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Associations Between Catecholaminergic and Serotonergic Genes and Persistent Arm Pain Severity Following Breast Cancer Surgery

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Abstract

Persistent arm pain is a common problem following breast cancer surgery. Little is known about genetic factors that contribute to this type of postsurgical pain. Study purpose was to explore associations between persistent arm pain phenotypes and genetic polymorphisms among fifteen genes involved in catecholaminergic and serotonergic neurotransmission. Women (n=398) rated the presence and intensity of arm pain monthly for six months following breast cancer surgery. Three distinct latent classes of patients were identified (i.e., No Arm Pain (41.6%), Mild Arm Pain (23.6%), and Moderate Arm Pain (34.8%). Logistic regression analyses were used to evaluate for differences between genotype or haplotype frequencies and the persistent arm pain classes. Compared to the No Arm Pain class, three SNPs and one haplotype, in four genes, were associated with membership in the Mild Arm Pain class: *COMT* rs4633, *HTR2A* haplotype B02 (composed of rs1923886 and rs7330636), *HTR3A* rs1985242, and *TH* rs2070762. Compared to the No Arm Pain class, four SNPs in three genes were associated with membership in the Moderate Arm Pain class: *COMT* rs165656, *HTR2A* rs2770298 and rs9534511, and *HTR3A* rs1985242. Findings suggest that variations in catecholaminergic and serotonergic genes play a role in the development of persistent arm pain.

Keywords

arm pain; persistent pain; post-surgical pain; polymorphisms; catecholaminergic genes; serotonergic genes; breast cancer

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INTRODUCTION

Surgery is the primary treatment for breast cancer. Unfortunately, 25% to 60% of patients will report persistent postsurgical pain following breast cancer surgery.[2,19] This pain usually occurs about twelve weeks post-surgery and is characterized by burning, throbbing, or aching in the ipsilateral chest, axilla, and/or arm. This pain is associated with other breast and arm symptoms, including swelling and weakness. While previous studies have identified various demographic and clinical risk factors,[1,5,31,40,42,57] as well as physiological factors (e.g., genetic variations[9,32,39,64]) associated with the development of persistent pain following breast cancer surgery, its exact etiology remains elusive. Inconsistencies exist in the characterization of this persistent postsurgical pain which hinder our understanding of underlying mechanisms.

Previously, we reported on distinct phenotypic characterizations of persistent breast[42] and arm[43] pain in a sample of 398 women who underwent breast cancer surgery. Using worst breast or arm/shoulder pain severity scores over 6 months, growth mixture modeling (GMM) identified four distinct persistent Breast Pain phenotypes (i.e., No Pain (31.7%), Mild Pain (43.4%), Moderate Pain (13.3%), and Severe Pain (11.6%)) and three distinct persistent Arm Pain phenotypes (i.e., No Pain (41.6%), Mild Pain (23.7%), and Moderate Pain (11.6%)). When these persistent breast and arm pain classes were compared, distinct differences in demographic and clinical characteristics between the two anatomic sites were identified.[30] For example, when compared to breast pain, arm pain was described more similarly to neuropathic pain and showed less variability in patterns of change over time.[30] These findings suggest that persistent arm pain represents a different pain condition from persistent breast pain.

Catecholamines (e.g., dopamine, norepinephrine, epinepherine) and serotonin modulate pain transmission in the peripheral and central nervous systems.[23,58,63,66] Alterations in these neurotransmitters are implicated in the development of persistent pain syndromes.[4,15,50] A number of reviews and meta-analyses have identified significant associations between polymorphisms in catecholaminergic (e.g., catechol-O-methyltransferase *(COMT))* and serotonergic genes (e.g., 5-hydroxytryptamine receptor 2A (*HTR2A*)) and persistent pain syndromes (e.g., chronic postsurgical pain,[22] migraine headaches,[35] fibromyalgia,[33] and lumbar radicular pain[6]). In a previous paper,[28] we reported on a number of polymorphisms in catecholaminergic and serotonergic genes that were associated with our persistent breast pain phenotypes. However, because no studies have evaluated for associations between these two groups of genes and the development of persistent arm pain following breast cancer surgery, in this study we extend our previous work and using an extreme phenotype approach evaluated for associations between our persistent arm pain phenotypes (i.e., No Pain versus Mild Pain and No Pain versus Moderate Pain) and genetic polymorphisms in the same fifteen candidate genes involved in catecholaminergic and serotonergic neurotransmission. We hypothesize that the genetic associations for persistent arm pain will differ from those identified for persistent breast pain.

METHODS

Patients and Settings

This analysis is part of a longitudinal study, funded by the National Cancer Institute, that evaluated for neuropathic pain and lymphedema in a sample of women who underwent breast cancer surgery. The methods used are described in detail elsewhere.[39,42]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 were known to have distant metastasis at the time of diagnosis. A total of 516 patients were approached and 410 enrolled in the study. For this analysis, 398 women completed study questionnaires and 310 provided blood samples for genetic analyses.

