CONTRIBUTION OF THE CANDIDATE GENES FOR PRIMARY LYMPHEDEMA TO SECONDARY LYMPHEDEMA AND CHRONIC PAIN IN POST-MASTECTOMY PATIENTS

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ABSTRACT

Secondary lymphedema is a common complication after surgical treatment, particularly after breast surgery, in which very little is understood about possible determinants. Multiple studies have been conducted to identify specific genes contributing to inherited primary lymphedema. Some of these causative genetic factors may also play a role in the development of secondary lymphedema. Among them, the *GJC2* and *MET* genes have been identified as being associated with primary lymphedema. Further investigation using common SNP analysis of the *GJC2* and *MET* genes was performed in patients post breast surgery, with and without secondary lymphedema to determine whether variants of either gene could be a determining factor for developing secondary lymphedema after surgical treatment. Survey data analysis addressing various psychosocial and bio-behavioral factors was also analyzed to indicate whether these candidate genes affect chronic pain and psychosocial traits in patients with secondary lymphedema compared to matched controls. Variants at rs11800309 of the *GJC2* gene and patients' Pain Catastrophizing Scores were significant (ά=.1) for predicting lymphedema. Variants of rs41737 of the *MET* gene was observed to have an effect on brief pain inventory, perceived stress scores, and depression scores. Variants at rs7539762 and rs11800309 of the

GJC2 gene were observed to effect anxiety levels. Age was observed to be inversely related to all psychosocial phenotype scores. This current study has public health significance because it can help identify women who may be at an increased risk for developing secondary lymphedema after breast surgery.

TABLE OF CONTENTS

PREFACE ..... **XII**

1.0 INTRODUCTION **1**

1.1 AIMS **2**

1.2 BACKGROUND AND SIGNIFICANCE **3**

1.2.1 The Lymphatic system and Lymphedema **3**

1.2.2 Genetics of Primary Lymphedema **6**

1.2.2.1 *GJC2* **7**

1.2.2.2 *MET* **8**

1.2.3 Secondary Lymphedema **9**

1.2.3.1 Causes of Secondary lymphedema **9**

1.2.3.2 Treatment **10**

1.2.4 Post Mastectomy Pain Study **11**

1.2.5 Significance **12**

2.0 MATERIALS AND METHODS **14**

2.1 DATA COLLECTION **14**

2.1.1 Patient Population **14**

2.1.2 Informed Consent **15**

2.1.3 Samples: DNA Extraction **15**

2.1.4 Survey 16

2.1.4.1 Distribution 16

2.1.4.2 Pain Catastrophizing Scale (PCS) 16

2.1.4.3 Perceived Stress Scale (PSS) 17

2.1.4.4 Brief Pain Inventory (BPI) 17

2.1.4.5 Emotional distress (Anxiety PROMIS) 18

2.1.4.6 Emotional Stability (Depression PROMIS) 18

2.1.4.7 Sleep Disturbances (PROMIS) 19

2.2 SNP GENOTYPING 19

2.2.1 SNP Selection 19

2.2.2 Setup of Plate 20

2.2.3 Genotyping 21

2.3 STATISTICAL ANALYSES 21

3.0 RESULTS 23

3.1 DESCRIPTIVES 23

3.2 ANALYSIS OF SNP GENOTYPE AND PSYCHOSOCIAL PHENOTYPES AS PREDICTORS OF SECONDARY LYMPHEDEMA 27

3.3 THE DEMOGRAPHIC EFFECT ON THE DEVELOPMENT OF LYMPHEDEMA 28

3.4 LINEAR REGRESSION AND SPEARMAN TEST: GENOTYPE AND DEMOGRAPHIC EFFECT ON PSYCHOSOCIAL PHENOTYPES 29

3.4.1 Brief Pain Inventory (BPI) 30

3.4.2 Perceived Stress Scale (PSS) 33

3.4.3 Pain Catastrophizing Scale (PCS) 36

3.4.4 Anxiety 38

3.4.5 Depression 41

3.4.6 Sleep Disturbances 44

3.5 RESULTS SUMMARY 46

4.0 DISCUSSIONS 48

4.1.1 Aim I 48

4.1.2 Aim II 50

4.1.3 Aim III 51

4.1.4 Public Health Significance 55

4.1.5 Limitations 57

4.1.6 Future Research 60

APPENDIX A: IRB APPROVAL 62

APPENDIX B: CONSENT FORM 64

APPENDIX C: STUDY SURVEY 72

BIBLIOGRAPHY 112

LIST OF TABLES

Table 1: Descriptive Statistics for age, body mass index, pain, and psychosocial measurement. 23

Table 2: Frequencies of lymphedema, menopause, and lymph node surgery 26

Table 3: Genotype Frequencies by SNP 27

Table 4: Logistic Regression: Lymphedema, Genotype, and Psychosocial Phenotype 28

Table 5: Univariate analysis: Demographics on lymphedema development..... 29

Table 6: Multivariable Analysis: Demographics effect on lymphedema development..... 29

Table 7: Linear Regression for BPI and Genotype..... 31

Table 8: Multivariable analysis Demographics on BPI 32

Table 9: Univariate analysis: Demographics on BPI..... 32

Table 10: Spearman Test: BPI 33

Table 11: Linear Regression for PSS and Genotype 34

Table 12: Multivariable analysis demographics on PSS..... 35

Table 13: Univariate analysis: Demographics on PSS 35

Table 14: Spearman Test: PSS..... 36

Table 15: Linear Regression for PCS and Genotype..... 36

Table 16: Multivariable analysis of demographics on PCS..... 37

Table 17: Univariate analysis of demographics on PCS..... 37

Table 18: Spearman Test on PCS 38

Table 19: Linear Regression for Anxiety and Genotype 39
Table 20: Multivariable analysis of demographics on Anxiety 40
Table 21: Univariate analysis of demographics on Anxiety 40
Table 22: Spearman Test: Anxiety 41
Table 23: Liner Regression for Depression and Genotype 42
Table 24: Multivariable analysis of demographics on Depression 43
Table 25: Univariate analysis of demographics on Depression 43
Table 26: Spearman Test: Depression 44
Table 27: Linear Regression for Sleep and Genotype 44
Table 28: Multivariable analysis of demographics on Sleep 45
Table 29: Univariate analysis of demographics on Sleep 45
Table 30: Spearman Test: Sleep 46

LIST OF FIGURES

Figure 1: Plate Setup 21

Figure 2: Psychosocial Phenotype Distribution 24

PREFACE

I would first like to thank the Principal Investigator of this project, my mentor, Dr. Inna Belfer, for giving me the opportunity to contribute to her Post-Mastectomy Pain Study and for allowing me to investigate Secondary Lymphedema in her patient population. I am very appreciative of her support, trust, and guidance with this project. I would also like to acknowledge my three other committee members, Dr. Michael Barmada, Dr. John Shaffer, and Dr. David Finegold for agreeing to serve on my committee. This project would not have been completed without their helpful suggestions, constant support, and enthusiasm throughout this project. I would like to recognize genetic counseling student, Kelly Johnson, for being so incredibly helpful with the statistical analysis for this project. In addition, I would like to thank the Magee Women's Foundation and Dr. Adam Brufsky for funding this project. I would also like to extend my gratitude to Dr. Robin Grubs, the director of University of Pittsburgh's Genetic Counseling program. It is because of her that I feel confident leaving Pittsburgh and ready to make great contributions to the field of Genetics. I would also like to thank my wonderful family for all of the love and support. They have all inspired me to work hard, never give up, and to always believe in myself. For those lessons I am eternally grateful. Finally, I would like to thank the University of Pittsburgh's Genetic Counseling Class of 2015. I am so thankful for the friendships and beautiful memories.

1.0 INTRODUCTION

Lymphedema is a condition in which lymph is unable to flow through the lymphatic vessels. This condition causes multiple physical and psychological symptoms for individuals that are affected. Physical symptoms of lymphedema include swelling of the limbs, restricted range of motion, discomfort and pain, fibrosis, and the possibility of recurrent infections. This condition may also have psychosocial effects on an affected individual. These psychosocial effects may include depression, anxiety, sleep disturbances, cosmetic concerns, lower self-esteem, and overall reduced quality of life.

There are two different types of lymphedema; primary lymphedema and secondary lymphedema (Mohler ER, et al). Although primary and secondary lymphedema have the same set of symptoms, the causes of both conditions are very different. Primary lymphedema is an inherited condition in which the lymphatic vessels of the body fail to develop or function properly. There are multiple genes that have been identified in which mutations can cause lymphedema. Primary lymphedema has varying ages of onset. Symptoms can occur soon after birth and have also been seen to develop early in childhood (Smeltzer et al., 1985). Secondary lymphedema is acquired due to damage to the lymphatic system which prevents lymph from flowing through the vessels. Secondary lymphedema may be caused from trauma, infection, radiation, or surgery. However, not all individuals who experience these triggers develop lymphedema. There is no current cure for lymphedema (Mohler ER, et al).

The main goal of this current study is to use genotyping technology to determine if common variants within two genes, *GJC2* and *MET* which are identified to cause primary lymphedema, cause a predisposition for secondary lymphedema development in a patient post-breast surgery. This current study also aims to analyze the association of common variants in *GJC2* and *MET* with chronic pain and psychological phenotypes.

1.1 AIMS

Aim 1: To measure participants' experience with chronic pain, perceived stress, sleep disturbance, depression, and anxiety after breast surgery by means of a telephone survey.

Aim 2: To genotype common Single Nucleotide Polymorphisms (SNPs) in *GJC2* and *MET*: Three common tagging SNPs in *GJC2* (rs7539762, rs7523917 and rs11800309) and two common tagging SNPs in *MET* (rs41737 and rs13223756) were selected based on reported minor allele frequencies.

Aim 3: To analyze the association of *GJC2* and *MET* common variation with secondary lymphedema, chronic pain and psychosocial variables.

1.2 BACKGROUND AND SIGNIFICANCE

1.2.1 The Lymphatic system and Lymphedema

The lymphatic system has many important functions, however it remains quite understudied compared to other body systems. The lymphatic system interacts with many different body systems. The lymphatic system removes interstitial fluid from body tissues and helps with the transportation of white blood cells to the lymph nodes and bones, providing fluid balance. The lymphatic system also interacts with the villi to assist the digestive system in the absorption and transportation of fatty acids. Another vital function of the lymphatic system is to transport antigen presenting cells to the lymph nodes in order to stimulate an immune response (Freeman, S, 2008).

Due to the high pressure of the circulatory system and the fact that capillaries are thin, fluid is able to leak from the capillaries into surrounding space. This fluid is called interstitial fluid because it is found between the cells of the body. The majority of interstitial fluid is able to diffuse back into the capillaries due to the osmotic differences of the fluid. The concentration difference exists because large proteins are retained in the blood capillaries due to their size inhibiting them from diffusing out of the capillaries. The remaining fluid that does not reenter the capillaries enters the lymphatic vessels as lymph. Lymph is a mixture of fluid and white blood cells which circulates through the lymphatic system. Only about 2-5 percent of plasma volume form lymph each day (Freeman, S, 2008).

The lymphatic system consists of lymphatic ducts and lymphatic vessels. Lymphatic ducts permeate all tissues in order to provide a channel for the lymph to flow. Lymph does not

have a specific organ designated to pump the fluid through the channels. Lymph is able to flow through these channels by the contraction of skeletal muscles, the contraction of smooth muscles in larger vessels, and thoracic pressure changes from respiration. The lymph can deliver nutrients and gases to the tissues. All of the tiny channels eventually combine to form large vessels. Lymph is able to reenter the circulatory system by means of the subclavian veins after it is filtered by lymph nodes (Sherwood, 2012).

The lymphatic system plays a role in the immune system and contributes to the body's protection against virus, bacteria, and cellular debris that can cause infections. Lymph nodes are small organs, consisting of masses of lymph tissue that are located throughout the body. These organs are responsible for filtering the lymph fluid and for detecting any possible pathogens. Lymph nodes are responsible for modifying lymphocytes to fight infections. Examples of lymph nodes include the tonsils located in the throat and Peyer's patches located in the small intestine (Sherwood, 2012).

Other specific structures of the lymphatic system also contribute to immune response. These structures include the spleen and the thymus gland. The spleen is similar to lymph nodes, however rather than filtering lymph fluid, the spleen filters blood. Pathogens that are present in the blood generate a response from lymph nodes when the blood passes through the spleen. The spleen also functions to filter older, non-working red blood cells from the blood and act as a reservoir for oxygen rich blood. The thymus gland is another important structure of the lymphatic system, however the entire function of the thymus gland remains unknown. The thymus gland size decreases as an individual ages. Before puberty this gland is made up of mostly lymphatic tissue. It is understood that the thymus gland provides a location for immature

T lymphocytes to be held after leaving the bone marrow. It is in the thymus gland that the immature T lymphocytes fully develop. T-lymphocytes that are beneficial to the immune system develop, while detrimental T-cells are rejected. This gland also functions to secrete hormones that may affect the body's immunological response. (Sherwood, 2012).

The lymphatic system plays an important role in the digestive system. A specific lymph vessel, lacteals, are located in the villi of the intestines to help with the absorption of fat. The liquid that contains the absorbed fat is called chyle. Chyle is also drained into the subclavian vein in order to enter the circulation to be transported to tissues.

Lymphedema is a disorder of the lymphatic system in which a block in lymphatic vessels inhibits fluid from flowing through the lymphatic channels. This leads to the progression of numerous symptoms. The most common physical features in the early stages of lymphedema is noticeable edema, otherwise known as swelling, and restricted movement which is most often located in the extremities. Some psychological features include feelings of heaviness in the areas of the edema as well has pain. Certain skin changes can also occur because of the edema. These skin change may include certain wart-like growths, thickening of the outer layer of the skin (hyperkeratosis), and papilloma growth in cases of severe lymphedema. Severe lymphedema may lead to significant deformity. A deformity of a particular area can effect function and mobility. A deformity may also have psychosocial implications. Body deformity can lead to depression, social anxiety, low self-esteem and overall lower quality of life in individuals. Increased risk for infections and low auto-immune response are other obvious symptoms of lymphedema because the immune system is unable to function properly (Mohler ER, et al).

Lymphedema may be inherited or acquired. The type of lymphedema that is inherited is described as primary lymphedema. Primary lymphedema is less common compared to secondary lymphedema. Primary lymphedema has a reported incidence of 1.5 per 100,000 individuals under the age of twenty in North America (Smeltzer et al, 1985). Primary lymphedema may be inherited in either a dominant or recessive manner, depending on which gene is responsible for symptoms. Multiple genes have been identified to cause primary lymphedema and onset of symptoms can vary from infancy, to childhood and adolescents, or even adulthood.

1.2.2 Genetics of Primary Lymphedema

The University of Pittsburgh Department of Human Genetics conducted a large family study to investigate the genetic causes of primary lymphedema. The genes were identified by interviewing families in which primary lymphedema was present. Blood samples were obtained from patients and their family members, both with and without primary lymphedema. This study revealed mutations in seven genes that cause primary lymphedema. These genes include *FLT4, FOXC2, HGF, MET, SOX18, CCBE1,* and *GJC2* (Ferrell, R. E., Finegold, D. N., & Levine, K).

The *FLT4* gene is located on chromosome 5 and codes for VEGFR3 (Ferrel et al., 1998). This gene is involved in the development of lymphatic vessels during fetal development. The *FOXC2* gene causes lymphedema-distichiasis syndrome, which causes lymphedema later in life (Traboulsi et al, 2002). *SOX18* is a gene on chromosome 20 which is involved in fetal development and associated with Hypotrichosis-Lymphedema-Telangiectasia syndrome (Irrthum et al., 2002). *HGF* and *MET* are located on chromosome 7 and play a role in the growth and development of the lymphatic vessels. *CCBE1* is also associated with lymphatic development

and Hennekam syndrome (Alders et al, 2009). *GJC2* is on chromosome 1 and affects the function of the lymphatic system rather than the development (Ferrell et al, 2010).

1.2.2.1 *GJC2*

Gap junction protein, gamma 2, 47kDa, *GJC2,* is a gene that provides the instruction for making a gap junction protein. Gap junction proteins are part of the connexin family of proteins, which play an essential role in cell-to-cell signaling, response to toxic substances, and transmembrane transport (NCBI, 2015). Connexins connect body cells and create channels for small ions, substances, and electrical signals to be transported from one cell to another. Connexins also have effects on non-connexin protein interactions within a cell flow (Merlijn J et al, 2014).

Although the relationship between connexins and the lymphatic system is a topic that remains understudied, some findings suggest that connexins and gap junction proteins play a significant role in the function and development of the lymphatic system. Early electron microscopy, immunohistochemistry, and pharmacological inhibitor studies all suggest the presence of gap junctions in lymphatic vessels. Functional studies on animal lymphatic systems observed that rhythmic contraction causes the lymph fluid to flow through the vessels. For cells to have a synchronized action, gap junction proteins would be essential in order to provide the communication and connection between the cells. Studies that measure the expression level of genes also suggest the importance of connexin. *GJC2* has been observed to be expressed in lymphatic endothelial cells and not blood endothelial cells. This suggests that *GJC2* plays a key role in the connection of lymphatic cells. Other evidence that connexins are essential for lymphatic system function comes from the observation of mutations. Studies have observed mutations in the *GJC2* gene in patients who have been diagnosed with primary lymphedema. Mutations were identified in the different regions of the gene. This indicates that the *GJC2* gene,

as well as other connexin and gap-junction proteins, provides assistance with lymphatic flow (Merlijn J et al, 2014).

Finegold *et al*. (2012) studied *GJC2* using biological samples collected at Magee hospital and identified four rare variants present in post mastectomy patients with secondary lymphedema that were not found in post mastectomy patients without secondary lymphedema symptoms. In addition, none of the non-breast cancer control group had these *GJC2* variants. This suggests that variants in this gene may increase an individual's risk for developing secondary lymphedema after surgical treatment.

