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EVIDENCE OF ASSOCIATIONS BETWEEN NEUROTRANSMITTER CANDIDATE GENES AND PERSISTENT ARM PAIN SEVERITY FOLLOWING BREAST CANCER SURGERY

by

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EVIDENCE OF ASSOCIATIONS BETWEEN NEUROTRANSMITTER CANDIDATE GENES AND PERSISTENT ARM PAIN SEVERITY FOLLOWING BREAST CANCER SURGERY

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ABSTRACT

Persistent arm pain, a distinct syndrome from persistent breast pain, is a considerable clinical problem following breast cancer surgery. The roles of neurotransmitters and neurotransmitter genes have been examined in persistent neuropathic pain; however, genetic associations have not been examined in the setting of breast cancer surgery. In this study, associations between previously identified arm pain classes (i.e., No Arm Pain vs. Mild Arm Pain and No Arm Pain vs. Moderate Arm Pain) and single nucleotide polymorphisms (SNPs) over 30 candidate neurotransmitter genes were evaluated. After multivariate logistic regression analyses for phenotypic characteristics, 4 SNPs and 1 haplotype remained significant between the No Arm Pain and Mild Arm Pain classes: 1 SNP in BDNF (i.e., rs11030102), 1 SNP in COMT (i.e., rs4633), 1 haplotype in HTR2A (i.e., Haplotype B02), 1 SNP for HTR3A (i.e., rs1985242), and 1 SNP in TH (i.e., rs2070762). Between the No Arm Pain and Moderate Arm Pain classes, 9 SNPs remained significant: 1 SNP in BDNF (i.e., rs2049046), 1 SNP in COMT (i.e., rs165656), 2 SNPs in HTR2A (i.e., rs2770298 and rs9534511), 1 SNP in HTR3A (i.e., rs1985242), 1 SNP in NOS2A (i.e., rs2248814), 1 SNP in NPY (i.e., rs16148), 1 SNP in SLC6A1 (i.e., rs2601126), and 1 SNP in TACR1 (i.e., rs4439987). These findings suggest meaningful impact of neurotransmitter genes on the development of persistent arm pain following breast cancer surgery.

TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

Surgery is the primary treatment for breast cancer. Following surgery, between 25% and 60% of patients report chronic, persistent pain.[1] This persistent pain syndrome is characterized by burning, throbbing, or aching in the ipsilateral chest, axilla, and/or arm. The syndrome is associated with other breast and arm symptoms, such as swelling and weakness. In a review of 60 studies,[1] Andersen and Kehlet examined preoperative, intraoperative, and postoperative factors associated with persistent pain after breast cancer surgery. While this review identified several demographic and clinical characteristics associated with the development of persistent pain, the authors did not distinguish between persistent breast and persistent arm pain. Only 13 studies were found that focused on the occurrence and predictors of persistent arm pain. In one study that segregated breast and ipsilateral arm pain,[2] 17% of patients reported persistent ipsilateral arm pain one year after surgery.

In a study conducted by our research team, patients (n=398) were evaluated prior to and for six months following breast cancer surgery. Separate phenotypic characterizations of persistent breast[3] and arm[4] pain were reported previously. In terms of persistent arm pain, four distinct persistent Arm Pain groups were identified. Patients in the No Arm Pain group (41.6%) did not report any arm/shoulder pain over the six months of the study. However, using growth mixture modeling (GMM), two distinct subgroups were identified (i.e. Mild Arm Pain (23.67%) and Moderate Arm Pain (34.8%)). When the persistent breast and arm pain classes were compared,[3,4] distinct demographics and clinical characteristics differentiated between the two anatomic sites. These findings suggest that persistent arm/shoulder pain represents a different pain condition from persistent breast pain.

A variety of neurotransmitters modulate pain transmission in the peripheral and central nervous systems.[5-8] A number of recent reviews have summarized the preclinical[9,10] and clinical[9,11,12] studies that have evaluated associations between polymorphisms in a number of

neurotransmitter genes and a variety of neuropathic pain conditions. Some of the most widely investigated neurotransmitter genes, that appear to play a role in the modulation of persistent pain, include catechol-O-methyltransferase (COMT) and the 5-hydroxytryptamine receptor (HTR) genes. To date, no studies were identified that evaluated the role of neurotransmitter genes in patients with persistent arm pain following breast cancer surgery. Therefore, building on our work that identified two persistent arm pain groups,[4] the purposes of this study in a sample of women (n=398) who were evaluated prior to and for six months after breast cancer surgery were to evaluate for associations between polymorphisms in a number of neurotransmitter genes and membership in the Mild Arm Pain class compared to the No Arm Pain class, as well as membership in the Moderate Arm Pain class compared to the No Arm Pain class.

MATERIALS AND METHODS

This study is part of a larger, longitudinal study that evaluated for neuropathic pain and lymphedema in a sample of women who underwent breast cancer surgery. The methods used in this study are described in detail elsewhere.[13,14]

Patients and Settings

In brief, patients were recruited from Breast Care Centers located in a Comprehensive Cancer Center, two public hospitals, and four community practices. Patients were eligible to participate if they: were an adult woman (≥18 years) who would undergo breast cancer surgery on one breast; were able to read, write, and understand English; agreed to participate; and gave written informed consent. Patients were excluded if they were having breast cancer surgery on both breasts and/or had distant metastasis at the time of diagnosis. A total of 516 patients were approached to participate and 410 were enrolled in the study (response rate 79.5%). The major reasons for refusal were: too busy, overwhelmed with the cancer diagnosis, or insufficient time available to do the assessment prior to surgery.

Instruments

The demographic questionnaire obtained information on age, education, ethnicity, marital status, employment status, living situation, and financial status. The Karnofsky Performance Status (KPS) scale is widely used to evaluate functional status in patients with cancer and has well established validity and reliability.[15,16] Patients rated their functional status using the KPS scale that ranged from 30 (I feel severely disabled and need to be hospitalized) to 100 (I feel normal; I have no complaints or symptoms). Patients were asked to indicate if they exercised on a regular basis (yes/no format).

The Self-Administered Comorbidity Questionnaire (SCQ) is a short and easily understood instrument that was developed to measure comorbidity in clinical and health service research settings.[17] The questionnaire consists of 13 common medical conditions that were

simplified into language that could be understood without any prior medical knowledge. Patients were asked to indicate if they had the condition; if they received treatment for it; and did it limit their activities. For each condition, a patient can receive a maximum of 3 points. The SCQ has well-established validity and reliability and was used in studies of patients with a variety of chronic conditions.[17-21]

Persistent and postsurgical pain were evaluated using the Arm/Shoulder Symptoms Questionnaire (ASQ) and Postsurgical Pain Questionnaire. The ASQ is an adaptation of the Brief Pain Inventory (BPI).[22] The ASQ consisted of two parts. Part 1 obtained information on the occurrence of pain in the arm and shoulder area. If the patient had pain in the shoulder, arm, or hand, they completed Part 2 of the ASQ. Patients were asked to rate the intensity of their average and worst pain using a numeric rating scale (NRS) that ranged from 0 (no pain) to 10 (worst imaginable pain).[23]

The Postsurgical Pain Questionnaire evaluated pain intensity in the first 24 to 48 hours after surgery. Average and worst pain were rated using a 0 (no pain) to 10 (worst imaginable pain) NRS. This questionnaire was completed during the month 1 study visit.

Study Procedures

The study was approved by the Committee on Human Research at the University of California, San Francisco and by the Institutional Review Boards at each of the study sites. During the patient's preoperative visit, a clinician explained the study to the patient and determined her willingness to participate. For those women who were willing to participate, the clinician introduced the patient to the research nurse. The research nurse met with the women, determined eligibility, and obtained written informed consent prior to surgery. After obtaining written informed consent, patients completed the enrollment questionnaires (Assessment 0).

Patients were contacted two weeks after surgery to schedule the first postsurgical appointment. The research nurse met with the patients either in their home or in the Clinical Research Center at 1, 2, 3, 4, 5, and 6 months after surgery. During each of the study visits, the

women completed the study questionnaires and provided information on new and ongoing treatments. Over the course of the study, patients' medical records were reviewed for disease and treatment information.

Characterization of the persistent arm pain phenotype

Characterization of the persistent arm pain phenotype used in this study was described previously.[4] Data were analyzed using SPSS Version 22[24] and Mplus Version 6.1.[25] Demographic and clinical characteristics and symptom severity scores were analyzed using descriptive statistics and frequency distributions.

Unconditional GMM with robust maximum likelihood estimation was carried out to identify latent classes of patients with distinct persistent arm pain trajectories. Arm/shoulder pain scores were assessed monthly for 6 months following breast cancer surgery. Prior to conducting the GMM analysis, patients who reported no pain in their affected arm/shoulder for all 6 assessments were identified (n = 164, 41.6%) and not included in the GMM analysis. The remaining 230 women's ratings of worst arm/shoulder pain were used in the GMM analysis. These methods are described in detail elsewhere.[26] In brief, a single growth curve that represented the "average" change trajectory was estimated for the sample. Then, the number of latent growth classes that best fit the data was identified using guidelines recommended in the literature.[27-29]

Descriptive statistics and frequency distributions for the No Arm pain, Mild Arm Pain, and Moderate Arm Pain classes were generated for demographic and clinical characteristics using SPSS version 22 and Stata version 13 (StataCorp, College Station, TX). Independent sample t-tests, Mann-Whitney U tests, Chi square tests, and Fisher's Exact tests were used to evaluate for differences in demographic and clinical characteristics between the No Arm Pain and the Mild Arm Pain and between the No Arm Pain and the Moderate Arm Pain classes. Logistic regression analyses were performed to evaluate the association between phenotypic characteristics and pain group membership. All phenotypic characteristics that were identified in

the bivariate analyses as being different between the No Arm Pain and each of the two persistent arm pain classes were evaluated for inclusion in the multivariate analysis. A backwards stepwise approach was used to create a parsimonious model. Only predictors with a p-value of <.05 were retained in the final model. These predictors were used in each of the logistic regression analyses to evaluate the associations between genotype and pain group membership.

Gene Selection

A total of 30 candidate genes involved in various aspects of neurotransmission, drug metabolism, or transport of molecules across cell membranes were evaluated. Genes involved in catecholaminergic neurotransmission included adrenergic, alpha-1D receptor (ADRA1D); adrenergic alpha-2A receptor (ADRA2A); adrenergic beta-2 receptor (ADRB2); adrenergic, beta-3 receptor (ADRB3); adrenergic, beta, receptor kinase 2 (ADRBK2); COMT; solute-like carrier (SLC) family 6 (neurotransmitter transporter, noradrenaline) member 2 (SLC6A2); and SLC family 6 (neurotransmitter transporter, dopamine) member 3 (SLC6A3). The gene involved in the GABAergic system was SLC family 6 (neurotransmitter transporter, GABA) member 1 (SLC6A1). Genes involved in serotonergic neurotransmission included: GTP cyclohydrolase 1 (GCH1); HTR 1A, G protein coupled (HTR1A); HTR 1B, G protein coupled (HTR1B); HTR 2A, G protein coupled (HTR2A); HTR 3A, G protein coupled (HTR3A); SLC family 6 (neurotransmitter transporter, serotonin) member 4 (SLC6A4); tyrosine hydroxylase (TH); and tryptophan hydroxylase 2 (TPH2). The two genes involved in molecular transport and drug metabolism that were evaluated were: ATP-binding cassette, subfamily B (MDR/TAP) member 1 (ABCB1) and cytochrome P450, family 3, subfamily A, polypeptide 4 (CYP3A4). A number of additional genes that are involved in various aspects of neurotransmission that were evaluated included: brain-derived neurotrophic factor (BDNF); galanin (GAL); galanin receptor 1 (GALR1); galanin receptor 2 (GALR2); nitric oxide synthase 1 (NOS1); nitric oxide synthase 2, inducible (NOS2A);

neuropeptide Y (NPY); neuropeptide Y receptor Y1 (NPYR1); prodynorphin (PDYN); tachykinin, precursor 1 (TAC1); and tachykinin receptor 1 (TACR1).

Blood collection and genotype

Genomic deoxyribonucleic acid (DNA) was extracted from peripheral blood mononuclear cells using the PUREGene DNA Isolation System (Invitrogen, Carlsbad, CA). DNA samples were quantitated with a Nanodrop Spectrophotometer (ND-1000; Nanodrop Products, Wilmington, DE) and normalized to a concentration of 50 ng/μL (diluted in 10 mM Tris/1 mM EDTA). Samples were genotyped using the Golden Gate genotyping platform (Illumina, San Diego, CA) and processed using GenomeStudio (Illumina, San Diego, CA). Two blinded reviewers visually inspected signal intensity profiles and resulting genotype calls for each single nucleotide polymorphism (SNP).

SNP selection

A combination of tagging SNPs and literature driven SNPs were selected for analysis. Tagging SNPs were required to be common (i.e., defined as having a minor allele frequency (MAF) of ≥.05) in public databases. In order to ensure robust genetic association analyses, quality control filtering of SNPs was performed. SNPs with call rates <95%, Hardy-Weinberg p <.001, and/or a MAF of <5% were excluded. As shown in Table 1, a total of 249 SNPs among the 30 candidate genes passed all quality control filters and are included in subsequent analyses. Potential functional roles of SNPs associated with persistent arm pain were examined using PUPASuite 2.0.[30]

Statistical analyses

Allele and genotype frequencies were determined by gene counting. Hardy-Weinberg equilibrium was assessed by the Chi-square test. Measures of linkage disequilibrium (i.e., D' and r^2) were computed with Haploview 4.2. Linkage disequilibrium (LD)-based haplotype block definition was based on the D' confidence interval method.[31]

For SNPs that were members of the same haploblock, haplotype analyses were conducted in order to localize the association signal within each gene and to determine if haplotypes improved the strength of the association with the phenotype. Haplotypes were constructed using the program PHASE version 2.1.[32] In order to improve the stability of haplotype inference, the haplotype construction procedure was repeated five times using different seed numbers with each cycle. Only haplotypes that were inferred with probability estimates of ≥.85, across the five iterations, were retained for downstream analyses.

