of women, mostly from minority ethnicities (African American, Hispanic and Asian women), do not return for their mammography screening as suggested by guidelines even though these women are at higher risk to be diagnosed with advanced breast cancer and an earlier age at death (Moy, Park, Feibelmann, Chiang, & Weissman, 2006). Moy et al. (2006) recruited 49 participants from minority ethnicities (14 Hispanic, 16 African American and 19 Asian) for the study. Results indicated African Americans and Asians women believed that insurance would not be a barrier to their return for mammography screenings. A few of the 16 African American women believed that screening methods would lead to breast cancer and cause death (fatalism); they

preferred to avoid the prevention strategy due to their health beliefs, as it was stated by Asian and Hispanic women as well (Moy et al., 2006).

Estrogen modulators (e.g., nalvodex and raloxifene) and aromatase inhibitors (e.g., exemestane and anastrazole) are other options for eligible women to use as risk reduction medications (NCCN, 2015b; Vogel, 2015). However, research reveals the interest and adherence to use the medications are low (0.2% of eligible high risk individuals in the United States and 3% in Australia) (Karmakar, 2013; Ralph et al., 2014). According to Karmakar, 38% of participants (n= 145) who had physician orders for adjuvant therapy were non-adherent to their aromatase inhibitors. The study sent questionnaires to the participants that were established based on PMT. Protection motivation scores showed significant correlation to adherence (r=0.31), but coping appraisal was a better predictor of adherence to aromatase inhibitors compared to threat appraisal in this study (Karmakar, 2013).

Based on the above discussion, the best approach would be an intervention using a prevention model based on PMT to educate and encourage women to follow the screening guidelines. The PMT, as mentioned earlier, consists of two cognitive processes, threat appraisal and coping appraisal, which lead to intention to change health behavior (Bui et al., 2013; Helms, 2002) and is used to develop and suggest this study.

Methods

Design and study samples

This is a focus group research pilot study applying constructs and concepts of Roger's (1975) PMT to educate and urge women with breast cancer to follow the strategies to prevent second primaries. According to several research studies, not all women adhere to the guidelines (ACS, 2015; Bui et al., 2013; Karmakar, 2013; Lee et al., 2007; Moy et al., 2006; Ralph et al., 2014; Vogel, 2015).

This study will invite 10 women with breast cancer and a positive mutation in hereditary breast cancer genes to a focus group meeting. They will be interviewed based on questions that applied PMT to assess their intention to change health behavior based on the genetic test results. The participants will learn about the study by a phone call contact by principle investigators of this study.

Instruments

PMT has been used in several studies (Cyrus-David, & Strom, 2001; Helms, 2002; Lee et al., 2007; Morrison et al., 2010; Ralph et al., 2014). Based on these research studies, this pilot study is suggested. The results from this pilot study focus group, which is based on interview questions using PMT, would assist us to develop prevention education for women with breast cancer who are at high risk for hereditary breast cancer syndromes.

The following measures will be used based on specific aims of the study:

- Participant demographics to include age, race, ethnicity, insurance, level of education, body mass index (BMI) and physical activity level (due to their associations with breast cancer risk).

- Family history of cancer is very important to determine their risk percentage based on NCCN guidelines/ Genetic/Familial High Risk Assessment: Breast and Ovarian.
- Interview questions based on each of PMT constructs:
    - Perceived risk measure can be taken by asking these questions:
        - Severity (Helms, 2002):
            - How serious do you feel you may be diagnosed with another type of cancer based on your high risk status?
            - How severe you think your disease outcome will be based on your genetic testing result?
        - Vulnerability (Helms, 2002; Lee et al., 2007)
            - What is the likelihood that you will be diagnosed with another cancer?
            - What are the chances a new cancer diagnosis would be due to your genetic mutation(s)?
            - Explain if you can control or prevent it from happening.
    - Perceived coping can be measured by following questions:
        - Response efficacy
            - Describe what genetic testing revealed about your health and well-being
            - State different strategies and explain how following the guidelines would change your risks?

- Discuss if you think adapting a healthy lifestyle would lower your risk of a secondary cancer?

- Self-efficacy (Helms, 2002; Lee et al., 2007)
  - Identify your personal cancer risk factors.
  - Discuss the actions you might take to reduce your risk.
  - Provide examples of risk reduction strategies you might use.
  - Would you prefer to have educational materials, clinician recommendations, or both?

- Participants' intention to change their behavior also can be assessed by the following question (Cyrus-David, & Strom, 2001; Helms, 2002; Lee et al., 2007):
  - How likely are you to take the prevention actions?
  - How much interest do you have to take action?
  - Discuss how the genetic testing would/could help you decide?
  - Explain if your decision would be changed if you did not have genetic testing?
  - How likely are you to recommend genetic testing to your friends and family?

Human subjects and research approval procedures

Prior to development of the study and interview questions, the study protocol will be reviewed by the Clemson University Institutional Review Board (IRB) to receive

approval. An informed consent is also developed and will be evaluated by IRB to explain the details about the purpose of the study, risks and potential benefits for participants to assess, ask questions and sign before the focus group meeting. All participants will be invited by study principal investigators and will be informed about the purpose of the study before scheduling the meeting.

Data collection procedures and data analysis

The total number of 10 participants will be invited to the study from a cancer clinic in Southeast region by study principal investigator, an advanced practitioner and genetic counselor. All invited participants will be informed about the details of the study and will be scheduled for a meeting at the cancer center.

At the focus group meeting, study coordinators will explain the study in details and consent form will be obtained from each participant. Each participant should be assigned with identification number following HIPPA regulations with no identifier and interview questions will be asked and all the answers would be recorded in paper. After the focus group meeting, participants' demographics and family history will be collected from their medical record. All data will be kept in password-protected computers.

The interview responses would be coded and transcribed in order to perform qualitative data analysis. All coded schemes should reflect the study purpose for intention to change a health behavior based on PMT application.

Limitations

This is a suggested focus group pilot and researchers are not able to determine limitations to the study.

Summary

Based on the review of the articles and examination of several studies using the PMT, it is concluded that this theory could be useful in today's research. The growing knowledge and evidence in practice makes healthcare genetics in breast cancer research important to reach the goal of personalized medicine with interdisciplinary research studies in collaboration with different disciplines and institutions.

The more rigorous attempt to create educational materials and deliver the designed questionnaire should be helpful and effective to encourage women to take the prevention and screening measures, as it was significant in the Ralph et al. (2014) study. However, there are limitations to this developing study, including a small sample size in terms of participants from different ethnic background and a lack of consistency in the population selection. These limitations make it harder to represent the population specifically and to generalize when it comes to minority races and ethnic groups. A further study with consideration of eliminating our limitations is suggested.

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Figure 4.1.

Protection Motivation Theory

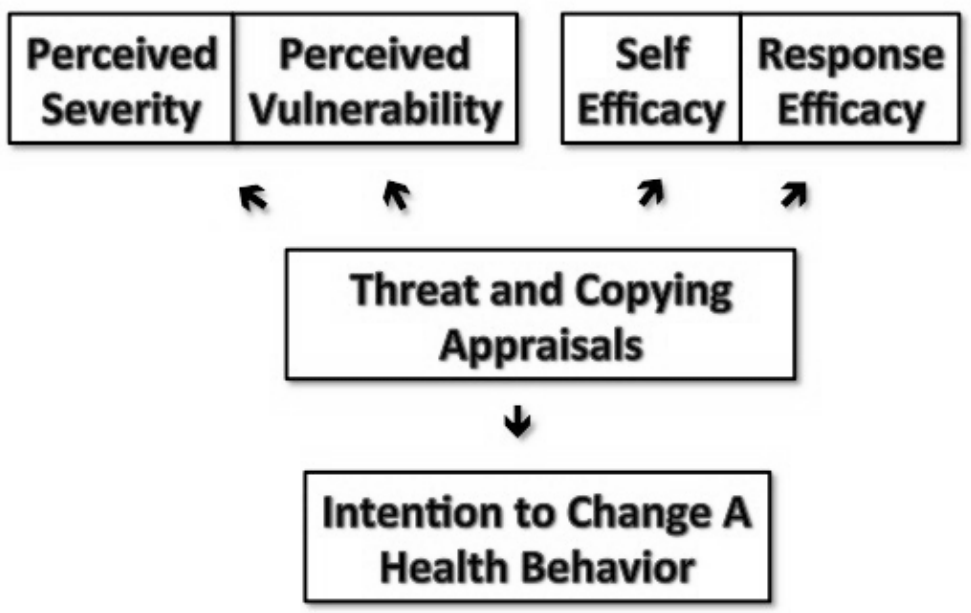


Figure 4.1. Protection Motivation Theory model illustrates two main constructs of the model and how they are interrelated to the main concepts, which eventually lead to intention to change a health behavior. Adapted from" The development of an arm activity survey for breast cancer survivors using the Protection Motivation Theory" by Lee, T. S., Kilbreath, S. L., Sullivan, G., Refshauge, K. M., & Beith, J. M. (2007). *BMC Cancer*, *7*(1), p.2.

CHAPTER FIVE

ASSOCIATIONS BETWEEN HEREDITARY BREAST CANCER SUSCEPTIBILITY GENE ALTERATIONS AND AGGRESSIVE TUMOR PHENOTYPE IN WOMEN DIAGNOSED WITH BREAST CANCER

Abstract

Purpose

The purpose of this study is to evaluate whether there is an association between breast cancer susceptibility gene alterations (GA) and aggressive tumor phenotypes using the molecular biomarkers of breast cancer. The specific variants will also be analyzed to determine if there is an association with the more aggressive tumors and their markers among ethnic groups.

Introduction

Almost 10% of 200,000 (approximately 20,000) of predicted new cases of invasive breast cancer are due to germline mutations, now including at least five newly identified breast cancer susceptibility gene mutations, such as *BRCA1* and *BRCA2* (American Cancer society, 2015; Tung et al., 2015). Technological advances in human genomics, in combination with faster and less costly results, have enhanced the research for the advancement of targeted therapy of molecular biomarkers (Grada, & Weinbrecht, 2013; Hawthorn, Luce, Stei, & Rothschild, 2010; Staren et al., 2014). Some of these new technologies require highly trained professionals to analyze the data, but may also have limitations for their usage such as inaccurate sequencing results and massive data analysis that can be difficult to manage (Grada, & Weinbrecht, 2013; Previati et al., 2013).