1.2.2.2 *MET*

MET proto-oncogene, receptor tyrosine kinase, *MET,* is a proto-oncogene. Proto-oncogenes help control both cellular division and apoptosis. These groups of genes are essential for regulating cell life and when mutated, play a role in the development of cancers. The product of this gene is the hepatocyte growth factor receptor and activates the tyrosine kinase signaling cascade. Kinases help regulate cell functions. Tyrosine kinase transfers a phosphate group from ATP to proteins, which can either initiate or terminate certain functions between cells.

MET has been identified as a proto-oncogene, and the *MET* pathway is involved in the formation of multiple forms of cancers (Peschard and Park, 2007, Mizuno S. and Nakamura. T, 2013). As a proto-oncogene, *MET* functions to regulate cell proliferation, scattering, morphogenesis and survival. During early embryonic development this gene regulates development and migration of muscles and neuronal precursors, angiogenesis and kidney formation. *MET* endorses differentiation and proliferation of hematopoietic cells which is essential in early development. Additionally, this gene contributes to organ regeneration, wound healing, and tissue remodeling throughout an individual's life.

Some studies suggest that *MET* is associated with lymphedema. Finegold *et al*. (2012) searched for variants in primary lymphedema genes in women who developed lymphedema after their breast surgery at Magee Womens Hospital by using collected biological samples. The study identified a rare *MET* variant in a single case. Finegold *et al.* (2008) identified four rare variants in *HGF* and *MET* that may be causative of developing secondary lymphedema. These truncation or missense mutations were only found in the cases with secondary lymphoma and not identified in any of the controls.

1.2.3 Secondary Lymphedema

Secondary lymphedema is an acquired condition. This complication occurs when there is damage to the lymphatic vessels or lymph nodes. This trauma to the lymphatic system causes the lymph fluid to be unable to flow properly throughout the body.

1.2.3.1 Causes of Secondary lymphedema

There are multiple known causes of secondary lymphedema; however, any trauma to the lymphatic system may lead to symptoms.

Surgery is a common cause of lymphedema, particular surgeries that include removal of a particular lymph node. Women diagnosed with breast cancer often undergo surgical procedures involving the sentinal or axillary lymph nodes. Examining the lymph nodes assists with determining if the cancer may metastasize to other regions of the body. Tumor cells can travel through the lymphatic system. Looking at lymph nodes nearby the cancer site can help determine the likelihood of metastasis. Sentinal lymph nodes are located near the breast and are the first

lymph nodes to which tumor cells may migrate to. Axillary lymph node are located near the breast and armpit. Axillary lymph node dissection usually removes between five and thirty nodes to determine the metastasis ability of the cancer (Lymph Node Removal for Invasive Breast Cancer, 2015).

Cancer and radiation treatment may also cause secondary lymphedema. Depending on the location of the tumor, cancer cells may grow to cause a block in the lymphatic vessels. Radiation may cause harm to lymph nodes. Radiation can cause both scarring and/or inflammation of lymphatic cells. This inflammation may lead to a block in normal lymph flow (Lymphedema (PDQ), 2014).

Certain types of infections may also lead to the development of secondary lymphedema. Whether bacteria, fungal, or parasitic, certain infections may lead to restriction of lymph flow. This is more common in developing countries, particularly those in tropical regions of the world. A common parasite that causes lymphedema is *Wuchereria bancrofti.* These worms are spread through mosquitos and cause a condition known as Elephantitis, a severe form of lymphedema.

1.2.3.2 Treatment

Currently there is no cure for lymphedema. Certain treatment is used in order to help manage the swelling and pain symptoms; however, nothing is available to completely eliminate this condition. Physical therapy is often recommended in order to encourage movement of the affected limb. This movement may assist with lymph drainage in order for help the patient to pursue every day activities. Wrapping the limb or wearing a compression garment may help force lymph to flow away from the blockage. Manual drainage of the lymph fluid may occur after massaging the limb; however, this technique may lead to other complications such as pain and infection. Pneumatic compression is another form of treatment for lymphedema. Pneumatic

compression treatment involves having the patient wear a sleeve on their affected limb. This sleeve is attached to a pump that sporadically fills with air in order to put pressure on the limb. This is done to divert the lymph from the blockage. These treatments may be combined to help reduce symptoms (Chiu, 2014).

1.2.4 Post Mastectomy Pain Study

In 2010 the University of Pittsburgh Department of Anesthesiology started a large pain phenotyping study in patients who had undergone a total or partial mastectomy at Magee Women's Hospital of UPMC, using the Breast Cancer Registry and Banking Study. Clinical/medical information and tissue, whole blood, or saliva sample were obtained for each patient. The patient was then interviewed on chronic pain and related symptoms by phone six months after their breast surgery. Pain and psychosocial data were collected using standardized validated questionnaires. In addition, a survey on lymphedema symptoms developed by University of Pittsburgh Department of Human Genetics and validated in Lymphedema Family Study was applied to each patient. A recently published paper from this study (using data from the first 600 subjects recruited) reported that psychosocial factors were more strongly related to persistent post mastectomy pain then the surgical treatment the patient initially received (Schreiber et al, 2012). The current study used the data already collected in the ongoing study (for 1300 subjects as of April 2014) to identify secondary lymphedema cases. Biological samples from these cases and matched lymphedema-free patients were already available (obtained from the Magee Tissue bank) and were used for DNA isolation and genetic data collection.

1.2.5 Significance

Approximately 300,000 new breast cancer cases are identified each year in the United States, the majority being invasive breast cancer (American cancer society, 2013) . Many women chose to have total or partial mastectomy to treat the diagnosed cancer or as a preventative option; however, having a breast surgery can cause other medical concerns. Post breast surgery patients are at risk for experiencing secondary lymphedema and chronic persistent pain, the reasons for which are still unexplained. A research proposal that aims to identify genetic factors associated with these significant complications will be greatly important for future breast cancer patients who are deciding which course of treatment is best. These risk factors could eventually predict which women are at risk for developing serious complications post-surgery. Knowledge of genetic predictors may affect clinical decision-making on optimal treatment strategies, and may lead to increased health and quality of life for patients at Magee and elsewhere.

Secondary lymphedema is a serious condition that commonly transpires in cancer patients, particularly breast cancer patients, due to the removal or damage of lymph nodes as a part of surgical treatment. Previous studies reported that the incidence of developing secondary lymphedema after partial or total mastectomy is up to 33% (Hayes et al., 2008) Patients that experience lymphedema can suffer from decreased flexibility, limited limb mobility, increased limb weight, and skin hypersensitivity in areas where swelling occurs. Common complaints in patients with lymphedema include chronic pain and poor quality of life (Shigaki et al, 2013). Although secondary lymphedema is a common complication among cancer patients, molecular mechanisms underlying this condition are understudied and not completely understood including the role of genetic factors. Furthermore, it is currently unclear if primary and secondary lymphedema share a genetic background or common pathways.

This study aims to evaluate the role of selected candidate genes, *GJC2* and *MET*, for developing secondary lymphedema in patients after breast surgery. Identifying genetic factors that may contribute to the susceptibility of secondary lymphedema has clinical relevance: if a screening procedure can be implicated to determine individuals that are at risk for developing secondary lymphedema, doctors can alter particular treatments, monitor patients more closely post-surgery, and implement novel preventive strategies in target patients.

Although some evidence points towards the impact of *GCJ2* and *MET* on the development of secondary lymphedema, it is currently unknown if their common SNPs contribute to secondary lymphedema and related phenotypes. These SNPs may potentially be used as predictive factors in screening testing. Understanding if these specific variants have a negative effect on an individual's psychosocial phenotype may provide information for patient and physician and allow for supportive mental health services to be recommended for patients.

2.0 MATERIALS AND METHODS

This thesis project was reviewed by the University of Pittsburgh's Institutional Review Board and approval was obtained (Appendix A).

2.1 DATA COLLECTION

2.1.1 Patient Population

The patient population consists of females from the Magee Women's Hospital Post Mastectomy Pain study. In order to be eligible for the Post Mastectomy Pain study women had to have undergone either a total or partial mastectomy at Magee Women's Hospital for the treatment of breast cancer. Participants in the secondary lymphedema project were selected from a larger sample population of approximately 1300 individuals who had completed a telephone survey six months after their breast surgery. The selection of cases and controls was made based on surgical and clinical information obtained through electronic records and patient answers to the study's validated survey questionnaire. The case group was defined as having secondary lymphedema symptoms. A diagnosis of secondary lymphedema was based on survey data obtained via structured phone interview or from electronic records. Matched controls for each individual case were then selected. The categories selected to match the cases to the controls were menopausal

status, age, body mass index (BMI), race, type of surgical treatment, type of adjuvant therapies. In cases where there was not a match in all of the five categories, age (menopausal status), node surgery, and treatment were prioritized.

2.1.2 Informed Consent

Informed consent was obtained for each participant involved in the Post Mastectomy Pain Study prior to any sample collection, survey, or review of medical records. In order to obtain consent from the participants the physicians performing the breast surgeries at Magee Women's Hospital of Pittsburgh described the goals and eligibility requirement for participation in the study. The patients were also informed of their rights to withdraw from the study at any time. Although the Post Mastectomy Pain Study had minimal risk, any risks and benefits associated with the study were explained in detail (Appendix B). The patient received a copy of the consent form for future reference and the contact information of the principle investigator, Dr. Inna Belfer. Only after receiving a signed consent form was the patient enrolled in the study, and a DNA sample requested from the participant or the Tissue Bank. Telephone surveys were conducted 6 months after the patient received their breast surgery.

2.1.3 Samples: DNA Extraction

DNA isolation and purification was performed on saliva, tissue, or blood samples by means of the protocol for the Qiagen extraction kits.

2.1.4 Survey

Each patient participated in a 30-60 minute telephone survey. A copy of the full survey can be found in Appendix C.

2.1.4.1 Distribution

All surveys were conducted via telephone interview no earlier than 6 months after the participant's breast surgery. The surveys were conducted by student researchers who had extensive training in conducting the phone interviews in order to maintain consistency. Each telephone interview was approximately 45 minutes in length.

2.1.4.2 Pain Catastrophizing Scale (PCS)

Multiple questions in the survey were asked in order to measure the emotional severity of an individual's pain after breast surgery. The validated Pain Catastrophizing Scale measures the catastrophic thinking regarding any experienced pain. A total of fourteen questions regarding an individual's thoughts and feelings regarding the experienced pain were asked to each participant. Each individual question was scored on a scale from 0 to 4. Scores of 0 indicated the most positive experience and scores of 4 indicated the most negative thoughts in regards to the pain. Each patient received an overall pain catastrophizing score ranging from 0 to 56. The score is directly related to the negative impact the pain has on the individual's emotions (Van Damme S et al, 2002).

2.1.4.3 Perceived Stress Scale (PSS)

The Perceived Stress Scale inquires about the frequency of instances in which an individual felt stressed within the past month prior to the survey. These specific questions are focused on understanding the level of stress that the participant is experiencing. This set of questions is a validated psychological instrument for measuring nonspecific stress in individual. The survey consists of ten questions all pertaining to participants' emotions and ability (or inability) to control certain aspects of their lives. Each individual question was scored on a scale from 0 to 4. Scores of 0 indicated the most positive experience and scores of 4 indicated the most negative thoughts in regard to the stress. Each patient received an overall PSS score ranging from 0 to 40. The score is directly related to the negative impact that such stress has on the individual's well-being.

2.1.4.4 Brief Pain Inventory (BPI)

BPI measures each participant's pain levels within the past week of the conducted interview. Questions include the present pain level, the average pain level, the lowest pain level, and the most intense pain level. The BPI score also consists of multiple questions pertaining to the effect that an individual's pain has on everyday activities including general activity, mood, walking ability, occupation, relationships, sleep, and enjoyment of life, recreational activities, self-care, and social activities. Each individual question was scored on a scale from 0 to 10. Scores of 0 indicated no pain or that the pain does not interfere with the specific activity while scores of 10 indicated the highest level of pain or that the pain completely interferes with the activity. Each participant received an overall BPI score ranging from 0 to 140. The number is directly related to the negative impact the pain has on the individual (Dworkin RH et al, 2005).

2.1.4.5 Emotional distress (Anxiety PROMIS)

The level of anxiety that an individual experienced after their breast surgery was measured based on the participants' answers to seven specific questions regarding anxious feelings and attention span. This questions were part of the short-form instruments from the National Institute of Health roadmap initiative, Patient Reported Outcome Measurement Information System (PROMIS). This set of questions that measures the anxiety level has been validated in previous studies of large sample populations. Each individual question was scored on a scale from 1 to 5. Scores of 1 indicated the most positive experience and scores of 5 indicated the most unsatisfying experience regarding anxiety. Each patient received an overall anxiety score ranging from 7 to 35. The number is directly related to the level of anxiety (Cella D, et al, 2010).

2.1.4.6 Emotional Stability (Depression PROMIS)

Depression levels were also measured by means of short-form instruments from the Patient Reported Outcome Measurement Information System, otherwise known as PROMIS, which was established as a National Institute of Health roadmap initiative. The individuals' experience with depression after their breast surgery was measured based on their answers to eight specific questions regarding specific emotions and self-worth. This set of questions that measure the depression are also well validated. Each individual question was scored on a scale from 1 to 5. Scores of 1 indicated the most positive experience and scores of 5 indicated the highest level of depression. Each patient received an overall depression score ranging from 8 to 40. The score is directly related to the level of anxiety (Cella D, et al, 2010).

2.1.4.7 Sleep Disturbances (PROMIS)

The quality of sleep that an individual experienced after their breast surgery was measured based on the participants answers to eight specific sleep-related questions. This questions were part of the short-form instruments from the National Institute of Health roadmap initiative, Patient Reported Outcome Measurement Information System (PROMIS). This set of questions that measure sleep disturbance has been well validated in previous studies of large sample populations. Some questions were regarding the general quality of the sleep and other questions regarded the length of sleep. Each individual question was scored on a scale from 1 to 5. Scores of 1 indicated the most positive experiences and scores of 5 indicated the most unsatisfied experiences regarding sleep. Each patient received an overall sleep disturbance score ranging from 8 to 40. The score is directly related to the negative quality of sleep experienced (Buysse, DJ et al, 2010) (Cella D, et al, 2010).

2.2 SNP GENOTYPING

2.2.1 SNP Selection

Three major criteria were used in order to select each SNP used in this current study. First the SNP had to be commercially available due to the study's financial limitations. The second criteria for selection of the SNPs was the reported minor allele frequency. The sample size only consisted of 163 participants; therefore, selecting a rare SNP with a very low minor allele frequency would be under powered to detect association. The final major criteria used to

determine which SNPs to select for the current study was the observed linkage disequilibrium with other SNPs in the gene in order to achieve adequate coverage for each gene.

Three SNPs of the *GJC2* gene were selected for the current study. SNP rs7523917, SNP rs11800309, and SNP rs7539762 all had reported minor allele frequencies higher than of .10 (.34, .18, and .30, respectively). In addition to the minor allele frequencies, this three SNP combination captures 17 of 33 sites (51% of the information) at $R^2 \geq 0.8$ according to the HapMap project.

SNP rs41737 and SNP rs13223756 were selected for the *MET* gene. The reported minor allele frequencies for SNP rs41737 and SNP rs13223756 were 0.3756 and 0.1873, respectively. When combining rs41737 and rs13223756 less than 30% of *MET* gene coverage was obtained according to the HapMap Proxy.

2.2.2 Setup of Plate

Each plate consisted of both samples from cases and controls. In order to ensure consistency, the plates were set up to have both inter-plate and intra-plate controls. Figure 1 shows the plate setup for this study. All shaded areas in Figure 1 represent samples that were used as inter-plate and intraplate controls. The two plates contained both cases and controls in order to ensure consistency between plates.

Plate 1	1	2	3	4	5	6	7	8	9	10	11	12
A	TP13-356	TP12-1186	TP08-1676	TP09-1412	TP08-958	1 / TP13-11	TP12-2061	CS1655-99	HS1416-80	TP09-1772	TP09-1103	TP11-1923
B	TP12-1391	TP12-1113	TP09-899	TP08-2089	TP08-595	TP13-257	TP12-1350	KC 1660-99	LO835-803	TP09-1014	TP07-119	TP05-346
C	TP12-2227	TP08-2201	TP11-102	80 / 07-162	TP07-119	TP09-2031	TP12-1225	MD291-698	LM794-625	TP07-1974	TP09-2031	TP09-560
D	TP11-1451	TP11-1839	TP08-2083	TP05-381	TP07-1632	TP07-1903	TP08-1500	B 0117-31	KD279-479	TP07-1745	LN1420-99	KC 1660-99
E	TP12-1686	TP12-199	TP08-1631	TP09-1515	TP07-414	LW2041-95	SW1194-99	W1178-48	PZ1633-99	TP06-980	MK2175-99	TP09-1877
F	12-135	TP12-1913	TP05-1207	TP10-1556	TP09-519	JT1751-99	DD250-474	NZ1254-354	TP08-683	TP07-157	TP08-2221	TP08-911
G	TP12-1738	3 / TP11-13	TP08-1104	TP06-861	TP07-722	PT1798-99	MB115-673	SR927-839	TP07-1072	TP09-1877	TP09-529	NTC
H	TP12-1460	3 / TP08-12	TP09-1813	TP09-11039	/ TP13-9	VC1687-99	M1391-77	LC1392-731	TP06-1642	TP07-2083	TP07-1188	NTC

Plate 2	1	2	3	4	5	6	7	8	9	10	11	12
A	TP08-2317	TP10-898	TP11-122	TP11-1967	TP06-1700	TP08-909	PD1583-99	CM1427-99	/R1372-676	TP08-354	TP09-1103	TP11-1923
B	TP12-503	TP08-525	TP08-2250	TP05-346	TP11-1986	TP10-607	LK1729-99	NP1428-99	AA1582-99	1289 HIGH	TP07-119	TP05-346
C	TP13-46	TP09-129	TP07-220	TP12-1231	TP12-854	TP11-1922	MC1495-99	MG385-361	MK2175-99	TP09-1786	TP09-2031	TP09-560
D	TP08-2009	TP12-2112	TP08-382	TP08-1322	TP10-4	TP06-1721	LK1673-99	MH499-729	SP893-99*	TP08-1293	LN1420-99	KC 1660-99
E	TP08-1840	TP11-1923	TP08-1938	TP10-635	TP07-1233	TP12-737	JJ539-99	MH506-688	TP13-740	TP07-242	MK2175-99	TP09-1877
F	TP08-339	TP06-957	TP08-1244	TP09-1745	TP07-1059	TP07-688	KC 177-99	TN826-220	TP13-501	TP06-1521	TP08-760	TP13-1533
G	5 / TP08-34	TP08-2095	TP08-1426	TP11-958	TP09-733	TP09-560	SG1575-99	MS1050-83	TP08-815	TP06-807	TP13-1308	NTC
H	TP13-117	TP11-31	TP08-2296	TP09-1430	TP08-1148	TP09-1059	LN1420-99	O1304-60	TP09-1613	TP06-1597	TP13-1423	NTC

Figure 1: Plate Setup

2.2.3 Genotyping

SNP Genotyping was conducted using the TaqMan method, with ABI pre-designed assays and an ABI StepOne machine.