Ancestry informative markers (AIMs) were used to minimize confounding due to population stratification.[33-35] Homogeneity in ancestry among patients was verified by principal component analysis[36] using Helix Tree (Golden Helix, Bozeman, MT). Briefly, the number of principal components (PCs) was sought which distinguished the major racial/ethnic groups in the sample by visual inspection of scatter plots of orthogonal PCs (i.e., PC 1 versus PC2, PC2 versus PC3). The first three PCs were selected to adjust for potential confounding due to population substructure (i.e., race/ethnicity) by including the three covariates in all regression models. One hundred and six AIMs were included in the analysis.

For association tests, three genetic models were assessed for each SNP: additive, dominant, and recessive. Barring trivial improvements (i.e., delta <10%), the genetic model that best fit the data, by maximizing the significance of the p-value, was selected for each SNP. Logistic regression analysis, that controlled for significant covariates, as well as genomic estimates of and self-reported race/ethnicity, was used to evaluate the associations between genotype and pain group membership. A backwards stepwise approach was used to create a parsimonious model. Except for genomic estimates of and self-reported race/ethnicity, only predictors with a p-value of <.05 were retained in the final model. Genetic model fit and both unadjusted and covariate-adjusted odds ratios were estimated using Stata version 13.0.

As was done in our previous studies,[14,37] based on recommendations in the literature,[38,39] as well as the implementation of rigorous quality controls for genomic data, the non-

independence of SNPs/haplotypes in LD, and the exploratory nature of the analyses, adjustments were not made for multiple testing. Significant SNPs identified in the bivariate analyses were evaluated further using regression analyses that controlled for differences in phenotypic characteristics, potential confounding due to population stratification, and variation in other SNPs/haplotypes within the same gene. Only those SNPs that remained significant are included in the final presentation of the results. Therefore, the significant independent associations reported are unlikely to be due solely to chance. Unadjusted associations are reported for all SNPs passing quality control criteria in Table 1 to allow for subsequent comparisons and meta-analyses.

RESULTS

Differences in Demographic and Clinical Characteristics between No Arm Pain and Mild Arm Pain Classes

As summarized in Table 2, a number of significant differences in demographical clinical characteristics were found between the No Arm Pain and Mild Arm Pain classes. Patients in the Mild Arm Pain class were significantly younger, had more education, had a lower KPS score, and were less likely to have comorbid high blood pressure. In addition, women in the Mild Arm Pain class had a more advanced stage of disease, had a higher number of breast biopsies, had an axillary lymph node dissection, and had a greater number of nodes removed during surgery. A greater percentage of women in the Mild Arm Pain class had pain in the breast prior to surgery, reported strange sensations in the affected breast, and had increased severity in average and worst postoperative pain. Women in the Mild Arm Pain class were more likely to have had a surgical drain either in the breast, axilla, or both; had a higher number of drains; were more likely to have received neoadjuvant chemotherapy; and a higher percentage had received a biologic therapy during the six months following surgery.

Candidate gene analyses of for the No Arm Pain versus Mild Arm Pain Classes

As shown in Table 1, genotype distributions differed between the No Arm Pain and Mild Arm Pain classes for 4 SNPs and 1 haplotype in BDNF; 5 SNPs and 2 haplotypes in COMT; 1 SNP in GAL; 2 SNPs in GCH1; 3 SNPs and 1 haplotype in HTR2A; 2 SNPs and 1 haplotype in HTR3A; 2 SNPs and 1 haplotype in NOS1; 1 haplotype in NOS2A; 1 SNP in SLC6A2; and 1 SNP in TH.

Regression Analyses for BDNF, COMT, HTR2A, HTR3A, and TH Genotypes and No Arm Pain versus Mild Arm Pain Classes

In order to better estimate the magnitude (i.e. odds ratio, OR) and precision (95% confidence interval, CI) of genotype on the odds of belonging to the No Arm Pain as compared

to the Mild Arm Pain class, multivariate logistic regression models were fit. In these regression analyses that included genomic estimates of and self-reported race/ethnicity, the phenotypic characteristics that remained significant were: functional status (KPS score in 10 unit increments), pain in the affected breast prior to surgery, and undergoing an ALND.

Five genetic associations remained significant in the multivariate logistic regression analyses: BDNF rs11030102, COMT rs4633, HTR2A Haplotype B02, HTR3A rs1985242, and TH rs2070762 (Table 4). In the regression analysis for BDNF rs11030102, carrying one or two doses of the rare G allele (i.e., CC versus CG+GG) was associated with a 64% decrease in the odds of belonging to the Mild Arm Pain class (p=.008). In the regression analysis for COMT rs4633, carrying two doses of the rare T allele (i.e., CC+CT versus TT) was associated with a 68% decrease in the odds of belonging to the Mild Arm Pain class (p=.012). In the regression analysis for HTR2A Haplotype B02, that is composed of alleles at two SNPs (i.e., rs1923886 [common T allele], rs7330636 [rare T allele]), each additional dose of HTR2A HapB02 was associated with a 51% decrease in the odds of belonging to the Mild Arm Pain class (p=.008). In the regression analysis for HTR3A rs1985242, carrying two doses of the rare A allele (i.e., TT+TA versus AA) was associated with a 90% decrease in the odds of belonging to the Mild Arm Pain class (p<.001).

Differences in Demographic and Clinical Characteristics between No Arm Pain and Moderate Arm Pain Classes

As summarized in Table 3, a number of significant differences in demographic and clinical characteristics were found between the No Arm Pain and the Moderate Arm Pain classes. Patients in the Moderate Arm Pain class were younger, with lower KPS scores, lower annual household incomes, higher BMI, higher SCQ scores, and more likely to be White. In addition, a higher percentage of women in the Moderate Arm Pain class reported comorbid anemia and were less likely to have breast fed. A higher percentage of patients in the Moderate Arm Pain class had advanced stage of disease, reported breast pain prior to surgery, reported

sensations of swelling, numbness, and hardness in the affected breast, had received neoadjuvant chemotherapy, and had a higher number of breast biopsies. A higher percentage of women in the Moderate Arm Pain class underwent a mastectomy; had a higher number of lymph nodes removed; had a drain placed either in the breast, axilla, or both; had a higher number of drains placed; had an ALND; and had the intercostobrachial nerve sacrificed. Post operatively, women in the Moderate Arm Pain class reported higher average and worst postoperative pain severity scores; were more likely to have had physical therapy within the six months post-surgery; have received biological therapy within the six months following surgery; and had more postoperative complications.

Candidate Gene Analyses for the No Arm Pain versus Moderate Arm Pain Classes

As shown in Table 1, genotype distributions differed between the No Arm Pain and Moderate Arm Pain classes for 1 SNP in ABCB1; 2 SNPs and 2 haplotypes in ADRA1D; 1 SNP in ADRBK2; 8 SNPs and 1 haplotype in BDNF; 5 SNPs and 4 haplotypes in COMT; 1 SNP in GALR2; 1 SNP in GCH1; 1 SNP in HTR1A; 7 SNPs and 3 haplotypes in HTR2A; 1 SNP and 1 haplotype in HTR3A; 2 SNPs and 1 haplotype in NOS2A; 1 SNP in NPY; 1 SNP in PDYN; 2 SNPs and 2 haplotypes in SLC6A1; 3 SNPs in SLC6A2; 1 SNP in SLC6A4; 7 SNPs in TACR1; and 1 SNP in TPH2.

Regression Analyses for BDNF, COMT, HTR2A, HTR3A, NOS2A, NPY, SLC6A1, and TACR1 Genotypes and No Arm Pain versus Moderate Arm Pain classes

In order to better estimate the magnitude (i.e. odds ratio, OR) and precision (95% confidence interval, CI) of genotype on the odds of belonging to the No Arm Pain as compared to the Moderate Arm Pain class, multivariate logistic regression models were fit. In these regression analyses that included genomic estimates of and self-reported race/ethnicity, the phenotypic characteristics that remained significant were: functional status (KPS score in 10 unit increments), pain in the affected breast prior to surgery, number of breast biopsies in the past year, placement of a surgical drain (i.e., no drain placed compared to drain placement only in

the breast, drain placement only in the axilla, or drain placement in both the breast and axilla), and receipt of physical therapy in the six months following surgery.

Nine genetic associations remained significant in the multivariate logistic regression analyses: BDNF rs2049046, COMT rs165656, HTR2A rs2770298, HTR2A rs9534511, HTR3A rs1985242, NOS2A rs2248814, NPY rs16148, SLC6A1 rs2601126, and TACR1 rs4439987 (Table 5). In the regression analysis for BDNF rs2049046, carrying two doses of the rare T allele (i.e., AA+AT versus TT) was associated with a 3.07-fold increase in the odds of belonging to the Moderate Arm Pain class (p=.009). In the regression analysis for COMT rs165656, carrying two doses of the rare G allele (i.e., CC+CG versus GG) was associated with a 63% decrease in the odds of belonging in the Moderate Arm Pain class (p=.027).

For HTR2A, two SNPs were associated with membership in the Moderate Arm Pain class (i.e., HTR2A rs2770298, HTR2A rs9534511). In the regression analysis, for HTR2A rs2770298, carrying two doses of the rare C allele (i.e., TT+TC versus CC) was associated with a 5.08-fold increase in the odds of belonging to the Moderate Arm Pain class (p=.028). In the same regression analysis, for HTR2A rs9534511, carrying one or two doses of the rare T allele (CC versus CT+TT) was associated with a 1.89-fold increase in the odds of belonging to the Moderate Arm Pain class (p=.019). In the regression analysis for HTR3A rs1985242, carrying two doses of the rare A allele (i.e., TT+TA versus AA) was associated with an 85% decrease in the odds of belonging to the Moderate Arm Pain class (p=.003).

In the regression analysis for NOS2A rs2248814, carrying one or two doses of the rare A allele (i.e., GG versus GA+AA) was associated with a 66% decrease in the odds of belonging to the Moderate Arm Pain class (p=.007). In the regression analysis for NPY rs16148, carrying one or two doses of the rare C allele (i.e., TT versus TC+CC) was associated with a 2.70-fold increase in the odds of belonging to the Moderate Arm Pain class (p=.021). In the regression analysis of SLC6A1 rs2601126, carrying one or two doses of the rare T allele (i.e., CC versus CT+TT) was associated with a 3.00-fold increase in the odds of belonging to the Moderate Arm

Pain class (p=.014). In the regression analysis of TACR1 rs4439987, carrying one or two doses of the rare G allele (i.e., AA versus AG+GG) was associated with a 60% decrease in the odds of belonging to the Moderate Arm Pain class (p=.025).

DISCUSSION

Phenotypic characteristics

A discussion of differences in phenotypic characteristics between the No Arm Pain and Mild Arm Pain classes, as well as between the No Arm Pain and Moderate Arm Pain classes are reported in detail elsewhere.[4] Therefore, this discussion will focus on differences in genotypic characteristics. The findings are grouped based on genes associated with membership in the Mild Arm Pain class, genes associated with membership in the Moderate Arm Pain class, and genes associated with membership in both persistent pain classes.

Genes Associated with Membership in the Mild Arm Pain class

Only one gene, namely TH, was uniquely associated with membership in the Mild Arm Pain class. TH is the enzyme that converts tyrosine to dopamine (DA). Mutations in the TH gene are associated with DA-related conditions, as well as psychiatric disorders (e.g., schizophrenia).[40] While the enzyme itself is not involved in pain, its effects on DA could influence pain mechanisms. Endogenous opioids are released in response to a noxious stimulus, stimulating the release of DA.[41] Stimulation of the DA receptors results in inhibition of nociception. In a review of the effects of DA,[41] studies of healthy samples found that participants with lower baseline levels of DA reported higher pain ratings during noxious stimulation. A higher level of DA during the noxious stimulus was associated with lower ratings of pain. DA levels and presynaptic activity has been examined in the setting of chronic pain (i.e., burning mouth syndrome and fibromyalgia). However, the study samples were small and the results are difficult to interpret. In a spared nerve injury (SNI) model of neuropathic pain that is used in rats to mimic neuropathic pain, the application of a DA-receptor agonist had an analgesic effect, while the application of a DA-receptor antagonist reversed this effect.[42] These results support DA-mediated antinociception in the experience of neuropathic pain.

In the current study, carrying one or two doses of the rare C allele at TH rs2070762 was associated with a 2.39-fold increase in the odds of belonging to the Mild Arm Pain class. While in one study, this polymorphism was associated with migraines,[43] this finding was not confirmed in a validation cohort.

Genes Associated with Membership in the Moderate Arm Pain class

Four genes, namely NOS2A, SLC6A1, TACR1, and NPY, were associated with membership in the Moderate Arm Pain class. NOS2A produces inducible nitric oxide (iNOS), a free radical, as an immune defense mechanism in response to tissue injury. Studies of skeletal muscle and peripheral nerve function have implicated iNOS in ischemia. Of note, inhibition of iNOS leads to improvements in the microcirculation and restitution of motor function.[44] In one preclinical study of neuropathic pain,[45] the administration of nitric oxide synthase inhibitors increased the analgesic effects of morphine.

