Musculoskeletal Symptoms with Endocrine Therapy for Breast Cancer: Trajectory and Predictors

by

Yehui Zhu

BSN, Sun Yat-sen University, 2010

MSN, Fudan University, 2013

Submitted to the Graduate Faculty of the

School of Nursing in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2019

UNIVERSITY OF PITTSBURGH

SCHOOL OF NURSING

This dissertation was presented

by

Yehui Zhu

It was defended on

December 13, 2019

and approved by

Yvette P. Conley, PhD, FAAN, Professor, School of Nursing

Susan M. Sereika, PhD, Professor, School of Nursing

Margaret Q. Rosenzweig, PhD, CRNP-C, AOCNP, Professor, School of Nursing

Michael S. Gold, PhD, Professor, School of Medicine

Dissertation Director: Catherine M. Bender, PhD, RN, FAAN, Professor, School of Nursing

Musculoskeletal Symptoms with Endocrine Therapy for Breast Cancer: Trajectory and Predictors

Yehui Zhu, PhD, MSN, BSN

University of Pittsburgh, 2019

Musculoskeletal symptoms (including arthralgias, myalgia, and muscle stiffness) are experienced by up to 85% of women undergoing aromatase inhibitor (AI) therapy for breast cancer, and are the number one contributor to the high treatment discontinuation rate. The purpose of this dissertation study was to examine the trajectories of musculoskeletal symptoms and related factors during the first 18 months of AI therapy for breast cancer. This is an ancillary study to a parent study, the Anastrozole Use in Menopausal Women (AIM). The AIM study provided data on pain, musculoskeletal symptoms, and candidate phenotypic factors for two cohorts of women (n=380) (cohort 1: women with early stage breast cancer who receive anastrozole; cohort 2: age- and education-matched women without cancer) at baseline (before initiation of AI therapy for breast cancer cohort), 6, 12, and 18 months after baseline. Based on the bio-banked DNA provided by a subgroup of participants (n=243), we genotyped 46 single nucleotide polymorphisms (SNPs) among the 25 candidate genes which were selected from a biological pathway analysis. Our results showed that a significant proportion of women experienced mild or moderate pain and musculoskeletal symptoms in a persistent or linearly increasing manner over the first 18 months of AI therapy. A profile of protective and risk factors across one or more phenotypes was identified. The protective phenotypic factors included older age, receipt of chemotherapy, older first menstrual period age, married/partnered, having an administrative level of occupation (vs unskilled/unemployed), having regular periods for most of one's life, greater numbers of pregnancies, and having a history of tubal ligation. The phenotypic risk factors included receipt of

AI therapy, greater anxiety, pain severity, depressive symptoms, fatigue at baseline, and having a history of arthritis, hysterectomy, or menopausal symptoms. A profile of protective and risk polymorphisms was identified. Variations in *CYP19A1* (rs1008805) and *NOS3* (rs1799983) were associated across phenotypes. The protective polymorphisms included *BDNF* rs6265, *COMT* rs4633 and rs887200, *CXCL8* rs4073, *ESR2* rs2772163, *IL1B* rs16944, *RANKL* rs1054016, *VDR* rs4516035 and rs731236. The risk polymorphisms included *CYP19A1* rs1008805, *CYP3A4* rs35599367, *COMT* rs165774, *NOS3* rs1799983, *OPG* rs2073618, *OPRM1* rs1799971, and *TCL1A* rs7158782 and rs7159713.

Table of Contents

List of Tables

List of Figures

Preface

Yehui would like to acknowledge her dissertation chair, committee members, family, peers, and the following support for her dissertation project: Musculoskeletal Pain Among Breast Cancer Survivors: Through Bio-behavioral and Imaging Lenses (F99CA234782), American Cancer Society Doctoral Degree Scholarship in Cancer Nursing (DSCN-17-073-01), Sigma Theta Tau International Eta Chapter Research Award, Margaret E. Wilkes Scholarship Fund Award, Newmeyer-Thompson Doctoral Student Research Award.

1.0 PROPOSAL INTRODUCTION AND SPECIFIC AIMS

Breast cancer is the most common cancer diagnosed in women in the United States. The National Comprehensive Cancer Network (NCCN) Clinical Practice Guideline for Breast Cancer recommends that women with hormone receptor positive disease, who are postmenopausal at diagnosis, receive 5-10 years of endocrine therapy with an aromatase inhibitor (AI). While AI therapy clearly improves the overall and disease-free survival in postmenopausal women with breast cancer, this treatment is associated with multiple symptoms that may have a detrimental impact on medication adherence and quality of life. Musculoskeletal symptoms including arthralgias, myalgia, and muscle stiffness, are the most commonly reported symptoms, effecting 3.6-89% of women receiving AI therapy. However, most clinical studies of musculoskeletal symptoms have only followed women with breast cancer through less than the first year of AI therapy; thus the trajectory of musculoskeletal symptoms after the first year is not known. In addition, little is known about the inter-individual variability of musculoskeletal symptoms and its associated phenotypic factors (demographic-, disease-, and individual-related characteristics) during AI therapy for breast cancer.

Moreover, the mechanisms underlying musculoskeletal symptoms during AI therapy are not completely understood. In the past decade, the etiology of musculoskeletal symptoms during AI therapy has been explored from perspectives of estrogen deprivation, pharmacogenetics of AI metabolism, and vitamin D deficiency. The genotypic factors associated with the inter-individual variability in musculoskeletal symptoms during AI therapy need to be further explored and examined. In addition, since musculoskeletal symptoms are widely prevalent among post-menopausal women due to menopausal status and comorbidities (e.g., osteoporosis, arthritis,

fibromyalgia etc.), there is a need to broaden the scope of investigation to explore the phenotypic and genotypic factors associated with potential mechanisms and to better understand the inter-individual variability of musculoskeletal symptoms during AI therapy.

The purpose of this dissertation study was to gain a greater understanding of trajectories of musculoskeletal symptoms associated with the first 18 months of anastrozole (the mostly used aromatase inhibitor) therapy among postmenopausal women with breast cancer. Phenotypic factors associated with the distinct trajectories were determined. The influence of genotypic factors on the distinct trajectories were explored.

The specific aims of the dissertation study were to:

Aim 1: Identify distinct latent classes of 18-month trajectories of musculoskeletal symptoms for two cohorts of postmenopausal women (cohort 1: women with early stage breast cancer who receive anastrozole; cohort 2: age- and education-matched women without cancer).

Aim 2: Determine phenotypic factors (demographic-, disease-, and individual-related characteristics) associated with the membership for the distinct latent classes of trajectories for musculoskeletal symptoms.

Aim 3: Explore the genotypic factors (DNA variation in genes related to estrogen biosynthesis and musculoskeletal pain) associated with the distinct latent classes of trajectories for musculoskeletal symptoms.

This dissertation study was an ancillary study to the Anastrozole Use in Menopausal Women (AIM) study (R01CA107408, PI: Dr. Catherine Bender). The AIM study was a prospective cohort study with repeated assessments at pre- and 6, 12, and 18 months post initiation of adjuvant therapy. The primary purpose of the AIM study was to examine and compare the effect of anastrozole on cognitive function among four groups of postmenopausal women: women with

early stage breast cancer who receive chemotherapy plus anastrozole (ChemoAnast), anastrozole alone (AnastAlone), chemotherapy only (ChemoOnly), and women without breast cancer who were matched on age, and years of education to the breast cancer cohorts. Data on sociodemographic status and clinical characteristics related to participants' breast cancer were collected at the baseline assessment. Assessments of pain, mood (depression and anxiety), fatigue, and other symptoms commonly experienced by women receiving endocrine therapy were performed at each time point. DNA samples were extracted from blood or saliva and banked for a subset of participants.

The dissertation study focused on three of the AIM study cohorts: ChemoAnast, AnastAlone, and healthy control. Data on musculoskeletal symptoms from the Brief Pain Inventory and Breast Cancer Prevention Trial Symptom Checklist at baseline (before initiation of AI therapy for the breast cancer cohorts) and 6, 12, and 18 months post baseline were analyzed to investigate the trajectory patterns for pain and musculoskeletal symptoms. Baseline demographic-, disease-, and individual characteristics, mood and fatigue were used to determine their associations with the membership for the distinct latent classes of trajectories for musculoskeletal symptoms. New genotype data of selected candidate genes were generated using the bio-banked DNA samples. The association between the DNA variation and the distinct latent classes of trajectories for musculoskeletal symptoms were explored.

1.1 VARIABLE FRAMEWORK

The framework of this dissertation study was adapted from the Symptom Experience Model (SEM), developed by Armstrong (Armstrong, 2003) and the NIH Symptom Science Model

(Cashion et al., 2015). In the SEM, symptom experience is a multi-dimensional concept defined as the "perception of the frequency, intensity, distress, and meaning of symptoms as they are produced and expressed" (Armstrong, 2003, P.602). Symptoms can be associated with demographic characteristics (e.g., age, gender, marital status, race, culture, role, education, and socioeconomic status), disease characteristics (e.g., type and state, type of treatment, and comorbid medical and clinical factors), and individual characteristics (e.g., health knowledge, values, past experience, etc.). The NIH Symptom Science Model is used to organize and guide biobehavioral symptom management. It starts with identifying a complex symptom, followed by phenotypic characterization, biomarker discovery, and clinical application (Cashion et al., 2015).

In this dissertation study, the musculoskeletal symptom experience was conceptualized as the perception of the occurrence, intensity, distress, and location occurring as symptoms are produced and expressed. Musculoskeletal symptoms included joint pain (arthralgia), muscle pain (myalgia), and muscle stiffness. Phenotypic factors associated with musculoskeletal symptoms include demographic-, disease-, individual-related characteristics adapted from the SEM. Genotypic factors (DNA variance in candidate genes) associated with musculoskeletal symptoms were added by adapting from the NIH Symptom Science Model and were explored as the basis for better understanding the inter-individual variability of musculoskeletal symptoms (Figure 1).

1.2 INNOVATION

This dissertation study is innovative in a number of ways.

- This study is a pioneering work because it is the first study to examine the inter-individual variability of musculoskeletal symptoms during AI therapy for breast cancer by identifying distinct latent classes (i.e., subgroup) of trajectories.
- This study is one of the first clinical studies to extend the description of pain and musculoskeletal symptoms through 18 months of AI therapy for breast cancer.
- This study explores genotypic factors associated with trajectory patterns for musculoskeletal symptoms, which has potential to expand the understanding of the mechanisms underlying musculoskeletal symptoms during AI therapy.

1.3 BACKGROUND AND SIGNIFICANCE

Breast cancer is the most commonly diagnosed cancer and the second leading cancer mortality among women worldwide (Torre et al., 2012). It is estimated that there will be 252,710 new breast cancer diagnoses and 40,610 deaths in 2017 in the United States (Siegel, Miller, & Jemal, 2017). Approximately 80% of post-menopausal women with breast cancer have hormone receptor (estrogen and/or progesterone receptor) positive disease (Osborne CK, 1998), with circulating estrogen levels influencing tumor growth and recurrence. With the application of third-generation aromatase inhibitors (AIs), including anastrozole, letrozole, and exemestane, the disease-free and overall survival rates have been significantly improved for post-menopausal women with breast cancer (Goss et al., 2016; Romera et al., 2011). In 1995, oral anastrozole, at a dose of one milligram daily, was initially approved by the United States Federal Drug Administration (FDA) (Drugs@FDA, 1995) for adjuvant therapy in postmenopausal women with early stage, hormone receptor-positive breast cancer and as first-line treatment of postmenopausal

women with hormone receptor-positive or hormone receptor unknown locally advanced or metastatic breast cancer. The National Comprehensive Cancer Network (NCCN) Clinical Practice Guideline for Breast Cancer recommends that women with hormone receptor positive disease, who are postmenopausal at diagnosis, receive at least 5 years of endocrine therapy with an aromatase inhibitor (AI) (NCCN, 2017). Furthermore, evidence shows that extension of adjuvant AI therapy to 10 years significantly improves disease-free survival and lowered contralateral breast cancer incidence (Goss et al., 2016). Therefore, 5-10 years of AI therapy is a vital component of systematic adjuvant treatment for postmenopausal women with hormone receptor positive breast cancer.

Musculoskeletal-related adverse events and symptoms were reported in early clinical trials of AI drugs. In the randomized phase III trials of AI, the incidence of musculoskeletal adverse events (e.g., fracture, osteoporosis, and osteopenia) and symptoms (e.g., arthralgias, myalgia, stiffness, etc.) were significantly higher compared to women who received the selective estrogen receptor modifier (SERM), tamoxifen or placebo. The occurrences of osteoporosis in the AI groups were 7.3% -11.0% and of fractures were 0.8% - 5.3% (Arimidex, 2006, 2008; Jakesz et al., 2005, 2007; Coates et al., 2007; Coombes et al., 2007; Goss et al., 2005). When extending AI adjuvant therapy to 10 years in the MA.17R trial, women who received 10 years of AI showed greater numbers of bone fractures and new-onset osteoporosis compared to women who received 5 years of AI plus 5 years of placebo (Goss et al., 2016). In terms of musculoskeletal symptoms, the occurrence of arthralgias, myalgia, and bone pain were reported 1% - 35.6%, 7.1% - 15%, and 5% - 19%, respectively (Arimidex, 2006; Boccardo et al., 2006; Coates et al., 2007; Coombes et al., 2007; Howell et al., 2005; Jakesz et al., 2005, 2007).

However, with wider clinical use of AI therapy, accumulating evidence suggests that symptoms associated with endocrine therapy were underestimated in the clinical trials perhaps due to the focus on efficacy and safety. Ruhstaller et al. reported that hot flashes/sweats (70% vs 38-40 in clinical trials), low energy (45% vs 9-15% in clinical trials), fluid retention (22% vs 7% in clinical trials), and vaginal dryness (30% vs. 3% in clinical trials) were significantly underrated in clinical trials of endocrine therapy (Ruhstaller et al., 2009). Moreover, the symptoms associated with AI treatment may have a detrimental impact on women's ability to adhere to therapy, as well as their functional status and quality of life (Aiello Bowles et al., 2012; Kidwell et al., 2014; Olufade, Gallicchio, MacDonald, & Helzlsouer, 2015). Significantly, 5% - 10% of AI users in clinical trials and 25% in clinical settings prematurely discontinued treatment due to musculoskeletal symptoms (Henry et al., 2012). Between 13% and 50% of the discontinuation of AI therapy is due to AI associated arthralgias (Crew et al., 2007; Dizdar et al., 2009; Henry et al., 2008). Therefore, it is important to gain a better understanding of these musculoskeletal symptoms during AI therapy in clinical settings.

1.3.1 Phenotype and phenotypic factors associated with musculoskeletal symptoms during AI therapy

A literature review on the occurrence, intensity, and distress of musculoskeletal symptoms during AI therapy among women with breast cancer was conducted. Studies published through February 2017 were searched using combination of key terms (Table 1) in MEDLINE®, PubMed, and CINAHL®.

Musculoskeletal symptoms have been shown to be a cluster of highly prevalent symptoms with moderate to severe intensity associated with aromatase inhibitor therapy for breast cancer.

Thirty-one studies reported musculoskeletal symptoms, including arthralgias (pain in joints), myalgia (pain in muscle), and muscle stiffness (Table 2) (Aiello Bowles et al., 2012; Boonstra et al., 2013; Brown et al., 2014; Chim et al., 2013; Crew et al., 2007; Dizdar et al., 2009; Egawa et al., 2016; Gallicchio et al., 2012; Garreau et al., 2006; Hadji et al., 2014; Horimoto et al., 2009; Hu et al., 2016; Kyvernitakis et al., 2014; Laroche et al., 2014; Lintermans et al., 2014; Lu et al., 2011; Mao et al., 2009, 2011; Napoli et al., 2010; Oberguggenberger et al., 2011; Ohsako et al, 2006; Olufade et al., 2015; Presant et al., 2007; Sagara et al., 2010; Servitja et al., 2012; Shi et al., 2013; Singer et al., 2012; Swenson et al., 2013; Waltman et al., 2009; Wang et al., 2013). The occurrence of musculoskeletal symptoms ranged widely from 3.6% to 89%; arthralgias and myalgia were the most prevalent symptoms with occurrences ranging from 3.6% to 89%. Between 25% and 72% of AI users reported joint/muscle stiffness (Table 3). Menas et al. and Mao et al. reported that 32% to 82% of arthralgias began in the first 6 months after initiation of AI treatment. An average of 8 joints were affected with arthralgias; the hands, knees, and wrists were the most common joints involved. Other joints that were reported to be affected included the shoulder, spine, fingers, elbows, and feet. Thirty-three percent of AI users experienced arthralgias all day (Mao et al., 2009; Menas et al., 2012). For those who experienced arthralgias and myalgia, 31.5%-46% reported moderate to severe intensity (Chim et al., 2013; Crew et al., 2007; Mao et al., 2009; Presant et al., 2007). The average intensity of arthralgias was moderate to severe and ranged from 4.9 to 7.5 out of 10 (as extreme severity) (Boonstra et al., 2013; Shi et al., 2013; Servitja et al., 2012; Present et al., 2007). The onset severity of arthralgia was 5.23 on a 10-point scale (Shi et al., 2013). Swenson et al. reported a trend toward increasing intensity of musculoskeletal symptoms during the first 6 months after initiation of AI treatment (Swenson et al., 2013). In terms of distress of musculoskeletal symptoms, 48% - 64.3% of AI users reported disturbances with daily activities

(Table 3) (Egawa et al., 2016; Waltman et al., 2009). However, the phenotype of musculoskeletal symptoms during AI therapy is not fully understood. Firstly, although the occurrence and severity of musculoskeletal symptoms are well-documented, there is a lack of research on the degree of distress and meaning associated with musculoskeletal symptoms. According to the Symptom Experience Model (Armstrong, 2003), symptom experience is a multi-dimensional concept with four domains: frequency (occurrence is a dichotomous concept of frequency), intensity (or severity), distress (or interference), and meaning. To date, only four studies (Egawa et al., 2016; Shi et al., 2013; Swenson et al., 2013; Waltman et al., 2009) investigated the distress associated with musculoskeletal symptoms; none of the studies examined the meaning of musculoskeletal symptoms S. However, the domains of distress and meaning of musculoskeletal symptoms may significantly influence adherence to therapy, treatment outcomes (e.g., quality of life) of breast cancer survivors and their ability to cope with musculoskeletal symptoms during AI therapy.

Furthermore, the trajectory of musculoskeletal symptoms during the course of AI therapy is not fully described. To date, most of the studies focusing on musculoskeletal symptoms adopted a cross-sectional design. And the follow-up period of the few longitudinal studies completed to date was no more than 12 months (from pre-therapy). Thus, there is a need to extend follow-up period beyond the first year of therapy to better describe the trajectories of musculoskeletal symptoms.

In addition, it is not clear whether there are subgroups of women who experience more severe musculoskeletal symptoms or who experience greater distress. Among breast cancer survivors, inter-individual variability in the experience of several common symptoms during surgery and adjuvant therapies have been reported, including the trajectories of fear of recurrence (Dunn et al., 2015), depressive symptoms after surgery (Dunn et al., 2011), pain after

chemotherapy (Langford et al., 2016), weight changes during chemotherapy (Liu et al., 2014), anxiety pre- and post-surgery (Kyranou et al., 2014), and fatigue during and after radiation therapy (Dhruva et al., 2010). The wide range of musculoskeletal symptoms prevalence (3.6% - 89%) suggests that there could be variability in the experience of these symptoms as well. However, no studies to date have examined variability in musculoskeletal symptoms during AI therapy to determine whether there are subgroups of women who are vulnerable to greater severity or distress of musculoskeletal symptoms.

The phenotypic factors associated with musculoskeletal symptoms during AI therapy are summarized in Table 4. Women with longer menopause duration, more severe breast symptoms, more joint-related comorbidity, presence of pain at pre-therapy, and vitamin D insufficiency were more likely to experience arthralgias (Castel et al., 2013; Crew et al.,2007; Laroche et al., 2014; Mao et al., 2011a; Shi et al., 2013; Waltman et al., 2009; Wang et al., 2013b). However, whether BMI, age and prior chemotherapy predicted arthralgias is not clear due to conflicting evidence (Castel et al., 2013; Crew et al., 2007; Mao et al., 2011a; Menas et al., 2012; Shi et al., 2013; Wang et al., 2013). The basis for some of this inconsistency may be due to methodological limitations (e.g., small samples, cross-sectional designs, heterogeneous measurement instrumentations, etc.) and diverse analytic methods used across studies (e.g., mean comparison, logistic regression, ANOVA, etc.) Additional longitudinal study is needed to confirm the phenotypic characteristics that are associated with musculoskeletal symptoms during AI therapy for breast cancer.

1.3.2 Hypothesized molecular basics of musculoskeletal symptoms during AI therapy

In the era of precision medicine, with the need for personalized symptom prediction and management, it is of utmost importance to explore the mechanisms underlying symptoms.

However, the underlying mechanism of musculoskeletal symptoms during AI therapy is largely unknown (Borrie & Kim, 2017). The molecular basis of musculoskeletal symptoms during AI therapy was hypothesized mainly from the perspectives of estrogen deprivation. Vitamin D deficiency, and pharmacogenetics of AI metabolism were also studied to uncover the inter-patient variability of musculoskeletal symptoms during AI therapy. Moreover, since musculoskeletal symptoms are widely experienced among post-menopausal women (hormone decline) and comorbidities (e.g., osteoporosis, arthritis, and fibromyalgia), there is a need to broaden the scope to explore the genetic factors associated with comorbidity-related musculoskeletal symptoms to better understand the individual variance in musculoskeletal symptoms during AI therapy.

1.3.2.1 Mechanism of action of the aromatase inhibitors

Estrone and estradiol are two major types of estrogen present in postmenopausal women. Aromatase is an enzyme transcribed from the *CYP19A1* gene that catalyzes estrogen biosynthesis through the conversion of testosterone to estradiol and androstenedione to estrone. In pre-menopausal women, estrogen is produced in the ovaries and adipose tissue. Among post-menopausal women, adipose tissue is the major source of estrogen synthesis (Simpson, 2003). Aromatase is expressed across multiple human tissues including the ovaries, testes, adipose tissue, brain, muscle, skin fibroblasts, and osteoblasts of bone (Czajka-Oraniec & Simpson, 2010). AIs block the activity of aromatase by reversibly binding (for letrozole and anastrozole) or irreversibly binding (for exemestane) to the enzyme. Letrozole and anastrozole bind to the AI substrate-binding site and prevent binding of androgens, thus limiting the catalytic conversion of androgens to estrogen. Exemestane binds irreversibly to the AI active site to inactivate the enzyme in a process commonly referred to as ‘suicide inhibition’ (Chumsri et al., 2011). Letrozole (2.5mg/day), anastrozole (1mg/day), and exemestane (25mg/day) inhibit estrogen biosynthesis by the rates of

99%, 97%, and 98%, respectively (Fabian, 2007). Use of AIs reduce estrogen synthesis and lower the circulating estrogens below the detectable level of most clinical assays (Geisler et al., 2002), and thereby reducing estrogen-dependent cellular proliferation (Campos, 2004).

1.3.2.2 Estrogen deprivation

Since 1925, estrogen decline has been reported to link to arthralgias among women without cancer (Cecil & Archer, 1925). Ho et al. reported that sudden estrogen decline can trigger arthralgias (Ho et al., 1999). The occurrence of increased joint pain and stiffness in peri-menopausal women (41%) was significantly higher than the occurrences among pre- and post-menopausal women (25% and 29%, respectively). Moreover, results from Women's Health Initiative (WHI) showed that hormonal replacement therapy (HRT) alleviated arthralgia and improved joint health among postmenopausal women (Cirillo et al., 2006). In addition, lower levels of estrogen were reported to be associated with arthralgias among women with breast cancer receiving AI therapy. Wang et al. found that women with breast cancer suffering from AI-related arthralgias had significantly lower levels of estradiol compared to those without arthralgias (Wang et al., 2015). With the accumulating evidence suggesting that arthralgias are associated with estrogen decline and lower levels of circulating estrogen, estrogen deprivation is the main hypothesis of the molecular basis of musculoskeletal symptoms during AI therapy.

However, there are conflicting results regarding the polymorphism of genes related in estrogen biosynthesis for musculoskeletal symptoms during AI therapy among women with breast cancer. For example, Park et al. reported a strong association between arthralgias and haplotype M_3_5 within *CYP19A1*, which contains 14 SNPs (Park et al., 2011). Lintermans et al. and Garcia-Giralt et al. failed to replicate this association with several SNPs from the M_3_5 haplotype

(Garcia-Giralt et al., 2013; Lintermans et al., 2016). More studies are needed to confirm the robustness of the identified associations.

1.3.2.3 Variation of AI metabolism

In clinical practice, switching from one AI to another is one of the methods used to manage intolerant musculoskeletal symptoms (e.g., discontinuation of AI therapy due to severe arthralgias), since research has showed that 71.5% of patients may continue AI therapy by switching from anastrozole to letrozole (Briot et al., 2010). Moreover, although the dosage of AIs is fixed (2.5mg/day for Letrozole, 1mg/day for anastrozole, and 25mg/day for exemestane), the drug plasma concentration of AIs has 10-12 fold' variation (Desta et al., 2011; Lazarus et al., 2010; Jeong et al., 2009; Kamdem et al, 2010, 2011). Therefore, variation in AI metabolism was hypothesized to be potentially associated with individual variability of musculoskeletal symptoms during AI therapy (Borrie & Kim, 2017; Gervasini et al., 2017). Genotype of five genes (*CYP2A6*, *CPY3A4*, *CYP3A5*, *CBCA1* and *UGT2B17*) has been reported to be potentially associated with plasma concentration or metabolism of AIs. (Desta et al., 2011; Wang, et al, 2016; Lamba et al., 2012; Sun, et al., 2010; Gervasini et al., 2017).

1.3.2.4 Vitamin D deficiency

Vitamin D deficiency has been linked to chronic pain. Increasing evidence suggests that pain pathways associated with cortical, immunological, hormonal, and neuronal changes are potentially influenced by Vitamin D levels (Shipton & Shipton, 2015). Furthermore, vitamin D deficiency has been associated with the occurrence and intensity of musculoskeletal symptoms during AI therapy. AI users who developed musculoskeletal symptoms were more likely to have vitamin D deficiency at pre-therapy (Singer et al. 2014; Waltman et al. 2009). Servitja et al

reported that lower vitamin D levels were significantly associated with worse intensity of musculoskeletal symptoms during AI therapy (Servitja et al., 2015). In addition, a phase II randomized controlled trial showed that daily supplementation with high dose vitamin D2 (50,000 IU) for 8-16 weeks significantly alleviated pain severity and interference for women with breast cancer who developed musculoskeletal symptoms during AI therapy (Rastelli et al., 2011). However, the effect of vitamin D supplementation on musculoskeletal symptoms during AI therapy was not confirmed by another randomized controlled trial of 4,000IU vitamin D3 supplement (Shapiro et al., 2016). Niravath et al reported that polymorphisms of the *VDR* (vitamin D receptor) may be associated with the occurrence of aromatase inhibitor associated arthralgias (Niravath et al., 2017).

1.4 PRELIMINARY STUDIES

1.4.1 Preliminary Study #1: A scoping review on symptoms with endocrine therapy for breast cancer

The purpose of this scoping review was to map the symptoms during endocrine therapy for breast cancer to provide implications for current practice and suggestions for future research. PubMed, CINAHL®, and China Science Periodical Databases (CSPD) were searched to identify related studies published in English and Chinese language. Of the 2,551 articles identified, 57 articles met inclusion criteria and were included in this scoping review (Zhu et al., 2019; Appendix C).

Main Results and Conclusions: Evidence for the 16 most studied symptoms and 15 most prevalent symptoms were synthesized. Five key symptoms associated with endocrine therapy were identified, including joint/muscle pain, hot flashes, low sexual interest/desire, joint/muscle stiffness, and fatigue/lack of energy. Future studies should focus on the domains of symptom intensity and distress, specific understudied symptoms, symptom clusters, and development of symptom assessment instruments specific to symptoms associated with endocrine therapy.

1.4.2 Preliminary study #2: A literature review with biological pathway analysis on genes associated with musculoskeletal pain (MSKP) during treatment with aromatase inhibitors for breast cancer

The goals of this literature review with biological pathway analysis were to 1) gain understanding of the genetic variation and biological mechanisms underlying MSKP with AI therapy, and 2) identify plausible biological pathways and candidate genes for future investigation. Genes associated with MSKP during AI therapy or genes involved in drug metabolism and drug response of AIs were identified from literature. Studies published through February 2019 were queried in PubMed®. The genes identified from the literature were entered into the QIAGEN's Ingenuity® Pathway Analysis (IPA) software to generate canonical pathways, upstream regulators, and networks through a core analysis (Zhu et al., 2019; Appendix D).

Main Results and Conclusions: Multiple genes and molecular-level etiologies may contribute to MSKP with AI therapy in women with breast cancer. Seventeen genes were identified, including *ABCB1, ABCG1, CYP17A1, CYP19A1, CYP27B1, CYP2A6, CYP3A4, CYP3A5, ESR1, OATP1B1, OPG, RANKL, SLCO3A1, TCL1A, UGT2A1, UGT2B17*, and *VDR*. These genes are involved in encoding bone remodeling regulators, drug metabolizing enzymes

(cytochrome P450 family, UGT family) or drug transporters (ABC transporters, OAT transporters). Multiple plausible biological pathways (e.g., nicotine degradation, melatonin degradation) and candidate genes (e.g., *NFKB, HSP90, AKT, ERK1/2, FOXA2*) were proposed for future investigation based on the IPA results.

1.5 DESIGN AND METHODOLOGY

1.5.1 Study design

This dissertation study was an ancillary study to the Anastrozole Use in Menopausal Women (AIM) study (R01CA107408, PI: Dr. Catherine Bender). The AIM study was a prospective cohort study with repeated assessments at pre- and 6, 12, and 18 months post initiation of adjuvant therapy. The primary aim of the AIM study was to examine and compare the effect of anastrozole on cognitive function among four groups of postmenopausal women: women with early stage breast cancer who receive chemotherapy plus anastrozole (ChemoAnast), anastrozole alone (AnastAlone), chemotherapy only (ChemoOnly), and women without breast cancer who were matched on age, and years of education to the breast cancer cohorts. Data on sociodemographic status and clinical characteristics related to participants' breast cancer were collected at the baseline assessment. Assessments of pain, mood (depression and anxiety), fatigue, and symptoms commonly experienced by women receiving endocrine therapy were performed at each time point. DNA samples were extracted from blood or saliva and banked for a subset of participants.

The dissertation study focused on three of the AIM study cohorts: ChemoAnast and AnastAlone, and women without cancer. Data on pain and musculoskeletal symptoms from the Brief Pain Inventory (Cleeland & Ryan, 1994) and Breast Cancer Prevention Trial Symptom Checklist (Stanton, Bernaards & Ganz, 2005) at baseline (before initiation of AI therapy for the breast cancer cohorts) and 6, 12, and 18 months post baseline were analyzed to investigate the trajectory patterns of musculoskeletal symptoms. Baseline phenotypic factors including demographic-, disease-, and individual characteristics were used for to examine the relationship between these factors and group membership of trajectories for musculoskeletal symptoms. New genotype data of selected candidate genes were generated using the banked DNA samples. The association between the DNA variation and the distinct latent classes of trajectories for musculoskeletal symptoms were explored.

1.5.2 Setting and sample

The participants were recruited from the Comprehensive Breast Care Program of the University of Pittsburgh Cancer Institute (UPCI), which was comprised of Magee Women's Hospital, Hillman Cancer Center, and Shadyside Hospital. Inclusion criteria for ChemoAnast and AnastOnly cohorts were: 1) female; 2) diagnosed with stage I, II, and IIIa breast cancer based on the Tumor, Node, Metastasis Classification System and confirmed by oncologist; 3) eligible to receive either chemotherapy + anastrozole, or anastrozole alone; 4) postmenopausal defined as amenorrhea persisting for an entire year, oophorectomy, or hysterectomy and age greater than 51 years; 5) maximum age of 75 years; 6) able to speak and read English; and 7) completed a minimum of 8 years of education. Exclusion criteria for all subjects were: 1) self-report of

hospitalization for psychiatric illness within the last 2 years; 2) a prior diagnosis of neurologic illness; 3) clinical evidence of distant metastases including the central nervous system, or 4) prior diagnosis of cancer.

The AIM study was approved by the University of Pittsburgh IRB, and all participants provided written informed consent. A subgroup of participants in the AIM study agreed to provide a blood or saliva sample. The DNA of these sample were extracted and bio-banked in a comprehensive Nursing and Basic Science Laboratory.

1.5.3 Measures and procedure

The baseline characteristics (demographic-, disease-, and individual-related characteristics) were recorded at the initial study time point. Depression, anxiety, and fatigue before adjuvant therapy were assessed by Beck Depression Inventory (second edition) (Groth-Marnat, 1990), Profile of Mood States Tension/Anxiety and Fatigue/Inertia subscale (Terry et al., 1999) respectively. Data on pain and musculoskeletal symptoms from the Brief Pain Inventory (Cleeland & Ryan, 1994) and Breast Cancer Prevention Trial Symptom Checklist (Stanton et al., 2005) were collected at pre- and 6, 12, and 18 months post initiation of adjuvant therapy.

The Brief Pain Inventory (BPI) short form was used to assess self-report of severity of pain and the impact of pain on daily functions, location of pain, and pain medications in the past week. The severity of pain (worst, least, average, and right now) was assessed by 4 items with a scale ranging from 0 (no pain) to 10 (pain as bad as you can imagine). Pain interference with general activity, mood, walking ability, normal work, relations with other people, sleep, and enjoyment of life are assessed by 7 items with a scale ranging from 0 (does not interfere) to 10 (completely

interferes). Cronbach's alpha reliability of BPI has been reported ranging from 0.77 to 0.91 (Cleeland & Ryan, 1994).

The musculoskeletal symptoms were assessed by the musculoskeletal component of the Breast Cancer Prevention Trial (BCPT) Symptom Checklist that include 5 items (general aches and pains, joint pains, muscle stiffness, swelling of hands or feet, and numbness or tingling). Participants were asked to indicate the presence or absence of each musculoskeletal symptom and rate its associated distress on a five-point Likert scale ranging from 0 (not at all) to 4 (extremely). Prior studies have demonstrated good reliability and validity with internal consistency reliability exceeding 0.70. The Cronbach's alpha for all items was reported as 0.81 (Stanton et al., 2005; Cella et al., 2008; Terhorst et al., 2011).

1.5.4 Selection of candidate genes and SNPs, and genotyping

A comprehensive, 3-step literature review with broadened scope was conducted to identify genes associated with musculoskeletal symptoms in 1) breast cancer, 2) cancer, and 3) a population without cancer (e.g., low back pain, fibromyalgia, arthritis-related pain, knee pain, widespread musculoskeletal pain etc.). Studies published through June 2017 were queried using combinations of key terms in PubMed. From the literature review, 13 genes were identified related to musculoskeletal symptoms with AI therapy for breast cancer, including *ESR1*, *CYP17A1*, *CYP19A1*, *CYP27B1*, *CYP2A6*, *CYP3A4*, *CYP3A5*, *IGF1*, *TCL1A*, *OPG*, *RANKL*, *UGT2B17*, and *VDR*. When broadening the review scope to include musculoskeletal symptoms in populations with/without cancer, the number of identified genes increased to 72.

The 72 genes identified from the literature review were entered into a gene-gene pathway analysis sing QIAGEN's Ingenuity® Pathway Analysis (IPA) software, and 23 genes were

selected based upon the current evidence of their association with musculoskeletal symptoms with AI therapy and the numbers of direct and indirect interactions (including binding, inhibiting, acting on, inhibiting and acting on, leading to, and translocating to) with other candidate genes in the IPA. The 23 candidate genes are *BDNF*, *CCL2, CXCL8*, *CYP17A1*, *CYP19A1, CYP27B1*, *CYP2A6, CYP3A4, CYP3A5, ESR1, ESR2, IGF1*, *IL1A, IL1B, IL1RN, LIF, MMP13, NOS3, TCL1A, OPG (TNFRSF11B), RANKL (TNFSF11), VDR*, and *WNT5A*. Three additional genes (*COMT*, *IL-6*, and *OPRM1*) that have been published to be closely associated with chronic pain were added to the candidate gene list (Knisely et al., 2019). A total of 26 candidate genes were initially selected. Tagging SNPs and literature-driven functional SNPs for these 26 candidate genes were selected for investigation. Tagging SNPs were selected using the Phase III HapMap database. Tagging SNPs were required to be common (minor allele frequency of ≥ 0.05) in public databases. The selection and prioritization of candidate genes, and the process of the ingenuity pathway analysis have been previously reported (Zhu et al., 2019, Appendix D).

Of the 283 participants who provided a blood or saliva sample from the AIM study, 243 who had complete phenotype and phenotypic factor profile were genotyped and included in the genetic analysis. Extracted DNA samples were genotyped with the Sequenom iPLEX MassARRAY platform or ABI TaqMan allelic discrimination. To ensure robust genetic association analyses, SNPs with call rates of <90%, minor allele frequency of >0.05, or Hardy-Weinberg estimates with $p<0.05$ were excluded. Table 5 lists the 46 SNPs among the 25 candidate genes that met all the quality control criteria and were included in the genetic analyses. (*CYP3A5* was removed from the candidate gene list because neither the tagging SNP nor functional SNP was identified).

1.6 DATA ANALYSIS

1.6.1 Descriptive statistics

Variables were described by descriptive statistics. For nominal variables, frequency, percentage, range, and mode will be counted and computed. For ordinal variables, frequency, percentage, mode, median, interquartile range, semi-interquartile range, and range were counted and computed. For ratio variables, mean, standard deviation, range were computed. If the distribution of ratio variables was very skewed, then non-parametric statistics (e.g., median, interquartile range, and semi-interquartile range) were further computed to better describe the skewed variables. Bar chart and pie chart were used to describe nominal and ordinal variables graphically. Histogram and box plot were used for ratio variables. Distribution of variables were described by cross-tabulation contingency table (nominal and ordinal variables), histogram, and scatterplots.

1.6.2 Data screening procedures

The data screening procedure were started with data accuracy screening through generating descriptive statistics and graphical plots for each variable. For descriptive statistics, whether minimum and maximum values are in a reasonable range and whether mean or median are plausible were checked. The out-of-range values were checked whether there are data entry error or the values are messed with missing value code. The consistency of similar variables among different measures were checked, for example, whether the pain occurrence was consistent between the BPI and BCPT instruments. In terms of graphical plots, scatterplot and histogram

were generated for continuous and categorical variables respectively. For genotype data, Hardy-Weinberg Equilibrium were checked to detect potential genotyping errors.

Data were further screened to check the outliers, missingness, satisfaction with underlying assumptions, and need of data transformation.

1.6.2.1 Outlier assessment

Univariate and multivariate outliers were assessed. For categorical variables, uneven category splits were identified using frequency distributions and contingency table. Histograms and boxplots were used to identify cases removed from the distribution. Z-scores were calculated and case with extreme values (absolute value > 3.29) were considered as potential outliers. The presence of multivariate outliers was assessed using bivariate scatterplots and Mahalanobis distances.

Outliers and influential values were also evaluated for regression model fitting. Outliers in Y were assessed using Jackknifed (deleted studentized) residuals, and outliers in X were assessed using leverage statistics. Boxplots of residual/leverage value and leverage plots of residual were generated to detect potential outliers. To explore influential values, the influence of the i-th observation on predicted values (DFFITS) and individual regression coefficients (DFBETAS) were calculated. To determine if the i-th observation exerts undue influence on a set of coefficients, Cook's distance were calculated. Additionally, covariance ratio (COVARATIO) were computed to determine if the i-th observation improves or worsens the estimation ability of the model.