2.3 STATISTICAL ANALYSES

Statistical analysis was carried out using the R-studio statistical program. Logistic regression was conducted in order to assess genetic SNP variants, demographic information, pain assessment, and psychosocial concerns in regards to the development of secondary lymphedema. The dependent variable was the development of secondary lymphedema and the independent variables included the genotype for each of the five SNPs, psychological pain scoring (brief pain inventory (BPI), sleep disturbances, perceived stress scale (PSS), anxiety, depression, sleep

disturbances, pain catastrophizing scale (PCS)), and demographic information (age, race, body mass index, and menopause status). Linear regression and Spearman correlation was performed to compare the effect of the genetic variants on the varying psychosocial phenotypes (Kuzma, J and Bohnenblust, S., 2005).

3.0 RESULTS

3.1 DESCRIPTIVES

Subjects were all Caucasian females between the ages 30-84 with 163 total participants. The age was defined as the age of the participant at the time of their breast surgery with an average age of 54.59 (SD =11.88). All participants participated in a telephone interview which measured different experiences with pain, stress, anxiety, depression, and sleep disturbances. Sample characteristics including means, ranges and standard deviations were calculated for age, body mass index, and brief pain inventory, perceived stress, pain catastrophizing, anxiety, depression, and sleep disturbance scores (Table 1).

Table 1: Descriptive Statistics for age, body mass index, pain, and psychosocial measurement

	Mean (SD)	*Range*
Age	54.59 (11.88)	30-84
BMI	29.91 (6.83)	17.48-49.32
BPI	17.90 (24.30)	0-112
PSS	10.61 (7.42)	0-39
PCS	5.28 (9.42)	0-47
Anxiety	12.52 (6.11)	7-32
Depression	11.63 (6.36)	8-40
Sleep Disturbances	21.81 (9.34)	8-40

All participants participated in the Post mastectomy Pain Survey conducted by means of a telephone interview which measured different experiences with pain, stress, anxiety, depression, and sleep disturbances. Body mass index was calculated by obtaining weight and height measurements form the electronic medical records.

The pain analysis and psychosocial measurements questions were separated into six different measurements (brief pain inventory (BPI), perceived stress scale (PSS), pain catastrophizing scale (PCS) anxiety PROMIS, depression PROMIS, and sleep disturbance PROMIS). For all of these, the higher values indicate a negative impact. The scores for the psychosocial phenotypes were not observed to follow a normal distribution pattern (Figure 2)

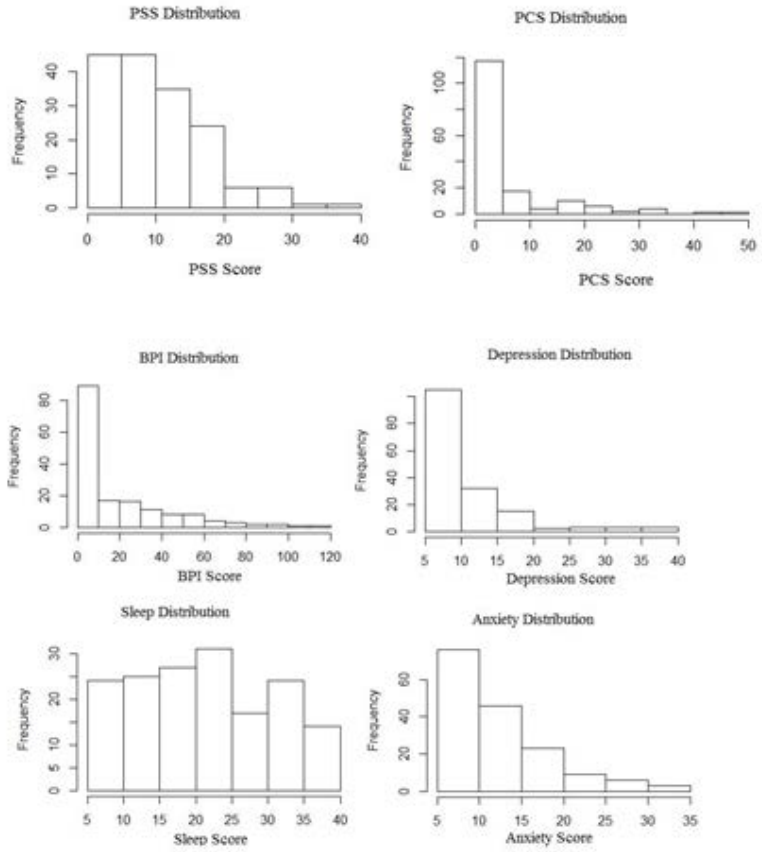

Figure 2: Psychosocial Phenotype Distribution

Sample frequencies were calculated for the development of lymphedema, onset of menopause, and type of node surgery (Table 2). Participants' type lymph node surgery and menopausal status were obtained via electronic medical records. The diagnosis of lymphedema was obtained from either electronic medical record or from the Post Mastectomy Pain Survey. The sample population consisted of 48.17% of individuals who developed secondary lymphedema symptoms and 51.83 % of individuals without the development of symptoms at the time of the survey. About 34.76% of patients were premenopausal or perimenopausal and 65.24% were post-menopausal from either natural menopause or as a result of surgery or chemotherapy. Approximately 30.67% of participants had axillary node dissection performed, 45.40% with sentinal node dissection performed, 10.43% had both sentinal and axillary node dissection, and 13.50% had no lymph node dissection.

Table 2: Frequencies of lymphedema, menopause, and lymph node surgery

Lymphedema Diagnosis	
Lymphedema symptoms	48.17% (n=79)
No Lymphedema symptoms	51.83% (n=85)
Menopause Status	
Pre/Perimenopausal	34.76% (n=57)
Postmenopausal	65.24% (n=107)
Lymph Node Surgery	
Axillary	30.67% (n=50)
Sentinal	45.40% (n=74)
Axillary AND Sentinal	10.43% (n=17)
No Lymph Node Surgery	13.50% (n=22)

Participants' genotype frequencies were calculated for each of the SNPs (Table 3). Genotypes were classified as 1/1 (homozygous for major allele), 1/2 (heterozygous), and 2/2 (homozygous for minor allele). For SNP rs41737 the frequencies of adenine homozygotes, adenine and guanine heterozygotes, and guanine homozygotes at that specific base pair location were 16.30%, 45.10%, and 38.60%, respectively. For SNP rs7523917 the frequencies of adenine homozygotes, adenine and guanine heterozygotes, and guanine homozygotes at that specific base pair location were 11.40%, 48.40%, and 40.20%, respectively. For SNP rs7539762 the frequencies of cytosine homozygotes, cytosine and thymine heterozygotes, and thymine homozygotes at that specific base pair location were 10.40%, 51.10%, and 38.50%, respectively. For SNP rs11800309 the frequencies of guanine homozygotes, guanine and thymine heterozygotes, and thymine homozygotes at that specific base pair location were 58.40%,

35.70%, and 5.90%, respectively. For SNP rs13223756 the frequencies of adenine homozygotes, adenine and guanine heterozygotes, and guanine homozygotes at that specific base pair location were 61.40%, 32.10%, and 6.50%, respectively.

Table 3: Genotype Frequencies by SNP

SNP	Gene	Allele 1	Allele 2	1/1	1/2	2/2
rs41737	*MET*	A	G	16.30%	45.10%	38.60%
rs7523917	*GJC2*	A	G	11.40%	48.40%	40.20%
rs7539762	*GJC2*	C	T	10.40%	51.10%	38.50%
rs11800309	*GJC2*	G	T	58.40%	35.70%	5.90%
rs13223756	*MET*	A	G	61.40%	32.10%	6.50%

3.2 ANALYSIS OF SNP GENOTYPE AND PSYCHOSOCIAL PHENOTYPES AS PREDICTORS OF SECONDARY LYMPHEDEMA

For each SNP logistic regression was performed in order to determine if the genotype of the participant is associated of the development of lymphedema (Table 4). For this calculation the development of lymphedema was the binary dependent variable. The independent or predictive variables include the genotype, age, menopause, sentinal lymph node surgery, axillary lymph node surgery, and BMI were adjusted for in the calculation that evaluation each SNP.

SNP rs41737, rs7523917, SNP rs7539762, SNP rs13223756 were not statistically shown to have a predictive influence on the development of lymphedema. SNP rs11800309 was significant (ά=.1) for being a predictor for the development of lymphedema in the sample

population. The beta coefficient (β) for SNP rs11800309 was calculated to be -0.5460, meaning that the development of lymphedema is indirectly related to the number of minor alleles present in an individual. The odds ratio was calculated to be .573.

The psychosocial phenotype scores of participants were also compared to the development of lymphedema after being adjusted for age, BMI, menopause status, and lymph node surgery. PSS, BPI, anxiety, depression, and sleep disturbances were all observe to have no significant effect on the development of lymphedema. PCS was found to be a statistically significant (ά=0.1) predictor of lymphedema in this sample population. The PCS Score in a patient was observed to be directly related (β= 0.043) to the development of lymphedema with an odd ratio of 1.0439.

Table 4: Logistic Regression: Lymphedema, Genotype, and Psychosocial Phenotype

PREDICTORS	POINT ESTIMATE	ODDS RATIO	STANDARD ERROR	P-VALUE
RS41737	.1856	1.2039	.2606	.4760
RS7523917	-.3410	.7111	.2730	.2120
RS7539762	-.2574	.7731	.2759	.3510
RS11800309	-.5460	.5793	.2958	.0649*
RS13223756	.4829	1.6208	.3195	.1310
PCS	.0430	1.0439	.0227	.0586*
PSS	-.0106	.9895	.0271	.6960
BPI	.0078	1.0078	.0073	.2890
ANXIETY	.0065	1.0065	.0315	.8360
DEPRESSION	.0129	1.0130	.0271	.6340
SLEEP	-.0245	.9758	.0209	.2410

-adjusted for age, menopause status, BMI, Lymph and node surgery

*significant at .01 level

3.3 THE DEMOGRAPHIC EFFECT ON THE DEVELOPMENT OF LYMPHEDEMA

Logistic regression was performed to observe the effect of the demographic information on the development of lymphedema. Univariate analysis (simple logistic regression) was performed for

each category of demographic information (age, menopause status, BMI, sentinal lymph node surgery, and axillary lymph node surgery) separately in order to observe the unadjusted effect (Table 5). Multivariable analysis was also performed in order to observe the adjusted effect that the demographic information has on the development of lymphedema (Table 6). For both models significant associations between demographic information and the outcome of lymphedema were not observed.

Table 5: Univariate analysis: Demographics on lymphedema development

PREDICTORS	POINT ESTIMATE	ODDS RATIO	STANDARD ERROR	P-VALUE
AGE	.0017	1.0017	.013230	.9000
MENOPAUSE	.0154	1.0155	.3301	.9630
BMI	.0337	1.0343	.0260	.1950
SENTINAL NODE	-.4100	.6636	.3171	.1960
AXILLARY NODE	.0535	1.0550	.3185	.867

Table 6: Multivariable Analysis: Demographics effect on lymphedema development

PREDICTORS	POINT ESTIMATE	ODDS RATIO	STANDARD ERROR	P-VALUE
AGE	.0070	1.0070	.0194	.7190
MENOPAUSE	-.0994	.9054	.5157	.8470
BMI	.0306	1.0311	.0268	.2530
SENTINAL NODE	-.5268	.5905	.4268	.2170
AXILLARY NODE	.0898	1.0940	.4311	.8350

3.4 LINEAR REGRESSION AND SPEARMAN TEST: GENOTYPE AND DEMOGRAPHIC EFFECT ON PSYCHOSOCIAL PHENOTYPES

This current study aimed to determine if the genotype and demographic information had an effect on the psychosocial phenotype. For evaluation of each psychosocial phenotype linear regression, demographic multivariable analysis, demographic univariate analysis, and the

Spearman Correlation test were performed. Linear regression was performed with the SNP genotypes being the predictor. These analyses were adjusted for the effects of age, menopause status, BMI, sentinal node surgery, and axillary node surgery. Univariate and Multivariable analysis were performed to determine the effect of the demographic information. The psychosocial phenotypes were not normally disturbed, which makes the reliability of the linear regression less than ideal. Spearman Correlation Test was also performed for each genotype and demographic information for each separate psychosocial feature in order to account for the screwed distributions.

3.4.1 Brief Pain Inventory (BPI)

For SNP rs7523917, SNP rs7539762, SNP rs11800309, and SNP rs13223756 no statistically significant predictive effects was observed when comparing these genotypes to the BPI scores in individuals. SNP rs41737 showed a statistically significant predictive effect on BPI scores ($\acute{\alpha}$=.05). The beta coefficient was observed to be 6.9165 indicating that individuals with two minor alleles are more likely to have higher BPI scores. Demographic information affect was adjusted for when using this static model (Table 7). The reliability and interpretation of this test is limited because the BPI score is not normally distributed.

Table 7: Linear Regression for BPI and Genotype

PREDICTORS	POINT ESTIMATE	STANDARD ERROR	P-VALUE
RS41737	6.9154	3.1164	.02835**
RS7523917	2.7938	3.3303	.4032
RS7539762	5.1469	3.2818	.1195
RS11800309	-4.1467	3.4901	.23709
RS13223756	-1.6112	8.8123	.67331

-adjusted for age, menopause status, BMI, Lymph and node surgery

**significant at .05 level

Linear regression was performed to observe the effect of the demographic information on the BPI scores. Univariate analysis (simple logistic regression) was performed for each category of demographic information (age, menopause status, BMI, sentinal lymph node surgery, and axillary lymph node surgery) separately in order to observe the unadjusted effect (Table 8). Multivariable analysis was also performed in order to observe the adjusted effect that the demographic information has on BPI scores (Table 9). Both different calculation failed to observe significant association between menopause status, BMI, sentinal Node lymph node surgery, and axillary lymph node surgery on BPI scores. Age was observed to be statistically significant for predicting BPI scores in both the Univariate (ά= .1) and Multivariable (ά= .05) statistic models. Both models showed that age was indirectly related to the BPI score (β=-.53449 and -2949). The reliability and interpretation of these tests is limited because the BPI score is not normally distributed.

Table 8: Multivariable analysis Demographics on BPI

PREDICTORS	POINT ESTIMATE	STANDARD ERROR	P-VALUE
AGE	-.53449	.23802	.02647**
MENOPAUSE	5.08393	6.36459	.42592
BMI	-.07167	.32864	.82771
SENTINAL NODE	5.41241	5.29088	.30828
AXILLARY NODE	5.15005	5.33880	33657

**significant at .05 level

Table 9: Univariate analysis: Demographics on BPI

PREDICTORS	POINT ESTIMATE	STANDARD ERROR	P-VALUE
AGE	-.2949	.1661	.07771*
MENOPAUSE	-.2015	4.1871	.962
BMI	-.1264	.3233	.6966
SENTINAL NODE	-.1568	4.0134	.969
AXILLARY NODE	3.493	4.043	.389

*significant at .01 level

A non-parametric test was performed because of the non-normal distribution of the BPI scores (Table 10). Spearman correlation test indicates that rs41737 genotype is correlated with BPI scores. The significance level for this comparison was observed to be .03932 and rho to be .167. All other data did not show significant correlations for BPI scores.

Table 10: Spearman Test: BPI

	P-VALUE	RHO
RS41737	.03932**	.1647
RS7523917	.7026	.03712
RS7539762	.6144	.0408
RS11800309	.8044	-.0199
RS13223756	.7089	.0300
AGE	.3900	-.0680
MENOPAUSE	.4711	.05702
BMI	.8188	.0201
SENTINAL NODE	.9632	-.0037
AXILLARY NODE	.1284	.1200

**significant at .05 level

3.4.2 Perceived Stress Scale (PSS)

For SNP rs7523917, SNP rs7539762, SNP rs11800309, and SNP rs13223756 no statistically significant predictive effect was observed when comparing these genotypes to the PSS scores in individuals. SNP rs41737 indicates a statistically significant predictive effect on PSS scores (ά=.05). The beta coefficient was observed to be 1.68709 indicating that individuals with a minor allele are more likely to have higher BPI scores. Demographic information effect was adjusted for when using this statistic model (Table 11). The reliability and interpretation of this test is limited because the PSS score is not normally distributed.