Integration of genetic information using new technologies is becoming essential in order to anticipate a problem, leading to earlier and potentially more specific detection of breast cancer. Newer technologies such as next generation sequencing (NGS) have recently been incorporated for oncology genetic multi-panel testing, however, as the guidelines are now used not everyone would be eligible for these tests. Multi-panel genetic testing offers the capacity to identify more than *BRCA* gene mutations in high risk populations thus providing future options for prevention and early detection of cancer in more than one generation of patients.

Background and Significance

A comprehensive literature search was conducted using specific search terms, including “breast cancer”, “high risk breast cancer”, “cancer genetics”, “breast cancer genes”, “breast cancer genomics”, aggressive breast cancer”, “triple negative breast cancer” and “hereditary breast cancer syndromes”. Database searches included PubMed, Google scholar, CINAL, Proquest Nursing and Allied Health, Cochrane's database and Web of Science. A total of 457 articles were identified between the years 2009 and 2015.The articles were screened for overlap between databases, quantitative versus qualitative research studies and relevance to specific aims of the research study described in this chapter. Original research articles with a focus on genes associated with hereditary breast cancer syndromes and their variants as well as molecular pathways and pathogenesis were included in the final number. Excluded were articles that were not in English, papers not related to breast cancer, research articles with lack of evidence and

animal studies. Finally, there were 44 studies identified having relevance to the purpose of the project.

The variables in this research study were chosen based on several essentials including risk factors for having a GA, elements associated with increased risk of breast cancer and endogenous factors affecting breast cancer. Commonly found factors of having a mutation associated with a hereditary breast cancer syndrome include: 1) family history of breast cancer with close blood relatives (three generations) who are diagnosed at younger age (under the age of 50) or at least two blood relatives diagnosed with breast cancer at any age; 2) one close family member with ovarian cancer; or 3) two blood relatives with pancreatic cancer (NCCN, 2015a).

Risk factors related with increased risk of developing breast cancer: 1) early onset menses; 2) late menopause; 3) increased breast density; 4) advancing age; 5) use of hormone replacement therapy; 6) having their first child after age 30; 7) higher body mass index (BMI); 7) history of radiation therapy to the chest, this can include thyroid cancer and acne radiation therapy; 8) low levels of physical activity; 9) poor nutrition; 10) smoking; or 11) alcohol consumption (with increased breast cancer risk of 10%) greater than one drink per day (Catsburg, Miller, & Rohan, 2014; Kushi et al., 2012; Li, 2009). Age similarly plays an important role in breast cancer development through mutation accumulation and decreased DNA damage repair. Estrogen Receptor (ER) positive breast cancer incidence increases with age as opposed to Estrogen Receptor (ER) negative cases, which are common in younger women (Gail, Anderson, Garcia-Closas, & Sherman, 2007). Patients between ages 80 and 85 have 15 times a greater risk of

experiencing breast cancer compared to younger women. This could be due to the increased risk of acquired mutations caused by defective DNA damage repair that occurs as a woman ages (Gail, et al.). DNA double break damage repair decreases as a woman ages; however, the mechanism remains unknown (Garm, et al., 2013).

Importantly, several recent studies revealed a link between aggressive tumor phenotypes and hereditary GAs in different cancer syndromes (Castro et al., 2013; Maier et al., 2014; Pern et al., 2012). *BRCA1* and *BRCA2* mutations in males have shown a more aggressive prostate cancer phenotype (Castro et al., 2013;Maier et al., 2014) and some patients with triple negative breast cancer, considered to be an aggressive phenotype, were found to have mutations not only in *BRCA* and, *BRCA2* but also the lesser known genes such as *PALB2* and *BRD7* genes (Pern et al., 2012).

Purpose of the Study

The purpose of this study was to:

1. Determine if identified breast cancer susceptibility GAs are associated with an aggressive tumor phenotype in women with a new diagnosis of breast cancer
2. Determine if the aggressive breast cancer tumor phenotype is associated with a specific gene variant(s)

Methods

This section focuses on the study subjects, procedures, hypotheses and the detail of the methods.

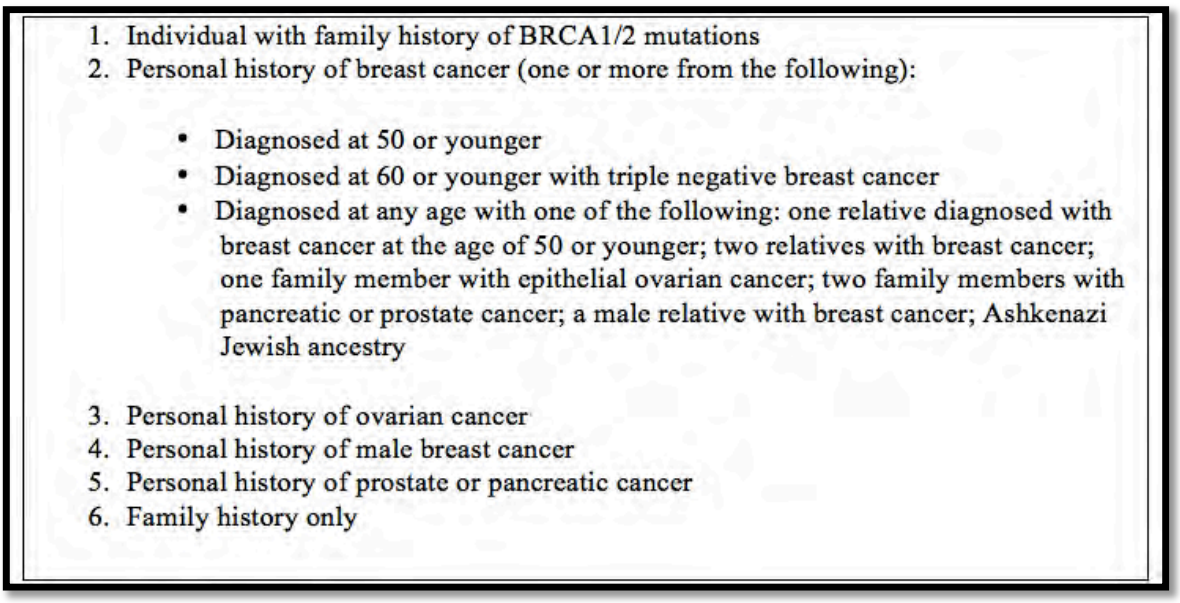
Subjects

A total of 257 electronic charts from women with newly diagnosed breast cancer who had also received genetic testing and counseling between October 2014 and August, 2015 were screened from Bon Secours St. Francis Hospital Cancer Center for study eligibility. Eligibility included women between the ages of 20 and 90 from diverse cultural and socioeconomic backgrounds, with and without insurance coverage. Non-eligibility included women with previously known breast cancer mutation(s), a diagnosis of lobular carcinoma in situ (LCIS) and benign breast biopsies. After meeting eligibility requirements, 101 women were invited to participate and determined to be interested in the study. These 101 participants have an elevated risk to carry a breast cancer susceptibility gene (based on NCCN guidelines, See Figure 5.1) and received genetic testing.

Power analysis was performed during the study design using G*Power 3.1 program to determine how many participants to include in the study (Faul, Erdfelder, Buchner, & Lang, 2009). The result from the power analysis indicated minimum sample size of 88 to conduct the study with 0.80 power and alpha of .05.

Figure 5.1

Hereditary Breast And Ovarian Cancer Eligibility Guidelines



Note. adapted from NCCN Guidelines, 2015a. Genetic/Familial High Risk Assessment: Breast and Ovarian. Version 2.

Procedures

Bon Secours St. Francis Hospital Cancer Center provided access to participants and an office with availability of the electronic health record for data collection. To promote collaboration and to clarify questions, the study protocol was presented to surgical, medical and radiation oncologists at a weekly tumor board meeting. The IRB approved consent form and protocol was also provided and an opportunity for questions and discussion was offered. An educational luncheon was used to educate all cancer clinic staff about the purpose of the study and the protocol. An opportunity for discussion was encouraged in order to make certain the best strategy was identified for participant accrual. IRB approved flyers to advertise the study were posted throughout the Cancer Center and distributed within the patient waiting areas.

Electronic health records of potential eligible participants (based on NCCN Guidelines) were reviewed and logged in a secure file. Participants were invited to join the study while at the Cancer Center for the genetics appointment or an oncologist clinic visit. If patients were not scheduled for genetic counseling, each medical oncologist was approached and asked to inform potential patients on the schedule for that day about the research study and to grant permission to meet with the team to introduce and explain the study. After obtaining verbal interest from the patients, the IRB approved consent (Appendix A-C) form was reviewed with the participant, questions answered and participant's signature obtained. To ensure participant confidentiality, a secure log was created in Dropbox. Each participant was assigned an ID number with no personal identifiers according to HIPPA regulations.

A 10-milliliter blood sample was collected from each participant, per protocol. Labeled vials of blood samples and a completed request form for genetic testing were transferred weekly to the laboratory facility for test completion. Samples were prepared for sequencing and identification of mutations. These included the *APC, ATM, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A (p14ARF and p16INK4a), CHEK2, EPCAM* (large rearrangements only*), MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, PTEN, RAD50, RAD51C, RAD51D, RINT1, SMAD4, STK11* and TP53 (NCCN, 2015b).

Technicians at the Greenwood Genetic Center performed the laboratory work. This included all the design and validation of the targeted NGS panel, NGS testing, plus bioinformatics review and resulting of the specimens. The NGS panel was performed using amplification based capture methodology (Wafergen, Inc.). Samples were multiplexed on the on MiSeq instrument (Illumina, Inc.). Bioinformatics experts validated the pathogenic sequence alterations and variants of uncertain significance using various databases. These databases included: National Center for Biotechnology Information (NCBI); ClinVar, cBioPortal of Cancer Genomics; Breast Cancer Information Core (BIC); Clinical Genome Resource (ClinGen); *BRCA* Mutation Database; and Breast Cancer genes IARC (International Agency for Research on Cancer) database.

The study also collected data from commercial genetic laboratories. Most of the participants previously had genetic testing done at a commercial laboratory.