1.6.2.2 Treatment of missing data

Missing values for all variables at each time point were quickly screened by frequencies and percentages. Whether the missing pattern is missing completely at random was examined by

the Little's MCAR test. If only a few cases (<5%) have missing data and appear to be at a random subset of the entire sample, simple deletion of missing cases was used to handle missingness. Alternatively, if data are missing from a considerable amount of cases or the pattern is not missing at random, appropriate strategies (e.g., multiple imputation, regression or expectation-maximization algorithm, etc.) were used to address the missing data based on its missing pattern.

1.6.2.3 Checking underlying assumptions

The Group-based Trajectory modeling assumes that repeated observations on the same individual are independent conditional on trajectory group, meaning that the within-person correlation structure is explained completed by the estimated trajectory curve for each person's group. Hence, this assumption of conditional independence for group-based trajectory modeling (GBTM) was examined.

For the multivariate multinomial logistic regression model, the assumptions of independent, linear relationship between the logit of the independent variables and dependent variable, multicollinearity, additivity of effects of the independent variables (predictors), and proportional odds assumption were examined.

1.6.3 Data analysis procedures

The analysis plans for each specific aim are demonstrated below.

Aim 1: Participants who 1) had completed more than two timepoint assessments with the BPI and BCPT, and 2) had complete profile of candidate risk factors, were included in the final analysis. Group-based trajectory modeling (GBTM) (Jones & Nagin, 2007; Nagin & Odgers, 2010) was performed using SAS (version 9.4, SAS Institute, Inc., Cry, NC) to identify distinct

latent classes of trajectories for pain experience over the first 18 months of adjuvant AI therapy and the corresponding time period for the control cohort. Using GBTM, the BPI pain severity (average), BPI pain at worst, BPI pain interference (average), subscale of BCPT musculoskeletal symptoms (average), BCPT joint pain, BCPT muscle stiffness were modeled as a function of time under a censored normal model to identify distinct latent classes of trajectories, separately. The usage of analgesics was modeled as function of time under a logit model to identify distinct latent classes of trajectory. The number of distinct latent classes of trajectories was determined by the Bayesian Information Criteria (BIC), where the best-fitting model has the greatest BIC when comparing model BIC using the Bayes factor (Nylund, Asparouhov & Muthen, 2007). The shape of each estimated representative average trajectory for each class was determined by fitting regression model as polynomials of time (constant [intercept only], linear, quadratic, and cubic) and testing whether regression coefficients differ from zero (significance testing with t-statistics).

Aim 2: Multivariate multinomial logistic regression was conducted to determine the associated phenotypic factors (demographic and clinical characteristics) of trajectory group membership. All factors are time invariant. Using predicted trajectory group membership as a categorical variable, all factors of interest were screened one at a time using analysis of variance (ANOVA) for continuous variables (or Kruskal-Wallis H test if homogeneity of variances was violated), and Chi-square test (or Fisher exact test if sparse cells are encountered) for categorical variables. Variables that were not significant at the 0.30 level in the univariate analysis were not included in the multivariate multinomial logistic regression. A backward stepwise approach was used to create a parsimonious model. Statistical tests were 2-sided, and only predictors with $p < 0.05$ were retained in the final model.

Aim 3: Allele and genotype frequencies were determined by gene counting. Hardy-Weinberg equilibrium was examined by the Chi-square test. Additive, dominant, and recessive genetic models were performed to assess the univariate association between each SNP and each trajectory group membership (phenotype) by Chi-square test (or Fisher exact test if sparse cells are encountered), individually. The genetic model that best fit the data, by maximizing the significance of the p-value, was selected for each SNP. Multinomial logistic regression analysis, which controlled for significant phenotypic factors (demographic and clinical characteristics) was conducted to examine the association between genotype of significant SNPs (selected from univariate analysis) and corresponding trajectory group membership (phenotype). A Backward stepwise approach was used to build a parsimonious model. Only predictors with $p < 0.05$ were retained in the final parsimonious model. Interactions between significant SNPs identified from multinomial logistic regression were further analyzed to explore the collective effects of significant SNPs.

1.7 SAMPLE SIZE JUSTIFICATION

The sample size of the parent study (AIM study) is fixed. Hence instead of determining sample size needs, we reported on the smallest effect sizes that would be detectable at a desired power of 0.80 at a level of significant of 0.05 for two-sided hypothesis testing given a fixed sample size of 380.

According to the literature reporting on trajectories of other symptoms (e.g., anxiety, pain), 2, 3 or 4 distinct classes of symptom trajectories were identified and with the 4 time points to be used in the proposed study up to a cubic polynomial trajectory shape may be estimated. For specific

aim 1 a cubic trajectory may be estimated with a minimum trajectory group size of 70 (20%) with at least 0.80 power.

For the specific aim 2 examining the associations between candidate time-invariant phenotypic factors and predicted trajectory group membership, minimum effect sizes were determined that would be detectable with 0.80 power when testing hypotheses at a significance level of 0.05 for two-sided hypothesis testing. For time-invariant categorical variables (mostly binary predictors), contingency tables and Chi-squared test statistics for independence will be used to examine associations. For binary categorical predictor variables (e.g., chemotherapy, hormonal replacement therapy use, etc.) with a sample size of 380, we can detect effect sizes (w) as small as 0.149, 0.165, and 0.176 for 2, 3, and 4 group trajectories, respectively. For continuous-type time-invariant variables (e.g., age, education, etc.) the minimum effect sizes (f) detectable at 0.80 power for 2, 3, and 4 trajectory groups would be 0.150, 0.166, and 0.177, respectively, when using one-way ANOVA.

For the specific aim 3 exploring the relationship between DNA variation in candidate genes and the distinct latent classes of trajectories for musculoskeletal symptoms, the sample size was not justified due to its exploratory nature.

1.8 HUMAN SUBJECTS

The dissertation study used de-identified collected data and banked DNA sample from a parent study (AIM study). The parent study has been approved by the University of Pittsburgh IRB and has completed patient recruitment and DNA banking. An application to the IRB for expedited classification of the proposed dissertation study has been approved by the University of Pittsburgh

Institutional Review Board (IRB) in August 2018 (PRO18070351; Appendix E). The student has completed the following online modules from the Collaborative Institutional Training Initiative (CITI) sponsored by the Research Conduct and Compliance Office of the University of Pittsburgh: 1) Responsible conduct of Research, 2) Human Subjects, and 3) Conflict of Interest. The student has received blood borne pathogen training and chemical hygiene training provided by University of Pittsburgh.

Potential risk of the proposed dissertation study includes break of confidentiality of data and to anonymity of subjects. In order to minimize the risk, all participants were assigned a unique code number under which all data are stored. Security of data was upheld through the use of password protection and restricted access to users. Banded DNA samples were assigned a unique code number and be de-identified also. DNA samples were stored and analyzed in an appropriately equipped laboratory.

2.0 SUMMARY OF STUDY

Musculoskeletal symptoms (including arthralgias, myalgia, and muscle stiffness) are common during adjuvant endocrine therapy using aromatase inhibitors (AI), which are generally prescribed for 5-10 years for postmenopausal women with hormone-sensitive breast cancer. Musculoskeletal symptoms are experienced by up to 85% of AI users, and are the number one contributor to the high treatment discontinuation rate (up to 73%). While strategies have been proposed to manage musculoskeletal symptoms with AI therapy, there are still no consistently effective interventions to prevent or manage the problem, due in large part to the fact that the phenotype of musculoskeletal symptoms has not been well-characterized, and the underlying mechanisms have not been clearly explicated.

No current studies have addressed the phenotype of musculoskeletal symptoms with AI therapy by examining the inter-individual variability of the problem, precluding the ability to screen for high-risk individuals. In addition, the long-term trajectory (after 12 months) at initiation of AI therapy is largely unknown.

Aromatase is expressed across multiple tissues including the brain, muscles, and osteoblasts of bone, etc. Estrogen suppression is the main hypothesized mechanism underlying musculoskeletal symptoms with AI therapy since AIs block the activity of aromatase and remove the protective effects of estrogen on the musculoskeletal system by inhibiting 97-99% of estrogen biosynthesis. Our preliminary literature reviews and a biological pathway analysis suggest multiple genetic variability in estrogen biosynthesis, AI metabolism, inflammation, and preexisting musculoskeletal disorders may all contribute to musculoskeletal symptoms with AI

therapy. However, inconsistent evidence about these identified genetic variabilities and the collective effects of multiple genetic variabilities requires further examination.

To address the identified gaps, we conducted this dissertation study to 1) characterize the inter-individual variability of the 18-month trajectories of pain and musculoskeletal symptoms, 2) determine the associated phenotypic factors (demographic-, clinical characteristics); and 3) evaluate the association between phenotypes (pain and musculoskeletal symptoms) and single nucleotide polymorphisms (SNPs) among 25 candidate genes (with 46 SNPs).

2.1 SUMMARY OF MAIN RESULTS, REMAINING GAPS AND FUTURE DIRECTION FOR SPECIFIC AIM 1

(Aim 1): Identify distinct latent classes of 18-month trajectories of pain and musculoskeletal symptoms for two cohorts of postmenopausal women (cohort 1: women with early stage breast cancer who receive anastrozole; cohort 2: age- and education-matched women without cancer).

Main results: Three trajectory subgroups were identified for pain severity, pain interference, musculoskeletal symptoms, joint pain, and muscle stiffness, respectively. Four distinct trajectory subgroups were identified for pain at worst. Two distinct trajectory subgroups were identified for the usage of analgesics.

Our results first supported the existence of inter-individual variability in pain and musculoskeletal symptoms with AI therapy for breast cancer. There were a significant proportion of women who experienced persistent/or increasing pain/musculoskeletal symptoms at the levels of mild/moderate severity. Pain/musculoskeletal symptoms before the initiation of AI therapy is a key factor to predict trajectory subgroups in the first 18-month of AI therapy.

Remaining gaps and future direction:

- The long-term changes in pain and musculoskeletal symptoms beyond the first 18 month of therapy to the completion of therapy (or even after the completion) are unknown. Extending the follow-up period beyond 18 months to after the completion of AI therapy is needed in future research.
- The associations of trajectory subgroup memberships among different phenotypes (e.g., pain severity and pain interference) were not examined in the dissertation study. Future studies/analysis are needed to further examine the association of subgroup memberships across different phenotypes.
- Several important characteristics (e.g., frequency, quality etc.) of pain and musculoskeletal symptoms were not assessed and included in this analysis due to the limitations of the data available for analysis. Future studies with a main focus on pain and musculoskeletal symptoms with more comprehensive assessment tools should be conducted to better phenotype the pain and musculoskeletal symptoms with AI therapy.
- The structural and functional alterations in deep tissue (joint and muscle) that are related to pain are unclear. Future studies are needed to describe the alterations in deep tissue and to examine the association between the alterations and patient-reported musculoskeletal pain with the AI therapy.

2.2 SUMMARY OF MAIN RESULTS, REMAINING GAPS AND FUTURE DIRECTION FOR SPECIFIC AIM 2

(Aim 2): Determine the phenotypic factors (demographic-, disease-, and individual-related characteristics) associated with the membership for the distinct latent classes of trajectories for pain and musculoskeletal symptoms.

Main results: We identified a profile of protective and risk factors across one or more phenotypes. The protective factors include older age, receipt of chemotherapy, older first menstrual period age, married/partnered, having an administrative level occupation (vs unskilled/unemployed), having regular periods for most of life, greater numbers of pregnancies, and having a history of tubal ligation. The risk factors include receipt of AI therapy, greater anxiety/pain severity/depressive symptoms/fatigue at baseline, and history of arthritis, hysterectomy, or menopausal symptoms.

Remaining gaps and future direction:

- The limitations of design (cross-sectional design, small sample size, less diverse population etc.) of current studies preclude drawing causal-effect relationship and consistent conclusion. Further longitudinal studies in a diverse sample with a larger sample size are needed.
- The collective effects of multiple phenotypic risk factors are not evaluated. A clinically useful algorithm to predict whether a patient will develop a persistent/increasing trajectory of pain/musculoskeletal symptoms need to be studied and developed in the future.

2.3 SUMMARY OF MAIN RESULTS, REMAINING GAPS AND FUTURE DIRECTION FOR SPECIFIC AIM 3

(Aim 3): Explore the genotypic factors (DNA variation in genes related to estrogen biosynthesis, AI metabolism, and musculoskeletal pain) associated with the distinct latent classes of trajectories for musculoskeletal symptoms.

Main results: We identified a profile of protective and risk polymorphisms that were associated with mild and/or moderate trajectories of pain and musculoskeletal symptoms (Table 7 in the manuscript). *BDNF* rs6265, *COMT* rs4633 and rs887200, *CXCL8* rs4073, *ESR2* rs2772163, *IL1B* rs16944, *RANKL* rs1054016, *VDR* rs4516035 and rs731236. The risk polymorphisms included: *CYP19A1* rs1008805, *CYP3A4* rs35599367, *COMT* rs165774, *NOS3* rs1799983, *OPG* rs2073618, *OPRM1* rs1799971, and *TCL1A* rs7158782 and rs7159713. We also found a significant interaction between *NOS3* rs1799983 and *OPG* rs2073618 when examining the genotypic factors of moderate class for musculoskeletal symptoms.

Remaining gaps and future direction:

- The majority of previous studies (including our study) on the association between genetic variance and pain/musculoskeletal symptoms during AI therapy only included a select number of candidate genes and SNPs. Examination of the whole genome level is optimal and needed to identify additional genes and polymorphisms associated with pain and musculoskeletal symptoms.
- Our study and previous studies did not examine the relationships between genetic variance and musculoskeletal pain at the levels of gene expression and epigenetics. Future studies are needed to further examine it.

2.4 STUDY STRENGTHS AND LIMITATIONS

This dissertation study adds to the science related to pain/musculoskeletal symptoms during AI therapy for breast cancer, including 1) first characterization of interindividual variability of pain and musculoskeletal symptoms during AI therapy and extend the follow-up period up to 18 months; 2) further identification and clarification of phenotypic risk factor with meaningful clinical implications; 3) further identification and clarification of a profile of protective and risk genetic variance.

Although the proposed dissertation study has significance for the science of musculoskeletal symptoms during AI therapy as well as the clinical application for women with breast cancer, there are some limitations. Firstly, the frequency of musculoskeletal symptoms during AI therapy was not included since it was not assessed in the parent study. The parent study focused on anastrozole and did not include other forms of AI therapy, which may have a different profile of musculoskeletal symptoms, although anastrozole is the most commonly prescribed form of AI therapy in clinical settings. Thus, the results of this study may not be fully generalized to other forms of AI therapy. Moreover, with the exploratory nature of specific aim 3, the sample size was not justified when exploring the relationship between DNA variation in candidate genes and distinct latent classes of trajectories for musculoskeletal symptoms during AI therapy. Therefore, the results for this specific aim will need to be interpreted with caution.

3.0 DATA-BASED MANUSCRIPT: PAIN WITH ENDOCRINE THERAPY FOR BREAST CANCER: 18-MONTH TRAJECTORIES AND PREDICTORS

3.1 ABSTRACT

Musculoskeletal pain (including arthralgias, myalgia, and muscle stiffness) is experienced by up to 85% of women undergoing aromatase inhibitor (AI) therapy for breast cancer, and is the number one contributor to the high treatment discontinuation rate. The purpose of this ancillary study was to examine the trajectories of musculoskeletal pain and related factors during the first 18 months of AI therapy among postmenopausal women with breast cancer. The parent study, Anastrozole Use in Menopausal Women (AIM) study, provided data on pain, musculoskeletal symptoms, and candidate phenotypic factors for two cohorts of women (n=380) (cohort 1: women with early stage breast cancer who receive anastrozole; cohort 2: age- and education-matched women without cancer) at baseline (before initiation of AI therapy for breast cancer cohort), 6, 12, and 18 months after baseline. Based on the bio-banked DNA provided by a subgroup of participants (n=243) in the parent study, we genotyped 46 single nucleotide polymorphisms (SNPs) among the 25 candidate genes which were selected from biological pathway analysis. Our results showed that a significant proportion of women experienced mild or moderate level of pain and musculoskeletal symptoms in a persistent or linearly increasing manner over the first 18 months of AI therapy. A profile of protective and risk factors across one or more phenotypes were identified. The protective phenotypic factors included older age, receipt of chemotherapy, older first menstrual period age, married/partnered, having an administrative level of occupation (vs unskilled/unemployed), having regular period for most of life, greater numbers of pregnancies,

and having a history of tubal ligation. The phenotypic risk factors included receipt of AI therapy, greater anxiety/pain severity/depressive symptoms/fatigue at baseline, and having history of arthritis, hysterectomy, or menopausal symptoms. Variations in *CYP19A1* (rs1008805) and *NOS3* (rs1799983) were associated with membership across pain and musculoskeletal symptoms. A profile of protective and risk polymorphisms was identified. The protective polymorphisms included: *BDNF* rs6265, *COMT* rs4633 and rs887200, *CXCL8* rs4073, *ESR2* rs2772163, *IL1B* rs16944, *RANKL* rs1054016, *VDR* rs4516035 and rs731236. The risk polymorphisms included: *CYP19A1* rs1008805, *CYP3A4* rs35599367, *COMT* rs165774, *NOS3* rs1799983, *OPG* rs2073618, *OPRM1* rs1799971, and *TCL1A* rs7158782 and rs7159713.

3.2 INTRODUCTION

Pain is a leading cause of physical disability among adults with cancer and can be triggered or exacerbated by systemic cancer treatments (Fallon et al., 2018). Musculoskeletal symptoms (including arthralgias, myalgia, and muscle stiffness) is particularly common during treatment with aromatase inhibitor (AI), which is generally prescribed for 5-10 years for postmenopausal women with hormone-sensitive breast cancer as an adjuvant endocrine therapy. Among women undergoing AI therapy, 48% - 64.3% reported musculoskeletal pain disturbances with daily activities (Egawa et al., 2016; Waltman et al., 2009). Our literature review (Zhu et al., 2019) on symptoms reported during endocrine therapy has shown that arthralgias and myalgia were the most commonly reported symptoms during AI therapy, with occurrences ranging widely from 3.6% to 89%. Between 25% and 72% of AI users reported joint/muscle stiffness. For those who experienced arthralgias and myalgia, 31.5%-46% reported moderate to severe intensity (Chim et

al., 2013; Crew et al., 2007; Mao et al., 2009; Presant et al., 2007). The average intensity of arthralgias was moderate to severe and ranged from 4.9 to 7.5 out of 10 (as extreme severity) (Boonstra et al., 2013; Shi et al., 2013; Servitja et al., 2012; Presant et al., 2007). The onset severity of arthralgias was 5.23 on a 10-point scale (Shi et al., 2013). Swenson et al. reported a trend toward increasing intensity of musculoskeletal symptoms during the first 6 months after initiation of AI treatment (Swenson et al., 2013). It is also reported that arthralgias is the number one contributor to the high treatment discontinuation rate; between 13% and 50% of the discontinuation of AI therapy is due to AI associated arthralgias (Crew et al., 2007; Dizdar et al., 2009; Henry et al., 2012). While strategies (e.g., acupuncture, vitamin D supplementation etc.) have been proposed to manage pain and musculoskeletal symptoms with AI therapy, there are still no consistently effective interventions to prevent or manage the problem, due in large part to the fact that the phenotype and risk factors of pain and musculoskeletal symptoms during AI therapy have not been well-characterized and its underlying biological mechanisms have not been clearly explicated.

Among the studies focusing on musculoskeletal symptoms during AI therapy, most adopted a cross-sectional design, and the follow-up period of the few longitudinal studies completed to date was no more than 12 months (from pre-therapy). There is a need to extend the follow-up period beyond the first year of AI therapy to better describe the trajectories of pain and musculoskeletal symptoms in women with breast cancer. In addition, among breast cancer survivors, inter-individual variability in the experience of several common symptoms during surgery and adjuvant therapies have been reported, for example, fear of recurrence (Dunn et al., 2015), depressive symptoms after surgery (Dunn et al., 2011), pain after chemotherapy (Langford et al., 2016), weight changes during chemotherapy (Liu et al., 2014), anxiety pre- and post-surgery (Kyranou et al., 2014), and fatigue during and after radiation therapy (Dhruva et al., 2010). The

wide range of the prevalence (3.6% - 89%) of musculoskeletal symptoms suggests the possibility of inter-individual variability with respect to this symptom as well. However, no studies, to date, have examined inter-individual variability in pain and musculoskeletal symptoms during AI therapy.

Estrogen suppression is the main hypothesized mechanism underlying the musculoskeletal symptoms associated with AI therapy. AIs block the activity of aromatase, expressed across multiple tissues including the brain, muscles, and osteoblasts of bone, etc. and remove the protective effects of estrogen on the musculoskeletal system by inhibiting 97-99% of estrogen biosynthesis (Fabian, 2007). Current literature reviews (Borrie & Kim, 2017; Sini et al., 2017) and our biological pathway analysis (Zhu et al., in press) suggest multiple genetic variabilities in estrogen biosynthesis, AI metabolism, inflammation, and preexisting musculoskeletal disorders may all contribute to the inter-individual variability of pain and musculoskeletal symptoms with AI therapy. However, inconsistent evidence about these identified genetic variabilities and the collective effects of multiple genetic variabilities require further examination (Borrie & Kim, 2017; Zhu et al., in press).

To address the above gaps, we conducted this study to 1) characterize the inter-individual variability of the 18-month trajectories of pain and musculoskeletal symptoms, 2) determine the associated phenotypic factors (demographic-, clinical characteristics), and 3) evaluate the association between phenotypes (pain and musculoskeletal symptoms) and single nucleotide polymorphisms (SNPs) among 25 candidate genes (with 46 SNPs).

3.3 METHODS

This is an ancillary study of the Anastrozole Use in Menopausal Women (AIM) study (R01CA107408, PI: Catherine Bender). The design and methods for the parent study have been described in detail in our previous publications (Bender et al., 2015). The AIM study was a prospective cohort study with a primary aim of examining and comparing the effect of anastrozole on cognitive function among four groups of postmenopausal women: women with early stage breast cancer who receive chemotherapy plus anastrozole (ChemoAnast), anastrozole alone (AnastAlone), chemotherapy only (ChemoOnly), and women without breast cancer who were matched on age, and years of education to the breast cancer cohorts. This secondary analysis focused on three of the AIM study cohorts: ChemoAnast, AnastAlone, and women without breast cancer (Control). Participants were evaluated at baseline (before the initiation of AI therapy for breast cancer cohorts), and at 6-, 12-, and 18-month after baseline assessment.

Inclusion criteria for ChemoAnast and AnastOnly cohorts were: 1) female; 2) diagnosed with stage I, II, and IIIa breast cancer based on the Tumor, Node, Metastasis Classification System and confirmed by oncologist; 3) eligible to receive either chemotherapy + anastrozole, or anastrozole alone; 4) postmenopausal defined as amenorrhea persisting for an entire year, oophorectomy, or hysterectomy and age greater than 51 years; 5) maximum age of 75 years; 6) able to speak and read English; and 7) completed a minimum of 8 years of education. Exclusion criteria for all subjects were: 1) self-report of hospitalization for psychiatric illness within the last 2 years; 2) a prior diagnosis of neurologic illness; 3) clinical evidence of distant metastases including the central nervous system, or 4) prior diagnosis of cancer. The participants were recruited from the Comprehensive Breast Care Program of the UPMC Hillman Cancer Center, an NCI-designated Comprehensive Cancer Center. The AIM study was approved by the University

of Pittsburgh IRB, and all participants provided written informed consent. A subgroup of participants (n=283) in the AIM study agreed to provide a blood or saliva sample. The DNA of these sample were extracted and bio-banked in a comprehensive Nursing and Basic Science Laboratory. New genetic data was generated from the bio-banked DNA samples.

3.3.1 Measures and instruments

The baseline characteristics (demographic and clinical) were recorded after the completion of surgery for the breast cancer cohorts and at the initial study time point for the control cohort. Depression, anxiety, and fatigue were assessed at baseline (pre-therapy) with the Beck Depression Inventory (second edition) (Beck, Steer & Carbin, 1988), Profile of Mood States Tension/Anxiety and Fatigue/Inertia subscale (Terry et al., 1999) respectively. Pain and musculoskeletal symptoms were assessed with the Brief Pain Inventory and Breast Cancer Prevention Trial Symptom Checklist at pre- and 6, 12, and 18 months post initiation of adjuvant AI therapy for breast cancer cohort and at corresponding timepoints for control cohort.

The Brief Pain Inventory (BPI) short form was used to assess self-report of severity of pain, interference of pain on daily functions, usage of analgesics (including both prescribed and over-the-counter analgesics) in the past week. The severity of pain (worst, least, average, and right now) was assessed by 4 items with a scale ranging from 0 (no pain) to 10 (pain as bad as you can imagine). Pain interference with general activity, mood, walking ability, normal work, relations with other people, sleep, and enjoyment of life are assessed by 7 items with a scale ranging from 0 (does not interfere) to 10 (completely interferes). Cronbach's alpha reliability of BPI has been reported ranging from 0.77 to 0.91 (Cleeland & Ryan, 1994).

Musculoskeletal symptoms were assessed by the musculoskeletal subscale of the Breast Cancer Prevention Trial (BCPT) Symptom Checklist that includes 5 items (general aches and pains, joint pains, muscle stiffness, swelling of hands or feet, and numbness or tingling). Participants were asked to indicate the presence or absence of each musculoskeletal symptom and rate its associated distress on a five-point Likert scale ranging from 0 (not at all) to 4 (extremely). Prior studies have demonstrated good reliability and validity with an internal consistency reliability exceeding 0.70. The Cronbach's alpha for all items was reported as 0.81 (Stanton et al., 2005; Cella et al., 2008; Terhorst et al., 2011).

3.3.2 Selection of candidate genes and SNPs, and genotyping

A comprehensive, 3-step literature review with broadened scope was conducted to identify genes associated with musculoskeletal symptoms in 1) breast cancer, 2) cancer, and 3) a population without cancer (e.g., low back pain, fibromyalgia, arthritis-related pain, knee pain, widespread musculoskeletal pain etc.). Studies published through June 2017 were queried using combinations of key terms in PubMed. From the literature review, 13 genes were identified related to musculoskeletal symptoms with AI therapy for breast cancer, including *ESR1*, *CYP17A1*, *CYP19A1*, *CYP27B1*, *CYP2A6*, *CYP3A4*, *CYP3A5*, *IGF1*, *TCL1A*, *OPG*, *RANKL*, *UGT2B17*, and *VDR*. When broadening the review scope to include musculoskeletal symptoms in populations with/without cancer, the number of identified genes increased to 72.

The 72 genes identified from the literature review were entered into a gene-gene pathway analysis sing QIAGEN's Ingenuity® Pathway Analysis (IPA) software, and 23 genes were selected based upon the current evidence of their association with musculoskeletal symptoms with AI therapy and the numbers of direct and indirect interactions (including binding, inhibiting, acting

on, inhibiting and acting on, leading to, and translocating to) with other candidate genes in the IPA. The 23 candidate genes are *BDNF, CCL2, CXCL8, CYP17A1, CYP19A1, CYP27B1, CYP2A6, CYP3A4, CYP3A5, ESR1, ESR2, IGF1, IL1A, IL1B, IL1RN, LIF, MMP13, NOS3, TCL1A, OPG (TNFRSF11B), RANKL (TNFSF11), VDR*, and *WNT5A*. Three additional genes (*COMT, IL-6,* and *OPRM1*) that have been published to be closely associated with chronic pain were added to the candidate gene list (Knisely et al., 2019). A total of 26 candidate genes were initially selected. Tagging SNPs and literature-driven functional SNPs for these 26 candidate genes were selected for investigation. Tagging SNPs were selected using the Phase III HapMap database. Tagging SNPs were required to be common (minor allele frequency of ≥ 0.05) in public databases. The selection and prioritization of candidate genes, and the process of the ingenuity pathway analysis have been previously reported (Zhu et al., 2019).

Of the 283 participants who provided a blood or saliva sample from the AIM study, 243 who had complete phenotype and phenotypic factor profile were genotyped and included in the genetic analysis. Extracted DNA samples were genotyped with the Sequenom iPLEX MassARRAY platform or ABI TaqMan allelic discrimination. To ensure robust genetic association analyses, SNPs with call rates of <90%, minor allele frequency of >0.05, or Hardy-Weinberg estimates with $p<0.05$ were excluded. **Table 5** lists the 46 SNPs among the 25 candidate genes that met all the quality control criteria and were included in the genetic analyses. (*CYP3A5* was removed from the candidate gene list because neither the tagging SNP nor functional SNP was identified).

3.3.3 Statistical analysis

3.3.3.1 Characterization of the inter-individual variability for the Pain and Musculoskeletal Symptoms (Phenotype)

Participants who 1) had completed more than two timepoint assessments with the BPI and BCPT, and 2) had complete profile of candidate risk factors, were included in the final analysis. Group-based trajectory modeling (GBTM) (Jones & Nagin, 2007; Nagin & Odgers, 2010) was performed using SAS (version 9.4, SAS Institute, Inc., Cry, NC) to identify distinct latent classes of trajectories for pain experience over the first 18 months of adjuvant AI therapy and the corresponding time period for the control cohort. Using GBTM, BPI pain at worst, BPI pain severity (average), BPI pain interference (average), subscale of BCPT musculoskeletal symptoms (average), BCPT joint pain, BCPT muscle stiffness were modeled as a function of time under a censored normal model to identify distinct latent classes of trajectories, separately. The usage of analgesics was modeled as function of time under a logit model to identify distinct latent classes of trajectory. The number of distinct latent classes of trajectories was determined by the Bayesian Information Criteria (BIC), where the best-fitting model has the greatest BIC when comparing model BIC using the Bayes factor (Nylund, Asparouhov & Muthen, 2007). The shape of each estimated representative average trajectory for each class was determined by fitting regression model as polynomials of time (constant [intercept only], linear, quadratic, and cubic) and testing whether regression coefficients differ from zero (significance testing with t-statistics).

3.3.3.2 Statistical Analyses of the Phenotypic Factors

Multivariate multinomial logistic regression was conducted to determine the associated phenotypic factors (demographic and clinical characteristics) of trajectory group membership. All factors are time invariant. Using predicted trajectory group membership as a categorical variable, all factors of interest were screened one at a time using analysis of variance (ANOVA) for continuous variables (or Kruskal-Wallis H test if homogeneity of variances was violated), and Chi-square test (or Fisher exact test if sparse cells are encountered) for categorical variables. Variables that were not significant at the 0.30 level in the univariate analysis were not included in the multivariate multinomial logistic regression. A backward stepwise approach was used to create a parsimonious model. Statistical tests were 2-sided, and only predictors with $p < 0.05$ were retained in the final model.

3.3.3.3 Statistical Analyses of the Genetic Data

Allele and genotype frequencies were determined by gene counting. Hardy-Weinberg equilibrium was examined by the Chi-square test. Additive, dominant, and recessive genetic models were performed to assess the univariate association between each SNP and each trajectory group membership (phenotype) by Chi-square test (or Fisher exact test if sparse cells are encountered), individually. The genetic model that best fit the data, by maximizing the significance of the p-value, was selected for each SNP. Multinomial logistic regression analysis, which controlled for significant phenotypic factors (demographic and clinical characteristics) was conducted to examine the association between genotype of significant SNPs (selected from univariate analysis) and corresponding trajectory group membership (phenotype). A Backward stepwise approach was used to build a parsimonious model. Only predictors with $p < 0.05$ were retained in the final parsimonious model. Interactions between significant SNPs identified from

multinomial logistic regression were further analyzed to explore the collective effects of significant SNPs.

3.4 RESULTS

3.4.1 Participant Characteristics

In total of 380 participants who 1) had completed more than two timepoints' assessments with the BPI and BCPT, and 2) had complete profile of candidate phenotypic risk factors, were included in the final analysis. The 380 women were on average 60.6 years of age, Caucasian (95.0%), and married/partnered (65.0%). Demographic and clinical characteristics of the 380 participants are detailed in the **Table 6.** The study flow diagram is displayed in **Figure 2**.

3.4.2 Inter-individual variability for pain and musculoskeletal symptoms

The GBTM identified three trajectory subgroups for pain severity, pain interference, pain at worst, musculoskeletal symptoms, joint pain, and muscle stiffness, respectively. Four distinct trajectory subgroups were identified for pain at worst. Two distinct trajectory subgroups were identified for the usage of analgesics. The observed and model-estimated subgroups of trajectories for pain and musculoskeletal symptoms are listed in the **Table 7**.

The three distinct trajectory subgroups for pain severity are: constant no pain (34.2%; b[intercept]=-3.47, $p<0.01$), constant mild pain (45.1%; b[intercept]=1.16, $p<0.01$), and moderate initial pain with linear increase (20.7%; b[intercept]=4.14, $p<0.01$; b[linear]=0.06, $p=0.01$). The

four distinct trajectory subgroups for pain at worst are: constant no pain (27.7%; b[intercept]=-4.37, $p<0.01$), initial no pain with linear increase (7.7%; b[intercept]=-9.69, $p<0.01$; b[linear]=0.82, $p<0.01$), constant mild pain (29.8%; b[intercept]=1.06, $p<0.01$), and constant moderate pain (34.8%; b[intercept]=3.82, $p<0.01$). For pain interference, three distinct trajectory subgroups are found: constant no interference (47.5%; b[intercept]=-2.55, $p<0.01$), constant mild interference (40.9%; b[intercept]=1.24, $p<0.01$), and constant moderate interference (11.6%; b[intercept]=5.09, $p<0.01$). For the usage of analgesics, two distinct trajectories are identified: constant no use (61.7%; b[intercept]=-2.22, $p<0.01$), and initial no use with linear increase (38.3%; b[intercept]=0.65, $p<0.01$; b[linear]=0.05, $p<0.01$).

The three distinct trajectory subgroups for joint pain are: constant no pain (20.1%; b[intercept]=-0.67, $p<0.01$), mild initial pain with quadratic change (61.2%; b[intercept]=0.89, $p<0.01$; b[linear]=0.08, $p<0.01$; b[quadratic]=-0.003, $p<0.01$), and moderate initial pain with linear increase (18.7%; b[intercept]=2.75, $p<0.01$; b[linear]=0.04, $p<0.01$). For muscle stiffness, three distinct trajectory subgroups are identified: constant no (22.3%; b[intercept]=-1.00, $p<0.01$), mild initial stiffness with linear increase (62.8%; b[intercept]=0.76, $p<0.01$; b[linear]=0.02, $p<0.01$), and moderate initial stiffness with linear increase (14.9%; b[intercept]=2.48, $p<0.01$; b[linear]=0.02, $p<0.01$). In terms of musculoskeletal symptoms, three distinct trajectory subgroups are identified: constant no (49.7%, b[intercept]=0.37, $p<0.01$), mild initial symptoms with linear increase (39.7%, b[intercept]=1.08, $p<0.01$; b[linear]=0.01, $p<0.01$), and constant moderate symptoms (10.5%, b[intercept]=2.37, $p<0.01$).

Figure 3 shows the patterns of trajectories for pain and musculoskeletal symptoms.

3.4.3 Phenotypic Predictors Associated with Membership in the Pain and Musculoskeletal Symptoms Subgroups

Table 8 exhibits the socio-demographic and clinical characteristics that differed significantly among subgroup membership for pain and musculoskeletal symptoms. **Table 9** summarizes significant phenotypic predictors across subgroup membership of pain and musculoskeletal symptoms.

For BPI pain severity, being a recipient of chemotherapy was associated with 54.2% lower odds of belonging to the mild pain severity class. Receipt of AI therapy, history of arthritis, and having greater depressive symptoms were associated with a 3.91-, 2.97-, and 1.11-fold increase in the odds of belonging in the mild pain severity class, respectively. Being married/partnered and having an administrative level of occupation (vs unskilled/unemployed) were associated with 55.6% and 70.4% lower odds of belonging to the moderate pain severity class. Recipient of AI therapy, history of hysterectomy, history of arthritis, greater depressive symptoms, and greater fatigue were associated with 5.37-, 3.10-, 10.58-, 1.15-, 1.14-fold increase in the odds of belonging in the moderate pain severity class, respectively.

For BPI worst pain, receipt of AI therapy was associated 4.18-fold increase in the odds of belonging in the initial no pain with linear increase subgroup. Greater numbers of pregnancies was associated with 28.6% lower odds of belong in the initial no pain with linear increase class. Receipt of AI therapy, history of arthritis, and greater depressive symptoms at baseline were associated with 3.34-, 1.67-, and 1.04-fold increase in the odds of belonging in the constant mild class, respectively. Receipt of AI therapy, history of arthritis, and greater depressive symptoms at baseline were associated with 4.83-, 11.23-, and 1.22-fold increase in the odds of belonging in the

constant moderate class, respectively. Having a history of tubal ligation was associated with 54.3% lower odds of belong in the constant moderate class.

For BPI pain interference, older age and having an administrative level of occupation (vs unskilled/unemployed) were associated with 5.5% and 72.7% lower odds of belonging to the mild class of pain interference. Receipt of AI therapy, history of hysterectomy, history of arthritis, greater pain severity at baseline, and greater fatigue were associated with 2.23-, 2.02-, 2.33-, 2.33-, and1.08-fold increase in the odds for belonging in the mild pain interference class, respectively. Older age and having an administrative level of occupation (vs unskilled/unemployed) were associated with 13.3% and 88.0% lower odds of belonging to the moderate class of pain interference. Receipt of AI therapy, history of hysterectomy, history of arthritis, greater depressive symptoms, greater anxiety, and greater pain severity at baseline were associated with 4.66-, 4.36-, 6.86-, 1.21-, 1.11-, and 3.93-fold increase in the odds for belonging in the moderate pain interference class, respectively.

For the usage of analgesics, being married/partnered, having an administrative level of occupation (vs unskilled/unemployed), and older age at menarche were associated with 50.2%, 63.6%, and 24.7% lower odds of belonging to the linear increase usage class. Receipt of AI therapy, greater anxiety, greater pain severity at baseline were associated with 4.68-, 1.09-, and 1.94-fold increase in the odds for belonging in the linear increase usage class.

For BCPT musculoskeletal symptoms, receipt of AI therapy, history of arthritis, greater depressive symptoms, and greater fatigue were associated with 1.93-, 3.23-, 1.08-, and 1.12-fold increase in the odds for belonging in the mild class. Having an administrative level of occupation (vs unskilled/unemployed) was associated with 74.9% lower odds of belonging to the moderate class. Having history of hysterectomy, history of arthritis, greater depressive symptoms, and

greater fatigue were associated with 2.89-, 10.10-, 1.16-, and 1.26-fold increase in the odds for belonging in the moderate class, respectively.

For BCPT joint pain, having an administrative level of occupation (vs unskilled/unemployed) and regular period for most of life were associated with 75.4% and 63.7% lower odds of belonging to the mild class. History of arthritis and greater fatigue were associated with 3.16-, and 1.22-fold increase in the odds of belonging in the mild class. Having an administrative level of occupation (vs unskilled/unemployed) and regular period for most of life were associated with 85.7% and 83.3% lower odds of belonging to the moderate class. History of menopausal symptoms, history of arthritis and greater fatigue were associated with 4.62-, 13.66-, and 1.40-fold increase in the odds for belonging in the moderate class, respectively.

For BCPT muscle stiffness, receipt of AI therapy, history of hysterectomy, and greater fatigue were associated with 1.96-, 2.64-, and 1.12-fold increase in the odds for belonging in the mild class. History of hysterectomy, history of arthritis, greater depressive symptoms, and greater fatigue were associated with 2.89-, 5.38-, 1.15-. and 1.29-fold increase in the odds of belonging in the moderate class, respectively.