Table 11: Linear Regression for PSS and Genotype

PREDICTORS	POINT ESTIMATE	STANDARD ERROR	P-VALUE
RS41737	1.68709	.84250	.0475**
RS7523917	1.24635	.89157	.1647
RS7539762	1.21889	.91058	.1832
RS11800309	-.48904	.94123	.6043
RS13223756	.59316	1.01987	.5619

-adjusted for age, menopause status, BMI, Lymph and node surgery

**significant at .05 level

Linear regression was performed to observe the effect of the demographic information on the PSS scores. Multivariable analysis was also performed in order to observe the adjusted effect that the demographic information has on the PSS scores (Table 12). Univariate analysis was performed for each category of demographic information separately in order to observe the unadjusted effect (Table 13). For both models significant association of BMI, sentinal lymph node surgery, and axillary lymph node surgery on PSS scores were not observed. Age was observed to be statistically significant for predicting PSS scores in both the Univariate and Multivariable (ά= .001) statistic models. Both models showed that age was indirectly related to the BPI score (β=-.53449 and -2949). Menopause was also shown to have a similar effect on PSS scores. Menopause was observed to have a significant effect on PSS in both models at a .05 ά level. When linear regression was performed for menopause as the only predictor a negative trend was observed (β= -2.1487), indicating that women who have not experienced menopause scored had higher stress levels. When age, BMI, and lymph node was adjusted for the calculation showed a positive trend for menopause (β=3.66098). The reliability and interpretation of these tests is limited because the BPI score is not normally distributed.

Table 12: Multivariable analysis demographics on PSS

PREDICTORS	POINT ESTIMATE	STANDARD ERROR	P-VALUE
AGE	-.28705	.06355	1.41e-07****
MENOPAUSE	3.66098	1.69852	.033**
BMI	.09429	.08774	.285
SENTINAL NODE	-.48952	1.41023	.729
AXILLARY NODE	-.50895	1.42300	.721

significant at .05 level, ** significant at .001

Table 13: Univariate analysis: Demographics on PSS

PREDICTORS	POINT ESTIMATE	STANDARD ERROR	P-VALUE
AGE	-.20123	.04661	2.75e-05****
MENOPAUSE	-2.1487	1.2165	.0793*
BMI	.09113	.09104	.3187
SENTINAL NODE	-8028	1.1729	.495
AXILLARY NODE	.007774	1.185512	.995

*significant at .1 level, **** significant at .001

A non-parametric test was performed because of the PSS scores do not follow normal distribution patterns (Table 14). Spearman correlation test indicates that age is correlated with PSS scores. The significance level for this comparison was observed to be 7.465e-05 and rho to be -.3052. All other data did not show significant correlation for PSS scores.

Table 14: Spearman Test: PSS

	P-VALUE	RHO
RS41737	.1073	.1286
RS7523917	.5324	.0500
RS7539762	.651	.0365
RS11800309	.3641	-.0725
RS13223756	.4580	.0595
AGE	7.465e-5***	-.3052
MENOPAUSE	.1514	-.1129
BMI	.3246	.0861
SENTINAL NODE	.5018	.0530
AXILLARY NODE	.9263	.0073

**** significant at .001

3.4.3 Pain Catastrophizing Scale (PCS)

All SNPs (SNP rs41737, SNP rs7523917, SNP rs7539762, SNP rs11800309, and SNP rs13223756) were not observed to be statistically significant predictors for PCS scores (Table 15). For this model the effect of demographic information was adjusted. The reliability and interpretation of this test is limited because the PCS scores do not follow normal distribution.

Table 15: Linear Regression for PCS and Genotype

PREDICTORS	POINT ESTIMATE	STANDARD ERROR	P-VALUE
RS41737	1.24999	1.14672	.2778
RS7523917	.9815	1.2126	.4199
RS7539762	1.62034	1.2332	.1914
RS11800309	-1.46191	1.26821	.25125
RS13223756	.233583	1.38260	.8648

**adjusted for age, menopause status, BMI, and lymph node surgery

Linear regression was performed to observe the effect of the demographic information on the PCS scores. Multivariable analysis was also performed in order to observe the adjust effect

that the demographic information has on the PCS scores (Table 16). Univariate analysis was performed for each category of demographic information separately in order to observe the unadjusted effect (Table 17). Age was observed to be statistically significant for predicting PCS scores in both the Univariate and Multivariable observations with ά= .01. Both models showed that age was indirectly related to the PCS scores (β= -.23038 and -.18011). Whether an individual had sentinal node surgery performed was observed to have a significant effect on the PCS scores when using the unadjusted univariate model (ά=.05). Sentinal lymph node surgery's effect on PCS was observed have a negative trend (β= -3.275). The reliability and interpretation of these tests are limited because the BPI score is not normally distributed.

Table 16: Multivariable analysis of demographics on PCS

PREDICTORS	POINT ESTIMATE	STANDARD ERROR	P-VALUE
AGE	-.23038	.08536	.0079***
MENOPAUSE	1.23633	2.28150	.5888
BMI	.09453	.11785	.4240
SENTINAL NODE	-.76075	1.89427	.6886
AXILLARY NODE	.55321	1.91142	.7727

*** significant at .01

Table 17: Univariate analysis of demographics on PCS

PREDICTORS	POINT ESTIMATE	STANDARD ERROR	P-VALUE
AGE	-.18011	.06101	.00363***
MENOPAUSE	-2.195	1.558	.161
BMI	.07473	.11766	.526
SENTINAL NODE	-3.275	1.471	.0274**
AXILLARY NODE	2.3515	1.4958	.118

significant at .05 level, * significant at .01

A non-parametric test was performed because of the non-normal distribution of the PCS scores (Table 18). Spearman correlation test indicates that axillary node dissection is correlated

with PSS scores. The significance level for this comparison was observed to be .003765 and rho to be .2264137. All other data did not show significant correlation for PCS scores.

Table 18: Spearman Test on PCS

	P-VALUE	RHO
RS41737	.9568	-.00435407
RS7523917	.9264	.007435468
RS7539762	.6297	.03902648
RS11800309	.2984	-.0832445
RS13223756	.3708	.0719315
AGE	.0119	-.1972205
MENOPAUSE	.4169	-.06421807
BMI	.4068	.07252584
SENTINAL NODE	.0105	-.2005482
AXILLARY NODE	.003765**	.2264137

** significant at .05

3.4.4 Anxiety

Linear regression was performed for each SNP to measure the predictability effect the genotypes have on anxiety scores. The effects of demographic information were adjusted for when using this statistic model (Table 19). Two SNPs (SNP rs7539762 and SNP rs11800309) were observed to have a significant effect on the anxiety score of an individual. SNP rs7539762 was significant (ά=.047501) and showed a positive trend with minor allele frequency (β=1.54650). SNP rs11800309 was significant (ά=.0987) and showed a negative trend with minor allele frequency (β=1.33464). SNP rs7523917, SNP rs13223756, and SNP rs41737 were not shown have statistically significant predictive effect on Anxiety scores in this sample population. The reliability and interpretation of this test is limited because the anxiety scores are not normally distributed.

Table 19: Linear Regression for Anxiety and Genotype

PREDICTORS	POINT ESTIMATE	STANDARD ERROR	P-VALUE
RS41737	.95913	.72884	.19065
RS7523917	.75036	.76943	.33138
RS7539762	1.54650	.77234	.047501**
RS11800309	-1.33465	.80214	.0987*
RS13223756	-.09059	.87664	.9179

Adjusted for age, menopause status, BMI, and lymph node surgery

significant at .05 level, ** significant at .001

For demographic information, linear regression was performed to observe their effect of on the anxiety scores. Multivariable analysis was also performed in order to observe the adjusted effect that the demographic information has on anxiety (Table 20). Simple logistic regression was performed for each category of demographic information separately in order to observe the unadjusted effect (Table 21). For both models significant association between menopause status, BMI, sentinal lymph node surgery, and axillary lymph node surgery on anxiety scores were not observed. Age was observed to be statistically significant for predicting anxiety scores in both the Univariate ($\acute{\alpha}$= .001) and Multivariable ($\acute{\alpha}$= .01) statistic models. Both models showed that age was indirectly related to the anxiety score (β=-.15757 (multivariable) and -.13214 (univariable)). Menopause was significant at a .1 significant and showed a negative trend when other demographics were unadjusted. The reliability and interpretation of these tests are limited because the anxiety score is not normally distributed.

Table 20: Multivariable analysis of demographics on Anxiety

PREDICTORS	POINT ESTIMATE	STANDARD ERROR	P-VALUE
AGE	-.15757	.05443	.00446***
MENOPAUSE	.85003	1.45479	.56006
BMI	.10843	.07515	.15153
SENTINAL NODE	.31961	1.20787	.79174
AXILLARY NODE	-.26344	1.21881	.82922

*** significant at .01

Table 21: Univariate analysis of demographics on Anxiety

PREDICTORS	POINT ESTIMATE	STANDARD ERROR	P-VALUE
AGE	-.13215	.03917	.000929****
MENOPAUSE	-1.817	1.001	.0713*
BMI	.08821	.07513	.242
SENTINAL NODE	-.7825	.9650	.419
AXILLARY NODE	-.02379	.97591	.981

*significant at .1 level, **** significant at .001

A non-parametric test was performed anxiety scores are not normal distribution (Table 22). Spearman correlation test indicates that age is correlated with anxiety scores. The significance level for this comparison was observed to be .0006 and rho to be -.2664. All other data did not show significant correlation for anxiety scores.

Table 22: Spearman Test: Anxiety

	P-VALUE	RHO
RS41737	.1252	.1225055
RS7523917	.7825	-.02213272
RS7539762	.97458	-.002443813
RS11800309	.4839	-.05591998
RS13223756	.6373	-.03779741
AGE	.0006****	-.2664
MENOPAUSE	.02662	-.1736715
BMI	.1628	.1217213
SENTINAL NODE	.3732	-.07021
AXILLARY NODE	.7248	-.02778109

**** significant at .001

3.4.5 Depression

SNP rs7523917, SNP rs7539762, SNP rs11800309, and SNP rs13223756 were observed to show no significant effect on predicting depression scores. SNP rs41737 was observed to have a statistically significant predictive effect on depression scores (ά=.0171). The beta coefficient was observed to be 2.0355 indicating that individuals' depression scores are directly related to the amount of minor alleles (Table 23). The reliability and interpretation of this test is limited because the depression scores do not follow normal distribution.

Table 23: Liner Regression for Depression and Genotype

PREDICTORS	POINT ESTIMATE	STANDARD ERROR	P-VALUE
RS41737	2.03350	.84106	.0171**
RS7523917	1.28855	.89703	.1534
RS7539762	1.37459	.91828	.137
RS11800309	-.58557	.94724	.53760
RS13223756	-.33327	1.02921	.74664

Adjusted for age, menopause status, BMI, and lymph node surgery

**significant at .05 level

Linear regression was performed to observe the effect of the demographic information on the depression scores. Multivariable analysis was also performed in order to observe the adjusted effect that the demographic information has on depression levels reported by the individual (Table 24). Univariate analysis was performed for each category of demographic information to determine the individual effect each categories has on depression (Table 25). Both models observe a significant effect of BMI on depression (ά=.0894 (multivariable) and ά=.08918 (univariate)). Both models also observed a positive trend between BMI and depression scores. For the univariate model, age also was observe to have an effect on depression scores (ά=.0903) and showed a negative trend between age and depression (β=-.07128). For both models significant association between menopause status, sentinal lymph node surgery, and axillary lymph node surgery on depression scores were not observed. The reliability and interpretation of these tests is limited because the BPI score is not normally distributed.

Table 24: Multivariable analysis of demographics on Depression

PREDICTORS	POINT ESTIMATE	STANDARD ERROR	P-VALUE
AGE	-.10185	.06355	.1115
MENOPAUSE	1.39367	1.69869	.4135
BMI	.15019	.08775	.0894*
SENTINAL NODE	.41326	1.41038	.7700
AXILLARY NODE	-.56274	1.42315	.6932

*significant at .1 level

Table 25: Univariate analysis of demographics on Depression

PREDICTORS	POINT ESTIMATE	STANDARD ERROR	P-VALUE
AGE	-.07128	.04183	.0903*
MENOPAUSE	-.4247	1.0523	.687
BMI	.14572	.08509	.08918*
SENTINAL NODE	-.3358	1.0065	.739
AXILLARY NODE	-.8195	1.0141	.42

*significant at .1 level

A non-parametric test was performed because of the non-normal distribution of the PSS scores (Table 26). Spearman correlation test indicates that SNP rs41737 and BMI are correlated with depression scores. The significance level for the correlation between SNP rs41737 and depression was observed to be .04701 and rho to be .1582705. The significance level for the correlation between BMI and depression was observed to be .0964 and rho to be .1447268. All other data did not show significant correlation with depression scores.

Table 26: Spearman Test: Depression

	P-VALUE	RHO
RS41737	.04701**	.1582704
RS7523917	.3929	.06844081
RS7539762	.3498	.07535713
RS11800309	.3105	-.0809357
RS13223756	.7215	.0285758
AGE	.06896	-.1428
MENOPAUSE	.3588	-.07233226
BMI	.0964*	.1447267
SENTINAL NODE	.7917	.02084828
AXILLARY NODE	.6627	-.03441947

**significant at .05 level, * significant at .1

3.4.6 Sleep Disturbances

Linear regression was performed to determine the predictive effect each SNP had on the sleep disturbance score for each participant. All SNPs (SNP rs41737, SNP rs7523917, SNP rs7539762, SNP rs11800309, and SNP rs13223756) were observed to show no statistically significant predictive effect on sleep disturbance. The effect of the demographic information was adjusted for when using this statistic model (Table 27). The reliability and interpretation of this test is limited because the sleep disturbance scores are normally distributed.

Table 27: Linear Regression for Sleep and Genotype

PREDICTORS	POINT ESTIMATE	STANDARD ERROR	P-VALUE
RS41737	1.44283	1.11098	.196518
RS7523917	-.508993	1.1180515	.66712
RS7539762	-.2884479	1.187383	.808460
RS11800309	1.39837	1.22055	.254167
RS13223756	.00602	1.33628	.996413

Adjusted for age, menopause status, BMI, lymph node surgery

Linear regression was performed to observe the effect of the demographic information on the sleep disturbance scores. Multivariable analysis was also performed in order to observe the

adjusted effect that the demographic information has on sleep (Table 28). Univariate analysis was performed for each category of demographic information in order to observe the unadjusted effect on sleep (Table 29). Age was observed to be statistically significant for predicting sleep disturbance scores in both the Univariate (ά= .00785) and Multivariable (ά= .000614) statistic models. The univariate and the multivariable models showed that age was indirectly related to the sleep score (β=-.20464 and -.29263 respectively). No significant association was observed between menopause status, BMI, sentinal lymph node surgery, and axillary lymph node surgery on sleep scores. The reliability and interpretation of these tests is limited because the sleep does not follow normal distribution.

Table 28: Multivariable analysis of demographics on Sleep

PREDICTORS	POINT ESTIMATE	STANDARD ERROR	P-VALUE
AGE	-.29263	.08328	.000614****
MENOPAUSE	2.79613	2.23194	.212606
BMI	.02328	.11519	.840191
SENTINAL NODE	-2.70886	1.84416	.135200
AXILLARY NODE	-2.70886	1.86283	.148388

**** significant at .001

Table 29: Univariate analysis of demographics on Sleep

PREDICTORS	POINT ESTIMATE	STANDARD ERROR	P-VALUE
AGE	-.20464	3.33981	.000785***
MENOPAUSE	-1.839	1.540	.234
BMI	.01901	.11813	.872
SENTINAL NODE	-1.883	1.473	.203
AXILLARY NODE	-.3205	1.4940	.83

**** significant at .001

A non-parametric test was performed because of the non-normal distribution of the sleep disturbance scores (Table 30). Spearman correlation test indicates that there is no significant

correlation between any of the genotype or demographic information with sleep disturbance scores.

Table 30: Spearman Test: Sleep

	P-VALUE	RHO
RS41737	.4271	.0628223
RS7523917	.5618	-.04664528
RS7539762	.5107	-.05322059
RS11800309	.09838	.1319599
RS13223756	.9976	.0002451708
AGE	.003091	-.231082
MENOPAUSE	.2681	-.08751388
BMI	.9719	.003092169
SENTINAL NODE	.2186	-.09718813
AXILLARY NODE	.9016	-.009792803

3.5 RESULTS SUMMARY

SNP rs11800309 and PCS scores were observed to be significant predictors for the development of secondary lymphedema symptoms at a 0.1 significance level. SNP rs41737 was observed to have significant effect on BPI scores when using both linear regression model and the Spearman Correlation test. Age was observed to be significantly related to BPI scores with the linear regression models. SNP rs41737, age, and menopause had significant at effect on PSS scores when using the linear regression models. Age was also observed to have significant effect on PSS with the Spearman Correlation test. Age was observed to have a significant effect on PCS scores when using multivariable and univariate linear regression models, as well as the Spearman Correlation test. Sentinal lymph node dissection was observed to be related to PCS

scores in the univariate linear regression model. Axillary lymph node dissection was observed to be related to PCS scores when using the Spearman Correlation test. SNPs rs7539762 and rs11800309, age, and menopause were observed to have a significant effect on anxiety scores when using the linear model. Age was also observed to have a significant effect on anxiety when using the Spearman Correlation test. SNP rs41737 and BMI was observed to be related to depression scores when using both the linear regression and the Spearman correlation test. Age was observed to effect depression scores when using the univariate liner regression model only. Only age was observed to have a significant effect on sleep scores in the multivariable and univariate linear regression models.

4.0 DISCUSSIONS

4.1.1 Aim I

Multiple studies have identified persistent pain after surgery as a probable concern for women who undergo breast cancer. The prevalence of pain after breast surgery is estimated to be experienced in up to 50% of patients. Side effects from cancer treatment have a negative impact on the quality of life in the majority of cancer survivors (Andersen et al, 2011; Chebille AL and Tchou J, 2007; Maunsell E et al, 1993). Looking at all the subjects in the current study, many conclusions can be drawn from the different psychosocial measurements obtained.

In the current study, the average brief pain inventory measurement was reported to be 17.90, with the BPI testing scale ranging from 0-140. This average measurement is associated with a low or mild experience with pain in this sample population. The brief pain inventory measures the present level of pain, the average intensity of pain, the lowest level of recent pain, the highest level of recent pain, and the recent effect the pain has had on the participants' daily activities. The majority of the participants in this study were observed not to be effected with recent pain, however, the overall range amongst those who participated was observed to be 0-112. This suggests that this measurement is quite variable, with the minority of patients being significantly affected with high levels of pain.