In our study, patients who were heterozygous or homozygous for the rare A allele in NOS2A rs2248814 had a 66% decreased likelihood of belonging to the Moderate Arm Pain class. NOS2A rs2248814 is located in the intron of the gene. While no studies were identified that evaluated this SNP in the context of persistent pain, associations were found with macular degeneration[46] and Parkinson's disease.[47] In one study,[46] an interaction was found between this SNP, smoking behavior, and the risk for macular degeneration. Specifically, individuals who were heterozygous or homozygous for the rare A allele and who smoked had an increased odds of developing age-related macular degeneration. In contrast, in a study of the association between this SNP and Parkinson's disease,[47] while a significant association was found between NOS2A rs2248814 and the occurrence of sporadic Parkinson's disease, no gene x smoking interaction was identified. Ayala-Haedo et al.[46] hypothesized that these inconsistent findings may be due to linkage disequilibrium, as the AA genotype is rare. When examining our findings in light of previous research, the presence of the rare A allele at rs2248814 may be associated

with decreased expression of NOS2A and iNOS, which may prevent nerve injury and associated neuropathic pain.

Gamma-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the CNS. GABA is implicated in a large number of disease states including anxiety and stress disorders, insomnia, epilepsy, cognitive and learning deficits, and pain.[48] GABA transporters clear GABA from the synapse, which regulates pain transmission. The primary transporter of GABA is GABA-transporter 1 (GAT-1). GAT-1 is encoded by the gene SLC6A1. Studies of GAT inhibitors[49] and GAT-1 knock-out mice[50] support a relationship between suppressed GAT-1 activity and higher levels of pain.

In our study, individuals who were homozygous for the rare T allele at SLC6A1 rs2601126 had a 3-fold increase in the likelihood of belonging to the Moderate Arm Pain class. This intronic SNP has no known function. Only two studies were identified that examined this polymorphism, focusing on its role in anxiety disorders.[51,52] In a case-control study of patients with anxiety disorders who did and did not have subsyndromal panic attacks,[52] no association was found with this SNP. In another study that evaluated the effects of kava, a plant-based medicine, in patients with generalized anxiety disorder (GAD),[51] for patients who received kava, each dose of the rare T allele was associated with significant decreases in patients' anxiety scores. Kava is known to effect anxiolytic activity from the effects of kavalactone constituents on GABA pathways. Findings from the study by Sarris et al.[53] suggest that polymorphism in SLC6A1 rs2601126 influences the transport of GABA and results in decreased anxiety. No studies were found that evaluated the relationship between polymorphisms in this SNP and persistent pain.

The neurokinin-1 receptor (NK1 receptor) is the primary target for Substance P and has a unique role in the development of persistent pain. Substance P is a tachykinin, released in the presence of a noxious stimulus. Binding of Substance P to the NK1 receptor increases the excitability of afferent neurons. Through NK1 receptor stimulation, *a*-amino-3-hydroxy-5-methyl-

4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptors are sensitized to glutamate, and cyclooxygenase (COX) synthesis of prostaglandin is promoted, which increases neurotransmitter release.[54] Rat studies found that the NK1 receptor is upregulated in the setting of nerve damage and persistent pain.[5,55] Prolonged stimulation with a noxious stimulus results in sustained binding of Substance P to the NK1 receptors.[54] Through these processes, Substance P and NK1 receptors perpetuate neuropathic pain.

In the current study, patients who carried one or two doses of the rare G allele at rs4439987 had a 60% decrease in the odds of belonging to the Moderate Arm Pain class. While TACR1 rs4439987 was evaluated in a study of alcohol dependence in Caucasians,[56] no associations were identified. This intronic SNP has not been studied in patients with acute or chronic pain.

The NPY gene encodes for NPY. Present on GABAergic neurons, NPY receptors (i.e., Y_1 and Y_2) are implicated in the inhibition of acute, inflammatory, and neuropathic pain states.[57] In animal models, reorganization of sensory pathways and upregulation of NPY occur after nerve injury.[5,57] In a mouse study,[58] the administration of NPY antagonists after nerve injury resulted in the resolution of behavioral signs of pain. The administration of NPY agonists restored signs of inflammatory and neuropathic pain. Escalation of receptor activation may lead to inhibition of GABA and glycine release through binding to Y_1 receptors and inhibition of excitatory neurotransmitter release through Y_2 receptor binding. The overall result is inhibition of spinal nociceptive transmission and inhibition of hyperalgesia, which prevents the transition from acute pain to chronic pain.

In the current study, carrying one or two doses of the rare C allele at NPY rs16148 was associated with a 2.70-fold increase in the odds of belonging to the Moderate Arm Pain class. This intronic SNP was not associated with the occurrence of atherosclerosis.[59] Solway et al.[58] inferred that failure of NPY upregulation after injury would cause susceptibility to chronic pain. Based on what is known about NPY and chronic pain, this hypothesis may explain the

association between the rs16148 polymorphism and membership in the Moderate Arm Pain group.

Genes Associated with Membership in Both the Mild and Moderate Arm Pain classes

Four genes, namely, BDNF, COMT, HTR2A, and HTR3A, were associated with membership in both the Mild and Moderate Arm Pain classes. BDNF has effects throughout the nervous system. BDNF is upregulated in the dorsal root ganglion during states of inflammation or injury. In persistent pain conditions, release of BDNF promotes excitatory, glutamatergic synaptic transmissions, which leads to central sensitization and hyperalgesia. In addition, BDNF suppresses the activity of inhibitory, GABAergic synapses.[60]

Consistent with previous reports of its role in the development of persistent pain,[61,62] two SNPs in BDNF remained significant after analysis: one in the Mild Arm Pain class (i.e., rs11030102) and one in the Moderate Arm Pain class (i.e., rs2049046). In our study, being heterozygous or homozygous for the G allele in BDNF rs11030102 was associated with a 64% decrease in the odds of belonging to the Mild Arm Pain class. This finding is consistent with work by Terracciano and colleagues,[63] who reported that individuals who carried the C allele for rs11030102 had higher serum levels of BDNF. These findings suggest that the G allele at rs11030102 may decrease BDNF expression and reduce the excitatory effects associated with release of this neurotransmitter.

Another polymorphism in the BDNF gene (i.e., rs2049046) was associated with membership in the Moderate Arm Pain class. Patients who were homozygous for the rare T allele were three times more likely to be in the Moderate Arm Pain class. An association between rs2049046 and an increased susceptibility to migraine was found in a retrospective study.[64] While no differences were found between cases and controls for the BDNF SNP alone, a significant interaction was found between the AT genotype in BDNF rs2049046 and a SNP in the calcitonin gene-related peptide gene (i.e., GC genotype in CGRP rs1553005).

COMT is an enzyme that is responsible for the metabolism of epinephrine, norepinephrine, and DA. Associations between polymorphisms in the COMT gene and pain mechanisms and management have been the subjects of intense investigations (for reviews see [9,65]). The results of a recent meta-analysis that focused primarily on studies of COMT rs4680 (Val158Met)[9] found a significant association between this SNP and fibromyalgia. In addition, the authors noted that COMT activity does not affect neuropathic or cancer pain. However, a decrease in COMT activity appears to enhance the efficacy of opioid analgesics and exacerbates the adverse effects of opioids in some patients with cancer. They acknowledged that the role of COMT in pain mechanisms and analgesic responses is extremely complex.

In our study, patients who were homozygous for the rare T allele in COMT rs4633 had a 68% decrease in the odds of belonging to the Mild Arm Pain class. In addition, women who were homozygous for the rare G allele in rs165656 had a 63% decrease in the odds of belonging in the Moderate Arm Pain class. Located in exon 3 of the COMT gene, rs4633 is a nonsynonymous SNP which was linked to pediatric postoperative pain,[66] pain after a motor vehicle accident,[67] pain associated with lumbar disc disease,[68] pain after lumbar spine surgery,[69] fibromyalgia,[70,71] pain in women with major depressive disorder,[72] and low back pain.[73]

Most often, rs4633 is studied as part of a haplotype. In combination with polymorphisms in rs6269, rs4818, and rs4680 (i.e. Val/Met), rs4633 was associated with low, average, and high pain sensitivity (i.e., LPS, APS, HPS, respectively) phenotypes. COMT rs4680 is the only SNP in this haplotype that changes an amino acid sequence and resulting protein. While in the bivariate analyses, the APS haplotype was significant for Mild Arm Pain and the APS and HPS haplotypes were significant for Moderate Arm Pain, these associations did not remain significant in the multivariate analyses.

While our results suggest a protective effect associated with the TT genotype at rs4633, as part of the COMT haplotype, the T allele at rs4633 is associated with APS. Conflicting evidence exists on the role of COMT rs4633 in pain. For example, in one study that evaluated

the frequency of the COMT haplotype in chronic widespread pain,[74] no differences in genotype frequencies were found between cases and controls. In another study,[75] the COMT haplotype was not associated with experimental pain thresholds in a sample of Chinese men.

The only study of COMT rs165656,[76] evaluated a sample of 44 patients with temporomandibular disorder (TMD) compared to healthy controls (n=182). Being heterogeneous for the G allele (likely referred to as the "C" allele in[76]) at rs165656, located in the promoter region of the COMT gene, was associated with an 80% decrease in the likelihood of having TMD (p=.001). This finding appears similar to our results, where the GG genotype at rs165656 was associated with a significant decrease in the likelihood of belonging to the Moderate Arm Pain class. Further study of the rs165656 in concordance with other polymorphisms in the COMT gene may increase our understanding of these results, as with the well-known haplotype associated with rs4633.

The HTR2A gene codes the $5HT_{2A}$ receptor. This receptor is highly expressed in dorsal root ganglion cells. In addition, $5HT_{2A}$ receptors are located in laminae I-IV of the dorsal horn, and in the nucleus raphe magnus, the thalamus, the cerebral cortex, and the limbic system. In the periphery, activation of $5HT_{2A}$ receptors during inflammation results in inhibition of sensitization of primary afferent neurons. In the spinal cord, the function of $5HT_{2A}$ receptors, particularly in neuropathic pain, is not well understood.

In the current study, polymorphisms in $5HT_{2A}$ were associated with membership in both the Mild and Moderate Arm Pain classes. In the No Arm Pain versus Mild Arm Pain analysis, for the HTR2A Haplotype B02, that is composed of alleles at two SNPs (i.e., rs1923886 [T common allele], rs7330636 [T rare allele]), each additional dose of HTR2A HapB02 was associated with a 51% decrease in the odds of belonging to the Mild Arm Pain class (p=.008). For Moderate Arm Pain, carrying two doses of the rare C allele at HTR2A rs2770298 was associated with a 5.08-fold increase in the odds of belonging to the Moderate Arm Pain class (p=.028). In

addition, carrying one or two doses of the rare T allele at HTR2A rs9534511 was associated with a 1.89-fold increase in the odds of belonging to the Moderate Arm Pain class (p=.019).

Our findings are consistent with a study of patients with chronic widespread pain (CWP) who were classified using the American College of Rheumatology's criteria.[77] This study used a discovery cohort (i.e., a population-based cohort of men and women from the Epidemiology of Functional Disorders (EPIFUND) study) and a validation cohort (i.e., a population-based cohort of men from the European Male Aging Study (EMAS)) to evaluate genetic associations with two phenotypes (i.e., CPW and maximum number of pain sites reported). One SNP in HTR2A (i.e., rs12584920) was associated with an increased odds of refractory CWP in both cohorts. In addition, HTR2A rs17289394 was associated with an increase in the odds of reporting a higher number of painful sites in both cohorts. In contrast to our data, HTR2A rs9534511 was associated with a decrease in the odds of reporting a higher number of painful sites. The authors suggested that the HTR2A receptor is involved in the development of musculoskeletal pain. The inconsistent findings may be related to differences in the pain phenotypes evaluated in the two studies.

The $5HT_3$ receptor is involved in pain, anxiety, and immunomodulatory processes. Located on primary afferent neurons, $5HT_3$ receptors in the peripheral nervous system alter pain transmission from the periphery.[78,79] Within the dorsal horn, the activation of $5HT_3$ receptors is associated with antinociceptive activity during acute pain. Stimulation of these receptors is thought to induce the release of GABA, which activates descending inhibitory pathways. The activation of this descending inhibitory system decreases sensory input from the peripheral nervous system.

Within the central nervous system, $5HT_3$ receptors are primarily located pre-synaptically and influence the release of neurotransmitters and neuropeptides.[78] In the setting of chronic pain, $5HT_3$ receptor antagonists inhibit the release of neurotransmitters like Substance P, neurokinin A, and calcitonin gene-related peptide from primary afferent neurons. In particular,

Substance P is implicated in the development of inflammation and chronic pain. $5HT_3$ receptor antagonists have been evaluated as treatments for chronic pain syndromes, including fibromyalgia and chronic back pain with positive results.[80] Inhibition of Substance P release may explain the analgesic effects of $5HT_3$ receptor antagonists.[78]

In the current study, carrying two doses of the rare A allele at HTR3A rs1985242 was associated with a 90% decrease in odds of belonging to Mild Arm Pain class. Carrying two doses of the rare A allele at HTR3A rs1985242 was associated with an 85% decrease in the odds of belonging to the Moderate Arm Pain class. The HTR3A gene encodes for the $5HT_3$ serotonin receptor. This intronic SNP has not been implicated in other persistent pain conditions.