3.4.4 Genotypic Predictors Associated with Membership in Pain and Musculoskeletal Symptoms Subgroups

Significant associations between SNPs and membership in the pain and musculoskeletal symptoms subgroups, adjusted by identified phenotypic factors, are shown in **Table 10**. **Table 11** summarizes significant genotypic predictors across membership in the pain and musculoskeletal symptoms subgroups.

For BPI pain severity, carrying 1 or 2 doses of the rare T allele (i.e., GT+TT vs GG) in *RANKL* rs1054016 was associated with 56.2% decrease in the odds of belonging in the mild class. Carrying 2 doses of the rare A allele (i.e., AA vs GG+GA) in *CYP19A1* rs1008805 was associated with 11.27-fold increase in the odds for belonging in the moderate class. Carrying 2 doses of the rare G allele (i.e., GG vs GT+TT) in *NOS3* rs1799983 was associated with 3.12-fold increase in the odds for belonging in the moderate class. For *COMT*, carrying 2 doses of the rare T allele (i.e., TT vs CT+CC) in rs887200 and rs4633 associated with 84.6% and 87.1% lower odds for belonging in the moderate class, respectively.

For BPI pain at worst, carrying 1 or 2 doses of the rare A allele (i.e., GA+AA vs GG) in *ESR2* rs2772163 was associated with a 90.8% decrease in the odds of belonging in the initial no pain with linear increase class. Carrying 1 or 2 doses of the rare A allele (i.e., GA+AA vs CC) in *LIF* rs737812 was associated with 3.78-fold increase in the odds of belonging in the initial no pain with linear increase class. Carrying 2 doses of the rare G allele (i.e., GG vs AA) in *TCL1A* rs7158782 and rs7159713 was associated with 23.9- and 25.07-fold increase in the odds of belonging in the initial no pain with linear increase class. Carrying 1 or 2 doses of the rare G allele (i.e., AG+GG vs AA) in *VDR* rs731236 was associated with 55.6% decrease in the odds of belonging in the constant mild pain class. Carrying 2 doses of the rare T allele (i.e., TT vs AA+AT) in *CXCL8* rs4073 was associated with 79.6% decrease in the odds of belonging in the constant moderate class. Carrying 2 doses of the rare A allele (i.e., AA vs GG+GA) in *CYP19A1* rs1008805 was associated with 4.32-fold increase in the odds of belonging in the constant moderate pain class. Carrying 2 doses of the rare C allele (i.e., CC vs TT) in *VDR* rs4516035 was associated with 78.2% decrease in the odds of belonging in the constant moderate pain class.

For BPI pain interference, carrying 1 or 2 doses of the rare C allele (i.e., TC+CC vs TT) in *VDR* rs4516035 was associated with 57.4% lower odds for belonging in the mild class.

For BCPT joint pain, carrying 1 or 2 doses of the rare T allele (i.e., CT+TT vs CC) in *BDNF* rs6265 was associated with 56.0% lower odds for belonging in the mild class.

For BCPT muscle stiffness, carrying 1 or 2 doses of the rare A allele (i.e., GA+AA vs GG) in *IL1A* rs3783521 was associated with 2-fold increase in odds for belonging in the mild class. Carrying 2 doses of the rare G allele (i.e., GG vs AG+AA) in *IL1B* rs16944 was associated with 55.2% lower in odds for belonging in the mild class. Carrying 1 or 2 doses of the rare G allele (i.e., AG+GG vs AA) in *OPRM1* rs1799971 was associated with 4.06-fold increase in odds for belonging in the mild class.

For BCPT musculoskeletal symptoms, carrying 2 doses of the rare A allele (i.e., AA vs GG) in *CYP3A4* rs35599367 was associated with 4.997-fold increase in odds for belonging in the mild class. Carrying 2 doses of the rare A allele (i.e., AA vs TA+TT) in *IL1RN* rs380092 was associated with 46.1% lower in odds for belonging in the mild class. Carrying 2 doses of the rare G allele (i.e., GG vs AG+AA) in *VDR* rs731236 was associated with 73.6% lower in odds for belonging in the mild class. Carrying 2 doses of the rare A allele (i.e., AA vs GG+GA) in *CYP19A1* rs1008805 was associated with 6.13-fold increase in the odds for belonging in the moderate class. Carrying 2 doses of the rare G allele (i.e., TG+GG vs TT) in *NOS3* rs1799983 was associated with 5.14-fold increase in the odds for belonging in the moderate class. Carrying 2 doses of the rare C allele (i.e., CC vs GC+GG) in *OPG* rs2073618 was associated with 4.50-fold increase in the odds for belonging in the moderate class. A significant interaction between *NOS3* rs1799983 and *OPG* rs2073618 (OR=4.77) was identified when exploring the genotypic factors of moderate class for musculoskeletal symptoms.

3.5 DISCUSSION

3.5.1 Inter-individual variability of pain and musculoskeletal symptoms (Phenotype)

Long-term longitudinal data are needed to understand the course and inter-individual variability of pain and musculoskeletal symptoms during the AI therapy. The number of longitudinal studies focusing on pain and musculoskeletal symptoms during AI therapy is very limited, as most of studies adopted a cross-sectional design. Previous longitudinal studies have relatively small sample sizes and a short follow-up period of 12 months post-initiation of therapy. Most previous studies focused on identifying the time of onset of pain and musculoskeletal symptoms, only a very few studies have reported pain severity and interference. Our study is the first to characterize the inter-individual variability of pain (severity and interference) and musculoskeletal symptoms among women with breast cancer undergoing the AI therapy. We extended the trajectories over the first 18 months of AI therapy, with a comparison cohort of postmenopausal women without breast cancer.

3.5.1.1 General pain

Only a few studies of pain with AI therapy have reported pain severity and interference, since most studies mainly focused on the onset of pain (occurrence). In our study, the group means of BPI pain severity and interference are mild and stable over the 18 months. The ranges of the group mean of pain severity and interference across the four-time assessment points are 1.56-1.92 and 1.22-1.40. This finding is consistent with other clinical studies that have reported the BPI pain scores, although the follow-up period of other studies is less than 12 months. Swenson et al. reported mild pain severity and interference at pretreatment (BPI severity [mean]=1.66 out of 10;

BPI interference [mean]=2.31) and at 6 months after AI initiation (BPI severity [mean] =1.08; BPI interference [mean]=1.75) (Swenson et al., 2013).

Except those who never developed pain during the AI therapy, the severity and interference of pain during AI therapy are persistent. We identified persistent mild and moderate trajectories for both pain severity and interference across the first 18 months of AI therapy. In our results, 45.1% of participants had constant mild pain severity, and 20.7% had moderate initial pain with linear increase over the 18 months. Although none of previous studies reported inter-individual trajectory patterns of pain, some demonstrated a similar trend. Laroche et al. also found that most of the pain (57%) that developed during the first 12 months of AI therapy was persistent and with high severity (60 out of 100) (Laroche et al., 2014). Singer et al. reported a mild increase trend in pain by a mean of 14.6mm (out of 100) (Singer et al., 2012). In addition, our study first examined the interindividual variability in the domain of pain severity at worst and identified a distinct trajectory of initial no pain with linear increase (7.7%) over the first 18 months. This subgroup trajectory is unique to the worst pain domain compared to the general pain severity domain. It is of utmost importance to identify this higher-risk subgroup in clinical practice. However, considering the relatively small percentage of participants in this class (7.7%), this finding needs to be further examined and confirmed by future research and clinical observation. For pain interference, we found 40.9% and 11.6% of participants reported constant mild and moderate interference respectively, which indicates that the pain interference during the AI therapy is persistent. Without effective strategies to manage pain, patients undergoing AI therapy may not effectively manage the pain and its related detrimental impact on daily lives by themselves.

In our study, 34.2% and 47.5% of participants never reported pain and interference with pain over the 18 months of therapy. In future research, it will be important to investigate why some

women may be free of pain during the AI therapy, and why a smaller subgroup of women who developed pain may experience no interference with pain. This finding also further introduces the importance of investigating a risk factor profile to predict the high-risk subgroup population.

3.5.1.2 Usage of analgesics

While analgesics (mostly nonsteroidal anti-inflammatory drugs) are authorized for use to manage pain with AI therapy, the outcome is not fully satisfactory. In our findings, 38.3% of participants used analgesics increasingly over the 18 months of therapy, nevertheless, we still identified a moderate trajectory of pain severity with linear increase trend and a moderate trajectory of pain interference remaining at a constant level. The percentage of our participants using analgesics was a bit less than the results from Shi's study, which reported that 50% of those with joint pain and 40% of those without joint pain used analgesia at 6 months after initiation of AI therapy (Shi et al., 2013). Concerns have been raised that degeneration of joints and deep tissues associated with AI use may be masked by opioid use (Cella & Fallowfield, 2008).

3.5.1.3 Musculoskeletal symptoms

In our study, the group means of BCPT musculoskeletal symptoms are mild and stable over the 18 months (Table 2). The ranges of group mean of BCPT musculoskeletal subscale across the four-time assessment points is 0.83-0.99. This finding is consistent but a bit lower than the results reported by Swenson. Swenson et al. reported mild musculoskeletal symptoms as measured by BCPT symptom checklist at pretreatment (mean=1.08±0.85 out of 4) and at 6 months after AI initiation (mean=1.7±1.18) (Swenson et al., 2013).

In our study, 79.1% of participants reported persistent joint pain over the course of 18 months: 61.2% of participants had mild joint pain at the initial assessment, prior to the initiation

of AI therapy with quadratic changes (increasing over the first 12 months and then decreasing from 12 to 18 months), and 18.7% had moderate initial joint pain with a linear increase over the 18 months post-initiation of therapy. Most previous studies reported that the average/median onset time of joint pain is before the first 3 months of AI initiation (6 weeks by Castel et al., 7 weeks by Shi et al. first 3 months by Mao et al.) (Castel et al., 2013; Shi et al., 2013; Mao et al., 2009). From the few studies that reported severity of pain, Mao reported that more than 60% of participants reported joint pain as moderate or severe (Mao et al., 2009). This is dramatically higher than our results related to pain severity (18.7%). Several reasons may explain this difference: 1) Mao adopted a cross-sectional design our study used a longitudinal design. During our longitudinal follow-up, those who experienced intolerable joint pain may be withdrawn from the parent study as they may have discontinued AI use; 2) in our study, including a subgroup of women without breast cancer who were not undergoing AI therapy may attenuate the proportion of subpopulation with more severe musculoskeletal symptoms. Castel reported that by week 6 of AI therapy, women experienced a significant increase in severity of their joint pain. Castel's trajectory analysis indicated that joint pain severity worsened over the first year of AI therapy, which is consistent with our findings (Castel et al., 2013). By extending the follow-up period to 18 months, we further demonstrated that, from 12 to 18 months, joint pain severity of 18.7% participants continued to worsen, while the pain severity of 61.2% of our participants decreased. Laroche et al., also found that some patients (22%) developed diffuse pain after 12 months of AI therapy, which leads to the hypothesis that estrogen deprivation may act on joints and tendons more rapidly than on the central nervous system, leading to a lag in the time for development of diffuse pain (Laroche et al., 2014). Both our findings and those of Laroche's suggest that women with AI therapy should undergo pain evaluation in a long-term manner over the course of AI therapy.

3.5.2 Phenotypic factors to pain and musculoskeletal symptoms

Phenotypic risk factors for pain and musculoskeletal symptoms are not well characterized. Current reported phenotypic risk factor profiles are mostly from cross-sectional studies and few longitudinal studies with relatively small sample sizes have been reported. There is inconclusive evidence for the reported risk factors. One of the main aims of our study was to examine the phenotypic risk factors for the individual variability of pain and musculoskeletal symptoms during AI therapy.

In our study, profiles of protective and risk factors across one or more phenotypes were identified. The protective factors include older age, receipt of chemotherapy, older age at first menstrual period, married/partnered, having an administrative level occupation (vs unskilled/unemployed), having regular periods for most of their lives, greater numbers of pregnancies, and having a history of tubal ligation. Risk factors include receipt of AI therapy, greater anxiety/pain severity/depressive symptoms/fatigue at baseline, and a history of arthritis, hysterectomy, or menopausal symptoms (Table 9).

In terms of demographic characteristics, we found that older age, currently married/partnered, and having an administrative level of occupation (vs unskilled/unemployed) were associated with an increase in odds of belonging to the class of constant no symptom in one or more phenotypes. Women who were older were less likely to belong to the mild or moderate trajectory of pain interference. This result is consistent with results from the studies of Menas et al. and Mao et al. 2011 (Menas et al., 2012; Mao et al, 2011), which showed that younger age was associated with less joint pain. In our results, women who were currently married were less likely to belong to the moderate trajectory of pain severity and more likely to not use analgesics during therapy. In our study, having an administrative level of occupation was a protective factor across

multiple phenotypes including pain severity, pain interference, usage of analgesics, joint pain, and musculoskeletal symptoms. However, Shi and Mao's studies reported that status of marriage and employment were not significantly associated with pain and musculoskeletal symptoms during AI therapy (Shi et al. 2013; Mao et al., 2009). The differences in demographic profiles between the studies may be the result of limited sample size of Shi's study and cross-sectional design for Mao's study. In addition, Shi and Mao did not examined the type of employment in their examination the employment as a predictor (Shi et al. 2013; Mao et al., 2009).

We found that receipt of chemotherapy was associated with increase in the odds of belonging to the constant no trajectory in pain severity. This result is different from others. Four studies reported that receipt of chemotherapy was a risk factor for the development of joint pain (Mao et al., 2011; Crew et al., 2007; Wang et al., 2013, Ohsako et al., 2006). However, three other studies demonstrated no significant association between joint pain and receipt of chemotherapy (Menas et al., 2012; Castel et al., 2013; Shi et al., 2013). Considering the relatively small sample size of these studies, these differences emphasize the need to further examine the effect of chemotherapy on pain and musculoskeletal symptoms in a diverse sample with a larger sample size.

Consistent with other studies, our findings confirmed that shorter hormonal exposure (older first menstrual period age) and stable hormonal status (regularity of periods for most of life, no history of menopausal symptoms, no history of hysterectomy) were associated with less pain and musculoskeletal symptoms. Mao and Castel's studies both reported that less time since last menstrual period (< 5 years) and less baseline severe menopausal symptoms were associated with less severe joint pain over the course of AI therapy (Mao et al., 2009; Castel et al., 2013). Laroche also reported that longer menopausal duration was a key predictive factor for the development of

pain during AI therapy (Laroche et al., 2014). Shi et al. reported that in the first 6 weeks of AI therapy, hormonal related symptoms (e.g., hot flashes, vaginal dryness, and decreased sexual activity) were positively associated with the emergence of joint pain (Shi et al., 2013). These findings confirmed the important role of estrogen suppression underlying the development and worsening the pain and musculoskeletal symptom during AI therapy.

We found that higher levels of psychological symptoms (anxiety, depression) and fatigue at baseline were associated with greater pain and musculoskeletal symptoms. Among the population of women with AI therapy, Laroche first demonstrated that psychological factors (e.g., greater anxiety at baseline, some personality traits) may be important predictors of pain (Laroche et al., 2014). In Shi et al.'s study, persistent fatigue was positively related to the presence of joint pain (Shi et al., 2013). Taken together, our findings reiterate the importance of assessment of pain within the context of psychological symptoms and fatigue in the clinical practice.

As all of our trajectory patterns in pain and musculoskeletal symptoms were distinctive from other since the baseline assessment, we observed that baseline pain and musculoskeletal symptoms were of utmost importance to determine subgroup membership. This observation is consistent with Shi and Henry's findings. In Shi's study, baseline pain was proven to be the only significant predictor of the development of joint pain during AI therapy, after adjustment for other covariates. Shi et al. reported that patients who experienced pain at baseline had 5 times the risk of developing joint pain (HR=5.55) compared to those who did not experience pain at baseline (Shi et al., 2013). Henry et al reported that patients with baseline pain were more likely to discontinue AI therapy due to intolerable symptoms (Henry et al., 2012). Additional research is needed to replicate this finding. This observation also has a particular implication for clinical practice, indicating assessment of pain and musculoskeletal symptoms before the initiation of AI

therapy is important to predict and select subgroups of women at higher risk to develop pain and musculoskeletal symptoms.

In our study, we found that history of arthritis (including osteoarthritis and rheumatoid arthritis) was a strong risk factor for the development of a mild and/or moderate trajectory across many phenotypes (worst pain severity, pain severity [mean], interference, join pain, muscle stiffness, and musculoskeletal symptoms). Castel also reported that preexisting joint-related comorbidity at baseline was a significant risk factor (OR=1.71) of more severe joint pain over the course of AI therapy (Castel et al., 2013).

3.5.3 Discussion on genotypic factors

The associations between variations in genes and inter-individual variability of pain and musculoskeletal symptoms are not well investigated. In our exploratory analysis, we identified a profile of protective and risk polymorphisms that are associated with mild and/or moderate trajectories of pain and musculoskeletal symptoms with AI therapy **(Table 11)**. The protective polymorphisms include: *BDNF* rs6265, *COMT* rs4633, *COMT* rs887200, *CXCL8* rs4073, *ESR2* rs2772163, *IL1B* rs16944, *RANKL* rs1054016, *VDR* rs4516035 and *VDR* rs731236. The risk polymorphisms include: *CYP19A1* rs1008805, *CYP3A4* rs35599367, *COMT* rs165774, *NOS3* rs1799983, *OPG* rs2073618, *OPRM1* rs1799971, and *TCL1A* rs7158782 and 7159713.

3.5.3.1 Polymorphisms associated with multiple phenotypes

In our study, variations in *CYP19A1* (rs1008805) and *NOS3* (rs1799983) were associated with membership across pain severity and musculoskeletal symptoms.

Gene *CYP19A1* is involved in regulation of the production and action of estrogen. The *CYP19A1* gene encodes aromatase, which is a critical enzyme to catalyze the biosynthesis of estrogen. Our findings indicate that carrying 2 doses of the rare A allele (i.e., AA vs GG+GA) in *CYP19A1* rs1008805 was associated with 11.27- and 6.13-fold increase in the odds for belonging in the moderate class in pain severity and musculoskeletal symptom, respectively. This result is consistent with Gervasini et al, who reported that *CYP19A1* rs1008805 was associated with arthralgias in 110 postmenopausal women with breast cancer treated with anastrozole (Gervasini et al., 2017). *CYP19A1* is a highly polymorphic gene. Other polymorphisms in *CYP19A1* have been associated with joint pain and musculoskeletal symptoms during AI therapy as well. From 390 Caucasian women with AI therapy for breast cancer, Mao et al. found that people who carry at least one $(TTTA)_7$ repeat alleles in rs60271534 were at higher risk of self-reported occurrence of arthralgias, whereas people carrying one or more $(TTTA)_8$ repeat alleles had lower risk (Mao et al., 2011). Other SNPs being examined but with no significant results in Mao et al.'s study included rs10046, rs749292, rs727479, and rs1157899. In a study of 109 Korean women with breast cancer under treatment with letrozole, Park et al. reported that the M_3_5 haplotype (composed of rs12148604, rs4646, rs10046, rs700519, rs4324076, rs700518, rs3759811, rs727479, rs4775936, rs10459592, rs767199, rs10519297, rs1062033, rs2008691, rs1008805, rs17523527) in *CYP19A1* was associated with self-reported occurrence of bone pain (Park et al., 2011). In a study of 737 Dutch patients under treatment with exemestane for breast cancer, homozygous *CYP19A1* rs934635-AA genotype was significantly associated with occurrence, but not severity, of musculoskeletal adverse events (including arthralgia, arthritis and osteoarthritis, myalgia, and other musculoskeletal problems) (Fontein et al., 2014).

Carrying 2 doses of the rare G allele (i.e., GG vs TT+TG) in *NOS3* rs1799983 was associated with 3.12- and 5.14-fold increase in the odds for belonging in the moderate class for pain severity and musculoskeletal symptoms, respectively. Variation in *NOS3* gene has not been reported to be associated with pain or musculoskeletal symptoms during AI therapy in current literature. *NOS3* gene encodes nitric oxide synthase 3 (NOS3), which is an important enzyme to synthesize nitric oxide (NO), primarily in the vascular endothelium, a monolayer of flat cells lining in the interior surface blood vessels. Therefore, the NOS is playing an essential role in the maintenance of function of the cardiovascular system. There is a growing body of evidence suggesting that NO is involved in skeletal muscle glucose uptake, control of skeletal muscle structure/function, skeletal muscle fiber type conversion, and mitochondrial ATP production and oxygen consumption in skeletal muscles (Gao, 2010; Martins et al., 2012). Although there is not a direct evidence to link the function of *NOS3* to pain and musculoskeletal symptoms, there is accumulative but inconclusive evidence to show the linkage between polymorphisms of *NOS3* and rheumatoid arthritis in both Caucasian and Chinese populations (Gonzalez-Gay et al., 2009; Nagy et al., 2010; Bunjevacki et al., 2016; An et al., 2012).

3.5.3.2 Synergetic effect between *NOS3* and *OPG*

We found a significant interaction between *NOS3* rs1799983 and *OPG* rs2073618 when exploring the genotypic factors of moderate class for musculoskeletal symptoms. We firstly found that carrying 2 doses of the rare C allele (i.e., CC vs GC+GG) in *OPG* rs2073618 was associated with 4.50-fold increase in the odds for belonging in the moderate class for musculoskeletal symptoms. This result is consistent with other studies. Litermans et al. found that *OPG* rs2073618 was significantly associated with the occurrence of musculoskeletal toxicity and severity of pain during AI therapy (Lintermans et al., 2016). This association was confirmed in Chinese Han

women with breast cancer as well (Wang et al., 2015). However, when we examined the interaction between *NOS3* rs1799983 and *OPG* rs2073618, their independent main effects became nonsignificant and a strong significance appeared. This finding suggests that some interactions between *NOS3* rs1799983 and *OPG* rs2073618 confer an increased risk of developing moderate trajectory of musculoskeletal symptoms. The synergetic effect between *NOS3* rs1799983 and *OPG* rs2073618 and its underlying mechanisms needs to be further examined in future studies.

3.6 CONCLUSION

Several limitations should be noted when interpreting the results of the present study. Firstly, with few African American women enrolled included in this analysis, lack of variation precluded examination of race as a risk factor and limited the generalizability of our results beyond the Caucasian population. Secondly, the use of analgesics was authorized in participants, thus our results may underestimate the actual severity of pain and musculoskeletal symptoms. Furthermore, the main focus of the parent study was not on the assessment of pain, thus the quality, severity and interference of pain at each specific body site was not phenotyped. In addition, this study included a select number of candidate genes and SNPs. Examination of the whole genome level is optimal and needed to replicate our findings and to identify additional genes and polymorphisms associated with pain and musculoskeletal symptoms.

This study is the first to characterize the inter-individual variability of pain and musculoskeletal symptoms during AI therapy for breast cancer. Our results show that a significant proportion of women experienced mild or moderate level of pain and musculoskeletal symptoms in a persistent manner over the first 18 months of AI therapy. A profile of protective and risk

factors across one or more phenotypes are identified. The protective phenotypic factors include older age, receipt of chemotherapy, older first menstrual period age, married/partnered, having an administrative level of occupation (vs unskilled/unemployed), having regular period for most of life, greater numbers of pregnancies, and having a history of tubal ligation. The phenotypic risk factors include receipt of AI therapy, greater anxiety/pain severity/depressive symptoms/fatigue at baseline, and history of arthritis/hysterectomy/menopausal symptoms. Variations in *CYP19A1* (rs1008805) and *NOS3* (rs1799983) were associated with membership across pain severity and musculoskeletal symptoms. A possible synergetic effect between *NOS3* rs1799983 and *OPG* rs2073618 was identified. Additional research is warranted to replicate this interaction among breast cancer survivors.

There are remaining gaps in the science of pain and musculoskeletal symptoms with AI therapy. Firstly, during the 5-10 years' course of AI therapy, the long-term changes of pain and musculoskeletal symptoms beyond the first 18 months to the completion (or even after the completion) are unknown. Extending the follow-up period beyond 18 months to after the completion of AI therapy is needed in the future study. Secondly, the limitation of design (cross-sectional design, small sample size, less diverse population etc.) of current studies preclude drawing causal-effect relationship and consistent conclusion. Further longitudinal studies in a diverse sample with a larger sample size are needed. In addition, the majority of previous studies (including our study) on the association between genetic variance and pain/musculoskeletal symptoms during AI therapy only included a select number of candidate genes and SNPs. Examination of the whole genome level is optimal and needed to identify additional genes and polymorphisms associated with pain and musculoskeletal symptoms.

Appendix A TABLES AND FIGURES IN PROPOSAL

Appendix Table 1 Search terms for literature review on musculoskeletal symptoms during AI therapy for breast cancer

Terms for Breast Cancer		Terms for Aromatase Inhibitor		Terms for Musculoskeletal Symptoms
• Breast Cancer • Breast Neoplasm	AND/ OR	• Aromatase Inhibitors • Antineoplastic Agents	AND/ OR	• Musculoskeletal Disease • Musculoskeletal Pain • Arthralgia • Myalgia • Stiffness

Appendix Table 2 Studies included in literature review on musculoskeletal symptoms for breast cancer

Author, Year	Country	Design	Instrument	Recall period	Domain
Aiello Bowles et al., 2012	USA	Cross-sectional (n=538)	Survey	At any point in endocrine therapy	Occurrence
Boonstra et al., 2013	Netherlands	Cross-sectional (n=57)	Rheumatoid Arthritis Disease Activity Index, FACT-ES	Past 7 days	Occurrence Intensity
Brown et al., 2014	USA	Cross-sectional (n=300)	WOMAC M-SACRAH Quick DASH	-	Occurrence Intensity
Chim et al., 2013	USA	Cross-sectional (n=437)	BPI	Past 24 hours	Occurrence Intensity Distress
Crew et al., 2007	USA	Cross-sectional (n=200)	Questionnaire adapted from BPI-SF	Past 7 days	Occurrence, Intensity
Dizdar et al., 2009	Turkey	Cross-sectional (n=92)	Patient Interview	Recently	Occurrence
Egawa et al., 2016	Japan	Longitudinal (pre- and 3, 6, 9, 12 months post-AI, n=391)	Questionnaire	-	Frequency Distress
Gallicchio et al., 2012	USA	Longitudinal (pre- and 3, 6 months post-AI, n=95)	Visual analog scale (VAS) Symptom checklist of 20 menopausal-type symptoms	Past 4 weeks	Occurrence Intensity Distress
Garreau et al., 2006	USA	Cross-sectional (n=452)	Questionnaire	-	Occurrence
Hadji et al., 2014	Germany	Longitudinal (pre- and 3, 6, 9 months post-study n=1916)	Rheumatoid Arthritis Symptom Questionnaire (RASQ)	-	Occurrence Intensity
Horimoto et al., 2009	Japan	Retrospective (n=329)	Chart Review	-	Occurrence
Hu et al., 2016	China	Retrospective review of case records (n=160)	Chart review	-	Occurrence
Kyvernitakis et al., 2014	Germany	Longitudinal (pre- and 12, 24 months post-AI, n=174)	Menopause rating scale (MRS)	-	Occurrence Intensity
Laroche et al., 2014	France	Longitudinal (pre- and 1, 3, 6, 12 months post-AI, n=135)	Visual analog scale (VAS), McGill Pain Questionnaire, Brief Pain Inventory (BPI)	-	Occurrence, Intensity, Distress
Lintermans et al., 2014	Belgium	Longitudinal (pre- and 3, 6, 12 months post-ET, n=292)	NSABP symptom checklist VAS Musculoskeletal questionnaire	Past 7 days	Occurrence intensity
Lu et al., 2011	China	Retrospective review of case records (n=271)	Telephone interview	-	Occurrence
Mao et al., 2009	USA	Cross-sectional (n=300)	Questionnaire	Past 7 days	Occurrence Intensity
Mao et al., 2011	USA	Cross-sectional (n=390)	Self-reported Arthralgia	-	Occurrence
Menas et al., 2012	USA	Cross-sectional (n=206)	Retrospective chart review	-	Occurrence

Napoli et al., 2010	USA	Cross-sectional (n=145)	Modified Leuven questionnaire	-	Occurrence Intensity
Obergugge nberger et al., 2011	Australia	Cross-sectional (n=280)	FACT-ES	Past 7 days	Occurrence Intensity
Ohsako et al., 2006	Japan	Longitudinal (n=53)	CTCAEver3.0	-	Occurrence Intensity
Olufade et al., 2015	USA	Cross-sectional (n=68)	Visual analog scale (VAS)	Past 4 weeks	Occurrence Intensity
Presant et al., 2007	USA	Semi-structured interview (n=56)	A linear analogue pain scale, location, character and treatment	-	Occurrence Intensity
Sagara et al., 2010	Japan	Longitudinal (n=656)	Symptom were collected retrospectively (no detail mentioned)	-	Occurrence
Servitja et al., 2012	Spain	Longitudinal (pre- and 3 months post-AI, n=343)	VAS	-	Occurrence Intensity
Shi et al., 2013	USA	Longitudinal (pre- and biweekly for 1 year, n=47)	BPI MDASI Joint Pain Assessment (JPA)	Past 24 hours Past 7 days	Occurrence Intensity Distress
Singer et al., 2012	USA	Longitudinal (pre- and 3, 6 months post-AI, n=52)	FACT-ES Global pain AUSCAN	Past 7 days	Occurrence Intensity
Swenson et al., 2013	USA	Longitudinal (pre- and 1, 3, 6 months post-AI, n=122)	BCPT Symptom Checklist AUSCAN WOMAC BPI QuickDASH	Past 24 hours Past 4 weeks	Occurrence Intensity Distress
Waltman et al., 2009	USA	Cross-sectional (n=29)	The Aromatase Inhibitor Questionnaire	Past 7 days	Occurrence Intensity Distress
Wang et al., 2013	China	Cross-sectional (n=436)	CTCAEver 3.0 WOMAC M-SACRAH BPI-SF	Past 7 days	Occurrence Intensity Distress

Appendix Table 3 Occurrence, intensity, and distress of musculoskeletal symptoms for breast cancer

Symptoms	Occurrence	Intensity	Distress
Joint/muscle pains	• 3.6% (Sagara, 2010) • 6% (Ohsako et al., 2006) • 27% (Horimoto, 2009) • 29.2% (Lu, 2011) • 31% (Waltman, 2009) • 32.6% (Dizdar, 2009) • 34% (Shi, 2013) • 36% (Laroche, 2014) • 44% (Hadji, 2014) • 46.3% (Brown, 2013) • 47% (Crew, 2007) (Mao, 2009) • 47.2% (Wang, 2013) • 48% (Menas, 2012) • 50.8% (Mao, 2011) • 54% (Singer, 2012) (Swenson, 2013) • 59.6% (Oberguggenberger, 2011) • 61% (Presant, 2007) • 61.3% (Napoli, 2010) • 64.7% (Olufade, 2015) • 65% (Boonstra, 2013) • 67.9% (Servitja, 2012) • 83.6% (Kyvernitakis, 2014) • 85.1% (Gallicchio, 2012_1) • 62.1%/45.7% (AI/TAM) (Aiello Bowles et al., 2012) • 22%/12% (AI/TAM) (Garreau, 2006) • 15%/25% (AI/TAM) (Hu, 2016)	• Mean=4.9 out of 10 (Boonstra, 2013) • Mean=5.23 out of 10 (Shi, 2013) • Median=7.5 out of 10 1-4 out of 10: 14% 5-7 out of 10: 16% 8-10 out of 10: 30% (Presant, 2007) • 34.6% have ≥ 4 (out of 10) pain at worst (Chim, 2013) • 31.5% moderate to severe (Crew, 2007) • 54 out of 100 (Kyvernitakis, 2014) • 31.5% ≥ moderate • 12.2% ≥ severe (Mao, 2009) • Grade 1: 4% Grade 2: 0% Grade 3: 2% (by CTCAE 3.0) (Ohsako et al., 2006) • Pre: mean=2.75 out of 10 (Servitja, 2012) • 1M: 0.341/0.284/0.219/0.227 • 6M: 0.552/0.476/0.467/0.368 (AUSCAN/WOMAC/QuickDASH/BPI: standardized mean difference to baseline) (Swenson, 2013)	• Mean=3.29 out of 10 (Shi, 2013) • Mild: 64% Moderate: 32.9% Severe: 3.1% (Egawa, 2016) • 1M: 0.274/0.272 (BCPT/BPI) 6M: 0.62/0.282 (BCPT/BPI) (standardized mean difference to baseline) (Swenson, 2013) • 48% 7% (interference only with athletic activity) 31% (interference with function but not ADLs) 10% (interference with ADLs) (Waltman, 2009)

	• Pre: 26.1% • 3M: 38.8% • 6M: 46.3% • 9M: 49.5% • 12M: 54.4% (Egawa, 2016) • 89%/65% (AI/TAM) (Litermans, 2014) • Pre: 66%/50% (AI/TAM) (Litermans, 2014)		
Joint/Muscle stiffness	• 25% (Ohsako et al., 2006) • 28% (Waltman, 2009) • 41% (Rosenberg et al., 2015) • 44% (Crew, 2007) • 72% (Boonstra, 2013) • Pre: 19.4% 3M: 39.1% 6M: 46.9% 9M: 50.2% 12M: 59.7% (Egawa, 2016)	• 29% ≥ moderate (Crew, 2007) • Grade 1: 17% Grade 2: 2% Grade 3: 6% (by CTCAE 3.0) (Ohsako et al., 2006)	• Mild: 63.2% Moderate: 31.6% Severe: 5.2% (Egawa, 2016)
Back Pain	• 2% (Hadji, 2014)		
Numbness or tingling	• 47%/32% (AI/TAM) (Lintermans, 2014_2)		

M: Month; TAM: Tamoxifen; AI: Aromatase Inhibitor

Appendix Table 4 Phenotypic factors associated with musculoskeletal symptoms for breast cancer

	Phenotypic factors		Study with Significant results	OR (95% CI)	Study with Non-significant results
Joint pain	BMI=25-30 (vs <25)	↓	Crew (2007)	0.33 (0.14, 0.74)	Shi (2013) Mao (2011)
	Prior tamoxifen therapy	↓	Crew (2007)	0.40 (0.19, 0.87)	
	Sexual functioning	↓	Laroche (2014)		
	Time since last menstrual period (<5 years)	↓	Mao (2009) Mao (2011)	3.39 (1.21, 9.44)	
	Age	↓	Menas (2012) Mao (2011)		Mao (2009) Shi (2013)
	Less baseline severe menopausal symptoms	↓	Castel (2013)	0.97 (0.95, 0.99)	
	Menopause duration	↑	Laroche (2014)		
	Severe breast symptoms	↑	Laroche (2014)		
	Prior chemotherapy	↑	Mao (2011) Crew (2007) Wang (2013)	4.08 (1.58, 10.57)	Mao (2009) Menas (2012) Castel (2013) Shi (2013)
	Joint-related comorbidity	↑	Castel (2013)	1.71 (1.12, 2.61)	
	Presence of pain at baseline	↑	Shi (2013)	10.66 (1.51, 75.89)	
	Vitamin D insufficiency	↑	Waltman (2009)		
	Weight gain since breast cancer	↑	Su (2010)	2.15 (1.04, 4.42)	Mao (2009)
	Race (White vs nonwhite)				Mao (2009) Shi (2013)
	Employment				Mao (2009) Shi (2013)
	# of comorbidities				Mao (2009)
	Marital status				Shi (2013)
	Education				Shi (2013)
Muscle pain	Prior taxane-based chemotherapy	↑	Crew (2007)	4.76 (1.84, 12.28)	
Joint stiffness	Prior chemotherapy	↑	Ohsako (2006)		

OR: Odds Ratio; ↑: risk factor; ↓: protective factor

Appendix Table 5 Candidate genes and SNPs

Gene	SNP	Position	Chr	MAF	Alleles
BDNF	rs6265	27658369	11	0.201	C>T
CCL2	rs4586	34256250	17	0.458	T>C
CXCL8	rs4073	73740307	4	0.478	A>T
CYP17A1	rs4919683	102825368	10	0.446	C>A
	rs4919687	102835491	10	0.187	G>A
CYP19A1	rs10046	51210789	15	0.362	G>A
	rs752760	51339282	15	0.368	C>T
	rs1008805	51257402	15	0.353	G>A
	rs934635	51186580	15	0.123	G>A
	rs4646	51210647	15	0.336	A>C
CYP27B1	rs4646536	57764205	12	0.410	A>G
	rs10877012	57768302	12	0.349	G>T
CYP2A6	rs28399433	40850474	19	0.128	A>C
	rs1801272	40848628	19	0.009	A>T
CYP3A4	rs35599367	99768693	7	0.015	G>A
ESR1	rs9322336	151879295	6	0.236	C>T
ESR2	rs4986938	64233098	14	0.260	C>T
	rs1152582	64225912	14	0.311	G>C
	rs2772163	64226667	14	0.202	G>A
IGF1	rs5742612	102481086	12	0.115	A>G
	rs6214	102399791	12	0.427	C>T
IL1A	rs3783521	112786000	2	0.339	G>A
IL1B	rs16944	112837290	2	0.491	A>G
	rs1143633	112832890	2	0.311	C>T
IL1RN	rs4251961	113116890	2	0.242	T>C
	rs380092	113131323	2	0.443	T>A
LIF	rs929271	30242237	22	0.294	T>G
	rs737812	30243121	22	0.279	C>A
MMP13	rs597315	102957055	11	0.325	A>T
NOS3	rs1799983	150999023	7	0.176	T>G
TCL1A	rs11849538	95709641	14	0.253	C>G
	rs7158782	95702794	14	0.366	A>G
	rs7159713	95703240	14	0.366	A>G
	rs2369049	95705514	14	0.324	A>G
OPG (TNFRSF11B)	rs2073618	118951813	8	0.333	G>C
	rs2073617	118952044	8	0.378	G>A
RANKL (TNFSF11)	rs1054016	42607866	13	0333	G>T
VDR	rs739837	47844438	12	0.494	G>T
	rs731236	47844974	12	0.277	A>G
	rs4516035	47906043	12	0.177	T>C
WNT5A	rs1829556	55467147	3	0.466	T>C
COMT	rs887200	19976143	22	0.366	C>T
	rs165774	19965038	22	0.203	G>A
	rs4633	19962712	22	0.371	C>T
IL-6	rs1800795	22727026	7	0.141	C>G
OPRM1	rs1799971	154039662	6	0.223	A>G

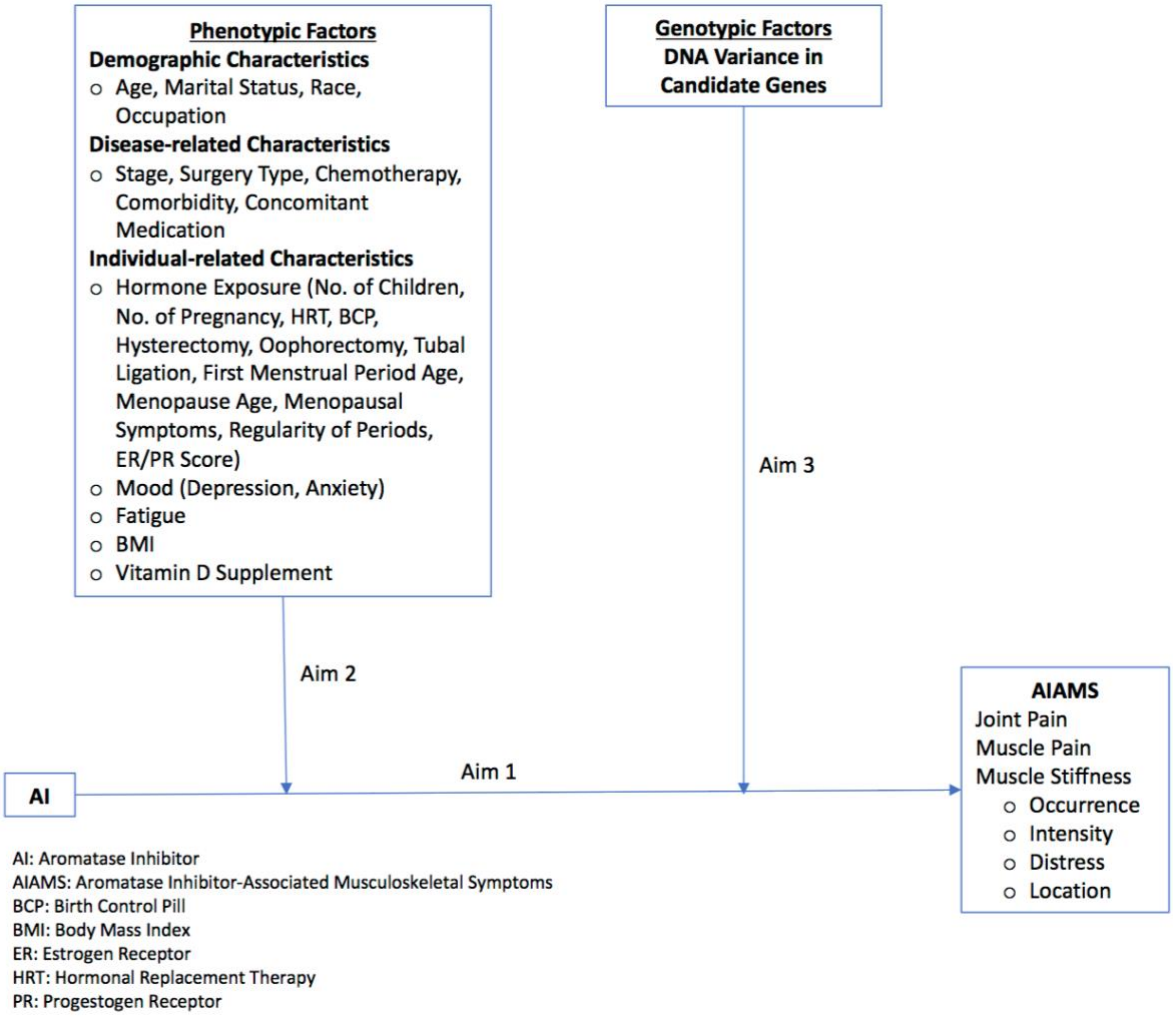


Figure 1. Variable Framework

Appendix B TABLES AND FIGURES IN MANUSCRIPT

Appendix Table 6 Socio-demographic and clinical characteristics of participants at baseline (n=380)

Characteristics	Breast cancer cohorts (n=250)	Healthy control (n=130)	All participants (n=380)
Age, mean (SD)	61.4 (6.1)	59.0 (5.8)	60.6 (6.07)
Caucasian (yes, %)	242 (96.8)	119 (91.5)	361 (95.0)
Currently Married/Partnered, (yes, %)	173 (69.2)	74 (56.9)	247 (65.0)
Occupation			
Level 1 (administrative)	137 (54.8)	103 (79.2)	240 (63.2)
Level 2 (skilled)	23 (9.2)	6 (4.6)	29 (7.7)
Level 3 (unskilled/unemployed)	90 (36.0)	21 (16.2)	111 (29.1)
First Menstrual period Age	12.3 (1.4)	12.7 (1.5)	12.4 (1.5)
# of Children, mean (SD)	2 (1)	2 (1)	2 (1)
# of Pregnant, mean (SD)	2 (2)	2 (2)	2 (2)
Hormonal Replacement Therapy (yes, %)	109 (43.6)	56 (43.1)	165 (43.4)
Birth Control Pills (yes, %)	175 (70.0)	97 (74.6)	272 (71.6)
Hysterectomy (yes, %)	63 (25.2)	25 (19.2)	88 (23.2)
Oophorectomy (yes, %)	46 (18.8)	21 (16.2)	67 (17.9)
History of Menopause Symptoms (yes, %)	200 (80.0)	106 (81.5)	306 (80.5)
Regularity of Periods (yes, %)	217 (86.8)	109 (83.8)	326 (85.8)
Pregnancy (yes, %)	206 (82.4)	109 (83.8)	315 (82.9)
Tubal Ligation (yes, %)	80 (32.0)	39 (30.0)	119 (31.3)
History of Arthritis (yes, %)	95 (38.0)	26 (20.0)	121 (31.8)
Baseline BDI II Total, mean (SD)	5.7 (5.4)	4.8 (5.8)	5.4 (5.5)
Baseline POMS Tension-Anxiety, mean (SD)	6.7 (5.1)	6.0 (5.0)	6.5 (5.1)
Baseline POMS Fatigue-Inertia, mean (SD)	6.3 (6.5)	5.1 (5.2)	5.9 (6.1)
Stage			
I	167 (66.8)	NA	NA
II	70 (28.0)		
IIIa	13 (5.2)		
Chemotherapy (yes, %)	77 (30.8)	NA	NA
Breast Cancer Surgery			
Modified Radical Mastectomy	11 (4.4)	NA	NA
Breast Conserving Surgery	239 (95.6)		

Abbreviations: BDI, Beck Depression Inventory; POMS, Profile of Mood States.