Instruments

A demographic questionnaire obtained information on age, education, ethnicity, marital status, employment status, living situation, and financial status. The Karnofsky Performance Status (KPS) scale was used to evaluate patients' functional status.[26,27] The Self-Administered Comorbidity Questionnaire (SCQ) was used to evaluate the occurrence of, treatment for, and impact of 13 common medical conditions.[10,11,37,56,59] Patients were asked to indicate if they exercised on a regular basis (yes/no format).

Upper extremity pain was evaluated using the Arm/Shoulder Symptoms Questionnaire (ASQ) and Postsurgical Pain Questionnaire. 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. 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).[25] The ASQ was completed monthly for six months following surgery. 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 Post-Surgical Pain Questionnaire was completed once 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 a research nurse. The research nurse met with the women, determined eligibility, and obtained written informed consent prior to surgery. After obtaining the 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 arm pain phenotype was described previously.[43] In summary, 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. Patients who reported no pain in their affected arm/shoulder for all 6 assessments (n = 164, 41.6%) were not included in the GMM analysis. These women comprised the "No Pain" group for the current analyses. For the remaining 230 women, six ratings of worst arm/shoulder pain were used in the GMM analysis to assign each patient into a latent class. The GMM analysis was performed using Mplus 6.1.[45]

Gene and SNP Selection

Fifteen candidate genes involved in various aspects of catecholaminergic and serotonergic neurotransmission 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*); SLC family 6 (neurotransmitter transporter, dopamine) member 3 (*SLC6A3*); and tyrosine hydroxylase (*TH).* Genes involved in serotonergic neurotransmission included: 5-hydroxytrypatimeine receptor (HTR)1A (*HTR1A*), *HTR1B, HTR2A, HTR3A*; SLC family 6 (neurotransmitter transporter, serotonin) member 4 (*SLC6A4*); and tryptophan hydroxylase 2 (*TPH2*). All genes were identified according to the approved symbol stored in the Human Genome Organization (HUGO) Gene Nomenclature Committee (HGNC) database (http://www.genenames.org). A combination of tagging single nucleotide polymorphisms (SNPs) and literature driven SNPs for these candidate genes were selected for analysis (Supplementary Table 1). Tagging SNPs were required to be common (i.e., defined as having a minor allele frequency ((MAF) of ≥05) in public databases.

Blood collection and genotype

Genotyping was completed on 310 women. 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 SNP. SNPs with call rates of <95% or

Hardy-Weinberg estimates with p-values of <0.001 were excluded. A total of 126 SNPs among the 15 candidate genes passed all the quality control filters and were included in the genetic analyses (Supplementary Table 1). Localization of SNPs on the human genome was performed using the GRCh37/hg19 human reference assembly. Regional annotations were identified using the University of California Santa Cruz (UCSC) Human Genome Browser GRCh37/hg 19 (http://genome.ucsc.edu/cgibin/hgTracks?db=hg19).

Statistical analyses

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 24 (IBM, Armonk, NY), 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. StataSE version 14 (StataCorp, College Station, TX) was used to conduct the logistic regression analyses to evaluate for associations 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 other 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 for associations between genotype and pain group membership.

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.[18]

For SNPs that were members of the same haploblock, haplotype analyses were conducted 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.[60] 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.[69]

Ancestry informative markers (AIMs) were used to minimize confounding due to population stratification.[20,2162] One hundred and six AIMs were included in the analysis. Homogeneity in ancestry among patients was verified by principal component analysis[51] using Helix Tree (Golden Helix, Bozeman, MT). The first three PCs were used as covariates in the regression analyses to adjust for potential confounding due to population substructure (i.e., race/ethnicity).

Three genetic models were assessed for each SNP (i.e., additive, dominant, recessive). The genetic model that best fit the data 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 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 StataSE version 14. Due to the exploratory nature of this study, a p-value of <.05 was considered significant.

RESULTS

Differences in demographic and clinical characteristics between arm pain classes

Table 1 summarizes the significant differences in demographic and clinical characteristics between the No Arm Pain and Mild Arm Pain classes. Compared to the No Arm Pain class, women in the Mild Arm Pain class were significantly younger, had more education, had a lower KPS score, and were less likely to have high blood pressure. These women had a more advanced stage of disease, had a higher number of breast biopsies, had an axillary lymph node dissection (ALND), and had a greater number of lymph nodes removed. 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 higher average and worst postoperative pain scores. These women were more likely to have had a surgical drain; 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.