Hard copy data collection sheets (Appendix D) were developed to record the study data for each participant. These hard copy data were coded for SPSS analysis and then transferred to an excel spreadsheet. To assure all data were correctly coded and included for analysis, coding errors were checked three times by two different researchers using comparison of the paper data collection sheet with the electronic medical records as well as for accuracy of transference from hard copy to excel spreadsheet.

Data analysis

SPSS® version 22 was used for data analysis. Descriptive analysis was performed for the participants (N=101) in order to explore the missing data. A table of descriptive data provided information regarding skewness and standard error; appropriate transformations were made to correct for skew. Data were cleaned with careful consideration for errors such as coding and outlier datum.

In order to examine the aims of the study, some variables were combined to form new variable clusters such as “GAs”, “triple negative breast cancer (TNBC)”, “aggressive phenotype” and “high-grade tumors”. GAs were defined as either having a positive genetic mutation or a variant of unknown significance (VUS); the TNBC variable was computed as negative status for all three tumor markers: estrogen receptors (ER), progesterone receptors (PR) and human epidermal growth factor 2 (HER2); high-grade tumors were computed as having grade 3 tumors in either breast tumor sites (right or left); and, the aggressive tumor phenotype described a TNBC tumor or a high-grade tumor.

Associations between the variables were investigated by observing Pearson 's Chi-Square or Fisher's Exact tests. For this statistical model, GA and aggressive tumor phenotype were assessed as well as other variables such as: age; ethnicity; insurance type; BMI; tumor markers (ER, PR, HER2 status); menopausal status; TNBC; and high-grade tumors. The bootstrapping method was used to estimate the population distribution based on resampling method.

A linear regression model was used to evaluate if the presence of a GA could predict aggressive breast cancer tumor phenotype. The data were also analyzed with regression analysis for other variables including age, ethnicity, insurance type, BMI, tumor markers, TNBC, menopausal status and high-grade tumors.

Results

The results section includes presentation of the descriptive statistics of the demographic variables followed by an exploration of the study variables. Relationships between the variables of interest are explored by Chi-Square or Fisher's exact tests and predictive measures are evaluated by linear regression model. Linear regression model is also used to evaluate if a GA predicts an aggressive breast cancer phenotype.

Participants

This section presents the demographic information of the data used for analysis, followed by the descriptive statistics of the study variables.

Demographic Information

A summary of demographic variables for participants enrolled in the study of associations between GA and aggressive breast cancer phenotype (N= 101) is presented

in Table 5.1. The demographics include: age, ethnicity, insurance type, BMI and level of education. Almost 80% of participants were white, 29.7% had 15 or more years of education and 37.6% were obese. Of the 101 participants enrolled on the study 67.3% had private insurance.

Clinical, pathological, and histological status of participant's tumors are shown in Table 5.2. Only 8.2% of the participant tumors were identified as TNBC while 26.7% of the TNBC were identified with high-grade tumors. Table 5.2 also shows 84.5% of the participants were found to have ER positive and 63.9% to have PR positive tumors. Only 23.7% of participants were diagnosed with HER2 positive tumors.

Table 5.1

Summary of Demographic Variables for 101 Women Enrolled in the Study

	Frequency	*Percent*
Age		
30 – 39	15	14.9
40 – 49	26	25.7
50 – 59	33	32.7
60 – 69	15	14.9
70 and older	12	11.5
Race/Ethnicity		
White	81	80.2
Non-White	20	19.8
Insurance Type		
Private	67.3	67.3
Medicare	21.8	21.8
Medicaid	10.9	10.9
Education		
9 – 12 Years	29	28.7
13 – 14 Years	42	41.6
15 or More	30	29.7
BMI		
≤ 24.9(Normal Weight)	29	28.7
25.0 – 29.9(Overweight)	34	33.7
≥ 30.0(Obese)	38	37.6

Note. This table shows descriptive detail of study participants.

Table 5.2

Data On Clinical, Histological, Pathological Status Of Tumors From 101 Women Enrolled In The Study

Parameters	Numbers (%)
Histology	
Low-Grade	33.3%
Intermediate-Grade	36.4%
High-Grade	26.7%
N/A	3.6%
ER	
Negative	15.5%
Positive	84.5%
PR	
Negative	35.1%
Positive	63.9%
N/A	1%
HER2	
Negative	63.9%
Positive	23.7%
N/A	12.4%
Sentinel Lymph Node	
Negative	73.5%
Positive	26.5%
TNBC	
No	91.8%
Yes	8.2%

Note. ER= estrogen receptor; PR= progesterone receptor; HER2=Human epidermal growth factor 2; TNBC- triple negative breast cancer; N/A- Not available.

Research Purpose and Hypotheses

The research purpose (RP) and its corresponding hypotheses (H) for this study are as follows:

RP 1: Determine if breast cancer susceptibility GAs are associated with an aggressive tumor phenotype in women with a new diagnosis of breast cancer and to assess if the GA is predictive of an aggressive phenotype.

H_0 1: There is no significant association between breast cancer susceptibility GAs and aggressive tumor phenotypes in women with a new diagnosis of breast cancer and the genetic variant does not predict an aggressive phenotype.

H_A 1: There is a significant association between breast cancer susceptibility GAs and aggressive tumor phenotypes in women with a new diagnosis of breast cancer or the GA predicts an aggressive phenotype.

RP 2: Determine if the aggressive breast cancer tumor phenotype is associated with a specific gene variant.

H_0 2: There is no significant relationship between the aggressive breast cancer tumor phenotype and a specific gene variant.

H_A 2: There is a significant relationship between the aggressive breast cancer tumor phenotype and a specific gene variant.

Description of Study Variables

The study variables of interest were breast cancer susceptibility GAs as identified by the NCCN Breast and Ovarian Cancer Genetic Assessment guidelines (NCCN, v2.2015b, page 29) and aggressive tumor phenotype according to molecular subtype of

breast cancer (Castro et al., 2013; Maier et al., 2014; Pern et al., 2012). Identified breast cancer susceptibility GAs used for analysis in the study were retrieved from GGC or commercial results. These DNA changes can be a mutation or VUS identified in any of the panel genes. The aggressive phenotype was defined as either TNBC or high-grade tumor (left or right breast).

Only 65 samples (N=101) included complete genetic results for the mutation analyses. All GGC results were from panel studies while 56.2% of commercial genetic testing results were from two-gene *BRCA* analysis and 43.6% from panel testing. The commercial lab testing decision was based on insurance reimbursement guidelines. Additionally, 66.7% of VUSs were detected using panel testing compared to 33.3% VUSs identified from two-gene *BRCA* analyses. This demonstrates that panel testing has the potential to reveal more variant results compared to gene specific analyses.

The TNBC and high-grade tumor data were analyzed separately to determine if associations existed among those variables and germline mutations. In a separate analysis using Chi-Square or Fisher's exact tests TNBC associations with GA (mutation and/or VUS) were assessed as well as linear regression analysis to see if GAs (mutation and/or VUS) predict TNBC. The same analyses were used for high-grade tumors and GAs to evaluate the association. They were also measured to evaluate if GAs in high risk breast cancer susceptibility genes could predict high-grade tumors in women with a breast cancer diagnosis.

Table 5.3 shows a summary of the study variables. For those with breast cancer susceptibility GAs, Table 5.4 shows the variety of gene names and variants identified in

the germline of study participants compared to the family history of hereditary breast and ovarian cancer syndromes in participants with a positive mutation or VUS result from genetic testing. Participant #58 has three VUSs (*SMAD4* c.606C>, *PALB2* c.1641C>T, *PALB2* c.2256A>G) and no family history indicated while participant #61 has two genetic mutations (*BRCA2*: c.6024G>C and *BRCA2*: c. 6252G>C) with a family history of breast, prostate and colorectal cancer. Participants #28 and #39, both with *APC* VUSs, have family history of colorectal cancer as well as breast cancer. Participant #70 has a mutation of *BRCA2* c.5621_5624delTTAA with a family history of breast and ovarian cancer. Two participants had a family history of pancreatic cancer: participant #66 with two VUSs (*BRCA1:* c.736T>G and *APC:* c.4905G>A) while participant # 96 was identified to have only one VUS in *RAD51C:* c.146C>T.

The results from Table 5.4 show the heterogeneous nature of breast cancer in population of women at high risk for hereditary breast and ovarian cancer. The data strongly suggest the importance of using a breast cancer panel for genetic testing, but also the need for assessing family history of other cancers such as pancreatic cancer (e.g., participants #96 and #66) and colorectal cancer (e.g., participants #61, #28 and #39) in conjunction with breast cancer history. These results also suggest that performing larger, more diverse testing panels than only breast cancer panels for these women may be essential for patient care and incorporation of personalized medicine for early detection and/or prevention of second primary breast cancers and other cancers for their family members.

Table 5.3

Summary of the Analysis of Susceptibility GAs and Aggressive Phenotype

	Frequency	*Percent*
GAs (mutation &/or VUS)		
No	51	49
Yes	18	17
Aggressive Phenotype		
No	66	68
Yes	31	32

Note. There were 65 complete GAs data samples out of 101 participants.