Appendix Table 7 Observed and model-estimated subgroups of trajectories for pain and musculoskeletal symptoms

	Baseline	6 months	12 months	18 months
BPI worst pain				
Group, mean (SD)	2.51 (3.09)	3.05 (3.43)	2.87 (3.30)	3.05 (3.37)
Constant no (observed)	0.37	0.02	0	0
Initial no with linear increase (observed)	0.01	0.24	2.08	5.01
Constant mild (observed)	2.15	2.76	2.37	1.97
Constant moderate (observed)	5.08	6.25	5.89	6.08
Constant no (estimated), mean (95% CI)	0.24 (0, 1.04)	0.01 (0, 0.61)	0 (0, 0.05)	0 (0, 0)
Initial no with linear increase (estimated)	0.01 (0, 0.54)	0.19 (0, 1.77)	1.58 (0.19, 2.97)	5.02 (1.25, 8.79)
Constant mild (estimated)	2.10 (1.26, 2.94)	2.10 (1.26, 2.94)	2.10 (1.26, 2.94)	2.10 (1.26, 2.94)
Constant moderate (estimated)	5.60 (4.54, 6.65)	5.60 (4.54, 6.65)	5.60 (4.54, 6.65)	5.60 (4.54, 6.65)
BPI severity				
Group, mean (SD)	1.56 (2.06)	1.92 (2.35)	1.86 (2.25)	1.92 (2.31)
Constant no (observed)	0.15	0.12	0.07	0.18
Constant mild (observed)	1.50	1.88	1.94	1.74
Moderate with linear increase (observed)	4.02	4.94	4.77	5.20
Constant no (estimated), mean (95% CI)	0.08 (0, 0.48)	0.08 (0, 0.48)	0.08 (0, 0.48)	0.08 (0, 0.48)
Constant mild (estimated)	1.66 (1.03, 2.28)	1.66 (1.03, 2.28)	1.66 (1.03, 2.28)	1.66 (1.03, 2.28)
Moderate with linear increase (estimated)	4.18 (3.22, 5.14)	4.54 (3.78, 5.29)	4.90 (4.15, 5.65)	5.27 (4.32, 6.21)
BPI interference				
Group, mean (SD)	1.22 (2.06)	1.38 (2.08)	1.36 (2.06)	1.40 (2.15)
Constant no (observed)	0.14	0.14	0.12	0.18
Constant mild (observed)	1.36	1.75	1.84	1.63
Constant moderate (observed)	5.11	5.12	4.95	5.26
Constant no (estimated), mean (95% CI)	0.14 (0, 0.34)	0.14 (0, 0.34)	0.14 (0, 0.34)	0.14 (0, 0.34)
Constant mild (estimated)	1.65 (1.16, 2.14)	1.65 (1.16, 2.14)	1.65 (1.16, 2.14)	1.65 (1.16, 2.14)
Constant moderate (estimated)	5.09 (4.34, 5.83)	5.09 (4.34, 5.83)	5.09 (4.34, 5.83)	5.09 (4.34, 5.83)
BPI usage of analgesics				
Group, yes (%)	117 (30.8)	121 (32.4)	108 (37.2)	93 (37.7)
Constant no (observed)	0.10	0.08	0.09	0.14
Linear Increase (observed)	0.64	0.72	0.83	0.79
Constant no (estimated), mean (95% CI)	0.10 (0.06, 0.13)	0.10 (0.06, 0.13)	0.10 (0.06, 0.13)	0.10 (0.06, 0.13)
Linear Increase (estimated)	0.66 (0.57, 0.74)	0.72 (0.66, 0.79)	0.78 (0.71, 0.85)	0.83 (0.75, 0.91)
BCPT musculoskeletal subscale (mean)				
Group, mean (SD)	0.83 (0.73)	0.97 (0.78)	0.97 (0.77)	0.99 (0.75)
Constant no (observed)	0.39	0.43	0.45	0.48
Mild linear increase (observed)	1.03	1.24	1.25	1.27
Constant moderate (observed)	2.18	2.42	2.52	2.40
Constant no (estimated), mean (95% CI)	0.44 (0.35, 0.52)	0.44 (0.35, 0.52)	0.44 (0.35, 0.52)	0.44 (0.35, 0.52)
Mild linear increase (estimated)	1.08 (0.96, 1.20)	1.16 (1.06, 1.26)	1.24 (1.14, 1.35)	1.32 (1.19, 1.45)
Constant moderate (estimated)	2.37 (2.26, 2.47)	2.37 (2.26, 2.47)	2.37 (2.26, 2.47)	2.37 (2.26, 2.47)
BCPT joint pain				
Group, mean (SD)	1.14 (1.05)	1.43 (1.16)	1.45 (1.19)	1.45 (1.14)
Constant no (observed)	0.21	0.16	0.18	0.30
Mild quadratic (observed)	1.00	1.36	1.35	1.37
Moderate linear increase (observed)	2.56	2.97	3.21	3.12
Constant no (estimated), mean (95% CI)	0.14 (0.08, 0.20)	0.14 (0.08, 0.20)	0.14 (0.08, 0.20)	0.14 (0.08, 0.20)
Mild quadratic (estimated)	0.98 (0.78, 1.18)	1.29 (1.08, 1.51)	1.42 (1.20, 1.64)	1.34(1.08, 1.59)
Moderate linear increase (estimated)	2.70 (2.36, 3.04)	2.92 (2.68, 3.17)	3.13 (2.88, 3.39)	3.32 (3.02, 3.62)
BCPT muscle stiffness				
Group, mean (SD)	0.94 (0.98)	1.10 (1.07)	1.10 (1.05)	1.16 (1.03)
Constant no (observed)	0.12	0.09	0.13	0.17
Mild linear increase (observed)	0.89	1.05	1.08	1.16
Moderate linear increase (observed)	2.35	2.73	2.88	2.87
Constant no (estimated), mean (95% CI)	0.08 (0, 0.19)	0.08 (0, 0.19)	0.08 (0, 0.19)	0.08 (0, 0.19)
Mild linear increase (estimated)	0.88 (0.70, 1.06)	0.97 (0.82, 1.12)	1.07 (0.90, 1.24)	1.17 (0.94, 1.40)
Moderate linear increase (estimated)	2.46 (2.06, 2.86)	2.65 (2.34, 2.94)	2.82 (2.51, 3.14)	2.99 (2.57, 3.42)

Abbreviations: BPI, Brief Pain Inventory; BCPT, Breast Cancer Prevention Trial Symptom Checklist.

Appendix Table 8 Significant phenotypic predictors to subgroup membership of pain and musculoskeletal symptoms (n=380)

Predictors	B	SE	OR	95% CI for OR	*p*
BPI Worst Pain: initial no with linear increase vs constant no					
AI therapy	1.433	0.508	4.189	1.547, 11.347)	0.005
Numbers of pregnancies	-0.337	0.162	0.714	0.519, 0.980)	0.037
BPI Worst Pain: constant mild vs constant no					
AI therapy	1.205	0.289	3.338	1.893, 5.887)	<0.001
History of arthritis	1.204	0.355	3.335	1.664, 6.684)	0.001
BDI II total (sum)	0.100	0.033	1.105	1.037, 1.178)	0.002
BPI Worst Pain: constant moderate vs constant no					
AI therapy	1.576	0.348	4.833	2.445, 9.554)	<0.001
History of tubal ligation	-0.783	0.363	0.457	0.224, 0.930)	0.031
History of arthritis	2.418	0.369	11.227	5.443, 23.155)	<0.001
BDI II total (sum)	0.195	0.035	1.215	1.135, 1.301)	<0.001
BPI Pain Severity: constant mild vs constant no					
Chemotherapy	-0.781	0.377	0.458	0.219, 0.960)	0.039
AI therapy	1.365	0.300	3.914	2.172, 7.052)	<0.001
History of arthritis	1.089	0.330	2.971	1.557, 5.668)	0.001
BDI II total (sum)	0.105	0.039	1.111	1.030, 1.199)	0.007
BPI Pain Severity: moderate linear increase vs constant no					
Currently married/partnered	-0.813	0.398	0.444	0.203, 0.968)	0.041
Occupation (level 1 vs 3)	-1.218	0.439	0.296	0.125, 0.700)	0.006
AI therapy	1.680	0.481	5.366	2.089, 13.785)	<0.001
History of hysterectomy	1.130	0.430	3.095	1.334, 7.184)	0.009
History of arthritis	2.359	0.412	10.584	4.716, 23.753)	<0.001
BDI II Total (sum)	0.143	0.046	1.154	1.055, 1.262)	0.002
POMS Fatigue-Inertia (sum)	0.130	0.039	1.139	1.055, 1.229)	0.001
BPI Pain Interference: constant mild vs constant no					
Occupation (level 1 vs 3)	-1.299	0.403	0.273	0.124, 0.601)	0.001
AI therapy	0.801	0.319	2.227	1.192, 4.160)	0.012
History of hysterectomy	0.702	0.344	2.017	1.028, 3.958)	0.041
History of arthritis	0.846	0.330	2.331	1.221, 4.449)	0.010
Age	-0.056	0.028	0.945	0.896, 0.998)	0.043
POMS Fatigue-Inertia (sum)	0.074	0.035	1.077	1.006, 1.153)	0.032
BPI severity at baseline	0.844	0.119	2.326	1.843, 2.935)	<0.001
BPI Pain Interference: constant moderate vs constant no					
Occupation (level 1 vs 3)	-2.117	0.668	0.120	0.032, 0.446)	0.002
AI therapy	1.540	0.653	4.663	1.296, 16.778)	0.018
History of hysterectomy	1.472	0.571	4.356	1.421, 13.351)	0.010
History of arthritis	1.926	0.573	6.859	2.231, 21.086)	0.001
Age	-0.143	0.052	0.867	0.784, 0.959)	0.006
BDI II Total (sum)	0.187	0.053	1.205	1.087, 1.337)	<0.001
POMS Tension-Anxiety (sum)	0.106	0.054	1.112	1.000, 1.237)	0.051
BPI severity at baseline	1.368	0.161	3.926	2.861, 5.388)	<0.001
BPI Usage of analgesics: linear increase vs constant no					
Currently married/partnered	-0.696	0.292	0.498	0.281, 0.884)	0.017
Occupation (level 1 vs 3)	-1.011	0.327	0.364	0.192, 0.691)	0.002
AI therapy	1.544	0.340	4.682	2.407, 9.108)	<0.001
First menstrual period age	-0.284	0.102	0.753	0.616, 0.920)	0.006
POMS Tension-Anxiety (sum)	0.084	0.028	1.087	1.030, 1.148)	0.002
BPI severity at baseline	0.665	0.083	1.944	1.652, 2.288)	<0.001
BCPT Musculoskeletal Symptoms: mild linear increase vs constant no					
AI therapy	0.661	0.275	1.937	1.129, 3.323)	0.016
History of arthritis	1.173	0.274	3.233	1.888, 5.535)	<0.001
BDI II Total (sum)	0.073	0.034	1.076	1.007, 1.150)	0.031
POMS Fatigue-Inertia (sum)	0.110	0.031	1.117	1.051, 1.186)	<0.001
BCPT Musculoskeletal Symptoms: constant moderate vs constant no					
Occupation (level 1 vs 3)	-1.383	0.516	0.251	0.091, 0.690)	0.007
History of hysterectomy	1.063	0.516	2.894	1.053, 7.955)	0.039
History of arthritis	2.312	0.474	10.095	3.990, 25.539)	<0.001
BDI II Total (sum)	0.149	0.045	1.161	1.063, 1.267)	0.001

Predictors	B	SE	OR	95% CI for OR	*p*
POMS Fatigue-Inertia (sum)	0.229	0.042	1.257	1.157, 1.366)	<0.001
BCPT Joint Pain: mild quadratic vs constant no					
Occupation (level 1 vs 3)	-1.403	0.447	0.246	0.102, 0.590)	0.002
Regularity of period for most of life	-1.012	0.492	0.363	0.139, 0.953)	0.040
History of arthritis	1.150	0.404	3.159	1.431, 6.970)	0.004
POMS Fatigue-Inertia (sum)	0.198	0.046	1.219	1.114, 1.335)	<0.001
BCPT Joint Pain: moderate linear vs constant no					
Occupation (level 1 vs 3)	-1.942	0.544	0.143	0.049, 0.416)	<0.001
History of menopausal symptoms	1.530	0.557	4.618	1.549, 13.770)	0.006
Regularity of period for most of life	-1.789	0.615	0.167	0.050, 0.559)	0.004
History of arthritis	2.614	0.488	13.660	5.253, 35.522)	<0.001
POMS Fatigue-Inertia (sum)	0.334	0.051	1.397	1.264, 1.543)	<0.001
BCPT Muscle Stiffness: mild linear increase vs constant no					
AI therapy	0.673	0.276	1.960	1.141, 3.368)	0.015
History of hysterectomy	0.971	0.380	2.640	1.253, 5.564)	0.011
POMS Fatigue-Inertia (sum)	0.110	0.041	1.116	1.029, 1.210)	0.008
BCPT Muscle Stiffness: moderate linear increase vs constant no					
History of hysterectomy	1.060	0.556	2.887	0.972, 8.578)	0.056
History of arthritis	1.683	0.476	5.382	2.118, 13.675)	<0.001
BDI II Total (sum)	0.135	0.051	1.145	1.035, 1.265)	0.008
POMS Fatigue-Inertia (sum)	0.257	0.050	1.293	1.173, 1.424)	<0.001

Abbreviations: BPI, Brief Pain Inventory; BCPT, Breast Cancer Prevention Trial Symptom Checklist; BDI, Beck Depression Inventory; POMS, Profile of Mood States.

Appendix Table 9 Summary of significant predictors to subgroup membership across pain and musculoskeletal symptoms

	BPI Worst Pain			BPI Pain Severity		BPI Pain Interference		Usage of Analgesics	BCPT Joint Pain		BCPT Muscle Stiffness		BCPT MSK symptoms	
	INCR	MID	MOD	MID	MOD	MID	MOD	Use	MID	MOD	MID	MOD	MID	MOD
Age						↓	↓							
Chemotherapy				↓										
First menstrual period age								↓						
Married/Partnered					↓			↓						
Occupation (admi vs unskilled)					↓	↓	↓	↓	↓					↓
Regularity of period for most of life									↓					
Numbers of pregnancies	↓													
History of tubal ligation			↓											
AI therapy	↑	↑	↑	↑	↑	↑	↑	↑			↑		↑	
Anxiety							B (↑)	↑						
BPI severity at baseline						↑	↑	↑						
Depressive symptoms		↑	↑	↑	↑		↑					↑	↑	↑
Fatigue					↑	↑			↑	↑	↑	↑	↑	↑
History of arthritis		↑	↑	↑	↑	↑	↑		↑	↑		↑	↑	↑
History of hysterectomy					↑	↑	↑				↑	B (↑)		↑
History of menopausal symptoms										↑				

B: borderline significant; **INCR**: increase; **MID**: mild; **MOD**: moderate; **MSK**: musculoskeletal.
↑: increased risk for mild or moderate trajectory; ↓: decreased risk for mild or moderate trajectory

Appendix Table 10 Multinomial logistic regression analyses for candidate genes and subgroup membership of pain and musculoskeletal symptoms (n=243)

Predictor	B	Standard Error	*p* value	Odds Ratio	95% CI
BPI Worst Pain: initial no with linear increase vs constant no					
ESR2 rs2772163 (Dom)	-2.385	0.821	0.004	0.092	0.018, 0.460
AI therapy	1.887	0.715	0.008	6.599	1.625, 26.791
Numbers of pregnancies	-0.733	0.283	0.010	0.480	0.276, 0.837
LIF rs737812 (Dom)	1.330	0.694	0.055	3.782	0.971, 14.724
AI therapy	1.542	0.673	0.022	4.673	1.249, 17.483
Numbers of pregnancies	-0.572	0.240	0.017	0.565	0.352, 0.904
TCL1A rs7158782 (Add)	3.174	1.550	0.041	23.904	1.146, 498.589
AI therapy	1.580	0.671	0.019	4.856	1.304, 18.086
Numbers of pregnancies	-0.566	0.241	0.019	0.568	0.354, 0.911
TCL1A rs7159713 (Add)	3.222	1.545	0.037	25.069	1.212, 518.296
AI therapy	1.495	0.676	0.027	4.459	1.184, 16.786
Numbers of pregnancies	-0.589	0.249	0.018	0.555	0.341, 0.905
BPI Worst Pain: constant mild vs constant no					
VDR rs731236 (Dom)	-0.812	0.398	0.041	0.444	0.203, 0.969
AI therapy	1.486	0.386	<0.001	4.419	2.073, 9.418
History of arthritis	1.678	0.544	0.002	5.355	1.842, 15.567
BDI II total (sum)	0.075	0.046	0.108	1.078	0.984, 1.180
BPI Worst Pain: constant moderate vs constant no					
CXCL8 rs4073 (Rec)	-1.588	0.673	0.018	0.204	0.055, 0.764
AI therapy	2.460	0.598	<0.001	11.709	3.626, 37.807
History of arthritis	3.705	0.737	<0.001	40.656	9.595, 172.257
History of tubal ligation	-1.156	0.583	0.047	0.315	0.100, 0.986
BDI II total (sum)	0.304	0.075	<0.001	1.356	1.171, 1.570
CYP19A1 rs1008805 (Rec)	1.463	0.588	0.013	4.318	1.364, 13.668
AI therapy	2.013	0.533	<0.001	7.487	2.635, 21.275
History of arthritis	3.383	0.664	<0.001	29.461	8.023, 108.174
History of tubal ligation	-1.152	0.576	0.046	0.316	0.102, 0.978
BDI II total (sum)	0.266	0.067	<0.001	1.304	1.143, 1.488
VDR rs4516035 (Add)	-1.524	0.597	0.011	0.218	0.068, 0.702
AI therapy	1.935	0.532	<0.001	6.926	2.440, 19.659
History of arthritis	3.322	0.661	<0.001	27.704	7.588, 101.149
History of tubal ligation	-1.522	0.596	0.011	0.218	0.068, 0.702
BDI II total (sum)	0.231	0.063	<0.001	1.260	1.114, 1.425
BPI Pain Severity: constant mild vs constant no					
RANKL rs1054016 (Dom)	-0.827	0.357	0.021	0.438	0.217, 0.881
Chemotherapy	-0.979	0.499	0.050	0.376	0.141, 0.999
AI therapy	1.330	0.377	<0.001	3.780	1.806, 7.910
History of arthritis	1.267	0.446	0.005	3.550	1.481, 8.509
BDI II total (sum)	0.149	0.048	0.002	1.161	1.057, 1.276
BPI Pain Severity: moderate linear increase vs constant no					
CYP19A1 rs1008805 (Rec)	2.422	0.862	0.005	11.271	2.080, 61.081
Occupation (level 1 vs 3)	-1.649	0.708	0.020	0.192	0.048, 0.770
AI therapy	1.386	0.731	0.058	3.999	0.954, 16.764
History of hysterectomy	2.582	0.882	0.003	13.223	2.345, 74.552
History of arthritis	4.135	0.899	<0.001	62.487	10.736, 363.679
BDI II Total (sum)	0.249	0.099	0.012	1.283	1.056, 1.559
POMS Fatigue-Inertia (sum)	0.202	0.084	0.016	1.224	1.038, 1.443
NOS3 rs1799983 (Rec)	1.137	0.578	0.049	3.116	1.003, 9.682
AI therapy	1.654	0.640	0.010	5.226	1.491, 18.319
History of hysterectomy	1.943	0.763	0.011	6.978	1.564, 31.135
History of arthritis	3.264	0.671	<0.001	26.150	7.021, 97.387
BDI II Total (sum)	0.227	0.083	0.006	1.255	1.067, 1.476
POMS Fatigue-Inertia (sum)	0.126	0.062	0.040	1.135	1.006, 1.280
COMT rs887200 (Rec)	-1.868	0.808	0.021	0.154	0.032, 0.753
Occupation (level 1 vs 3)	-1.667	0.684	0.015	0.189	0.049, 0.721
AI therapy	1.112	0.680	0.102	3.041	0.802, 11.531
History of hysterectomy	2.038	0.820	0.013	7.677	1.539, 38.302

Predictor	B	Standard Error	*p* value	Odds Ratio	95% CI
History of arthritis	3.828	0.799	<0.001	45.968	9.604, 220.016
BDI II Total (sum)	0.236	0.101	0.019	1.266	1.039, 1.544
POMS Fatigue-Inertia (sum)	0.162	0.075	0.030	1.176	1.016, 1.361
COMT rs4633 (Rec)	-2.046	0.865	0.018	0.129	0.024, 0.705
Occupation (level 1 vs 3)	-1.731	0.692	0.012	0.177	0.046, 0.687
History of hysterectomy	2.724	0.849	0.001	15.245	2.886, 80.522
History of arthritis	3.803	0.813	<0.001	44.821	9.107, 220.595
BDI II Total (sum)	0.225	0.099	0.023	1.252	1.031, 1.520
POMS Fatigue-Inertia (sum)	0.193	0.079	0.015	1.212	1.038, 1.416
BPI Pain Interference: constant mild vs constant no					
VDR rs4516035 (Dom)	-0.853	0.398	0.032	0.426	0.195, 0.929
Occupation (level 1 vs 3)	-1.265	0.473	0.008	0.282	0.112, 0.714
AI therapy	0.948	0.401	0.018	2.580	1.176, 5.662
History of hysterectomy	0.850	0.461	0.065	2.340	0.948, 5.776
Age	-0.089	0.035	0.011	0.915	0.854, 0.980
BPI severity at baseline	1.117	0.187	<0.001	3.056	2.118, 4.408
BCPT Joint Pain: mild quadratic vs constant no					
BDNF rs6265 (Dom)	-0.821	0.395	0.038	0.440	0.203, 0.955
Occupation (level 1 vs 3)	-1.230	0.512	0.016	0.292	0.107, 0.797
Regularity of period for most of life	-1.279	0.627	0.041	0.278	0.081, 0.951
History of arthritis	1.170	0.540	0.030	3.220	1.117, 9.289
POMS Fatigue-Inertia (sum)	0.215	0.058	<0.001	1.240	1.106, 1.390
BCPT Muscle Stiffness: mild linear increase vs constant no					
IL1A rs3783521 (Dom)	0.693	0.367	0.059	2.000	0.974, 4.107
History of hysterectomy	1.733	0.647	0.007	5.658	1.593, 20.096
POMS Fatigue-Inertia (sum)	0.181	0.056	0.001	1.198	1.074, 1.337
IL1B rs16944 (Rec)	-0.804	0.360	0.026	0.448	0.221, 0.907
History of hysterectomy	1.403	0.576	0.015	4.069	1.317, 12.574
POMS Fatigue-Inertia (sum)	0.192	0.057	0.001	1.211	1.083, 1.355
OPRM1 rs1799971 (Dom)	1.400	0.571	0.014	4.056	1.324, 12.426
History of hysterectomy	1.434	0.571	0.012	4.193	1.369, 12.842
POMS Fatigue-Inertia (sum)	0.180	0.056	0.001	1.197	1.072, 1.337
POMS Fatigue-Inertia (sum)	0.180	0.056	0.001	1.197	1.072, 1.337
BCPT Musculoskeletal Symptoms: mild linear increase vs constant no					
CYP3A4 rs35599367 (Add)	1.609	0.757	0.034	4.997	1.133, 22.034
AI therapy	0.888	0.353	0.012	2.431	1.217, 4.856
History of arthritis	0.906	0.355	0.011	2.474	1.234, 4.959
BDI II Total (sum)	0.091	0.043	0.036	1.095	1.006, 1.192
POMS Fatigue-Inertia (sum)	0.110	0.039	0.005	1.116	1.034, 1.205
IL1RN rs380092 (Rec)	-0.618	0.319	0.053	0.539	0.289, 1.008
AI therapy	0.756	0.342	0.027	2.131	1.090, 4.165
History of arthritis	0.959	0.354	0.007	2.609	1.303, 5.225
BDI II Total (sum)	0.098	0.045	0.029	1.103	1.010, 1.205
POMS Fatigue-Inertia (sum)	0.102	0.039	0.008	1.108	1.027, 1.195
VDR rs731236 (Rec)	-1.332	0.506	0.009	0.264	0.098, 0.712
AI therapy	0.905	0.346	0.009	2.472	1.254, 4.873
History of arthritis	1.026	0.358	0.004	2.789	1.383, 5.623
BDI II Total (sum)	0.104	0.045	0.020	1.110	1.017, 1.212
POMS Fatigue-Inertia (sum)	0.115	0.040	0.004	1.122	1.037, 1.214
BCPT Musculoskeletal Symptoms: constant moderate vs constant no					
CYP19A1 rs1008805 (Rec)	1.814	0.787	0.021	6.133	1.313, 28.656
History of arthritis	2.630	0.775	0.001	13.874	3.037, 63.388
BDI II Total (sum)	0.170	0.080	0.033	1.185	1.014, 1.385
POMS Fatigue-Inertia (sum)	0.225	0.069	0.001	1.252	1.093, 1.433
NOS3 rs1799983 (Rec)	1.636	0.739	0.027	5.135	1.206, 21.868
History of hysterectomy	1.533	0.859	0.074	4.634	0.861, 24.940
History of arthritis	2.500	0.723	0.001	12.180	2.952, 50.255
BDI II Total (sum)	0.135	0.075	0.072	0.144	0.988, 1.325
POMS Fatigue-Inertia (sum)	0.262	0.067	<0.001	1.299	1.140, 1.482
OPG rs2073618 (Rec)	1.504	0.733	0.040	4.497	1.069, 18.921
History of arthritis	2.635	0.737	<0.001	13.946	3.289, 59.136

Predictor	B	Standard Error	*p* value	Odds Ratio	95% CI
BDI II Total (sum)	0.186	0.080	0.020	1.204	1.030, 1.408
POMS Fatigue-Inertia (sum)	0.190	0.062	0.002	1.210	1.070, 1.367
NOS3 rs1799983 (Rec)	0.134	0.895	0.881	1.143	0.198, 6.607
OPG rs2073618 (Rec)	-0.368	1.163	0.752	0.692	0.071, 6.769
NOS3 rs1799983**OPG* rs2073618	3.779	1.681	0.025	43.773	1.623, 1180.890
History of arthritis	2.810	0.819	0.001	16.615	3.337, 82.721
BDI II Total (sum)	0.175	0.081	0.031	1.191	1.107, 1.396
POMS Fatigue-Inertia (sum)	0.244	0.072	0.001	1.276	1.107, 1.470

Appendix Table 11 Summary of SNPs across phenotypes

	BPI Worst Pain			BPI Severity		BPI Interference		Analgesics Usage	BCPT Joint Pain		BCPT Muscle Stiffness		BCPT MSK	
	INCR	MID	MOD	MID	MOD	MID	MOD	Use	MID	MOD	MID	MOD	MID	MOD
BDNF rs6265									↓					
CCL2 rs4586			R											
COMT rs4633					↓									
COMT rs887200					↓			R						
COMT rs165774	↑													
CYP17A1 rs4919683													R	
CYP19A1 rs1008805			↑		↑	R		R		B				↑
CYP27B1 rs10877012										R				
CYP27B1 rs4646536			R							B				
CYP3A4 rs35599367													↑	
CXCL8 rs4073	R		↓											
ESR1 rs9322336		R	R											
ESR2 rs2772163	↓												R	
IGF1 rs6214											R			
IL-6 rs1800795			R			R								
IL1A rs3783521											B			
IL1B rs16944											↓			
IL1RN rs380092													B	
LIF rs929271	R													
LIF rs737812	R													
MMP13 rs597315			R											
NOS3 rs1799983					↑									↑
OPG rs2073617												R		R
OPG rs2073618							R					R		↑
OPRM1 rs1799971											↑			
RANKL rs1054016				↓										
TCL1A rs11849538	R						R							
TCL1A rs7158782	↑													
TCL1A rs7159713	↑													
TCL1A rs2369049	R													
VDR rs4516035	R	R	↓			↓			R				R	
VDR rs731236	R	↓											↓	
WNT5A rs1829556													R	

R: significant in univariate analysis, but removed from final model; **B**: borderline significance; **INCR**: increase; **MID**: mild; **MOD**: moderate; **MSK**: musculoskeletal.
↑: increased risk for mild or moderate trajectory; ↓: decreased risk for mild or moderate trajectory

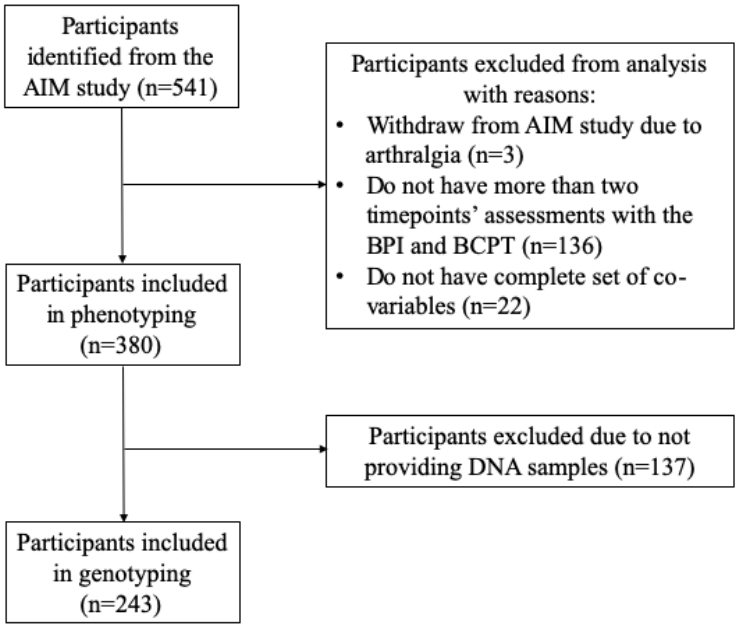


Figure 2. Study flow diagram.

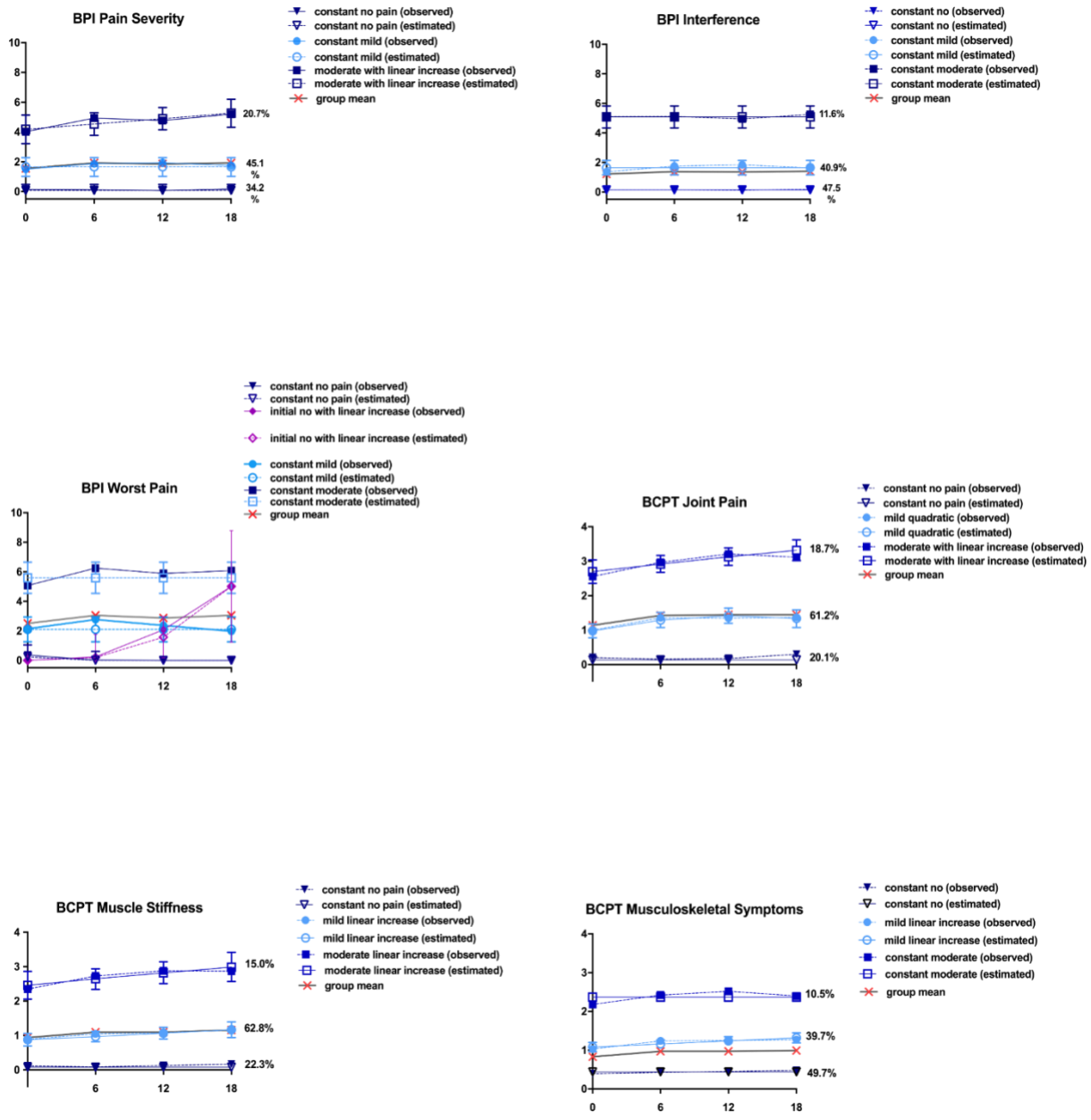
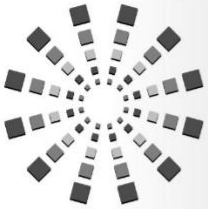


Figure 3. Patterns of Trajectories for Pain and Musculoskeletal Symptoms (n=380)

Appendix C PRELIMINARY WORK #1: SYMPTOM MAP OF ENDOCRINE THERAPY FOR BREAST CANCER: A SCOPING REVIEW



Yehui Zhu, PhD(c), MSN, BSN
Susan M. Cohen, PhD, CRNP, FAAN
Margaret Q. Rosenzweig, PhD, CRNP, AOCN, FAAN
Catherine M. Bender, PhD, RN, FAAN

Symptom Map of Endocrine Therapy for Breast Cancer: A Scoping Review

KEY WORDS
Aromatase inhibitors
Breast neoplasms
Scoping review
Signs and symptoms
Tamoxifen

Background: Multiple symptoms associated with endocrine therapy have a detrimental impact on medication adherence and quality of life. **Objective:** The purpose of this scoping review is to map the symptoms during endocrine therapy for breast cancer to provide implications for current practice and suggestions for future research. **Methods:** The PubMed, CINAHL, and China Science Periodical Databases were searched to identify related studies published in English and Chinese languages. References of included articles were reviewed for additional eligible studies. Of the 2551 articles identified, 57 articles met inclusion criteria and were included in this scoping review. **Results:** Evidence for the 16 most studied symptoms and 15 most prevalent symptoms were synthesized. Five key symptoms associated with endocrine therapy were identified, including joint/muscle pain, hot flashes, low sexual interest/desire, joint/muscle stiffness, and fatigue/lack of energy. Rarely studied but highly prevalent symptoms and other gaps in the symptom science during endocrine therapy for breast cancer were identified. **Conclusion:** Nurses caring for women receiving endocrine therapy for breast cancer should assess the 5 key symptoms identified. There remain substantial gaps in the science related to the symptom experience during endocrine therapy for breast cancer. Future studies should focus on the domains of symptom intensity and distress, specific understudied symptoms, symptom clusters, and development of symptom assessment instruments specific to symptoms associated with endocrine therapy. **Implications for**

Author Affiliations: School of Nursing, University of Pittsburgh, Pennsylvania.
Y.Z. is supported by a Doctoral Degree Scholarship in Cancer Nursing, DSCN-17-073-01, from the American Cancer Society.
The authors have no conflicts of interest to disclose.
Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.cancernursingonline.com).