Table 2 summarizes the significant differences in demographic and clinical characteristics between the No Arm Pain and the Moderate Arm Pain classes. Compared to the No Arm Pain class, women in the Moderate Arm Pain class were younger, had lower KPS scores and annual household incomes, higher BMI and SCQ scores, and were less likely to be White. A higher percentage of women in this class reported comorbid anemia; were less likely to have breast fed; had more advanced disease; reported breast pain prior to surgery; reported sensations of swelling, numbness, and hardness in the affected breast; had received neoadjuvant chemotherapy; had a higher number of breast biopsies; underwent a mastectomy; had a higher number of lymph nodes removed; had a surgical drain; had a higher number of drains placed; had an ALND; and had the intercostobrachial nerve sacrificed. Women in the Moderate Arm Pain class reported higher average and worst postoperative pain severity scores; were more likely to have had physical therapy and received biological therapy within the six months following surgery; and had more postoperative complications.

Candidate gene analyses: No Arm Pain versus Mild Arm Pain classes

Genotype distributions differed between the No Arm Pain and Mild Arm Pain classes for: 5 SNPs and 2 haplotypes in *COMT*; 3 SNPs and 1 haplotype in *HTR2A*; 2 SNPs and 1 haplotype in *HTR3A*; 1 SNP in *SLC6A2*; and 1 SNP in *TH* (Supplementary Table 1).

Multivariate logistic regression models were fit to determine the phenotypic and genotypic predictors for membership in the Mild Arm Pain class. In addition to self-reported race/

ethnicity and AIMs, the significant covariates included in these analyses were: functional status (KPS), pain in the affected breast prior to surgery, and having had an ALND.

Three SNPs and one haplotype in four different genes remained significant in the multivariate analyses: *COMT* rs4633, *HTR2A* haplotype B02, *HTR3A* rs1985242, and *TH* rs2070762 (Table 3). Figures 1a through 1d illustrate the differences between the No Arm Pain and Mild Arm Pain classes in the percentage of patients who were homozygous for the common allele or heterozygous or homozygous for the rare allele for each of the significant polymorphisms or dose of the haplotype. 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. For *HTR2A* haplotype B02 (composed of rs1923886 [common T allele], rs7330636 [rare T allele]), each additional dose of the haplotype was associated with a 51% decrease in the odds of belonging to the Mild Arm Pain class. 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. For *TH* rs2070762, carrying one or two doses of the rare C allele (i.e., TT versus TC+CC) was associated with a 2.39-fold increase in the odds of belonging to the Mild Arm Pain class.

Candidate gene analyses: No Arm Pain versus Moderate Arm Pain classes

Genotype distributions differed between the No Arm Pain and Moderate Arm Pain classes for: 2 SNPs and 2 haplotypes in *ADRA1D*; 1 SNP in *ADRBK2*; 5 SNPs and 4 haplotypes in *COMT*; 1 SNP in *HTR1A*; 7 SNPs and 3 haplotypes in *HTR2A*; 1 SNP and 1 haplotype in *HTR3A*; 3 SNPs in *SLC6A2*; 1 SNP in *SLC6A4*; and 1 SNP in *TPH2* (Supplementary Table 1).

Multivariate logistic regression models were fit to determine the phenotypic and genotypic predictors for membership in the Moderate Arm Pain class. In addition to self-reported race/ethnicity and AIMs, the significant covariates included in these analyses were: functional status (i.e., KPS), pain in the affected breast prior to surgery, number of breast biopsies in the past year, placement of a surgical drain, and receipt of physical therapy in the six months following surgery.

Four SNPs in three different genes remained significant in the multivariate logistic regression analyses: *COMT* rs165656, *HTR2A* rs2770298 and rs9534511, and *HTR3A* rs1985242 (Table 4). Figures 2a through 2d show the differences between the No Arm Pain and Moderate Arm Pain classes in the percentage of patients who were homozygous for the common allele or heterozygous or homozygous for the rare allele for each of the significant polymorphisms. 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. Two SNPs in *HTR2A* were associated with membership in the Moderate Arm Pain class. For *HTR2A* rs2770298, carrying two doses of the rare G allele (i.e., CC +CG versus GG) was associated with a 5.08-fold increase in the odds of belonging to the Moderate Arm Pain class. 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. 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.

DISCUSSION

This exploratory study evaluated for associations between variations in genes involved in the catacholaminergic and serotonergic pathways and persistent arm pain following breast cancer surgery. Our findings suggest that several genetic variations in these two pathways may play a role in the occurrence and severity of persistent arm pain. A discussion of the differences in demographic and clinical characteristics among the Arm Pain classes was reported in detail elsewhere.[43] This discussion will focus on the genetic findings.