Perceived stress was an additional measurement obtained from conducting the study survey. In the current study the average PSS measurement was reported to be 10.60, with the PSS testing scale ranging from 0-40. The sample mean suggest that the sample population "almost never" felt nervous, incapable of control, or overwhelmed within a month of the survey being conducted. Although average participants in this study were observed not to be stressed the overall range amongst participated was observed to be 0-36. This again suggests that this measurement is quite variable among the sample population, with the minority of patient often affected with severe stress.

Pain catastrophizing scale was an essential measurement to understand the patients' general feeling, thought, and emotions regarding the effect of the experienced pain. The average score observed in this sample population was 5.28. This suggests that the participants were having negative feelings or thoughts about their pain either not at all or to a slight degree. The PCS range observed in this sample population is quite variable, ranging from 0-47. This suggests that some outliers in the sample experience negative thoughts to a great degree.

Anxiety was also measured by means of the Anxiety short form. These seven questions measured the patients' level of fear, worry, focus, and vulnerability within a week of the survey date. The results of the study show a mean score of 12.52, the testing range for anxiety was 7-35. This showed that the average patient rarely felt anxious feelings. The survey results were variable with a range from 7-32. Only a minority of participants were observed to have severe anxiety.

Depression scores were calculated by means of an eight question survey. These questions measured negative feeling within the past week of the survey date. The results showed a mean score of 11.63, with the possible test scores ranging from 0-40. The observed mean of 11.63

indicated that the participants rarely experienced these negative feelings of depression. Ranges were very variable, however, with some participants reporting always having these feelings.

Sleep quality and disturbances were also measured by means of an eight question survey. Although the ranges were variable, the average sample score indicates that participants are only somewhat satisfied with their sleep quality. When compared to the other psychosocial phenotypes, sleep disturbance was the measurement that was observed to have the majority of participants being not completely satisfied.

Overall, these scores were lower than hypothesized in a sample population of women that have recently undergone breast cancer surgery; however, since both the cases and controls had undergone breast surgery it is impossible to say whether each specific mean score was higher than that of the general population. It is apparent that each score showed quite variable ranges. It would be both helpful and have an impact in the field of public health to determine the variables that cause differences in psychosocial phenotypes amongst the population in order to provide treatment, management, and care for the outliers in the population.

4.1.2 Aim II

SNP genotyping of common Single Nucleotide Polymorphisms (SNPs) in *GJC2 and MET* was performed on our sample population. For all of the SNPs in the current study, we observed participants that were homozygous for the major allele, homozygous for the minor allele, and individuals that were heterozygotes. The fact that both alleles were observed when genotyping made it possible to draw proper conclusions of genetic variant's effect on the development of lymphedema and psychosocial phenotypes.

4.1.3 Aim III

The analysis of information was broken down into three separate sections. The first part of the analysis was to determine if the SNPs genotypes were determining factors in the development of lymphedema. The second part of the analysis was to determine if the psychosocial phenotypes were associated of the development of lymphedema. The last part of the data analysis for the current study was to determine if genotype variation had an influence on the presenting psychosocial phenotypes. Demographic information was also considered in order to adjust for the effect of age, menopause, lymph surgery, and body mass index.

Although no specific demographic feature was statistically significant at .05 level in contributing to the development of lymphedema, a few interesting observations can be drawn from this analysis. First, previous studies have observed that individuals who had axillary dissection or axillary sampling had an increased risk for developing lymphedema symptoms (Liljegren G and Holmberg, 1997). The current study did not find any correlation between lymphedema and axillary surgery. Perhaps this can be explained by the improvement of surgical techniques over time. Past studies have also reported a correlation with the development and the severity of lymphedema with obesity (Ridner SH et al, 2011). Again no significant correlation was observed between the development of lymphedema and an increased BMI. Although not significant as being a predictor, the data indicates age, BMI, and axillary node dissection follow the expected positive trends (increased BMI and age, and a history of axillary node surgery was observed to increase the risk of lymphedema).

Another interesting conclusion drawn from the logistical regression analysis for the development of lymphedema and the genotype, is the results yielded from SNP rs1800309 of the *GJC2* gene. Although not statistically significant at a .05 level, the p-value was observed to be

.0649 and the greatest predictor. Decreasing the confidence interval to 90%, SNP rs11800309 was observed to have a significant effect. The beta coefficient was observed to be a negative value, indicating that the minor allele is in protective for developing symptoms of lymphedema. For this particular SNP located within the *GJC2* gene at position 228391808, individuals with genotype G/T or T/T are less likely to develop lymphedema symptoms than individuals with the genotype G/G. The odds ratio for this association was observed to be .5793 indicating that individuals that have the G/T have approximately a half-fold decrease for developing lymphedema when compared to individuals with a G/G phenotype. These results also indicate that individuals that have the T/T have a half-fold decrease for developing lymphedema when compared to individuals with a G/T phenotype. With the limited sample size and some of the other study limitations, this particular SNP may require further studies in order to determine whether variation in this SNP is significant in the development of lymphedema.

Interesting results were also observed when determining if the psychosocial phenotype is associated with the development of lymphedema. The Pain catastrophizing score was found to be significantly associated with lymphedema development. The PCS scores were positively associated with lymphedema, meaning that individuals with high PCS scores were more likely to have lymphedema symptoms. The odds ratio for this association was 1.0439, indicating for each increase in PCS score the odds of developing lymphedema increased by 1.0439. This data suggests that individuals that are experiencing high levels of severe emotional response to pain should be observed closely by their physician for lymphedema. Also, patients with lymphedema may benefit from counseling services. Further studies, with a larger sample size, may be warranted to better understand the relationship between PCS and lymphedema.

When observing the relationship between lymphedema, BPI, PSS, anxiety, sleep, and depression, no significant relationships were identified. Although not significant, positive trends were observed for BPI, depression, and anxiety while negative trends were observed for PSS scores and sleep disturbances.

The last section of the current study aimed to determine whether the genotype had an influence on the psychosocial phenotypes of an individual. Multiple interesting conclusions were observed from this comparison.

SNP rs41737 was observed to be statistically significant in predicting BPI, PSS, and depression scores at a .05 significance level. The trends for BPI, PSS, and depression scores were observed to be positive, indicating that the minor allele is associated with increased pain, stress, and depression. This positive association was observed in the linear regression models, which assumes normal distribution. All of these psychosocial phenotypes do not follow normal distribution; the psychosocial scores are all positively skewed. Spearman Correlation was also performed and SNP rs41737 showed significance, indicating the minor allele is correlated with increased pain, stress, and depression.

SNP rs753962 was observed to be statistically significantly associated with anxiety scores at a .05 significance level. The trend for anxiety and SNP rs753962 was observed to be positive, indicating that the minor allele is associated with increased anxiety. This positive association was observed in the linear regression models, which assumes normal distribution. Anxiety does not follow normal distribution; it is skewed right. Spearman Correlation was also performed and did not show a significant correlation between SNP rs753962 and anxiety scores.

SNP rs1180039 was observed to be statistically significant in predicting anxiety scores at a .1 significance level. The trend for anxiety and SNP rs1180039 was observed to be negative,

indicating that the minor allele is associated with decreased anxiety. This negative association was observed in the linear regression models, which assumes normal distribution; however, anxiety does not follow normal distribution. Spearman Correlation was also performed and no statistically significant correlation between SNP rs1180039 and anxiety scores was observed.

Effects of the demographic information on the psychosocial phenotypes were also observed in the current study. Age had a significant effect on all of the psychosocial phenotypes. Data indicates that the older an individual was at the time of their breast surgery, the less negative experience the individual will have with pain, stress, anxiety, depression, and sleep. It is challenging to interpret the explanation behind this observation. One possibility is that older individuals are less sensitive to pain. Another explanation may be that older generations are less inclined to express their true pain level due to cultural differences and fear of appearing more vulnerable. This correlation between age and all psychosocial phenotypes was observed when using the linear regression models. This correlation between age and psychosocial phenotypes was observed when using Spearman Correlation test for BPI, PSS, anxiety and depression.

Women with a history of sentinal node dissection were statistically observed to have lower PCS scores. This observation was only statistically significant when using the univariate model. When adjusting for age, axillary surgery, BMI, and menopause status this association was not observed. When using the Spearman Correlation test, women with a history of axillary node dissection were statistically observed to have higher PCS scores. The interpretation of the effect of lymph node surgery is challenging. Axillary and sentinal node dissection seem to have no effect on any of the other psychosocial phenotypes. Perhaps a possible explanation for this observation is that women who had lymph node surgery were originally diagnosed with more aggressive invasive cancer than those who did not have lymph node surgery. Perhaps having a

more invasive cancer increases one's PCS score because the individual is more fearful of future diagnosis. Further investigation should be conducted to add more insight to this observation.

BMI was observed to be associated with higher depression scores at a significance level of .1. This was observed with the multivariable linear regression, univariate linear aggression, and Spearman correlation test. Multiple studies suggest that depression is more prevalent in individuals with higher BMI scores (Onyike CU et al, 2003). This study adds more evidence that BMI has an impact on the quality of life in individuals that are overweight.

4.1.4 Public Health Significance

The current study had identified information that may be relevant in identifying women who may be at an increased risk for developing secondary lymphedema after breast surgery. The data shows that women who have high PCS scores were likely to also experience lymphedema symptoms. Whether lymphedema development is causative of developing this psychosocial phenotype is unknown. The correlation between pain catastrophizing and lymphedema is evidence that women with lymphedema have a lower quality of life than that of unaffected individuals. This data suggests the possible need for psychological services and resources to be offered to patients that have breast surgery, specifically individuals that go on to develop lymphedema.

This study also identified the genotype at SNP rs41737 to affect the pain, stress and depression levels. SNP rs41737 genotype was also identified to effect anxiety scores. Mental health concerns are a colossal challenge that effects the American population. According to the Center of Disease Control, approximately 8% of the American population suffers from depression in any two-week period (QuickStats: Prevalence of Current Depression , 2012). Also,

according to the National Ambulatory Medical Care Survey and National Hospital Ambulatory Medical Care Survey, it is also estimated that major depressive disorders cost the American healthcare system 8.0 million dollars per year. Mental health illness is a subject where not much is understood about the genetic contributions to this group of disorders. A larger study is warranted to see if variations within this gene increase the susceptibility for developing mental health issues, such as depression and stress. If larger studies can further validate these findings perhaps screening for higher susceptibility of depression can be implemented in order to assist individuals in receiving counseling services and resources.

Another significant finding in the current study was the common observation that BMI may lead to depression. BMI was not seen to affect anxiety levels, pain levels, stress levels, or the development of lymphedema. However, BMI measurements were observed to have an impact on higher depression scores. Whether societies' discrimination tendencies and stereotypes cause individuals that are obese to be more likely to experience symptoms of depression or whether depressed individuals are more likely to live unhealthy life styles that may lead to obesity is not indicated for this current study. Perhaps a third, unknown factor causes both depression and obesity. Causation cannot be determined from the current study; however, an association between BMI scores and depression was observed. Studies are consistently observing this same result. It is important that public awareness programs be initiated in order to educate the public about the effect obesity has on not just physical health concerns, but mental health concerns as well. Also, because of the increased risk for depression, counseling services should be offered to patients who are obese, especially in school settings, so that individuals can learn coping mechanisms earlier in life.

4.1.5 Limitations

Throughout the course of the project, multiple limitations that may have impacted the outcome of the study were identified. These limitations include the sample size, case definition, selections of SNPs, selection of matched controls, limited information for some participants, and timing of the survey for certain patients.

One of the major limitations of this study was the small sample size. Although the Post mastectomy Pain study database consisted of approximately 1,300 participants that completed surveys, the actual cases of physician diagnosed lymphedema was relatively small. Only a total of 85 participants were identified to have developed secondary lymphedema after their breast surgery based on physician diagnosis or self-reported treatment of lymphedema. 85 controls were included in the study making the total sample population 170 participants. When it came time to perform the data analysis, because the number of African American and/or Hispanic individuals was small and would be an unnecessary cofounding variable, these participants were eliminated from the study for simplicity. This decreased our total sample size to 164 participants (79 cases, 85 controls). Epidemiological studies estimate the incidence of lymphedema to range from 8-20% (Paskett ED, et al, 2007). Based on this estimate, it was expected that more cases would be identified.

Another limitation to this study was the case definition. In ideal circumstances, the cases for this study would have included individuals with a physician confirmed diagnoses of secondary lymphedema. In order to have a large enough sample size for any results to be significant, the case definition was expanded to include individuals that had either a physician confirmed diagnoses of secondary lymphedema or experienced self-reported symptoms of lymphedema. It is challenging to predict the overall effect this limitation had on defining the

cases. Although lymphedema symptoms are often quite apparent to a patient, the study could not rule out false reports of lymphedema in the sample population. Another essential consideration when reviewing the case definition in secondary lymphedema cases after breast surgery is that these symptoms may not become apparent until years after the surgical therapy. These surveys were conducted after 6-months post breast surgery. Because it could take years to develop symptoms, it is possible that some of the controls in our study may have a diagnosis of lymphedema later in life. This would obviously have a large effect on the results of the study, particularly the genotype data. The main goal of the study was to identify a genetic or psychosocial difference between the case and control groups. To examine and interpret any difference it is essential that no cases pollute the control group.

Identifying a match control for each specific case was also another limitation for the study. The Post Mastectomy Pain Study database provided a multitude of possible cases, which allowed for the majority of the cases to be matched based on seven categories (menopausal status, age at surgery, body mass index, race, type of breast surgery, type of node dissection, and treatment). These particular categories were matched to each patient to limit the effect that cofounding variables would have on both the genetic and psychosocial results. A small proportion of cases did not have a perfect match in all of the seven categories. In these cases, age, menopausal status, type of surgery, and treatment were prioritized when selecting a match because these categories are major contributing factors to the development of secondary lymphedema.

Another limitation in the study design was the fact that SNP genotyping was performed with a limited amount of SNPs for each gene. Due to limited funding, sequencing of the entire *GJC2* and *MET* genes was not a viable option. According to NCBI, *GJC2* is a gene that is made

up of 10,113 bases and the *MET* gene is made up of 125,997 bases. In the current study, only three of these bases for *GJC2* and two bases for *MET* were examined. The HapMap Proxy estimated gene coverage by considering linkage disequilibrium. For *GJC2* only 50% of the gene was covered and less than 30% for *MET*. The low coverage is a major limitation in predicting whether or not these genes influence the development of secondary lymphedema or have an effect on the psychosocial phenotypes.

Concerns involved in performing multiple comparison are another limitation of this current study. The psychosocial data did not follow normal distribution patterns; therefore, the linear regression results and interpretations are not reliable. The Spearman Correlation test was performed to determine if genotype and demographic information were correlated with psychosocial scores; however these results are not adjusted. The trends identified in this study are not significant when viewed in context of the whole project2.

The time in which the su[r]vey was conducted was another inconsistency in the study design. An attempt to contact each patient was made six months after receiving breast surgery; however, not all participants were available at the time the student researcher made the telephone call. Some of the participants were not interviewed until much later than six months after their breast surgery. Although this inconsistency may not have had a substantial effect on the study, it is important to recognize this inconsistency as having a possible effect on the pain and psychosocial phenotypes.

Missing survey information for certain participants was another set-back for the project. This was in relatively rare situations; however, certain parts of the survey were unanswered on occasion. This drawback was limited due to the consistency of the interviewers. When a psychosocial question was not answered by a participant the scoring was adjusted in order to

compensate for the limited information. This compensation was implemented by multiplying the total score of each measurement by the total amount of answered questions. This number was than divided by the total amount of questions for that part of the survey. This method ensured that each score containing unanswered questions would be adjusted.

4.1.6 Future Research

Lymphedema and the lymphatic system are, in general, understudied. More studies are needed in order to help determine which patients are susceptible to this condition and which are not. Full sequencing of all the genes known to cause primary lymphedema should be performed on participants that are affected with secondary lymphedema to better determine if any of these genes contribute to the development of symptoms. Full sequencing of these genes would allow coverage of the entire gene and would be able to identify if any specific variant is evident in lymphedema patients. It would also be beneficial to have a larger sample to increase statistical power to detect associations. This would allow a more detailed examination of these genes' contribution to lymphedema.

This current study identified certain SNPs being correlated to a negative psychosocial phenotype. Mental health illness is a major concern in the American population. It would be interesting to perform a similar study with the sample consisting of participants from the general population (not suffering from breast cancer). Particularly, SNP rs41737 was observed to be statistically significant in predicting BPI, PSS, and depression scores at a .05 significance level. It would be interesting to examine the general population for this same variant and measure the depression score to determine if this gene is associated with higher depression rates. SNP

rs1180039 and SNP rs753962 were associated with higher levels of anxiety. It would be valuable to observe whether or not these symptoms contribute to psychosocial phenotypes in other populations. Perhaps these studies may provide information for screening individuals for mental health concerns in order to ensure people receive proper medical care.

APPENDIX A: IRB APPROVAL

3500 Fifth Avenue
University of Pittsburgh Pittsburgh, PA 15213(412) 383-1480
Institutional Review Board (412) 383-1508 (fax) http://www.irb.pitt.edu

Memorandum

To: Inna Belfer

From: IRB Office

Date: 11/28/2014

IRB#: REN14110253 / PRO09090125

Subject: Cross-sectional Study on Postmastectomy Pain Genetics and Prognostic Value of Therapeutic Procedures

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Your renewal for the above referenced research study has received expedited review and approval from the Institutional Review Board under:

45 CFR 46.110.(4)
45 CFR 46.110.(5)
45 CFR 46.110.(7)

Please note the following information:

Approval Date: 11/28/2014
Expiration Date: 11/27/2015

Please note that it is the investigator's responsibility to report to the IRB any unanticipated problems involving risks to subjects or others [see 45 CFR 46.103(b)(5) and 21 CFR 56.108(b)]. Refer to the IRB Policy and Procedure Manual regarding the reporting

requirements for unanticipated problems which include, but are not limited to, adverse events. If you have any questions about this process, please contact the Adverse Events Coordinator at 412-383-1480.