Table 5.4

Family History Of Hereditary Breast And Ovarian Cancer Syndromes In Participants With A GA Result From Genetic Testing

ID- Numbers	Genetic Testing	Family History of HBOC and Other Common Cancers
	Commercial Mutation	
#20	*BRCA2*: 4355del4	Breast cancer
#31	*PALB2*: c.172_175del	Breast, ovarian, CRC and other GI
#41	*CHEK2*: c.1265del	Breast, ovarian, prostate and CRC
#47	*BRCA1*: c.5319dupC	Prostate and gastric cancer
#51	*ATM*	Breast and uterine cancer
#61	*BRCA2*: c.6024G>C	Breast, prostate and CRC
#61	*BRCA2*: c. 6252G>C	Breast, prostate and CRC
#70	*BRCA2* c.5621_5624delTTAA	Breast and ovarian cancer
#87	*BRCA1* c. 4035delA	Ovarian, prostate and gastric cancer
#90		
	Commercial VUS	
#21	*PMS2*: c.2317A>G	Breast and brain cancer
#23	*BRIP*: c.550G>T	Breast and gastric cancer
#31	*ATM*: c.6919C>T	Breast, ovarian, CRC and other GI
#32	*MSH6*: c.3961A>G	Breast cancer and sarcoma
#51	*ATM*	Breast and uterine cancer
#94	*BRCA2:* c. 714_716dup	Breast and thyroid cancer
#96	*RAD51C:* c.146C>T	Pancreatic cancer
#106	*BRCA1:* p.E755K	Breast cancer
#118	*BRCA1:* p.E755K	None
	GGC Mutation Type	
#29	*PTEN* c. 1176delT	None
#47	BRCA1: c.5382dupC	Prostate and gastric cancer
	GGC VUS	
#21	*PMS2:* c.2317A>G	Breast and brain cancer
#22	*CDH1:* c.892G>A	Prostate and CRC
#27	*BRCA1:* c. 4039A>G	Breast cancer
#28	*APC:* c. 7514G>A	Breast and CRC
#39	*APC:* c.6921G>A	Breast, prostate, CRC and uterine cancer
#41	*MLH1:* c. 2252A>G	Breast, ovarian, prostate and CRC
#58	*SMAD4:* c.606C>	None
#58	*PALB2:* c.1641C>T	None
#58	*PALB2:* c.2256A>G	None
#66	*BRCA1:* c.736T>G	Pancreatic cancer
#66	*APC:* c.4905G>A	Pancreatic cancer

Note. Participants are identified by study number with their GA (mutation &/or VUS), and family history of cancer including hereditary breast and ovarian cancer syndrome-cancers, colorectal cancer (CRC), Gastrointestinal (GI), variant of unknown significant. c. = coding DNA, p. = protein sequence.

Statistical Results

For Hypothesis 1 and 2, non-parametric Chi-Square and Fisher's Exact analyses as well as linear regression model were used to observe the association between breast cancer susceptibility GAs and aggressive tumor phenotype according to molecular subtype of breast cancer.

Research Hypothesis 1

Hypothesis one seeks to determine if breast cancer susceptibility GAs are associated with an aggressive tumor phenotype in women with a new diagnosis of breast cancer. To assess this question, a Pearson's chi-square test was used to explore the association between breast cancer susceptibility GAs with an aggressive tumor phenotype in women with a new diagnosis of breast cancer. Results of the Fisher's Exact test showed that breast cancer susceptibility GAs were not associated with an aggressive tumor phenotype in women in this study with a new diagnosis of breast cancer, $\chi^2(1) = 2.33$, $p = 0.1$. Therefore, the null hypothesis is retained, concluding that there is no significant association between breast cancer susceptibility GAs and aggressive tumor phenotypes in women with a new diagnosis of breast cancer in the upstate of SC.

The study originally defined aggressive phenotype to be considered as anyone with TNBC or high-grade tumors. The results from the analysis showed no significant results with either Pearson's chi-square, $\chi^2(1) = 2.33$, $p = 0.1$ or regression analysis, $F(1/ 64) = 1.119$, $p = .29$. However, when the study analyzed only high-grade tumors (df=1 and 95.0% confidence interval) it found significant results, $F(1/ 64) = 4.40$, $p=. 036$,

association of high-grade tumors to GA (Tables 5.5a and 5.5b) was considered. This shows that a GA predicts a high-grade tumor status.

Table 5.5a

*Crosstabulation of GAs and High-Grade Tumor**

	Breast Cancer Susceptibility GAs (Mutations and/or VUS)	
	No	Yes
High-Grade tumors		
No	39 (81.3%)	9 (18.8%)
Yes	12 (57.1%)	9 (42.9%)

Note. $\chi^2 = 4.40$, df = 1. Numbers in parentheses indicate column percentages. (*p*=.036)

Table 5.5b

*Regression Analysis of GA and High-Grade Tumor**

Model	Sum of Squares	df	F	Sig.
Regression		1	4.567	.036

Note. *p*= .036, df = 1, 95.0% Confidence Interval. Dependent Variable: High-grade tumor and predictor: Genetic Alterations (GA).
**p*<.05

Research Hypothesis 2

Research hypothesis 2 sought to determine if the aggressive breast cancer tumor phenotype is associated with a specific gene variant**.** Since there were no associations between aggressive phenotype and specific GA in this study and the null hypothesis is retained, it is concluded there is no specific GA associated with an aggressive breast cancer tumor phenotype among 65 women.

Additional Analyses

High-grade breast tumors have be associated with inherited *BRCA1* and *BRCA2* mutations **(**Agnarsson, Jonasson, Björnsdottir, Barkardottir, Egilsson, & Sigurdsson, 1998). To further explore the study variables, the association of tumor markers with high-grade tumors in women with a new diagnosis of breast cancer was noted in this study. To analyze data for this question, a Fisher's Exact test was used and the results are shown in Tables 5.6 through 5.8.

Additional data analysis based on ER percentage status showed significant results, $\chi^2(1) = 19.5$, $p <$. 05, which indicated that the tumors with ER "negative" status or "low" ER percentage tumors were associated with high-grade tumors when compared to "high" ER percentage tumors (ER<1%: Negative, ER= 1-32%: Low, ER= 33% or higher: High). Table 5.6 shows a summary of significant association between ER status and high-grade tumors in this participant population, $\chi^2(1) = 19.5$, $p = .001$. The results from the crosstab analysis are displayed in Table 5.7; the results showed that the tumors with PR "positive" status are associated with not having high-grade tumors, $\chi^2(1) = 11.07$ $p = .004$. There is

no significant difference between high-grade tumor and HER2 status as presented in Table 5.8.

The data were also analyzed assessing the association between aggressive tumor phenotype and GAs using Crosstabulation with bootstrapping option which showed non-significant results in accordance with what the analyses showed previously, $\chi^2(1) = 2.32$, $p = .11$.

This research was also designed to analyze the associations of the demographic variables by aggressive tumor phenotype in women with a new diagnosis of breast cancer, as well as the family history by aggressive phenotype. Table 5.9 shows a summary of each demographic variable by aggressive tumor phenotype. Results of the crosstab tests show that Race/Ethnicity was significantly different between aggressive phenotypes (no vs. yes), $\chi^2(1) = 6.15$, $p = .013$.

Research studies have shown that patients with germ-line mutations tend to have more aggressive cancer phenotypes (Castro et al., 2013; Maier et al., 2014; Pern et al., 2012). Since TNBC, high-grade tumor and disease stage are typically associated in the clinical setting with aggressive breast cancer, these variables were analyzed to determine if there was a significant difference among TNBC, high-grade tumor, or disease stage. Chi-square analysis found no significant differences in any of these variables related to their GAs except for high-grade tumors, $\chi^2(1) = 4.40$, $p = .038$ and linear regression analysis result, $F(1/64) = 4.40$, $p = .036$.

Table 5.6

Crosstabulation of ER Status and Presence of High-Grade Tumor

	ER % Status		
	Negative*	Low*	High
High-Grade Tumor			
No	4 (33.3%)	11 (50%)	55 (88.7%)
Yes	8 (66.7%)	11 (50%)	7 (11.3%)

Note. χ^2 = 19.5, df = 1. Numbers in parentheses indicate column percentages.
ER<1%: Negative, ER= 1-32%: Low, ER= 33% or higher: High
*p < .05

Table 5.7

Crosstabulation of PR Status and Presence of High-Grade Tumor

	PR Status*	
	Negative	Positive
High-Grade Tumor		
No	18 (52.9%)	52 (83.9%)
Yes	16 (47.1%)	10 (16.1%)

Note. χ^2 = 11.07, df = 1. Numbers in parentheses indicate column percentages.
*p < .05

Table 5.8

Crosstabulation of HER2 Status and Presence of High-Grade Tumor

	HER2 Status		
	Negative	Positive	Not Done
High-Grade Tumor			
No	46 (74.2%)	18 (78.3%)	7 (58.3%)
Yes	16 (25.8%)	5 (21.7%)	5 (41.7%)

Note. χ^2 = 1.68, df = 1. Numbers in parentheses indicate column percentages.

Table 5.9

Crosstabulation of Demographic Variables and Presence of Aggressive Phenotype

	Aggressive Phenotype		
	No	Yes	χ^2
Age			2.92
30 – 39	7 (10.6%)	7 (22.6%)	
40 – 49	18 (27.3%)	6 (19.4%)	
50 – 59	22 (33.3%)	10 (32.3%)	
60 – 69	10 (15.1%)	5 (16.1%)	
70 and Older	9 (13.6%)	3 (9.7%)	
Race/Ethnicity			6.15**
White	57 (74%)	20 (26%)	
Non-White	9 (45%)	11 (55%)	
Insurance Type			3.48
Private	41 (62.1%)	24 (77.4%)	
Medicare	16 (22.9%)	6 (19.4%)	
Medicaid	9 (13.3%)	1 (3.2%)	
Education			0.71
9 – 12 Years	17 (25.7%)	10 (32.3%)	
13 – 14 Years	27 (41%)	13 (41.9%)	
15 or More	22 (33.3%)	8 (25.8%)	
BMI			0.19
≤ 24.9(Normal Weight)	20 (30.3%)	8 (25.8%)	
25.0 – 29.9(Overweight)	23 (34.8%)	11 (35.5%)	
≥ 30.0(Obese)	23 (34.8%)	12 (38.7%)	

*$p < .05$

Limitations

Although this research was meticulously prepared, there are some limitations to the study which include:

- only 101 participants were accrued due to time limitations. Since this was a non-probability sample, the results may not be generalized;
- because of the absence of genetic testing lab results for all participant samples, the complete data on GAs were not available for analysis;
- commercial panel results were not complete due to personal preference to not join the study, insurance authorization requirements and/or the financial status of participants. The study included all eligible women regardless of their insurance status;
- due to the study's small sample size, a single variable made as "GA" which defined as either having a genetic mutation or VUS. These variables were not analyzed separately;
- specific population of participants seeking treatments for breast cancer at the cancer center;
- potential confounding factors not accounted for due to the small sample size. These confounding factors could include age distributions, ethnicity, inconsistent information in the family history, requirement of hospital use by insurance companies, insurance requirements for genetic testing and use of different laboratories for the genetic testing.

Conclusions

This research study has the potential to provide several contributions to patient care. Because of the low sample size some results are difficult to discuss in light of the population from which the sample was drawn but there are hints at potential application to patient care.