Variations in three genes (i.e., *COMT, HTR2A,* and *HTR3A)* were associated with membership in both the Mild and Moderate Arm Pain classes. COMT is an enzyme that effects the metabolism of epinephrine, norepinephrine, and dopamine.[3,61] In this study, women who were homozygous for the rare T allele in *COMT* rs4633 had a decreased odds of belonging to the Mild Arm Pain class. Located in exon 3 of the *COMT* gene, rs4633 is a nonsynonymous SNP that is implicated in pediatric postoperative pain,[55] pain after a motor vehicle accident,[7] pain associated with lumbar disc disease,[13] pain after lumbar spine surgery,[54] fibromyalgia,[38,65] pain in women with major depressive disorder,[17] and low back pain.[49]

In many studies,[3,7,13,15] rs4633 is evaluated as part of a “pain 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 demonstrated a significant association with the Mild Arm Pain phenotype and the APS and HPS haplotypes demonstrated a significant association with the Moderate Arm Pain phenotype, these associations did not remain significant in the multivariate analyses. Conflicting evidence exists on the associations between various pain conditions and the *COMT* pain haplotype. For example, in one study,[47] no differences in the frequencies of the *COMT* haplotype were found between patients with chronic widespread pain and controls. In addition, the *COMT* haplotype was not associated with experimental pain thresholds in a sample of Chinese men.[68]

Women who were homozygous for the rare G allele in *COMT* rs165656 had a decreased odds of belonging to the Moderate Arm Pain class. Of note, in a small study, *COMT* rs165656, located in the promoter region of the *COMT* gene, was associated with an 80% decrease in the likelihood of having a temporomandibular disorder.[44] Additional research on rs165656 and other polymorphisms in the *COMT* gene may increase our understanding of the role of this gene in persistent arm pain following breast cancer surgery.

One haplotype and two SNPs in *HTR2A* were associated with membership in the Mild or Moderate Arm Pain classes. *HTR2A* encodes for the G protein-coupled serotonin receptor 2A. Normal neurotransmission can be disrupted by variations in the density of this receptor

which alters the activity of serotonergic neurons.[8] Polymorphisms in this gene were associated with persistent breast pain in our previous report[28] and chronic widespread pain,[48] as well as with the regulation of mood,[8] responses to antidepressant treatments,[34,52] and alterations in cognitive function.[41] In the current study, each additional dose of *HTR2A* Haplotype B02, which is composed of two SNPs (i.e., rs1923886 [T common allele], rs7330636 [T rare allele]), was protective of belonging to the Mild Arm Pain class. In contrast, carrying two doses of the rare G allele at rs2770298 and carrying one or two doses of the rare T allele at rs9534511 was associated with increased odds of belonging to the Moderate Arm Pain class. All these SNPs on *HTR2A* are intron variants that have not been associated with other pain phenotypes.

One SNP in the *HTR3A* gene was associated with membership in both the Mild and Moderate Arm Pain classes. *HTR3A* encodes for the subunit A of the type 3 receptor for 5-hydroxytryptamine (serotonin), a receptor that is involved in pain, anxiety, and immunomodulatory processes.[36] These receptors mediate pain transmission in both the peripheral and central nervous systems. After activation, these receptors are responsible for fast and depolarizing responses in neurons.[16,36,46] Our findings suggest that carrying two doses of the rare A allele at *HTR3A* rs1985242 is associated with a decrease in the odds of belonging to Mild and Moderate Arm Pain classes. However, this intronic SNP has not been associated with other pain conditions.

A SNP in the gene that encodes for TH was associated with membership in only the Mild Arm Pain class. TH is the enzyme that converts tyrosine to dopamine. While the enzyme itself is not involved in pain, its effects on dopamine could influence pain mechanisms. For example, endogenous opioids are released in response to a noxious stimulus, which stimulates the release of dopamine.[24] Stimulation of dopamine receptors results in the inhibition of nociception.[24] Our findings suggest that carrying one or two doses of the rare C allele at *TH* rs2070762 is associated with an increased odds of belonging to the Mild Arm Pain class. The C allele of this functional intronic polymorphism likely serves as a functional enhancer element that regulates gene expression.[67] While initial work suggested that this SNP was associated with migraine, this finding was not confirmed in a validation cohort.[12] In another study, this SNP was associated with an increased risk for opioid addiction.[53]

As noted above, our previous work suggests that persistent breast and arm pain following breast cancer surgery represent distinct pain phenotypes.[30,42,43] As summarized in Table 5, findings from our previous genetic association study of catecholaminergic and serotonergic genes and persistent breast pain[28] and findings from the current study support this hypothesis. The only genes that were associated with both persistent breast and arm pain following breast cancer surgery were *HTR2A* and *HTR3A.* In subsequent studies of post-surgical pain, detailed information should be collected for breast pain and for arm pain.

Several study limitations need to be acknowledged. While our sample was adequate in size and representative of breast cancer patients in the United States, our findings warrant replication before any definitive conclusions can be drawn about the genomic findings. Furthermore, additional latent classes and significant gene polymorphisms may be identified

with a larger and more diverse sample. In addition, women were recruited through referrals from twenty surgeons at seven different sites, to enhance generalizability of the study's findings. An evaluation of how surgical and postoperative pain management protocols impact the development of persistent postsurgical pain and genetic associations may increase our understanding of the mechanisms that underlies this persistent pain condition. Finally, variations in catecholaminergic and serotonergic genes are associated with depression[14] and anxiety.[29] Future studies should control for these symptoms in any genomic analyses.