The protocol and consent forms, along with a brief progress report must be resubmitted at least **one month** prior to the renewal date noted above as required by FWA00006790 (University of Pittsburgh), FWA00006735 (University of Pittsburgh Medical Center), FWA00000600 (Children's Hospital of Pittsburgh), FWA00003567 (Magee-Womens Health Corporation), FWA00003338 (University of Pittsburgh Medical Center Cancer Institute).

Please be advised that your research study may be audited periodically by the University of Pittsburgh Research Conduct and Compliance Office.
https://www.osiris.pitt.edu/osiris/Doc/0/GHC2S2JQEUC4521C20H2G4M3D9/fromString....
2/25/2015

APPENDIX B: CONSENT FORM

UPMC

University of Pittsburgh
Medical Center

CONSENT TO ACT AS A PARTICIPANT IN A RESEARCH STUDY

TITLE: Cross-sectional Study on Postmastectomy Pain Genetics and Prognostic Value of Therapeutic Procedures

PRINCIPAL INVESTIGATOR:

Inna Belfer, M.D., Ph.D.
Associate Professor of Anesthesiology
University of Pittsburgh
3550 Terrace Street
Pittsburgh, PA 15261
Telephone: 412-648-1342

CO-INVESTIGATORS:

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Fax: 412-623-6414

Jodi Martin, BS
Clinical Research Coordinator

Department of Anesthesiology
Magee-Women's Hospital
300 Halket St, Room 3402
Pittsburgh, PA 15213
Phone: 412-641-2179

SOURCE OF SUPPORT: Department of Anesthesiology

Why is this research being done?

We are interested in learning why many patients who have undergone treatment for breast cancer (breast surgery followed by adjuvant therapy) develop Post Mastectomy Pain Syndrome (PMPS). We know that surgery and associated therapies cause tissue and nerve damage producing inflammation and other changes, so it is not surprising that breast cancer patients have pain. However, pain sometimes continues when the inflammation has calmed down and there is no obvious reason for continued pain. For this reason we will examine whether the type and sequence of treatments for breast cancer are associated with the severity and duration of PMPS. We will also examine if mastectomy has changed the way body processes information about painful stimulation or whether concerns and anxiety about bodily functions and pain make patients more likely to develop PMPS. To help us understand the complex relationship between breast cancer-related therapy, psychological factors, response to pain stimulation and PMPS, we would like to measure individual responses to several harmless, but uncomfortable stimuli that test pain thresholds. In the end, we hope to more effectively treat the many patients who suffer from chronic post mastectomy pain, and predict/prevent PMPS development and chronicity.

Who is being asked to take part in this research study?

You are being invited to take part in this research study because you participated in the Magee Breast Cancer Registry and Banking Study, and agreed to complete the telephone interview. We asked over 1300 women who were treated for breast cancer at the Magee Women's Hospital to complete the telephone interview, and at that time, you agreed to participate in follow-up visits of pain assessment and DNA collection. We will ask at least 200 women to participate in this portion of our research.

What procedures will be performed for research purposes?

If you qualify to take part in this research study, you will undergo the experimental procedures listed below, including DNA collection (saliva, frozen tissue or blood sample). These procedures will take place at the Anesthesia Research unit located at Magee Women's Hospital. The procedures will take approximately 1.5 hours to complete.

TEST PROCEDURES:

Testing threshold for superficial pain with small plastic filaments over hand and breast (2 min)

Testing pain threshold for pressure pain with a small plate (size of a fingertip) over forearm and shoulder (2 min)

Testing heat pain by briefly warming a small plate up to 47^{o} C over hand (10 min).

Testing cold pain by placing your hand in ice-cold water for up to 200 seconds (5 min).

Testing 'ischemic' pain caused by exercise during brief stop of blood flow to the arm for up to 200 seconds (5 min).

Testing smell sensitivity using standard smell identification kit (Sensonic, Inc.)

Collecting one DNA specimen in the form of saliva in Oragene Kit, previously donated frozen tissue or blood depending on availability.

Testing autonomic function (blood pressure and heart rate)

You will be asked to answer questions regarding your mood, sleep, quality of life and functioning. You will be asked to answer these questions using laptop-based scales during your visit.

We will record information about your breast cancer and any related treatment by reviewing your medical chart associated with your Registry entry. We will not use your name, date of birth or medical record number to identify you, and we do not anticipate that this information can be used by others in a way that could affect you

DNA GENOTYPING

For the genetic part of this study, we are going to use DNA samples extracted from frozen blood samples you kindly donated previously. However, if, for any reason, blood is not available for DNA extraction, we are going to ask you to donate a saliva sample using a standard Oragene saliva kit. After your DNA will be extracted from your saliva sample, it will be genotyped for genetic markers (e.g., DNA sequences with a known location on a chromosome and associated with a particular gene or trait. We genotype single nucleotide polymorphisms – particular variation, which may arise due to mutation or alteration in the genomic loci, that can be observed in some people) in genes known to be related to human pain. The remaining DNA will be stored for future research involving painful disorders. Length of storage is indefinite. Your biologic samples will be under the control of the principal investigator of this research project. To protect your confidentiality, all personal identifiers (i.e., name, social security number, and birth date) will be removed (de-identified) and replaced with a specific code number. The information linking these code numbers to the corresponding subjects' identities will be kept in a separate, secure location. Your biologic de-identified samples may be given to investigators outside of UPMC to be utilized in future studies of human pain.

What are the possible risks, side effects, and discomforts of this research study?

There is a potential risk of an accidental or inadvertent breach of confidentiality. We have taken steps to guard against this risk. The information recorded for this research will not be

identified with your name or any other information that could identify you. All information obtained will be identified with a code number.

As we are interesting in understanding why people experience pain, the study will include several tests to determine pain thresholds. All of these tests are often performed and do not cause harm or lasting pain. However, you will at least briefly experience discomfort, at which point each of the tests is ended. Any discomfort or pain will resolve completely within less than 5 minutes.

As part of the examinations, we will test the effect of heat. To eliminate the chance of localized burns, we will stop raising the temperature, once we reached to 47^{o} C. The maximal exposure time to this temperature is limited to 3 seconds. While you may experience pain, the pain should not be intense and the heat should not cause any tissue damage, as we will avoid conditions that can cause even minor burns. The exposure to cold water will cause pain, which may be followed by tingling or a burning sensation that can last for about 1 min. Your hand may become visibly red as it warms up after the cold exposure. We will ask you to exercise at keep a blood pressure cuff inflated to a high pressure (220 mmHg). This pressure will exceed your own blood pressure. Therefore, blood will briefly not reach your arm. The pressure of the cuff and briefly blocked blood flow can cause a tingling sensation and will induce pain. However, these feelings will disappear within 1 min after we remove the blood pressure cuff. Similar to the cold exposure, your hand may become red after blood flow to your arm is allowed again.

What are possible benefits from taking part in this study?

You will likely receive no direct benefit from taking part in this research study. However the data obtained from this research will help to identify the genetic determinants of PMPS and human pain in general that will lead to better understanding of pain mechanisms and personalized pain medicine.

Will my insurance provider or I be charged for the costs of any procedures performed as part of this research study?

Neither you, nor your insurance provider, will be charged for the costs of any of the procedures performed for the purpose of this research study. You will be charged, in the standard manner, for your routine medical care (e.g., regular visits to your doctor for which you were already scheduled).

Will I be paid if I take part in this research study?

You will receive a payment of $50 to reimburse you for time and effort if you complete the study.

Who will pay if I am injured as a result of taking part in this study?

If you believe that the research procedures have resulted in an injury to you, immediately contact the Principal Investigator who is listed on the first page of this form. Emergency medical treatment for injuries solely and directly related to your participation in this research study will be provided to you by the hospitals of UPMC. Your insurance provider may be billed for the costs of this emergency treatment, but none of those costs will be charged directly to you. If your research-related injury requires medical care beyond this emergency treatment, you will be

responsible for the costs of this follow-up care. At this time, there is no plan for any additional financial compensation.

Who will know about my participation in this research study?

Any information about you obtained from this research will be kept as confidential (private) as possible. All records related to your involvement in this research study will be stored in a locked file cabinet. Your identity on these records will be indicated by a case number rather than by your name, and the information linking these case numbers with your identity will be kept separate from the research records. You will not be identified by name in any publication of the research results unless you sign a separate consent form giving your permission (release).

Will this research study involve the use or disclosure of my identifiable medical information?

This research study will involve the recording of current identifiable medical information from your medical records. The information that will be recorded will be limited to information concerning post mastectomy painful sensations (the duration, nature, severity and treatment of PMPS if you have it) and information about other diseases associated with pain (sleep or mood disorders). This research study will not result in identifiable information that will be placed into your medical records held at UPMC Magee Women's Hospital.

Who will have access to identifiable information related to my participation in this research study?

If you have a high score on the depression and/or related scale, your information may be released to a clinical psychologist who is a co-investigator on this study; and you will be provided with a referral sheet to take home.

In addition to the investigators listed on the first page of this authorization (consent) form and their research staff, the following individuals will or may have access to identifiable information (which may include your identifiable medical information) related to your participation in this research study:

Authorized representatives of the University of Pittsburgh Research Conduct and Compliance Office may review your identifiable research information (which may include your identifiable medical information) for the purpose of monitoring the appropriate conduct of this research study.

In unusual cases, the investigators may be required to release identifiable information (which may include your identifiable medical information) related to your participation in this research study in response to an order from a court of law. If the investigators learn that you or someone with whom you are involved is in serious danger or potential harm, they will need to inform the related authorities, as required by Pennsylvania law.

For how long will the investigators be permitted to use and disclose identifiable information related to my participation in this research study?

The investigators may continue to use and disclose, for the purposes described above, identifiable information (which may include your identifiable medical information) related to

your participation in this research study for a minimum of 5 years after final reporting or publication of a project.

Is my participation in this research study voluntary?

Your participation in this research study, to include the use and disclosure of your identifiable information for the purposes described above, is completely voluntary. (Note, however, that if you do not provide your consent for the use and disclosure of your identifiable information for the purposes described above, you will not be allowed to participate in the research study). Whether or not you provide your consent for participation in this research study will have no effect on your current or future relationship with the University of Pittsburgh. Whether or not you provide your consent for participation in this research study will have no effect on your current or future medical care at a UPMC hospital or affiliated health care provider or your current or future relationship with a health care insurance provider.

Your doctor is involved as an investigator in this research study. As both your doctor and a research investigator, s/he is interested both in your medical care and the conduct of this research study. Before agreeing to participate in this research study, or at any time during your study participation, you may discuss your care with another doctor who is not associated with this research study. You are not under any obligation to participate in any research study offered by your doctor.

May I withdraw, at a future date, my consent for participation in this research study?

You may withdraw, at any time, your consent for participation in this research study, to include the use and disclosure of your identifiable information for the purposes described above. (Note, however, that if you withdraw your consent for the use and disclosure of your identifiable medical record information for the purposes described above, you will also be withdrawn, in general, from further participation in this research study.) Any identifiable research or medical information recorded for, or resulting from, your participation in this research study prior to the date that you formally withdrew your consent may continue to be used and disclosed by the investigators for the purposes described above.

To formally withdraw your consent for participation in this research study you should provide a written and dated notice of this decision to the principal investigator of this research study at the address listed on the first page of this form. Your decision to withdraw your consent for participation in this research study will have no effect on your current or future relationship with the University of Pittsburgh. Your decision to withdraw your consent for participation in this research study will have no effect on your current or future medical care at a UPMC Health System hospital or affiliated health care provider or your current or future relationship with a health care insurance provider.

If I agree to take in this research study, can I be removed from the study without my consent?

It is possible that you may be removed from the research study by the researchers if, for example, if your self-described symptoms do not meet inclusion criteria for the study. If you are withdrawn from participation in this research study, you will continue to undergo testing and receive treatment as recommended by your physician.

****

VOLUNTARY CONSENT

All of the above has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions about any aspect of this research study during the course of this study, and that such future questions will be answered by the researchers listed on the first page of this form. Any questions which I have about my rights as a research participant will be answered by the Human Subject Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668).

By signing this form, I agree to participate in this research study. A copy of this consent form will be given to me.

_____ _____

Participant's Signature Date

CERTIFICATION of INFORMED CONSENT

I certify that I have explained the nature and purpose of this research study to the above-named individual(s), and I have discussed the potential benefits and possible risks of study participation. Any questions the individual(s) have about this study have been answered, and we will always be available to address future questions as they arise."

_____ _____

Printed Name of Person Obtaining Consent Role in Research Study

_____ _____

Signature of Person Obtaining Consent Date

APPENDIX C: STUDY SURVEY

Post-Mastectomy Pain and Genetics Questionnaire

1. Demographic Information

1. Today's date

Today's M M / D D / YYYY

2. What is your age? (years)

3. What is your race?

White or Caucasian (1) Black or African American (2) Asian (3) Pacific Islander (4) American Indian (5)

Other (please specify)

4. Are you Hispanic?

Yes (1) No (0)

5. What was race/ethnicity of your father?

White or Caucasian (1) Black/African American (2) Asian (3) Pacific Islander (4) American Indian (5)

Other (please specify)

6. What was race/ethnicity of your mother?

White or Caucasian Black or African American Asian Pacific Islander American Indian

Other (please specify)

7. Is your natural hair color red?

Yes No

8. Are you right or left hand dominant?

Right Left Ambidextrous (eat with my Right Hand) Ambidextrous (eat with my Left Hand)

Post-Mastectomy Pain and Genetics Questionnaire

9. Please indicate your height and weight. Enter numeric value only. (Use 999 code to denote N/A status)

Height (ex. 5'6)

Weight (lbs)

10. What is your marital status?

Never Married Separated Divorced Divorced and married Widowed Remarried after death of spouse

11. How many children do you have?

0 1 2 3 4 or more

Post-Mastectomy Pain and Genetics Questionnaire

2.

3.

1. Did you breastfeed your child(ren)?

Yes (all children) No, used formula (all children) Some were breastfed, some were fed with formula

2. What is the highest degree you have obtained? (check highest degree)

Never graduated High school diploma Trade school degree College/university Advanced degree from high school (less (12 years) beyond high school (12- degree (16 years) (16-23 years) than 12 years) 14 years)

3. Which statement best describes your current work status?

Working Working Working Working Retired Retired, Housekeeper, Disabled:

full-time, part-time, full-time, part-time, because of but not homemaker unable to work unable t OUTSIDE the OUTSIDE the Inside the Inside the chronic because of because of because home home home home disease chronic chronic disease other rea

disease than a disease Other (please specify)

4. Which answer best describes how often you exercise?

I never exercise I exercise less I exercise 1 or 2 I exercise 3 or 4 I exercise nearly I exercise every

than once per week times per week times per week every day day

5. Do you currently smoke?

Yes No

Dis c

Post-Mastectomy Pain and Genetics Questionnaire

4.

1. If yes, how many cigarettes per day? (20 cigarettes = 1 pack)

2. If No, have you ever smoked?

Yes No

Post-Mastectomy Pain and Genetics Questionnaire

5. 6

1. What type of breast surgery did you undergo?

Biopsy only Breast surgery (mastectomy, lumpectomy) with lymph nodes removal

Breast surgery (mastectomy, lumpectomy) without lymph nodes removal

Breast surgery with Biopsy Only

Other (please specify)

Post-Mastectomy Pain and Genetics Questionnaire

6.

1. On which side was the surgery performed?

Left Only Right Only Bilateral (one surgery)

Left, then Right Right, then Left

2. Record date of breast surgery listed above (mm/yyyy).

3. On which side was the surgery performed?

Left Only Right Only Bilateral (one surgery)
Left, then Right Right, then Left

4. Date(s) of breast surgeries (MM/YEAR Format) FOR MULTIPLE ENTRIES, USE ";"

5. What type(s) of therapies did you complete/in process of completing? Check all that apply.

Chemotherapy Radiation
Hormone (Femara, Arimidex, Tamoxifen, Aromasin) None

Post-Mastectomy Pain and Genetics Questionnaire

7.

1. Which of these therapies were completed pre-op or post-op?

	Pre-operative	Post-operative	Both pre/post-op	None
Chemotherapy				
Radiation				
Hormone				

2. Did you have a reconstructive surgery?

Yes No Planning on one in the future

Post-Mastectomy Pain and Genetics Questionnaire

8.

1. What type of reconstructive procedure did you have?

Implant (saline, silicone) Tissue Flap (TRAM, Latissimus Dorsi) Flap(TRAM, DIEP, etc.) + Implant (Saline, Silicone)

Post-Mastectomy Pain and Genetics Questionnaire

9.

1. Have you suffered from pain in the breast area since the reconstructive surgery?

Yes No

Post-Mastectomy Pain and Genetics Questionnaire

10. Pain before breast surgery

1. Did you suffer from pain before surgery (breast surgery area only)?

Yes No

Post-Mastectomy Pain and Genetics Questionnaire

11.

1. Please describe pain that you experienced before the breast surgery.

	None (0)	Mild (1)	Moderate (2)	Severe (3)
Throbbing				
Shooting				
Stabbing				
Sharp				
Cramping				
Gnawing				
Hot-Burning				
Aching				
Heavy				
Tender				

Splitting				
Tiring-Exhausting				
Sickening				
Fearful				
Punishing-Cruel				

2. Please rate the intensity of pain before the breast surgery.

0-no pain 1 2 3 4 5 6 7 8 9 10-possible pain

3. What was the location of your pain before the breast surgery?

Entire Breast Nipple Region No Specific Location

Other (please specify)

Post-Mastectomy Pain and Genetics Questionnaire

12. Brief Pain Inventory (BPI)

Throughout our lives, most of us have had pain from time to time (such as toothache, minor headaches, sprains). Please rate your pain OTHER than everyday kind of pain on a "0" to "10" scale in the last week.

1. What is your pain level at the PRESENT time, that is, right now?

0-no pain 1 2 3 4 5 6 7 8 9 10-pain as bad as could be

13.

1. During the PAST week, ON AVERAGE, how INTENSE was your pain?

0-no pain 1 2 3 4 5 6 7 8 9 10-pain as bad as could be

14.