Several study limitations need to be acknowledged. The sample was adequate in size and representative of breast cancer patients in the United States. However, additional latent classes and significant neurotransmitter gene polymorphisms may have been defined from a larger, more diverse sample, including a larger percentage of non-white, older patients, or those who had more advanced disease or more extensive surgery. This study was limited to the selected candidate genes. As technology evolves, examination of the full genome may elucidate additional genes and polymorphisms associated with persistent pain. Additionally, serum levels of the various neurotransmitters were not measured to support the gene associations that were identified. Patients were recruited through referrals from twenty surgeons at seven different sites, to enhance generalizability of the study's findings. Evaluating how surgical and postoperative pain management protocols impact persistent postoperative pain and SNP interactions will add another dimension to future studies.

This study is the first prospective, longitudinal study to examine the prevalence of persistent arm pain following breast cancer surgery and its association with neurotransmitter genes. The elucidation of genetic factors that predispose patients to persistent arm pain will

change how we treat breast cancer patients and improve postoperative outcomes. Further study is needed to confirm our findings in varied populations and in other persistent pain conditions.

DISCLOSURES

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Table 1 - Summary of Single Nucleotide Polymorphisms Analyzed for Neurotransmitter Genes and the Growth Mixture Model Analyses for Mild and Moderate Arm Pain

Gene	SNP	Position	Chr	MAF	Alleles	None to Mild Pain			None to Moderate Pain		
						Chi Square	p-value	Model	Chi Square	p-value	Model
ATP-BINDING CASSETTE, SUBFAMILY B (MDR/TAP) MEMBER 1											
ABCB1	rs2235048	86976447	7	.471	T>C	0.951	.621	A	2.262	.323	A
ABCB1	rs6961419	87010072	7	.400	T>C	0.149	.928	A	0.654	.721	A
ABCB1	rs1128503	87017537	7	.433	C>T	1.619	.445	A	0.298	.861	A
ABCB1	rs1922241	87023830	7	.299	G>A	0.222	.895	A	0.715	.699	A
ABCB1	rs10264990	87040551	7	.293	T>C	1.132	.568	A	0.058	.971	A
ABCB1	rs1989830	87043599	7	.309	C>T	2.176	.337	A	0.965	.617	A
ABCB1	rs1858923	87059152	7	.445	T>C	1.497	.473	A	FE	.048	D
ABCB1	rs9282564	87067376	7	.089	A>G	2.734	.255	A	4.026	.134	A
ABCB1	rs13233308	87082896	7	.438	C>T	0.454	.797	A	1.983	.371	A
ABCB1	rs10267099	87116696	7	.213	A>G	2.865	.239	A	0.244	.885	A
ABCB1	HapA01					1.885	.390		0.421	.810	
ABCB1	HapA05					0.161	.923		0.600	.741	
ABCB1	HapB01					0.745	.689		1.929	.381	
ABCB1	HapB02					0.581	.748		2.365	.307	
ALPHA-1D ADRENERGIC RECEPTOR											
ADRA1D	rs3787441	4153060	20	.268	T>C	0.246	.884	A	FE	.035	D
ADRA1D	rs6084664	4155930	20	.159	T>C	1.154	.562	A	1.987	.370	A
ADRA1D	rs2326478	4156247	20	.326	C>T	4.280	.118	A	2.069	.355	A
ADRA1D	rs835880	4156895	20	.225	A>G	0.920	.631	A	2.748	.253	A
ADRA1D	rs8183794	4158448	20	.182	C>T	2.892	.236	A	0.306	.858	A
ADRA1D	rs6116268	4159440	20	.480	C>T	1.819	.403	A	2.208	.332	A
ADRA1D	rs946188	4163316	20	.236	A>G	1.032	.597	A	2.125	.346	A
ADRA1D	rs1556832	4163557	20	.461	C>T	1.102	.576	A	0.840	.657	A
ADRA1D	rs8118409	4164663	20	.229	G>A	0.044	.978	A	1.766	.414	A
ADRA1D	rs4815670	4164864	20	.467	G>A	1.907	.385	A	FE	.015	R
ADRA1D	rs6076639	4167258	20	.206	C>T	0.933	.627	A	2.012	.366	A
ADRA1D	rs4815675	4171454	20	.423	T>C	0.471	.790	A	0.982	.612	A

ADRA1D	HapA01					1.754	.416		6.769	.034	
ADRA1D	HapA03					1.008	.604		2.902	.234	
ADRA1D	HapB02					1.032	.597		1.907	.385	
ADRA1D	HapB03					1.677	.432		2.044	.360	
ADRA1D	HapC01					1.857	.395		6.721	.035	
ADRA1D	HapC02					2.815	.245		0.421	.810	
ADRA1D	HapC03					0.044	.978		1.766	.414	
ADRA1D	HapD01					0.538	.764		0.552	.759	
ADRA1D	HapD02					0.233	.890		0.684	.710	
ALPHA 2A ADRENERGIC RECEPTOR											
ADRA2A	rs521674	112825580	10	.364	A>T	n/a	n/a	n/a	n/a	n/a	n/a
ADRA2A	rs3750625	112829591	10	.079	C>A	FE	.077	A	4.334	.115	A
BETA 2 ADRENERGIC RECEPTOR											
ADRB2	rs2400707	148185245	5	.401	G>A	4.094	.129	A	2.204	.332	A
ADRB2	rs11168070	148186120	5	.357	C>G	3.582	.167	A	0.790	.674	A
ADRB2	rs1042718	148187110	5	.203	C>A	4.738	.094	A	2.626	.269	A
ADRB2	rs1042719	148187640	5	.315	G>C	1.542	.463	A	2.030	.362	A
ADRB2	HapA01					1.129	.569		0.158	.924	
ADRB2	HapA02					4.633	.099		2.711	.258	
ADRB2	HapA05					4.090	.129		1.279	.528	
BETA 3 ADRENERGIC RECEPTOR											
ADRB3	rs4994	37942955	8	.092	T>C	0.493	.781	A	0.823	.663	A
BETA ADRENERGIC RECEPTOR KINASE 2											
ADRBK2	rs1008673	24324013	22	.148	A>G	0.777	.678	A	3.220	.200	A
ADRBK2	rs3817819	24405188	22	.421	C>T	3.060	.217	A	1.488	.475	A
ADRBK2	rs5761159	24432308	22	.438	G>T	3.889	.143	A	1.206	.547	A
ADRBK2	rs9608416	24441018	22	.468	A>G	5.645	.059	A	FE	.018	D
ADRBK2	HapA01					5.502	.064		4.994	.082	
ADRBK2	HapA04					3.780	.151		2.962	.227	
BRAIN DERIVED NEUROTROPHIC FACTOR											
BDNF	rs7124442	27633617	11	.290	T>C	3.043	.218	A	2.071	.355	A
BDNF	rs6265	27636492	11	.222	G>A	0.068	.967	A	FE	.038	R
BDNF	rs11030101	27637320	11	.409	A>T	FE	.035	R	FE	.004	R
BDNF	rs11030102	27638172	11	.205	C>G	FE	.038	D	FE	.009	D

BDNF	rs11030104	27641093	11	.233	A>G	0.457	.796	A	3.224	.200	A
BDNF	rs2049045	27650817	11	.156	G>C	0.009	.996	A	3.388	.184	A
BDNF	rs11030107	27651411	11	.205	A>G	FE	.027	D	FE	.009	D
BDNF	rs7103411	27656701	11	.243	T>C	0.875	.646	A	FE	.014	R
BDNF	rs16917237	27658959	11	.231	G>T	0.598	.742	A	FE	.024	R
BDNF	rs6484320	27659764	11	.243	A>T	0.875	.646	A	FE	.014	R
BDNF	rs7127507	27671460	11	.295	T>C	4.893	.087	A	3.078	.215	A
BDNF	rs2049046	27680351	11	.464	A>T	FE	.008	R	FE	.001	R
BDNF	HapA01					6.494	.039		9.766	.008	
CATECHOL-O-METHYLTRANSFERASE											
COMT	rs5748489	18307146	22	.388	C>A	4.205	.122	A	3.415	.181	A
COMT	rs2020917	18308884	22	.263	C>T	0.815	.665	A	0.143	.931	A
COMT	rs737866	18310109	22	.265	A>G	0.769	.681	A	0.088	.957	A
COMT	rs1544325	18311668	22	.397	G>A	4.431	.109	A	1.594	.451	A
COMT	rs5993882	18317533	22	.234	T>G	FE	.029	D	3.178	.204	A
COMT	rs5993883	18317638	22	.495	T>G	3.578	.167	A	0.738	.691	A
COMT	rs740603	18325177	22	.495	G>A	2.055	.358	A	0.293	.864	A
COMT	rs4646312	18328337	22	.371	T>C	3.666	.160	A	0.267	.875	A
COMT	rs165656	18328863	22	.489	C>G	FE	.024	R	FE	.015	R
COMT	rs6269	18329952	22	.391	A>G	4.259	.119	A	0.053	.974	A
COMT	rs4633	18330235	22	.472	C>T	FE	.007	R	FE	.008	R
COMT	rs6267	18330263	22	.002	G>T	n/a	n/a	n/a	n/a	n/a	n/a
COMT	rs740601	18330763	22	.399	A>C	3.856	.145	A	0.416	.812	A
COMT	rs5031015	18331103	22	.001	G>A	n/a	n/a	n/a	n/a	n/a	n/a
COMT	rs4818	18331207	22	.387	C>G	3.399	.183	A	0.058	.972	A
COMT	rs4680	18331271	22	.475	G>A	FE	.032	R	FE	.012	R
COMT	rs165774	18332561	22	.288	G>A	FE	.045	D	FE	.003	R
COMT	rs174699	18334458	22	.098	T>C	4.417	.110	A	0.610	.737	A
COMT	rs9332377	18335692	22	.129	T>C	0.018	.991	A	0.767	.681	A
COMT	rs165599	18336781	22	.338	A>G	1.581	.454	A	FE	.047	D
COMT	HapA01					4.027	.134		2.710	.258	
COMT	HapA06					6.654	.036		2.942	.230	
COMT	HapA10					0.815	.665		0.215	.898	

COMT	HapB02					5.924	.052		8.689	.013	
COMT	HapB20					3.668	.160		0.125	.939	
COMT	HapC01					1.581	.454		4.271	.118	
COMT	HapC02					2.868	.238		3.355	.187	
COMT	PAIN LPS					3.342	.188		0.075	.963	
COMT	PAIN APS					7.278	.026		6.602	.037	
COMT	PAIN HPS					1.399	.497		9.679	.008	
COMT	PAIN DIPLO					8.053	.153		13.076	.023	
COMT	PAIN RECODE A					FE	.139		FE	1.000	
CYTOCHROME P450, FAMILY 3, SUBFAMILY A, POLYPEPTIDE 4											
CYP3A4	rs4646437	99203019	7	.163	C>T	0.215	.898	A	3.751	.153	A
GALANIN											
GAL	rs694066	68209561	11	.104	G>A	0.201	.905	A	1.052	.591	A
GAL	rs3136540	68212986	11	.249	C>T	FE	.044	D	5.190	.075	A
GAL	rs1042577	68215046	11	.334	G>A	2.467	.291	A	1.382	.501	A
GAL	HapA01					2.892	.235		1.449	.485	
GAL	HapA04					4.490	.106		5.274	.072	
GALANIN RECEPTOR 1											
GALR1	rs949060	73087926	18	.381	G>C	0.058	.972	A	0.036	.982	A
GALANIN RECEPTOR 2											
GALR2	rs2443168	71578042	17	.443	T>A	0.165	.921	A	5.145	.076	A
GALR2	rs2598414	71578694	17	.391	C>T	0.056	.972	A	FE	.026	R
GALR2	HapA01					0.056	.972		5.789	.055	
GALR2	HapA03					0.104	.949		5.683	.058	
GTP CYCLOHYDROLASE 1											
GCH1	rs7142517	54376554	14	.297	C>A	0.722	.697	A	3.055	.217	A
GCH1	rs841	54380242	14	.236	C>T	0.080	.961	A	0.108	.947	A
GCH1	rs752688	54381319	14	.236	C>T	0.080	.961	A	0.108	.947	A
GCH1	rs7155309	54392601	14	.234	T>C	0.080	.961	A	0.224	.894	A
GCH1	rs12587434	54395333	14	.236	T>G	0.057	.972	A	0.351	.839	A
GCH1	rs9671371	54398385	14	.337	C>T	0.069	.966	A	2.297	.317	A
GCH1	rs2183081	54406501	14	.409	T>C	0.709	.701	A	0.906	.636	A
GCH1	rs17128050	54413629	14	.148	T>C	6.129	.047	A	2.755	.252	A
GCH1	rs3783637	54417868	14	.155	C>T	5.403	.067	A	4.623	.099	A