Correspondence: Yehui Zhu, PhD(c), MSN, BSN, School of Nursing, University of Pittsburgh, 415 Victoria Bldg, 3500 Victoria St, Pittsburgh, PA 15261 (zhuyehui09@gmail.com; yez7@pitt.edu).
Accepted for publication April 4, 2018.
DOI: 10.1097/NCC.0000000000000632

APPENDIX B

Practice: This scoping review identified 5 well-studied and highly prevalent symptoms that should be assessed in women with breast cancer receiving endocrine therapy.

Breast cancer is the most commonly diagnosed cancer among women. It is estimated that there were 252 710 new breast cancer diagnoses and 40 610 deaths in 2017 in the United States.[1] In China, 15% of all new cancer diagnoses in women are breast cancer, and the disease is the leading cause of cancer deaths in women younger than 45 years.[2] Globally, with the application of tamoxifen, the breast cancer recurrence and mortality rates were decreased by 41% and 34%, respectively.[3] Third-generation aromatase inhibitors (AIs), including anastrozole, letrozole, and exemestane, are associated with a significant improvement in disease-free and overall survival for postmenopausal women with breast cancer.[4,5] Therefore, the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines of Breast Cancer (version 2.2017) recommend that women with early-stage, hormone receptor–positive breast cancer receive at least 5 years of endocrine therapy generally consisting of tamoxifen for premenopausal women and an AI for postmenopausal women.[6] In addition, except for immediately life-threatening cases, endocrine therapy alone or in combination has been recommended as an initial treatment for women with hormone receptor–positive metastatic breast cancer by the American Society of Clinical Oncology.[7]

Although endocrine therapy significantly improves the overall and disease-free survival in women with breast cancer, this treatment is associated with multiple symptoms that may have a detrimental impact on medication adherence, functional status, and quality of life.[8–10] Co-occurring symptoms associated with endocrine therapy were reported as one of the most common reasons for treatment discontinuation (66.7% of AI discontinuers and 59.1% of tamoxifen discontinuers).[8] Moreover, endocrine therapy–related symptoms are more likely to be neglected by both healthcare providers and patients because of less frequent follow-up visits, compared with follow-ups for other forms of adjuvant therapy, such as chemotherapy and radiation therapy.[11]

Although assessment of adverse events is essential in clinical trials of endocrine therapy development mainly for the purpose of safety, evidence now suggests that endocrine therapy–associated symptoms were underestimated. Ruhstaller et al[12] reported that hot flashes/sweats (70% vs 38%-40% in clinical trials), low energy (45% vs 9%-15% in clinical trials), fluid retention (22% vs 7% in clinical trials), and vaginal dryness (30% vs 3% in clinical trials) were significantly underrated in clinical trials of endocrine therapy. Therefore, having a comprehensive understanding of the symptom experience associated with endocrine therapy is urgently needed, as it will serve as the bases for development of interventions to manage those symptoms. The purpose of this scoping review is to map the occurrence (frequency), intensity, and distress of symptoms during endocrine therapy for breast cancer.

■ Methods

This scoping review was conducted under the framework proposed by Khalil et al[13] and the Joanna Briggs Institute methods of evidence synthesis, as detailed hereinafter.

Step 1: Identify the Research Question

The research question for this scoping review was "What is the symptom(s) experience during endocrine therapy for breast cancer that has been reported?" The Joanna Briggs Institute suggests using population, concept, and context to construct a clear and meaningful scoping review. Therefore, we further defined the population, concept, and context of this scoping review as follows.

1. Population

Participants in the included studies in this scoping review are female adults (18 years or older), who were diagnosed with breast cancer and receiving oral endocrine therapy. Both observational studies describing the symptom(s) experience and experimental studies comparing the symptom experience among different types of endocrine therapies were eligible. Studies with samples that were undividable from other types of cancer or other types of treatment were excluded from this review because they precluded the ability to discern symptoms specifically related to endocrine therapy.

2. Concept

Endocrine therapy and symptom experience are 2 key concepts in this scoping review. Endocrine therapy refers to oral adjuvant endocrine therapy currently recommended by the NCCN Guideline for Breast Cancer, including selective estrogen receptor modulators such as tamoxifen (Nolvadex and Soltamox) and AIs including anastrozole (Arimidex), letrozole (Femara), and exemestane (Aromasin). Symptom experience is defined as the "perception of the frequency, intensity, distress, and meaning of symptoms as they are produced and expressed" in accordance with the Symptom Experience Model (SEM).[14]

3. Context

In this scoping review, the symptom(s) experience is determined within the context of endocrine therapy for breast cancer in clinical studies. Excluded are clinical trials or studies using endocrine therapy to prevent breast cancer or chemoprevention.

Step 2: Identify Relevant Studies

Studies published in English and Chinese languages before February 2017 were comprehensively searched. A 3-step search

strategy was used. An initial scoping search was conducted in PubMed and China Science Periodical Databases (CSPD) to identify key terms. Then, comprehensive searches were performed in the following databases: PubMed, CINAHL, and CSPD. The following search terms were combined: "breast," "neoplasm," "endocrine therapy," "hormonal therapy," "antineoplastic agents," "aromatase inhibitor," "tamoxifen," "symptom," and "adverse effects." The search string in PubMed is: ((((("Antineoplastic Agents, Hormonal/adverse effects"[Majr]) OR "Aromatase Inhibitors/adverse effects"[Majr]) OR "Tamoxifen/adverse effects"[Majr])) AND "Breast Neoplasms"[Mesh:NoExp]. Finally, additional pertinent studies were identified by reviewing the bibliographies of included studies.

Step 3: Study Selection

The initial search revealed 2551 references (PubMed, 1489; CINAHL, 822; CSPD, 236; other recourses, 4). After removal of 70 duplicated references, 2481 (2245 English and 236 Chinese) were screened by title and abstract for eligibility. Figure 1 summarizes the details of study selection. Studies were reviewed by 2 researchers for determination of eligibility. A third researcher adjudicated situations in which there was a disagreement. Eventually, 53 clinical studies were identified from 57 articles (54 in English and 3 in Chinese) and were included in this scoping review (Table 1)

Step 4: Charting the Data

Data charting includes the process of data extraction and describing the data both narratively and in tabular form. The SEM was used to guide the data charting, the process of data extraction. We synthesized the symptom experience based on each domain (frequency, intensity, and distress). Because most studies reported symptom occurrence (a dichotomized variable of frequency), we integrated occurrence into a frequency domain. Because of the heterogeneity of study design (eg, cross-sectional or longitudinal) and characteristics of participants across studies (eg, tamoxifen or AI users, white or African American), data on each characteristic at every time point were charted as an independent report if they were available in the original articles, to facilitate comparison across studies. For example, a 6-month longitudinal study on the occurrence of joint pain could have 12 occurrence reports based on the combination of time point (0, 3, or 6 months), agent (tamoxifen or AI), and ethnicity (white or African American). In terms of intensity and distress, some studies reported the percentage of people who experience different levels of symptoms among the entire sample, whereas some reported the percentage

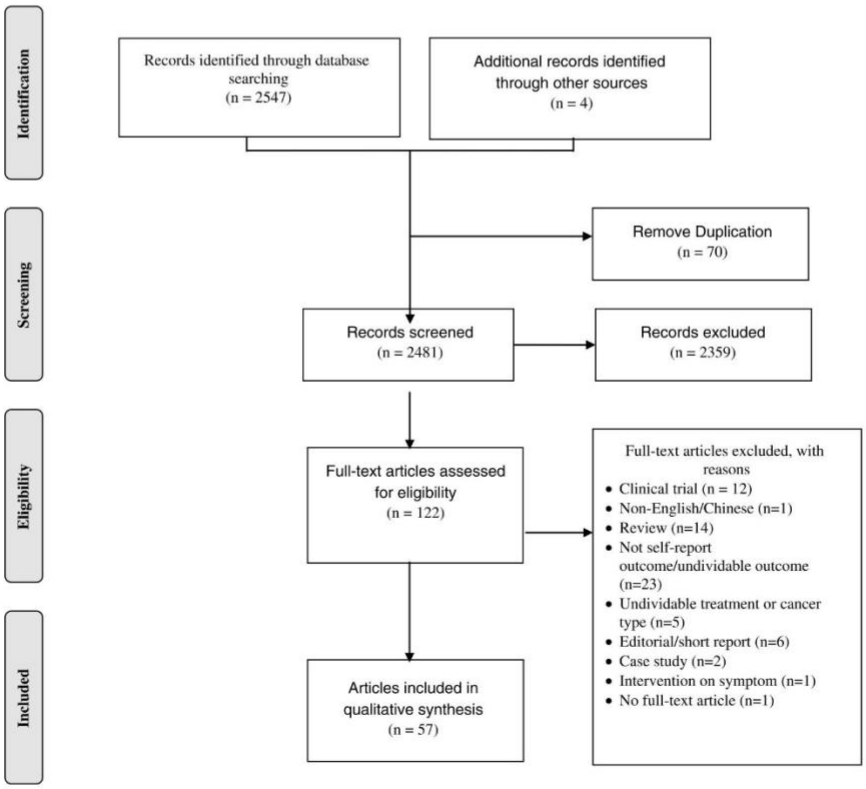


Figure 1 ■ The process of selecting studies.

Table 1 • Studies Included in This Scoping Review

Author(s), Year	Country	Agent	Design	Instrument	Recall Period	Domain	Symptoms
Ashraf et al,[15] 2009	India	TAM	Retrospective review of case records (n = 3000)	Medical records	—	Occurrence, intensity	TAM-related side effects
Baumgart et al,[16] 2011	Sweden	ET	Cross-sectional (n = 97)	FACT-ES, IIQ-7, UDI-6	—	Occurrence, intensity	Urogenital symptoms, ET-related symptoms
Baumgart et al,[17] 2013	Sweden	ET	Cross-sectional (n = 97)	Standardized questionnaire	Past 12 mo	Occurrence, frequency, distress	Sexual dysfunction symptoms
Baxter et al,[18] 2014	Canada	TAM	Cross-sectional (n = 132)	Survey	Past 7 d	Occurrence, frequency, intensity	Hot flashes and TAM-related symptoms
Boehm et al,[19] 2009	Germany	TAM	Cross-sectional (n = 136)	Questionnaire	Past 7 d	Occurrence, frequency	AI-related symptoms
Boonstra et al,[20] 2013	The Netherlands	AI	Cross-sectional (n = 57)	Rheumatoid Arthritis Disease Activity Index, FACT-ES	Past 7 d	Occurrence, intensity	Arthralgia/stiffness/AI-related symptoms
Aiello Bowles et al,[8] 2012	United States	ET	Cross-sectional (n = 538)	Survey	At any point in ET	Occurrence	Adverse effects of ET
Brown et al,[21] 2014	United States	AI	Cross-sectional (n = 300)	WOMAC, M-SACRAH, Quick DASH	—	Occurrence, intensity	Musculoskeletal symptoms
Castel et al,[22] 2013	United States	AI	Longitudinal (pre-AI and 2, 4, 6, 8, 12, and 52 wk post-AI, n = 91)	FACT-ES, PRAI	—	Occurrence, intensity	Arthralgia, ET-related symptoms
Chim et al,[23] 2013	United States	AI	Cross-sectional (n = 437)	BPI	Past 24 h	Occurrence, intensity, distress	Joint pain
Chin et al,[24] 2009	Canada	ET	Cross-sectional (n = 251)	FACT-ES, sexual activity questionnaire	Past 7 d	Occurrence, intensity	Vulvovaginal and urinary symptoms, ET-related symptoms
Crew et al,[25] 2007	United States	AI	Cross-sectional (n = 200)	Questionnaire adapted from BPI-SF	Past 7 d	Occurrence, intensity	Joint symptoms
Desai et al,[26] 2013	United States	AI	Cross-sectional (n = 413)	Insomnia Severity Index	Current	Occurrence, intensity, distress	Insomnia
Dizdar et al,[27] 2009	Turkey	AI	Cross-sectional (n = 92)	Patient interview	Recently	Occurrence	Arthralgia
Egawa et al,[28] 2016	Japan	AI	Longitudinal (pre-AI and 3, 6, 9, and 12 mo post-AI, n = 391)	Questionnaire	—	Frequency, distress	Joint symptoms
Frechette et al,[29] 2013	Canada	ET	Longitudinal (pre-ET and 6 mo post-ET, n = 66)	Female sexual function index, FSDS-R, FACT-ES	Past 30 d (FSDS-R)/ 7 d (FACT-ES)	Occurrence, intensity, distress	Sexual dysfunction, ET-related symptoms
Gallicchio et al,[30,31] 2012	United States	AI	Longitudinal (pre-AI and 3 and 6 mo post-AI, n = 95)	VAS Symptom Checklist of 20 menopausal-type symptoms	Past 4 wk	Occurrence, intensity, distress	Musculoskeletal pain, menopausal-type symptoms
Gallicchio et al,[32] 2013	United States	ET	Survey (n = 851)	Hospital registry-based survey	Past 4 wk	Distress	Hair loss and hair thinning

(continues)

Table 1 • Studies Included in This Scoping Review, Continued

Author(s), Year	Country	Agent	Design	Instrument	Recall Period	Domain	Symptoms
Garreau et al,[33] 2006	United States	ET	Cross-sectional (n = 452)	Questionnaire	—	Occurrence	ET-related symptoms
Hadji et al,[34] 2014	Germany	AI	Longitudinal (prestudy and 3, 6, and 9 mo poststudy, n = 1916)	RASQ	—	Occurrence, intensity	Arthralgia
Horimoto et al,[35] 2009	Japan	AI	Retrospective (n = 329)	Chart review	—	Occurrence	Arthralgia
Hu et al,[36] 2016	China	ET	Retrospective review of case records (n = 160)	Chart review	—	Occurrence	ET-related symptoms
Huang et al,[37] 2010	China	ET	Cross-sectional (n = 315)	VAS	—	Occurrence, intensity	Cancer-related fatigue
Inglis et al,[38] 2015	Australia	AI	Cross-sectional (n = 93)	OSDI, FACT-ES	Past 2 wk	Occurrence, intensity, distress	Dry eye syndrome, ET-related symptoms
Kanti et al,[39] 2016	Germany	TAM	Longitudinal (1 d pretherapy up to 28 wk posttherapy) (n = 17)	Diary, modified hairdex questionnaire, and the SF-MPQ	Past day	Occurrence, distress	Trichodynia (hair pain)
Kyvernitakis et al,[40] 2014	Germany	AI	Longitudinal (pre-AI and 12 and 24 mo post-AI, n = 174)	MRS	—	Occurrence, intensity	Menopausal symptoms
Laroche et al,[41] 2014	France	AI	Longitudinal (pre-AI and 1, 3, 6, and 12 mo post-AI, n = 135)	VAS, McGill Pain Questionnaire, BPI	—	Occurrence, intensity, distress	Pain
Lintermans et al,[42,43] 2014	Belgium	ET	Longitudinal (pre-ET and 3, 6, and 12 mo post-ET, n = 292)	NSABP symptom checklist, VAS, musculoskeletal questionnaire	Past 7 d	Occurrence, intensity	Musculoskeletal pain
Lu et al,[44] 2011	China	AI	Retrospective review of case records (n = 271)	Telephone interview	—	Occurrence	ET-related symptoms
Mao et al,[45] 2009	United States	AI	Cross-sectional (n = 300)	Questionnaire	Past 7 d	Occurrence, intensity	Arthralgia
Mao et al,[46] 2011	United States	AI	Cross-sectional (n = 390)	Self-reported arthralgia	—	Occurrence	Arthralgia
Menas et al,[47] 2012	United States	AI	Cross-sectional (n = 206)	Retrospective chart review	—	Occurrence	Arthralgia
Mortimer et al,[48] 1999	United States	TAM	Cross-sectional (n = 57)	BCPT Symptom Checklist	Past 4 wk	Occurrence, distress	ET-related symptoms
Mortimer et al, 2008[49]	United States	TAM	Baseline data of RCT (n = 864)	"Thoughts and Feelings" questionnaire	Past 4 wk	Occurrence, intensity	Physical/psychological symptoms
Napoli et al,[50] 2010	United States	AI	Cross-sectional (n = 145)	Modified Leuven Questionnaire	—	Occurrence, intensity	Musculoskeletal symptoms
Oberguggenberger et al,[51] 2011	Australia	AI	Cross-sectional (n = 280)	FACT-ES	Past 7 d	Occurrence, intensity	ET-related symptoms
Ochayon et al,[52] 2014	Israel	ET	Cross-sectional (n = 210)	MDASI, BCPT Symptom Checklist	Past 24 h, past 4 wk	Occurrence, intensity, distress	ET-related symptoms
Ohsako et al,[53] 2006	Japan	ANA	Longitudinal (n = 53)	CTCAEver3.0	—	Occurrence, intensity	Musculoskeletal symptoms

(continues)

Table 1 • Studies Included in This Scoping Review, Continued

Author(s), Year	Country	Agent	Design	Instrument	Recall Period	Domain	Symptoms
Olufade et al,[10] 2015	United States	AI	Cross-sectional (n = 68)	VAS	Past 4 wk	Occurrence, intensity	Musculoskeletal pain
Presant et al,[54] 2007	United States	AI	Semistructured interview (n = 56)	A linear analog pain scale; location, character, and treatment	—	Occurrence, intensity	Arthralgia
Ribi et al,[55] 2007; Ruhstaller et al,[12] 2009	Switzerland	ET	Cross-sectional (n = 373)	Checklist for Patients with Endocrine Therapy (C-PET)	—	Occurrence, frequency	ET-related symptoms
Rosenberg et al,[56] 2015	United States	ET	Cross-sectional (n = 2086)	BCPT Symptom Checklist	Past 4 wk	Occurrence, distress	ET-related symptoms
Sagara et al,[57] 2010	Japan	AI	Longitudinal (n = 656)	Symptoms were collected retrospectively (no detail mentioned).	—	Occurrence	ANA-related adverse events
Schover et al,[58] 2014	United States	AI	Cross-sectional (n = 129)	FSFI, MSIQ, FSDS-R, BESS	Past 4 wk	Occurrence, distress	Sexual function
Servitja et al,[59] 2012	Spain	AI	Longitudinal (pre-AI and 3 mo post-AI, n = 343)	VAS	—	Occurrence, intensity	Arthralgia
Shi et al,[60] 2013	United States	AI	Longitudinal (pre- and biweekly for 1 y, n = 47)	BPI, MDASI, Joint Pain Assessment	Past 24 h, past 7 d	Occurrence, intensity, distress	Arthralgia, ET-related symptoms
Singer et al,[61] 2012	United States	AI	Longitudinal (pre-AI and 3 and 6 mo post-AI, n = 52)	FACT-ES, global pain, AUSCAN	Past 7 d	Occurrence, intensity	Arthralgia, ET-related symptoms
Su et al,[62] 2010	United States	AI	Cross-sectional (n = 300)	Questionnaire	Past 7 d	Occurrence, frequency, intensity	Hot flashes, weight gain
Swenson et al,[63] 2013	United States	AI	Longitudinal (pre-AI and 1, 3, and 6 mo post-AI, n = 122)	BCPT Symptom Checklist, AUSCAN, WOMAC, BPI, Quick DASH	Past 24 h, past 4 wk	Occurrence, intensity, distress	Musculoskeletal symptoms
Waltman et al,[64] 2009	United States	AI	Cross-sectional (n = 29)	The Aromatase Inhibitor Questionnaire	Past 7 d	Occurrence, intensity, distress	Musculoskeletal symptoms
Wang et al,[65] 2013	China	AI	Cross-sectional (n = 436)	CTCAEver 3.0, WOMAC, M-SACRAH, BPI-SF	Past 7 d	Occurrence, intensity, distress	Musculoskeletal symptoms
Xu et al,[66] 2014	China	ET	Cross-sectional (n = 122)	Retrospective telephone interview	—	Occurrence	Musculoskeletal symptoms
Zhan et al,[67] 2007	United States	TAM	Cross-sectional (n = 138)	Symptom checklist	—	Occurrence	TAM-related side effects
Zhou et al,[68] 2011	China	ET	Retrospective review of case records (n = 50)	CTCAEver3.0	—	Occurrence	ET-related symptoms

Abbreviations: AI, aromatase inhibitor; ANA, anastrozole; AUSCAN, Australian/Canadian Hand Osteoarthritis Index; BCPT, Breast Cancer Prevention Trial; BESS, Breast Cancer Prevention Trial Eight Symptom Scale; BPI, Brief Pain Inventory; CTCAEver3.0, Common Terminology Criteria for Adverse Events v3.0; ET, endocrine therapy; FACT-ES, Functional Assessment of Cancer Therapy–Endocrine Symptom; FSDS-R, Female Sexual Distress Scale–Revised; FSFI, Female Sexual Function Index; IIQ-7, Incontinence Impact Questionnaire; MDASI, MD Anderson Symptom Inventory; MRS, Menopause Rating Scale; M-SACRAH, Modified Score for the Assessment and Quantification of Chronic Rheumatoid Affections of the Hands; MSIQ, Menopausal Sexual Interest Questionnaire; NSABP, National Surgical Adjuvant Breast and Bowel Project; OSDI, Ocular Surface Disease Index; PRAI, Patient-reported Arthralgia Inventory; Quick DASH, Quick Disabilities of the Arm, Shoulder and Hand Questionnaire; SF-MPQ, Short Form of the McGill Pain Questionnaire; TAM, tamoxifen; UDI-6, Urogenital Distress Inventory-6; VAS, visual analog scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

of participants who developed symptoms. To make the data comparable, we recalculated the percentage for the latter situation so that the percentages were referring to proportion among all the participants. For example, when a study reported that 30% of 50 patients of 100 research participants had severe pain, we recalculated the percentage of patients who had severe pain as $50 \times 30\% / 100 = 15\%$.

Step 5: Collating, Summarizing, and Reporting the Results

Symptom data from individual studies were collated after being extracted. Summary and interpretation of data were demonstrated in the Results session. The implications of the findings for clinical practice and future research were further detailed in the Discussion session.

■ Results

Since 2006, the number of studies on symptoms associated with endocrine therapy fluctuated with an increasing trend, reaching its peak in 2013 (n = 11), and dropped dramatically in 2015 and 2016 (Figure 2). More studies focused on AIs than tamoxifen (34 vs 7). The sample sizes varied considerably ranging from 17 to 3000. Most of the studies used a cross-sectional design (n = 33). The longest follow-up period for symptom assessment in the longitudinal studies was 24 months (see Table 1).

Most studies assessed symptoms by using self-report questionnaires or symptom checklists. Retrospective medical record reviews or telephone interviews was adopted in 8 studies.[15,35,36,44,47,57,66,68] Two studies conducted retrospective semistructured interviews[54] and patient interviews.[27] The recall period ranged from 24 hours to 12 months, with recall for the past 7 days and 4 weeks most commonly adopted. Twenty-three studies did not report recall period (see Table 1).

The mostly used symptom assessments used were the Breast Cancer Prevention Trial (BCPT) Symptom Checklist, Functional Assessment of Cancer Therapy (FACT-ES), and the MD Anderson Symptom Inventory (MDASI). Symptom intensity and distress were quantified using Likert scales. Investigator-developed symptom questionnaires and checklists adapted from visual analog scales were commonly used as well (see Table 1).

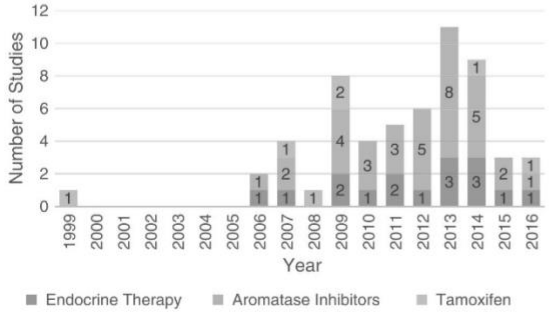


Figure 2 ■ Number of studies on endocrine therapy for breast cancer over time.

In this scoping review, individual symptoms identified were categorized into cognitive, musculoskeletal, vasomotor, gastrointestinal, urogenital, mood-related, sleep-related, and sexual symptoms, adapted from the subscales of BCPT Symptom Checklist. Symptoms that did not fall into these categories were grouped into a separate category labeled "others." Symptom occurrence, intensity, and distress reported by each study are exhibited in Table 1, Supplemental Digital Content, http://links.lww.com/CN/A18.

Mostly Studied Symptoms

On the basis of the numbers of studies that report symptom occurrence, the 16 mostly studied symptoms were joint/muscle pain, hot flashes, vaginal dryness/insufficient lubrication, sleep disorder/insomnia, fatigue/lack of energy, nausea, vaginal bleeding or spotting, headaches/migraines, irritability, joint/muscle stiffness, weight gain, vaginal discharge, depression/depressive mood, low sexual interest/desire, difficulty breathing/short of breath, and dizziness/faintness (see Figure 3). Far fewer studies reported symptom intensity and distress. In the 16 mostly reported symptoms, results related to the occurrence, intensity, and distress domains were only reported for 8 symptoms including joint/muscle pain, hot flashes, vaginal dryness/insufficient lubrication, sleep disorder/insomnia, fatigue/lack of energy, irritability, joint/muscle stiffness, and depression/depressive mood. Intensity was reported by more than 1 study on only 10 symptoms: joint/muscle pain (10), hot flashes (4), vaginal dryness/insufficient lubrication (4), vaginal discharge (3), joint/muscle stiffness (2), genital itching/irritation (2), vaginal bleeding/spotting (2), incontinence (2), sleep disorder/insomnia (2), and fatigue/lack of energy (2). Distress was reported by more than 1 study on only 10 symptoms: joint/muscle pain (5), hot flashes (3), pain with intercourse (3), forgetfulness (2), general aches and pains (2), joint/muscle stiffness (2), unhappy with the appearance of body (2), irritability (2), headaches/migraines (2), and loss of hair/hair thinning (2).

Symptoms With the Highest Occurrence, Intensity, and Distress

After extracting the symptom occurrences (the percentage of people who reported the symptom) from included studies (see Table 1, Supplemental Digital Content, http://links.lww.com/CN/A18), we sorted the occurrences from low to high for each symptom and identified the median occurrence for each symptom. On the basis of the median of occurrence of individual symptoms, we identified 15 symptoms with the highest occurrences (the most prevalent symptoms). From high to low, these 15 most prevalent symptoms include cramps, hot flashes, fatigue/lack of energy, eye irritation, heart discomfort, joint/muscle pain, night sweats, sexual arousal problem/orgasmic dysfunction, anxiety, dyspareunia, low sexual interest/desire, joint/muscle stiffness, urinary urgency, numbness or tingling, and dry eye syndrome (see Figure 4). Notably, 6 of these 15 symptoms (including

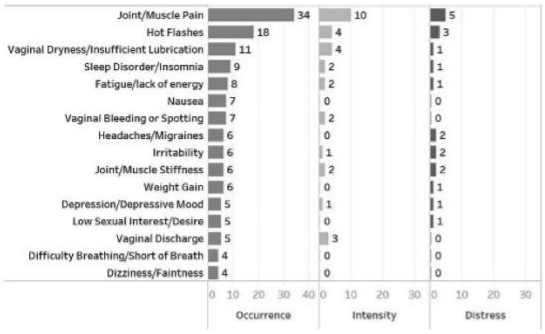


Figure 3 ■ Number of studies on the occurrence, intensity, and distress of symptoms during endocrine therapy (top 16).

cramps, eye irritation, heart discomfort, anxiety, dyspareunia, urinary urgency, numbness and tingling, and dry eye syndrome) were reported by only 1 study. Sexual arousal problem/orgasmic dysfunction was reported by only 2 studies.

Five of the 15 most prevalent symptoms overlapped with the most studied symptoms, including joint/muscle pain, hot flashes, low sexual interest/desire, joint/muscle stiffness, and fatigue/lack of energy (see Figures 3 and 4). Interestingly, these 5 symptoms had the top 5 highest maximum symptom occurrences, suggesting that these 5 symptoms are particularly relevant to women receiving endocrine therapy for breast cancer.

Intensity and distress were assessed using visual analog scales in several studies. The proportion of participants who rated symptoms as mild, moderate, severe, or extremely severe and distressful was also reported (see Table 1, Supplemental Digital Content, http://links.lww.com/CN/A18). Intensity of only 4 symptoms (joint/muscle pain, hot flashes, vaginal dryness/insufficient lubrication, and vaginal discharge) was reported by more than 2 studies. Moderate to severe joint/muscle pain was reported by 31.5% to 46% of participants.[23,25,45,54] The range of mean intensity scores for joint/muscle pain was 4.9 to 5.4 of 10.[20,40,60] There were reports that 19.7% to 53% of participants reported moderate to severe hot flashes.[16,18,40,62] There were 20.6% to 32.8% of participants who reported moderate to severe vaginal dryness/insufficient lubrication.[16,24,40] Moderate to severe vaginal discharge was reported by 4% to 17.6% of participants across studies.[16,24] Only 3 symptoms have more than 2 studies reporting distress including joint/muscle pain, hot flashes, and pain with intercourse. Moderate or greater distress associated with joint/muscle pain was reported by 36% of participants.[28] The mean distress with joint/muscle pain was 3.29 of 10.[60] The mean distress of hot flashes was 1.41 of 4, and that of pain with intercourse was 1.17 of 4.[52]

■ Discussion

Symptoms are increasingly important self-reported outcomes during cancer treatment. Symptom science and self-management are listed as research priorities of both the National Institutes of Health and National Institute of Nursing Research. However, the science of the symptom experience during endocrine therapy remains underdeveloped. Heterogeneity across symptom assessment instruments and methodological limitations across completed studies underscore the inability to integrate the evidence and better understand the phenomenon of symptom experience during endocrine therapy for breast cancer.

Instrumentations for Symptoms During Endocrine Therapy

In this scoping review, considerable methodological heterogeneity was identified across the included 57 articles, including variance in study design, symptom assessment instruments, symptom measurement recall period, data collection procedures, and sample characteristics (eg, ethnicity, menopausal status, previous treatments, cancer stage). The biggest barrier to the

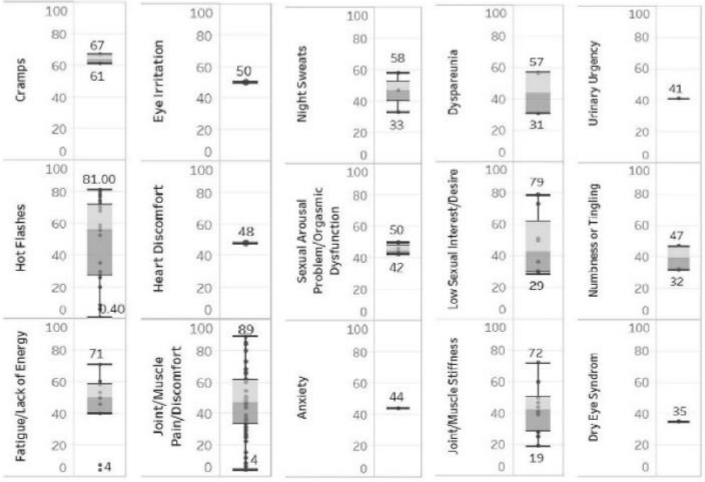


Figure 4 ■ Top 15 prevalent symptoms (based on median) reported by current studies on endocrine therapy for breast cancer.

comparison of results across studies is the heterogeneity of symptom assessment instruments. Given the consensus related to the experience of multiple co-occurring symptoms, a self-reported symptom questionnaire/checklist is a plausible approach to efficient assessment of concurrent symptoms. Unfortunately, although the 3 most commonly used self-reported symptom questionnaires/checklists (BCPT, FACT-ES, and MDASI) have been reported to be reliable and valid,[69–71] none of them assesses symptoms associated with endocrine therapy comprehensively in terms of the types of symptoms experienced and the occurrence, intensity, and distress associated with those symptoms. Table 2 shows the coverage of the 16 most commonly studied symptoms among these 3 commonly used symptom questionnaires/checklists. The FACT-ES covers 14 of 16 symptoms, the BCPT Symptom Checklist covers 12 of 16 symptoms, and the MDASI covers 5 of 16 symptoms. Table 3 shows the coverage of the 15 symptoms with the highest occurrences among the 3 symptom checklists. The BCPT and FACT-ES both cover 6 of 15 symptoms. The MDASI covers 2 of 15 symptoms. Compared with the BCPT and FACT-ES, the MDASI covers far fewer symptoms as illustrated in Tables 2 and 3. This is most probably due to the fact that the MDASI is not an endocrine therapy–specific symptom assessment. However, only the MDASI comprehensively assesses the 3 domains of symptoms. The BCPT assesses symptom distress, the FACT-ES assesses occurrence and intensity, and the MDASI assesses occurrence, intensity, and distress. In addition, 6 symptoms with high occurrence rates in women receiving endocrine therapy are not included in any of the 3 instruments (Table 3), including eye irritation,[19] heart discomfort,[40] sexual arousal problem/orgasmic dysfunction,[17,29] dyspareunia,[17] urinary urgency,[24] and dry eye syndrome.[38] Interestingly, each of these 6 nonincluded symptoms was reported by only 1 or 2 studies. Given the high occurrence rates, future studies should ensure the inclusion of these 6 symptoms. In addition, more studies are needed to confirm the robustness of the high occurrence of these 6 symptoms.

Table 2 • Coverage of 16 Mostly Studied Symptoms in BCPT Symptom Checklist, FACT-ES, and MDASI

	BCPT	FACT-ES	MDASI
1. Joint/muscle pain	√	√	-
2. Hot flashes	√	√	-
3. Vaginal dryness/insufficient lubrication	√	√	-
4. Sleep disorder/insomnia	-	√	√
5. Fatigue/lack of energy	-	√	√
6. Nausea	√	√	√
7. Vaginal bleeding or spotting	√	√	-
8. Headaches/migraines	√	√	-
9. Irritability	√	√	-
10. Joint/muscle stiffness	√	-	-
11. Weight gain	√	√	-
12. Vaginal discharge	√	√	-
13. Depression/depressive mood	-	√	√
14. Low sexual interest/desire	-	√	-
15. Difficulty breathing/short of breath	√	-	√
16. Dizziness/faintness	√	√	-

Abbreviations: BCPT, Breast Cancer Prevention Trial; FACT-ES, Functional Assessment of Cancer; MDASI, MD Anderson Symptom Inventory.
√, included in the instrumentation; -, not included in the instrumentation.

Methodological Limitations in the Current Studies

In this scoping review, we identified several methodological issues that preclude comprehensive understanding of symptoms during endocrine therapy for breast cancer.

First, most of the current studies used cross-sectional designs. A cross-sectional design precludes the possibility of examining causal relationships related to factors that may be associated with symptoms during endocrine therapy. Moreover, the onset time and shape of trajectories of symptoms remained understudied because of the lack of longitudinal studies. In addition, the few longitudinal studies that have been reported only included follow-up periods up to 24 months,[40] a relatively short time frame relative to the 5 to 10 years of endocrine therapy typically recommended by the NCCN Guideline. Because of the relative short follow-up time, the trajectories of symptoms during the course of endocrine therapy are not fully described.

Second, there is a considerable variance in recall period. The recall period can affect the accuracy and comparability of symptom outcomes. However, the optimal recall period of symptoms is still under controversy; a shorter recall period (eg, 3 days in children and 4 days in adults) may help assess symptom occurrence accurately[72] but may underestimate symptom distress when symptoms have diurnal fluctuation.[73]

Third, there is a lack of definitions of symptoms in the current studies. The wording of 1 symptom varies among different studies. For example, lack of energy, low energy, feeling tired, physical and mental exhaustion, and fatigue are used by different studies.[8,12,18,34,37,40,44,51,55] Without a clear definition, it is not rigorous to treat them as 1 symptom. Moreover, it also remains arguable whether or not the outcomes from 1 item of a symptom checklist and a series of items of a questionnaire for 1 symptom are equivalent. In addition, the definitions of the extents of intensity/distress (eg, mild, moderate, severe, very severe) are not defined in most of the current studies, especially in the studies using symptom checklists to assess multiple concurrent symptoms.

Finally, there is a lack of theoretical guidance for the symptom-related studies during endocrine therapy. Theoretical frameworks established for examining symptoms (eg, the University of California San Fransisco Symptom Management Theory, SEM, National Institutes of Health Symptoms Science Model) should be encouraged in future studies.[14,74,75]

Other Gaps of the Current Studies

None of the included studies adopted common data elements (CDEs). The National Institute of Nursing Research recommended 6 symptoms (pain, fatigue, sleep disturbance, mood, anxiety, and cognitive disturbance) as CDEs for symptoms

Table 3 • Coverage of 15 Most Prevalent Symptoms in BCPT Symptom Checklist, FACT-ES, and MDASI

	BCPT	FACT-ES	MDASI
1. Cramps	√	-	-
2. Hot flashes	√	√	-
3. Fatigue/lack of energy	-	√	√
4. Eye irritation	-	-	-
5. Heart discomfort	-	-	-
6. Joint/muscle pain	√	√	-
7. Night sweats	√	√	-
8. Sexual arousal problem/orgasmic dysfunction	-	-	-
9. Anxiety	-	√	-
10. Dyspareunia	-	-	-
11. Low sexual interest/desire	-	√	-
12. Joint/muscle stiffness	√	-	-
13. Urinary urgency	-	-	-
14. Numbness or tingling	√	-	√
15. Dry eye syndrome	-	-	-

Abbreviations: BCPT, Breast Cancer Prevention Trial; FACT-ES, Functional Assessment of Cancer; MDASI, MD Anderson Symptom Inventory.
√, included in the instrumentation; -, not included in the instrumentation.

studies.[76] However, these symptoms were not well assessed and reported in the studies on symptoms during endocrine therapy. This impedes further comparison of symptom results both among studies for endocrine therapy and also among different types of cancer treatments.

Symptom cluster and trajectory patterns are understudied in symptoms during endocrine therapy for breast cancer. The numbers of research on symptom clusters in patients with cancer are exponentially increasing.[77] However, the symptom cluster in endocrine therapy for breast cancer is poorly studied. None of the identified studies in this scoping review is aiming to identify symptom clusters. The vast majority of current studies on symptom clusters is focusing on patients with cancer in the period of surgery, chemotherapy, and radiation therapy. Patients under endocrine therapy are rarely included. It is the same situation in the research focusing on trajectory patterns and high-risk subgroup membership. With an insufficient understanding of phenotypic characteristics of symptoms during endocrine therapy, there is a lack of studies further exploring the underlying mechanisms of symptom clusters and phenotypic variance associated with endocrine therapy.