This study is the first prospective, longitudinal study to examine associations between the occurrence and severity of persistent arm pain following breast cancer surgery and catecholaminergic and serotonergic genes. The elucidation of genetic factors that predispose patients to persistent arm pain has the potential to identify high risk patients who warrant more aggressive postoperative pain management. Our findings warrant replication in women with breast cancer and in patients with other persistent postsurgical pain conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Persistent arm pain following breast cancer surgery represents a distinct phenotype
- Distinct polymorphisms are associated with mild versus moderate arm pain
- Serotonergic and catecholaminergic genes are involved in persistent arm pain

PERSPECTIVE

Limited information is available on genetic factors that contribute to persistent arm pain following breast cancer surgery. Genetic polymorphisms in genes involved in catecholaminergic and serotonergic neurotransmission were associated with two persistent arm pain phenotypes. Findings may be used to identify patients are higher risk for this common pain condition.

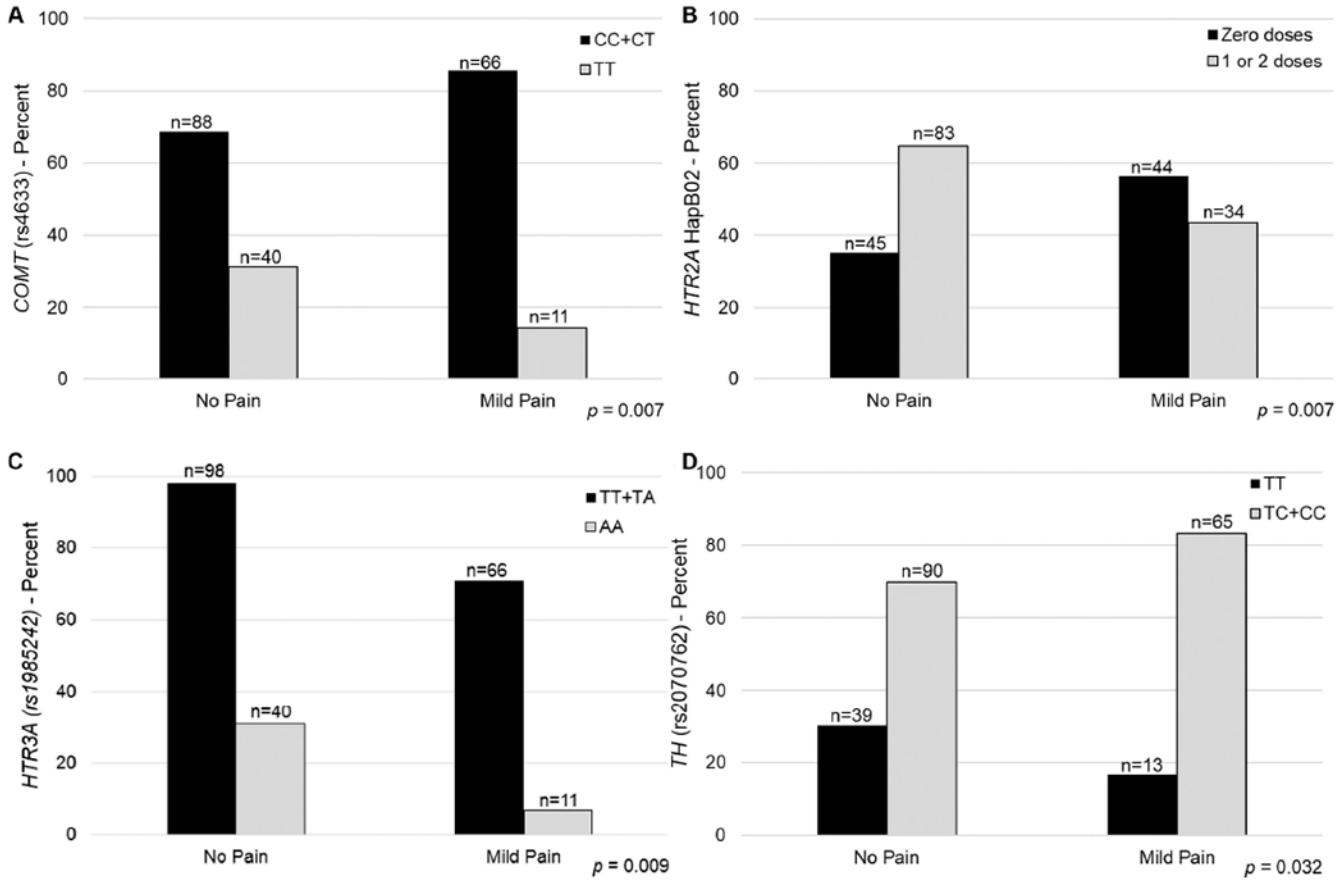

Figure 1.

a through d – Differences between No Arm Pain and Mild Arm Pain classes in the percentage of patients who were homozygous for the common allele or heterozygous or homozygous for the rare allele for each significant polymorphism or number of doses of haplotypes identified. Values are plotted as unadjusted proportions with corresponding *p*-value.