Overall this study aimed to determine the associations between aggressive breast cancer phenotype and breast cancer susceptibility gene mutations and their variants. There was a significant difference between high-grade tumors and GAs, $F\ (1/\ 64) = 4.40$, p=. 036, among study participants emphasizing that GAs are associated with high-grade tumors as it was reported by this research and other similar research studies (Castro et al., 2013; Maier et al., 2014; Pern et al., 2012).

Additionally, this study revealed ER "negative" status or "low" ER percentage tumors were associated with high-grade tumors when compared to "high" ER percentage tumors (ER<1%: Negative, ER= 1-32%: Low, ER= 33% or higher: High), $\chi^2(1) = 19.5$, p <. 05. It was also noted the population of non-white women have a significantly more aggressive tumor phenotype when compared to other ethnicities $\chi^2(1) = 6.15$, p =. 013. However, the results showed no significant differences between aggressive tumor phenotypes (high-grade tumors and/or TNBC) and breast cancer GAs (commercial or GGC mutation and/or VUS) in women at high risk for hereditary breast cancer syndromes, $\chi^2(1) = 2.33$, $p = 0.1$. This could be due to the limitations of this study such as small sample size, missing genetic testing results from the laboratories and the population

of participants seeking treatments for breast cancer at the cancer center.

The study results also hint at the future potential for multi-panel genetic testing not only for the women at high risk for germline mutations, but also for women with high-grade breast tumor histology. Due to the study results an emphasis for considering family history of cancer during decision-making regarding genetic testing continues to be necessary for women at high risk for germline variants as well as their tumor biomarkers. Some of the participants with GAs in this research had family history of other cancers (not just breast and ovarian cancer) such as pancreatic cancer (participants #66 and # 96) and colorectal cancer (participants #31, #61, #22, #28 and # 39). These results, although small (43.6% of mutations and 66.7% of VUSs were from panel testing), indicate that including multi-panel genetic testing that includes related cancer predisposition genes, not just *BRCA1* and *BRCA2* genes, is essential to achieve better patient outcomes. Currently, only women meeting the strict requirements for being at high risk for hereditary breast and ovarian cancer are eligible for breast cancer genetic testing (NCCN, 2015a). Expansion of the scope of genetic testing eligibility based on tumor histology as well as ER/PR/ HER2 biomarkers status brings the future of personalized treatment to better patient outcome.

Future Research

While this dissertation reveals interesting phenomena associated with hereditary breast cancer and the approach introduced providing a natural guide to future research, there are still many unanswered questions to investigate. To enhance understanding of the aggressive breast cancer associated with GAs and to determine future clinical application,

the recommended future studies are summarized as follows:

- evaluating the recognized association between the grade of tumors and germ-line variants further to be confirmed with larger number of participants first;
- determining if there are more GAs in a larger population of women with breast cancer that could predict the growth and progression of future breast cancers in a woman;
- developing another study to follow up on the incidental findings such as participants with pancreatic cancer, participants #66 (with double VUS) and # 96;
- investigating if socioeconomic factors affecting genetic testing and how to improve the quality of care for uninsured or underinsured patients;
- comparing other important variables such as mammography reports, social behavior (smoking and drinking habit) and lifestyle would be beneficial;
- investigating the association between high-grade tumors and GAs in more diverse population;
- revising eligibility criteria for the future study to include patients from diverse population as well as considering tumor biomarkers (ER, PR and HER2 status) and tumor grade to eligibility criteria;
- developing a mixed methods research study applying PMT is highly recommended to be able to motivate the population of women to follow

the prevention strategies to reduce their risk of another primary cancer diagnosis specifically in women with diverse ethnic background. As noted by this study previously, non-white women had a more aggressive phenotype when compared to white women and this could be due to several factors including lack of education about prevention and early detection strategies. In order to educate and motivate the population of women, applying four concepts of PMT is suggested for future studies (severity of the health problem, vulnerability or risk of the threat, self-efficacy and response to the protective measures) (Helmes, 2002).

Summary

Breast cancer genetic testing guidelines established by NCCN suggest testing only for the patients at high risk for hereditary breast and ovarian cancer based on very specific guidelines (NCCN, 2015a). Although there are several research studies on cancer and its epidemiology that have led to guidelines for early detection and risk reduction, a low percentage of people (less than 0.2% of women in United States use Tamoxifen and approximately 3% of Australian women follow the prevention guidelines) follow the suggested strategies; especially those from diverse ethnicities (Ralph et al., 2014).

In summary, the study did predict associations between breast cancer susceptibility GAs and high-grade tumors in women with a new diagnosis of breast cancer and at high risk for hereditary breast cancer syndromes, $\chi^2(1) = 4.40$, $p =. 038$. Having a high-grade breast tumor could be important in breast cancer genetic testing

guidelines since results from this research study suggest that ER and PR markers as well as ethnicity are predictive of aggressive tumor phenotype in the population of women with breast cancer.

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CHAPTER SIX

SYNTHESIS

The American Cancer Society anticipates more than 200,000 new cases of invasive breast cancer in 2015 (American Cancer Society, 2015) with 10% of the cases believed to be the result of germline mutations (Tung et al., 2015). Family history of breast cancer, ovarian cancer, pancreatic cancer and prostate cancer are all classified as frequently identified in families with hereditary breast cancer syndromes (NCCN, 2015). Moreover, breast cancer is influenced by ethnicity and race where African American women in the United States tend to be diagnosed with a more aggressive type of breast cancer as compared to Caucasian women (Boone, et al., 2014; Iqbal, et al., 2015).

Healthcare professionals have a critical role in care for patients in regards to screening, diagnosis, treatment and translation of current genomic knowledge into practice to disseminate better outcomes. Through extensive biomedical research, advances in DNA sequencing have helped clinicians make progress in cancer screening, prevention and treatment (Chin, Hahn, Getz, & Meyerson, 2011; Grada, & Weinbrecht, 2013; Hawthorn, Luce, Stei, & Rothschild, 2010; Staren, et al., 2014).

All chapters of this body of work present various aspects about aggressive breast cancer; *ATM* and *PALB2*, two new high risk gene mutations that may cause malignant disease much like the *BRCA* genes, aggressive inflammatory breast cancer (IBC), application of a health promotion theory targeting women who need a plan to prevent or detect another cancer at an early stage and multi-gene panel research to identify a high susceptibility GA for breast cancer. The purpose of the dissertation work was to

determine if breast cancer susceptibility genetic alterations (GA) could predict an aggressive phenotype.

In order to better understand the new GAs associated with hereditary breast cancer, the first manuscript (Chapter two) took an analytical look at breast cancer through genomic investigation of two newly recognized genes*;* the *ATM* and *PALB2* genes. A comprehensive review and synthesis of current literature was completed to discuss updates on guidelines for genetic testing and prevention strategies. According to the newest version of the National Comprehensive Cancer Network (NCCN) guidelines (Genetic/Familial High Risk Assessment: Breast and Ovarian, version 2.2015), patients must meet one or more breast cancer risk assessment criteria in order to be eligible for genetic testing for the hereditary breast and ovarian cancer (HBOC) syndrome (See Figure 5.1) (NCCN, 2015). This review of literature concluded that multi-gene panels testing to detect *ATM* and *PALB2* gene mutations would be appropriate for the population of individuals considered to be "high risk" for hereditary breast cancer syndromes, even though the prevalence appears to be low; meaning there are other genes besides the well known *BRCA1* and *BRCA2.*

After careful consideration of multi-gene panel genetic testing, the second manuscript took an in depth look at IBC (an aggressive type of breast cancer), the mechanism of inflammation and potential causes of the aggressive IBC. The purpose of the literature synthesis was to discuss IBC and explain disease pathogenesis, new genomic discoveries, diagnosis and treatment. In the United States, 2.5 percent of new breast cancer diagnoses are the rare and aggressive type of IBC with specific features

such as edema, swelling, erythema, triple negative tumor markers, rapid metastasis and poor prognosis (Fernandez, et al., 2013; Makower & Sparano, 2013; Robertson, et al., 2010; Shkurnikov, et al, 2013). The results from this comprehensive synthesis of literature based on the GA of IBC indicate that IBC has a heterogeneous and complex nature. Further investigation and new personalized drug developments are critical to achieve better prognosis and overall survival rate. Increased collaborative interdisciplinary research is recommended to improve IBC awareness as well as educating the general public about the disease that could improve patient outcomes.

Because women with germline mutations and breast cancer have a higher risk of being diagnosed with another primary breast cancer as well as ovarian cancer, pancreatic cancer, or melanoma, prevention from further cancer diagnosis is very critical for these women. Considering the aggressive nature of breast cancer and the fact that the percentage of individuals who follow the prevention guidelines are low (10 percent based on chemoprevention studies) (Evans, Lalloo, Shenton, Boggis, & Howell, 2001; NCCN, 2015; Ralph et al., 2014), it is important to educate patients in prevention and early detection strategies.

The dichotomy between the necessity and actual usage of screening and prevention methods in high risk patients has led to incorporation of the Protection Motivation Theory (PMT) into the third manuscript of this dissertation. This theory could be used as a tool to offer education for women with breast cancer and have positive breast cancer susceptibility GA. PMT is comprised of four concepts that effect decision making: 1) severity of the health problem; 2) vulnerability or risk of the threat 3) self-

efficacy; and, 4) response to protective measures that promote a health behavior (See Figure 4.1) (Helmes, 2002; Maddux & Rogers, 1983; Ralph et al., 2014; Rogers, 1975). Several studies have applied PMT to a health behavior using the same concepts including research on breast cancer genetic testing, life style change and obesity, physical activity adherence and skin cancer screening (Baghianimoghadam, Mohammadi, Noorbala, & Mahmoodabad, 2011; Cyrus-David, & Strom, 2001; Frosch, Mello, & Lerman, 2005; Grindley, Zizzi, & Nasypany, 2008; Helms, 2002; Lee et al., 2007; Morrison et al., 2010; Ralph et al., 2014). The suggested pilot study would utilize the instruments applied to these studies to assess the application of PMT to motivate patients with GAs to follow screening and prevention strategies. This is a new approach with the theory for patients with a diagnosis of aggressive breast cancer and a GA.

According to several research studies, there is a link between aggressive tumor phenotype and GAs in different hereditary cancer syndromes (Castro et al., 2013; Maier et al., 2014; Pern et al., 2012). Studies have shown triple negative breast cancer, an aggressive phenotype, is seen in patients with mutations in *BRCA1, BRCA2, PALB2* and *BRD7* genes (Pern et al., 2012).