■ Strengths and Limitations

To the authors' knowledge, this is the first scoping review to map the multiple symptoms experienced during endocrine therapy for breast cancer. Furthermore, the methodology and data charting process were both framework guided, which ensured the rigorousness of this scoping review. In addition, including both English- and Chinese-language published articles facilitates broadening the scope of the results of related studies.

However, this review should be taken within the context of several limitations. The limitations are as follows: (1) studies published in languages other than English and Chinese and unpublished studies were not included, and (2) by only focusing on quantitative research, the meaning domain of the symptom experience was not included and discussed in this scoping review.

■ Implication for Clinical Practice and Future Research

For the implications for clinical practice, this scoping review identified 5 well-studied and highly prevalent symptoms that should be assessed in women with breast cancer receiving endocrine therapy. These 5 symptoms are joint/muscle pain, hot flashes, low sexual interest/desire, joint/muscle stiffness, and fatigue/lack of energy. Moreover, some rarely studied but highly prevalent symptoms should also be assessed, including cramps, eye irritation, heart discomfort, anxiety, dyspareunia, urinary urgency, numbness and tingling, and dry eye syndrome. When assessing symptoms, nurses should evaluate the frequency of occurrence, intensity, and distress of key symptoms to have a clear and comprehensive understanding of the symptom experience during endocrine therapy in women with breast cancer. Nurses should also assess the influence of symptoms experienced on the quality of life and functional ability of women receiving endocrine therapy.

Given the state of the science related to symptoms experienced by women with breast cancer receiving endocrine therapy, there are several implications for future research. First, compared with the occurrence domain, there is a dearth of research addressing the intensity and distress domains of symptoms. There is a considerable need for studies to comprehensively determine the frequency of occurrence, intensity of symptoms, symptom distress, and the impact of symptoms on functional ability and quality of life. Second, because the heterogeneity of instruments significantly affects the comparison of results across studies, a symptom questionnaire/checklist encompassing multiple domains of endocrine therapy–specific symptoms is urgently needed. Meanwhile, use of CDEs should be encouraged in future studies on symptoms during endocrine therapy. An optimal recall period and clear definitions of symptoms should be studied and standardized in future studies. Third, this scoping review indicates that more research is needed, investigating rarely studied but highly prevalent symptoms, such as cramps, eye irritation, heart discomfort, anxiety, dyspareunia, urinary urgency, numbness and tingling, and dry eye syndrome, to confirm the robustness of the current evidence. Finally, more studies are needed to determine the symptoms clusters that occur in women receiving endocrine therapy and the trajectory patterns of symptoms (such as joint pain) during endocrine therapy. In addition, more studies to determine the mechanisms underlying the symptoms/symptom clusters and phenotypic variance should be conducted to gain a deeper understanding of symptoms during endocrine therapy as the basis for the development of symptom management interventions.

■ Conclusions

In this scoping review, 5 key symptoms associated with endocrine therapy were identified, including joint/muscle pain, hot flashes, low sexual interest/desire, joint/muscle stiffness, and fatigue/lack of energy. These symptoms should be included in clinical practice and future studies of endocrine therapy for breast cancer. There remain substantial gaps in the science related to the symptom experience during endocrine therapy for breast cancer, especially for the domains of symptom intensity and distress, specific understudied symptoms, and symptom clusters. Investigations examining rarely studied but highly prevalent symptoms (eg, cramps, eye irritation, heart discomfort, anxiety, dyspareunia, urinary urgency, numbness and tingling, and dry eye syndrome) are needed. Future studies on symptom clusters, individual variants of certain symptoms, and focused symptom assessment instruments are urgently needed.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. *CA Cancer J Clin.* 2017;67(1):7–30.
2. Chen W, Zheng R, Baade PD, et al. Cancer statistics in China, 2015. *CA Cancer J Clin.* 2016;66(2):115–132.
3. Early Breast Cancer Trialists' Collaborative Group. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet.* 2005; 365(9472):1687–1717.
4. Goss PE, Ingle JN, Pritchard KI, et al. Extending aromatase-inhibitor adjuvant therapy to 10 years. *N Engl J Med.* 2016;375(3):209–219.
5. Lao Romera J, Puertolas Hernández TJ, Peláez Fernández I, et al. Update on adjuvant hormonal treatment of early breast cancer. *Adv Ther.* 2011;28(suppl 6):1–18.
6. National Comprehensive Cancer Network. NCCN Clinical practice guidelines of breast cancer (version 2.2017). https://www.nccn.org/professionals/physician_gls/PDF/breast.pdf.
7. Rugo HS, Rumble RB, Burstein HJ. Endocrine therapy for hormone receptor positive metastatic breast cancer: American Society of Clinical Oncology guideline summary. *J Oncol Pract.* 2016;12(6):583–587.
8. Aiello Bowles EJ, Boudreau DM, Chubak J, et al. Patient-reported discontinuation of endocrine therapy and related adverse effects among women with early-stage breast cancer. *J Oncol Pract.* 2012;8(6):e149–e157.
9. Kidwell KM, Harte SE, Hayes DF, et al. Patient-reported symptoms and discontinuation of adjuvant aromatase inhibitor therapy. *Cancer.* 2014; 120(16):2403–2411.
10. Olufade T, Gallicchio L, MacDonald R, Helzlsouer KJ. Musculoskeletal pain and health-related quality of life among breast cancer patients treated with aromatase inhibitors. *Support Care Cancer.* 2015;23(2):447–455.
11. Fallowfield L, Atkins L, Catt S, et al. Patients' preference for administration of endocrine treatments by injection or tablets: results from a study of women with breast cancer. *Ann Oncol.* 2005;17(2):205–210.
12. Ruhstaller T, Von Moos R, Rufibach K, et al. Breast cancer patients on endocrine therapy reveal more symptoms when self-reporting than in pivotal trials: an outcome research study. *Oncology.* 2009;76(2):142–148.
13. Khalil H, Peters M, Godfrey CM, McInerney P, Soares CB, Parker D. An evidence-based approach to scoping reviews. *Worldviews Evid Based Nurs.* 2016;13(2):118–123.
14. Armstrong TS. Symptoms experience: a concept analysis. *Oncol Nurs Forum.* 2003;30(4):601–606.
15. Ashraf M, Biswas J, Majumdar S, et al. Tamoxifen use in Indian women—adverse effects revisited. *Asian Pac J Cancer Prev.* 2009;10(4):609–612.
16. Baumgart J, Nilsson K, Stavreus-Evers A, et al. Urogenital disorders in women with adjuvant endocrine therapy after early breast cancer. *Am J Obstet Gynecol.* 2011;204(1):26.e1–26.e7.
17. Baumgart J, Nilsson K, Evers AS, Kallak TK, Poromaa IS. Sexual dysfunction in women on adjuvant endocrine therapy after breast cancer. *Menopause.* 2013;20(2):162–168.
18. Baxter SD, Teft WA, Choi YH, Winquist E, Kim RB. Tamoxifen-associated hot flash severity is inversely correlated with endoxifen concentration and CYP3A4* 22. *Breast Cancer Res Treat.* 2014;145(2): 419–428.
19. Boehm DU, Lebrecht A, Eckhardt T, et al. Quality of life and adjuvant tamoxifen treatment in breast cancer patients. *Eur J Cancer Care.* 2009; 18(5):500–506.
20. Boonstra A, van Zadelhoff J, Timmer-Bonte A, Ottevanger PB, Beurskens CH, van Laarhoven HW. Arthralgia during aromatase inhibitor treatment in early breast cancer patients: prevalence, impact, and recognition by healthcare providers. *Cancer Nurs.* 2013;36(1):52–59.
21. Brown JC, Mao JJ, Stricker C, Hwang WT, Tan KS, Schmitz KH. Aromatase inhibitor associated musculoskeletal symptoms are associated with reduced physical activity among breast cancer survivors. *Breast J.* 2014;20(1):22–28.
22. Castel LD, Hartmann KE, Mayer IA, et al. Time course of arthralgia among women initiating aromatase inhibitor therapy and a postmenopausal comparison group in a prospective cohort. *Cancer.* 2013;119(13): 2375–2382.
23. Chim K, Xie SX, Stricker CT, et al. Joint pain severity predicts premature discontinuation of aromatase inhibitors in breast cancer survivors. *BMC Cancer.* 2013;13(1):401.
24. Chin SN, Trinkaus M, Simmons C, et al. Prevalence and severity of urogenital symptoms in postmenopausal women receiving endocrine therapy for breast cancer. *Clin Breast Cancer.* 2009;9(2):108–117.
25. Crew KD, Greenlee H, Capodice J, et al. Prevalence of joint symptoms in postmenopausal women taking aromatase inhibitors for early-stage breast cancer. *J Clin Oncol.* 2007;25(25):3877–3883.
26. Desai K, Mao JJ, Su I, et al. Prevalence and risk factors for insomnia among breast cancer patients on aromatase inhibitors. *Support Care Cancer.* 2013;21(1):43–51.
27. Dizdar O, Özçakar L, Malas FÜ, et al. Sonographic and electrodiagnostic evaluations in patients with aromatase inhibitor–related arthralgia. *J Clin Oncol.* 2009;27(30):4955–4960.
28. Egawa C, Hirokaga K, Takao S, et al. Risk factors for joint symptoms in postmenopausal Japanese breast cancer patients treated with anastrozole: a prospective multicenter cohort study of patient-reported outcomes. *Int J Clin Oncol.* 2016;21(2):262–269.
29. Frechette D, Paquet L, Verma S, et al. The impact of endocrine therapy on sexual dysfunction in postmenopausal women with early stage breast cancer: encouraging results from a prospective study. *Breast Cancer Res Treat.* 2013;141(1):111–117.
30. Gallicchio L, MacDonald R, Wood B, Rushovich E, Fedarko NS, Helzlsouer KJ. Changes in bone biomarker concentrations and musculoskeletal symptoms among breast cancer patients initiating aromatase inhibitor therapy and women without a history of cancer. *J Bone Miner Res.* 2012; 27(9):1959–1966.
31. Gallicchio L, MacDonald R, Wood B, Rushovich E, Helzlsouer KJ. Menopausal-type symptoms among breast cancer patients on aromatase inhibitor therapy. *Climacteric.* 2012;15(4):339–349.
32. Gallicchio L, Calhoun C, Helzlsouer KJ. Aromatase inhibitor therapy and hair loss among breast cancer survivors. *Breast Cancer Res Treat.* 2013; 142(2):435–443.
33. Garreau JR, DeLaMelena T, Walts D, Karamlou K, Johnson N. Side effects of aromatase inhibitors versus tamoxifen: the patients' perspective. *Am J Surg.* 2006;192(4):496–498.
34. Hadji P, Jackisch C, Bolten W, et al. COMPliance and Arthralgia in Clinical Therapy: the COMPACT trial, assessing the incidence of arthralgia, and compliance within the first year of adjuvant anastrozole therapy. *Ann Oncol.* 2014;25(2):372–377.
35. Horimoto Y, Saito M, Kasumi F. Arthralgia in 329 patients taking aromatase inhibitors. *Breast Care.* 2009;4(5):319–323.
36. Hu G, Zhan H, Hu R. Side effects associated with endocrine therapy in premenopausal patients with breast cancer-related research outcomes. *Shanxi Med J.* 2016;45(9):1007–1010.

37. Huang X, Zhang Q, Kang X, Song Y, Zhao W. Factors associated with cancer-related fatigue in breast cancer patients undergoing endocrine therapy in an urban setting: a cross-sectional study. *BMC Cancer*. 2010; 10(1):453.
38. Inglis H, Boyle FM, Friedlander ML, et al. Dry eyes and AIs: if you don't ask you won't find out. *Breast*. 2015;24(6):694–698.
39. Kanti V, Nuwayhid R, Lindner J, et al. Evaluation of trichodynia (hair pain) during chemotherapy or tamoxifen treatment in breast cancer patients. *J Eur Acad Dermatol Venereol*. 2016;30(1):112–118.
40. Kyvernitakis I, Ziller V, Hars O, Bauer M, Kalder M, Hadji P. Prevalence of menopausal symptoms and their influence on adherence in women with breast cancer. *Climacteric*. 2014;17(3):252–259.
41. Laroche F, Coste J, Medkour T, et al. Classification of and risk factors for estrogen deprivation pain syndromes related to aromatase inhibitor treatments in women with breast cancer: a prospective multicenter cohort study. *J Pain*. 2014;15(3):293–303.
42. Lintermans A, Van Asten K, Wildiers H, et al. A prospective assessment of musculoskeletal toxicity and loss of grip strength in breast cancer patients receiving adjuvant aromatase inhibitors and tamoxifen, and relation with BMI. *Breast Cancer Res Treat*. 2014;146(1):109–116.
43. Lintermans A, Vanderschueren D, Verhaeghe J, et al. Arthralgia induced by endocrine treatment for breast cancer: a prospective study of serum levels of insulin like growth factor-I, its binding protein and oestrogens. *Eur J Cancer*. 2014;50(17):2925–2931.
44. Lu HH, Li Q, Xu BH, et al. Effects of anastrozole on lipid metabolism in Chinese postmenopausal women with breast cancer. *Chinese J Oncol*. 2011;33(7):520–525.
45. Mao JJ, Stricker C, Bruner D, et al. Patterns and risk factors associated with aromatase inhibitor-related arthralgia among breast cancer survivors. *Cancer*. 2009;115(16):3631–3639.
46. Mao JJ, Su HI, Feng R, et al. Association of functional polymorphisms in CYP19A1 with aromatase inhibitor associated arthralgia in breast cancer survivors. *Breast Cancer Res*. 2011;13(1):R8.
47. Menas P, Merkel D, Hui W, Lawton J, Harper A, Carro G. Incidence and management of arthralgias in breast cancer patients treated with aromatase inhibitors in an outpatient oncology clinic. *J Oncol Pharm Pract*. 2012;18(4):387–393.
48. Mortimer JE, Boucher L, Baty J, Knapp DL, Ryan E, Rowland JH. Effect of tamoxifen on sexual functioning in patients with breast cancer. *J Clin Oncol*. 1999;17(5):1488.
49. Mortimer JE, Flatt SW, Parker BA, et al. Tamoxifen, hot flashes and recurrence in breast cancer. *Breast Cancer Res Treat*. 2008;108(3):421–426.
50. Napoli N, Vattikuti S, Ma C, et al. High prevalence of low vitamin D and musculoskeletal complaints in women with breast cancer. *Breast J*. 2010;16(6):609–616.
51. Oberguggenberger A, Hubalek M, Sztankay M, et al. Is the toxicity of adjuvant aromatase inhibitor therapy underestimated? Complementary information from patient-reported outcomes (PROs). *Breast Cancer Res Treat*. 2011;128(2):553–561.
52. Ochayon L, Tunin R, Yoselis A, Kadmon I. Symptoms of hormonal therapy and social support: is there a connection? Comparison of symptom severity, symptom interference and social support among breast cancer patients receiving and not receiving adjuvant hormonal treatment. *Eur J Oncol Nurs*. 2015;19(3):260–267.
53. Ohsako T, Inoue K, Nagamoto N, Yoshida Y, Nakahara O, Sakamoto N. Joint symptoms: a practical problem of anastrozole. *Breast Cancer*. 2006; 13(3):284–288.
54. Presant CA, Bosserman L, Young T, et al. Aromatase inhibitor–associated arthralgia and/or bone pain: frequency and characterization in non–clinical trial patients. *Clin Breast Cancer*. 2007;7(10):775–778.
55. Ribi K, Bernhard J, Rufibach K, et al. Endocrine symptom assessment in women with breast cancer: what a simple "yes" means. *Support Care Cancer*. 2007;15(12):1349–1356.
56. Rosenberg SM, Stanton AL, Petrie KJ, Partridge AH. Symptoms and symptom attribution among women on endocrine therapy for breast cancer. *Oncologist*. 2015;20(6):598–604.
57. Sagara Y, Kosha S, Baba S, et al. Adverse events and bone health during anastrozole therapy in postmenopausal Japanese breast cancer patients. *Breast Cancer*. 2010;17(3):212–217.
58. Schover LR, Baum GP, Fuson LA, Brewster A, Melhem-Bertrandt A. Sexual problems during the first 2 years of adjuvant treatment with aromatase inhibitors. *J Sex Med*. 2014;11(12):3102–3111.
59. Servitja S, Nogués X, Prieto-Alhambra D, et al. Bone health in a prospective cohort of postmenopausal women receiving aromatase inhibitors for early breast cancer. *Breast*. 2012;21(1):95–101.
60. Shi Q, Giordano SH, Lu H, Saleeba AK, Malveaux D, Cleeland CS. Anastrozole-associated joint pain and other symptoms in patients with breast cancer. *J Pain*. 2013;14(3):290–296.
61. Singer O, Cigler T, Moore AB, et al. Defining the aromatase inhibitor musculoskeletal syndrome: a prospective study. *Arthritis Care Res (Hoboken)*. 2012;64(12):1910–1918.
62. Su HI, Sammel MD, Springer E, Freeman EW, DeMichele A, Mao JJ. Weight gain is associated with increased risk of hot flashes in breast cancer survivors on aromatase inhibitors. *Breast Cancer Res Treat*. 2010;124(1): 205–211.
63. Swenson KK, Nissen MJ, Henly SJ, et al. Identification of tools to measure changes in musculoskeletal symptoms and physical functioning in women with breast cancer receiving aromatase inhibitors. *Oncol Nurs Forum*. 2013;40(6):549–557.
64. Waltman NL, Ott CD, Twiss JJ, Gross GJ, Lindsey AM. Vitamin D insufficiency and musculoskeletal symptoms in breast cancer survivors on aromatase inhibitor therapy. *Cancer Nurs*. 2009;32(2):143–150.
65. Wang J, Lu K, Song Y, et al. Indications of clinical and genetic predictors for aromatase inhibitors related musculoskeletal adverse events in Chinese Han women with breast cancer. *PLoS One*. 2013;8(7):e68798.
66. Xu L, Wang J, Xue DD, He W. Aromatase inhibitors associated musculoskeletal disorders and bone fractures in postmenopausal breast cancer patients: a result from Chinese population. *Med Oncol*. 2014;31(9):128.
67. Zhan M, Flaws JA, Gallicchio L, Tkaczuk K, Lewis LM, Royak-Schaler R. Profiles of tamoxifen-related side effects by race and smoking status in women with breast cancer. *Cancer Detect Prev*. 2007;31(5):384–390.
68. Zhou LH, Liu GY, Di GH, Lu JS, Shen ZZ, Shao ZM. Safety of sequential exemestane therapy after 2–3 years' tamoxifen treatment in postmenopausal women with hormone sensitive breast cancer. *Tumor*. 2011;31(4):354–358.
69. Cleeland CS, Mendoza TR, Wang XS, et al. Assessing symptom distress in cancer patients: the M.D. Anderson Symptom Inventory. *Cancer*. 2000; 89(7):1634–1646.
70. Fallowfield LJ, Leaity SK, Howell A, Benson S, Cella D. Assessment of quality of life in women undergoing hormonal therapy for breast cancer: validation of an endocrine symptom subscale for the FACT-B. *Breast Cancer Res Treat*. 1999;55(2):189–199.
71. Terhorst L, Blair-Belansky H, Moore PJ, Bender C. Evaluation of the psychometric properties of the BCPT Symptom Checklist with a sample of breast cancer patients before and after adjuvant therapy. *Psychooncology*. 2011;20(9):961–968.
72. Feikin DR, Audi A, Olack B, et al. Evaluation of the optimal recall period for disease symptoms in home-based morbidity surveillance in rural and urban Kenya. *Int J Epidemiol*. 2010;39(2):450–458.
73. Norquist JM, Girman C, Fehnel S, DeMuro-Mercon C, Santanello N. Choice of recall period for patient-reported outcome (PRO) measures: criteria for consideration. *Qual Life Res*. 2012;21(6):1013–1020.
74. Cashion AK, Grady PA. The National institutes of Health/National Institutes of Nursing Research intramural research program and the development of The National institutes of health symptom science model. *Nurs Outlook*. 2015;63(4):484–487.
75. Dodd M, Janson S, Facione N, et al. Advancing the science of symptom management. *J Adv Nurs*. 2001;33(5):668–676.
76. Redeker NS, Anderson R, Bakken S, et al. Advancing symptom science through use of common data elements. *J Nurs Scholarsh*. 2015;47(5):379–388.
77. Miaskowski C. Future directions in symptom cluster research. *Semin Oncol Nurs*. 2016;32(4):405–415.

Appendix D PRELIMINARY WORK #2: GENETIC UNDERPINNINGS OF MUSCULOSKELETAL PAIN DURING TREATMENT WITH AROMATASE INHIBITOR FOR BREAST CANCER: A BIOLOGICAL PATHWAY ANALYSIS

Methodological Paper

Biological Research for Nursing
1-14

DOI: 10.1177/1099800419895114
journals.sagepub.com/home/brn
SAGE

Genetic Underpinnings of Musculoskeletal Pain During Treatment With Aromatase Inhibitors for Breast Cancer: A Biological Pathway Analysis

Yehui Zhu, PhD, MSN, BSN[1], Theresa A. Koleck, PhD, RN[2], Catherine M. Bender, PhD, RN, FAAN[1], and Yvette P. Conley, PhD, FAAN[1]

Abstract

Background: Musculoskeletal pain (MSKP) is the most reported symptom during treatment with aromatase inhibitors (AIs) for breast cancer. The mechanisms underlying MSKP are multidimensional and not well understood. The goals of this biological pathway analysis were to (1) gain an understanding of the genetic variation and biological mechanisms underlying MSKP with AI therapy and (2) identify plausible biological pathways and candidate genes for future investigation. **Method:** Genes associated with MSKP during AI therapy or genes involved in drug metabolism of and response to AIs were identified from the literature. Studies published through February 2019 were queried in PubMed®. The genes identified from the literature were entered into QIAGEN's Ingenuity® Pathway Analysis (IPA) software to generate canonical pathways, upstream regulators, and networks through a core analysis. **Results:** The 17 genes identified were *ABCB1*, *ABCG1*, *CYP17A1*, *CYP19A1*, *CYP27B1*, *CYP2A6*, *CYP3A4*, *CYP3A5*, *ESR1*, *OATP1B1*, *OPG*, *RANKL*, *SLCO3A1*, *TCL1A*, *UGT2A1*, *UGT2B17*, and *VDR*. These genes are involved in encoding bone-remodeling regulators, drug-metabolizing enzymes (cytochrome P450 family, UDP-glucuronosyltransferases family), or drug transporters (ATP-binding cassette transporters, organic anion transporters). Multiple plausible biological pathways (e.g., nicotine degradation, melatonin degradation) and candidate genes (e.g., *NFKB*, *HSP90*, *AKT*, *ERK1/2*, *FOXA2*) are proposed for future investigation based on the IPA results. **Conclusion:** Multiple genes and molecular-level etiologies may contribute to MSKP with AI therapy in women with breast cancer. Our innovative combination of gene identification from the literature plus biological pathway analysis allowed for the emergence of novel candidate genes and biological pathways for future investigations.

Keywords

breast neoplasms, musculoskeletal pain, gene, biological pathway analysis, aromatase inhibitors

Breast cancer is the most common newly diagnosed cancer and the second-leading cause of cancer mortality among women worldwide (Torre et al., 2015). Approximately, 80% of post-menopausal women with breast cancer have hormone receptor–positive disease (i.e., estrogen and/or progesterone receptor; Osborne, 1998). Overall and disease-free survival rates are significantly improved in these cases with 5–10 years of aromatase inhibitor (AI) therapy (Goss et al., 2016; Romera et al., 2011); nevertheless, this AI therapy has a high discontinuation rate. Musculoskeletal pain (MSKP), including arthralgias, myalgia, and joint/muscle stiffness, has been implicated as the number one reason for the high AI therapy discontinuation rate, with 13–50% of women on AI therapy reporting that MSKP was the reason they discontinued it (Chim et al., 2013; Crew et al., 2007; Mao et al., 2009; Presant et al., 2007). Additionally, a woman's quality of life and physical functioning deteriorate with AI therapy: 48–64.3% of AI users report a decline in their ability to carry out their daily activities due to MSKP (Egawa et al., 2016; Waltman, Ott, Twiss, Gross, & Lindsey, 2009). While several strategies have been proposed to manage MSKP with AI therapy, there are still no conclusively effective interventions to prevent or manage the problem. One of the main barriers is the lack of understanding of the multifactorial biological mechanisms underlying MSKP (Borrie & Kim, 2017).

Aromatase is an enzyme that catalyzes a critical step of estrogen biosynthesis (i.e., aromatization of testosterone to estradiol and androstenedione to estrone). An AI blocks the

[1] School of Nursing, University of Pittsburgh, PA, USA
[2] School of Nursing, Columbia University, New York, NY, USA

Corresponding Author:
Yehui Zhu, PhD, MSN, BSN, School of Nursing, University of Pittsburgh, 415 Victoria Building, 3500 Victoria Street, Pittsburgh, PA 15261, USA.
Emails: zhuyehui09@gmail.com; yez7@pitt.edu

Table 1. Search Terms for Candidate-Gene Identification in the Literature.

Terms for Breast Cancer		Terms for AI		Terms for Genetics		Terms for MSKP
"Breast Neoplasm" [Mesh]	AND/ OR	"Aromatase Inhibitor" [Mesh]	AND	• "Genetics" [Mesh] • "Biology" [Mesh] • "Genotype" [Mesh] • "Pharmacogenetics" [Mesh]	OR	• "Musculoskeletal Disease" [Mesh:NoExp] • "Musculoskeletal Pain" [Mesh:NoExp] • "Arthralgia" [Mesh] • "Myalgia" [Mesh]

Note. Search combination 1: (Breast cancer) and MSKP and genetics. Search combination 2: AI and genetics. AI = aromatase inhibitor; MSKP = musculoskeletal pain.

activity of aromatase by either reversibly binding (e.g., anastrozole and letrozole) or irreversibly binding (e.g., exemestane) to the enzyme. The three most commonly prescribed AIs for breast cancer, letrozole (2.5 mg/day), anastrozole (1 mg/day), and exemestane (25 mg/day), inhibit estrogen biosynthesis by rates of 99%, 97%, and 98%, respectively (Fabian, 2007). Consequently, circulating estrogen levels and estrogen-dependent cellular proliferation are reduced (Campos, 2004; Geisler, Haynes, Anker, Dowsett, & Lonning, 2002). Because estrogen had been reported to have a complex modulatory function for nociceptive effects through the nervous, immune, and skeletal systems (Craft, 2007), estrogen suppression/deprivation became the early hypothesis for the mechanism underlying development of MSKP during AI therapy for breast cancer (Felson & Cummings, 2005). This hypothesis was reinforced by the finding that women experiencing arthralgias have a significantly lower estradiol level than those without arthralgias during AI therapy for breast cancer (J. Wang et al., 2013). Thus, genes involved in the biosynthesis and action of estrogen (e.g., *CYP19A1*, *ESR1*) have been the main focus for studying the genetic underpinnings of MSKP with AI therapy for breast cancer (Fontein et al., 2014; Henry et al., 2013; Mao et al., 2011; Park et al., 2011; J. Wang et al., 2013).

Recent advances in pharmacogenetics and pharmacogenomics have provided new insights related to the genetic underpinnings of MSKP with AI therapy by enabling the investigation of inherited and acquired genetic variation in drug metabolism and drug response at individual and population levels (L. Wang, McLeod, & Weinshilboum, 2011; Wilkinson, 2005). With a flat-fixed dosing (2.5 mg/day for letrozole, 1 mg/day for anastrozole, and 25 mg/day for exemestane), researchers have observed a 10- to 12-fold variation in plasma concentration for both letrozole and anastrozole (Desta et al., 2011; Kamdem et al., 2010). The variation in drug concentration suggests that genetic variation involved in drug metabolism may contribute to variability in the onset or severity of drug side effects (such as MSKP with AI therapy). At the population level, genomic variation has also been associated with drug response (e.g., efficacy, side effects). In the MA.27 study, a large multisite clinical trial of AIs, a case–control genome-wide association study (GWAS) identified an association between susceptibility to MSKP and single-nucleotide polymorphisms (SNPs) in the *TCL1A* gene (Ingle et al, 2010). With this remarkable finding and the hope of fully uncovering the etiologies of MSKP with AI therapy for breast cancer, more researchers began to investigate the role of genetic variation in drug metabolism of and response to AIs.

Despite the previous research, the candidate biological pathways already being investigated do not fully explain the variability in the MSKP phenotype (e.g., occurrence, intensity). Therefore, we conducted the present biological pathway analysis, aiming to (1) gain an understanding of the genetic variation and biological mechanisms underlying MSKP with AI therapy in breast cancer and (2) identify plausible candidate genes and biological pathways of MSKP for future investigation.

Method

Candidate-Gene Identification

We identified an initial list of candidate genes that have been associated with MSKP during AI therapy or genes involved in drug metabolism of and response to AIs from the literature. We searched for studies published through February 2019 using two combinations of key term categories in PubMed®: (1) breast cancer and MSKP and genetics and (2) AI and genetics (Table 1). We also reviewed reference lists of selected articles to obtain additional relevant articles. Participants in selected studies were adults (18 years or older) who received oral AIs (i.e., anastrozole, letrozole, or exemestane) to prevent or treat breast cancer. We excluded studies that included treatment with Tamoxifen or were not published in English. Figure 1 displays the detailed screening and selection processes. A total of 18 articles, featuring 17 genes, met inclusion criteria (Borrie et al., 2018; Desta et al., 2011; Fontein et al., 2014; Garcia-Giralt et al., 2013; Gervasini et al., 2017; Gregory, Chen, Murphy, Atchley, & Kamdem, 2017; Henry et al., 2013; Ingle et al., 2010; Jeong, Woo, Flockhart, & Desta 2009; Kamdem, David, & Zeruesenay, 2014; Lintermans et al., 2016; Liu et al., 2012; Mao et al., 2011; Park et al., 2011; Rumiato et al., 2016; Sun, Chen, Dellinger, Sharma, & Lazarus, 2010; J. Wang et al., 2013, J. Wang et al., 2015).

Ingenuity Pathway Analysis

Ingenuity Pathway Analysis (IPA) is an up-to-date, web-based biological analysis tool for omics data, including genetic/

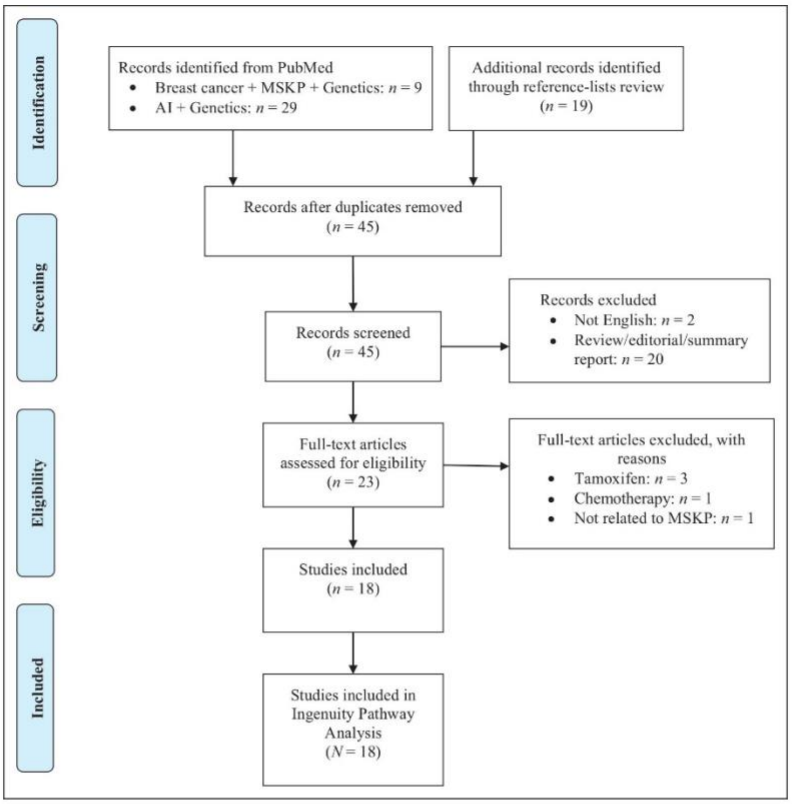


Figure 1. Flowchart depicting selection of studies for identifying candidate genes. AI = aromatase inhibitor; MSKP = musculoskeletal pain.

genomic data. IPA allows researchers to quickly gain knowledge and understanding of the functions of genes and their relevant biological pathways using the Ingenuity Knowledge Base. The Ingenuity Knowledge Base is a manually curated repository of biological relationships. Based on a list of user-defined genes, IPA generates potentially relevant pathways, regulators, and networks and informs testable hypotheses for future investigation. Researchers have previously used IPA as an innovative method to identify and prioritize candidate genes and biological pathways in genetic studies in symptom science (Koleck & Conley, 2016; Livingston et al., 2015).

We entered the 17 genes that we identified from the literature into QIAGEN's IPA software (IPA®, QIAGEN, Redwood City, CA, https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis/) and performed a core analysis that included both direct and indirect relationships between genes. The relationships were limited to human studies. The IPA software returned the overlapping canonical pathways, upstream regulators, and mechanistic/causal networks.

Results

According to our literature review, the genes with evidence of a relationship to MSKP during AI treatment for breast cancer are *ABCB1*, *CYP17A1*, *CYP19A1*, *CYP27B1*, *ESR1*, *OPG*, *RANKL*, *TCL1A*, and *VDR*. Genes reported to be involved in drug metabolism and drug response of AIs include *ABCB1*, *ABCG1*, *CYP2A6*, *CYP3A4*, *CYP3A5*, *OATP1B1*, *SLCO3A1*, *UGT2A1*, and *UGT2B17*. The functions and references of these 17 genes are detailed in Table 2. Of these 17 genes, 8 play important roles in the production of systemic (estrogen, vitamin D, etc.) and local regulators (receptor activator of nuclear factor kappa-B ligand [RANKL] and osteoprotegrin [OPG]) for bone remodeling, including *CYP17A1*, *CYP19A1*, *ESR1*, *TCL1A*, *VDR*, *CYP27B1*, *OPG*, and *RANKL*, while the remaining 9 (*CYP2A6*, *CYP3A4*, *CYP3A5*, *UGT2B17*, *UGT2A1*, *ABCB1*, *ABCG1*, *OATP1B1*, and *SLCO3A1*) belong to two families of drug-metabolizing enzymes (P450 family and UDP-glucuronosyltransferases family [UGT] family) and two families of drug transporters (ATP-binding cassette [ABC]

Table 2. Genes Associated With MSKP With AI Therapy or Drug Metabolism and Drug Response for AIs.

Gene Symbol	Name	Function[a]	References
Bone remodeling			
CYP17A1	Cytochrome P450 family 17 subfamily A member 1	Encodes a member of the cytochrome P450 superfamily of enzymes, which catalyze many reactions in drug metabolism and synthesis of cholesterol, steroids, and other lipids. It is a key enzyme in the steroidogenic pathway that produces progestin, mineralocorticoids, glucocorticoids, androgens, and estrogens.	Garcia-Giralt et al. (2013)
CYP19A1	Cytochrome P450 family 19 subfamily A member 1	Encodes a member of the cytochrome P450 superfamily of enzymes, which catalyze the last steps of estrogen biosynthesis.	Fontein et al. (2014), Gervasini et al. (2017), Mao et al. (2011), and Park et al. (2011)
CYP27B1	Cytochrome P450 family 27 subfamily B member 1	Encodes a member of the cytochrome P450 superfamily of enzymes. The enzyme encoded by this gene regulates the level of biologically active vitamin D and plays an important role in calcium homeostasis.	Garcia-Giralt et al. (2013)
ESR1	Estrogen receptor 1	Encodes an estrogen receptor that is essential for sexual development and reproductive function and plays a role in other tissues such as bone. Estrogen receptors are involved in pathological processes including breast cancer, endometrial cancer, and osteoporosis.	Henry et al. (2013) and Wang et al. (2013)
OPG (TNFRSF11B)	Osteoprotegerin, an alias for TNF receptor superfamily member 11B	Encodes a protein that is a member of the TNF-receptor superfamily. The protein is an osteoblast-secreted decoy receptor that functions as a negative regulator of bone resorption.	Lintermans et al. (2016) and Wang et al. (2015)
RANKL (TNFSF11)	Receptor activator of Nuclear factor kappa-B ligand, an alias for TNF superfamily member 11	Encodes a member of the TNF cytokine family that is a ligand for osteoprotegerin and functions as a key factor for osteoclast differentiation and activation.	Wang et al. (2015)
TCL1A	T-cell leukemia/lymphoma 1A	Encodes a protein, T-cell leukemia/lymphoma protein 1A, that functions as a coactivator of the cell survival kinase Akt.	Ingle et al. (2010) and Liu et al. (2012)
VDR	Vitamin D receptor	Encodes the nuclear hormone receptor for vitamin D3.	Garcia-Giralt et al. (2013)
Drug metabolism			
ABCB1	ATP-binding cassette subfamily B member 1	Encodes an ABC transporter. ABC protein transports various molecules across extra- and intracellular membranes.	Gervasini et al. (2017)
ABCG1	ATP-binding cassette subfamily G member 1	Encodes an ABC transporter.	Rumiato et al. (2016)
CYP2A6	Cytochrome P450 family 2 subfamily A member 6	Encodes a member of the cytochrome P450 superfamily of enzymes. The enzyme encoded by this gene metabolizes nicotine, aflatoxin B1, nitrosamines, and some pharmaceuticals.	Borrie et al. (2018), Desta et al. (2011), and Jeong et al. (2009)
CYP3A4	Cytochrome P450 family 3 subfamily A member 4	Encodes a member of the cytochrome P450 superfamily of enzymes. The enzyme encoded by this gene is involved in the metabolism of approximately half the drugs in use today, including acetaminophen, codeine, cyclosporine A, diazepam, and erythromycin. It metabolizes some steroids and carcinogens.	Kamdem, David, and Zeruesenay (2014)

(continued)

Table 2. (continued)

Gene Symbol	Name	Function[a]	References
CYP3A5	Cytochrome P450 family 3 subfamily A member 5	Encodes a member of the cytochrome P450 superfamily of enzymes. Its encoded protein metabolizes drugs and the steroid hormones testosterone and progesterone.	Kamdem et al. (2014)
OATP1B1 (SLCO1B1)	Organic anion–transporting polypeptide 1B1, an alias for solute carrier organic anion transporter family member 1B1	Encodes a liver-specific member of the organic anion transporter family. The encoded protein is a transmembrane receptor.	Gregory, Chen, Murphy, Atchley, and Kamdem (2017)
SLCO3A1	Solute carrier organic anion transporter family member 3A1	Encodes an organic anion transporter, which transports glucose and other sugars, vitamins, nucleosides, and so on	Rumiato et al. (2016)
UGT2A1	UDP glucuronosyltransferase family 2 member A1 complex locus	Encodes a protein that belongs to the Uridine 5′-diphospho-glucuronosyltransferase (UDP glucuronosyltransferase, UGT) family of proteins, which catalyze biotransformation reactions.	Rumiato et al. (2016)
UGT2B17	UDP glucuronosyltransferase family 2 member B17	Encodes a member of the UDP-glycosyltransferase (UGT) family of proteins. The encoded enzyme catalyzes glucuronidation, which is an intermediate step in the metabolism of steroids.	Sun, Chen, Dellinger, Sharma, and Lazarus (2010)

Note. ABC = ATP-binding cassette; AI = aromatase inhibitor; Akt = protein kinase B; ATP = adenosine triphosphate; MSKP = musculoskeletal pain; TNF = tumor necrosis factor.
[a]The gene function was extracted from the GeneCards® Human Gene Database at www.genecards.org.

transporters and organic anion transporters [OAT]). We further examine the influence of these genes on MSKP with AI therapy in the Discussion section.