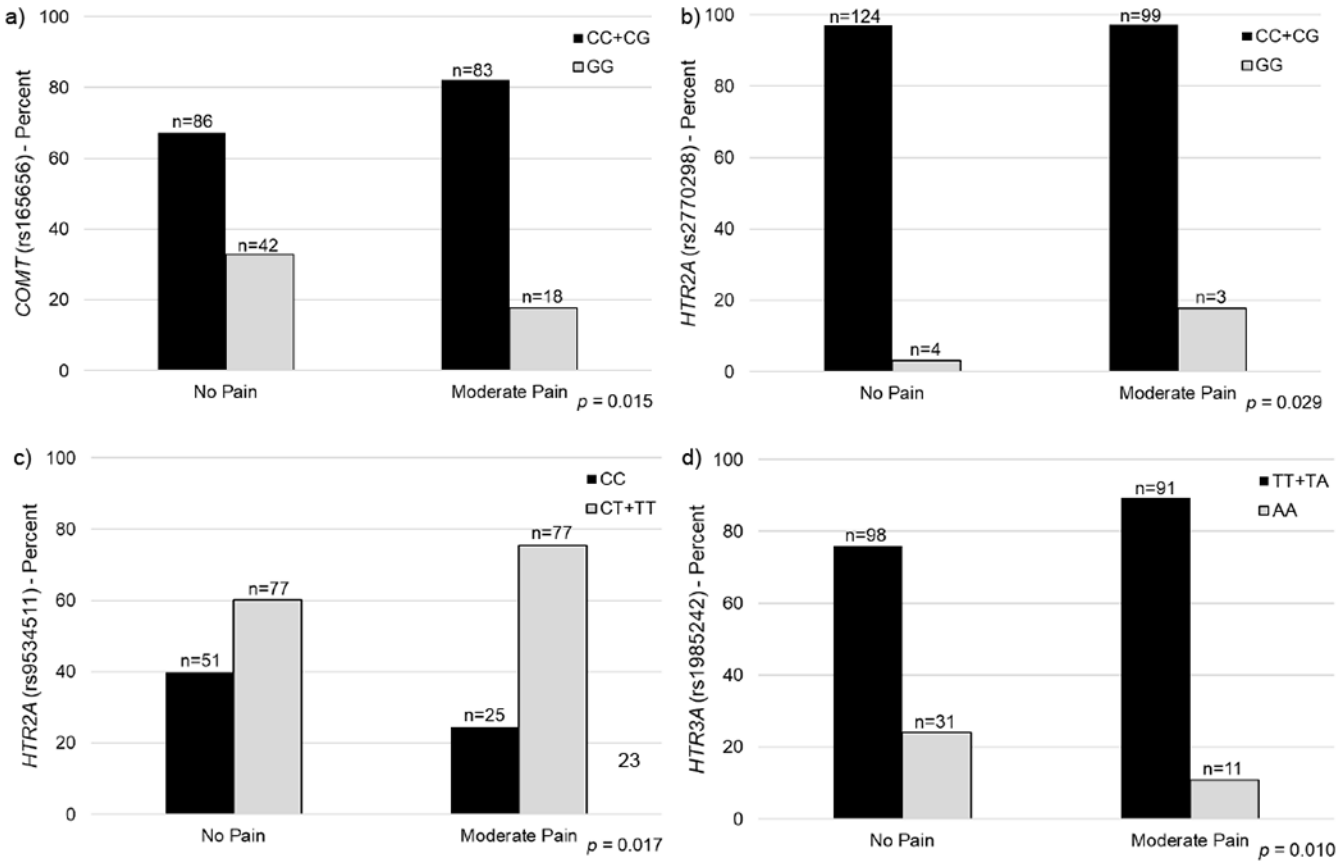

Figure 2.

a through d – Differences between No Arm Pain and Moderate Arm Pain classes in the percentage of patients who were homozygous for the common allele or heterozygous or homozygous for the rare allele for each significant polymorphism or number of doses of haplotypes identified. Values are plotted as unadjusted proportions with corresponding *p*-value.

Table 1.

Significant differences in demographic, clinical, and surgical characteristics between women in the No Arm Pain (n=164) and Mild (n=93) Arm Pain classes