GCH1	rs3783638	54418123	14	.187	G>A	FE	.030	R	FE	.012	R
GCH1	rs998259	54424781	14	.168	C>T	4.000	.135	A	0.795	.672	A
GCH1	rs3783642	54429953	14	.461	T>C	0.674	.714	A	1.342	.511	A
GCH1	HapA01					2.583	.275		0.119	.942	
GCH1	HapA05					0.080	.961		0.172	.918	
GCH1	HapA06					0.722	.697		3.166	.205	
GCH1	HapB01					1.029	.598		1.621	.445	
GCH1	HapB03					0.315	.854		2.235	.327	
5-HYDROXYTRYPTAMINE RECEPTOR 1A											
HTR1A	rs6449693	63291774	5	.437	A>G	1.955	.376	A	FE	.008	R
5-HYDROXYTRYPTAMINE RECEPTOR 1B											
HTR1B	rs6296	78228979	6	.313	G>C	1.488	.475	A	0.567	.753	A
5-HYDROXYTRYPTAMINE RECEPTOR 2A											
HTR2A	rs6314	46307035	13	.078	C>T	5.763	.056	A	FE	.719	A
HTR2A	rs7322347	46308104	13	.420	T>A	FE	.022	D	2.746	.253	A
HTR2A	rs1923882	46309662	13	.223	C>T	4.360	.113	A	0.451	.798	A
HTR2A	rs7997012	46309986	13	.380	G>A	3.022	.221	A	2.978	.226	A
HTR2A	rs3742278	46317578	13	.189	A>G	1.745	.418	A	0.649	.723	A
HTR2A	rs1923884	46319837	13	.167	C>T	FE	.032	D	0.529	.768	A
HTR2A	rs1923886	46321292	13	.427	T>C	2.791	.248	A	3.291	.193	A
HTR2A	rs7330636	46321593	13	.364	C>T	FE	.004	D	1.165	.559	A
HTR2A	rs9567739	46322945	13	.374	G>C	1.503	.472	A	0.548	.760	A
HTR2A	rs2296972	46326472	13	.330	G>T	2.802	.246	A	1.020	.600	A
HTR2A	rs9534495	46327229	13	.114	A>G	FE	1.000	A	FE	.200	A
HTR2A	rs9534496	46329109	13	.182	G>C	0.183	.913	A	1.881	.390	A
HTR2A	rs4942578	46330611	13	.264	G>T	0.241	.886	A	0.832	.660	A
HTR2A	rs2770292	46333107	13	.162	C>G	0.071	.965	A	3.327	.189	A
HTR2A	rs1928042	46335217	13	.218	A>C	1.813	.404	A	0.619	.734	A
HTR2A	rs2770293	46336975	13	.376	C>T	3.475	.176	A	9.020	.011	A
HTR2A	rs1328674	46339708	13	.044	G>A	n/a	n/a	n/a	n/a	n/a	n/a
HTR2A	rs2770298	46344848	13	.260	T>C	3.279	.194	A	FE	.029	R
HTR2A	rs1928040	46345237	13	.480	T>C	1.214	.545	A	FE	.045	R
HTR2A	rs972979	46347165	13	.373	G>A	2.619	.270	A	2.376	.305	A

HTR2A	rs731779	46350039	13	.171	T>G	3.285	.193	A	1.915	.384	A
HTR2A	rs2770304	46353366	13	.333	A>G	2.938	.230	A	4.311	.116	A
HTR2A	rs927544	46354052	13	.255	T>C	3.611	.164	A	FE	.006	R
HTR2A	rs594242	46356053	13	.169	C>G	0.164	.921	A	2.866	.239	A
HTR2A	rs4941573	46362858	13	.447	A>G	1.926	.382	A	7.845	.020	A
HTR2A	rs1328684	46364231	13	.314	T>C	0.912	.634	A	3.253	.197	A
HTR2A	rs6304	46364550	13	.010	A>G	n/a	n/a	n/a	n/a	n/a	n/a
HTR2A	rs2296973	46364782	13	.281	G>T	1.919	.383	A	2.545	.280	A
HTR2A	rs2070037	46365071	13	.216	T>C	5.155	.076	A	3.124	.210	A
HTR2A	rs9534511	46366581	13	.445	C>T	1.888	.389	A	FE	.017	D
HTR2A	rs6313	46367941	13	.450	C>T	2.383	.304	A	FE	.023	D
HTR2A	HapA03					4.204	.122		0.386	.824	
HTR2A	HapA07					2.312	.315		3.668	.160	
HTR2A	HapB01					5.229	.073		0.688	.709	
HTR2A	HapB02					9.889	.007		1.327	.515	
HTR2A	HapB03					2.791	.248		3.291	.193	
HTR2A	HapC01					1.157	.561		0.269	.874	
HTR2A	HapC05					3.219	.200		1.200	.549	
HTR2A	HapD01					0.241	.886		0.832	.660	
HTR2A	HapD02					3.623	.163		3.461	.177	
HTR2A	HapE01					1.813	.404		0.619	.734	
HTR2A	HapF01					1.331	.514		5.503	.064	
HTR2A	HapF02					0.914	.633		0.491	.782	
HTR2A	HapF03					3.279	.194		5.936	.051	
HTR2A	HapG01					3.637	.162		2.319	.314	
HTR2A	HapH01					1.470	.479		6.990	.030	
HTR2A	HapH06					3.216	.200		8.455	.015	
HTR2A	HapI01					1.903	.386		6.558	.038	
5-HYDROXYTRYPTAMINE RECEPTOR 3A											
HTR3A	rs1985242	113353483	11	.370	T>A	FE	.009	R	FE	.010	R
HTR3A	rs11214796	113359889	11	.261	T>C	FE	.032	R	1.930	.381	A
HTR3A	rs10160548	113361891	11	.378	T>G	4.999	.082	A	1.587	.452	A
HTR3A	HapA01					7.137	.028		6.565	.038	

HTR3A	HapA04					4.976	.083		2.015	.365	
NITRIC OXIDE SYNTHASE 1											
NOS1	rs2682826	116137221	12	.311	C>T	5.573	.062	A	0.756	.685	A
NOS1	rs816361	116139514	12	.318	C>G	4.711	.095	A	0.827	.661	A
NOS1	rs816363	116144850	12	.458	C>G	1.142	.565	A	0.198	.906	A
NOS1	rs9658498	116152908	12	.409	T>C	1.108	.575	A	0.313	.855	A
NOS1	rs1353939	116159736	12	.261	G>A	4.889	.087	A	0.840	.657	A
NOS1	rs1047735	116169653	12	.346	C>T	2.796	.247	A	1.859	.395	A
NOS1	rs12829185	116178403	12	.243	C>T	8.762	.013	A	1.795	.407	A
NOS1	rs2293054	116186097	12	.299	G>A	0.131	.936	A	2.112	.348	A
NOS1	rs6490121	116192578	12	.364	A>G	0.182	.913	A	0.976	.614	A
NOS1	rs2293052	116200003	12	.358	C>T	4.012	.135	A	1.895	.388	A
NOS1	rs7977109	116214723	12	.418	A>G	0.477	.788	A	1.511	.470	A
NOS1	rs3782206	116229472	12	.116	C>T	0.618	.734	A	0.824	.662	A
NOS1	rs7295972	116231751	12	.445	G>A	1.907	.385	A	2.161	.339	A
NOS1	rs11068447	116232070	12	.124	C>T	0.314	.855	A	0.701	.704	A
NOS1	rs547954	116238889	12	.206	C>T	3.646	.162	A	3.820	.148	A
NOS1	rs3782212	116239785	12	.270	C>T	FE	.025	R	2.558	.278	A
NOS1	rs12578547	116247730	12	.266	T>C	0.945	.623	A	3.361	.186	A
NOS1	rs471871	116249901	12	.246	A>T	3.447	.178	A	3.073	.215	A
NOS1	rs545654	116261432	12	.496	T=C	1.015	.602	A	0.800	.670	A
NOS1	rs1552227	116263418	12	.257	C>T	0.557	.757	A	2.030	.362	A
NOS1	rs10507279	116264657	12	.122	G>A	0.987	.610	A	2.338	.311	A
NOS1	rs693534	116269101	12	.382	G>A	1.558	.459	A	2.907	.234	A
NOS1	rs1123425	116270488	12	.439	A>G	0.688	.709	A	1.587	.452	A
NOS1	rs3782221	116280264	12	.270	G>A	0.132	.936	A	0.629	.730	A
NOS1	HapA02					0.838	.658		0.031	.985	
NOS1	HapA04					5.473	.065		0.483	.785	
NOS1	HapB02					4.889	.087		0.840	.657	
NOS1	HapB03					1.108	.575		0.313	.855	
NOS1	HapC01					2.796	.247		1.859	.395	
NOS1	HapC03					8.762	.013		1.795	.407	
NOS1	HapD01					3.571	.168		1.497	.473	

NOS1	HapD02					1.735	.420		4.142	.126	
NOS1	HapD03					0.484	.785		1.711	.425	
NOS1	HapE01					2.155	.340		1.382	.501	
NOS1	HapE03					1.907	.385		2.161	.339	
NOS1	HapF01					0.528	.768		0.542	.763	
NOS1	HapF02					0.283	.868		0.611	.737	
NOS1	HapF04					1.192	.551		0.934	.627	
NOS1	HapF06					1.099	.577		3.170	.205	
NITRIC OXIDE SYNTHASE 2											
NOS2A	rs9906835	23113501	17	.413	A>G	1.803	.406	A	0.569	.752	A
NOS2A	rs2297512	23116682	17	.385	A>G	1.515	.469	A	FE	.014	D
NOS2A	rs2297516	23119857	17	.416	A>C	1.437	.488	A	0.355	.837	A
NOS2A	rs2297518	23120724	17	.145	G>A	0.016	.992	A	2.650	.266	A
NOS2A	rs2248814	23124448	17	.393	G>A	0.486	.784	A	FE	.004	D
NOS2A	rs1137933	23130059	17	.170	C>T	1.123	.570	A	3.410	.182	A
NOS2A	rs4795067	23130802	17	.278	A>G	0.031	.985	A	0.628	.731	A
NOS2A	rs3729508	23133157	17	.422	G>A	1.788	.409	A	3.684	.158	A
NOS2A	rs944725	23133698	17	.382	C>T	3.189	.203	A	3.495	.174	A
NOS2A	rs3730013	23150045	17	.342	C>T	1.546	.462	A	1.135	.567	A
NOS2A	rs10459953	23151645	17	.366	G>C	1.084	.582	A	1.744	.418	A
NOS2A	rs2779248	23151959	17	.347	T>C	0.721	.697	A	2.300	.317	A
NOS2A	HapA01					1.261	.532		0.295	.863	
NOS2A	HapA04					1.433	.488		6.802	.033	
NOS2A	HapB01					6.528	.038		2.552	.279	
NOS2A	HapB02					1.827	.401		4.114	.128	
NOS2A	HapC01					1.726	.422		0.617	.734	
NOS2A	HapC02					1.013	.603		1.733	.420	
NOS2A	HapC03					1.682	.431		1.153	.562	
NEUROPEPTIDE Y											
NPY	rs16148	24288863	7	.424	T>C	1.199	.549	A	FE	.012	D
NPY	rs16147	24289935	7	.496	A>G	0.353	.838	A	0.090	.056	A
NPY	rs16478	24291133	7	.290	C>T	1.712	.425	A	3.743	.154	A
NPY	rs16139	24291404	7	.029	A>G	n/a	n/a	n/a	n/a	n/a	n/a

NPY	rs1468271	24293506	7	.027	A>G	n/a	n/a	n/a	n/a	n/a	n/a
NPY	rs5574	24295658	7	.429	C>T	0.486	.784	A	2.268	.322	A
NPY	HapA01					0.573	.751		1.785	.410	
NPY	HapA04					1.131	.568		5.149	.076	
NPY	HapA05					1.712	.425		3.743	.154	
NEUROPEPTIDE Y RECEPTOR Y1											
NPYR1	rs9764	164464855	4	.282	T>C	2.710	.258	A	2.661	.264	A
NPYR1	rs7687423	164470247	4	.410	G>A	2.864	.239	A	3.150	.207	A
NPYR1	HapA01					2.710	.258		3.018	.221	
NPYR1	HapA04					2.864	.239		2.868	.238	
PRODYNORPHIN											
PDYN	rs6045868	1915278	20	.334	G>A	0.168	.919	A	1.762	.414	A
PDYN	rs2235751	1917934	20	.361	G>A	1.079	.583	A	FE	.001	D
SOLUTE CARRIER FAMILY 6 MEMBER 1 – GABA TRANSPORTER											
SLC6A1	rs2697149	11011480	3	.221	T>G	1.696	.428	A	0.865	.649	A
SLC6A1	rs2601126	11011624	3	.407	C>T	0.230	.892	A	FE	.017	D
SLC6A1	rs1710885	11013807	3	.192	T>C	0.824	.662	A	0.851	.653	A
SLC6A1	rs1710886	11014655	3	.333	G>C	2.664	.264	A	0.507	.776	A
SLC6A1	rs1710887	11014960	3	.395	G>T	0.915	.633	A	1.784	.410	A
SLC6A1	rs9990174	11015439	3	.326	G>T	2.544	.280	A	2.783	.249	A
SLC6A1	rs1568072	11016606	3	.220	C>T	0.392	.822	A	0.980	.613	A
SLC6A1	rs1728811	11016870	3	.426	C>T	1.676	.433	A	0.846	.655	A
SLC6A1	rs11718132	11020020	3	.134	G>T	1.200	.549	A	3.454	.178	A
SLC6A1	rs2697144	11026099	3	.251	A>G	2.716	.257	A	FE	.037	R
SLC6A1	rs2928079	11030114	3	.425	A>T	1.742	.419	A	2.795	.247	A
SLC6A1	rs1170695	11030338	3	.309	T>C	1.485	.476	A	0.123	.940	A
SLC6A1	rs2933308	11030624	3	.366	G>A	1.432	.489	A	4.429	.109	A
SLC6A1	rs10510403	11041670	3	.141	A>G	2.202	.333	A	3.568	.168	A
SLC6A1	rs2675163	11050014	3	.231	T>C	1.710	.425	A	3.592	.166	A
SLC6A1	rs10514669	11050912	3	.194	C>T	0.460	.795	A	0.193	.908	A
SLC6A1	rs2697138	11051907	3	.145	C>A	3.625	.163	A	0.702	.704	A
SLC6A1	rs1062246	11055169	3	.417	A>G	1.805	.405	A	1.302	.522	A
SLC6A1	HapA01					0.117	.943		6.580	.037	