Overlapping Canonical Pathways

Figure 2 displays canonical pathways from the 17-gene set that share one or more genes in common. The 15 most significant canonical pathways of the 17-gene set were nicotine degradation III, nicotine degradation II, melatonin degradation I, superpathway of melatonin degradation, bupropion degradation, acetone degradation I, lipopolysaccharide (LPS) /interleukin-1 (IL-1)-mediated inhibition of retinoid X receptors (RXR) function, estrogen biosynthesis, xenobiotic metabolism signaling, hepatic cholestasis, pregnane X receptor (PXR)/RXR activation, vitamin D receptor (VDR)/RXR activation, IL-6 (interleukin-6) signaling, thyroid hormone metabolism II, and serotonin degradation. Inclusion of the 17 genes in the 15 most significant canonical pathways is detailed in Table 3.

Networks

Our analysis identified two unique networks associated with the identified genes. The main associated functions for Network A are connective-tissue development and function, skeletal- and muscular-system development and function, and tissue development and for Network B are lipid metabolism, small-molecule biochemistry, and vitamin and mineral metabolism (Figure 3).

Upregulators

The top five upstream transcription regulators of the 17-gene set are *NR1I2* (ligand-dependent nuclear receptor), *FOXA2* (transcription regulator), *Histone h4* (DNA binding, protein domain–specific binding), *BRCA1* (transcription regulator), and *NR1I3* (ligand-dependent nuclear receptor).

Discussion

We identified 17 genes from the literature and gained further knowledge about their function and relevant biological pathways using IPA. We found that multiple genes reported to be relevant to MSKP during AI therapy play critical regulatory roles in bone remodeling.

Bone Remodeling

Bone remodeling, which maintains the homeostasis of bone, is a highly coordinated and continuous process involving the resorption of mature bone (by osteoclasts) and the formation of new bone (by osteoblasts; Raggatt & Partridge, 2010). The products of multiple genes we identified from the literature are either systemic (e.g., estrogen, vitamin D, insulin-like growth factor [IGF]-1) or local regulators (e.g., RANKL and OPG) for bone remodeling.

Estrogen. In adults, estrogen is one of the most important endocrine regulators of bone turnover via the role it plays in determining the rate of bone remodeling (Walsh, 2017). At the cellular level, estrogen decreases osteoclast formation

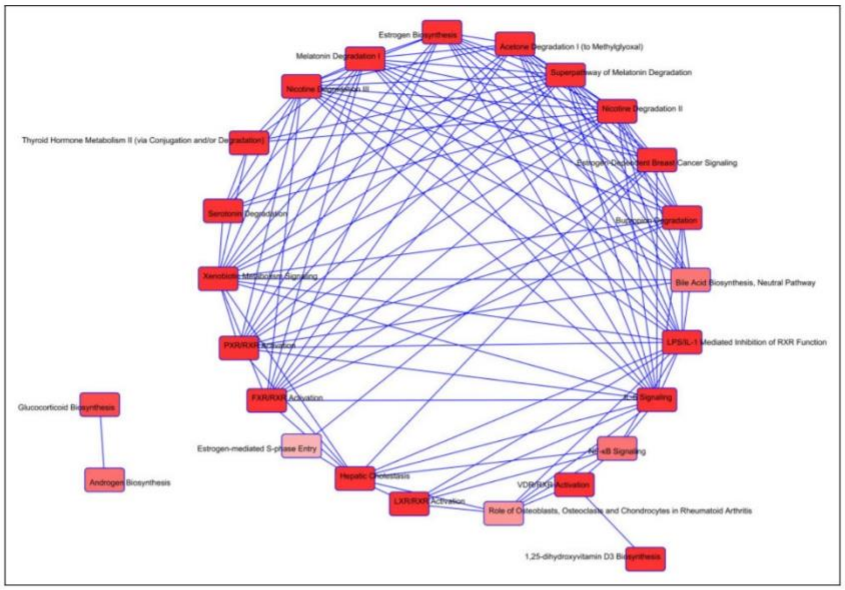


Figure 2. Overlapping canonical pathways map representing shared biology among the identified 17-gene set. The network of overlapping canonical pathways shows each pathway as a single "node" colored proportionally to the Fisher's exact test *p* value, where brighter red means greater significance. A line connects any two pathways that share at least one gene in common.

(Srivastava et al., 2001) and reduces osteoclast life span (Kameda et al., 1997). Moreover, estrogen stimulates osteoblast proliferation and prevents apoptosis of osteoblasts. In menopausal women, estrogen deficiency may cause loss of bone, decreased bone mass, disturbed architecture, and reduced bone strength.

CYP17A1, *CYP19A1*, and *ESR1* are involved in regulation of the production and action of estrogen. Enzymes transcribed from *CYP17A1* are requisite for the biosynthesis of androgen (i.e., the primary precursor of estrogen; Chung et al., 1987; Matteson, Picado-Leonard, Chung, Mohandas, & Miller, 1986; Picado-Leonard & Miller, 1987). Garcia-Giralt et al. (2013) reported that the *CYP17A1* SNPs rs4919686, rs4919683, rs4919687, rs3781287, rs10786712, rs6163, and rs743572 were significantly associated with worsening pain intensity in joints at 12 months after initiation of AI therapy. The *CYP19A1* gene encodes aromatase, which is a critical enzyme for catalyzing the biosynthesis of estrogen. *CYP19A1* is a highly polymorphic gene. Gervasini et al. (2017) reported that *CYP19A1* rs1008805 was associated with arthralgias in 110 postmenopausal women with breast cancer treated with anastrozole. In a study among 390 Caucasian women undergoing AI therapy for breast cancer, Mao et al. (2011) found that women who carry at least one $(TTTA)_7$ repeat allele in rs60271534 were at higher risk of self-reported occurrence of arthralgias, whereas people carrying one or more $(TTTA)_8$ repeat alleles had lower risk. Mao et al. also examined rs10046, rs749292, rs727479, and rs1157899 but found no significant results. In a study of 109 Korean women under treatment with letrozole, Park et al. (2011) reported that the M_3_5 haplotype (composed of rs12148604, rs4646, rs10046, rs700519, rs4324076, rs700518, rs3759811, rs727479, rs4775936, rs10459592, rs767199, rs10519297, rs1062033, rs2008691, rs1008805, and rs17523527) in *CYP19A1* was associated with self-reported occurrence of bone pain. In a study of 737 Dutch patients under treatment with exemestane, homozygous *CYP19A1* rs934635-AA genotype was significantly associated with occurrence, but not severity, of adverse musculoskeletal events (including arthralgia, arthritis and osteoarthritis, myalgia, and other musculoskeletal problems; Fontein et al., 2014).

The *ESR1* gene encodes an estrogen receptor, which is a ligand-activated transcription factor for hormone binding, DNA binding, and activation of transcription. In a study among 436 postmenopausal Chinese Han women who received letrozole or anastrozole, J. Wang et al. (2013) found two SNPs (rs2234693 and rs9340799) in *ESR1* that were associated with adverse musculoskeletal events (including joint pain, muscle pain, bone pain, arthritis, diminished joint function, or other musculoskeletal problems). Henry et al. (2013) reported that rs9322336 in *ESR1* was associated with the discontinuation of exemestane due to musculoskeletal toxicity.

In a GWAS study, four SNPs (rs11849538, rs7158782, rs7159713, and rs2369049) close to *TCL1A* were associated

Table 3. Fifteen Most Significant Canonical Pathways Across the Entire Data Set and Their Overlap With the 17-Gene Set.

Canonical Pathway	Sig. Value	Overlap (Ratio)	Group	*ABCB1*	*ABCG1*	*CYP17A1*	*CYP19A1*	*CYP27B1*	*CYP2A6*	*CYP3A4*	*CYP3A5*	*ESR1*	*OATP1B1*	*OPG*	*RANKL*	*SLCO3A1*	*TCL1A*	*UGT2A1*	*UGT2B17*	*VDR*
Nicotine degradation III	11.78	6/48 12.5%	Metabolic pathway				X		X	X	X							X	X	
Nicotine degradation II	11.46	6/54 11.1%	Metabolic pathway				X		X	X	X							X	X	
Melatonin degradation I	11.31	6/57 10.5%	Metabolic pathway				X		X	X	X							X	X	
Superpathway of melatonin degradation	11.08	6/62 9.7%	Metabolic pathway				X		X	X	X							X	X	
Bupropion degradation	8.43	4/24 16.7%	Metabolic pathway				X		X	X	X									
Acetone degradation I (to methylglyoxal)	8.08	4/29 13.8%	Metabolic pathway				X		X	X	X									
LPS/IL-1-mediated inhibition of RXR function	7.86	6/210 2.9%	Signaling pathway	X	X				X	X	X			X						
Estrogen biosynthesis	7.54	4/39 10.3%	Metabolic pathway				X		X	X	X									
Xenobiotic metabolism signaling	7.10	6/282 2.1%	Signaling pathway	X	X					X	X							X	X	
Hepatic cholestasis	6.78	5/158 3.2%	Metabolic pathway	X	X							X		X		X				
PXR/RXR activation	6.69	4/63 6.3%	Signaling pathway	X					X	X	X									
VDR/RXR activation	4.45	3/77 3.9%	Signaling pathway					X							X					X
IL-6 signaling	3.71	3/136 2.2%	Signaling pathway	X			X							X						
Thyroid hormone metabolism II	3.38	2/36 5.6%	Metabolic pathway															X	X	
Serotonin degradation	2.91	2/62 3.2%	Metabolic pathway															X	X	

Note. The significance (Sig.) values for the canonical pathways are −log of *p* value, which is calculated by Fisher's exact test right-tailed. The significance indicates the probability of association of molecules from the 17-gene set with the canonical pathway by random chance alone. Greater significance value indicates smaller *p* value. VDR = vitamin D receptor.

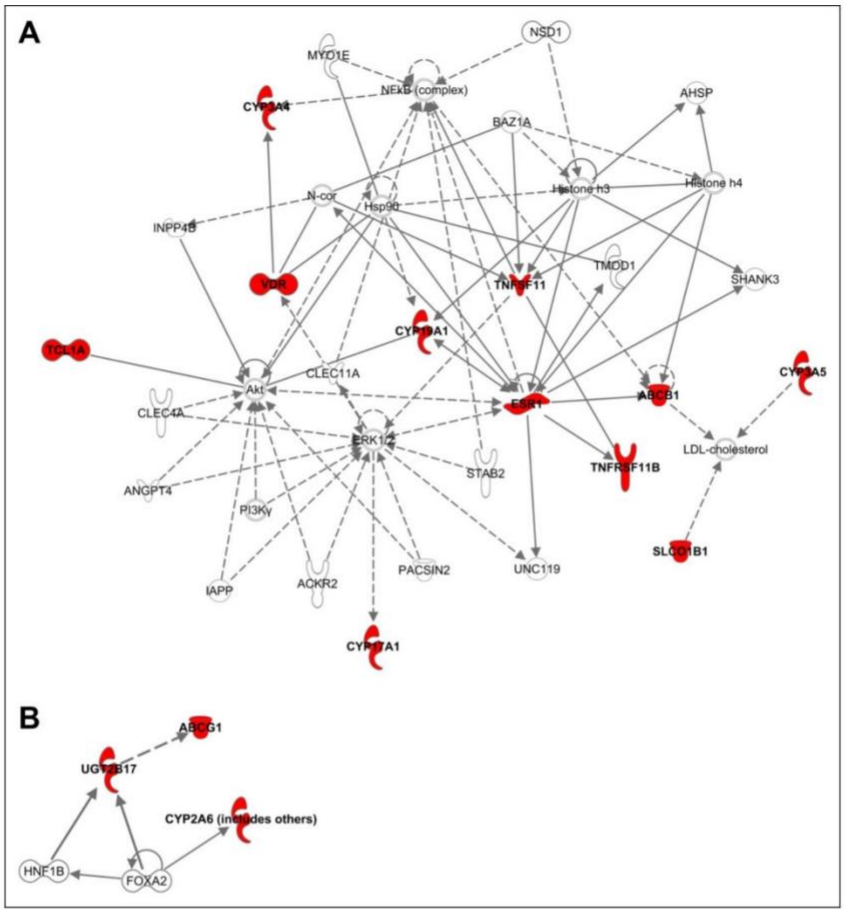


Figure 3. Gene–gene networks generated by pathway analysis from the 17-gene set. The main associated functions of networks are (A) connective-tissue development and function, skeletal- and muscular-system development and function, and tissue development and (B) lipid metabolism, small-molecule biochemistry, and vitamin and mineral metabolism. Genes identified from the literature are marked in red. Solid lines represent direct interactions. Dashed lines represent indirect interactions. The Ingenuity® Pathway Analysis networks legend can be found at http://qiagen.force.com/KnowledgeBase/articles/Basic_Technical_Q_A/Legend

with adverse musculoskeletal events, with rs11849538 found to be associated with increased *TCL1A* expression after exposure to estrogen in further functional genomic studies (Ingle et al., 2010). The estrogen-induced *TCL1A* expression altered the expression of IL-17, IL-17RA, IL-12, IL-12RB2, and IL-1R2 and increased nuclear factor-κB transcriptional activity (Liu et al., 2012). These results provide evidence of a relationship between estrogen and arthralgias (L. Wang et al., 2011).

Vitamin D. The storage of excess calcium in bones can trigger bone remodeling, which enables removal of calcium from bones. Vitamin D facilitates intestinal calcium and phosphorus absorption to enhance bone mineralization, and it has been observed to inhibit osteoclastogenesis under the regulation of *OPG* in mature osteoblasts (Baldock et al., 2006). Among women with breast cancer receiving AIs, vitamin D deficiency is related to the occurrence and intensity of arthralgia (Servitja et al., 2015; Singer et al., 2014). Moreover, supplementation with a high dose of vitamin D eliminated arthralgia during AI therapy in a double-blind, randomized clinical trial (Rastelli et al., 2011). *VDR* encodes the nuclear hormone receptor for vitamin D3. Garcia-Giralt et al. (2013) reported that rs11568820 in *VDR* was associated with the development of arthralgia in AI therapy during the first 12 months of AI use.

CYP27B1 encodes an enzyme that converts vitamin D into its activated form (i.e., vitamin D3 or calcitriol). Garcia-Giralt et al. (2013) reported that rs4646536 in *CYP27B1* was associated with development of arthralgia in the first 3 and 12 months of AI therapy, and rs10877012 in *CYP27B1* was associated with arthralgia in the first 12 months of AI therapy.

IGF-1. IGF-1 plays an important in skeletal growth, especially at the cartilaginous end plates and during endochondral bone formation, by increasing osteoblast activity and bone formation. IGF-1 also assists in the maintenance of bone mineral density in adults (J. Wang, Zhou, Cheng, Kopchick, & Bondy, 2004). Gallicchio, MacDonald, and Helzlsouer (2013) found that increases in IGF-1 concentration in the blood over the first 6 months of AI therapy were significantly associated with the development and intensity of MSKP among women with breast cancer. The association between genetic variants in *IGF-1* and MSKP has not been reported in the current literature.

RANK/RANKL/OPG system. The rate and equilibrium of bone remodeling is controlled by local regulators such as the RANK/RANKL/OPG system. RANK is a receptor expressed on the cell membrane of osteoclasts, and its binding to RANK ligand (RANKL) activates a number of intracellular signaling pathways involved in osteoclast formation, activity, and survival. OPG is a soluble decoy receptor for RANKL and prevents the interaction of RANK and RANKL, inhibiting osteoclast function and bone resorption (Kearns, Khosla, & Kostenuik, 2007; Kwan Tat et al., 2009). The equilibrium of OPG and RANKL levels is important in bone pathophysiology and serves as a drug target for the treatment of bone metastases and osteoarthritis (Tat, Pelletier, Velasco, Padrines, & Martel-Pelletier, 2009). Lintermans et al. (2016) found that *OPG* rs2073618 was significantly associated with the occurrence of musculoskeletal toxicity and severity of pain during AI therapy. This association was confirmed in Chinese Han women with breast cancer in another study in which results also demonstrated an association between *RANKL* rs7984870 and adverse musculoskeletal events and RANK/OPG ratio (J. Wang et al., 2015).

Metabolism of and Response to AIs

In terms of genes involved in metabolism of and response to AIs, we targeted two families of drug-metabolizing enzymes (P450 family and UGT family) and two families of drug transporters (ABC transporters and OAT transporters).

Cytochrome P450s. CYPs are the most prominent Phase I drug-metabolizing enzymes, metabolizing more than 90% of drugs (Prakash et al., 2015). Multiple genes in the P450 family have been investigated to predict the phenotype of MSKP with AI therapy, such as *CYP1A2*, *CYP2A6*, *CYP2B6*, *CYP2C8*, *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP2E1*, *CYP3A4*, *CYP3A5*, and *CYP4A11*. Studies have shown only *CYP2A6* and *CYP3A4/5* to have significant associations with MSKP with AI therapy. Jeong et al. (2009) provided in vitro evidence that letrozole was a strong inhibitor of *CYP2A6*. Desta et al. (2011) and Borrie et al. (2018) found that plasma letrozole concentration was highly variable (>10-fold) and significantly associated with *CYP2A6* genotype (*CYP2A6*2*, *CYP2A6*4*, *CYP2A6*9*, *CYP2A6*12*). However, Borrie et al. also found no significant association between *CYP2A6* variation and arthralgias. Kamdem, David, and Zeruesenay (2014) provided in vivo evidence that *CYP3A* played a major role in the metabolism of exemestane.

UGT family. UDP-glucuronosyltransferases comprise another important family of metabolizing enzymes in the deactivation and clearance of AIs (Lazarus & Sun, 2010). Sun, Chen, Dellinger, Sharma, and Lazarus (2010) provided in vitro evidence that *UGT2B17* was a strong inhibitor of 17-dihydroexemestan (i.e., the major metabolite of exemestane). Deletion of *UGT2B17* was significantly associated with overall exemestane metabolism. Packard et al. (2018) examined and confirmed the correlation between deletion of *UGT2B17* and joint pain in treatment with exemestane for breast cancer. Rumiato et al. (2016) found that *UGT2A1* rs4148304 was significantly associated with response to AI therapy in elderly breast cancer patients.

ABC transporter family. P-glycoprotein (*ABCB1*) is a member of the ABC transporter family that is responsible for cellular homeostasis (Jones & George, 2004). Anastrozole is a substrate for P-glycoprotein (Miyajima et al., 2013). Gervasini et al. (2017) reported that SNPs in *ABCB1* were significantly associated with higher plasma anastrozole concentration (rs2677-TT) and lower occurrence of arthralgia (rs3435-TT) for postmenopausal women with breast cancer. Two SNPs (rs3788007 and rs914189) in *ABCG1*, which encodes another ABC transporter, were associated with drug response to AIs in a study among elderly breast cancer patients (Rumiato et al., 2016).

OAT transporter family. OATs are a family of transporter proteins that play an important role in drug disposition in the liver, kidneys, and intestines (Marzolini, Tirona, & Kim, 2004; Nigam et al., 2015). *OATP1B1* encodes a transporter of steroidal AIs, including exemestane. Gregory, Chen, Murphy, Atchley, and Kamdem (2017) observed that *OATP1B1* rs4149056 was significantly associated with plasma exemestane concentration and further hypothesized that this SNP may influence the pharmacokinetics of exemestane. However, this finding and hypothesis require further replication and examination as the sample size of that study was very small ($N = 14$). Rumiato et al.'s (2016) findings indicate that three SNPs (rs2283458, rs960440, and rs2190748) in *SLCO3A1*, that encode OATs, also play a role in the response to AIs.

Plausible Candidate Genes and Biological Pathways Proposed by the IPA for Future Investigation

Candidate genes. Our IPA analysis identified several additional biologically plausible candidate genes from the networks and upregulators, including *NFKB*, *HSP90*, *AKT*, *ERK1/2*, and *FOXA2*. In Network A (Figure 3), *NFKB* indirectly affects *CYP3A4* and is directly associated with *TNFSF11* (*RANKL*). The nuclear factor kappa-light-chain-enhancer of activated B cells (NFKB) complex plays a critical role in regulating the immune response to infection (Liang, Zhou, & Shen, 2004). Examining the association between genetic variation in *NFKB* and NFKB signaling may help further elucidate the proinflammatory component of the biological mechanism underlying MSKP with AI therapy. Network A also shows that *HSP90* indirectly affects *CYP19A1* and has a direct relationship with *VDR*. *HSP90* is an important gene for stabilizing proteins and has been studied for the development of anticancer drugs (Calderwood, Khaleque, Sawyer, & Ciocca, 2006). *AKT*, an oncogene, has direct relationships with *TCL1A* and *CYP19A1* as well as an indirect relationship with *ESR1* in Network A. *ERK1/2* indirectly affects *ESR1* and *CYP17A1* and is indirectly affected by *TNFSF11* (*RANKL*). *ERK1/2* encodes extracellular protein-serine/threonine kinases, which are involved in cell differentiation and regulation of meiosis and mitosis (Rubinfeld & Seger, 2005). In Network B, *FOXA2* directly affects *CYP2A6* and *UGT2B17*. The IPA also suggests that *FOXA2* is one of the top upregulators among the 17-gene list. *FOXA2* encodes DNA-binding proteins. There is in vivo genetic evidence showing that *FOXA2* is required for formation of intervertebral discs (Maier, Lo, & Harfe, 2013).

Candidate pathways. Through this biological pathway analysis, we also uncovered several candidate pathways for future research to further clarify the mechanisms underlying MSKP in women with breast cancer receiving AIs, including nicotine degradation and melatonin degradation. Interestingly, investigators have studied the effects of nicotine and melatonin on pain (particularly chronic pain) and found associations; however, there have been no studies hypothesizing or testing their linkage to MSKP during AI therapy.

Nicotine degradation. Nicotine degradation is the most significant canonical pathway for the 17-gene set (Table 3). Nicotine is the principal alkaloid and the addictive compound in commercially used tobacco and a commonly used psychoactive drug. Nicotine has complicated physiological effects and is soluble and transferable across cell membranes and the blood–brain barrier. In humans, the primary site of nicotine metabolism is the liver, and its metabolites are excreted in urine.

Cigarette smoking (including secondhand smoke) is the main way for nicotine to enter into the human body. The influence of nicotine on pain is paradoxical and not fully understood. Nicotine has analgesic properties that have been observed in multiple in vivo and in vitro studies (Christensen & Smith, 1990; Kanarek & Carrington, 2004). However, epidemiologic evidence repeatedly shows that smoking is a risk factor for chronic pain (Mikkonen et al., 2008). Furthermore, cigarette smoking jeopardizes the musculoskeletal system directly and indirectly. In vitro studies have shown that nicotine may have direct toxic effects on bone metabolism by affecting osteoblast/osteoclast activity (i.e., proliferation and osteoblast differentiation; Kim et al., 2012; Walker, Preston, Magnay, Thomas, & El Haj, 2001). Smoking has been associated with osteoporosis via decrease of bone mineral content (Gerdhem & Obrant, 2002; Rudang et al., 2012). Postmenopausal women who are smokers (including passive smoking) have significantly more bone loss than nonsmoking controls. Kim et al. (2012) reported a positive relationship between bone loss and daily number of cigarettes smoked and years of exposure, controlling for sex, age, weight, body mass index, and unhealthy lifestyles (e.g., lack of physical activity and sun exposure, low calcium intake, alcohol and caffeine use). It has also been suggested that smoking has indirect effects on MSKP by influencing sex and adrenocortical hormones, vitamin D, intestinal calcium absorption, and vessels and oxygen supply (Abate, Vanni, Pantalone, & Salini, 2013). Researchers have also reported that smoking has negative influences on muscles and tendons. The associations among cigarette smoking, genetic variance related to response to nicotine and its degradation, and MSKP during AI therapy for breast cancer, however, have not been studied.

Melatonin degradation. Melatonin is a neurohormone secreted by the pineal gland. It not only regulates circadian and seasonal rhythms but also has other roles, such as antioxidant and immune-modulating functions. Moreover, accumulated evidence suggests a correlation between melatonin and pain (e.g., fibromyalgia, headaches, irritable bowel syndrome, chronic back pain, and rheumatoid arthritis). Melatonin can relieve pain by restoring circadian rhythms and decreasing anxiety. Moreover, researchers have suggested that it has a separate analgesic effect on the melatonin receptors in some areas of the brain related to pain perception and processing (Danilov & Kurganova, 2016). Although the mechanisms of the analgesic effect of melatonin are not fully understood and remain under debate, supplementation with melatonin has shown promising effects on the management of MSKP (e.g., fibromyalgia) in preclinical studies (Favero et al., 2017). Effects of the genetic variability involved in the production, action, and degradation of melatonin on MSKP have not been studied in AI therapy for breast cancer.

Finally, we also noticed that the estrogen biosynthesis canonical pathway shares at least one gene with 13 other signal or metabolic pathways (Figure 2), including IL-6 signaling, LPS/IL-1-mediated inhibition of RXR function, PXR/RXR activation, bupropion degradation, xenobiotic metabolism signaling, bile acid biosynthesis/neutral pathway, superpathway of melatonin degradation, nicotine degradation II, melatonin degradation I, nicotine degradation III, FXR/RXR activation, and estrogen-dependent breast cancer signaling. Given the overlap

with estrogen biosynthesis, the question of whether or not these pathways play a role in MSKP with AI therapy warrants further exploration.

Conclusion

The literature identifies 17 genes that are associated with MSKP during AI treatment of breast cancer or are involved in metabolism of and response to AIs. Prior studies have shown that genetic variation in multiple systemic and local regulators of bone remodeling plays an important role in the phenotype of MSKP with AI therapy. Genes that encode metabolizing enzymes (cytochrome P450 family and UGT family) and drug transporters (ABC transporters and OAT transporters) contribute to plasma AI concentration, but whether they also contribute to MSKP with AI treatment needs further examination. Our findings from the present biological pathway analysis indicate that multiple, molecular-level etiologies may contribute to MSKP in AI therapy for breast cancer. Nicotine degradation and melatonin degradation are two plausible biological pathways for future investigation. Our findings also have implications for nursing practice, as incorporating an understanding of the multiple biological mechanisms that may underlay MSKP during AI therapy into patient education and communication could help patients grasp the interindividual variability of MSKP and seek out more individualized coping strategies.

Acknowledgment

We would like to express our very great appreciation to Jennifer A. Marx for her helpful assistance with the article tables and for the Undergraduate Research Mentorship Program at the School of Nursing, University of Pittsburgh, which provided support for her work on this project.

Author Contributions

Yehui Zhu contributed to conception and design, acquisition, analysis, and interpretation; drafted the manuscript; critically revised the manuscript; gave final approval; and agrees to be accountable for all aspects of work ensuring integrity and accuracy. Theresa A. Koleck contributed to conception and design, acquisition, analysis, and interpretation; critically revised the manuscript; gave final approval; and agrees to be accountable for all aspects of work ensuring integrity and accuracy. Catherine M. Bender contributed to conception and design, acquisition, and interpretation; critically revised the manuscript; gave final approval; and agrees to be accountable for all aspects of work ensuring integrity and accuracy. Yvette P. Conley contributed to conception and design, acquisition, and interpretation; critically revised the manuscript; gave final approval; and agrees to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: National Cancer Institute (1F99CA234782-01) and American Cancer Society Doctoral Degree Scholarship in Cancer Nursing (DSCN-17-073-01).

ORCID iD

Yehui Zhu https://orcid.org/0000-0002-8115-0080
Yvette P. Conley https://orcid.org/0000-0002-1784-6067

References

Abate, M., Vanni, D., Pantalone, A., & Salini, V. (2013). Cigarette smoking and musculoskeletal disorders. *Muscles, Ligaments and Tendons Journal*, *3*, 63.

Baldock, P. A., Thomas, G. P., Hodge, J. M., Baker, S. U., Dressel, U., O'Loughlin, P. D., ... Gardiner, E. M. (2006). Vitamin D action and regulation of bone remodeling: Suppression of osteoclastogenesis by the mature osteoblast. *Journal of Bone and Mineral Research*, *21*, 1618–1626.

Borrie, A. E., & Kim, R. B. (2017). Molecular basis of aromatase inhibitor associated arthralgia: Known and potential candidate genes and associated biomarkers. *Expert Opinion on Drug Metabolism & Toxicology*, *13*, 149–156.

Borrie, A. E., Rose, R. V., Choi, Y. H., Perera, F. E., Read, N., Sexton, T., ... Younus, J. (2018). Letrozole concentration is associated with CYP2A6 variation but not with arthralgia in patients with breast cancer. *Breast Cancer Research and Treatment*, *172*, 371–379.

Calderwood, S. K., Khaleque, M. A., Sawyer, D. B., & Ciocca, D. R. (2006). Heat shock proteins in cancer: Chaperones of tumorigenesis. *Trends in Biochemical Sciences*, *31*, 164–172.

Campos, S. M. (2004). Aromatase inhibitors for breast cancer in postmenopausal women. *The Oncologist*, *9*, 126–136.

Chim, K., Xie, S. X., Stricker, C. T., Li, Q. S., Gross, R., Farrar, J. T., ... Mao, J. J. (2013). Joint pain severity predicts premature discontinuation of aromatase inhibitors in breast cancer survivors. *BMC Cancer*, *13*, 401.

Christensen, M. K., & Smith, D. F. (1990). Antinociceptive effects of the stereoisomers of nicotine given intrathecally in spinal rats. *Journal of Neural Transmission/General Section JNT*, *80*, 189–194.

Chung, B. C., Picado-Leonard, J., Haniu, M., Bienkowski, M., Hall, P. F., Shively, J. E., & Miller, W. L. (1987). Cytochrome P450c17 (steroid 17 alpha-hydroxylase/17, 20 lyase): Cloning of human adrenal and testis cDNAs indicates the same gene is expressed in both tissues. *Proceedings of the National Academy of Sciences*, *84*, 407–411.

Craft, R. M. (2007). Modulation of pain by estrogens. *Pain*, *132*, S3–S12.

Crew, K. D., Greenlee, H., Capodice, J., Raptis, G., Brafman, L., Fuentes, D., ... Hershman, D. L. (2007). Prevalence of joint symptoms in postmenopausal women taking aromatase inhibitors for early-stage breast cancer. *Journal of Clinical Oncology*, *25*, 3877–3883.

Danilov, A., & Kurganova, J. (2016). Melatonin in chronic pain syndromes. *Pain and Therapy*, *5*, 1–17.

Desta, Z., Kreutz, Y., Nguyen, A. T., Li, L., Skaar, T., Kamdem, L. K., ... Hoffmann, E. (2011). Plasma letrozole concentrations in postmenopausal women with breast cancer are associated

with CYP2A6 genetic variants, body mass index, and age. *Clinical Pharmacology & Therapeutics*, *90*, 693–700.

Egawa, C., Hirokaga, K., Takao, S., Yamagami, K., Miyashita, M., Baba, M., . . . Okuno, T. (2016). Risk factors for joint symptoms in postmenopausal Japanese breast cancer patients treated with anastrozole: A prospective multicenter cohort study of patient-reported outcomes. *International Journal of Clinical Oncology*, *21*, 262–269.

Fabian, C. J. (2007). The what, why and how of aromatase inhibitors: Hormonal agents for treatment and prevention of breast cancer. *International Journal of Clinical Practice*, *61*, 2051–2063.

Favero, G., Trapletti, V., Bonomini, F., Stacchiotti, A., Lavazza, A., Rodella, L. F., & Rezzani, R. (2017). Oral supplementation of melatonin protects against fibromyalgia-related skeletal muscle alterations in reserpine-induced myalgia rats. *International Journal of Molecular Sciences*, *18*, 1389.

Felson, D. T., & Cummings, S. R. (2005). Aromatase inhibitors and the syndrome of arthralgias with estrogen deprivation. *Arthritis & Rheumatism*, *52*, 2594–2598.

Fontein, D. B., Houtsma, D., Nortier, J. W., Baak-Pablo, R. F., Kranenbarg, E. M. K., van der Straaten, T. R., . . . Guchelaar, H. J. (2014). Germline variants in the CYP19A1 gene are related to specific adverse events in aromatase inhibitor users: A substudy of Dutch patients in the TEAM trial. *Breast Cancer Research and Treatment*, *144*, 599–606.

Gallicchio, L., MacDonald, R., & Helzlsouer, K. J. (2013). Insulin-like growth factor 1 and musculoskeletal pain among breast cancer patients on aromatase inhibitor therapy and women without a history of cancer. *Journal of Cancer Research and Clinical Oncology*, *139*, 837–843.

Garcia-Giralt, N., Rodríguez-Sanz, M., Prieto-Alhambra, D., Servitja, S., Torres-del Pliego, E., Balcells, S., . . . Nogués, X. (2013). Genetic determinants of aromatase inhibitor-related arthralgia: The B-ABLE cohort study. *Breast Cancer Research and Treatment*, *140*, 385–395.

Geisler, J., Haynes, B., Anker, G., Dowsett, M., & Lonning, P. E. (2002). Influence of letrozole and anastrozole on total body aromatization and plasma estrogen levels in postmenopausal breast cancer patients evaluated in a randomized, cross-over study. *Journal of Clinical Oncology*, *20*, 751–757.

Gerdhem, P., & Obrant, K. J. (2002). Effects of cigarette-smoking on bone mass as assessed by dual-energy X-ray absorptiometry and ultrasound. *Osteoporosis International*, *13*, 932–936.

Gervasini, G., Jara, C., Olier, C., Romero, N., Martínez, R., & Carrillo, J. A. (2017). Polymorphisms in ABCB1 and CYP19A1 genes affect anastrozole plasma concentrations and clinical outcomes in postmenopausal breast cancer patients. *British Journal of Clinical Pharmacology*, *83*, 562–571.

Goss, P. E., Ingle, J. N., Pritchard, K. I., Robert, N. J., Muss, H., Gralow, J., . . . Sturtz, K. (2016). Extending aromatase-inhibitor adjuvant therapy to 10 years. *New England Journal of Medicine*, *375*, 209–219.

Gregory, B. J., Chen, S. M., Murphy, M. A., Atchley, D. H., & Kamdem, L. K. (2017). Impact of the OATP 1B1 c. 521T> C single nucleotide polymorphism on the pharmacokinetics of exemestane in healthy post-menopausal female volunteers. *Journal of Clinical Pharmacy and Therapeutics*, *42*, 547–553.

Henry, N. L., Skaar, T. C., Dantzer, J., Li, L., Kidwell, K., Gersch, C., . . . Philips, S. (2013). Genetic associations with toxicity-related discontinuation of aromatase inhibitor therapy for breast cancer. *Breast Cancer Research and Treatment*, *138*, 807–816.

Ingle, J. N., Schaid, D. J., Goss, P. E., Liu, M., Mushiroda, T., Chapman, J. A. W., . . . Pater, J. (2010). Genome-wide associations and functional genomic studies of musculoskeletal adverse events in women receiving aromatase inhibitors. *Journal of Clinical Oncology*, *28*, 4674.

Jeong, S., Woo, M. M., Flockhart, D. A., & Desta, Z. (2009). Inhibition of drug metabolizing cytochrome P450s by the aromatase inhibitor drug letrozole and its major oxidative metabolite 4, 4′-methanol-bisbenzonitrile in vitro. *Cancer Chemotherapy and Pharmacology*, *64*, 867–875.

Jones, P. M., & George, A. M. (2004). The ABC transporter structure and mechanism: Perspectives on recent research. *Cellular and Molecular Life Sciences CMLS*, *61*, 682–699.

Kamdem, L. K., David, F. A., & Zeruesenay, D. (2014). 17-Hydroexemestane: A potent inhibitor of CYP19 (aromatase) and substrate of CYP3A. *Journal of Drug Metabolism & Toxicology*, *5*, 2.

Kamdem, L. K., Liu, Y., Stearns, V., Kadlubar, S. A., Ramirez, J., Jeter, S., . . . Flockhart, D. A. (2010). In vitro and in vivo oxidative metabolism and glucuronidation of anastrozole. *British Journal of Clinical Pharmacology*, *70*, 854–869.

Kameda, T., Mano, H., Yuasa, T., Mori, Y., Miyazawa, K., Shiokawa, M., . . . Yang, N. N. (1997). Estrogen inhibits bone resorption by directly inducing apoptosis of the bone-resorbing osteoclasts. *Journal of Experimental Medicine*, *186*, 489–495.

Kanarek, R. B., & Carrington, C. (2004). Sucrose consumption enhances the analgesic effects of cigarette smoking in male and female smokers. *Psychopharmacology*, *173*, 57–63.

Kearns, A. E., Khosla, S., & Kostenuik, P. J. (2007). Receptor activator of nuclear factor κB ligand and osteoprotegerin regulation of bone remodeling in health and disease. *Endocrine Reviews*, *29*, 155–192.

Kim, B. S., Kim, S. J., Kim, H. J., Lee, S. J., Park, Y. J., Lee, J., & You, H. K. (2012). Effects of nicotine on proliferation and osteoblast differentiation in human alveolar bone marrow-derived mesenchymal stem cells. *Life Sciences*, *90*, 109–115.

Koleck, T. A., & Conley, Y. P. (2016). Identification and prioritization of candidate genes for symptom variability in breast cancer survivors based on disease characteristics at the cellular level. *Breast Cancer: Targets and Therapy*, *8*, 29–37.

Kwan Tat, S., Amiable, N., Pelletier, J. P., Boileau, C., Lajeunesse, D., Duval, N., & Martel-Pelletier, J. (2009). Modulation of OPG, RANK and RANKL by human chondrocytes and their implication during osteoarthritis. *Rheumatology*, *48*, 1482–1490.

Lazarus, P., & Sun, D. (2010). Potential role of UGT pharmacogenetics in cancer treatment and prevention: Focus on tamoxifen and aromatase inhibitors. *Drug Metabolism Reviews*, *42*, 182–194.

Liang, Y., Zhou, Y., & Shen, P. (2004). NF-kappaB and its regulation on the immune system. *Cellular & Molecular Immunology*, *1*, 343–350.

Lintermans, A., Van Asten, K., Jongen, L., Van Brussel, T., Laenen, A., Verhaeghe, J., ... Neven, P. (2016). Genetic variant in the osteoprotegerin gene is associated with aromatase inhibitor-related musculoskeletal toxicity in breast cancer patients. *European Journal of Cancer*, *56*, 31–36.

Liu, M., Wang, L., Bongartz, T., Hawse, J. R., Markovic, S. N., Schaid, D. J., ... Goss, P. E. (2012). Aromatase inhibitors, estrogens and musculoskeletal pain: Estrogen-dependent T-cell leukemia 1A (TCL1A) gene-mediated regulation of cytokine expression. *Breast Cancer Research*, *14*, R41.

Livingston, W. S., Rusch, H. L., Nersesian, P. V., Baxter, T., Mysliwiec, V., & Gill, J. M. (2015). Improved sleep in military personnel is associated with changes in the expression of inflammatory genes and improvement in depression symptoms. *Frontiers in Psychiatry*, *6*, 59.

Maier, J. A., Lo, Y., & Harfe, B. D. (2013). Foxa1 and Foxa2 are required for formation of the intervertebral discs. *PLoS One*, *8*, e55528.