Characteristics	No Pain	Mild Pain	Statistics
	Mean (SD)	Mean (SD)	
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
Karnofsky Performance Status (KPS) score	96.7 (6.8)	93.1 (10.0)	t=3.12; p=.002
Number of breast biopsies	1.3 (0.6)	1.6 (0.9)	U; p=.007
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
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)	
Occurrence of high blood pressure	35.4 (58)	22.6 (21)	FE; p=.036
Received neoadjuvant chemotherapy	8.0 (13)	23.7 (22)	FE; p=.001
Stage of disease			
Stage 0	24.4 (40)	18.3 (17)	
Stage 1	45.1 (74)	34.4 (32)	U; p=.008
Stage IIA and IIB	28.7 (47)	38.7 (36)	
Stage IIIA, IIIB, IIIC, and IV	1.8 (3)	8.6 (8)	
Pain in breast prior to surgery	15.0 (24)	35.2 (32)	FE; p<.0001
Strange sensations in affected breast	20.1 (33)	34.4 (32)	FE; p=.016
Axillary lymph node dissection	19.6 (32)	47.3 (44)	FE; p<.0001
Placement of surgical drain			
No drain	75.0 (123)	57.0 (53)	
Only in the breast	17.7 (29)	16.1 (15)	X^2=19.91; p<.0001
Only in the axilla	6.7 (11)	20.4 (19)	
Both in the breast and axilla	0.6 (1)	6.5 (6)	
Received biological therapy during the 6 months	5.5 (9)	17.2 (16)	FE; p=.004

Abbreviations: FE = Fisher's Exact; SD = standard deviation; U = Mann-Whitney U test; X^2 = Chi square test

Table 2.

Significant differences in demographic, clinical, and surgical characteristics between women in the No Arm Pain (n=164) and Moderate Arm Pain (n=137) Classes

Characteristics	No Pain	Moderate Pain	Statistics
	Mean (SD)	Mean (SD)	
Age (years)	58.0 (12.1)	52.9 (11.3)	t=3.74; p<.0001
Body mass index (kg/m^2)	26.1 (5.2)	28.1 (7.0)	t=−2.79; p=.006
Karnofsky Performance Status (KPS) score	96.7 (6.8)	89.3 (12.4)	t=6.27; p<.0001
Self-Administered Comorbidity Questionnaire (SCQ) 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
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
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)	
Ethnicity			
White	75.5 (123)	50.0 (68)	
Black	4.3 (7)	19.1 (26)	X^2=25.63; p<.0001
Asian/Pacific Islander	9.2 (15)	14.0 (19)	
Hispanic/mixed ethnic background/other	11.0 (18)	16.9 (23)	
Total annual household income			
<$30,000	15.4 (21)	29.9 (32)	
$30,000 to $99,000	44.1 (60)	42.1 (45)	X^2=8.44; p=.015
≥ $100,000	40.4 (55)	28.0 (30)	
Occurrence of anemia	4.9 (8)	11.7 (16)	FE; p=.034
Ever breast fed	54.0 (88)	41.6 (57)	FE; p=.037
Received neoadjuvant chemotherapy	8.0 (13)	31.4 (43)	FE; p=<.0001
Stage of disease			
Stage 0	24.4 (40)	11.7 (16)	
Stage 1	45.1 (74)	32.1 (44)	U; p<.0001
Stage IIA and IIB	28.7 (47)	40.9 (56)	
Stage IIIA, IIIB, IIIC, and IV	1.8 (3)	15.3 (21)	
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
Hardness in affected breast	14.0 (23)	24.1 (33)	FE; p=.037
Type of surgery			
Breast conserving	86.0 (141)	74.5 (102)	FE; p=.013
Mastectomy	14.0 (23)	25.5 (35)	
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

Characteristics	No Pain	Moderate Pain	Statistics
Placement of surgical drain			
No drain	75.0 (123)	48.9 (67)	
Only in the breast	17.7 (29)	13.1 (18)	X^2=43.15; p<.0001
Only in the axilla	6.7 (11)	27.7 (38)	
Both in the breast and axilla	0.6 (1)	10.2 (14)	
Received biological therapy during the 6 months	5.5 (9)	12.4 (17)	FE; p=.040
Received physical therapy during the 6 months	10.4 (17)	24.8 (34)	FE; p=.001

Abbreviations: FE = Fisher's Exact; kg = kilogram; m^2 = meters squared; SD = standard deviation; U = Mann-Whitney U test; X^2 = Chi square test

Table 3.

Multiple logistic regression analyses for *COMT, HTR2A, HTR3A,* and *TH* candidate genes and membership in the No Arm Pain (n = 129) versus Mild Arm Pain (n = 78) classes

Predictor	Odds Ratio	Standard Error	95% CI	Z	p-value
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: $X^2 = 45.49$, 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: $X^2 = 46.77$, 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: $X^2 = 57.51$, 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: $X^2 = 43.78$, p <.0001 $R^2 = 0.1697$					

Multiple logistic regression analyses of candidate gene associations with No Arm Pain versus Mild Arm Pain classes. 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). Predictors evaluated in each model included genotype (*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 underwent an axillary lymph node dissection.

Abbreviations: ALND = axillary lymph node dissection; 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 4.