SLC6A1	HapA02					0.183	.912		8.465	.015	
SLC6A1	HapA04					2.022	.364		1.125	.570	
SLC6A1	HapB01					1.592	.451		0.928	.629	
SLC6A1	HapB03					1.676	.433		0.846	.655	
SLC6A1	HapC01					1.432	.489		4.429	.109	
SLC6A1	HapC02					0.287	.866		0.794	.672	
SLC6A1	HapC03					1.485	.476		0.123	.940	
SLC6A1	HapD01					0.995	.608		1.445	.485	
SLC6A1	HapD02					1.421	.491		0.720	.698	
SOLUTE CARRIER FAMILY 6 MEMBER 2 – NORADRENALINE TRANSPORTER											
SLC6A2	rs2242446	54247926	16	.242	T>C	2.772	.250	A	0.437	.804	A
SLC6A2	rs17841327	54251754	16	.321	C>A	0.772	.680	A	1.312	.519	A
SLC6A2	rs3785143	54252607	16	.087	C>T	2.416	.299	A	1.597	.450	A
SLC6A2	rs192303	54257725	16	.291	G>C	5.949	.051	A	2.290	.318	A
SLC6A2	rs6499771	54258172	16	.155	A>G	1.793	.408	A	1.944	.378	A
SLC6A2	rs36027	54260281	16	.439	A>G	1.584	.453	A	0.654	.721	A
SLC6A2	rs36024	54263892	16	.403	C>T	1.386	.500	A	0.966	.617	A
SLC6A2	rs36021	54269451	16	.416	T>A	0.902	.637	A	1.466	.480	A
SLC6A2	rs40147	54274341	16	.323	C>T	1.048	.592	A	1.097	.578	A
SLC6A2	rs1814270	54274578	16	.404	T>C	FE	.001	R	FE	.046	R
SLC6A2	rs36017	54276319	16	.438	C>G	3.849	.146	A	FE	.003	R
SLC6A2	rs3785155	54279891	16	.138	G>A	4.249	.119	A	2.932	.231	A
SLC6A2	rs47958	54283963	16	.433	C>A	3.562	.168	A	5.500	.064	A
SLC6A2	rs5568	54287625	16	.315	A>C	2.086	.352	A	2.899	.235	A
SLC6A2	rs1566652	54289076	16	.321	G>T	5.460	.065	A	FE	.011	R
SLC6A2	rs5569	54289336	16	.303	C>T	1.584	.453	A	4.325	.115	A
SLC6A2	rs998424	54289447	16	.303	C>T	2.184	.336	A	3.595	.166	A
SLC6A2	HapA01					0.844	.656		1.588	.452	
SLC6A2	HapC01					0.028	.986		1.846	.397	
SLC6A2	HapC10					2.818	.244		5.049	.080	
SLC6A2	HapD01					1.642	.440		4.325	.115	
SLC6A2	HapD04					2.022	.364		3.370	.185	
SOLUTE CARRIER FAMILY 6 MEMBER 3 – DOPAMINE TRANSPORTER											

SLC6A3	rs3863145	1445711	5	.219	C>T	1.484	.476	A	3.859	.145	A
SLC6A3	rs40184	1448077	5	.419	G>A	2.643	.267	A	2.416	.299	A
SLC6A3	rs11564773	1449813	5	.052	A>G	FE	1.000	A	FE	.832	A
SLC6A3	rs6876225	1459036	5	.035	C>A	n/a	n/a	n/a	n/a	n/a	n/a
SLC6A3	rs6347	1464412	5	.265	A>G	0.509	.775	A	1.233	.540	A
SLC6A3	rs37022	1468629	5	.216	T>A	0.181	.914	A	1.091	.579	A
SLC6A3	rs2975292	1472932	5	.447	C>G	1.127	.569	A	0.571	.752	A
SLC6A3	rs11564758	1473588	5	.323	G>C	0.693	.707	A	0.708	.702	A
SLC6A3	rs464049	1476905	5	.465	T>C	1.094	.579	A	0.619	.734	A
SLC6A3	rs10053602	1481135	5	.213	T>C	1.810	.404	A	0.924	.630	A
SLC6A3	rs463379	1484164	5	.253	C>G	0.602	.740	A	0.168	.920	A
SLC6A3	rs403636	1491354	5	.207	G>T	0.798	.671	A	1.079	.583	A
SLC6A3	rs6350	1496199	5	.060	C>T	FE	.830	A	FE	.537	A
SLC6A3	rs2937639	1496728	5	.471	G>A	0.954	.621	A	1.374	.503	A
SLC6A3	HapA01					0.638	.727		0.472	.790	
SLC6A3	HapA07					0.621	.733		0.326	.850	
SLC6A3	HapA09					1.719	.423		2.061	.357	
SLC6A3	HapA10					0.598	.741		0.743	.690	
SOLUTE CARRIER FAMILY 6 MEMBER 4 – SEROTONIN TRANSPORTER											
SLC6A4	rs3813034	25548930	17	.476	A>C	1.468	.480	A	5.742	.057	A
SLC6A4	rs1042173	25549137	17	.478	T>G	1.468	.480	A	5.197	.074	A
SLC6A4	rs4325622	25550601	17	.473	T>C	1.704	.427	A	6.351	.042	A
SLC6A4	rs3794808	25555919	17	.469	G>A	2.040	.361	A	2.562	.278	A
SLC6A4	rs140701	25562658	17	.464	G>A	1.538	.463	A	1.538	.463	A
SLC6A4	rs140700	25567515	17	.089	G>A	1.759	.415	A	FE	.732	A
SLC6A4	rs2020942	25571040	17	.346	G>A	0.538	.764	A	2.163	.339	A
SLC6A4	rs8076005	25571336	17	.214	A>G	1.073	.585	A	1.121	.571	A
SLC6A4	rs6354	25574024	17	.180	A>C	0.573	.751	A	0.106	.949	A
SLC6A4	rs2066713	25575791	17	.345	C>T	1.388	.500	A	2.195	.334	A
SLC6A4	HapA01					3.153	.207		3.665	.160	
SLC6A4	HapA11					0.116	.944		1.830	.401	
SLC6A4	HapB01					2.629	.269		4.513	.105	
SLC6A4	HapB04					0.458	.795		1.985	.371	

TACHYKININ PRECURSOR 1											
TAC1	rs7793277	97197521	7	.267	C>G	1.545	.462	A	3.818	.148	A
TAC1	rs2072100	97199720	7	.476	A>G	1.447	.485	A	0.923	.630	A
TAC1	rs1229434	97203778	7	.429	A>G	2.319	.314	A	0.364	.834	A
TAC1	rs4526299	97205565	7	.195	C>T	2.367	.306	A	1.039	.595	A
TAC1	HapA01					2.319	.314		0.364	.834	
TAC1	HapA05					2.703	.259		1.328	.515	
TAC1	HapA06					1.502	.472		3.723	.155	
TACHYKININ RECEPTOR 1											
TACR1	rs1106855	75131495	2	.243	G>A	3.903	.142	A	FE	.014	R
TACR1	rs4439987	75140614	2	.385	A>G	5.526	.063	A	FE	.030	D
TACR1	rs11688000	75146665	2	.390	A>G	2.122	.346	A	4.879	.087	A
TACR1	rs6546952	75155271	2	.399	T>C	1.788	.409	A	3.038	.219	A
TACR1	rs17564182	75155814	2	.224	C>G	4.318	.115	A	0.899	.638	A
TACR1	rs3771810	75161161	2	.167	T>C	0.302	.860	A	FE	.025	R
TACR1	rs34242711	75174688	2	.199	G>A	0.581	.748	A	0.621	.733	A
TACR1	rs2111378	75208112	2	.315	C>T	1.191	.551	A	0.254	.881	A
TACR1	rs3771825	75208988	2	.197	C>T	1.078	.583	A	4.575	.102	A
TACR1	rs3771827	75215372	2	.453	T>C	n/a	n/a	n/a	n/a	n/a	n/a
TACR1	rs741418	75216694	2	.440	A>G	0.026	.987	A	2.969	.227	A
TACR1	rs9808455	75223077	2	.479	T>C	0.058	.971	A	1.655	.437	A
TACR1	rs3771836	75234460	2	.484	T>G	0.690	.708	A	0.999	.607	A
TACR1	rs759588	75238057	2	.378	C>T	0.653	.721	A	FE	.038	R
TACR1	rs3821318	75240819	2	.458	C>T	2.531	.282	A	FE	.026	D
TACR1	rs6733933	75241342	2	.189	A>G	0.695	.706	A	0.778	.678	A
TACR1	rs13428269	75249287	2	.169	C>T	0.115	.944	A	8.232	.016	A
TACR1	rs3771853	75255122	2	.407	C>T	2.564	.278	A	1.597	.450	A
TACR1	rs12477554	75255573	2	.462	G>A	2.719	.257	A	4.255	.119	A
TACR1	rs4853116	75264786	2	.334	A>G	0.370	.831	A	0.086	.958	A
TACR1	rs3821320	75267600	2	.410	A>G	0.590	.744	A	FE	.020	R
TACR1	rs4853119	75269804	2	.229	T>C	2.417	.299	A	1.767	.413	A
TACR1	rs3771863	75273222	2	.195	C>T	0.358	.836	A	2.725	.256	A
TACR1	HapA01					0.125	.939		0.266	.876	

TACR1	HapA04					1.788	.409		3.020	.221	
TACR1	HapB01					1.191	.551		0.254	.881	
TACR1	HapB02					0.422	.810		4.145	.126	
TACR1	HapB03					1.078	.583		4.575	.102	
TACR1	HapC01					0.026	.987		2.399	.301	
TACR1	HapC04					0.058	.971		2.049	.359	
TACR1	HapD03					1.886	.389		6.006	.050	
TACR1	HapD05					0.653	.721		5.229	.073	
TACR1	HapE01					3.155	.206		4.303	.116	
TACR1	HapE04					2.397	.302		2.436	.296	
TYROSINE HYDROXYLASE											
TH	rs2070762	2142911	11	.500	T>C	FE	.032	D	1.573	.456	A
TH	rs6357	2144814	11	.243	G>A	4.028	.133	A	2.107	.349	A
TH	rs6356	2147527	11	.403	G>A	1.743	.418	A	0.939	.625	A
TH	HapA01					2.214	.331		1.218	.544	
TH	HapA02					0.601	.741		4.530	.104	
TH	HapA04					FE	.083		FE	.284	
TRYTOPHAN HYDROXYLASE 2											
TPH2	rs11179000	70624895	12	.268	A>T	2.633	.268	A	FE	.046	D
TPH2	rs7955501	70636293	12	.357	A>T	1.077	.584	A	3.144	.208	A
TPH2	rs1487275	70696559	12	.259	T>G	3.434	.180	A	2.011	.366	A

A = additive model, ABCB = ATP-binding cassette, subfamily B (MDR/TAP) member 1, ADRA1D = adrenergic, alpha-1D receptor, ADRA2A = adrenergic, alpha-2A receptor, ADRB2 = adrenergic, beta-2 receptor, surface, ADRB3 = adrenergic, beta 3 receptor, ADRBK2 = adrenergic, beta, receptor kinase 2, BDNF = brain derived neurotrophic factor, Chr = chromosome, COMT = catechol-O-methyltransferase, CYP3A4 = cytochrome P450, family 3, subfamily A, polypeptide 4, D = dominant model, FE = Fisher's Exact GAL = galanin, GALR1 = galanin receptor 1, GALR2 = galanin receptor 2, GCH1 = GTP cyclohydrolase 1, Hap = haplotype, HTR1A = 5-hydroxytryptamine receptor 1A, G protein coupled, HTR1B = 5-hydroxytryptamine receptor 1B, G protein coupled, HTR2A = 5-hydroxytryptamine receptor 2A, G protein coupled, HTR3A = 5-hydroxytryptamine receptor 3A, ionotropic, MAF = minor allele frequency, n/a = not assayed because SNP violated Hardy-Weinberg expectations (p<.001) or because MAF was <.05, NOS1 = nitric oxide synthase 1, NOS2A = nitric oxide synthase 2, inducible, NPY = neuropeptide Y, NPYR1 = neuropeptide Y receptor Y1, PDYN = prodynorphin; R = recessive model, SLC6A1 = solute carrier family 6 (neurotransmitter transporter, GABA) member 1, SLC6A2 = solute carrier family 6 (neurotransmitter transporter, noradrenaline) member 2, SLC6A3 = solute carrier family 6 (neurotransmitter transporter, dopamine) member 3, SLC6A4 = solute carrier family 6 (neurotransmitter transporter, serotonin) member 4, SNP= single nucleotide polymorphism, TAC = tachykinin, precursor 1, TACR1 = tachykinin receptor 1, TH = tyrosine hydroxylase, TPH2 = tryptophan hydroxylase 2

Table 2 - Differences in Demographic and Clinical Characteristics Between the No Pain (n=164) and Mild Arm (n=93) Pain Classes Prior to Surgery