Mao, J. J., Stricker, C., Bruner, D., Xie, S., Bowman, M. A., Farrar, J. T., ... DeMichele, A. (2009). Patterns and risk factors associated with aromatase inhibitor-related arthralgia among breast cancer survivors. *Cancer*, *115*, 3631–3639. doi:10.1002/cncr.24419

Mao, J. J., Su, H. I., Feng, R., Donelson, M. L., Aplenc, R., Rebbeck, T. R., ... DeMichele, A. (2011). Association of functional polymorphisms in CYP19A1 with aromatase inhibitor associated arthralgia in breast cancer survivors. *Breast Cancer Research*, *13*, R8.

Marzolini, C., Tirona, R. G., & Kim, R. B. (2004). Pharmacogenomics of the OATP and OAT families. *Pharmacogenomics*, *5*, 273–282.

Matteson, K. J., Picado-Leonard, J., Chung, B. C., Mohandas, T. K., & Miller, W. L. (1986). Assignment of the gene for adrenal P450c17 (Steroid 17α-HYDR0XYLASE/17, 20 Lyase) to human chromosome 10. *The Journal of Clinical Endocrinology & Metabolism*, *63*, 789–791.

Mikkonen, P., Leino-Arjas, P., Remes, J., Zitting, P., Taimela, S., & Karppinen, J. (2008). Is smoking a risk factor for low back pain in adolescents? A prospective cohort study. *Spine*, *33*, 527–532.

Miyajima, M., Kusuhara, H., Takahashi, K., Takashima, T., Hosoya, T., Watanabe, Y., & Sugiyama, Y. (2013). Investigation of the effect of active efflux at the blood–brain barrier on the distribution of nonsteroidal aromatase inhibitors in the central nervous system. *Journal of Pharmaceutical Sciences*, *102*, 3309–3319.

Nigam, S. K., Bush, K. T., Martovetsky, G., Ahn, S. Y., Liu, H. C., Richard, E., ... Wu, W. (2015). The organic anion transporter (OAT) family: A systems biology perspective. *Physiological Reviews*, *95*, 83–123.

Osborne, C. K. (1998). Steroid hormone receptors in breast cancer management. In G. Gasparini (Ed.), *Prognostic variables in node-negative and node-positive breast cancer* (pp. 35–46). Springer.

Packard, E., Campbell-Baird, C., Leitzel, K., Luo, S., Chen, G., Zhu, J., ... Truica, C. I. (2018). UGT2B17 deletion polymorphism and exemestane-induced toxicity. *Journal of Clinical Oncology*, *36*, e12534.

Park, I. H., Lee, Y. S., Lee, K. S., Kim, S. Y., Hong, S. H., Jeong, J., ... Nam, B. H. (2011). Single nucleotide polymorphisms of CYP19A1 predict clinical outcomes and adverse events associated with letrozole in patients with metastatic breast cancer. *Cancer Chemotherapy and Pharmacology*, *68*, 1263–1271.

Picado-Leonard, J. A. M. E. S., & Miller, W. L. (1987). Cloning and sequence of the human gene for P450cl7 (steroid 17α-hydroxylase/17, 20 lyase): Similarity with the gene for P450c21. *Deoxyribonucleic Acid*, *6*, 439–448.

Prakash, C., Zuniga, B., Song, C. S., Jiang, S., Cropper, J., Park, S., & Chatterjee, B. (2015). Nuclear receptors in drug metabolism, drug response and drug interactions. *Nuclear Receptor Research*, *2*, 101178.

Presant, C. A., Bosserman, L., Young, T., Vakil, M., Horns, R., Upadhyaya, G., ... Howard, F. (2007). Aromatase inhibitor-associated arthralgia and/ or bone pain: Frequency and characterization in non-clinical trial patients. *Clinical Breast Cancer*, *7*, 775–778.

Raggatt, L. J., & Partridge, N. C. (2010). Cellular and molecular mechanisms of bone remodeling. *Journal of Biological Chemistry*, *285*, 25103–25108.

Rastelli, A. L., Taylor, M. E., Gao, F., Armamento-Villareal, R., Jamalabadi-Majidi, S., Napoli, N., & Ellis, M. J. (2011). Vitamin D and aromatase inhibitor-induced musculoskeletal symptoms (AIMSS): A phase II, double-blind, placebo-controlled, randomized trial. *Breast Cancer Research and Treatment*, *129*, 107–116.

Romera, J. L., Hernández, T. D. J. P., Fernández, I. P., Gimeno, T. S., Martínez, R. F., Pérez, I. F., ... Gil, E. M. C. (2011). Update on adjuvant hormonal treatment of early breast cancer. *Advances in Therapy*, *28*, 1.

Rubinfeld, H., & Seger, R. (2005). The ERK cascade. *Molecular Biotechnology*, *31*, 151–174.

Rudäng, R., Darelid, A., Nilsson, M., Nilsson, S., Mellström, D., Ohlsson, C., & Lorentzon, M. (2012). Smoking is associated with impaired bone mass development in young adult men: A 5-year longitudinal study. *Journal of Bone and Mineral Research*, *27*, 2189–2197.

Rumiato, E., Brunello, A., Ahcene-Djaballah, S., Borgato, L., Gusella, M., Menon, D., ... Zagonel, V. (2016). Predictive markers in elderly patients with estrogen receptor-positive breast cancer treated with aromatase inhibitors: An array-based pharmacogenetic study. *The Pharmacogenomics Journal*, *16*, 525.

Servitja, S., Martos, T., Rodriguez Sanz, M., Garcia-Giralt, N., Prieto-Alhambra, D., Garrigos, L., ... Tusquets, I. (2015). Skeletal adverse effects with aromatase inhibitors in early breast cancer: Evidence to date and clinical guidance. *Therapeutic Advances in Medical Oncology*, *7*, 291–296.

Singer, O., Cigler, T., Moore, A. B., Levine, A. B., Do, H. T., & Mandl, L. A. (2014). Hypovitaminosis D is a predictor of aromatase inhibitor musculoskeletal symptoms. *The Breast Journal*, *20*, 174–179.

Srivastava, S., Toraldo, G., Weitzmann, M. N., Cenci, S., Ross, F. P., & Pacifici, R. (2001). Estrogen decreases osteoclast formation by down-regulating receptor activator of NF-κB ligand (RANKL)-induced JNK activation. *Journal of Biological Chemistry*, *276*, 8836–8840.

Sun, D., Chen, G., Dellinger, R. W., Sharma, A. K., & Lazarus, P. (2010). Characterization of 17-dihydroexemestane glucuronidation:

Potential role of the UGT2B17 deletion in exemestane pharmacogenetics. *Pharmacogenetics and Genomics*, *20*, 575.

Tat, S. K., Pelletier, J. P., Velasco, C. R., Padrines, M., & Martel-Pelletier, J. (2009). New perspective in osteoarthritis: The OPG and RANKL system as a potential therapeutic target? *The Keio Journal of Medicine*, *58*, 29–40.

Torre, L. A., Bray, F., Siegel, R. L., Ferlay, J., Lortet-Tieulent, J., & Jemal, A. (2015). Global cancer statistics, 2012. *CA: A Cancer Journal for Clinicians*, *65*, 87–108.

Walker, L. M., Preston, M. R., Magnay, J. L., Thomas, P. B. M., & El Haj, A. J. (2001). Nicotinic regulation of c-fos and osteopontin expression in human-derived osteoblast-like cells and human trabecular bone organ culture. *Bone*, *28*, 603–608.

Walsh, J. S. (2017). Normal bone physiology, remodelling and its hormonal regulation. *Surgery (Oxford)*, *36*, 1–6.

Waltman, N. L., Ott, C. D., Twiss, J. J., Gross, G. J., & Lindsey, A. M. (2009). Vitamin D insufficiency and musculoskeletal symptoms in breast cancer survivors on aromatase inhibitor therapy. *Cancer Nursing*, *32*, 143–150.

Wang, J., Lu, K., Song, Y., Xie, L., Zhao, S., Wang, Y., . . . Zhang, Q. (2013). Indications of clinical and genetic predictors for aromatase inhibitors related musculoskeletal adverse events in Chinese Han women with breast cancer. *PLoS One*, *8*, e68798.

Wang, J., Lu, K., Song, Y., Zhao, S., Ma, W., Xuan, Q., . . . Zhang, Q. (2015). RANKL and OPG polymorphisms are associated with aromatase inhibitor-related musculoskeletal adverse events in Chinese Han breast cancer patients. *PLoS One*, *10*, e0133964.

Wang, J., Zhou, J., Cheng, C. M., Kopchick, J. J., & Bondy, C. A. (2004). Evidence supporting dual, IGF-I-independent and IGF-I-dependent, roles for GH in promoting longitudinal bone growth. *Journal of Endocrinology*, *180*, 247–255.

Wang, L., McLeod, H. L., & Weinshilboum, R. M. (2011). Genomics and drug response. *New England Journal of Medicine*, *364*, 1144–1153.

Wilkinson, G. R. (2005). Drug metabolism and variability among patients in drug response. *New England Journal of Medicine*, *352*, 2211–2221.

Appendix E UNIVERSITY OF PITTSBURGH INSTITUTIONAL REVIEW BOARD APPROVAL LETTER

University of Pittsburgh
Institutional Review Board

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

Memorandum

To: Yehui Zhu
From: IRB Office
Date: 8/21/2018
IRB#: PRO18070351
Subject: Musculoskeletal Pain with Endocrine Therapy for Breast Cancer: Trajectory and Predictors

---

The University of Pittsburgh Institutional Review Board reviewed and approved the above referenced study by the expedited review procedure authorized under 45 CFR 46.110 and 21 CFR 56.110. Your research study was approved under:

45 CFR 46.110.(7)

The risk level designation is Minimal Risk.

Approval Date: 8/21/2018
Expiration Date: 8/20/2019

For studies being conducted in UPMC facilities, no clinical activities can be undertaken by investigators until they have received approval from the UPMC Fiscal Review Office.

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.

BIBLIOGRAPHY

Aiello Bowles, E. J., Boudreau, D. M., Chubak, J., Yu, O., Fujii, M., Chestnut, J., & Buist, D. S. (2012). Patient-reported discontinuation of endocrine therapy and related adverse effects among women with early-stage breast cancer. *Journal of oncology practice*, *8*(6), e149-e157.

An, J. D., Li, X. Y., Yu, J. B., Yu, Z. H. A. O., & Jin, Z. S. (2012). Association between the eNOSgene polymorphisms and rheumatoid arthritis risk in a northern Chinese population. *Chinese Medical Journal*, *125*(8), 1496-1499.

Arimidex, T. (2006). Comprehensive side-effect profile of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: long-term safety analysis of the ATAC trial. *The lancet oncology*, *7*(8), 633-643.

Arimidex, T. (2008). Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 100-month analysis of the ATAC trial. *The lancet oncology*, *9*(1), 45-53.

Armstrong, T. S. (2003). Symptoms experience: a concept analysis. *Oncology Nursing Forum*, 30(4), 601–606.

Beck, A. T., Steer, R. A., & Carbin, M. G. (1988). Psychometric properties of the Beck Depression Inventory: Twenty-five years of evaluation. *Clinical Psychology Review*, *8*(1), 77-100.

Bender, C. M., Merriman, J. D., Gentry, A. L., Ahrendt, G. M., Berga, S. L., Brufsky, A. M., ... & McAuliffe, P. F. (2015). Patterns of change in cognitive function with anastrozole therapy. *Cancer*, *121*(15), 2627-2636.

Boccardo, F., Rubagotti, A., Guglielmini, P., Fini, A., Paladini, G., Mesiti, M., ... & Restuccia, N. (2006). Switching to anastrozole versus continued tamoxifen treatment of early breast cancer. Updated results of the Italian tamoxifen anastrozole (ITA) trial. *Annals of Oncology*, *17*(suppl_7), vii10-vii14.

Boonstra, A., van Zadelhoff, J., Timmer-Bonte, A., Ottevanger, P. B., Beurskens, C. H., & van Laarhoven, H. W. (2013). Arthralgia during aromatase inhibitor treatment in early breast cancer patients: prevalence, impact, and recognition by healthcare providers. *Cancer nursing*, *36*(1), 52-59.

Borrie, A. E., & Kim, R. B. (2017). Molecular basis of aromatase inhibitor associated arthralgia: known and potential candidate genes and associated biomarkers. *Expert opinion on drug metabolism & toxicology*, *13*(2), 149-156.

Briot, K., Tubiana-Hulin, M., Bastit, L., Kloos, I., & Roux, C. (2010). Effect of a switch of aromatase inhibitors on musculoskeletal symptoms in postmenopausal women with hormone-receptor-positive breast cancer: the ATOLL (articular tolerance of letrozole) study. *Breast cancer research and treatment*, *120*(1), 127-134.

Brown, J. C., Mao, J. J., Stricker, C., Hwang, W. T., Tan, K. S., & Schmitz, K. H. (2014). Aromatase inhibitor associated musculoskeletal symptoms are associated with reduced physical activity among breast cancer survivors. *The Breast Journal*, 20(1), 22-28.

Bunjevacki, V., Maksimovic, N., Jekic, B., Milic, V., Lukovic, L., Novakovic, I., ... & Damnjanovic, T. (2016). Polymorphisms of the eNOS gene are associated with disease activity in rheumatoid arthritis. *Rheumatology International*, *36*(4), 597-602.

Campos, S. M. (2004). Aromatase inhibitors for breast cancer in postmenopausal women. *The Oncologist*, *9*(2), 126-136.

Cashion, A. K., & Grady, P. A. (2015). The National Institutes of Health/National Institutes of Nursing Research intramural research program and the development of the National Institutes of Health Symptom Science Model. *Nursing outlook*, *63*(4), 484-487.

Castel, L. D., Hartmann, K. E., Mayer, I. A., Saville, B. R., Alvarez, J., Boomershine, C. S., ... & Cella, D. F. (2013). Time course of arthralgia among women initiating aromatase inhibitor therapy and a postmenopausal comparison group in a prospective cohort. *Cancer*, *119*(13), 2375-2382.

Cecil, R. L., & Archer, B. H. (1925). Arthritis of the menopause: a study of fifty cases. *Journal of the American Medical Association*, *84*(2), 75-79.

Cella, D., & Fallowfield, L. J. (2008). Recognition and management of treatment-related side effects for breast cancer patients receiving adjuvant endocrine therapy. *Breast Cancer Research and Treatment*, *107*(2), 167-180.

Cella, D., Land, S. R., Chang, C. H., Day, R., Costantino, J. P., Wolmark, N., & Ganz, P. A. (2008). Symptom measurement in the Breast Cancer Prevention Trial (BCPT)(P-1): psychometric properties of a new measure of symptoms for midlife women. *Breast Cancer Research and Treatment*, *109*(3), 515-526.

Chim, K., Xie, S. X., Stricker, C. T., Li, Q. S., Gross, R., Farrar, J. T., ... & Mao, J. J. (2013). Joint pain severity predicts premature discontinuation of aromatase inhibitors in breast cancer survivors. *BMC Cancer*, *13*(1), 401.

Chumsri, S., Howes, T., Bao, T., Sabnis, G., & Brodie, A. (2011). Aromatase, aromatase inhibitors, and breast cancer. *The Journal of steroid biochemistry and molecular biology*, *125*(1), 13-22.

Cirillo, D. J., Wallace, R. B., Wu, L., & Yood, R. A. (2006). Effect of hormone therapy on risk of hip and knee joint replacement in the Women's Health Initiative. *Arthritis & Rheumatology*, *54*(10), 3194-3204.

Cleeland, C. S., & Ryan, K. M. (1994). Pain assessment: global use of the Brief Pain Inventory. *Annals of the Academy of Medicine, Singapore*, *23*(2), 129-138.

Coates, A. S., Keshaviah, A., Thürlimann, B., Mouridsen, H., Mauriac, L., Forbes, J. F., ... & Láng, I. (2007). Five years of letrozole compared with tamoxifen as initial adjuvant therapy for postmenopausal women with endocrine-responsive early breast cancer: update of study BIG 1-98. *Journal of clinical oncology*, *25*(5), 486-492.

Coombes, R. C., Kilburn, L. S., Snowdon, C. F., Paridaens, R., Coleman, R. E., Jones, S. E., ... & Del Mastro, L. (2007). Survival and safety of exemestane versus tamoxifen after 2–3 years' tamoxifen treatment (Intergroup Exemestane Study): a randomised controlled trial. *The Lancet*, *369*(9561), 559-570.

Crew, K. D., Greenlee, H., Capodice, J., Raptis, G., Brafman, L., Fuentes, D., ... & Hershman, D. L. (2007). Prevalence of joint symptoms in postmenopausal women taking aromatase inhibitors for early-stage breast cancer. *Journal of Clinical Oncology*, *25*(25), 3877-3883.

Czajka-Oraniec, I., & Simpson, E. R. (2010). Aromatase research and its clinical significance. *Endokrynologia Polska*, *61*(1), 126-134.

Desta, Z., Kreutz, Y., Nguyen, A. T., Li, L., Skaar, T., Kamdem, L. K., ... & Hoffmann, E. (2011). Plasma letrozole concentrations in postmenopausal women with breast cancer are associated with CYP2A6 genetic variants, body mass index, and age. *Clinical Pharmacology & Therapeutics*, *90*(5), 693-700.

Dhruva, A., Dodd, M., Paul, S. M., Cooper, B. A., Lee, K., West, C., ... & Miaskowski, C. (2010). Trajectories of fatigue in patients with breast cancer before, during, and after radiation therapy. *Cancer Nursing*, *33*(3), 201.

Dizdar, O., Özçakar, L., Malas, F. Ü., Harputluoglu, H., Bulut, N., Aksoy, S., ... & Altundag, K. (2009). Sonographic and electrodiagnostic evaluations in patients with aromatase inhibitor–related arthralgia. *Journal of Clinical Oncology*, *27*(30), 4955-4960.

Dunn, L. B., Cooper, B. A., Neuhaus, J., West, C., Paul, S., Aouizerat, B., ... & Miaskowski, C. (2011). Identification of distinct depressive symptom trajectories in women following surgery for breast cancer. *Health Psychology*, *30*(6), 683.

Dunn, L. B., Langford, D. J., Paul, S. M., Berman, M. B., Shumay, D. M., Kober, K., ... & Miaskowski, C. (2015). Trajectories of fear of recurrence in women with breast cancer. *Supportive Care in Cancer*, *23*(7), 2033-2043.

Egawa, C., Hirokaga, K., Takao, S., Yamagami, K., Miyashita, M., Baba, M., . . . Miyoshi, Y. (2016). Risk factors for joint symptoms in postmenopausal Japanese breast cancer patients treated with anastrozole: a prospective multicenter cohort study of patient-reported outcomes. *International Journal of Clinical Oncology*, *21*(2), 262-269.

Fabian, C. J. (2007). The what, why and how of aromatase inhibitors: Hormonal agents for treatment and prevention of breast cancer. *International Journal of Clinical Practice, 61*(12), 2051-2063.

Fallon, M., Giusti, R., Aielli, F., Hoskin, P., Rolke, R., Sharma, M., ... & ESMO Guidelines Committee. (2018). Management of cancer pain in adult patients: ESMO Clinical Practice Guidelines. *Annals of Oncology*, *29*(Supplement_4), iv166-iv191.

Fontein, D. B., Houtsma, D., Nortier, J. W., Baak-Pablo, R. F., Kranenbarg, E. M. K., van der Straaten, T. R., ... & Guchelaar, H. J. (2014). Germline variants in the CYP19A1 gene are related to specific adverse events in aromatase inhibitor users: a substudy of Dutch patients in the TEAM trial. *Breast Cancer Research and Treatment*, *144*(3), 599-606.

Gallicchio, L., MacDonald, R., Wood, B., Rushovich, E., Fedarko, N. S., & Helzlsouer, K. J. (2012). Changes in bone biomarker concentrations and musculoskeletal symptoms among breast cancer patients initiating aromatase inhibitor therapy and women without a history of cancer. *Journal of Bone and Mineral Research*, 27(9), 1959-1966.

Gao, Y. (2010). The multiple actions of NO. *Pflügers Archiv-European Journal of Physiology*, *459*(6), 829-839.

Garcia-Giralt, N., Rodríguez-Sanz, M., Prieto-Alhambra, D., Servitja, S., Torres-del Pliego, E., Balcells, S., ... & Nogués, X. (2013). Genetic determinants of aromatase inhibitor-related arthralgia: The B-ABLE cohort study. *Breast cancer research and treatment*, *140*(2), 385-395.

Garreau, J. R., Delamelena, T., Walts, D., Karamlou, K., & Johnson, N. (2006). Side effects of aromatase inhibitors versus tamoxifen: the patients' perspective. *The American Journal of Surgery*, 192(4), 496-498.

Geisler, J., Haynes, B., Anker, G., Dowsett, M., & Lønning, P. E. (2002). Influence of letrozole and anastrozole on total body aromatization and plasma estrogen levels in postmenopausal breast cancer patients evaluated in a randomized, cross-over study. *Journal of Clinical Oncology*, *20*(3), 751-757.

Gervasini, G., Jara, C., Olier, C., Romero, N., Martínez, R., & Carrillo, J. A. (2017). Polymorphisms in ABCB1 and CYP19A1 genes affect anastrozole plasma concentrations and clinical outcomes in postmenopausal breast cancer patients. *British Journal of Clinical Pharmacology, 83*(3), 562-571.

Gonzalez-Gay, M. A., Llorca, J., Palomino-Morales, R., Gomez-Acebo, I., Gonzalez-Juanatey, C., & Martin, J. (2009). Influence of nitric oxide synthase gene polymorphisms on the risk of cardiovascular events in rheumatoid arthritis. *Clinical & Experimental Rheumatology*, *27*(1), 116.

Goss, P. E., Ingle, J. N., Martino, S., Robert, N. J., Muss, H. B., Piccart, M. J., ... & Livingston, R. B. (2005). Randomized trial of letrozole following tamoxifen as extended adjuvant therapy in receptor-positive breast cancer: updated findings from NCIC CTG MA. 17. *Journal of the National Cancer Institute*, *97*(17), 1262-1271.

Goss, P. E., Ingle, J. N., Pritchard, K. I., Robert, N. J., Muss, H., Gralow, J., ... & Sturtz, K. (2016). Extending aromatase-inhibitor adjuvant therapy to 10 years. *New England Journal of Medicine*, *375*(3), 209-219.

Groth-Marnat, G. The handbook of psychological assessment. 1990.

Hadji, P., Jackisch, C., Bolten, W., Blettner, M., Hindenburg, H. J., Klein, P., . . . Harbeck, N. (2014). COMPliance and Arthralgia in Clinical Therapy: the COMPACT trial, assessing the incidence of arthralgia, and compliance within the first year of adjuvant anastrozole therapy. *Annals of Oncology*, 25(2), 372-377.

Henry, N. L., Azzouz, F., Desta, Z., Li, L., Nguyen, A. T., Lemler, S., ... & Stearns, V. (2012). Predictors of aromatase inhibitor discontinuation as a result of treatment-emergent symptoms in early-stage breast cancer. *Journal of Clinical Oncology*, *30*(9), 936-942.

Ho, S. C., Chan, S. G., Yip, Y. B., Cheng, A., Yi, Q., & Chan, C. (1999). Menopausal symptoms and symptom clustering in Chinese women. *Maturitas*, *33*(3), 219-227.

Horimoto, Y., Saito, M., & Kasumi, F. (2009). Arthralgia in 329 Patients Taking Aromatase Inhibitors. *Breast Care*, *4*(5), 319-323.

Howell, A., Cuzick, J., Baum, M., Buzdar, A., Dowsett, M., Forbes, J. F., ... & Tobias, J. S. (2005). Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years' adjuvant treatment for breast cancer. *Lancet*, *365*(9453), 60-62.

Hu, G., Zhan, H., Hu, R. (2016). Side effects associated with endocrine therapy in premenopausal patients with breast cancer-related research outcomes. *Shanxi Medical Journal*, 45(9), 1007-1010.

Jakesz, R., Greil, R., Gnant, M., Schmid, M., Kwasny, W., Kubista, E., ... & Renner, K. (2007). Extended adjuvant therapy with anastrozole among postmenopausal breast cancer patients: results from the randomized Austrian Breast and Colorectal Cancer Study Group Trial 6a. *Journal of the National Cancer Institute*, *99*(24), 1845-1853.

Jakesz, R., Jonat, W., Gnant, M., Mittlboeck, M., Greil, R., Tausch, C., ... & Seifert, M. (2005). Switching of postmenopausal women with endocrine-responsive early breast cancer to

anastrozole after 2 years' adjuvant tamoxifen: combined results of ABCSG trial 8 and ARNO 95 trial. *The Lancet*, *366*(9484), 455-462.

Jeong, S., Woo, M. M., Flockhart, D. A., & Desta, Z. (2009). Inhibition of drug metabolizing cytochrome P450s by the aromatase inhibitor drug letrozole and its major oxidative metabolite 4, 4′-methanol-bisbenzonitrile in vitro. *Cancer chemotherapy and pharmacology*, *64*(5), 867-875.

Jones, B. L., & Nagin, D. S. (2007). Advances in group-based trajectory modeling and an SAS procedure for estimating them. *Sociological Methods & Research*, *35*(4), 542-571.

Kamdem, L. K., Flockhart, D. A., & Desta, Z. (2011). In vitro cytochrome P450-mediated metabolism of exemestane. *Drug Metabolism and Disposition*, *39*(1), 98-105.

Kamdem, L. K., Liu, Y., Stearns, V., Kadlubar, S. A., Ramirez, J., Jeter, S., ... & Flockhart, D. A. (2010). In vitro and in vivo oxidative metabolism and glucuronidation of anastrozole. *British journal of clinical pharmacology*, *70*(6), 854-869.

Kidwell, K. M., Harte, S. E., Hayes, D. F., Storniolo, A. M., Carpenter, J., Flockhart, D. A., ... & Henry, N. L. (2014). Patient-reported symptoms and discontinuation of adjuvant aromatase inhibitor therapy. *Cancer*, *120*(16), 2403-2411.

Knisely, M. R., Maserati, M., Heinsberg, L. W., Shah, L. L., Li, H., Zhu, Y., ... & Conley, Y. P. (2019). Symptom Science: Advocating for Inclusion of Functional Genetic Polymorphisms. *Biological Research for Nursing*, 1099800419846407.

Kyranou, M., Puntillo, K., Dunn, L. B., Aouizerat, B. E., Paul, S. M., Cooper, B. A., ... & Miaskowski, C. (2014). Predictors of Initial Levels and Trajectories of Anxiety in Women Prior to and For Six Months Following Breast Cancer Surgery. *Cancer nursing*, *37*(6), 406.

Kyvernitakis, I., Ziller, V., Hars, O., Bauer, M., Kalder, M., & Hadji, P. (2014). Prevalence of menopausal symptoms and their influence on adherence in women with breast cancer. *Climacteric*, 17(3), 252-259.

Lamba, J., Hebert, J. M., Schuetz, E. G., Klein, T. E., & Altman, R. B. (2012). PharmGKB summary: very important pharmacogene information for CYP3A5. *Pharmacogenetics and genomics*, *22*(7), 555.

Langford, D. J., Paul, S. M., Cooper, B., Kober, K. M., Mastick, J., Melisko, M., ... & Lee, K. A. (2016). Comparison of subgroups of breast cancer patients on pain and co-occurring symptoms following chemotherapy. *Supportive Care in Cancer*, *24*(2), 605-614.

Laroche, F., Coste, J., Medkour, T., Cottu, P. H., Pierga, J. Y., Lotz, J. P., ... & Bouhassira, D. (2014). Classification of and risk factors for estrogen deprivation pain syndromes related to aromatase inhibitor treatments in women with breast cancer: a prospective multicenter cohort study. *The Journal of Pain*, *15*(3), 293-303.

Lazarus, P., & Sun, D. (2010). Potential role of UGT pharmacogenetics in cancer treatment and prevention: focus on tamoxifen and aromatase inhibitors. *Drug metabolism reviews*, *42*(1), 182-194.

Lintermans, A., Van Asten, K., Jongen, L., Van Brussel, T., Laenen, A., Verhaeghe, J., ... & Neven, P. (2016). Genetic variant in the osteoprotegerin gene is associated with aromatase inhibitor-related musculoskeletal toxicity in breast cancer patients. *European Journal of Cancer*, *56*, 31-36.

Lintermans, A., Van Asten, K., Wildiers, H., Laenen, A., Paridaens, R., Weltens, C., . . . Neven, P. (2014). A prospective assessment of musculoskeletal toxicity and loss of grip strength in breast cancer patients receiving adjuvant aromatase inhibitors and tamoxifen, and relation with BMI. *Breast Cancer Research and Treatment*, 146(1), 109-116.

Liu, L. N., Wen, F. H., Miaskowski, C., Lin, Y. C., Wang, J. S., Jeng, C., & Chen, M. L. (2014). Weight change trajectory in women with breast cancer receiving chemotherapy and the effect of different regimens. *Journal of Clinical Nursing*, *23*(19-20), 2757-2768.

Lu, H., Li, Q., Xu, B., Zhang, P., Yuan, P., Wnag, J., Cai, R. (2011). Effects of anastrozole on lipid metabolism in Chinese postmenopausal women with breast cancer. *Chinese Journal of Oncology*, 33(7), 520-525.

Mao, J. J., Stricker, C., Bruner, D., Xie, S., Bowman, M. A., Farrar, J. T., . . . DeMichele, A. (2009). Patterns and risk factors associated with aromatase inhibitor-related arthralgia among breast cancer survivors. *Cancer*, 115(16), 3631-3639.

Mao, J. J., Su, H. I., Feng, R., Donelson, M. L., Aplenc, R., Rebbeck, T. R., . . . DeMichele, A. (2011). Association of functional polymorphisms in CYP19A1 with aromatase inhibitor associated arthralgia in breast cancer survivors. *Breast Cancer Research*, 13(1), R8.

Martins, K. J., St-Louis, M., Murdoch, G. K., MacLean, I. M., McDonald, P., Dixon, W. T., ... & Michel, R. N. (2012). Nitric oxide synthase inhibition prevents activity-induced calcineurin–NFATc1 signalling and fast-to-slow skeletal muscle fibre type conversions. *The Journal of physiology*, *590*(6), 1427-1442.

Menas, P., Merkel, D., Hui, W., Lawton, J., Harper, A., & Carro, G. (2012). Incidence and management of arthralgias in breast cancer patients treated with aromatase inhibitors in an outpatient oncology clinic. *Journal of Oncology Pharmacy Practice*, 18(4), 387-393.

Nagin, D. S., & Odgers, C. L. (2010). Group-based trajectory modeling in clinical research. *Annual Review of Clinical Psychology*, *6*, 109-138.

Nagy, G., Koncz, A., Telarico, T., Fernandez, D., Érsek, B., Buzás, E., & Perl, A. (2010). Central role of nitric oxide in the pathogenesis of rheumatoid arthritis and sysemic lupus erythematosus. *Arthritis Research & Therapy*, *12*(3), 210.

Napoli, N., Vattikuti, S., Ma, C., Rastelli, A., Rayani, A., Donepudi, R., . . . Armamento-Villareal, R. (2010). High prevalence of low vitamin D and musculoskeletal complaints in women with breast cancer. *The Breast Journal*, 16(6), 609-616.

National Comprehensive Cancer Network. (2017). Retrieved December 17, 2017, from https://www.nccn.org/professionals/physician_gls/default.aspx#site

Niravath, P., Chen, B., Chapman, J. A. W., Agarwal, S. K., Welschhans, R. L., Bongartz, T., ... & Gelmon, K. (2017). Vitamin D levels, vitamin D receptor polymorphisms, and inflammatory cytokines in aromatase inhibitor-induced arthralgias: An analysis of CCTG MA. 27. *Clinical Breast Cancer*,*18*(1), 78-87.

Nylund, K. L., Asparouhov, T., & Muthén, B. O. (2007). Deciding on the number of classes in latent class analysis and growth mixture modeling: A Monte Carlo simulation study. *Structural Equation Modeling*, *14*(4), 535-569.

Oberguggenberger, A., Hubalek, M., Sztankay, M., Meraner, V., Beer, B., Oberacher, H., . . . Holzner, B. (2011). Is the toxicity of adjuvant aromatase inhibitor therapy underestimated? Complementary information from patient-reported outcomes (PROs). *Breast Cancer Research and Treatment*, 128(2), 553-561.

Ohsako, T., Inoue, K., Nagamoto, N., Yoshida, Y., Nakahara, O., & Sakamoto, N. (2006). Joint symptoms: A practical problem of anastrozole. *Breast Cancer*, *13*(3), 284-288.

Olufade, T., Gallicchio, L., MacDonald, R., & Helzlsouer, K. J. (2015). Musculoskeletal pain and health-related quality of life among breast cancer patients treated with aromatase inhibitors. *Supportive Care in Cancer*, *23*(2), 447-455.

Osborne, C. K. (1998). Steroid hormone receptors in breast cancer management. In Prognostic variables in node-negative and node-positive breast cancer (pp. 35-46). Springer US.

Park, I. H., Lee, Y. S., Lee, K. S., Kim, S. Y., Hong, S. H., Jeong, J., ... & Nam, B. H. (2011). Single nucleotide polymorphisms of CYP19A1 predict clinical outcomes and adverse events associated with letrozole in patients with metastatic breast cancer. *Cancer Chemotherapy and Pharmacology*, *68*(5), 1263-1271.

Presant, C. A., Bosserman, L., Young, T., Vakil, M., Horns, R., Upadhyaya, G., . . . Howard, F. (2007). Aromatase inhibitor-associated arthralgia and/ or bone pain: frequency and characterization in non-clinical trial patients. *Clinical Breast Cancer*, *7*(10), 775-778.

Rastelli, A. L., Taylor, M. E., Gao, F., Armamento-Villareal, R., Jamalabadi-Majidi, S., Napoli, N., & Ellis, M. J. (2011). Vitamin D and aromatase inhibitor-induced musculoskeletal symptoms (AIMSS): a phase II, double-blind, placebo-controlled, randomized trial. *Breast Cancer Research and Treatment*, *129*(1), 107-116.

Romera, J. L., Hernández, T. D. J. P., Fernández, I. P., Gimeno, T. S., Martínez, R. F., Pérez, I. F., ... & Gil, E. M. C. (2011). Update on adjuvant hormonal treatment of early breast cancer. *Advances in Therapy*, *28*(6), 1.

Ruhstaller, T., Von Moos, R., Rufibach, K., Ribi, K., Glaus, A., Spaeti, B., ... & Boehme, C. (2009). Breast cancer patients on endocrine therapy reveal more symptoms when self-reporting than in pivotal trials: an outcome research study. *Oncology*, *76*(2), 142-148.

Sagara, Y., Kosha, S., Baba, S., Dokiya, F., Tamada, S., Sagara, Y., . . . Douchi, T. (2010). Adverse events and bone health during anastrozole therapy in postmenopausal Japanese breast cancer patients. *Breast Cancer*, *17*(3), 212-217.

Servitja, S., Nogues, X., Prieto-Alhambra, D., Martinez-Garcia, M., Garrigos, L., Pena, M. J., . . . Tusquets, I. (2012). Bone health in a prospective cohort of postmenopausal women receiving aromatase inhibitors for early breast cancer. *Breast*, *21*(1), 95-101.

Shapiro, A. C., Adlis, S. A., Robien, K., Kirstein, M. N., Liang, S., Richter, S. A., & Lerner, R. E. (2016). Randomized, blinded trial of vitamin D3 for treating aromatase inhibitor-associated musculoskeletal symptoms (AIMSS). *Breast Cancer Research and Treatment*, *155*(3), 501-512.

Shi, Q., Giordano, S. H., Lu, H., Saleeba, A. K., Malveaux, D., & Cleeland, C. S. (2013). Anastrozole-associated joint pain and other symptoms in patients with breast cancer. *Journal of Pain*, *14*(3), 290-296.

Shipton, E. E., & Shipton, E. A. (2015). Vitamin D deficiency and pain: clinical evidence of low levels of vitamin D and supplementation in chronic pain states. *Pain and Therapy*, *4*(1), 67-87.

Siegel, R. L., Miller, K. D., & Jemal, A. (2017). Cancer statistics, 2017. *CA: A Cancer Journal for Clinicians*, *67*(1), 7-30.

Simpson, E. R. (2003). Sources of estrogen and their importance. *The Journal of Steroid Biochemistry and Molecular Biology*, *86*(3), 225-230.

Singer, O., Cigler, T., Moore, A. B., Levine, A. B., Hentel, K., Belfi, L., ... & Mandl, L. A. (2012). Defining the aromatase inhibitor musculoskeletal syndrome: a prospective study. *Arthritis Care & Research*, *64*(12), 1910-1918.

Sini, V., Botticelli, A., Lunardi, G., Gori, S., & Marchetti, P. (2017). Pharmacogenetics and aromatase inhibitor induced side effects in breast cancer patients. *Pharmacogenomics*, *18*(8), 821-830.

Stanton, A. L., Bernaards, C. A., & Ganz, P. A. (2005). The BCPT symptom scales: a measure of physical symptoms for women diagnosed with or at risk for breast cancer. *Journal of the National Cancer Institute*, *97*(6), 448-456.

Sun, D., Chen, G., Dellinger, R. W., Sharma, A. K., & Lazarus, P. (2010). Characterization of 17-dihydroexemestane glucuronidation: potential role of the UGT2B17 deletion in exemestane pharmacogenetics. *Pharmacogenetics and Genomics*, *20*(10), 575.

Swenson, K. K., Nissen, M. J., Henly, S. J., Maybon, L., Pupkes, J., Zwicky, K., . . . Shapiro, A. C. (2013). Identification of tools to measure changes in musculoskeletal symptoms and physical functioning in women with breast cancer receiving aromatase inhibitors. *Oncology Nursing Forum*, 40(6), 549-557.

Terhorst, L., Blair-Belansky, H., Moore, P. J., & Bender, C. (2011). Evaluation of the psychometric properties of the BCPT Symptom Checklist with a sample of breast cancer patients before and after adjuvant therapy. *Psycho-Oncology*, *20*(9), 961-968.

Terry, P. C., Lane, A. M., Lane, H. J., & Keohane, L. (1999). Development and validation of a mood measure for adolescents. *Journal of Sports Sciences*, *17*(11), 861-872.

Torre, L. A., Bray, F., Siegel, R. L., Ferlay, J., Lortet-Tieulent, J., & Jemal, A. (2015). Global cancer statistics, 2012. *CA: A Cancer Journal for Clinicians*, *65*(2), 87-108.

Waltman, N. L., Ott, C. D., Twiss, J. J., Gross, G. J., & Lindsey, A. M. (2009). Vitamin D insufficiency and musculoskeletal symptoms in breast cancer survivors on aromatase inhibitor therapy. *Cancer Nu*rsing, *32*(2), 143-150.

Wang, J., Lu, K., Song, Y., Xie, L., Zhao, S., Wang, Y., ... & Ma, W. (2013). Indications of clinical and genetic predictors for aromatase inhibitors related musculoskeletal adverse events in Chinese Han women with breast cancer. *PloS one*, *8*(7), e68798.

Wang, J., Lu, K., Song, Y., Zhao, S., Ma, W., Xuan, Q., ... & Zhang, Q. (2015). RANKL and OPG polymorphisms are associated with aromatase inhibitor-related musculoskeletal adverse events in Chinese Han breast cancer patients. *PloS one*, *10*(7), e0133964.

Zhu, Y., Cohen, S. M., Rosenzweig, M. Q., & Bender, C. M. (2019). symptom map of endocrine therapy for breast cancer: A scoping review. *Cancer Nursing*, *42*(5), E19-E30.

Zhu, Y., Koleck, T. A., Bender, C. M., & Conley, Y. P. (2019). Genetic underpinnings of musculoskeletal pain during treatment with aromatase inhibitors for breast cancer: A biological pathway analysis. *Biological Research for Nursing*, 1099800419895114.