1. During the PAST week, what was the LOWEST level of your pain?

0-no pain 1 2 3 4 5 6 7 8 9 10-pain as bad as could be

15.

1. During the PAST week, how INTENSE was your WORST pain?

0-no pain 1 2 3 4 5 6 7 8 9 10-pain as bad as could be

Post-Mastectomy Pain and Genetics Questionnaire

16. Brief Pain Inventory (BPI)

For the next ten questions, check the one number that describes how, during the PAST WEEK, PAIN has interfered with your:

1. General Activity

0- 1 2 3 4 5 6 7 8 9 10-

does not completely interfere interferes

2. Mood

0- 1 2 3 4 5 6 7 8 9 10-

does not completely interfere interferes

3. Walking Ability

0- 1 2 3 4 5 6 7 8 9 10-

does not completely interfere interferes

4. Normal Work (includes both work outside the home and housework)

0- 1 2 3 4 5 6 7 8 9 10-

does not completely interfere interferes

5. Relations with other people

0- 1 2 3 4 5 6 7 8 9 10-

does not completely interfere interferes

6. Sleep

0- 1 2 3 4 5 6 7 8 9 10-

does not completely interfere interferes

7. Enjoyment of Life

0- 1
2 3 4 5 6 7 8 9
10-

does not completely interfere interferes

Post-Mastectomy Pain and Genetics Questionnaire

8. Recreational activities

0- 1
2 3 4 5 6 7 8 9
10-

does not completely interfere interferes

9. Self-care (eating, dressing, etc.)

0- 1
2 3 4 5 6 7 8 9
10-

does not completely interfere interferes

10. Social activities

0- 1
2 3 4 5 6 7 8 9
10-

does not completely interfere interferes

Post-Mastectomy Pain and Genetics Questionnaire

17. Phantom Breast Pain

1. Have you experienced PHANTOM BREAST PAIN since the surgery(i.e pain in the breast that has been removed)?

Yes No Not Applicable (Biopsy only)

Post-Mastectomy Pain and Genetics Questionnaire

18.

1. When did you first notice post-surgery phantom breast pain (i.e. pain in the breast that

has been removed)?

Within a week after Within one month Within first 3 months Three to 12 months Over a year after breast surgery after breast surgery after breast surgery after breast surgery breast surgery

2. Are you CURRENTLY experiencing phantom breast pain?

Yes No

Post-Mastectomy Pain and Genetics Questionnaire

19.

1. Please describe the type of pain that you experience now.

	None (0)	Mild (1)	Moderate (2)	Severe (3)
Throbbing				
Shooting				
Stabbing				
Sharp				
Cramping				
Gnawing				
Hot/Burning				
Aching				
Heavy				
Tender				
Splitting				
Exhausting				
Sickening				
Fearful				
Punishing/Cruel				

2. If No, how long did the pain last?

0-3 months 3-6 months 6-12

months over 12 months

3. Rate the average intensity of phantom breast pain that you PRESENTLY have OR PREVIOUSLY experienced on the 0-10 scale. "0" is "no pain" and "10" is "worst possible pain.

0-no 1 2 3 4 5 6 7 8 9 10-

pain worst

possible pain

4. How long do/did individual episodes of phantom breast pain USUALLY last?

Few seconds Few minutes Longer than few minutes Constant Pain

5. How often do/did you USUALLY experience episodes of phantom breast pain?

Every day Every week Every month Less than once a month

Post-Mastectomy Pain and Genetics Questionnaire

6. What are some factors that affect(ed) your phantom breast pain (i.e. pain in the breast that has been removed)?

	Yes	No
Menstruation		
Change in weather		
Time of the day		
Sexual arousal		
Touch or pressure		
Psychological Stress		
Physical Stress		

Other (please specify)

Post-Mastectomy Pain and Genetics Questionnaire

20. Pain Assessment

We are interested to learn about the pain, if any, that you may be experiencing in the area you had surgery. We are also interested in pain on the operated side in your chest, armpit or arm.

By pain we mean that an area of your body is the cause of discomfort or hurts. We are interested in all pain, from a light discomfort to severe pain.

1. Within the LAST 3 MONTHS, have you experienced pain the area of the breast, armpit, arm, or chest on the operated side(s)? Please account for ongoing pain as well as pain evoked by activity.

Yes No

Post-Mastectomy Pain and Genetics Questionnaire

21.

1. Type of pain in the past 3 months

Random Ongoing Evoked (activity, etc.)

Post-Mastectomy Pain and Genetics Questionnaire

22.

1. Please select area(s) where you experienced pain in the last 3 months (since the breast surgery)

Breast Arm Armpit Side of the Upper Back Neck

body/Chest

Post-Mastectomy Pain and Genetics Questionnaire

23.

1. Please select where in the BREAST area you experience pain.

RUQ RLQ LUQ LLQ Above Below Side of the

Nipple Nipple Nipple

2. When you experience pain in/around breast, please indicate AVERAGE SEVERITY of pain in the last 3 months.

0-no 1 2 3 4 5 6 7 8 9 10-

pain severe

pain

3. When you experience pain in/around the breast, how often does this occur?

Constant Daily Weekly Monthly Seasonal

4. For each pain description, please select AVERAGE INTENSITY of breast pain in the past 3 months.

	None (0)	Mild (1)	Moderate (2)	Severe (3)
Throbbing				
Shooting				
Stabbing				
Sharp				
Cramping				
Gnawing				
Hot-Burning				
Aching				
Heavy				
Tender				
Splitting				
Tiring-Exhausting				
Sickening				
Fearful				
Punishing-Cruel				

5. Rank Overall Pain Intensity (PPI) in/around area of the breast based on pain description(s) above.

No Pain (0) Mild (1)

Discomforting (2) Distressing (3) Horrible (4) Excruciating (5)

Post-Mastectomy Pain and Genetics Questionnaire

6. When you experience pain in/around SIDE OF THE CHEST, please indicate AVERAGE SEVERITY of pain in the last 3 months.

0-no pain most 1 2 3 4 5 6 7 8 9 10-severe

7. When you experience pain in/around side of the chest, how often does this occur?

Constant Daily Weekly Monthly Seasonal

8. For each pain description, please select AVERAGE INTENSITY of pain on the side of the chest in the past 3 months.

	None (0)	Mild (1)	Moderate (2)	Severe (3)
Throbbing				
Shooting				
Stabbing				
Sharp				
Cramping				
Gnawing				
Hot-Burning				
Aching				
Heavy				
Tender				
Splitting				
Tiring-Exhausting				
Sickening				
Fearful				

Punishing-Cruel				

9. Rank overall pain intensity (PPI) in/around the side of the chest based on pain description(s) above.

No Pain (0) Mild (1) Discomforting (2) Distressing (3) Horrible (4) Excruciating (5)

10. When you experience pain in/around ARMPIT, please indicate AVERAGE SEVERITY of pain in the last 3 months.

0-no pain most severe 1 2 3 4 5 6 7 8 9 10-

Post-Mastectomy Pain and Genetics Questionnaire

11. When you experience pain in/around the armpit, how often does this occur?

Constant Daily Weekly Monthly Seasonal

12. For each pain description, please select AVERAGE INTENSITY of armpit pain in the past 3 months.

	None (0)	Mild (1)	Moderate (2)	Severe (3)
Throbbing				
Shooting				
Stabbing				
Sharp				
Cramping				
Gnawing				
Hot-Burning				
Aching				
Heavy				
Tender				
Splitting				

Tiring-Exhausting				
Sickening				
Fearful				
Punishing-Cruel				

13. Rank overall pain intensity (PPI) in/around the armpit based on pain description(s) above.

No Pain (0) Mild (1) Discomforting (2) Distressing (3) Horrible (4) Excruciating (5)

14. When you experience pain in the ARM, please indicate AVERAGE SEVERITY of pain in the last 3 months.

0-no pain most severe 1 2 3 4 5 6 7 8 9 10-

15. When you experience pain in the arm, how often does this occur?

Constant Daily Weekly Monthly Seasonal

Post-Mastectomy Pain and Genetics Questionnaire

16. For each pain description, please select AVERAGE INTENSITY of arm pain in the past 3 months.

	None (0)	Mild (1)	Moderate (2)	Severe (3)
Throbbing				
Shooting				
Stabbing				
Sharp				
Cramping				
Gnawing				
Hot-Burning				
Aching				

Heavy				
Tender				
Splitting				
Tiring-Exhausting				
Sickening				
Fearful				
Punishing-Cruel				

17. Rank overall pain intensity (PPI) in the arm based on pain description(s) above.

No Pain (0) Mild (1)
Discomforting (2) Distressing (3) Horrible (4) Excruciating (5)

Post-Mastectomy Pain and Genetics Questionnaire

24. Pain Medication

1. Do you take painkillers (Tylenol, Vicodin, Fentanyl) for pain in the area of breast surgery (breast, armpit, arm, chest)?

Yes No

Post-Mastectomy Pain and Genetics Questionnaire

25.

1. Please select the most appropriate choice(s) for pain relief

Over the counter meds (ibuprofen, tylenol, aspirin)
Opioids (morphine, oxycodone, codeine)
Special pain meds (gabapentin, lyrica, lamictal, tramadol, etc.)
Other (please specify)

2. How effective are painkillers in relieving your breast surgery pain?

0-not 1 2 3 4 5 6 7 8 9 10-

at all complete

pain relief

3. How often do you take painkillers?

(part of routine Weekly Monthly Occasionally Constantly (more Daily

than suggested daily meds regimen) (seasonal/few times dose) throughout the year)

4. Please list pain medication dose below. (FORMAT: mg x daily frequency)

Advil/Ibuprofen 200 mg

Tylenol/Acetaminophen Regular -325 mg, Extra Strength -500 mg

Aspirin Regular -325 mg, Extra Strength -500 mg

Post-Mastectomy Pain and Genetics Questionnaire

26. Pain in other places in the body.

We are interested to learn about the pain, if any, you are experiencing in places other than breast surgery.

1. We are interested to learn about the pain, if any, you are experiencing in other places than where you were operated. This pain does not have to be related to your breast surgery. Do you experience pain in any other area of the body than the area where you had surgery?

Yes No

Post-Mastectomy Pain and Genetics Questionnaire

27.

1. Has the pain begun before/after therapy(ies)?

Before After Currently completing Pain was present

I did not complete

therapy/ only present throughout (before and any therapies during therapy after)

Post-Mastectomy Pain and Genetics Questionnaire

28.

1. Did you ever notice pain in any other area of the body following breast surgery? Area(s) other than the site of breast surgery.

Yes No

Post-Mastectomy Pain and Genetics Questionnaire

29.

1. Did the pain begin before or after therapy(ies)?

Before After Currently completing Pain has been there I did not have to

therapies/ pain was only throughout (before and complete any follow-up

present during therapies after) therapies

Post-Mastectomy Pain and Genetics Questionnaire

30.

1. Have you had a pre-existing chronic pain condition (prior to breast surgery)?

Yes No

Post-Mastectomy Pain and Genetics Questionnaire

31.

1. Yes, please select pain problem(s) area(s)

Head/ Neck/Shoulders

Low back/ Knees Ankles Abdomen Hands/Wrists

Sinuses Hips

Other (please specify)

2. On the scale 0-10, please indicate the severity of pain in the area(s) that gives you the most trouble.

0-no 1

2 3 4 5 6 7 8 9

10-

pain severe

pain

3. How often do you experience pain in the located area(s). Indicate frequency of pain occurrence in the area that gives you the most trouble.

Constantly Daily Weekly Monthly Seasonal

4. For each pain description, please indicate AVERAGE SEVERITY of pain in the area that gives you the most trouble.

	None (0)	Mild (1)	Moderate (2)	Severe (3)
Throbbing				
Shooting				
Stabbing				
Sharp				
Cramping				
Gnawing				
Hot-Burning				
Aching				
Heavy				
Tender				
Splitting				
Tiring-Exhausting				
Sickening				
Fearful				
Punishing-Cruel				

Post-Mastectomy Pain and Genetics Questionnaire

5. Please indicate overall pain intensity in the area that gives you most trouble.

No Pain (0) Mild (1)

Discomforting (2) Distressing (3) Horrible (4) Excruciating (5)

Post-Mastectomy Pain and Genetics Questionnaire

32. Sensory disturbances

We would like to learn if you are experiencing sensory disturbances such as pins and needles or prickling sensations in the area of your breast surgery. WITHIN THE PAST 3 MONTHS, HAVE YOU...

1. ...had pins and needles, shooting or stabbing sensations in or around the area of your surgery?

Yes No

2. ...experienced an electric shock like sensation or jabbing feelings in the skin area in or around the area of your surgery?

Yes No

3. ...experienced hot or burning sensations in or around the area of breast surgery?

Yes No

4. ...had numbness or decreased sensitivity in or around the area of your surgery?

Yes No

5. ...had the lightest of touches cause pain in or around the area of breast surgery? (e.g. clothes)

Yes No

6. ...cold temperatures been the cause of pain in or around the area of breast surgery?

Yes No

7. ...experienced a painful itch in or around the area of your surgery?

Yes No

8. If you have experienced any of the sensory disturbances, where have they originated from? Please check all that apply.

Breast Armpit Arm Side of the chest None

Other (please specify)

Post-Mastectomy Pain and Genetics Questionnaire

33. Lymphedema Assessment

1. Within the PAST 3 MONTHS, have you experienced swelling, tensions or heaviness in the breast, armpit, arm, or back of your hand on the side of breast surgery?

Yes No

Post-Mastectomy Pain and Genetics Questionnaire

34.

1. If yes, I have a SENSATION of heaviness, swelling, or tension in...

N/A Breast Armpit Arm Side of chest Back of the hand

2. Please indicate how severe the sensation has been

0-not at all 1 2 3 4 5 6 7 8 9 10-very severe

3. If there is a VISIBLE DIFFERENCE between the operated and non-operated sides, the operated side is noticeably bigger. Please indicate location of the swelling.

N/A Breast Armpit Arm Side of chest Back of the hand

Post-Mastectomy Pain and Genetics Questionnaire

35.

1. Please indicate how visible the difference is between the operated and non-operated sides.

0-not 1

2 3 4 5 6 7 8 9 10-

at all very severe

2. Have you undergone treatment for lymphedema?

No Yes, lymphatic drainage by a physical therapist Yes, arm and wrist bandages Yes, physical therapy exercises

Other (please specify)

Post-Mastectomy Pain and Genetics Questionnaire

36. Daily Activities

We are interested to learn how breast surgery has affected your daily activities. During the PAST 3 MONTHS how were the following activities affected...

1. Carrying and/or lifting (grocery bags, luggage, children)

Not relevant, I don't do this I can do this without any problem I can do this, but with difficulties I can't do this

Post-Mastectomy Pain and Genetics Questionnaire

37.

1. Is the cause of your limitation(s) due to ongoing/evoked pain in (or around) the area of the breast surgery?

Ongoing Evoked (pressure, exercise,etc.) N/A Ongoing and evoked

2. Reaching and/or raising your arms above your head

Not relevant, I don't do this I can do this without any problem I can do this but difficulties I can't do this because

Post-Mastectomy Pain and Genetics Questionnaire

38.

1. Is the cause of your limitation(s) due to ongoing/evoked pain in (or around) the area of the breast surgery?

Ongoing Evoked N/A Ongoing and evoked

2. Pushing a heavy door

Not relevant, I don't do this I can do this without any problem I can do this, but with difficulties because I can't do this

39.

1. Is the cause of your limitation(s) due to ongoing/evoked pain in (or around) the area of the breast surgery?

Ongoing Evoked N/A Ongoing and evoked

2. Pulling a heavy door

Not relevant, I don't do this I can do this without any problem I can do this, but with difficulties because I can't do this

Post-Mastectomy Pain and Genetics Questionnaire

40.

1. Is the cause of your limitation(s) due to ongoing/evoked pain in (or around) the area of the breast surgery?

Ongoing Evoked N/A Ongoing and evoked

2. Bending over

Not relevant, I don't do this I can do without any problem I can do this, but with difficulties because I can't do this

41.

1. Is the cause of your limitation(s) due to ongoing/evoked pain in (or around) the area of the breast surgery?

Ongoing Evoked N/A Ongoing and evoked

2. Walking

Not relevant, I don't do this I can do this without any problem I can do this, but with difficulties because I can't do this

42.

1. Is the cause of your limitation(s) due to ongoing/evoked pain in (or around) the area of the breast surgery?

Ongoing Evoked N/A Ongoing and evoked

2. Exercising/ Sports activities

Not relevant, I don't do this I can do this without any problem I can do this, but with difficulties because I can't do this

43.

1. Is the cause of your limitation(s) due to ongoing/evoked pain in (or around) the area of the breast surgery?

Ongoing Evoked N/A Ongoing and evoked

2. Driving

Not relevant, I don't do this I can do this without any problem I can do this, but with difficulties because I can't do this

Post-Mastectomy Pain and Genetics Questionnaire

44.

1. Is the cause of your limitation(s) due to ongoing/evoked pain in (or around) the area of the breast surgery?

Ongoing Evoked N/A Ongoing and evoked

2. Self-Care (putting on a bra, washing hair, taking off a sweater)

Not relevant, I don't do this I can do this without any problem I can do this, but with difficulties because I can't do this

Post-Mastectomy Pain and Genetics Questionnaire

45.

1. Is the cause of your limitation(s) due to ongoing/evoked pain in (or around) the area of the breast surgery?

Ongoing Evoked N/A Ongoing and evoked

2. Having Sexual Intercourse

Not relevant I can do this without any I can do this, but with problems I can't do this limitations

Post-Mastectomy Pain and Genetics Questionnaire

46.

1. Is the cause of your limitation(s) due to ongoing/evoked pain in (or around) the area of the breast surgery?

Ongoing Evoked N/A Ongoing and evoked

Post-Mastectomy Pain and Genetics Questionnaire

47. Emotional Stability Form

Below is a list a of common human traits. Select a response that best describes how you see yourself at the PRESENT time, not as you wish to be in the future. Describe yourself as you are generally or typically, as compared with other persons you know of the same sex and of roughly the same age.