Demographic Characteristics	No Pain n=164 Mean (SD)	Mild Pain n=93 Mean (SD)	Statistics
Age (years)	58.0 (12.1)	52.7 (9.7)	t=3.84; p<.0001
Education (years)	15.6 (2.6)	16.3 (2.7)	t=-2.00; p=.046
	% (N)	% (N)	
Ethnicity			
White	75.5 (123)	68.8 (64)	
Black	4.3 (7)	7.5 (7)	x^2=2.83; p=.419
Asian/Pacific Islander	9.2 (15)	14.0 (13)	
Hispanic/mixed ethnic background/other	11.0 (18)	9.7 (9)	
Lives alone	25.3 (41)	19.4 (18)	FE; p=.355
Marital status			
Married/partnered	43.2 (70)	35.5 (33)	FE; p=.236
Single/separated/widowed/divorced	56.8 (92)	64.5 (60)	
Currently working for pay	49.4 (80)	53.3 (49)	FE; p=.602
Total annual household income			
< $30,000	15.4 (21)	18.1 (15)	x^2=1.80; p=.407
$30,000 to $99,000	44.1 (60)	34.9 (29)	
≥ $100,000	40.4 (55)	47.0 (39)	
Clinical Characteristics	Mean (SD)	Mean (SD)	
Body mass index (kg/m^2)	26.1 (5.2)	26.3 (6.7)	t=-0.38; p=.701
Karnofsky Performance Status score	96.7 (6.8)	93.1 (10.0)	t=3.12; p=.002
Self-Administered Comorbidity Scale score	3.9 (2.7)	3.8 (2.3)	t=0.42; p=.677
Number of breast biopsies	1.3 (0.6)	1.6 (0.9)	U; p=.007
	% (N)	% (N)	
Occurrence of comorbid conditions (% and number of women who reported each comorbid condition from the Self-Administered Comorbidity Questionnaire)			
Heart disease	4.3 (7)	3.2 (3)	FE; p=1.000
High blood pressure	35.4 (58)	22.6 (21)	FE; p=.036
Lung disease	1.8 (3)	2.2 (2)	FE; p=1.000
Diabetes	5.5 (9)	6.5 (6)	FE; p=.786
Ulcer	2.4 (4)	5.4 (5)	FE; p=.291
Kidney disease	0.6 (1)	0.0 (0)	FE; p=1.000
Liver disease	1.2 (2)	3.2 (3)	FE; p=.356
Anemia	4.9 (8)	7.5 (7)	FE; p=.414
Depression	22.0 (36)	14.0 (13)	FE; p=.138
Osteoarthritis	20.1 (33)	12.9 (12)	FE; p=.173
Back pain	24.4 (40)	24.7 (23)	FE; p=1.000
Rheumatoid arthritis	2.4 (4)	1.1 (1)	FE; p=.656
Diagnosed with mastitis	15.4 (25)	10.9 (10)	FE; p=.349
Diagnosed with fibrocystic disease	17.2 (27)	22.8 (21)	FE; p=.319
Ever breast fed	54.0 (88)	43.0 (40)	FE; p=.119
Surgery to affected breast unrelated to cancer	11.0 (18)	10.8 (10)	FE; p=1.000
Surgery to affected arm unrelated to cancer	4.3 (7)	1.1 (1)	FE; p=.265
Post-menopausal	69.6 (112)	56.7 (51)	FE; p=.053
Received neoadjuvant chemotherapy	8.0 (13)	23.7 (22)	FE; p=.001
On hormonal replacement therapy prior to surgery	22.1 (36)	12.9 (12)	FE; p=.095

Stage of disease Stage 0 Stage 1 Stage IIA and IIB Stage IIIA, IIIB, IIIC, and IV	 24.4 (40) 45.1 (74) 28.7 (47) 1.8 (3)	 18.3 (17) 34.4 (32) 38.7 (36) 8.6 (8)	U; p=.008
Pain in breast prior to surgery	15.0 (24)	35.2 (32)	FE; p<.0001
Swelling in affected breast	4.3 (7)	5.4 (5)	FE; p=.761
Numbness in affected breast	1.8 (3)	4.3 (4)	FE; p=.258
Strange sensations in affected breast	20.1 (33)	34.4 (32)	FE; p=.016
Hardness in affected breast	14.0 (23)	16.1 (15)	FE; p=.715
Surgical Characteristics	Mean (SD)	Mean (SD)	
Number of lymph nodes removed	3.3 (4.6)	6.6 (5.9)	t=-4.53; p<.0001
Number of drains placed during surgery	0.3 (0.6)	0.5 (0.7)	t=-2.43; p=.016
	% (N)	% (N)	
Type of surgery Breast conserving Mastectomy	 86.0 (141) 14.0 (23)	 79.6 (74) 20.4 (19)	FE; p=.219
Sentinel lymph node biopsy	79.9 (131)	86.0 (80)	FE; p=.240
Axillary lymph node dissection	19.6 (32)	47.3 (44)	FE; p<.0001
Intercostobrachial nerve sacrificed	0.6 (1)	3.2 (3)	x^2=2.80; p=.246
Reconstruction at the time of surgery	20.7 (34)	20.7 (19)	FE; p=1.000
Placement of surgical drain No drain Only in the breast Only in the axilla Both in the breast and axilla	 75.0 (123) 17.7 (29) 6.7 (11) 0.6 (1)	 57.0 (53) 16.1 (15) 20.4 (19) 6.5 (6)	x^2=19.91; p<.0001
Postoperative Characteristics	Mean (SD)	Mean (SD)	
Number of postoperative complications	0.2 (0.5)	0.2 (0.4)	t=-0.15; p=.877
Severity of average postoperative pain	3.0 (2.3)	3.7 (2.3)	t=-2.10; p=.037
Severity of worst postoperative pain	4.2 (2.7)	5.0 (2.6)	t=-2.34; p=.020
	% (N)	% (N)	
Received radiation therapy during the 6 months	59.1 (97)	54.8 (51)	FE; p=.514
Received adjuvant chemotherapy during the 6 months	27.4 (45)	38.7 (36)	FE; p=.070
Received hormonal therapy during the 6 months	45.1 (74)	45.2 (42)	FE; p=1.000
Received biological therapy during the 6 months	5.5 (9)	17.2 (16)	FE; p=.004
Received complementary therapy during the 6 months	25.6 (42)	29.0 (27)	FE; p=.561
Received physical therapy during the 6 months	10.4 (17)	12.9 (12)	FE; p=.544
Had breast reconstruction during the 6 months	6.1 (10)	7.5 (7)	FE; p=.795
Had re-excision or mastectomy during the 6 months	24.4 (40)	24.7 (23)	FE; p=1.000

Abbreviations: FE = Fisher's Exact; SD = standard deviation; kg = kilogram; m^2 = meters squared

Table 3 - Differences in Demographic and Clinical Characteristics Between the No Pain (n=164) and Moderate Arm (n=137) Pain Classes Prior to Surgery

Demographic Characteristics	No Pain n=164 Mean (SD)	Moderate Pain n=137 Mean (SD)	Statistics
Age (years)	58.0 (12.1)	52.9 (11.3)	t=3.74; p<.0001
Education (years)	15.6 (2.6)	15.3 (2.7)	t=0.88; p=.378
	% (N)	% (N)	
Ethnicity			
White	75.5 (123)	50.0 (68)	x^2=25.63; p<.0001
Black	4.3 (7)	19.1 (26)	
Asian/Pacific Islander	9.2 (15)	14.0 (19)	
Hispanic/mixed ethnic background/other	11.0 (18)	16.9 (23)	
Lives alone	25.3 (41)	24.6 (33)	FE; p=1.000
Marital status			
Married/partnered	43.2 (70)	43.0 (58)	FE; p=1.000
Single/separated/widowed/divorced	56.8 (92)	57.0 (77)	
Currently working for pay	49.4 (80)	43.1 (59)	FE; p=.296
Total annual household income			
< $30,000	15.4 (21)	29.9 (32)	x^2=8.44; p=.015
$30,000 to $99,000	44.1 (60)	42.1 (45)	
≥ $100,000	40.4 (55)	28.0 (30)	
Clinical Characteristics	Mean (SD)	Mean (SD)	
Body mass index (kg/m^2)	26.1 (5.2)	28.1 (7.0)	t=-2.79; p=.006
Karnofsky Performance Status score	96.7 (6.8)	89.3 (12.4)	t=6.27; p<.0001
Self-Administered Comorbidity Scale score	3.9 (2.7)	5.0 (3.1)	t=-3.09; p=.002
Number of breast biopsies	1.3 (0.6)	1.6 (0.9)	U; p=.002
	% (N)	% (N)	
Occurrence of comorbid conditions (% and number of women who reported each comorbid condition from the Self-Administered Comorbidity Questionnaire)			
Heart disease	4.3 (7)	3.6 (5)	FE; p=1.000
High blood pressure	35.4 (58)	31.4 (43)	FE; p=.540
Lung disease	1.8 (3)	4.4 (6)	FE; p=.309
Diabetes	5.5 (9)	11.7 (16)	FE; p=.061
Ulcer	2.4 (4)	4.4 (6)	FE; p=.521
Kidney disease	0.6 (1)	1.5 (2)	FE; p=.593
Liver disease	1.2 (2)	2.9 (4)	FE; p=.417
Anemia	4.9 (8)	11.7 (16)	FE; p=.034
Depression	22.0 (36)	27.0 (37)	FE; p=.345
Osteoarthritis	20.1 (33)	17.5 (24)	FE; p=.658
Back pain	24.4 (40)	34.3 (47)	FE; p=.074
Rheumatoid arthritis	2.4 (4)	5.8 (8)	FE; p=.150
Diagnosed with mastitis	15.4 (25)	8.9 (12)	FE; p=.063

Diagnosed with fibrocystic disease	17.2 (27)	18.3 (24)	FE; p=.877
Ever breast fed	54.0 (88)	41.6 (57)	FE; p=.037
Surgery to affected breast unrelated to cancer	11.0 (18)	9.5 (13)	FE; p=.707
Surgery to affected arm unrelated to cancer	4.3 (7)	4.4 (6)	FE; p=1.000
Post-menopausal	69.6 (112)	62.9 (83)	FE; p=.263
Received neoadjuvant chemotherapy	8.0 (13)	31.4 (43)	FE; p=.000
On hormonal replacement therapy prior to surgery	22.1 (36)	14.0 (19)	FE; p=.074
Stage of disease Stage 0 Stage 1 Stage IIA and IIB Stage IIIA, IIIB, IIIC, and IV	 24.4 (40) 45.1 (74) 28.7 (47) 1.8 (3)	 11.7 (16) 32.1 (44) 40.9 (56) 15.3 (21)	U; p<.0001
Pain in breast prior to surgery	15.0 (24)	38.5 (52)	FE; p<.0001
Swelling in affected breast	4.3 (7)	13.9 (19)	FE; p=.004
Numbness in affected breast	1.8 (3)	6.6 (9)	FE; p=.042
Strange sensations in affected breast	20.1 (33)	26.3 (36)	FE; p=.218
Hardness in affected breast	14.0 (23)	24.1 (33)	FE; p=.037
Surgical Characteristics	Mean (SD)	Mean (SD)	
Number of lymph nodes removed	3.3 (4.6)	8.0 (8.2)	t=-5.94; p<.0001
Number of drains placed during surgery	0.3 (0.6)	0.7 (0.8)	t=5.06; p<.0001
	% (N)	% (N)	
Type of surgery Breast conserving Mastectomy	 86.0 (141) 14.0 (23)	 74.5 (102) 25.5 (35)	FE; p=.013
Sentinel lymph node biopsy	79.9 (131)	83.9 (115)	FE; p=.374
Axillary lymph node dissection	19.6 (32)	51.1 (70)	FE; p<.0001
Intercostobrachial nerve sacrificed	0.6 (1)	6.6 (9)	x^2=8.49; p=.014
Reconstruction at the time of surgery	20.7 (34)	24.1 (33)	FE; p=.491
Placement of surgical drain No drain Only in the breast Only in the axilla Both in the breast and axilla	 75.0 (123) 17.7 (29) 6.7 (11) 0.6 (1)	 48.9 (67) 13.1 (18) 27.7 (38) 10.2 (14)	x^2=42.15; p<.0001
Postoperative Characteristics	Mean (SD)	Mean (SD)	
Number of postoperative complications	0.2 (0.5)	0.3 (0.6)	t=-2.36; p=.019
Severity of average postoperative pain	3.0 (2.3)	5.0 (2.2)	t=-7.46; p<.0001
Severity of worst postoperative pain	4.2 (2.7)	6.6 (2.4)	t=-7.91; p<.0001

	% (N)	% (N)	
Received radiation therapy during the 6 months	59.1 (97)	54.7 (75)	FE; p=.483
Received adjuvant chemotherapy during the 6 months	27.4 (45)	38.0 (52)	FE; p=.063
Received hormonal therapy during the 6 months	45.1 (74)	38.0 (52)	FE; p=.241
Received biological therapy during the 6 months	5.5 (9)	12.4 (17)	FE; p=.040
Received complementary therapy during the 6 months	25.6 (42)	28.5 (39)	FE; p=.603
Received physical therapy during the 6 months	10.4 (17)	24.8 (34)	FE; p=.001
Had breast reconstruction during the 6 months	6.1 (10)	8.0 (11)	FE; p=.651
Had re-excision or mastectomy during the 6 months	24.4 (40)	33.6 (46)	FE; p=.096

Abbreviations: FE = Fisher's Exact; SD = standard deviation; kg = kilogram; m^2 = meters squared

Table 4 - Multiple Logistic Regression Analyses for Neurotransmitter Genes and None Versus Mild Arm Pain