Considering the importance of understanding the genetic mechanisms of breast cancer and associations to phenotypic outcomes, the fifth chapter, a quantitative research study, was developed. The purpose of this study was to determine (1) if breast cancer susceptibility GAs are associated with an aggressive tumor phenotype in women with a new diagnosis of breast cancer and (2) if the aggressive breast cancer tumor phenotype is associated with a specific gene variant (s).

The results predicted the association between breast cancer GA and high-grade tumor phenotype in women with breast cancer, $F\ (1/\ 64) = 4.40$, p=. 036 (See Table 5.5a and 5.5b). The aggressive phenotype in this research is defined as either having triple negative marker status or a high-grade tumor and GA is described as having a designated mutation or variant of unknown significance (VUS) result from either Greenwood Genetic Center (GGC) or commercial genetic testing laboratories. The results also revealed that estrogen receptor (ER) or progesterone receptor (PR) status is associated with breast cancer tumor grade status in a separate analysis (See Tables 5.6 through 5.8).

Despite an increasing knowledge in breast cancer genomics and studies evaluating the disease based on different ethnic backgrounds, the guidelines for breast cancer genetic testing eligibility are not focused on ethnicity (NCCN, 2015). This study revealed that aggressive tumor phenotype is associated with non-white population of women participated in the study, $\chi^2(1) = 6.15$, p=. 013. This suggests the need for recommendations for genetic testing guidelines to be revised to include multi-panel genetic testing for all eligible individuals as well as inclusion of tumor biomarkers and ethnicity in eligibility criteria.

Summary

This dissertation reviewed and analyzed different aspects of aggressive breast cancer such as *ATM* and *PALB2* gene mutations, aggressive IBC, application of PMT and multi-gene panel testing for breast cancer. Even though a small percentage of breast cancer cases are the result of GAs (Tung et al., 2015), there are several guidelines and protocols available to encourage the use of screening tools for early detection and

prevention of developing breast cancer in women at high risk. However, current use of the diagnostics and the chemoprevention agents is low (e.g., almost ten percent in chemoprevention studies) (Evans et al., 2001; NCCN, 2015; Vogel, 2010).

This research analyzed ER, PR status and breast cancer tumor grades. Currently genetic testing eligibility criteria are based on age, ethnicity, family history and negative ER/PR/human epidermal growth factor 2 (HER2) status. This study suggests multi-panel genetic testing based on tumor histology according to significant association between having breast cancer GAs and an aggressive high-grade tumor phenotype, $\chi^2(1) = 4.40$, p=. 038 might also be important.

After reviewing breast cancer genomics, aggressive IBC, PMT application in breast cancer studies and multi- panel genetic testing, there are hints for the need to change guidelines that could affect women at high risk to develop an aggressive breast cancer. A synthesis of the work suggests the need for genetic testing for all women at high risk for breast cancer. This should be in a timely manner to better guide treatment and to implement strategies toward more personalized medicine. Applying PMT would also be beneficial to educate patients, particularly in low income and minority ethnic groups, to change their intention toward a healthy behavior.

Future research should: 1) include more participants in a replicative study with complete genetic results to determine if there are more GAs in a larger population; 2) develop another study to follow incidental findings; 3) investigate if socioeconomic factors affecting genetic testing; 4) compare other essential variables; 5) revise eligibility criteria for breast cancer genetic testing; 6) apply PMT to educate patients; and, 7)

investigate more individuals from diverse populations.

With advances in genomic technology, the body of work of this dissertation represents the urgent need to support similar studies. More studies would provide more evidence to pave the path toward personalized medicine, particularly in breast cancer research.

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APPENDICES

Appendix A

Informed Consent

Informed Consent Form
Clemson University/Greenwood Genetic Center/St Francis Hospital

TITLE OF THE STUDY: Collaborative Oncology Testing for Breast Cancer: A New Model for South Carolina

INVESTIGATOR: Julie Eggert, PhD, GNP-BC, AOCN®

STUDY SPONSOR(S): School of Nursing
College of HEHD, Clemson University
Greenwood Genetic Center

OTHER INVESTIGATORS: Alka Chaubey, PhD
Mike Friez, PhD

Participant's Printed Name: _____

Introduction
We invite you to take part in a research study "Collaborative Oncology Testing for Breast Cancer: A New Model For South Carolina" at Bon Secours St. Francis Hospital, which seeks to identify a more effective means of diagnosing breast cancer. Taking part in this study is entirely voluntary. We urge you to discuss any questions about the study with our staff. Talk to family and friends and take your time to make a decision. If you decide to participate you must sign this form to show that you want to take part.

Purpose of the Research Study
You are being asked to take part in this study because you have breast cancer and will be receiving treatment. The purpose of this research study is to validate the ability of a new genetic diagnostic panel developed by the Greenwood Genetic Center (GGC) Diagnostic Laboratory, to identify known breast cancer-causing DNA changes (mutations) present in South Carolina women; especially diverse populations. In addition, this research will determine if the new genetic diagnostic panel can identify other currently unknown DNA changes in other breast cancer-related genes.
After diagnostics are complete, stored tumor tissue will be obtained from St. Francis Hospital and sent to the laboratory in Greenwood to see if there is a link between germline DNA changes and gene alterations.

The sponsor expects to enroll at least 200 South Carolina participants in this research study: 100 participants diagnosed with leukemia and 100 separate individuals with a breast cancer diagnosis.

Procedures

If you are interested in participating in the research study your medical record will be reviewed to find out if you meet the study requirements; a new diagnosis of breast cancer.

You will have your blood drawn one time only at the same time as your typical laboratory studies requested by your treatment doctor. Approximately two tablespoonsful will be drawn for this study. The blood sample will be delivered to the Diagnostic Laboratory of GGC where experimental procedures will be used to examine the deoxyribonucleic acid (DNA) and determine the state of any genetic changes associated with breast cancer.

After diagnostics are completed, your previously-stored breast cancer tumor tissue will be also collected based on St. Francis Hospital policies and procedures, delivered to the GGC Diagnostic Laboratory and stored as de-identified tissue following established policies and procedures.

The study coordinators will collect demographic and breast cancer-related information from your laboratory results included in your electronic health record (chart).

This is a Pilot Study (initial research) funded through the Self Family Foundation to determine if a larger study is feasible for submission to a national funding agency.

Time Duration of Study and Procedures
If you agree to take part in this study, your involvement will last approximately 15 minutes. You will not be asked to return to the clinic for a follow-up visit.

Risks and Discomforts
While participating in the study, approximately two tablespoonsful of extra blood will be collected once at the time of your routine lab visit for treatment. The removal of the two tablespoonsful for purposes of the study will not cause a risk to you. There is a risk to confidentiality, but the Privacy and Confidentiality section below describes those actions the researchers will take to minimize that risk during the research study.

Potential Benefits
While you will receive the results of your diagnostic testing, you will not be notified of the results from this test, so you may not experience benefit from this research other than believing you are helping persons in the future with a diagnosis similar to yours. The sponsor believes the results of the research will lead to a better understanding of breast cancer and ultimately may be used to guide the future treatment of others with this diagnosis.

Alternatives
Persons may choose not to participate in the study and blood samples will be used only for routine lab-work requested by the treatment doctors. Tumor tissue would not be collected.

Privacy and Confidentiality
After consent, you will be assigned a study number. The Principal Investigator will maintain the only copy of a coded list with participant name and study number through the end of the study. This list will be secured in a password-protected laptop in a locked room. Once accrual numbers are met and the study is completed the coded list will be destroyed.

When retrieved from the electronic health record, all study records will be provided the assigned study number and de-identified with removal of name, date of birth, social security number, address or phone number. Records that are reviewed, stored, and analyzed at Upstate Oncology Associates or Clemson University will be kept in a password protected laptop computer; secured in a locked office. In addition the de-identified information will be available to all sites via a password protected and secure "cloud" account.

In addition, only the designated study number and the date of collection will identify samples sent to GGC for use in diagnostics. All identifiers such as name, date of birth, social security number, address, or phone number will be removed prior to transport from St Francis Hospital to the GGC lab. The numbered samples will be refrigerated (blood) or frozen (tumor) in a laboratory prior to use. Once the study is completed all stored samples will be destroyed and disposed of, according to protocol, as biohazardous waste, except for those stored samples you agree to have stored for future research in the Optional Tissue Storage for Future Use section and consent.

All data will be kept for five years.

In the event of any publication or presentation resulting from this research, no personally identifiable information will be shared. We will keep your participation in this study confidential to the extent permitted by law. However, it is possible that other people may become aware of your participation in this study. For example, the following people/groups may inspect and copy records pertaining to this research:

- The Office of Human Research Protections in the U. S. Department of Health and Human Services;
- The Bon Secours Richmond Health System (BSRHS) Institutional Review Board (IRB); and/or
- The study sponsor or any agency/individuals that would have access to data.

Some of these records could contain information that personally identifies you. Reasonable efforts will be made to keep the personal information in your research record private and confidential but absolute confidentiality cannot be guaranteed.

Use of Protected Health Information:
Protected Health Information (PHI) about you will be collected if you choose to be part of this research study. Protected Health Information is protected by law. At Bon Secours Health System your PHI will only be used or shared as explained and authorized in this consent form or when required by law. It is possible that some of the other people/groups who receive your PHI may not be required by Federal privacy laws to protect your PHI and may share it without your permission. However, this team of research professionals/group of researchers are committed to keeping your PHI confidential.

To participate in this research you must sign this form and allow the investigator to use your PHI. If you do not want the investigator to use your PHI, you may not participate in this study.

If you choose to participate, you are free to withdraw your permission for the use and sharing of your PHI at any time. You must do this in writing to Dr. Eggert and inform her that you are withdrawing from the study. Her mailing address is 528a Edwards Hall, Clemson University, Clemson, SC 29634.

If you withdraw your permission:
You will not be able to continue in this study. However, we are unable to take back anything we have already done or any PHI already shared. We may continue using and sharing PHI obtained prior to your withdrawal if necessary for the soundness of the research. We will keep our records of your participation in this study as long as the law requires.

The research team may use the following sources of PHI; blood and tumor samples and related tests, medical history as it relates to the research study, x-rays, MRIs, questionnaires, and other diagnostics as related to the research study during the first three months of diagnosis and treatment.