Multiple logistic regression analyses for *COMT, HTR2A,* and *HTR3A* candidate genes and membership in the No Arm Pain (n = 129) versus Moderate Arm Pain (n = 102) classes

Predictor	Odds Ratio	Standard Error	95% CI	Z	p-value
COMT rs165656	0.37	0.166	0.153, 0.893	−2.21	0.027
KPS score	0.47	0.102	0.305, 0.719	−3.47	0.001
Preoperative breast pain	3.83	1.649	1.646, 8.906	3.12	0.002
Number of breast biopsies	1.86	0.466	1.141, 3.042	2.49	0.013
Surgical drain placement					
Breast only	0.95	0.466	0.360, 2.486	−0.11	0.910
Axilla only	10.46	6.067	3.353, 32.605	4.04	<0.0001
Breast and axilla	19.44	22.521	2.007, 188.276	2.56	0.010
Any physical therapy	2.94	1.408	1.150, 7.518	2.25	0.024
Overall model fit: $X^2 = 106.70$, $p < .0001$ $R^2 = 0.3581$					
HTR2A rs2770298	5.08	3.752	1.193, 21.613	2.20	0.028
HTR2A rs9534511	1.89	0.513	1.110, 3.217	2.34	0.019
KPS score	0.44	0.103	0.281, 0.698	−3.51	<0.0001
Preoperative breast pain	4.44	1.972	1.861, 10.602	3.36	0.001
Number of breast biopsies	1.84	0.460	1.131, 3.008	2.45	0.014
Surgical drain placement					
Breast only	0.90	0.455	0.334, 2.426	−0.21	0.835
Axilla only	9.27	5.389	2.965, 28.966	3.83	<0.0001
Breast and axilla	18.27	23.297	1.502, 222.344	2.28	0.023
Any physical therapy	3.25	1.602	1.239, 8.541	2.39	0.017
Overall model fit: $X^2 = 113.38$, $p < .0001$ $R^2 = 0.3800$					
HTR3A rs1985242	0.15	0.096	0.046, 0.520	−3.01	0.003
KPS score	0.44	0.104	0.280, 0.701	−3.48	0.001
Preoperative breast pain	3.76	1.650	1.593, 8.889	3.02	0.003
Number of breast biopsies	1.83	0.459	1.117, 2.988	2.40	0.016
Surgical drain placement					
Breast only	0.90	0.449	0.340, 2.395	−0.21	0.837
Axilla only	13.02	7.738	4.064, 41.733	4.32	<0.0001
Breast and axilla	26.33	30.918	2.637, 262.982	2.79	0.005
Any physical therapy	2.40	1.159	0.930, 6.183	1.81	0.070
Overall model fit: $X^2 = 114.11$, $p < .0001$ $R^2 = 0.3809$					

Multiple logistic regression analyses of candidate gene associations with No Arm Pain versus Moderate Arm Pain classes. 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). Predictors evaluated in each model included genotype (*COMT* rs165656: CC+CG versus GG; *HTR2A* rs2770298: CC+CG versus GG; *HTR2A* rs9534511: CC versus CT+TT; *HTR3A* rs1985242: TT+TA versus AA), functional status (KPS score in 10 unit increments), number of breast biopsies in the past year, placement of a surgical drain (no drain placed compared to drain placement only in the breast, drain placement only in the axilla, or drain placement in both in the breast and axilla), and receipt of physical therapy in the six months following surgery. Abbreviations: CI = confidence interval; *COMT* = catechol-O-methyltransferase; *HTR2A* = 5-hydroxytryptamine receptor 2A, G protein coupled; *HTR3A* = 5-hydroxytryptamine receptor 3A, ionotropic; KPS = Karnofsky Performance Status

Table 5.

Comparison of genomic markers for no pain versus mild or moderate pain in the breast versus the arm

Gene	SNP or Haplotype	Breast Pain	Arm Pain
NO PAIN VERSUS MILD PAIN			
ADRB2	rs2400707	↑	--
ADRBK2	Hap A04	↑	--
COMT	rs4633	---	↓
HTR2A	Hap B02	---	↓
HTR3A	rs10160548 rs1985242	↓ ---	--- ↓
SLC6A2	rs1566652	↑	---
TH	rs2070762	---	↑
TPH2	rs11179000	↑	---
NO PAIN VERSUS MODERATE PAIN			
COMT	rs165656	---	↓
HTR2A	rs2296972 rs2770298 rs9534511	↓ --- ---	--- ↑ ↑
HTR3A	rs1985242	---	↓
SLC6A2	rs17841327	↑	---
SLCA3	rs403636	↑	---

Abbreviations: *ADRB2* = beta-2-adrenergic receptor; *ADRBK2* = beta adrenergic receptor kinase 2; *COMT* = catechol-O-methyltransferase; Hap = haplotype; *HTR2A* = 5-hydroxytryptamine receptor 2A; *HTR3A* = 5-hydroxytryptamine receptor 3A; SNP = single nucleotide polymorphism; *SLC6A2* = solute-like carrier (SLC) family 6 member 2-noradrenaline transporter; *SLCA3* = SLC family 6 member 3-dopamine transporter; *TH* = tyrosine hydroxylase; *TPH2* = tryptophan hydroxylase 2

Legend: ↑ = increased risk for mild or moderate pain; ↓ = decreased risk for mild or moderate pain; --- = no genetic association identified