Predictor	Odds Ratio	Standard Error	95% CI	Z	p-value
BDNF rs11030102	0.36	0.138	0.167, 0.763	-2.66	.008
KPS score	0.62	0.137	0.403, 0.956	-2.16	.031
Preoperative breast pain	3.73	1.476	1.715, 8.098	3.32	.001
ALND	4.60	1.777	2.156, 9.809	3.95	<.0001
Overall model fit: $\chi^2 = 46.82$, $p < .0001$ $R^2 = 0.1795$					
COMT rs4633	0.32	0.144	0.129, 0.773	-2.52	.012
KPS score	0.66	0.142	0.436, 1.011	-1.91	.056
Preoperative breast pain	3.41	1.323	1.592, 7.294	3.16	.002
ALND	4.51	1.743	2.118, 9.623	3.90	<.0001
Overall model fit: $\chi^2 = 45.49$, $p < .0001$ $R^2 = 0.1757$					
HTR2A Haplotype B02	0.49	0.132	0.288, 0.832	-2.64	.008
KPS score	0.62	0.134	0.407, 0.948	-2.21	.027
Preoperative breast pain	3.06	1.197	1.418, 6.587	2.85	.004
ALND	4.67	1.809	2.186, 9.978	3.98	<.0001
Overall model fit: $\chi^2 = 46.77$, $p < .0001$ $R^2 = 0.1793$					
HTR3A rs1985242	0.10	0.061	0.030, 0.331	-3.77	<.0001
KPS score	0.52	0.123	0.323, 0.821	-2.79	.005
Preoperative breast pain	3.84	1.567	1.728, 8.546	3.30	.001
ALND	6.74	2.868	2.927, 15.520	4.48	<.0001
Overall model fit: $\chi^2 = 57.51$, $p < .0001$ $R^2 = 0.2205$					
TH rs2070762	2.39	1.024	1.035, 5.535	2.04	.041
KPS score	0.63	0.133	0.416, 0.953	-2.19	.029
Preoperative breast pain	3.09	1.186	1.453, 6.556	2.93	.003
ALND	4.53	1.732	2.141, 9.584	3.95	<.0001
Overall model fit: $\chi^2 = 43.78$, $p < .0001$ $R^2 = 0.1697$					

Multiple logistic regression analyses of candidate gene associations with no arm pain versus mild arm pain classes (n=196). For each model, the first three principal components identified from the analysis of ancestry informative markers, as well as self-reported race/ethnicity, were retained in all models to adjust for potential confounding due to race/ethnicity (data not shown). For the regression analyses, predictors evaluated in each model included: genotype (BDNF rs11030102: CC versus CG+GG; COMT rs4633: CC+CT versus TT; HTR2A HapB02 composed of the rs1923886 common T allele and the rs7330636 rare T allele; HTR3A rs1985242: TT+TA versus AA; TH rs2070762: TT versus TC+CC), functional status (KPS score in 10 unit increments), pain in the affected breast prior to surgery, and undergoing an axillary lymph node dissection.

Abbreviations: ALND = axillary lymph node dissection; BDNF = brain derived neurotrophic factor; CI = confidence interval; COMT= catechol-O-methyltransferase; Hap = haplotype; HTR2A = 5-hydroxytryptamine receptor 2A, G protein coupled; HTR3A = 5-hydroxytryptamine receptor 3A, ionotropic; KPS = Karnofsky Performance Status; TH = tyrosine hydroxylase

Table 5 - Multiple Logistic Regression Analyses for Neurotransmitter Genes and None Versus Moderate Arm Pain

Predictor	Odds Ratio	Standard Error	95% CI	Z	p-value
BDNF rs2049046	3.07	1.324	1.321, 7.151	2.61	.009
KPS score	0.50	0.116	0.317, 0.789	-2.98	.003
Preoperative breast pain	3.20	1.370	1.386, 7.408	2.72	.006
Number of breast biopsies	1.75	0.430	1.085, 2.837	2.29	.022
Surgical drain placement					
Breast only	0.97	0.476	0.374, 2.536	-0.05	.958
Axilla only	10.68	6.191	3.430, 33.262	4.09	<.0001
Breast and axilla	23.86	27.719	2.449, 232.536	2.73	.006
Any physical therapy	3.22	1.520	1.274, 8.120	2.47	.013
Race/ethnicity					
African American	9.04	12.577	0.591, 138.229	1.58	.114
Asian	2.78	3.918	0.176, 43.945	0.73	.467
Hispanic/mixed/other	5.27	3.684	1.337, 20.747	2.37	.018
Principal components					
PC1	0.98	0.194	0.667, 1.448	-0.09	.930
PC2	0.91	0.154	0.653, 1.269	-0.56	.579
PC3	0.99	0.150	0.736, 1.332	-0.07	.947
Overall model fit: χ^2 = 110.01, p <.0001 R^2 = 0.3672					
COMT rs165656	0.37	0.166	0.153, 0.893	-2.21	.027
KPS score	0.47	0.102	0.305, 0.719	-3.47	.001
Preoperative breast pain	3.83	1.649	1.646, 8.906	3.12	.002
Number of breast biopsies	1.86	0.466	1.141, 3.042	2.49	.013
Surgical drain placement					
Breast only	0.95	0.466	0.360, 2.486	-0.11	.910
Axilla only	10.46	6.067	3.353, 32.605	4.04	<.0001
Breast and axilla	19.44	22.521	2.007, 188.276	2.56	.010
Any physical therapy	2.94	1.408	1.150, 7.518	2.25	.024
Race/ethnicity					
African American	14.32	19.176	1.037, 197.664	1.99	.047
Asian	3.14	4.419	0.200, 49.467	0.81	.416
Hispanic/mixed/other	6.69	4.675	1.699, 26.322	2.72	.007
Principal components					
PC1	0.90	0.168	0.623, 1.295	-0.58	.565
PC2	0.88	0.150	0.630, 1.230	-0.75	.455
PC3	0.94	0.143	0.699, 1.268	-0.39	.693
Overall model fit: χ^2 = 106.70, p <.0001 R^2 = 0.3581					
HTR2A rs2770298	5.08	3.752	1.193, 21.613	2.20	.028
HTR2A rs9534511	1.89	0.513	1.110, 3.217	2.34	.019
KPS score	0.44	0.103	0.281, 0.698	-3.51	<.0001
Preoperative breast pain	4.44	1.972	1.861, 10.602	3.36	.001
Number of breast biopsies	1.84	0.460	1.131, 3.008	2.45	.014
Surgical drain placement					
Breast only	0.90	0.455	0.334, 2.426	-0.21	.835
Axilla only	9.27	5.389	2.965, 28.966	3.83	<.0001
Breast and axilla	18.27	23.297	1.502, 222.344	2.28	.023
Any physical therapy	3.25	1.602	1.239, 8.541	2.39	.017
Race/ethnicity					

African American	10.08	14.822	0.565, 179.827	1.57	.116
Asian	1.19	1.810	0.060, 23.454	0.11	.909
Hispanic/mixed/other	4.62	3.276	1.150, 18.545	2.16	.031
Principal components					
PC1	1.00	0.209	0.666, 1.507	0.01	.995
PC2	0.98	0.180	0.680, 1.401	-0.13	.896
PC3	0.97	0.148	0.716, 1.306	-0.22	.828
Overall model fit: χ^2 = 113.38, p <.0001 R^2 = 0.3800					
HTR3A rs1985242	0.15	0.096	0.046, 0.520	-3.01	.003
KPS score	0.44	0.104	0.280, 0.701	-3.48	.001
Preoperative breast pain	3.76	1.650	1.593, 8.889	3.02	.003
Number of breast biopsies	1.83	0.459	1.117, 2.988	2.40	.016
Surgical drain placement					
Breast only	0.90	0.449	0.340, 2.395	-0.21	.837
Axilla only	13.02	7.738	4.064, 41.733	4.32	<.0001
Breast and axilla	26.33	30.918	2.637, 262.982	2.79	.005
Any physical therapy	2.40	1.159	0.930, 6.183	1.81	.070
Race/ethnicity					
African American	12.78	19.930	0.600, 271.822	1.63	.102
Asian	2.87	3.943	0.193, 42.493	0.77	.444
Hispanic/mixed/other	5.20	3.555	1.361, 19.862	2.41	.016
Principal components					
PC1	1.02	0.232	0.657, 1.596	0.11	.916
PC2	0.90	0.156	0.641, 1.262	-0.61	.539
PC3	1.00	0.154	0.740, 1.351	-0.00	.997
Overall model fit: χ^2 = 114.11, p <.0001 R^2 = 0.3809					
NOS2A rs2248814	0.34	0.136	0.156, 0.746	-2.69	.007
KPS score	0.48	0.111	0.304, 0.753	-3.19	.001
Preoperative breast pain	3.48	1.474	1.514, 7.979	2.94	.003
Number of breast biopsies	1.96	0.487	1.209, 3.192	2.73	.006
Surgical drain placement					
Breast only	1.31	0.656	0.491, 3.495	0.54	.590
Axilla only	12.96	7.567	4.129, 40.701	4.39	<.0001
Breast and axilla	25.33	30.465	2.397, 267.600	2.69	.007
Any physical therapy	2.54	1.235	0.983, 6.590	1.92	.054
Race/ethnicity					
African American	14.13	20.609	0.810, 246.451	1.82	.069
Asian	6.08	8.697	0.369, 100.314	1.26	.207
Hispanic/mixed/other	6.44	4.673	1.553, 26.699	2.57	.010
Principal components					
PC1	0.84	0.176	0.558, 1.270	-0.82	.412
PC2	0.84	0.145	0.600, 1.178	-1.01	.313
PC3	0.87	0.137	0.641, 1.186	-0.87	.384
Overall model fit: χ^2 = 107.95, p <.0001 R^2 = 0.3637					
NPY rs16148	2.70	1.164	1.163, 6.285	2.31	.021
KPS score	0.45	0.105	0.287, 0.712	-3.42	.001
Preoperative breast pain	3.83	1.628	1.662, 8.811	3.15	.002
Number of breast biopsies	2.01	0.501	1.236, 3.279	2.81	.005
Surgical drain placement					
Breast only	1.26	0.623	0.477, 3.318	0.46	.643
Axilla only	12.91	7.616	4.061, 41.026	4.34	<.0001
Breast and axilla	19.99	22.712	2.157, 185.298	2.64	.008
Any physical therapy	2.91	1.400	1.132, 7.469	2.22	.027

Race/ethnicity					
African American	10.82	14.713	0.754, 155.407	1.75	.080
Asian	3.88	5.513	0.240, 62.816	0.95	.340
Hispanic/mixed/other	5.35	3.818	1.319, 21.674	2.35	.019
Principal components					
PC1	0.89	0.171	0.615, 1.301	-0.58	.559
PC2	0.83	0.143	0.589, 1.161	-1.10	.272
PC3	0.93	0.144	0.692, 1.263	-0.44	.661
Overall model fit: χ^2 = 104.52, p <.0001 R^2 = 0.3541					
SLC6A1 rs2601126	3.00	1.341	1.247, 7.202	2.45	.014
KPS score	0.51	0.112	0.334, 0.786	-3.06	.002
Preoperative breast pain	4.19	1.820	1.790, 9.817	3.30	.001
Number of breast biopsies	1.89	0.467	1.164, 3.066	2.57	.010
Surgical drain placement					
Breast only	1.21	0.603	0.455, 3.214	0.38	.720
Axilla only	10.86	6.204	3.544, 33.271	4.17	<.0001
Breast and axilla	35.62	43.298	3.288, 385.826	2.94	.003
Any physical therapy	3.02	1.444	1.184, 7.710	2.31	.021
Race/ethnicity					
African American	13.66	19.225	0.865, 215.612	1.86	.063
Asian	4.18	6.048	0.245, 71.244	0.99	.323
Hispanic/mixed/other	5.55	4.002	1.350, 22.814	2.38	.018
Principal components					
PC1	0.88	0.177	0.594, 1.305	-0.63	.526
PC2	0.89	0.154	0.629, 1.245	-0.70	.483
PC3	0.99	0.152	0.729, 1.334	-0.09	.927
Overall model fit: χ^2 = 109.50, p <.0001 R^2 = 0.3655					
TACR1 rs4439987	0.40	0.163	0.183, 0.891	-2.25	.025
KPS score	0.45	0.100	0.292, 0.695	-3.60	<.0001
Preoperative breast pain	3.77	1.595	1.643, 8.640	3.13	.002
Number of breast biopsies	1.90	0.472	1.164, 3.090	2.57	.010
Surgical drain placement					
Breast only	0.84	0.412	0.319, 2.199	-0.36	.719
Axilla only	9.52	5.461	3.094, 29.303	3.93	<.0001
Breast and axilla	26.04	30.378	2.647, 256.197	2.79	.005
Any physical therapy	3.51	1.716	1.348, 9.152	2.57	.010
Race/ethnicity					
African American	12.14	17.566	0.713, 206.888	1.73	.084
Asian	5.16	7.324	0.320, 83.210	1.16	.247
Hispanic/mixed/other	5.87	3.977	1.553, 22.150	2.61	.009
Principal components					
PC1	0.85	0.177	0.567, 1.279	-0.78	.438
PC2	0.84	0.146	0.601, 1.182	-0.99	.322
PC3	0.99	0.154	0.733, 1.347	-0.04	.968
Overall model fit: χ^2 = 108.20, p <.0001 R^2 = 0.3612					

Multiple logistic regression analyses of candidate gene associations with no arm pain versus moderate arm pain classes (n=218). For each model, the first three principal components identified from the analysis of ancestry informative markers, as well as self-reported race/ethnicity, were retained in all models to adjust for potential confounding due to race/ethnicity. For the regression analyses, predictors evaluated in each model included genotype (BDNF rs2049046: AA+AT versus TT; COMT rs165656: CC+CG versus GG; HTR2A rs2770298: TT+ CT versus CC; HTR2A rs9534511: CC versus CT+TT; HTR3A rs1985242: TT+TA versus AA; NOS2A rs2248814: GG versus GA+AA; NPY rs16148: TT versus

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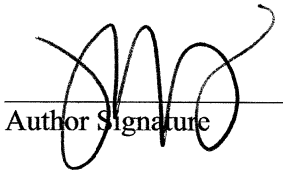

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