1. Anxious

Not at all accurate (0) A little accurate (1) Moderately accurate (2) Quite a bit accurate (3) Extremely accurate (4)

2. Touchy (sensitive)

Not at all accurate (0) A little accurate (1) Moderately accurate (2) Quite a bit accurate (3) Extremely accurate (4)

3. Nervous

Not at all accurate A little accurate (1)

Moderately accurate Quite a bit accurate Extremely accurate

(0) (2) (3) (4)

4. Tense

Not at all accurate A little accurate (1)
Moderately accurate Quite a bit accurate Extremely accurate

(0) (2) (3) (4)

5. Irritable

Not at all accurate A little accurate (1)
Moderately accurate Quite a bit accurate Extremely accurate

(0) (2) (3) (4)

6. Sad

Not at all accurate A little accurate (1)
Moderately accurate Quite a bit accurate Extremely accurate

(0) (2) (3) (4)

7. Happy

Not at all accurate A little accurate (1)
Moderately accurate Quite a bit accurate Extremely accurate

(0) (2) (3) (4)

8. Resentful

Not at all accurate A little accurate (1)
Moderately accurate Quite a bit accurate Extremely accurate

(0) (2) (3) (4)

9. Relaxed

Not at all accurate A little accurate (1)
Moderately accurate Quite a bit accurate Extremely accurate

(0) (2) (3) (4)

10. Depressed

Not at all accurate A little accurate (1)
Moderately accurate Quite a bit accurate Extremely accurate

(0) (2) (3) (4)

Post-Mastectomy Pain and Genetics Questionnaire

48. Emotional Distress - Anxiety Short form

Please respond to each item by selecting only one response. IN THE PAST 7 DAYS...

1. I felt fearful...

Never (1) Rarely (2) Sometimes (3) Often (4) Always (5)

2. I felt anxious...

Never (1) Rarely (2) Sometimes (3) Often (4) Always (5)

3. I felt worried...

Never (1) Rarely (2) Sometimes (3) Often (4) Always (5)

4. I found it hard to focus on anything other than my anxiety...

Never (1) Rarely (2) Sometimes (3) Often (4) Always (5)

5. I felt nervous...

Never (1) Rarely (2) Sometimes (3) Often (4) Always (5)

6. I felt uneasy...

Never (1) Rarely (2) Sometimes (3) Often (4) Always (5)

7. I felt tense...

Never (1) Rarely (2) Always (5)
Sometimes (3) Often (4)

Post-Mastectomy Pain and Genetics Questionnaire

49. Emotional Distress - Depression Short Form

1. I felt worthless...	Please respond to each item by selecting only one response. IN THE PAST 7 DAYS...			Often (4)	
Never (1)					
2	Rarely (2)			Often (4)	
Never (1)					
3. I felt helpless...	**. I felt that I had nothing to look forward to...**		Sometimes (3)	Often (4)	Always (5)
Never (1)					
4. I felt sad...	Rarely (2)		Sometimes (3)	Often (4)	Always (5)
Never (1)					
5. I felt like a failure...	Rarely (2)		Sometimes (3)	Often (4)	Always (5)
Never (1)					
6. I felt depressed...	Rarely (2)		Sometimes (3)	Often (4)	Always (5)
Never (1)					
7. I felt unhappy...	Rarely (2)		Sometimes (3)		Always (5)
Never (1)					
8. I felt hopeless...	Rarely (2)		Sometimes (3)		Always (5)

Never (1) Rarely (2) Always (5)
Sometimes (3) Often (4)

Post-Mastectomy Pain and Genetics Questionnaire

50. Sleep Disturbance - Short Form

Please respond to each item by selecting one response. IN THE PAST 7 DAYS...

1. My sleep was restless...

Not at all (1) A little bit (2) Somewhat (3) Quite a bit (4) Very much (5)

2. I had difficulty falling asleep...

Not at all (5) A little bit (4) Somewhat (3) Quite a bit (2) Very much (1)

3. I had trouble staying asleep...

Never (1) Rarely (2) Sometimes (3) Often (4) Always (5)

4. I had trouble sleeping...

Never (1) Rarely (2) Sometimes (3) Often (4) Always (5)

5. I was satisfied with my sleep...

Not at all (5) A little bit (4) Somewhat (3) Quite a bit (2) Always (1)

6. My sleep was refreshing...

Not at all (5) A little bit (4) Somewhat (3) Quite a bit (2) Very much (1)

7. I got enough sleep...

Never (5) Rarely (4) Sometimes (3) Often (2) Very much (1)

8. My sleep quality was...

Sometimes (3) Always (1)

Very poor (5)
Poor (4) Fair (3) Good (2) Very good (1)

Post-Mastectomy Pain and Genetics Questionnaire

51. Perceived Stress Scale (PSS)

The questions in this scale ask you about your feelings and thoughts during the last month. In each case, you will be asked to indicate HOW OFTEN you felt or thought a certain way. Although some of the questions are similar, there are differences between them and you should treat each one as a separate question. The best approach is to answer fairly quickly. That is, don't try to count up the number of times you felt a particular way; rather indicate the alternative that seems like a reasonable estimate.

Give the choice that best fits how you have been feeling in the PAST MONTH.

1. ...been upset because of something that happened unexpectedly?

Never (0) Almost never (1)
Sometimes (2) Fairly often (3) Often (4)

2. ...felt unable to control the important things in your life?

Never (0) Almost never (1)
Sometimes (2) Fairly often (3) Often (4)

3. ...felt nervous and "stressed"?

Never (0) Almost never (1)
Sometimes (2) Fairly often (3) Often (4)

4. ...felt confident about your ability to handle your personal problems?

Never (4) Almost never (3)
Sometimes (2) Fairly often (1) Often (0)

5. ...felt that things were going your way?

Never (4) Almost never (3)
Sometimes (2) Fairly often (1) Often (0)

6. ...found that you could not cope with all the things that you had to do?

Never (0) Almost never (1)
Sometimes (2) Fairly often (3) Often (4)

7. ...been able to control irritations in your life?

Never (4) Almost never (3)
Sometimes (2) Fairly often (1) Often (0)

8. ...felt that you were on top of things?

Never (0) Almost never (1)
Sometimes (2) Fairly often (3) Often (4)

9. ...been angered because of things that happened that were outside of your control?

Never (0) Almost never (1)
Sometimes (2) Fairly often (3) Often (4)

10. ...felt that difficulties were piling up so high that you could not overcome them?

Never (0) Almost never (1)
Sometimes (2) Fairly often (3) Often (4)

Post-Mastectomy Pain and Genetics Questionnaire

52. Concentration and Memory

We would like to know how your memory and energy have been affected since the surgery. Please indicate how you have been feeling during the LAST MONTH.

1. I have difficulty concentrating

Never To some degree Quite a bit Very much

2. I have more difficulty concentrating now than before my surgery

Never To some degree Quite a bit Very much

3. I feel that I don't have the energy to solve problems

Never To some degree Quite a bit Very much

4. I feel that I quickly get tired in my head since the surgery

Never To some degree Quite a bit Very much

Post-Mastectomy Pain and Genetics Questionnaire

53. Pain Catastrophizing Scale (PCS)

Everyone experiences painful situations at some point in their lives. Such experiences may include headaches, tooth pain, joint or muscle pain. People are often exposed to situations that may cause pain such as illness, injury, dental procedures, or surgery. WE ARE INTERESTED IN THE TYPES OF THOUGHTS AND FEELINGS THAT YOU HAVE WHEN YOU ARE IN PAIN. Listed below are 14 statements describing different thoughts and feelings that may be associated with pain. Using the following scale, please indicate the degree to which you have these thoughts and feelings when you are experiencing pain.

1. I worry all the time about whether it will end.

Not at all (0) To a slight degree To a moderate To a great degree All the time (4)

(1) degree (2) (3)

2. I feel I cannot go on.

Not at all (0) To a slight degree To a moderate To a great degree All the time (4)

(1) degree (2) (3)

3. It's terrible and I think it's never going to get any better.

Not at all (0) To a slight degree To a moderate To a great degree All the time (4)

(1) degree (2) (3)

4. It's awful and I feel that it overwhelms me.

Not at all (0) To a slight degree To a moderate To a great degree All the time (4)

(1) degree (2) (3)

5. I feel I can't stand it anymore.

Not at all (0) To a slight degree To a moderate To a great degree All the time (4)

(1) degree (2) (3)

6. I become afraid that the pain will get worse.

Not at all (0) To a slight degree To a moderate degree To a great degree All the time (4)

(1) (2) (3)

7. I keep thinking of other painful events.

Not at all (0) To a slight degree To a moderate degree To a great degree All the time (4)

(1) (2) (3)

8. I anxiously want the pain to go away.

Not at all (0) To a slight degree To a moderate degree To a great degree All the time (4)

(1) (2) (3)

Post-Mastectomy Pain and Genetics Questionnaire

9. I can't seem to get it out of my mind.

Not at all (0) To a slight degree To a moderate To a great degree All the time (4)

(1) (2) (3)

10. I keep thinking about how much it hurts.

Not at all (0) To a slight degree To a moderate To a great degree All the time (4)

(1) (2) (3)

11. I keep thinking about how badly I want the pain to stop.

Not at all (0) To a slight degree To a moderate To a great degree All the time (4)

(1) (2) (3)

12. There's nothing I can do to reduce the intensity of the pain.

Not at all (0) To a slight degree To a moderate To a great degree All the time (4)

(1) (2) (3)

13. I wonder whether something serious may happen.

Not at all (0) To a slight degree To a moderate To a great degree All the time

(1) (2) (3)

14. I feel my life isn't worth living. (4)

Not at all (0)	To a slight degree (1)	To a moderate degree (2)	To a great degree (3)	All the time (4)

Post-Mastectomy Pain and Genetics Questionnaire

54. Follow-Up Contact

Thank you very much for your time, Ms._____(Last Name)! I really appreciate your help with the study!

1. May we contact you again in the future?

Yes

No

BIBLIOGRAPHY

American Cancer Society.Breast Cancer Facts & Figures 2013-2014. Atlanta: American Cancer Society, Inc.2013.

Andersen KG, Kehlet H. Persistent pain after breast cancer treatment: a critical review of risk factors and strategies for prevention. J Pain. 2011;12:725–46

Alders M, Hogan BM, Gjini E, Salehi F, Al-Gazali L, et al. Mutations in CCBE1 cause generalized lymph vessel dysplasia in humans. Nat Genet. 2009;41:1272–1274.

Cella D, Riley W, Stone A, Rothrock N, Reeve B, Yount S, Amtmann D, Hays R, Buysse D, Choi S, Cook K, Devellis R, DeWalt D, Fries JF, Gershon R, Hahn EA, Lai JS, Pilkonis P, Revicki D, Rose M, Weinfurt K. PROMIS cooperative group.

Cella, D et al (2010). The Patient-Reported Outcomes Measurement Information System (PROMIS) developed and tested its first wave of adult self-reported health outcome item banks: 2005–2008. Journal of Clinical Epidemology, 63(11), 1179-1194. Retrieved from http://www.jclinepi.com/article/S0895-4356(10)00173-3/abstract?cc=yDworkin RH, Turk DC, Farrar JT, Haythornthwaite JA, Jensen MP, Katz NP. Core outcome measures for chronic pain clinical trials: IMMPACT recommendations. PAIN® 2005;113:9–19.

Cheville AL, Tchou J. Barriers to rehabilitation following surgery for primary breast cancer. J Surg Oncol. 2007;95:409–18

Chiu TW. Management of secondary lymphoedema. Hong Kong Medical Journals. 2014;20:1.

Ferrell et al., GJC2 Missense Mutations Cause Human Lymphedema, The American Journal of Human Genetics (2010), doi:10.1016/j.ajhg.2010.04.010

Ferrell, R. E., Finegold, D. N., & Levine, K. (n.d.). Lymphedema family study. Retrieved from University of Pittsburgh website: http://lymphedema.pitt.edu/

Ferrell RE, Levinson KL, Esman JH, Kimak MA, Lawrence EC, Barmada MM, Finegold DN (1998) Hereditary lymphedema: evidence for linkage and genetic heterogeneity. Hum Mol Genet 7:2073–2078.

Finegold D, Baty C, Knickelbein K, Perschke S, Noon S, et al. Connexin 47 mutations increase risk for secondary lymphedema following breast cancer treatment. Clin Cancer Res. 2012;18:2382–2890

Finegold DN, Schacht V, Kimak MA, Lawrence EC, Foeldi E, et al. (2008) HGF and MET mutations in primary and secondary lymphedema. Lymphat Res Biol 6: 65–68.

Freeman, S. (2008). Biological Science (3rd ed., pp. 996-998, 1108-1109). San Francisco, CA: Pearson Benjamin Cummings.

Gartner R, Jensen MB, Nielsen J, Ewertz M, Kroman N, Kehlet H. Prevalence of and factors associated with persistent pain following breast cancer surgery. JAMA. 2009;302:1985–92.

Hayes SC, Janda M, Cornish B, Battistutta D, Newman B. Lymphedema after breast cancer: incidence, risk factors. And effect on upper body function. J Clin Oncol 2008; 26:3536-42.

Irrthum A, Devriendt K, Chitayat D, Matthijs G, Glade C, et al. Mutations in the transcription factor gene SOX18 underlie recessive and dominant forms of hypotrichosis-lymphedema-telangiectasia. Am J Hum Genet. 2003;72:1470–1478.

Kuzma, J., & Bohnenblust, S. (2005). Basic Statistics for the Health Sciences (5th ed., pp. 244-271). New York, New York: McGraw-Hill.

Liljegren G, Holmberg L: Arm morbidity after sector resection and axillary dissection with or without postoperative radiotherapy in breast cancer stage I. Results from a randomized trial. Uppsala-Orebro Breast Cancer Study Group. Eur J Cancer 33 (2): 193-9, 1997.

Lymph Node Removal for Invasive Breast Cancer. (2015, March 5). Retrieved March 10, 2015, from http://www.breastcancer.org/treatment/surgery/lymph_node_removalLymphedema. (2014, October 1). Retrieved March 11, 2015, from http://www.mayoclinic.org/diseases-conditions/lymphedema/basics/definition/con-20025603

Lymphedema (PDQ): Health professional version. National Cancer Institute. http://www.cancer.gov/cancertopics/pdq/supportivecare/lymphedema/healthprofessional/AllPages/Print. Accessed Sept. 16, 2014

Maunsell E, Brisson J, Deschênes L. Arm problems and psychological distress after surgery for breast cancer. Can J Surg. 1993;36:315–20.

Melzack R. The short-form McGill Pain Questionnaire. PAIN® 1987;30:191–7.

Merlijn J. Meens, Amélie Sabine, Tatiana V. Petrova, Brenda R. Kwak, Connexins in lymphatic vessel physiology and disease, FEBS Letters, Volume 588, Issue 8, 17 April 2014, Pages 1271-1277, ISSN 0014-5793, http://dx.doi.org/10.1016/j.febslet.2014.01.011.

Mizuno S., Nakamura T. Hgf-met cascade, a key target for inhibiting cancer metastasis: The impact of nk4 discovery on cancer biology and therapeutics. Int. J. Mol. Sci. 2013;14:888–919. doi: 10.3390/ijms14010888.

Mohler ER, et al. Clinical manifestations and diagnosis of lymphedema. http://www.uptodate.com/home. Accessed Sept. 16, 2014.

NCBI [GJC2 gap junction protein, gamma 2, 47kDa [**Homo sapiens** (human)] Bethesda (MD): National Center for Biotechnology Information (US); 2005-. Updated 2015 April 5. Available from: http://www.ncbi.nlm.nih.gov/gene/57165

Onyike CU, Crum RM, Lee HB, Lyketsos CG, Eaton WW. Is Obesity Associated with Major Depression? Results from the Third National, Health and Nutrition Examination Survey. American Journal of Epidemiology. 2003;158(12):1139–1147.

Paskett ED, Naughton MJ, McCoy TP, et al.: The epidemiology of arm and hand swelling in premenopausal breast cancer survivors. Cancer Epidemiol Biomarkers Prev 16 (4): 775-82, 2007.

Peschard P, Park M. From Tpr-Met to Met tumorigenesis and tubes. Oncogene. 2007;26:1276–1285.

QuickStats: Prevalence of Current Depression* Among Persons Aged ≥12 Years, by Age Group and Sex — United States, National Health and Nutrition Examination Survey, 2007–2010. (2012, January 6). Retrieved March 15, 2015, from http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6051a7.htm

Ridner SH, Dietrich MS, Stewart BR, et al.: Body mass index and breast cancer treatment-related lymphedema. Support Care Cancer 19 (6): 853-7, 2011.

Schreiber KL, Martel MO, Shnol H, et al. Persistent pain in postmastectomy patients: comparison of psychophysical, medical, surgical, and psychosocial characteristics between patients with and without pain. Pain. 2012Selected patient and provider characteristics for ambulatory care visits to physician offices and hospital outpatient and emergency departments: United States, 2009-2010

Sherwood, L. (2012). Human physiology: From cells to systems (8th ed.). Pacific Grove, Calif.: Cengage Learning.

Shigaki CL, Madsen R, Wanchai A, Stewart BR, Armer JM. (2013) Upper extremity lymphedema: presence and effect on functioning five years after breast cancer treatment. Rehabilitation Psycholchology 58(4):342-9.

Smeltzer DM, Stickler GB, Schirger A. Primary lymphedema in children and adolescents: a follow-up study and review. Pediatrics 76(2), 206-218 (1985).

Traboulsi EI, Al-Khayer K, Matsumoto M, Kimak MA, Crowe S, Wilson SE, Finegold DN, Ferrell RE, Meisler DM. (2002) Lymphedema-distichiasis syndrome and FOXC2 gene mutation. Am J Opthalmol 134(4)592-6

Van Damme S, Crombez G, Bijttebier P, Goubert L, Van Houdenhove B. A confirmatory factor analysis of the Pain Catastrophizing Scale: invariant factor structure across clinical and non-clinical populations. PAIN® 2002;96:319–24.