Representatives of the following people/groups within Bon Secours Health System may use your PHI and share it with other specific groups in connection with this research study. This includes the principal investigator, Dr. Julie Eggert, study coordinators and doctoral students, Sourat Darabi and Matt Tedder, and BSRHS Institutional Review Board.

The above people/groups may share your PHI with the following people/groups outside

Bon Secours Health System who may, in the course of monitoring the study, review or copy your records.

- The Office of Human Research Protections in the U. S. Department of Health and Human Services;
- Clemson University IRB; and/or,
- Co-Investigators Drs. Alka Chaubey and Mike Friez (Greenwood Genetics).

Refusal or Withdrawal without Penalty
Taking part in this study is your choice. There will be no penalty if you decide not to be in the study. If you refuse to participate or withdraw from the study, you will not be penalized or lose any benefits and your decision will not affect your relationship with your doctor or hospital. You are free to withdraw from this research with this institution. You may be removed from the study without your consent if the sponsor ends the study or if you are not following the study rules.

Costs for Participation
There are no costs to you to participate in this research study. There is no anticipated injury since the blood draw is part of your treatment standard of care.

You will not lose any legal rights by signing this form.

Compensation for Participation
You will not receive any compensation for being in this research study.

Research Funding
Clemson University and GGC are receiving funding from the Self Family Foundation for this research study. Supplies for blood draws and sample transport will be provided by GGC. Healthcare Genetics doctoral students working as study coordinators will be reimbursed by Clemson University for work completed on this research project.

Voluntary Participation
Participation in this research study is voluntary. You may refuse to participate or withdraw from the study at any time. If you refuse to participate or withdraw from the study, you will not be penalized or lose any benefits and your decision will not affect your relationship with your doctor or hospital or your treatment.

You will receive a copy of this consent form. You may also receive a copy of the protocol (full study plan) upon request.

Contact Information for Questions and Concerns
For more information concerning this research study or to express concerns or complaints, you may contact the principal investigator (study doctor), Dr. Julie Eggert, (864)-640-1869.

If you have questions regarding your rights as a research participant or general questions about the research or your privacy, please contact:

- Bon Secours Richmond Health System Institutional Review Board, 8580 Magellan Parkway, Richmond, VA 23227
  (804) 627-5157

Signature and Consent/Permission to be in the Research

Before deciding to enroll in this research study you should have:

- Discussed this study with an investigator,
- Reviewed the information reviewed in this form, and
- Had the opportunity to ask any questions you may have.

Your signature below means that you have received this information, have asked any questions you have about the research and your questions have been answered. You will receive a copy of this signed and dated form to keep for future reference.

Participant: By signing this consent form, you are voluntarily choosing to take part in this research.

_____	_____	_____
Signature of Participant	Date	Printed Name

Participant's Legally Authorized Representative: By signing below, you indicate that you give permission for the participant to take part in this research.

_____	_____	_____
Signature of Participant's Legally Authorized Representative	Date	Printed Name

Person Explaining the Research: Your signature means that you have explained the research to the participant/participant representative and have answered any questions he/she has about the research.

Signature of person who explained this research	Date	Printed Name
Signature of the Principal Investigator	Date	Printed Name

Optional Tissue Storage for Future Use

Your participation in this research study does not require you to provide breast cancer tumor tissue, other than the previously-stored tumor tissue as described in the main consent form and to be used as a part of this study. If you agree, the researchers will store a leftover sample of your tumor so that it may be studied in the future after this study is over for other purposes. These future studies may provide additional information that will be helpful in understanding breast cancer, but it is unlikely that these studies will have a direct benefit to you. The results of these tests will not have an effect on your care. You will not receive results of these future research tests, nor will the results be put in your health record. Sometimes tissue is used for genetic research about diseases that are passed on in families. Even if your sample is used for this kind of research, the results will not be placed in your health records. It is possible that your tumor tissue might be used to develop products or tests that could be patented and licensed. There are no plans to provide financial compensation to you should this occur. If you have any questions, you should contact Dr. Julie Eggert at (864) 640-1869

Your optional breast cancer tumor sample(s) will not be labeled with any of your personal information, such as your name. Once you give your permission to have your leftover samples stored, they will be available for use in future research studies indefinitely and cannot be removed due to the inability to identify them.

Please initial below to indicate your preferences regarding the optional storage of leftover tissue for future research studies.

a. Sample(s) of your blood/tumor tissue may be stored and used for future research studies to learn about, prevent, treat or cure breast cancer. _____ Yes _____ No

b. Sample(s) may be stored and used for research about other health problems. _____ Yes _____ No

c. Sample(s) without any identifying information may be shared with other investigator/groups. _____ Yes _____ No

Participant: By signing below, you indicate that you have read the information written above and have indicated your choices for the optional storage of tissue for future research studies.

Signature of Participant	Date	Printed Name

Participant's Legally Authorized Representative: By signing below, you indicate that you have read the information written above and have indicated your choices for the optional storage of tissue for future research studies.

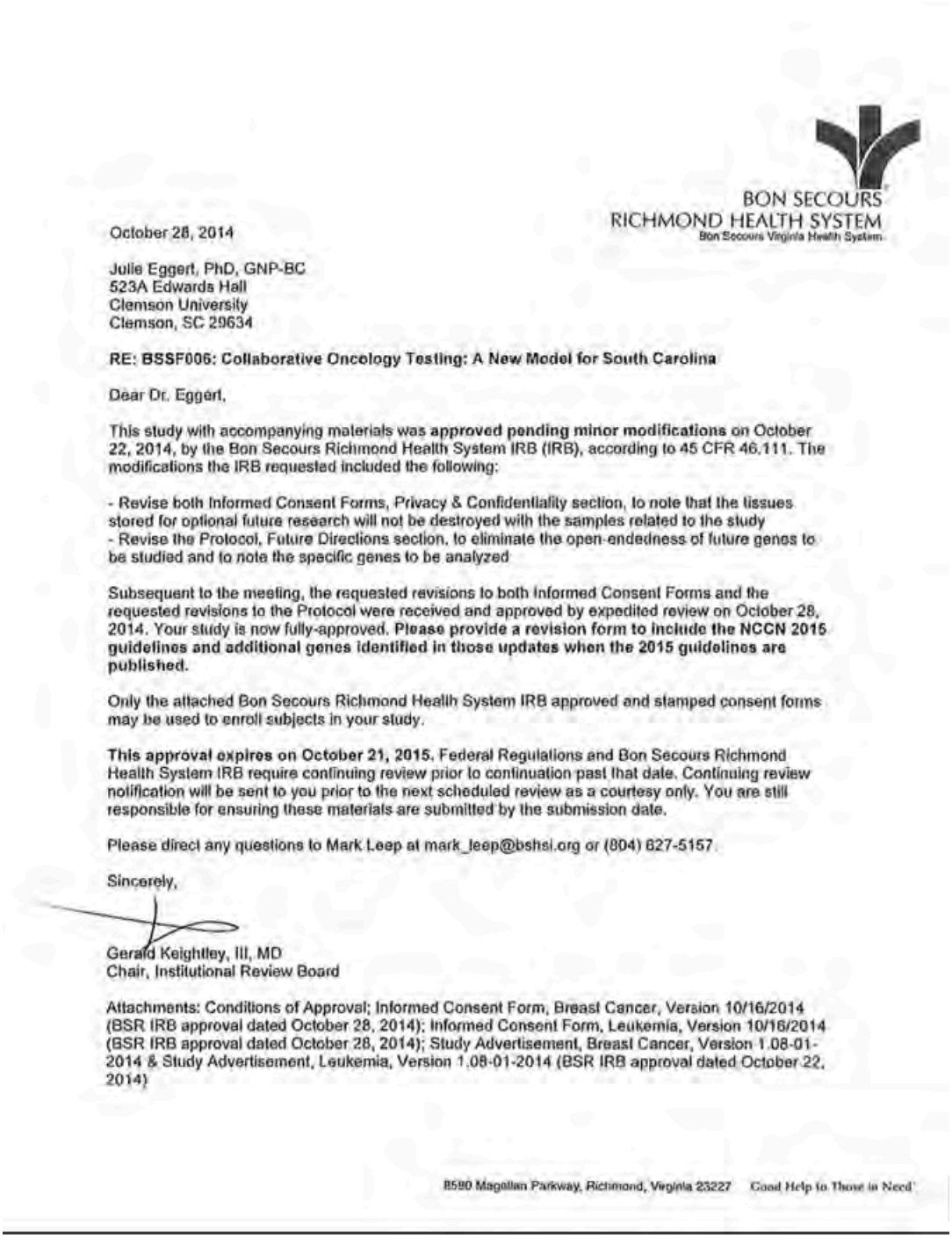
Signature of Participant's Legally Authorized Representative	Date	Printed Name

Person Explaining the Optional Tissue Storage: Your signature means that you have explained the optional tissue storage to the participant/participant representative and have answered any questions he/she has about the optional tissue storage.

Signature of person who explained this optional tissue storage	Date	Printed Name

Appendix B

St. Francis IRB Approval



BON SECOURS
RICHMOND HEALTH SYSTEM
Bon Secours Virginia Health System

October 28, 2014

Julie Eggert, PhD, GNP-BC
523A Edwards Hall
Clemson University
Clemson, SC 29634

RE: BSSF006: Collaborative Oncology Testing: A New Model for South Carolina

Dear Dr. Eggert,

This study with accompanying materials was **approved pending minor modifications** on October 22, 2014, by the Bon Secours Richmond Health System IRB (IRB), according to 45 CFR 46.111. The modifications the IRB requested included the following:

- Revise both Informed Consent Forms, Privacy & Confidentiality section, to note that the tissues stored for optional future research will not be destroyed with the samples related to the study
- Revise the Protocol, Future Directions section, to eliminate the open-endedness of future genes to be studied and to note the specific genes to be analyzed

Subsequent to the meeting, the requested revisions to both Informed Consent Forms and the requested revisions to the Protocol were received and approved by expedited review on October 28, 2014. Your study is now fully-approved. **Please provide a revision form to include the NCCN 2015 guidelines and additional genes identified in those updates when the 2015 guidelines are published.**

Only the attached Bon Secours Richmond Health System IRB approved and stamped consent forms may be used to enroll subjects in your study.

This approval expires on October 21, 2015. Federal Regulations and Bon Secours Richmond Health System IRB require continuing review prior to continuation past that date. Continuing review notification will be sent to you prior to the next scheduled review as a courtesy only. You are still responsible for ensuring these materials are submitted by the submission date.

Please direct any questions to Mark Leep at mark_leep@bshsi.org or (804) 627-5157.

Sincerely,

Gerald Keightley, III, MD
Chair, Institutional Review Board

Attachments: Conditions of Approval; Informed Consent Form, Breast Cancer, Version 10/16/2014 (BSR IRB approval dated October 28, 2014); Informed Consent Form, Leukemia, Version 10/16/2014 (BSR IRB approval dated October 28, 2014); Study Advertisement, Breast Cancer, Version 1.08-01-2014 & Study Advertisement, Leukemia, Version 1.08-01-2014 (BSR IRB approval dated October 22, 2014)

8590 Magellan Parkway, Richmond, Virginia 23227 Good Help to Those in Need

Appendix C

Clemson University Administrative Review

Clemson University's Administrative Review of IRB2015-284 (BSSF006): "Collaborative Oncology Testing: A New Model for SC"

2 messages

Nalinee Patin <npatin@clemson.edu> Fri, Sep 25, 2015 at 11:22 AM
To: JULIA A EGGERT <jaegger@clemson.edu>
Cc: Sourat Darabi <sdarabi@g.clemson.edu>, Matthew Tedder <mltedde@g.clemson.edu>

Dear Dr. Eggert,

The Clemson University Office of Research Compliance conducted an administrative review of the research project identified and found the materials to be satisfactory. You may begin your study as approved by Bon Secours Richmond Health System.

Please forward copies of any continuing review or amendment requests to our office. Also, please notify us when the study has been completed and we will close the file.

The Clemson University IRB is committed to facilitating ethical research and protecting the rights of human subjects. Please contact the Office of Research Compliance if you have any questions.

Sincerely,
Nalinee

Nalinee D. Patin
IRB Administrator
Clemson University
Office of Research Compliance
Institutional Review Board (IRB)
223 Brackett Hall
Voice: (864) 656-0636
Fax: (864) 656-4475
E-mail: npatin@clemson.edu
Web site: http://www.clemson.edu/research/compliance/irb/
IRB E-mail: irb@clemson.edu

Appendix D

Data Collection Sheet

Participant #:

Demographics

AGE 20-29 (0) 30-39 (1) 40-49 (2) 50-59 (3) 60-69 (4) 70-79 (5) 80-89 (6)	
SEX M (0) F (1)	
RACE White (0) Black (1) Hispanic (2) Other (3)	
INSURANCE Private (0) Medicare (1) Medicaid (2) Uninsured (3)	
COLLECTION DATE	
SPECIMEN	
EDUCATION 9-12 years (0) 13-14 years (1) 15 or more (2)	

HEIGHT	
WEIGHT	
BMI ≤24.9 (0) 25-29.9 (1) ≥30	

BREAST CANCER

MENOPAUSE Pre menopausal (0) Post menopausal (1)	
CANCER SITE Right breast (0) Left breast (1) Bilateral (2)	
SENTINEL NODE Sentinel Node Negative (0) Sentinel Node Positive (1)	
TUMOR SIZE Size of malignancy 0-0.9 cm (0) Size of malignancy > 1 cm (1)	

ER MARKER ER - marker (0) ER + marker (1) Not done (2)	
PR MARKER PR – marker (0) PR + marker (1) Not done (2)	
HER2 MARKER HER2 – marker (0) HER2 + marker (1) Not done (2)	
SURGICAL INTERVENTION No surgery (0) Lumpectomy (1) Mastectomy (2) Neoadjuvant + Unknown (3) Neoadjuvant + Lumpectomy (4) Neoadjuvant + Mastectomy (5) Neoadjuvant only (6)	
STAGE Stage 0 (0) Stage I (1) Stage II A (2) Stage II B (3) Stage III A (4) Stage III B (5) Stage IV (6)	
FAMILY HISTORY of BC No (0) Yes (1)	
Siblings with Breast Cancer No (0) Yes (1)	
Breast Cancer Paternal 1st generation (0) 2nd generation (1) 3rd generation (2) Blended generation (3)	

Breast Cancer Maternal 1st generation (0) 2nd generation (1) 3rd generation (2) Blended generation (3)	
Family HX of Ovarian Cancer No (0) Yes (1)	
Ovarian Cancer Paternal 1st generation (0) 2nd generation (1) 3rd generation (2) Blended generation (3)	
Ovarian Cancer maternal 1st generation (0) 2nd generation (1) 3rd generation (2) Blended generation (3)	
Family HX of Prostate Cancer No (0) Yes (1)	
Prostate Cancer Paternal 1st generation (0) 2nd generation (1) 3rd generation (2) Blended generation (3)	
Prostate Cancer maternal 1st generation (0) 2nd generation (1) 3rd generation (2) Blended generation (3)	
Family HX of Pancreatic Cancer No (0) Yes (1)	
Pancreatic Cancer Paternal 1st generation (0) 2nd generation (1) 3rd generation (2) Blended generation (3)	
Pancreatic Cancer maternal 1st generation (0) 2nd generation (1) 3rd generation (2)	

Blended generation (3)	
Family HX of Male Breast Cancer No (0) Yes (1)	
Male Breast Cancer Paternal 1st generation (0) 2nd generation (1) 3rd generation (2) Blended generation (3)	
Male Breast Cancer maternal 1st generation (0) 2nd generation (1) 3rd generation (2) Blended generation (3)	
Family HX of Gastric Cancer No (0) Yes (1)	
Gastric Cancer Paternal 1st generation (0) 2nd generation (1) 3rd generation (2) Blended generation (3)	
Gastric Cancer maternal 1st generation (0) 2nd generation (1) 3rd generation (2) Blended generation (3)	
Family HX of Colorectal Cancer No (0) Yes (1)	
Colorectal Cancer Paternal 1st generation (0) 2nd generation (1) 3rd generation (2) Blended generation (3)	
Colorectal Cancer maternal 1st generation (0) 2nd generation (1) 3rd generation (2) Blended generation (3)	

Family HX of Thyroid Cancer No (0) Yes (1)	
Thyroid Cancer Paternal 1st generation (0) 2nd generation (1) 3rd generation (2) Blended generation (3)	
Thyroid Cancer maternal 1st generation (0) 2nd generation (1) 3rd generation (2) Blended generation (3)	
Family HX of Uterine Cancer No (0) Yes (1)	
Uterine Cancer Paternal 1st generation (0) 2nd generation (1) 3rd generation (2) Blended generation (3)	
Uterine Cancer maternal 1st generation (0) 2nd generation (1) 3rd generation (2) Blended generation (3)	
Family HX of Kidney Cancer No (0) Yes (1)	
Kidney Cancer Paternal 1st generation (0) 2nd generation (1) 3rd generation (2) Blended generation (3)	
Kidney Cancer maternal 1st generation (0) 2nd generation (1) 3rd generation (2) Blended generation (3)	
Family HX of Sarcoma Cancer No (0) Yes (1)	

Sarcoma Cancer Paternal 1st generation (0) 2nd generation (1) 3rd generation (2) Blended generation (3)	
Sarcoma Cancer maternal 1st generation (0) 2nd generation (1) 3rd generation (2) Blended generation (3)	
Family HX of Brain Cancer No (0) Yes (1)	
Brain Cancer Paternal 1st generation (0) 2nd generation (1) 3rd generation (2) Blended generation (3)	
Brain Cancer maternal 1st generation (0) 2nd generation (1) 3rd generation (2) Blended generation (3)	
Family HX of Leukemia No (0) Yes (1)	
Leukemia Paternal 1st generation (0) 2nd generation (1) 3rd generation (2) Blended generation (3)	
Leukemia maternal 1st generation (0) 2nd generation (1) 3rd generation (2) Blended generation (3)	
Family HX of Gastrointestinal Cancer No (0) Yes (1)	
Gastrointestinal Cancer Paternal 1st generation (0) 2nd generation (1) 3rd generation (2)	

Blended generation (3)	
Gastrointestinal Cancer maternal 1st generation (0) 2nd generation (1) 3rd generation (2) Blended generation (3)	
Family HX of Optic Glioma Cancer No (0) Yes (1)	
Optic Glioma Cancer Paternal 1st generation (0) 2nd generation (1) 3rd generation (2) Blended generation (3)	
Optic Glioma Cancer maternal 1st generation (0) 2nd generation (1) 3rd generation (2) Blended generation (3)	
Sum of Family HX of all cancer types	
Oncotype DX Not done (0) Low risk (1) Intermediate risk (2) High risk (3)	
Other Signature Studies	

GGC Test Result Negative (0) Positive (1) VUS (2) Positive and VUS (3)	
GGC MUTATION TYPE	
GGC VUS TYPE	
Commercial Genetic Testing None (0) Myriad BRCAnalysis (1) Myriad Panel (2) GeneDX (3) Ambry: BRCAplus (4) Ambry: GYNplus (5) Ambry: BreastNext (6) Ambry: Ovanext (7) Ambry: Pancnext (8) Ambry: Cancernext (9) Ambry: Cancernext expanded (10) Ambry: BRCA deletion/duplication analysis (11) Ambry: gene sequence and deletion/duplication analysis (12) Integrated Genetics: Comprehensive BRCA 1/2 Analysis (13) Ambry: Colonext (14) BreastAssure LabCorp (15) Unknown (16)	
Commercial Genetic Testing Result Negative (0) Positive (1) VUS (2) Positive & VUS (3)	

Commercial Genetic Testing Variant Identified	
VUS identified	
Left Breast Tumor 1 grade None (0) Low (1) Intermediate (2) High (3)	
Left Breast Tumor 2 grade None (0) Low (1) Intermediate (2) High (3)	
Right Breast Tumor 1 grade None (0) Low (1) Intermediate (2) High (3)	
Right Breast Tumor 2 grade None (0) Low (1) Intermediate (2) High (3)	

Second Primary No (0) Yes (1)	
Second Primary Type	
Primary 2 ER ER – (0) ER + (1)	
Primary 2 PR PR – (0) PR + (1)	
Primary 2 HER2 HER2 – (0) HER2 + (1)	